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ORIGINAL ARTICLE

Persistence of ultrasound alterations after antibiotic treatment with levofloxacin in patients with male accessory gland infection

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No studies have evaluated the ultrasound features of the male sex accessory glands in infertile patients with bacterial male accessory gland infection (MAGI) according to the microbiological outcomes of bacterial cultures (absent, partial or complete) following antibiotic therapy administration. Therefore, the aim of this study was to evaluate the ultrasound characteristics of the prostate, seminal vesicles, and epididymal tracts after treatment with levofloxacin (a common quinolone antibiotic), in patients with infections caused by *Escherichia coli* (a Gram-negative bacterium) according to the Naber's classification, which includes the following categories: eradication, eradication with superinfection, persistence and persistence with superinfection. The study was conducted in 100 patients aged 25±8 years (range: 20–40 years) with bacterial MAGI and bacterial cultures positive only for *E. coli* (colony forming units $\geqslant 10^6$ per ml). Retrospective analysis was conducted only on patients treated with oral levofloxacin (500 mg) administered once daily for 28 days who were recruited over the last 5 years. Following antibiotic treatment, patients with microbiological persistence or persistence with superinfection had a significantly higher percentage of ultrasound abnormalities suggestive of prostato-vesiculitis (PV) (30.2% and 36.0%, respectively) or prostato-vesiculo-epididymitis (PVE) (60.2% and 70.0%, respectively) compared with patients with microbiological eradication (PV=10.2% and PVE=8.2%, respectively) or eradication with superinfection (PV=18.8% and PVE=21.2%, respectively). In conclusion, patients with microbiological persistence or persistence plus superinfection showed the highest prevalence of complicated forms of MAGI (PV and PVE), compared with patients with microbiological eradication or eradication with superinfection.

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INTRODUCTION

Male accessory gland infection (MAGI) is a consequence of canalicular spreading of agents *via* the urethra, prostate gland, seminal vesicles, deferent ducts, epididymis and testes.[1,2] The following parameters are considered diagnostic criteria suggestive of MAGI: a clinical history of urogenital infection and/or abnormal signs in one or more accessory glands after a physical examination, including digital rectal exploration; significant alterations in the expressed prostatic fluid and/or urinary sediment after prostatic massage; uniform growth of more than 10^3 pathogenic bacteria or more than 10^4 non-pathogenic bacteria per millilitre, in culture of diluted seminal plasma; the presence of more than 10^6 (peroxidase positive) leukocytes per millilitre of ejaculate; or signs of altered secretory function of the prostate or seminal vesicles. The criteria for MAGI are reported in **Figure 1**.

For the microbiological point of view, MAGI can be distinguished into bacterial and abacterial (no bacteria in the semen sample) forms;[1–4] moreover, there is a high prevalence of MAGI in patients with bacteria that cause sexually transmitted infections. In particular, a recent retrospective study explored the prevalence of sexually transmitted infection bacteria in semen samples from asymptomatic male infertile patients with and without leukocytospermia, showing that the DNA of sexually transmitted infection bacteria was detectable in 45/241 (18.7%) of the samples, with no difference in prevalence between the leukocytospermic and non leukocytospermic groups.[5]

Bacteriological analyses more frequently reveal different types of microorganisms considered to be the aetiological agents isolated in different biological samples, including urine, expressed prostatic secretions and semen, and on urethral swabs. These germs are *Escherichia coli*, *Klebsiella*, *Proteus* spp., *Enterococcus*, *Staphylococcus* spp., *Mycobacterium tuberculosis*, *Neisseria gonorrhea, Chlamydia trachomatis* and *Ureaplasma urealyticum*.[6] Moreover, the presence of leukocytes in semen samples can also occur in association with the presence of a viral infection.[7]

Generally, Nabers's definitions[8] have been adopted to report the microbiological response to antibacterial treatment in patients with chronic bacterial prostatitis, that is, the condition most investigated up to now. In more details, this classification includes: (i) eradication—baseline pathogen eradicated to less than 10^3 colony forming

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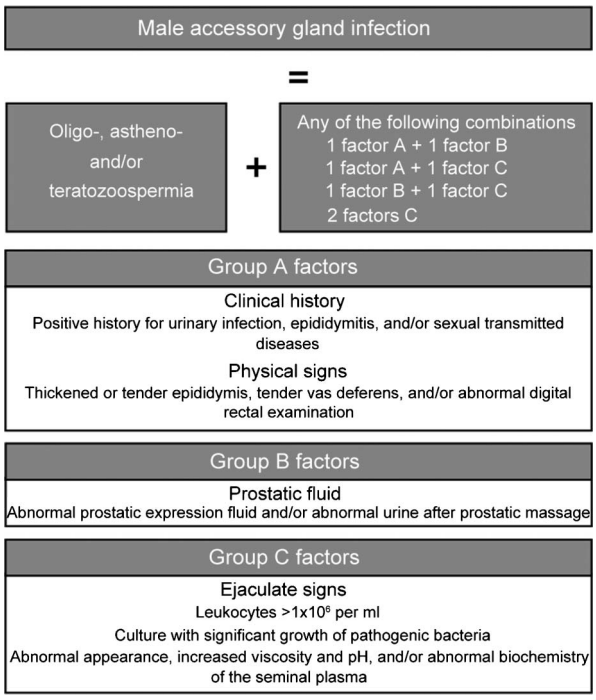

Figure 1 Diagnostic criteria for male accessory gland infection (MAGI).

units (CFU) per millilitre; (ii) eradication with superinfection—baseline pathogen eradicated ($<10^3$ CFU ml^{-1}) with the appearance of a new pathogen ($\geqslant 10^3$ CFU ml^{-1}); (iii) persistence—baseline pathogen not eradicated ($\geqslant 10^3$ CFU ml^{-1}); and (iv) persistence with superinfection—baseline pathogen persistent ($\geqslant 10^3$ CFU ml^{-1}) with the appearance of a new pathogen ($\geqslant 10^3$ CFU ml^{-1}).

Additionally, there is growing interest in the ultrasound characterisation of MAGI. On first evaluation, only calcifications and dilatation of the venous plexus are generally reproducible, whereas other ultrasound abnormalities of the prostate and seminal vesicles are poorly reproducible.[9] Dohle *et al.*[10] reported that in patients with a history of MAGI, 10% had abnormalities found on transrectal ultrasound of the prostate, such as oedema, dilatation of the seminal vesicles and ejaculatory ducts, intraprostatic calcifications and dilatation of the periprostatic venous plexus. Other studies by our group have shown that scrotal and transrectal prostate-vesicular ultrasound examination can detect three diagnostic categories of MAGI — prostatitis (P, prostate-vesciculitis (PV) and prostate-vesciculo-epididymitis (PVE) — and their unilateral or bilateral extensions.[11–13] The ultrasound criteria for the diagnosis of MAGI are reported in **Table 1**.

There are no data from studies that have evaluated the ultrasound characterisation of infertile patients affected by bacterial MAGI according to microbiological cure (absent, partial or complete) after antibiotic treatment. Therefore, based on the aforementioned evidence, the aim of this retrospective study was to evaluate the ultrasound characteristics of the prostate, seminal vesicles and epididymal tracts after treatment with a specific quinolone (levofloxacin) of patients with infections caused by *Escherichia coli* (a Gram-negative bacterium), according to the Naber's classification.

MATERIALS AND METHODS

Patient selection

A retrospective analysis was conducted of a clinical database of more than 600 infertile patients with diagnoses of microbial MAGI, who were evaluated over the last 5 years. One hundred patients aged 25±8 years (range: 20–40 years), with bacterial cultures positive only for *E. coli* (CFU $\geqslant 10^6$ ml^{-1}) and treated with 500 mg levofloxacin orally once daily for 28 consecutive days, were included in the analysis.

Table 1 Ultrasound criteria for the diagnosis of male accessory gland infection

The classification of male accessory gland infection	*Ultrasound criteria for the diagnosis*
Prostatitis (>2 criteria simultaneously present among the following)	(a) asymmetry of the gland volume
	(b) areas of low echogenicity
	(c) areas of high echogenicity
	(d) dilatation of the peri-prostatic venous plexus
	(e) single or multiple internal similar cystic areas
	(f) area(s) of moderate increase in vascularity (focal or multiple)
Vesiculitis (>2 criteria simultaneously present among the following)	(a) increased (>14 mm) anteroposterior diameter, mono- or bilateral
	(b) asymmetry >2.5 mm (normal 7–14 mm) compared with the contralateral vesicle
	(c) reduced (<7 mm) anteroposterior diameter, mono- or bilateral
	(d) thickened and/or calcified glandular epithelium
	(e) polycyclic areas separated by hyperechoic septa in one or both vesicles
	(f) fundus-to-body ratio >2.5
	(g) fundus-to-body ratio <1
	(h) anteroposterior diameter unchanged after recent immediate ejaculation
Epididymitis (>2 criteria simultaneously present among the following)	(a) increase in size of the head (craniocaudal diameter >12 mm) and/or of the tail (craniocaudal diameter >6 mm) (finding single or bilateral)
	(b) presence of multiple microcystis in the head and/or tail (finding single or bilateral)
	(c) low echogenicity or high echogenicity, mono- or bilateral
	(d) large hydrocele, mono- or bilateral
	(e) enlargement of the superior part of the cephalic tract and a superior-to-inferior part ratio >1
	(f) unchanged anteroposterior diameter of tail after ejaculation

Prostatitis: a–d=conventional ultrasound criteria;[11] e, f=additional ultrasound criteria.[13]
Vesiculitis: a–e=conventional ultrasound criteria;[11] f–h=additional ultrasound criteria.[13]
Epididymitis: a–d=conventional ultrasound criteria;[11] e, f=additional ultrasound criteria.[13]

All of the patients, before and after therapy, completed a specific questionnaire for MAGI[14] and underwent a physical examination and digital rectal exploration, semen analysis, cytological and bacteriological examinations of prostate secretions, a semen sample microbiological evaluation, and finally, scrotal plus prostate-vesicular ultrasound scans (see below). The diagnosis of bacterial MAGI was made in the presence of the following criteria:

- 1 factor **A** (altered anamnesis and/or physical examination) plus 1 factor **B** (altered prostatic secretion)
- 1 factor **A** plus 1 factor **C** (inflammatory signs of seminal plasma)
- 1 factor **B** plus 1 factor **C**
- 2 factor **C**s.

The infection was diagnosed in the presence of uniform growth of more than 10^3 CFU ml^{-1} of pathogenic bacteria (*E. coli*) in cultures of diluted seminal plasma and/or secretions obtained after prostatic massage. The samples were seeded using a calibrated loop on agar plates and were incubated overnight at 37 °C in normal air with 5% CO_2. The microorganisms were identified by gram stain, oxidase and catalase tests. Bacteriological examinations of prostate secretions and microbiological evaluations of semen samples were repeated 1 week after treatment. The data for this study were collected only from patients with evidence of colony-forming units $\geqslant 10^6$ CFU ml^{-1}.

Patients who received antibiotic therapy during the 3 months before this evaluation were excluded from this study.

The protocol was approved by the Institutional Review Board, and an informed written consent was obtained from each patient.

Sperm evaluation

Semen analyses were performed according to the WHO criteria (WHO, 1999)[15] and the methods in the Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction, 4th edition (1999).[15]

Ultrasound evaluation assessment

The didimo-epididymal regions were carefully assessed with scrotal ultrasonography by means of a scanner with a 7.5–11 MHz linear transducer. Scrotal ultrasonographic ultrasound was performed systematically in various longitudinal, transverse and oblique scans with the patients lying in the supine position. The examination always included measurement of testicular volume, documentation of testicular homogeneity and echogenicity, epididymal morphometry, including the cranio-caudal diameter of the caput and cauda epididymis, and echogenicity evaluation of multiple cysts and/or of a large hydrocele. The prostate-vesicular region was assessed with rectal ultrasonography using a 7.5 MHz biplan biconvex transducer with transverse and longitudinal scans. The prostate volume was measured using the planimetric method by scanning the organ at 5-mm intervals in transverse sections. Pathological seminal vesicles were either increased in diameter (with thickness >14 mm), asymmetric, hypoplastic (with thickness <7 mm) or atrophic. The ultrasound equipment used was the Esaote Megas GPX (Esaote SPA, Genoa, Italy). The examinations were conducted randomly by three different operators. The ultrasound abnormalities used to make a diagnosis of prostatitis, PV or PVE have been reported elsewhere (**Table 1**). The scores collected were incorporated into an electronic database for subsequent statistical processing. The study was approved by the internal committee of the Institute. All of the patients signed informed consents.

Statistical analysis

The data were analysed by one-way analysis of variance or Student's *t*-test, as appropriate. Statistical analysis was conducted using SPSS for Windows (version 10.0; SPSS Inc., Chicago, IL, USA). A *P* value less than 0.05 was accepted as statistically significant.

RESULTS

Microbiological findings after antibiotic administration

Fifty-two patients (52%) had microbiological eradication, 20 patients (20%) had microbiological eradication with superinfection, 16 patients (16%) had microbiological persistence and 12 patients (12%) had microbiological persistence with superinfection.

Sperm parameters

Sperm parameters are reported in **Table 2**. The sperm concentrations, progressive motility and normal morphologies were significantly lower ($P<0.05$) in patients with persistence or persistence with superinfection compared to patients with eradication or eradication with superinfection after therapy.

Ultrasound findings

The results of the ultrasound evaluations obtained after antibiotic treatment are reported in **Figure 2**. Before treatment, 35 patients (35%) showed ultrasound abnormalities suggestive of prostatitis, 40 (40%) had ultrasound abnormalities suggestive of PV and 25 (25%) had ultrasound criteria positive for PVE. In addition, among the patients with ultrasound diagnoses of PV and PVE ($n=65$), 40 patients showed ultrasound abnormalities suggestive of bilateral involvement, while the other 25 showed ultrasound signs suggestive of unilateral involvement.

Table 2 Sperm parameters related to microbiological response (according to Naber's classification) before and after levofloxacin (500 mg per day for 28 days) administration in patients with bacterial male accessory gland infection positive for *E. coli* ($n=100$)

Microbiological response	*Sperm concentration ($\times 10^6$ ml^{-1})*		*Progressive motility (%)*		*Normal morphology (%)*	
	Before	*After*	*Before*	*After*	*Before*	*After*
Eradication	12.0 (2–60)	32.0* (12–140)	19.0 (12–23)	33.0* (16–44)	4.0 (2–12)	12.0* (6–30)
Eradication with superinfection	14.0 (4–80)	18.0** (10–120)	16.0 (11–20)	18.0** (14–40)	6.0 (2–14)	8.0** (3–16)
Persistence	11.0 (4–70)	10.0 (6–70)	16.0 (10–21)	12.0 (4–15)	5.0 (4–12)	4.0 (2–12)
Persistence with superinfection	16.0 (4–80)	15.0 (4–70)	18.0 (6–21)	11.0 (3–12)	7.0 (4–14)	5.0 (2–10)

Values are expressed as median (range).

*$P<0.05$ *vs.* other groups and eradication group at baseline.

**$P<0.05$ *vs.* persistence and persistence with superinfection.

Semen analyses were performed according to the WHO criteria (WHO, 1999)[15] from the Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction, 4th edition (1999).[15]

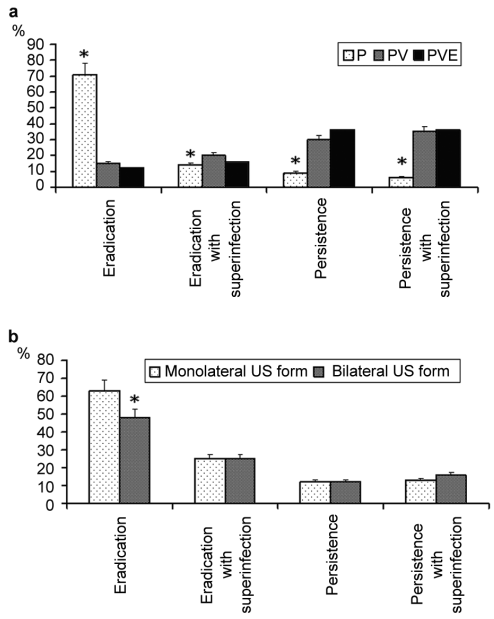

Figure 2. Ultrasonographic (US) categories detected after therapy. (**a**) The different extensions of US findings (in the prostate alone (P); in the prostate and seminal vesicles (PV); in the prostate, seminal vesicles and epididymis (PVE)). (**b**) The percentages of mono- or bilateral US forms. $P<0.05$ *vs.* PV and PVE (a); $P<0.05$ *vs.* bilateral US form (b).

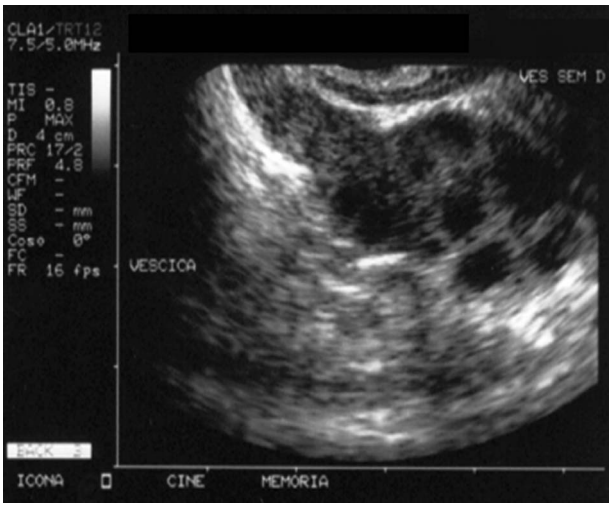

Figure 3 Ultrasound alterations more frequently detected in patients with bacterial male accessory gland infection.

Patients with ultrasound alterations limited to the prostate showed an eradication rate significantly higher ($P<0.05$) compared to other groups; moreover, patients with the bilateral ultrasound form of inflammatory involvement showed a significantly lower eradication rate compared to patients with the unilateral form ($P<0.05$).

Among the patients with microbiological persistence or persistence with superinfection, the percentage of ultrasound findings suggestive of PV (30% and 35%, respectively) or PVE (36% and 36%, respectively) was significantly higher ($P<0.05$) compared to patients with microbiological eradication (PV=15% and PVE=12%, respectively) or eradication with superinfection (PV=20% and PVE=16%, respectively).

Finally, after levofloxacin administration, among the patients with microbiological persistence or persistence plus superinfection, the ultrasound sign most frequently detected was the presence of polycyclic areas separated by hyperechoic septa in one or both seminal vesicles (**Figure 3**). In particular, this ultrasonographic feature was detected in 60% of the patients with persistence and in 70% of the patients with persistence associated with superinfection.

DISCUSSION

This study examined the ultrasound characterisation of infertile patients with bacterial male accessory gland infections associated with positive cultures for *E. coli*, treated for 28 days with 500 mg levofloxacin once daily. The results showed that, in this study population, the eradication rate was 52%; however, 20% of the patients treated showed microbiological eradication with superinfection, 16% of the patients showed persistence of infection, and 12% of them showed microbiological findings of persistence plus superinfection. Moreover, after therapy, patients with microbiological findings of eradication showed improved sperm quality compared to patients with microbiological persistence or persistence plus superinfection. Finally, ultrasound examinations revealed that the patients with microbiological persistence or persistence plus superinfection had a higher incidence of ultrasound-complicated forms (PV and PVE) compared to the patients with microbiological eradication or eradication plus superinfection.

The results of our study suggest us two important observations.

First, the prevalence of fluoroquinolone-resistant *E. coli* was higher than reported in other studies. In fact, Minamida and colleagues[16] recently showed that in 100 patients, 13 (13%) had stool cultures positive for fluoroquinolone-resistant *E. coli*. The data obtained in our study do not explain this difference. In addition, the ultrasound examinations could help to explain the different susceptibility of the host's response to chronic infection. There are three possible interpretations of this finding: (i) unmasking of fluoroquinolone-resistant organisms present in low numbers before therapy but undetected by the methodology; (ii) acquisition of an exogenous, resistant strain during treatment; and (iii) selection in the host of highly resistant mutations from pre-existing, intermediately susceptible organisms.[17]

Second, the data from our study suggest that therapy protocols should be adequately differentiated with regard to the different ultrasound patterns observed before and after antibiotic therapy. In particular, it would be important to evaluate or to subject to a longer duration of therapy forms limited to ultrasound complications or to add other standard therapies to the antibacterial treatment; in fact, with regard to the treatment of infertility, antibiotics play a role in bacterial prostatitis, whereas in abacterial prostatitis, other treatments, such as antioxidant compounds, sacral nerve stimulation and anti-inflammatory treatments, are worth being considered.[18]

In the first study by Comhaire *et al.*,[19] the author concluded that features of MAGI in semen can regress spontaneously and are not influenced by therapy;[19] more recently, the consideration of specific antibiotics has been necessary to treatment.[1] In particular, for treatment, the fluoroquinolones are considered the drugs of choice because of their favourable pharmacokinetic properties and their antimicrobial spectra. In chronic bacterial prostatitis, antibiotic treatment seems to demonstrate both antimicrobial effectiveness and reduction of reactive oxygen species production, with values similar to those of control groups;[4] however, long-term amtolmetin-guacyl administration demonstrated efficacy and safety in the treatment of amicrobial

MAGI, exhibiting a positive impact on all of the sperm parameters studied and no side effects.[11] In patients with PVE, antimicrobials and/or antiphlogistic drugs obtain a full, positive antimicrobial response, but only a partial antioxidative response, which seems to be potentiated by the addition of antioxidative agents (carnitines). Furthermore, it is important to underline that antioxidative treatment with carnitines, administered along with anti-infectious agents, is less effective, and finally, this treatment is unsuccessful without the eradication of pro-oxidant (germs and white blood cells) agents.[20]

Patients with persistent male accessory gland infection after six cycles are defined as non-responders,[21] and although antibiotic therapy is considered suitable when microbial MAGI is suspected, it is not possible to anticipate a poor response to antibiotics merely on the basis of conventional criteria (clinical history, physical and ejaculate signs). Thus, transrectal ultrasound evaluation can be helpful in the follow-up of these patients; in fact, in our recent study, we defined prostate and seminal vesicle abnormalities in patients with MAGI who failed to respond to antibacterial treatment. In particular, in that study, we selected patients with MAGI and persistently elevated bacteriospermia after three antibiotic courses, and transrectal ultrasound revealed multiple abnormalities indicative of: (i) bilaterally extended PV (nine of these patients also had micro-emphysematous prostate abscesses); and (ii) PV with unilateral or bilateral sub-obstruction of the ejaculatory ducts.[22] Moreover, the importance of ultrasound evaluation concerns also the characterisation of unilateral forms, rather than bilateral extension, of inflammatory processes, as confirmed by our recent article, in which it was shown that sperm parameter abnormalities, low seminal fructose and reactive oxygen species overproduction do not discriminate between patients with unilateral or bilateral post-infectious inflammatory PVE.[12] Finally, the evaluation of oxidative status during medical therapy could be useful for optimising antimicrobial effects;[4] in fact, persistent infertility, abnormal semen analysis due to quantitative sperm parameters alterations and sperm-generated reactive oxygen species overproduction in MAGI might be related to a significant percentage of antibiotic-independent re-infections and/or to low antioxidative epididymal properties, which can persist following antimicrobial treatment.[23]

In conclusion, this study showed that among patients with persistent bacterial MAGI, there is a high prevalence of ultrasonographic complicated forms (PV and PVE). Because only ultrasound examination can confirm the true anatomical extension of inflammation, it should be recommended before and after medical therapy. In particular, before therapy, it is important to identify different categories of patients, and after treatment, it is helpful for the evaluation of the effective reduction of inflammatory signs.

AUTHOR CONTRIBUTIONS

SLV, AEC and EV carried out the study, analysed the data and wrote the manuscript. SLV, AEC, SB, MS, RAC and EV were involved in the study design, data management and analysis of the study.

COMPETING FINANCIAL INTERESTS

The authors declare no conflicts of interest.

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