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ASSESSMENT IN THE DIAGNOSIS OF MALE CHRONIC GENITAL TRACT INFECTION

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Abstract Different methodologies have been proposed to interpret the microbiological findings associated with contaminating, indigenous microbiota of the anterior urethra. In order to solve the controversy related to the diagnosis of chronic seminal infections in asymptomatic young adults, the results applying Stamey and Meares' criteria were compared with those obtained when semen cultures were studied for significant bacteriospermia. A total of 218 consecutive asymptomatic male partners of infertile couples were evaluated by the four-specimen technique described by Stamey and Meares' with the addition of semen (SM). Infection was detected in 46% by SM, while semen cultures (SC) showed a prevalence of infection of 41%; 73 patients were positive by both criteria and 102 negative; 27 patients were positive by SM technique in prostate fluid while their semen cultures were negative; 16 patients had positive semen cultures and were considered negative by SM. The kappa statistic indicated a good degree of agreement between both methodologies ($\kappa = 0.61$, $z = 8.68$, $p < 0.001$). The estimated risk of being considered negative attributable to the semen culture (27 patients) was 25% (attributable risk = $\gamma_{SC^-} = 0.2550$), and of being considered positive attributable to the semen culture (16 patients) was 26% ($\gamma_{SC^+} = 0.2579$). The 95% confidence limits were estimated in 12 to 39%, and in 13 to 31%, respectively. In view of these results, to establish the diagnosis of chronic prostatitis, the addition of prostatic fluid or voided urine cultures after prostatic massage, must be performed. Semen culture confronted with first-voided urine avoid overestimating seminal infection.

Resumen ***Evaluación del diagnóstico de las infecciones crónicas del tracto genital masculino.*** Diferentes metodologías han sido propuestas para evitar las causas de error más importantes en la interpretación de los cultivos microbiológicos; es decir la presencia de la flora uretral normal y colonizante. Para determinar acuerdos y desacuerdos en el diagnóstico de las infecciones seminales crónicas en pacientes asintomáticos, los resultados obtenidos aplicando los criterios de Stamey y Meares' fueron comparados con aquellos logrados a través del cultivo del semen sólo. Se evaluaron 218 pacientes consecutivos de parejas infértiles, por la técnica de las 4 muestras descripta por Stamey y Meares', con el agregado de semen (SM). La infección fue detectada en el 46% por SM, mientras que el cultivo de semen (SC) mostró una prevalencia del 41%. Fueron positivos por ambos criterios 73 pacientes y otros 102 fueron negativos. Con la técnica de SM, 27 pacientes tuvieron el líquido prostático positivo y los cultivos de semen negativos. Dieciséis pacientes presentaron los cultivos de semen positivos y fueron considerados negativos por SM. La kappa estadística indicó un buen grado de acuerdo entre ambas metodologías ($\kappa = 0.61$, $z = 8.68$, $p < 0.001$). El riesgo estimado de considerarlos negativos al estudiar el semen sólo (27 pacientes) fue del 25% ($\gamma_{SC^-} = 0.2550$) y de considerarlos positivos (16 pacientes) fue del 26% ($\gamma_{SC^+} = 0.2579$). El límite de confianza del 95% fue estimado entre 12 a 39% y entre 13 a 31%, respectivamente. Los resultados obtenidos muestran que el dignóstico de certeza de prostatitis crónica, requiere el cultivo del líquido prostático o de la orina post masaje prostático. El agregado de la primera porción de orina al cultivo de semen evita sobrestimar infección seminal.

Key words: bacteriospermia, semen culture, chronical bacterial prostatitis

Although detailed microbiological diagnostic procedures are routinely carried out in male chronic genital tract infection, the reliable diagnosis and localization of infection is often difficult. Different methodologies have been proposed to overcome the most important problem related to the interpretation of the microbiological findings, that is, the presence of contaminating, indigenous microbiota. The ideal specimen should be uncontaminated, and this only can be achieved through invasive techniques such as biopsies, and needle aspiration biopsies, from where tissue samples are cultured. As most patients refuse these proceedings, the only way to obtain a diagnosis is by means of two indirect methods such as the four-specimen technique described by Stamey and Meares[1, 2] or semen culture[3, 4].

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Stamey and Meares' sustain that quantitative bacteriological cultures clearly localize pathogenic bacteria in the prostate when they are confronted with culture of the first-voided 10 ml of urine[1, 2]. This method is still considered by many to be the "gold standard" for localizing infection to the prostate gland in spite of its labor intensity and overall costs[5]. Mobley, subsequently demonstrated the usefulness of semen cultures in the diagnosis of prostatic infection, and he stated that the correlation between prostatic secretion culture and semen culture is excellent when the urine is sterile[6]. Other authors, such as McGowan et al.[4], Witkin et al.[7], Naessens et al.[8] and Jarvi et al.[9], usually based the diagnosis of significant bacteriospermia in infertile men through semen cultures.

Therefore, it is important to assess the role of urethral microbiota in male chronic seminal infection, and their participation in the misinterpretation of genital cultures. In order to determine the best and most specific method for the detection of chronic seminal infection in young adults, which is usually asymptomatic and insidious, a prospective study was carried out using the four-specimen technique with the addition of semen (SM) to evaluate the agreements and disagreements between the results applying Stamey and Meares' criteria with those obtained when the semen culture is performed according to McGowan for significant bacteriospermia[4].

Materials and Methods

Patients: A group of 218 asymptomatic male partners of infertile couples were evaluated. Their age ranged from 20 to 38 years with a median of 26 years. The patients were studied according to the following procedure: they were asked to produce the samples in the laboratory after three days of sexual abstinence and should have full bladder and desire to void. Prostatic massage procedures were carried out by a physician and semen samples were obtained by masturbation.

Hands and penis were washed with bactericidal soap and rinsed with sterile water. Full retraction of foreskin throughout all collection was maintained and the following samples were obtained: first 10 ml voided urine (VB_1), midstream aliquot (VB_2), expressed prostatic secretions from prostatic massage (EPS), first 10 ml of urine voided immediately after prostatic massage (VB_3), and semen (S). None of the subjects presented urethral discharge or urinary tract infections at the time of cultures.

Laboratory procedures. All specimens were cultured within one hour in the following way: –Qualitative identification of microorganisms was performed from the surface of plates streaked on the following media: chocolate agar, Thayer-Martin agar, V medium (incubated in CO_2 enriched atmosphere), blood agar, eosin methylene blue agar, Sabouraud agar (incubated in air at 35°C), and supplemented Brucella agar (incubated in anaerobic atmosphere). About 0.1 ml of each sample was placed into supplemented thioglycolate medium and brain heart infusion broth, incubated in anaerobic atmosphere and air respectively, up to 10 days. Gram and Giemsa stains were obtained for all specimens and Ziehl Neelsen only for semen.

All samples were assessed for normal genital tract organisms and sexually transmitted pathogens, including aerobic and anaerobic bacteria, fungus, yeasts, and *Ureaplasma urealyticum* by standard procedures[10, 11, 12, 13].

For quantitative colony counts 0.1 ml of VB_1, VB_2, EPS, VB_3 and S (dilution 1/2 with saline solution), and dilutions up to 10^{-3} were mixed with melted blood agar, with the addition of 2-3 drops of sheep blood, and poured into Petri dishes. They were incubated for 5 days at 35°C in aerobic and anaerobic conditions. On the other hand, 0.2 ml of VB_1, EPS or VB_3 and S were mixed with 1.8 ml of Mycoplasma urease broth. Dilutions up to 10^{-3} were made and incubated for 7 days at 35°C in CO_2 enriched atmosphere.

The concentration of seminal polymorphonuclear neutrophil white blood cells (PMN) was measured by the peroxidase stain method according to WHO[3]. The threshold of 0.5 x 10^6 PMN/ml was used to define leukocytospermia[14].

Definitions: prostatic fluid or semen were considered infected if: 1. their colony counts were ≥ 1 $\log_{10}$ with regard to VB_1, or 2. EPS was not obtained, VB_3 colony counts were ≥ 1 $\log_{10}$ with regard to VB_1, including the prostatic dilution factor.

According to McGowan[4] a semen culture alone was considered positive (SC^+), when its colony count was $\geq 10^3$ CFU/ml of pathogenic bacteria or $\geq 10^4$ CFU/ml of non-pathogenic bacteria, and only one specie was isolated.

Statistical analysis: The kappa statistic (Cohen, 1968)[15] was used as a measure of interrater agreement and the degree of agreement between both laboratory methods was determined with a one-tailed rater independence test, assuming kappa equal zero (Fleiss, Cohen & Fueritt, 1969)[15]. The Levin's attributable risk[15] was estimated for both risk situations: to consider a patient positive when he is not and viceversa. A 95% confidence interval for the attributable risk was calculated for each situation. The risk factor indicates how much the occurrence of a result would be reduced when this risk can be avoided.

Results

A total of 218 patients were studied. Infection was detected by the Stamey and Meares' methodology (SM^+) in 46% (100 patients): 44 localized in prostate gland, and 56 in other sites of the male genital tract different from the urethra.

Leukocytospermia was found in 54/100 infected patients, as well as in 17/118 non infected ones. When the results of 218 semen cultures according to McGowan (SC^+) were analyzed, a prevalence of infection of 41% (89 patients) was found. By both criteria 73 patients were considered positive (SM^+ and SC^+) and 102 negative (SM^- and SC^-).

Twenty seven patients (SM^+ and SC^-) had positive cultures in EPS or VB_3 with colony counts ≥ 1 $\log_{10}$ than VB_1 (SM^+), but their semen cultures were considered negative (SC^-) with colony counts $< 10^3$ CFU/ml (pathogenic bacteria) or $< 10^4$ CFU/ml (non-pathogenic bacteria). Sixteen patients (SM^- and SC^+) with colony counts $> 10^3$ CFU/ml of pathogenic bacteria or $\geq 10^4$ CFU/ml of non pathogenic bacteria, had equal or more colony counts in VB_1, and for this reason they were considered negative according to Stamey and Meares' criteria (SM^-). Table 1 shows the agreements and disagreements between both criteria in cultures of 218 patientes studied.

TABLE 1.– Agreements and disagreements between the results obtained by Stamey and Meares' technique and semen culture alone in 218 patients (k = 0.61, p < 0.001)

	SM^+	SM^-	Total
SC^+	73	16	89
SC^-	27	102	129
Total	100	118	218

SM^+: Positive results by Stamey and Meares' technique with addition of semen.
SM^-: Negative results by Stamey and Meares' technique with addition of semen.
SC^+: Positive results by semen culture.
SC^-: Negative results by semen culture.

The value obtained for the Kappa statistic was κ = 0.61 and resulted statistically significant (z = 8.68, p < 0.001). It indicates a good degree of agreement between both criteria. The estimated risk of being considered negative attributable to the SC criterion (27 patients) was 25% (attributable risk = γ_{SC^-} = 0.25250) and of being considered positive attributable to the SC (16 patientes) was 26% (γ_{SC^+} = 0.2579).

The 95% confidence limits were estimated in 12 and 39% for SC^-, and in 13 and 31% for SC^+.

Discussion

Semen cultures and the four-specimen technique described by Stamey and Meares' with the addition of semen are useful for microbiological diagnosis in asymptomatic young men of infertile couples. These are indirect reflections of the bacteriological milieu of the prostate and other sites of the male genital tract, and only through rigid adherence to both diagnostic regimes, these infections can be diagnosed[16, 17].

Cultures of tissue samples obtained by prostatectomy assess directly the bacteriological status of the prostate[16] but they are not feasible in these patients. The needle aspiration biopsy is useful for assessing prostatic inflammation, but not always for detecting microorganisms because of focal involvement of the gland[16, 17].

The authors have been involved in the diagnosis of seminal infections in infertile men since 1979. Previously a prevalence of infection around 45% had been observed in a large population of infertile men[18]. *Enterococcus faecalis* was the most frequent etiological agent followed by *Ureaplasma urealyticum.* At the present time *U. urealiticum* is the principal etiologic agent observed (unpublished data).

The finding of *Chlamydia trachomatis* in semen samples by immunofluorescence does not allow differential colony counts, therefore its diagnosis and localization were not considered in this paper. However, this microorganism must always be investigated.

According to the results obtained we found significant agreement between both criteria (κ = 0.61, z = 8.68, p < 0.001). Other authors described similar rates of infection such as McGowan, 36% in sub-fertile males[4], and Witkin, 48% in asymptomatic patients[7].

Although Mobley reported that semen cultures alone can be used to establish or to eliminate a diagnosis of bacterial prostatitis[6], we have shown that 27/100 patients SM^+ and SC^- had positive cultures in EPS or VB_3, which were not detected by SC. The 95% confidence limits of attributable risk SC^- (γ_{SC^-} = 0.2550), indicated that between 12 and 39% of all patients would be considered negative when the semen culture is performed exclusively. When the semen is used in the diagnosis of prostatitis, according to Meares' the clinician must simultaneously examine urethral and bladder samples to rule out inflammation or infection at these sites[2].

On the other hand 16/89 patients SC^+ were considered SM^- because their counts in VB_1 exceeded by tenfold or more the counts in their semen. The 95% limits of attributable risk to SC^+ (γ_{SC^+} = 0.2579) indicated that between 13 and 31% of patients would be considered positive when semen culture is performed.

The addition of the first voided urine to semen culture does not increase significantly the overall costs and labor intensity, but could avoid overestimating seminal infections and unnecessary prolonged antibiotic therapy[19]. In view of these results, we consider that quantitative cultures of semen and VB_1, should be performed.

Leukocytospermia was not always associated with infection. We found and inflammatory reaction in 54% of infected patients and in 14.4% of non-infected ones. We believe that leukocytospermia (defined by PMN peroxidase +) is a poor marker of infection. Gorelick et al. found that only 18% of their patients with positive prostatic cultures of tissue samples presented an inflammatory reaction[16]. In view of these results, leukocytospermia should not be used as a criterion of exclusion to avoid performing a microbiological study of the seminal tract in these patients.

We conclude that semen culture confronted with the first voided urine, with rigid adherence to laboratory procedures, is a better method than semen culture alone to detect male genital tract infections. When a chronic prostatitis is suspected or when it is necessary to establish or to discard this diagnosis, cultures of prostatic fluid or voided urine after prostatic massage, must be performed.

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Scepticaemia: An uncommon generalised disorder of low infectivity. Medical school education is likely to confer life-long immunity.

Escepticemia: Una poco común afección generalizada de baja infectividad. La educación en la escuela de medicina es proclive a conferir inmunidad de por vida.

Petr Skrabanek and James McCormick

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