Hypertrophic-congestive and fibro-sclerotic ultrasound variants of male accessory gland infection have different sperm output

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ABSTRACT. ***Introduction:*** **Male accessory gland infection (MAGI) exerts a negative influence on male fertility which depends upon its extension. Indeed, we have shown that patients with MAGI involving prostate, seminal vesicles and epididymis have worse sperm parameters compared with patients with prostatitis alone or prostate-vesiculitis. Similarly, MAGI extending bilaterally is associated with a worse sperm output. The aims of this study were to evaluate the prevalence of two different additional ultrasound (US) findings (hypertrophic-congestive and a fibro-sclerotic US form) and to evaluate their semen quality.** ***Materials and methods:*** **One hundred infertile patients with MAGI, diagnosed according to the World Health Organization (WHO) 1993 criteria, were evaluated by scrotal and transrectal ultrasound scans. The control group consisted of 100 healthy, age-matched men.** ***Results:*** **The ultrasound examination confirms two separate US variants of MAGI: a hypertrophic-congestive (prevalence of 56%) and a fibro-sclerotic form (prevalence of 29%). Patients with hypertrophic-congestive MAGI showed higher sperm concentration, motility and normal forms, but also higher sperm leukocytes concentration and seminal reactive oxygen species compared to patients with fibro-sclerotic MAGI. However, all these parameters were significantly worse than those observed in the control group.** ***Discussion:*** **Infertile patients with hypertrophic-congestive MAGI have a better sperm quality compared with patients with fibro-sclerotic MAGI; however, they showed higher oxidative stress in semen.**

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INTRODUCTION

The acronym of MAGI refers to inflammation of the male sex accessory glands. There are two main definitions. The first is that proposed by the World Health Organization (WHO) in 1993 (1), the second, concerning prostatitis, was proposed by the National Insitutes of Health (NIH) in 1995 (2). According to the WHO, the diagnosis of MAGI is based on the presence of sperm alterations (oligo-, astheno- and/or terathospermia) associated with two or more of the following factors: a) history of urogenital infection, sexually transmitted infection and/or presence of post-inflammatory alterations in physical examination of testicular and epididymal region and/or digito-rectal exploration of the prostate and the seminal vesicles; b) cytological signs, microbiological infection and/or inflammation on the secretion obtained after prostatic massage; c) signs of infection in the ejaculate (leukocytospermia >1x10^6/ml); semen cultures positive for significant presence of pathogenic bacteria; alterations of physical-chemical properties and/or biochemistry of seminal plasma. According to the NIH Chronic Prostatitis Collaborative Research Network, which produced the 1995 National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)/NIH classification, published by Krieger (2), prostatitis includes: acute (category I) and chronic (category II) bacterial infection of the prostate; chronic abacterial prostatitis/chronic pelvic pain syndrome: inflammatory (category IIIa) and non-inflammatory (category IIIb); and asyntomatic inflammation of the prostate (histological prostatitis) (category IV). Although the WHO recognizes the MAGI among diagnostic categories responsible for male infertility, various studies to determine the extent of the negative impact of these clinical conditions on the reproductive function report a variable frequency (1.5-16%). The wide range of prevalence reflects several issues: a) incomplete and not well characterized diagnosis; b) higher prevalence reported in cohorts of infertile patients or persistent infection (poor-responder antibiotic therapy) and/or past failures of *in vitro* fertilization programs (IVF) (3-7).

Ultrasound evaluation of epididymis, prostate and seminal vesicles is important to evaluate the extension of MAGI. We found that the negative impact of the inflammatory process on sperm quality and consequently, fertility, is higher in clinical forms that simultaneously involve prostate, seminal vesicles and epididymis, compared to clinical forms limited to only prostate. Recently, we have also reported that only the ultrasound evaluation is able to discriminate bilateral from unilateral forms of MAGI. Thus we have proposed the following criteria for ultrasound diagnosis of MAGI:

- prostatitis is suspected in the presence of >2 of the following ultrasound signs: a) asymmetry of the gland volume [in our experience: >5 mm in transverse or antero-posterior diameter (APD) (unpublished data)]; b) areas of ipoechogenicity; c) areas of iperechogenicity; d) dilatation of peri-prostatic venous plexus [in our experience: >4 mm in APD (unpublished data)];
- vesiculitis is suspected in the presence of >2 of the

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following ultrasound signs: a) increased (>14 mm) mono- or bilateral APD; b) asymmetry >2.5 mm (normal 7-14 mm) compared to the contralateral vesicle; c) reduced (<7 mm) mono- or bilateral APD; d) glandular epithelium thickened and/or calcified; 5) polycyclic areas separated by hyperechoic septa in one or both vesicles;
- epididymitis is suspected in the presence of >2 of the following ultrasound signs: a) mono- or bilateral increased head (>12 mm) and/or tail (>6 mm) cranio-caudal diameter; b) presence of multiple microcystis in the head and/or tail (finding single or bilateral); c) mono- or bilateral ipoechogenicity or iperechogenicity; d) mono- or bilateral large hydrocele (8-10).

There are no studies about the true reproducibility of these criteria and evidence about other possible signs after several years of their publication. We have tested other possible signs detected with high frequency in these patients. These ultrasound signs, defined additional criteria, are: for prostate: a) single or multiple internal cyst-like areas, b) area(s) of moderate increased vascularity (focal or multiple); for seminal vesicles: a) fundus/body ratio >2.5; b) fundus/body ratio <1; c) unchanged APD just after ejaculation; for epididimys: a) enlargment in the superior part of the cephalic tract and superior/inferior part ratio >1; b) unchanged tail APD after ejaculation (11, 12).

In our experience, based on different combinations of these ultrasound criteria, we identify two different ultrasound forms of MAGI, arbitrarily called "hypertrophic-congestive" and a "fibro-sclerotic" form. The hypertrophic-congestive ultrasound form (HCUF) is characterized by the simultaneous presence of the following ultrasound criteria:
- prostate: increase of volume, areas of ipoechogenicity, dilatation of peri-prostatic venous plexus, single or multiple internal similar cystis areas;
- seminal vesicles: increase of volume, mono- or bilateral increased (>14 mm) APD, polycyclic areas separated by hyperechoic septa in both vesicles, fundus/body ratio >2.5;
- epididymis: increased (>6 mm) tail cranio-caudal diameter, bilateral head and tail areas of ipoechogenicity, unchanged tail APD just after ejaculation.

The fibro-sclerotic ultrasound form (FSUF) is characterized by the simultaneous presence of following ultrasound criteria:
- prostate: areas of hyperechogenicity, asimmetry of the gland volume;
- seminal vesicles: reduced (<7 mm) mono- or bilateral APD, thickened and/or calcified glandular epithelium, fundus/body ratio <1;
- epididymis: bilateral head and tail areas of hyperechogenicity.

Therefore, on this basis, the aims of this study were: to evaluate the prevalence of these separate ultrasound variants in patients with diagnosis of MAGI and to evaluate their semen characterization.

MATERIALS AND METHODS

Patient selection

One hundred consecutive patients with MAGI were evaluated. Their mean age was 28.5±3.2 yr (range: 24-32 yr). The diagnosis of MAGI was made according to the WHO criteria (1993). One hundred age-matched healthy men (29.6±3.5 yr; range: 25-34 yr) were selected as control group. The controls were selected with the following characteristics: they were normospermic subjects with a pregnancy in the last year and with negative history of seminal infections; they were recruited among subjects afferent to our Institute for moderate pelvic pain (in the absence of diagnostic criteria suggestive for MAGI) or atypical sexual dysfunction (hypospermia) such as loss of erection maintenance.

Moreover, patients and controls were excluded in the presence of any of the following: endocrine disorders (hormonal parameters of patients and controls are shown in Table 1), history positive for cigarette smoking, alcohol consumption, occupational chemical exposure, fever or drugs taken within 3 months before the enrolment in this study, azoospermia, testicular volume <15 ml [testicular volume correlated significantly with testicular function, in particular sperm parameters are subnormal in patients with total testicular volume (right plus left testicular volume) <30 ml by orchidometry] (13), past or present cryptorchidism, varicocele.

The protocol was approved by the Ethics Committe and an informed written consent was obtained from each men.

Ultrasound examination

All patients and controls underwent an accurate anamnesis, physical examination, scrotal ultrasound evaluation using a linear probe (7.5-11 MHz) and prostato-vesicular ultrasound evaluation using an end-fire transrectal probe (7.5 MHz) before (with 4 days of sexual abstinence) and immediately after ejaculation. The prostate volume was estimated by applying the formula to calculate the ellipsoid volume (14). The volume of the seminal vesicles as represented by the maximum horizontal area of the seminal vesicles (MHA) (15). In addition, the APD of the seminal vesicles was calculated before and immediately after ejaculation. The ultrasound equipment used was the Megas GPX (Esaote, Genoa, Italy). The ultrasound examination was conducted randomly by 3 different operators, to ascertain the reproducibility of these ultrasound criteria.

Finally, controls underwent transrectal ultrasound examination, because they reported chronic pelvic pain and/or atypical sexual dysfunction (hypospermia and/or anorgasmia) and/or loss of erection maintenance.

Semen analysis and sperm preparation

All semen specimens were collected by masturbation into sterile containers after 3-5 days of sexual abstinence. Sperm analysis was conducted according to the WHO (1999) guidelines (15). The concentration of semen leukocytes was also determined in the 45% Percoll fraction by immunocytochemical staining (15). To evaluate the activity of these leukocytes, the production of reactive oxygen species (ROS) was evaluated in 45% Percoll fraction.

Leukocyte morphological identification

In whole semen and in 45% Percoll fraction, the leukocyte concentration was determined by morphological identification using conventional immunocytochemical staining (16).

Determination of leukocyte-specific ROS production

ROS production was measured using the chemiluminescence. The probe Luminol (Sigma Chemical Co., St. Louis, MO, USA;

Table 1 - *Demographics characteristics and hormonal parameters of patients and controls.*

Groups	Age (yr)	BMI (kg/m²)	FSH (mUI/ml)	LH (mUI/ml)	Testosterone (ng/ml)	Estradiol (pg/ml)	Prolactin (ng/ml)
MAGI	28.5±3.2	23.5±5.0	3.2±3.0	2.2±4.0	7.2±3.0	11.9±20.0	8.0±6.0
HCUF	28.0±3.0	23.0±5.0	3.9±4.0	3.3±3.0	6.7±2.0	15.2±7.0	7.6±4.0
FSUF	27.0±2.0	22.5±4.0	2.8±3.0	2.6±3.0	7.4±3.0	12.0±10.0	6.2±6.0
Controls	29.6±3.5	24.0±3.5	4.0±3.0	3.1±4.0	7.0±2.0	15.5±8.0	8.5±4.0

BMI: body mass index; MAGI: male accessory gland infection; HCUF: hypertrophic-congestive ultrasound form; FSUF: fibro-sclerotic ultrasound form. Values are mean±SEM. The hormone assays were performed by electrochemiluminescence with Hitachi-Roche (Cobas 6000) of Roche Diagnostics, Indianapolis, USA. The reference intervals were: LH: 1.6-9-0 mUI/ml, FSH: 2-12 mUI/ml, estradiol: 8-43 pg/ml, total testosterone: 2.8-8 ng/ml, prolactin: 4-15 ng/ml.

4µl of a 25 mM stock solution in dimethylsulphoxide, DMSO) containing 8.5 U horseradish peroxidase (Type VI, 310 U/mg, Sigma) to sensitize the assay for the generation of extracellular hydrogen peroxide was added to 400 µl cell suspension. The resultant chemiluminescent signal was measured on a Berthold LB 9505 luminometer at a chamber temperature of 37 C. After 15 min, once the steady state signal was established, 2 µl (final concentration 50 µM) of the leukocyte chemoattractant peptide formyl-leucyl-phenylalanine (fMLP, Sigma), dissolved in DMSO, were added and the leukocyte-dependent chemiluminescence was monitored for further 15 min. The scores collected were incorporated into an electronic database for subsequent statistical processing.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test and Student's t test for direct comparison of the two groups. Statistical analysis was performed using SPSS 9.0 for Windows. A *p*-value <0.05 was accepted as statistically significant.

RESULTS

No statistically significant difference between patients and controls was detected for age, body mass index and hormonal parameters (Table 1).

Altogether 20 ultrasound signs suggestive for MAGI were detected, 6 for the diagnosis of prostatitis (4 conventional and 2 additional), 8 for the diagnosis of vesicles (5 conventional and 3 additional), 6 for the diagnosis of epididymitis (4 conventional and 2 additional). According to the frequency of a different combination of conventional and additional ultrasound criteria in infertile patients with MAGI the following combinations were observed:

- HCUF was detected in 56% of patients with MAGI (Fig. 1);
- FSUF was detected in 29% of patients with MAGI (Fig. 1).

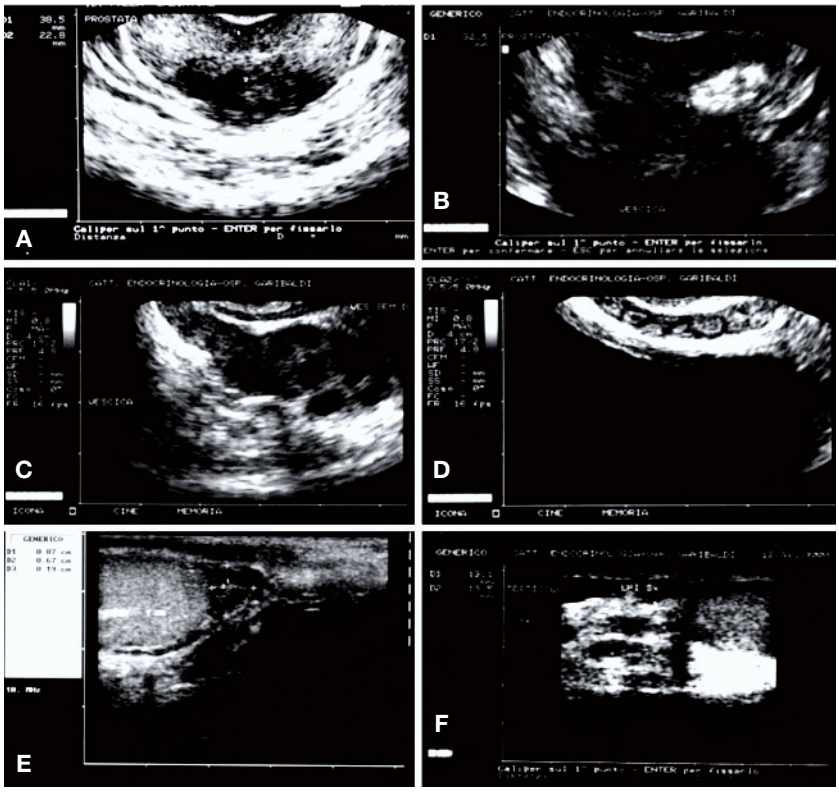

Fig. 1 - *Representative examples of patients with male accessory gland infection with ultrasound features indicating the presence of hypertrophic-congestive or sclero-fibrotic variants. A) Hypertrofic-congestive variant of prostatitis; B) sclero-fibrotic variant of prostatitis; C) hypertrofic-congestive variant of vesciculitis; D) sclero-fibrotic variant of vesciculitis; E) hypertrofic-congestive variant of epididymitis; F) sclero-fibrotic variant of epididymitis.*

Table 2 - *Semen analysis parameters from patient and controls.*

Groups	Sperm concentration (x10^6/ml)	Total sperm number (x10^6/ejaculated)	Motility (% grade a+b, after 1 h)	Morphology (% normal forms)
MAGI	23.1 (2.3-130.0)*	69.5 (3.2-502)*	16.5 (5-37)*	21.5 (12-38)*
HCUF	35.2 (9.2-130.0)*	106.0 (28.2-502)*	22.2 (13-37)*	30 (20-38)*
FSUF	11.0 (2.3-56.2)*,**	33.3 (3.2-110)*,**	11.2 (5-20)*,**	13 (12-30)*,**
Controls	60.2 (24.6-142.2)	134.2 (50.2-567)	37.8 (27-52)	43.0 (33-50)

MAGI: male accessory gland infection; HCUF: hypertrophic-congestive ultrasound form; FSUF: fibro-sclerotic ultrasound form. Values are median and 10th-90th percentiles are given in parentheses. *p<0.05 *vs* controls; **p<0.05 *vs* HCUF.

The remaining 15% of patients with MAGI not included in these combinations of signs showed a different, but not significant ultrasound combination of these criteria with observational frequency always <1%.
No subject in the control group showed sufficient ultrasound criteria of MAGI or combination of ultrasound criteria compatible with HCUF or FSUF.
No statistically significant difference between HCUF and FSUF was detected for age, body mass index and hormonal parameters (Table 1).
Patients with HCUF showed a significantly higher (36.5±2.5 cm^3) prostatic volume, estimated with transrectal ultrasonography by calculating the ellipsoid volume (12, 14), compared to patients with FSUF (20.5±2.8 cm^3). In addition HCUF patients showed a significantly higher (4.5±1.2 cm^3) volume of seminal vesicles, estimated with transrectal ultrasonography by calculating the MHA (15), compared to FSUF patients (1.2±0.6 cm^3).
Results on conventional semen analyses in the various groups of patients and in controls are summarized in Table 2. Semen samples in patients with FSUF exhibited significantly (p<0.05) lower values than those in HCUF patients and in the control group. Seminal white blood cell (WBC) concentrations in HCUF patients were significantly (p<0.05) higher than those found in FSUF patients and in the control group. HCUF patients showed significantly (p<0.05) higher basal and fMLP-stimulated ROS production than those found in FSUF and control group (Table 3).

DISCUSSION

Previously we reported that the inflammation extension to all sexual accessory glands (prostate, seminal vesicles and epididymis) results in higher alterations of sperm parameters compared to prostatitis alone (8, 9).
We also described that the presence of bilateral inflammation (bilateral MAGI) has a severer impact on fertility compared to unilateral form (10). We also described the ultrasound prostatic alteration in persistent MAGI (17).
In the present study, the ultrasound evaluation of selected infertile patients with MAGI showed a different characterization. There is a frequent ultrasound form showing the following combination of signs: a) prostate: areas of ipoechogenicity, dilatation of peri-prostatic venous plexus, single or multiple internal similar cystis areas; b) seminal vesicles: increased (>14 mm) mono- or bilateral APD, polycyclic areas separated by hyperechoic septa in both vesicles, fundus/body ratio >2.5; c) epididymis: increased (>6 mm) tail cranio-caudal diameter, head and tail bilateral areas of ipoechogenicity, unchanged post-ejaculatory tail APD. This form of MAGI showed a higher prostate and seminal vesicles volume and may be arbitrarily defined as HCUF of MAGI.
There is another, less prevalent ultrasound form of MAGI with the following combination of signs: a) prostate: areas of hyperechogenicity, asimmetry of the gland volume; b) seminal vesicles: reduced (<7 mm) mono- or bilateral APD, thickened and/or calcified glandular epithelium, fundus/body ratio <1; c) epididymis: bilateral head and tail areas of hyperechogenicity. This form showed lower prostate volume and may be arbitrarily defined as FSUF of MAGI.
The HCUF of MAGI had better conventional sperm parameters compared to the FSUF form, but worse than those found in controls. These patients had a higher concentration of WBC and ROS compared to the FSUS of MAGI and control group. This study suggests that hypertrophic-congestive MAGI is a clinical condition associated with progressive alteration of sperm parameters, which is less severe compared to the fibro-sclerotic form

Table 3 - *White blood cell (WBC) concentration in semen and 45% Percoll fraction; basal and fMLP-stimulated WBC-specific reactive oxygen species (ROS) generation in the 45%-Percoll fraction from patient and control group.*

Groups	Seminal WBC concentration (x10^6/ml)	WBC concentration 45% Percoll fraction (x10^4/10^7 spermatozoa)	Basal ROS production (x10^3 cpm)	fMLP-stimulated ROS production (x10^3 cpm)
MAGI	3.2 (0.6-9.8)*	240.0 (70-900)*	297.5 (60-990)*	585.0 (180-1000)*
HCUF	4.2 (2.0-9.8)*,**	300.0 (150-900)*,**	400.0 (120-990)*,**	800.0 (350-1000)*,**
FSUF	2.2 (0.6-1.8)*	180.0 (70-400)*	195.0 (60-450)*	370.0 (180-860)*
Controls	1.0 (0.6-1.4)	80.9 (50.0-133.2)	35.0 (10-40.6)	60.0 (23.2-87.1)

MAGI: male accessory gland infection; HCUF: hypertrophic-congestive ultrasound form; FSUF: fibro-sclerotic ultrasound form.Values are median and 10th-90th percentiles are given in parentheses. *p<0.05 *vs* controls; **p<0.05 *vs* FSUF.

but with higher semen levels of oxidative stress; this aspect is probably caused by higher semen concentrations of leukocytes (18) and/or ultrasound presence of stagnation and congestive areas in prostate and seminal vesicles wich express a major inflammation of these organs (16). There are not many comparative studies between prostate-vesicular ultrasound and histological analysis, for example, Hendrikx et al. showed that the hyperechoic areas represent calcifications, while hypoechoic areas are small gland ectasia, infectious areas and atrophy of tissue (19).

A recent and very interesting study conducted on selected patients with MAGI assessed the association between seminal plasma interleukin-8 (sIL-8) and color-Doppler ultrasound. After adjusting for age, sIL-8 in patients with MAGI was significantly related to several abnormal semen and CDU parameters. In particular, leucocytospermia was closely associated with sIL-8. Ejaculate volume, unlike other semen or hormonal parameters, was negatively associated with sIL-8. When scrotal CDU was performed, sIL-8 was positively related to CDU in homogeneous, hypo-echoic, hyper-echoic epididymis and to epididymal calcifications. In addition, a positive correlation among sIL-8, hyperemic epididymis and an increased size of epididymal tail was found. When transrectal CDU was performed, an association among sIL-8 and hyper-echoic seminal vesicles, dilated ejaculatory ducts and duct calcifications was also observed. Finally, sIL-8 was positively related to prostate CDU abnormalities such as calcifications, inhomogeneous/hypo-echoic texture, hyperaemia and high arterial blood flow. No association was found with testis parameters (20).

The present study confirms the role of the ultrasound scan in male infertile patients (21), in fact scrotal ultrasound evaluation is considered critical in the assessment and clinical management of male infertility (6). Scrotal ultrasound is aimed mainly at the finding of non-palpable lesions or the differential diagnosis between processes of various etiology when physical examination is not clarifying. Scrotal ultrasonography is an indication when the clinical conditions make the clinical uro-genital examination difficult, inappropriate or suspect for testicular mass (22-24), while it is a relative indication in certain categories of patients with infertility, where the ultrasound becomes a second level examination such as inflammatory alterations of the proximal post-testicular tract (6, 25).

The indications for transrectal ultrasonography are an initial clinical spermatic obstructive pathology, doubt digital anorectal exploration and before starting treatment and during monitoring of treatment with testosterone in a patient with hypogonadism (8, 9, 25-27). Transrectal ultrasonography is an indication in some categories of patients with post-testicular causes of infertility (17). Among the post-testicular causes include inflammation of the male accessory glands or, ejaculatory disorders as anejaculation, hematospermia, painful ejaculation.

In conclusion, this study identified two different ultrasound forms of MAGI by scrotal and prostate-vesicular ultrasound scans which impact differently on sperm output. Indeed, the so-called congestive-hypertrophic form has a less negative impact on sperm conventional parameters compared with the fibro-sclerotic form. This study adds further evidence on the importance of ultrasound evaluation for a better characterization of MAGI.

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