Male accessory gland infection and subfertility: a diagnostic challenge
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Chapter 10

Summary, conclusions and implications for future research

The number of leukocytes was assessed to discriminate between healthy and infected individuals. The diagnostic value of a positive culture for bacterial infection or a positive serology for salmonellosis was evaluated. The positive culture rate was 85% (CI: 0.74-0.93) for a positive culture indicating a high sensitivity. Serology positive rates were 58% (CI: 0.50-0.67). The sensitivity and specificity of the antibody test were 95% (CI: 0.87-0.99) and 98% (CI: 0.95-0.99), respectively. The efficacy of the antibody test for detecting M. avium-intracellulare was 95% (CI: 0.92-0.98).
SUMMARY

*Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Mycoplasma hominis* may cause non-gonococcal Male Accessory Gland Infection (MAGI). Convincing evidence that MAGI constitutes a common cause of male subfertility is not available. Detection, however, is important since these pathogens are sexually transmitted and may cause severe damage in the female genital tract.

Due to the paucity of clinical symptoms and the large variety of characteristics in the definition, the diagnosis of MAGI is a major problem in clinical practice.

This thesis presents the results from research on the accuracy of various laboratory and ultrasonographic tests in the diagnosis of MAGI compared to the urethral swab culture as reference strategy. In addition the prevalence and significance of actual and previous sexually transmitted pathogens in a male infertility population was studied. The aim of the thesis as outlined in Chapter 1, was to answer six questions.

Chapter 2 focuses on the first and part of the second question:

*What is the prevalence of sexually transmitted microorganisms in the genital tract of a male subfertility population?*

*What is the value of leukocyte count in semen in the diagnosis of bacterial MAGI, compared to urethral swab culture after digital prostatic massage as the reference strategy?*
To answer the first question, 184 men attending our Center for Reproductive Medicine were included in a prospective clinical study. All men underwent a culture of the urethra after digital prostatic massage since this is advocated as the most reliable way to detect sexually transmitted microorganisms. Furthermore, all men underwent serologic testing to detect a sexually transmitted active cytomegalovirus (CMV), Epstein-Barr virus (EBV) or hepatitis B virus (HBV) infection.

The prevalence of the different microorganisms was: *U. urealyticum* 36%, *M. hominis* 7.6%, *C. trachomatis* 0.5%, CMV 8.7%, EBV 0.5%, HBV 2.2%. The overall sexually transmitted bacterial infection was 39% and the overall sexually transmitted viral infection was 10.9%.

Urethral swab culture after digital prostatic massage is expensive and time consuming and is reported as a negative experience by most men. Men without clinical symptoms are not likely to undergo this test. The quantification of leukocytes as a less expensive and more acceptable test in the diagnosis of MAGI, was evaluated to answer the second question.

The number of leukocytes was assessed in three semen samples of all men included in the study. The diagnostic value of detecting leukocytes in sperm for identifying men with a positive culture for microbial infection or a positive serology for viral infection, was assessed with a receiver operating characteristic curve (ROC). The area under the ROC was 0.55 (95% CI, 0.46-0.63) for a positive culture indicating a bacterial MAGI. The area under the ROC was 0.56 (95% CI, 0.44-0.78) for a positive serology indicating a viral MAGI.

The sensitivity and specificity of leukocytospermia for detecting *U. urealyticum* infection were 65% (95% CI, 57-74) and 40% (95% CI, 28-54), respectively. The sensitivity and specificity of leukocytospermia for detecting *M. hominis* infection were 93% (95% CI, 88-
97) and 10% (95% CI, 4-21), respectively. The sensitivity and specificity of leukocytospermia in detecting a *C. trachomatis* infection could not be calculated because of the low prevalence of this organism.

There is a poor correlation between leukocytospermia and MAGI. Moreover, leukocytospermia itself is episodic and disappears over time, as is demonstrated by an intraclass correlation coefficient of only 0.38 (95%CI, 0.27-0.55).

The quantification of leukocytospermia in the routine semen analysis is therefore of no diagnostic value in selecting patients with bacterial MAGI who can benefit from antibiotic treatment. WBC count in the routine semen analysis cannot replace culture of the distal uterthra.

**Chapter 3** focuses on the third question:

*What is the value of the PACE2 DNA hybridization assay relative to a polymerase chain reaction in the diagnosis of *Chlamydia trachomatis* infection?*

Nowadays the polymerase chain reaction (PCR) is the reference standard to detect the presence of *C. trachomatis*. Preliminary studies using PCR for detection of *C. trachomatis* infections among healthy sperm donors and male members of subfertile couples reported prevalences of 16% to 39.3%. In the large study among male subfertility patients described in chapter 2 using the PACE 2 hybridization assay, we found a prevalence of only 0.5% of *C. trachomatis* infection. The question therefore arose whether this percentage reflected a genuine low prevalence among the study population or pointed to differences in accuracy between the PCR and the PACE2 hybridization assay detection methods.
Ninety-nine men enrolled the study. Eighty three men attending the Center for Reproductive Medicine and 16 partners of women who presented at the department of gynecology and who had tested positive for *C. trachomatis*.

The discriminative capacity of the PACE2 hybridization assay to detect a *C. trachomatis* infection in these men was as follows: sensitivity 100% (95%CI, 0.75-1), specificity 99% (95%CI, 0.94-1). When choosing a test for screening asymptomatic persons in a population with a low prevalence of infection, the positive predictive value of the test will be high when the specificity of the test is good. This study showed the widely used PACE2 hybridization assay to be an excellent test for the detection of *C. trachomatis*. Furthermore when large volumes have to be tested, costs become an important issue. The cost of the DNA hybridization assay is about half the cost of PCR.

Chapter 4 focuses on part of the second question:

*What is the value of cervical culture of female partners, in the diagnosis of bacterial MAGI, compared to urethral swab culture after digital prostatic massage as the reference strategy?*

To answer this question a prospective clinical trial was initiated. One hundred and eighty-four men were screened for the presence of *N. gonorrhoeae, C. trachomatis, U. urealyticum* and *M. hominis*. In none of the men *N. gonorrhoeae* was detected. *C. trachomatis* was present in two cases (1%), *U. urealyticum* in 67 cases (36%) and *M. hominis* in 14 cases (8%). The overall prevalence of bacterial infection was 39%. All female partners were
screened for the presence of *C. trachomatis*. Sixty-seven female partners (94%) of men with a positive culture for mycoplasmas were available for analysis. The sensitivity and specificity of the PACE2 DNA hybridization assay in women to detect a *C. trachomatis* infection in their male partners was 100% (95% CI: 0.16 to 1) and 100% (95% CI: 0.98 to 1) respectively. The sensitivity for the cervical culture for the detection of *M. hominis* and *U. urealyticum* in the male partners was 100% (95% CI: 0.95 to 1).

When choosing a test for screening asymptomatic persons in a population with a high prevalence of disease, the sensitivity of the test must be good.

In view of the high prevalence of *U. urealyticum* and *M. hominis* in the male genital tract and the role these sexually transmitted pathogens may play in infertility, one might consider whether all couples should be screened for the presence of these pathogens.

Transurethral swab culture after digital prostatic massage is disincentive to men. The cervical culture in their female partner, performed as part of the routine fertility work-up, is a suitable alternative to detect the presence of these microorganisms in the male genital tract.

**Chapter 5** focuses on part of the second question:

*What is the value of cytokine measurement in semenplasma in the diagnosis of bacterial MAGI, compared to urethral swab culture after digital prostatic massage as the reference strategy?*
To answer this question, semen of 30 subjects with MAGI was compared with semen of 23 men with a negative urethral swab culture after digital prostatic massage. The urethral culture was positive for *U. urealyticum* in 24 cases and *M. hominis* was cultured in 6 cases. Numerous studies clearly indicate that mycoplasmas are able to modulate the activities of monocytes/macrophages and natural killer cells, thus trigger the production of a wide variety of up and down regulating cytokines. Some authors found elevated concentrations of cytokines in seminal fluids of men with bacterial infections while other investigators found no differences in cytokine concentrations between infected and non-infected men. Comparison of these data is however hampered because different patient populations were studied and, in neither of these studies the relative contribution of the different bacterial species to cytokine concentration was analyzed. In this study we found that the levels of IL-6, IL-8, TNFalpha and IFNgamma were not significantly different in seminal plasma of men with a positive culture for *U. urealyticum* or *M. hominis* and of men who tested negative for these mycoplasmas (p>0.05). Measurement of these cytokines in seminal fluid is therefore of no value in selecting men with MAGI. A wide range of overlapping cytokine concentrations was found between the infected and control group, suggesting that the presence and concentrations of cytokines do not seem to be related to the presence of mycoplasmas in the male genital tract.

The absence of any inflammatory indication on the cytokine level, and the absence of genito-urinary complaints in the infected group, strongly point to the view that the presence of mycoplasma in the male urethra reflects colonization rather than infection.
Chapter 6 focuses on the fourth question:

*What is the role of scrotal ultrasonography with color Doppler flow in the diagnosis of varicocele?*

To answer the question a prospective clinical trial was initiated comparing the value of palpation, varicoscreen contact thermography and Color Doppler Ultrasonography versus spermatic venography in the diagnosis of varicocele.

The role a varicocele may play in male subfertility is still a subject of debate. The reference strategy to detect a varicocele is spermatic venography. The hypothesis was that SUS with color Doppler flow, because it is noninvasive, might be a preferable diagnostic tool.

Sixty-three men attending the Center for Reproductive Medicine were included in the study. The prevalence of varicocele in this group of subfertile men was 49%. Of the various non-invasive techniques only SUS with color Doppler flow had a good accuracy: sensitivity 97% (95%CI, 0.83-1) and specificity 94% (95%CI, 0.79-0.99).

SUS with color Doppler flow proved to be a good screening method to detect varicocele. We suggest that all male subfertility patients undergo a SUS with color Doppler flow.
Chapter 7 and 8 focus on the fifth question:

*What is the role of transrectal and scrotal ultrasonography in the diagnosis of MAGI?*

Both transrectal ultrasonography (TRUS) and scrotal ultrasonography (SUS) have proven their usefulness in the diagnosis of prostatic hyperplasia, prostatic carcinoma, testicular tumors and assessment of testicular volume. In male subfertility TRUS can be used to detect and evaluate the treatment of ejaculatory duct obstruction and to demonstrate congenital hypoplasia of the seminal vesicles.

Many ultrasonographic abnormalities like capsular thickening, calcifications, dilatation of the prostatic venous plexus, edema of the bladderneck, enlargement and cystic formation in the seminal vesicles are said to be characteristic findings in MAGI.

Before the accuracy of ultrasonography in the diagnosis of MAGI could be studied, an intra- and inter-observer variation of the above mentioned ultrasonographic abnormalities had to be determined.

Chapter 7 describes the results of an intra- and inter-observer study. Only criteria as calcifications, dilatation of the venous plexus and cysts had a good reproducibility.

Chapter 8 focuses on the accuracy of ultrasonography using the reproducible criteria in the diagnosis of MAGI.

One hundred and eighty-four men of subfertile couples were included in this prospective clinical trial. A subclinical bacterial infection which was diagnosed with the urethral swab culture after digital prostatic massage as reference strategy, was present in 39% of men.
Reproducible ultrasonographic features associated with MAGI were seen in 94.4% of men. None of these ultrasonographic features were associated with the presence of microorganisms in the genital tract. Ultrasonography of the male genital tract is therefore of no diagnostic value with regard to selection of men with MAGI.

Chapter 9 focuses on the sixth question:

_Does a previous Chlamydia trachomatis infection lead to anatomical changes in the male genital tract?_

To answer this question a group of 147 men attending the Center for Reproductive Medicine were asked to participate in this prospective clinical trial. A previous _C. trachomatis_ infection in women may cause severe damage in the female genital tract, leading to tubal infertility. The hypothesis was that a previous _C. trachomatis_ in men would lead to anatomical changes in the male accessory glands as seen with ultrasonography.

All men were tested for the presence of _C. trachomatis_ specific antibodies in serum. A previous _C. trachomatis_ infection was diagnosed in 47.6% of men. Transrectal ultrasonographic abnormalities were observed in 70% of men. Scrotal ultrasonographic abnormalities were observed in 65.3% of men. There was no correlation between any observed ultrasonographic features of the prostate, seminal vesicles and scrotal contents and a previous _C. trachomatis_ infection. The results of this study, however, do not exclude the possibility that a _C. trachomatis_ infection might lead to more discrete abnormalities, which cannot be detected with present day ultrasonography.
Conclusions:

1. There is a high prevalence of sexually transmitted mycoplasmas (*U. urealyticum*, *M. hominis*) in male subfertility patients and their female partners.

2. The prevalence of an actual *C. trachomatis* infection in subfertile males is low. However, half of the men have serologic evidence of a previous *C. trachomatis* infection.

3. MAGI can be diagnosed by culture of the male urethra after digital prostatic massage or cervical culture of their female partner.

4. Ultrasonography may be of use in the diagnosis of some conditions possibly related to male subfertility like varicocele, but is of no value in the diagnosis of MAGI.

5. A previous *C. trachomatis* infection does not lead to anatomical changes in the male genital tract that can be detected with present date ultrasonography.

Implications for further research

With the results of this thesis at hand, culture of bacterial microorganisms of the urethra after digital prostatic massage remains the cornerstone in the diagnosis of MAGI. However, recent studies report that the sensitivity and specificity of urethral swabs by nucleic acid tests like ligase chain reaction assay are greater than that of culturing urethral specimens (1,2). Urethral swab testing after digital prostatic massage is disincentive to men. Especially men without symptoms of an infection are not likely to be screened this way. Fortunately, nucleic acid detection methods are commercially available for the dia-
gnosis of *C. trachomatis* or *N. gonorrhoeae* in urine nowadays. These are almost as sensitive as those of urethral specimens (1,2). *C. trachomatis* can be subclinically present in the accessory glands as testes, epididymides, prostate and vesiculae seminales (3,4).

Theoretically, these pathogens might not be detectable in urine, but only in semen as being the ultimate "endproduct" of the male accessory glands.

Therefore, future studies should focus on nucleid acid tests that can be used in semen for the detection of *C. trachomatis, N. gonorrhoeae* and mycoplasmas in the genital tract without the need of obtaining urethral specimens.

So far, no consensus has been reached as to whether the presence of microorganisms like *C. trachomatis, U. urealyticum* and *M. hominis* may lead to male subfertility. Further research is needed to elucidate this matter. The prevalence of mycoplasmas in the male and female genital tract of patients attending an infertility clinic is high. Whether antibiotic treatment will improve pregnancy rate and pregnancy outcome has to be subject of a randomized placebo controlled clinical trial.

References


