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Effectiveness of antibiotic treatment in infertile patients with sterile leukocytospermia induced by tobacco use

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ABSTRACT

Our study investigated whether antibiotic therapy in infertile tobacco users with sterile leukocytospermia and a history of former sexual transmitted disease (STD) has an advantage over cessation of smoking alone. For this study, 80 male infertile smokers were divided into two subgroups. The first group numbered 40 patients treated with a two week course of doxycycline, 100 mg, twice daily, while the second group simply ceased smoking. A control group of 20 patients with chronic abacterial prostatitis (CPPS III a) were treated with the same antibiotic for two weeks. Patients from the first group exhibited significantly higher values in semen volume, total motility, and progressive motility of spermatozoa, when compared with the second group, two weeks post treatment. The latter two measures persisted during the 12 week follow up period. In both groups of patients, the correlation coefficient between the number of leukocytes and progressive motility was determined as statistically significant. However, in the control group the correlation was not statistically significant; however progressive motility, total motility, and vitality were significantly improved after a two week course of antibiotics. Our study implies that antibiotic treatment is perhaps effective in treating sterile leukocytospermia in smokers with a former STD and is a more effective treatment option in improving sperm parameters over smoking cessation alone.

Abbreviations: STD: sexual transmitted disease; CPPS IIIa: chronic abacterial prostatitis; PSA: prostatic specific antigen; EPS: expressed prostatic secretion; WBC: white blood count; LUTS: lower urinary tract symptoms; PPMT: pre-massage and post-massage urine test; VAS: visual analogue scale

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Introduction

Leukocytospermia, defined by the World Health Organization (WHO) as >1 million white blood cells per mL of semen, is found in about 20% of infertile men but its influence on semen quality is unclear [Lackner et al. 2006]. Numerous studies have demonstrated that leukocytes present in ejaculates have a physiological effect on sperm functions, which may further affect male infertility [Pentyala et al. 2007]. The origin of leukocytes in ejaculate is often unclear. They mostly originate from the epididymis [Wolf 1995] and are associated with infection. However, most infertile men attending andrology clinics do not have an apparent infection [Lackner et al. 2006]. Recent meta-analysis showed that toxins from tobacco smoking may potentially affect sperm development and function, resulting in a negative effect of semen parameters [Sharma et al. 2016]. Cigarette smoking may be associated with reduced fertility in males, which may possibly be attributed to decreased sperm concentration, lower sperm motility, and a reduced percentage of morphologically normal sperm [Asare-Anane et al. 2016]. However, not all studies have the same conclusions [Harlev et al. 2015]. Some authors have found positive effects of smoking on sperm motility [Lewin et al. 1991] and no association with DNA damage in sperm [Sergerie et al. 2000]. Significantly, even studies reporting the effect of smoking on semen parameters have not clearly proven any effect of smoking on male fertility [Marinelli et al. 2004] although a meta-analysis suggests an effect [Sharma et al 2016]. It is often assumed that leukocytospermia is an indication of an underlying genitourinary infection. However, it is questionable whether hidden urogenital infections could be simply one reason for the development of sterile leukocytospermia. Chlamydia trachomatis is the most common notifiable sexual transmitted disease (STD), and extragenital sites are believed to serve as hidden reservoirs of the continuing transmission of infection [Gratrix et al. 2015]. Thus, the possibility of undiagnosed Chlamydia infections may be a significantly

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influential, or at the minimum an additional, factor in the infertility of patients with sterile leukocytospermia. This study investigated whether antibiotic therapy in conjunction with the cessation of smoking may improve the semen parameters in infertile men with sterile leukocytospermia and a history of STD, when compared with smoking cessation alone.

Results

The average age of the patients was 35.69 ± 8.90 years (30 to 50 years). In both groups of patients, microbiologic cultures and microscopic analysis of urine confirmed the absence of infection and inflammation before and after prostatic massage; inflammation was present in the control group of patients. Nevertheless, none of the patients had a PSA level > 4 ng/mL [Pajovic et al. 2016]. During rectal palpation, patients reported the presence and degree of pain. The first group of patients reported slight discomfort or a low degree of pain during palpation (2.1 ± 1.1), while the second group of patients denied painful sensations; the control group of examinees initially reported moderate pain (6.1 ± 2.7), which persisted after the 12 week follow-up period (5.8 ± 1.5), with no significant difference (p>0.05).

Table 1 displays the mean values and standard deviations of the measured parameters of the two groups of patients, as well as of the first and control group prior to beginning the treatment, along with the parameters at the end of the treatment period and during the 12 week post-treatment follow up period. Initial values of sperm parameters (except sperm concentration), did not show a significant difference between the two groups of patients and control group of examinees. However, the table indicates some statistically significant differences between the two groups in total motility and progressive motility, and also between the first and control group in sperm concentration and vitality. Table 2 reveals a statistically significant negative correlation between the number of leukocytes and the total and progressive motility in the first and second groups of patients, while in the control group there was no significant correlation.

Table 2. Correlation coefficients between the initial number of leukocytes and the total and progressive motility in first, second, and control group of patients.

			Number of leukocytes (x10^6)
First group	Total motility (%)	Correlation Coefficient	-.682*
	Progressive motility (%)	Correlation Coefficient	-.683*
Second group	Total motility (%)	Correlation Coefficient	-.781*
	Progressive motility (%)	Correlation Coefficient	-.861*
Control group	Total motility (%)	Correlation Coefficient	-.476
	Progressive motility (%)	Correlation Coefficient	-.424

Group I: 40 patients who received antibiotic therapy and quit smoking; Group II: 40 patients who simply ceased smoking; Control group: 20 infertile, non-smoking patients with leukocytospermia and CPPS IIIa, treated with antibiotic therapy.
*Correlation is significant at the 0.01 level. With an asterisk (*) we marked parameters for which there were statistically significant differences in distribution.

Discussion

It is well known that leukocytospermia has multifactorial causes, including inflammation, with no bacteria growing

Table 1. Comparison of sperm parameters and white blood cells (WBC) between two groups and between first and control group, before and after applied treatment.

	Mean (SD)						
Group I	Semen volume (ml)	Sperm conc. (x10^6 per ml)	Total motility (%)	Progressive motility (%)	Vitality (%)	Morphology (normal forms %)	WBC (Mio/mL)
Initial	2.98 (0.81)	51.21 (20.46)	29.32 (5.05)	22.86 (4.78)	67.42 (7.48)	6.70 (1.21)	2.34 (0.33)
2 weeks	3.28 (0.70)[b]	54.68 (28.26)[a]	32.62 (5.93)[a,b]	24.20 (4.67)[a,b]	67.73 (8.12)	7.48 (1.93)	2.03 (0.41)
12 weeks	3.33 (0.69)	52.75 (25.76)	34.84 (5.48)[a,c]	26.42 (4.29)[a,c,D]	66.25 (8.07)	7.51 (2.0)	0.85 (0.7)[c]
p value	0.04	0.032	<0.05	<0.05			<0.05
Group II							
Initial	2.99 (0.84)	46.77 (11.08)	30.84 (6.68)	23.07 (6.37)	64.90 (6.79)	7.15 (1.30)	1.75 (0.47)
2 weeks	2.89 (0.72)	48.59 (15.53)[a]	28.99 (6.40)	21.34 (6.08)	65.94 (7.09)	6.97 (1.27)	1.69 (0.31)
12 weeks	3.21 (0.73)	70.60 (28.88)[a]	29.86 (5.41)	21.42 (3.58)	69.6 (8.03)[a]	7.45 (1.89)	1.79 (0.34)
p value		<0.05			0.041		
Control group							
Initial	2.98 (0.66)	60.90 (26.52)	28.09 (5.46)	20.14 (4.35)	68.5 (7.71)	7.0 (1.61)	2.85 (1.12)
2 weeks	3.14 (0.78)	49.55 (19.32)[a]	32.70 (6.41)[a]	24.63 (5.70)[a]	65.53 (7.35)[a]	7.32 (1.65)	2.91 (1.34)
12 weeks	3.21 (0.73)[a]	70.60 (28.88)[a,D]	29.86 (5.41)	21.42 (3.58)	69.6 (8.03)[D]	7.45 (1.89)	1.10 (0.71)[a]
p value	0.35	<0.05	0.042	0.023	<0.05		0.042

The first and control group: antibiotic therapy; the second group: only cessation of smoking. Semen was analyzed at the time zero, two weeks and 12 weeks afterwards. Group I: 40 patients who received antibiotic therapy and quit smoking; Group II: 40 patients who simply ceased smoking; Control group: 20 infertile, non-smoking patients with leukocytospermia and CPPS IIIa, treated with antibiotic therapy. [a]Statistically significant difference compared with initial values among patients in the same group. [b]Statistically significant difference between Group I and Group II after 2 weeks treatment period. [c]Statistically significant difference between Group I and Group II after 12 weeks treatment period. [D]Statistically significant difference between Group I and Control group after 12 weeks treatment period.

in reproductive tract fluids (semen, urine, EPS). In addition, the presence of leukocytes is a poor predictor for the presence of bacteria [Rodin et al. 2003]. According to a previous study [Pentyala et al. 2007], the etiology of non-infective pyospermia can be classified into several categories: varicocele, chronic prostatitis, smoking, drug abuse, alcohol, toxins, abstinence, and several others. The latest studies showed that smokers have significantly lower semen volumes, sperm concentrations, sperm motility, total sperm counts, and significant sperm morphologies. Smokers are at a higher risk of developing oligozoospermia, asthenozoospermia, and teratozoospermia ($p<0.05$) than non-smokers [Asare-Anane et al. 2016]. Our study relied on smoking and hidden urogenital infection as a potential cause of leukocytospermia and whether antibiotic therapy could have a better impact on sperm parameters when compared to smoking cessation alone. As a further test of our hypothesis, we used a control group of patients with chronic abacterial prostatitis in order to compare the effect of antibiotic treatment on semen. According to our results, patients from the first group, treated with antibiotic therapy, showed significantly higher values of semen volume, total motility, and progressive motility, over the second group, initially after the two-week treatment period. However, after the 12 week follow-up period, total motility and progressive motility were still significantly higher in the first group, where patients received a two week course of doxycycline, compared with the second group of patients, whose treatment consisted of cessation of smoking alone. Nevertheless, patients from the second group exhibited WBC in a concentration above 1 Mi/ml, 12 weeks after the treatment, which significantly differed from patients within the first group. It is important to mention that, among patients in the second group, sperm concentration and vitality significantly improved after smoking cessation, at the end of the 12 week follow-up period, but total motility and progressive motility exhibited no significant change, as well as WBC count. This means that over extended periods, antibiotic treatment could be more effective than smoking cessation alone, although patients had no proven urogenital infection. The contemporary literature shows conflicting data, which do not always resemble our results. Recent meta-analysis has shown that cigarette smoking acts initially on bio-functional parameters, whereas the effects of cigarette smoke on conventional parameters require a longer time [Sharma et al. 2016; Harlev et al. 2015] as well as the effects after cessation of smoking. Nevertheless, cessation of smoking should certainly be recommended to any male smoker, especially if he is trying to conceive with his partner [Harlev et al. 2015].

Treating patients with non-infective leukocytospermia with different antibioticregimens could reduce the white blood cell count in the semen and improve male fertility only temporarily, with no evidence of long-term effects [Jarvi and Noss 1994]. There is evidence that doxycycline alone or in combination with ceftriaxone could significantly and equally improve the white blood cell counts in men with leukocytospermia [Erel et al. 1997]. Nevertheless, it is doubtful that antibiotic therapy should be recommended for asymptomatic leukocytospermia, since no study confirmed complete treatment of leukocytospermia, i.e., the count below the limit of one million WBC/ml. On the one hand, our study may provide evidence of a long-term positive antibiotic effect on male fertility potential, although we did not determine fertility rates in the examinees. On the other hand, clinical trials have shown no significant beneficial effect of doxycycline because of high rates of spontaneous resolution in untreated leukocytospermic men and the relative toxicity of antibiotics on spermatogenesis and spermatozoa function [Pentyala et al. 2007]. Nevertheless, some authors found [Yanushpolsky et al. 1995] no positive effects of different 14-day courses of antibiotics (doxycycline, trimetoprim-sulfomethoxasole) over no treatment trials, in patients with leukocytospermia of unknown etiology. However, they did not declare whether they included smokers, or patients with other chronic, non-infective, inflammatory genitourinary diseases; also, the followup period was only four weeks, which is likely too short a time frame to observe semen improvement. Close et al. [1999] reported an increased number of leukocytes and a significant decrease in penetration assay score in smokers with leukocytospermia. Nevertheless, they reported no decrease in sperm count or motility, with no difference in prevalence of antisperm antibodies compared to non-users. This is the opposite of our results, where a significantly negative correlation between number of leukocytes and total and progressive motility, which indicates a clear negative influence of leukocytospermia on sperm parameters, was observed.

When we compared the first group of patients and the control group, after two weeks of antibiotic treatment and 12 weeks afterwards, we realized that in the first group of patients significant improvements were seen regarding progressive motility and total motility at the end of the treatment as well as 12 weeks afterwards, while in the control group only semen volume and sperm concentration were improved after the 12 week follow-up period. Although, progressive motility, total motility, and vitality were significantly improved after a two week course of antibiotic treatment, but without significant, steady, long-term improvement. This probably advocates the attitude of long-term antibiotic regimen for patients with chronic prostatitis (CPPS), instead of shorter therapy time [Weidner 2004]. Nevertheless, improvement in sperm

parameters was evident after a short course of antibiotic therapy, although not in sperm motility. Also, antibiotic treatment could only be an additional therapy for patients with CPPS, since they still had symptoms of lower urinary tract symptoms (LUTS) after the antibiotic trial. The main purpose of our study was to examine whether antibiotic treatment could have a positive impact on sterile leukocytospermia. The first and the control groups of patients had positive responses to antibiotic treatment. However, one significant difference between them consisted of sperm concentration and progressive motility. Also, the correlation coefficient between the number of leukocytes and total and progressive motility was not significant in the control group.

It is still unclear why smokers from the first group and examinees from the control group responded positively to doxycycline treatment. It is evident that antibiotic therapy has advantages over simple smoking cessation in regard to semen parameters and improvement of leukocytospermia, but it is questionable whether other antibiotic regimens (e.g., fluoroquinolones) would have the same impact on non-infective leukocytospermia. The possibility of hidden Chlamydia infection, as a potential influential factor in infertility of patients with sterile leukocytospermia, was prompted after isolation of Chlamydia trachomatis from a gonad biopsy specimen in a man with sterile leukocytospermia [Hartmann et al. 1986]. Anamnestic data from our examinees that confirms a previous urogenital infection supports this presumption. However, more comprehensive studies are warranted.

In summary, antibiotic therapy appears effective and more reliable for treating sterile leukocytospermia in infertile smokers over smoking cessation alone. It may be effective in other non-infective cases of male infertility where leukocytospermia is presented. What the exact mechanism by which antibiotics could improve sperm parameters in absence of bacteria is still controversial, but possible hidden infections, non-detectable in conventional microbiological tests, could be basic promoter. Further analysis and more comprehensive microbiological tests should be incorporated in every day praxis to resolve this enigma.

Materials and methods

This study was performed using 80 infertile tobacco users (aged 30 - 50 y) with leukocytospermia. Each subject signed the acceptance of the study protocol consent form, in which the Ethical Principles for Medical Research Involving Human Subjects (The Helsinki Declaration) were clearly stated. The patients were divided into two groups, according to treatment regimes. The first group of 40 patients received antibiotic therapy and also quit smoking, while the second group of 40 patients simply ceased smoking, according to anamnestic data. Patients were suspected of suffering hidden urogenital infection, since each patient experienced at least one episode of a sexually transmitted disease during their lifetime. A control group of 20 infertile, non-smoking patients with leukocytospermia, classified as having Category IIIa CPPS, according to the National Institutes of Health (NIH) were also included. They were treated with antibiotic therapy and their semen values were compared with the semen of the other groups of patients, to investigate potential correlations between pre- and post-treatment semen analysis. All patients were followed-up and semen parameters were measured before treatment, at the end of the two w treatment trial, and at 12 w post-treatment (follow-up period).

Patients were interviewed about their clinical and family history, encompassing the most common risk factors in male infertility including diabetes, hypertension, allergy, cancer, etc. [Collodel et al. 2009]. All patients had a minimum of a three-year history of infertility. The information on smoking habits included the number of cigarettes smoked daily and the number of years of smoking. We considered 'smokers' to be those who had smoked cigarettes for > 5 years and 'non-smokers' to be those who had never smoked [Collodel et al. 2009]. All 'smokers' smoked at least 10 cigarettes per d. Semen analysis of the participants in the first and control groups were performed before and after antibiotic therapy and compared with semen of the patients from the second group, after cessation of smoking. According to our prediction, of hidden *Chlamydia trachomatis* infections, we used polymerase chain reaction (PCR), nucleic acid amplification techniques, and prepared sperm cultures for direct visualization of specific inclusions, and, by doing so, the specificity of the tests increased to 100%, instead of 98% [Pajovic et al. 2013]. We searched mainly for Chlamydia germs and semen samples were collected during the period from September 2010 to May 2015. Patients had been at least one y without clinical or subjective signs of urogenital infection.

Inclusion and exclusion criteria

Eligibility criteria for each patient's inclusion in study groups were: history of infertility which had lasted for at least three y; smoking at least 10 cigarettes per d for >5 years; sterile leukocytospermia; and at least one episode of urogenital infection during the patient's lifetime, however none within last year. We excluded patients using the following criteria: clinical varicocele; urogenital infection within the last year; evidence of bacteria in seminal culture tests; chronic prostatic inflammation; LUTS; PSA values > 4 ng/ml [Pajovic et al. 2016]; chromosomal abnormalities; sperm autoantibodies; testicular tumors; systemic diseases

(including diabetes mellitus and autoimmune diseases), and exposure to irritants, toxins, or alcohol abuse.

Examinees from the control group were non-smokers, with LUTS and proven CPPS (IIIa). Prior to beginning the antibiotic treatment or smoking cessation and after 5 d of sexual abstinence, patients provided a spermogram sample through self-administered masturbation. A subsequent sample was given two and 14 w after initiation of the antibiotic therapy or cessation of smoking, observing a 5 d abstinence rule, used to provide the first sample [Pajovic et al. 2013].

Spermogram

After 5 d of sexual abstinence, patients provided a sperm sample through self-administered masturbation, collected into sterile sample cups without the use of a lubricant. Samples were taken in our facility. The sperm samples were analyzed using a Sperm Quality Analyzer (SQA IIC-P; Medical Electronic Systems, Perchtoldsdorf, Austria), after 20 min of liquefaction [Pajovic et al. 2013]. Sperm concentration, progressive motility, total motility, viability. and morphology were determined. The reference values used, with the lower reference limit (95% confidence intervals) of above mentioned semen parameters, were taken from the criteria given by the EAU guidelines [Jungwirth et al. 2012].

Volume

The volume of the ejaculate, primarily determined by the accessory sex gland's function, was measured in graduated tubes. The reference limit used was > 1.5 ml [WHO 2010].

Leukocytospermia

Leukocytospermia was diagnosed by counting the leukocytes (WBC) in the chamber after treatment with hydrogen peroxide on the enzymes of the leucocytes, in haematoxylin – eosin stained sections. The reference number of leucocytes was < 10^6 per ml (< 1.00 Mio/mL). All semen samples where the number of leucocytes was above the baseline were considered to be evidence of leukocytospermia [WHO 2010].

Microbiological analysis

Microbiological tests included taking urethral smears and a sample of semen. The main focus was on possible leukocytospermia caused by *Chlamydia trachomatis*, but we also considered *Ureaplasma urealyticum* and *Mycoplasma genitalium* as possible hidden causes of leukocytospermia. Any pathogens were identified using the PCR nucleic acid amplification technique, with a specificity of 98%. Since we looked for asymptomatic Chlamydial leukocytospermia, we prepared sperm cultures for direct visualization of specific inclusions [Pajovic et al. 2013]. We used a real–time PCR system (Applied Biosystems 7300 Real Time PCR System, California 94404, USA), for antigen detection. Once again, a five d abstinence rule was used when providing the samples.

Prostate examination and pre-massage and post-massage urine test (PPMT)

Patients were carefully examined by inspection and palpation of external genitalia, groin, perineum, coccyx, external anal sphincter (tone), and internal pelvic floor and side walls for prominent areas of pain or discomfort. Digital rectal examination was performed after the patient had produced preprostatic massage urine specimens [Nickel et al. 2006]. The level of pain during examination was evaluated using a visual analogue scale (VAS, 1-10) [Teillac et al. 2004]. Prostate cancer was excluded both clinically and serologically. The total PSA was determined in all examinees before treatment.

Segmented urine collection was used and the PPMT was made according to the '2–glass test' method [Nickel et al. 2006]. Expressed prostatic secretion (EPS) was available and microscopy and culture of secretion was performed as described by Maeres and Stamey [1968].

Antibiotic therapy

Patients were treated with an antibiotic therapy according to recommendations from the EAU and from previous studies, concerning leucocytospermia of unknown etiology [Johansen et al. 2011; Yanushpolsky et al. 1995; Berger et al. 1983]. The first group of patients received a 14 d course of oral doxycycline, 100 mg twice per d [Yanushpolsky et al. 1995]. The same treatment regimen was applied in the control group [Berger et al. 1983]. Semen samples were analyzed after treatment and 12 w post treatment.

Statistical analysis

Statistical analysis started with the evaluation of parameters for normality of distribution using the Shapiro-Wilk normality test. We applied Independent Sample t-Tests for parameters that are normally distributed and Independent sample Kruskal - Wallis tests for the other, non-parametric samples, in order to see whether the parameters distributions were the same across categories. The significance level was set to 0.05. We also calculated correlation coefficients between the number of leukocytes and total and progressive motility among these groups of patients using Sperman's

rank correlation, with consideration that the number of leukocytes is not normally distributed.

Declaration of interests

Authors did not have any support funding of the paper. The authors of this paper hereby declare that they have not received nor shall receive any financial benefits from publishing the paper, neither have they received any financial incentive from a third party. We, the authors, hereby solemnly declare that we are not in any situation which could give rise to a conflict of interest.

Notes on contributors

Each author has contributed to the research process. Conceived and designed the experiments: PB, VM; Performed the experiments: PB, VM; Analyzed the data: PLj. Wrote and revised the paper, read and approved the final manuscript: All authors.

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