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# Male accessory gland inflammation, infertility, and sexual dysfunctions: a practical approach to diagnosis and therapy

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## SUMMARY

The role of urogenital inflammation in causing infertility and sexual dysfunctions has long been a matter of debate in the international scientific literature. The most recent scientific evidences show that male accessory gland infection/inflammation could alter, with various mechanisms, both conventional and biofunctional sperm parameters, and determine worst reproductive outcome. At the same time, the high prevalence of erectile dysfunction and premature ejaculation in patients with male accessory gland infection/inflammation underlines the close link between these diseases and sexual dysfunctions. The aim of this review was to provide the reader the basis for a correct diagnosis of male accessory gland infection/inflammation and a subsequent appropriate therapeutic approach, particularly in patients with infertility and/or sexual dysfunction.

## INTRODUCTION

Male accessory gland infection/inflammation (MAGI) is a generic acronym that indicates a set of inflammatory diseases of the male accessory sexual glands. The first definition of MAGI was proposed by Comhaire *et al.* (1980). In 1993, WHO elaborated the first diagnostic algorithm (WHO, 1993). According to this algorithm, the diagnosis of MAGI can be established when a patient has oligo-, astheno-, and/or teratozoospermia associated with at least one factor A: history of genitourinary infection or physical signs plus one factor B (abnormal prostate fluid expression and/or abnormal urine after prostatic massage), one factor A plus one factor C (leukocytospermia, bacteriospermia, or alteration in seminal biochemistry) (Table 1).

In 1999, the National Institutes of Health (NIH) Chronic Prostatitis Collaborative Network developed a new classification of prostatitis, which includes four diagnostic categories: acute bacterial prostatitis (I), chronic bacterial prostatitis (II), inflammatory (IIIa) or non-inflammatory (IIIb) chronic pelvic pain syndrome (CPPS), and asymptomatic/histological inflammatory prostatitis (IV) (Krieger *et al.*, 1999).

While the diagnostic algorithm developed by the WHO considers the presence of sperm abnormalities a key prerequisite for the diagnosis of MAGI, the NIH classification of prostatitis does not consider sperm analysis a crucial step in the diagnostic process. This has, in the past, been responsible for an underestimation of the prevalence of sperm parameter abnormalities in patients with prostatitis. Similarly (Giamarellou *et al.*, 1984; Weidner *et al.*, 1999), the Chronic Prostatitis Symptom Index of the NIH (NIHCPSI), the standardized questionnaire most commonly used for identifying prostatic symptoms, takes into account only irritative urinary tract symptoms and does not investigate the presence of sexual dysfunction (Litwin *et al.*, 1999), which today considered an important symptom/consequence of MAGI.

## DIAGNOSIS OF MAGI

The frequency of MAGI reported in the literature ranges from 5% to 30% (La Vignera *et al.*, 2011b; La Vignera *et al.*, 2015) because the diagnostic criteria are not always correctly applied and it is probably underestimated because these conditions are often asymptomatic or paucisymptomatic.

The most common symptoms of MAGI are urinary disorders, particularly nocturia, pollachiuria, decreased urinary strength, and incomplete bladder emptying; chronic pelvic pain, which can manifest itself as pain in scrotal, penile, inguinal, suprapubic, and anal region; finally, sexual dysfunctions, including erectile dysfunction, premature ejaculation, and decreased libido, are present in approximately 50% of patients (La Vignera *et al.*, 2012a).

**Table 1** Clinical criteria adopted for the diagnosis of MAGI (WHO, 1993)

Oligo-, astheno- and/or teratozoospermia associated with the following:

- One factor A + one factor B
- One factor A + one factor C
- One factor B + one factor C
- Two factors C

| Factors | Description |
|---|---|
| A | *History:* positive for urinary infection, epididymitis, and/or sexually transmitted disease<br>*Physical signs:* thickened or tender epididymis, tender vas deferens, and/or abnormal digital rectal examination |
| B | *Prostatic fluid:* abnormal prostate fluid expression and/or abnormal urine after prostatic massage |
| C | *Ejaculate signs:* leukocyte > 1 million per ml, culture with significant growth of pathogenic bacteria, abnormal appearance, increased viscosity, increased pH, and/or abnormal biochemistry of the seminal plasma |

To address the diagnosis, a semi-structured interview has been development, called SI-MAGI, which aids the clinician in identifying patients with signs and/or symptoms of MAGI. SI-MAGI includes three domains: irritable and obstructive symptoms of the low urinary tract; spontaneous and/or post-ejaculation pain or discomfort and comorbidities (irritable bowel syndrome); sexual dysfunction, mainly erectile dysfunction, premature and delayed ejaculation (La Vignera, 2012a,b).

From a microbiological point of view, MAGIs are classified into microbial and inflammatory forms (La Vignera *et al.*, 2014). There are much microorganisms potentially responsible of MAGI; the most common are *Enterobacteriaceae* (such as *Escherichia coli* and *Klebsiella* species), *Neisseria gonorrhoeae, Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma hominis, Candida albicans, and Trichomonas vaginalis.*

The Meares and Stamey test is currently considered the most important test for the diagnosis of bacterial prostatitis (Nickel *et al.*, 2006). To increase its sensitivity, the test could be associated with an ultrasound examination of the prostate to allow the physician to massage more energetically the prostate zone mostly affected by the inflammatory process. Other important microbiological tests for the diagnosis of MAGI are urine culture, bacterial culture of the semen (both with bacterial colonies count), and urethral swab. To make a diagnosis of bacterial prostatitis, the quantitative bacterial culture must show, after dilution of seminal plasma with saline, a concentration $>10^3$ c.f.u/ml for pathogenic bacteria and $>10^4$ for non-pathogens (Comhaire *et al.*, 1980). In MAGI, the quantitative threshold value that best correlates with other clinical and laboratory signs (flogosis and symptomatology) is $10^5$ c.f.u./ml (Vicari, 1999).

Evaluation of viral causes, such as HPV infection, is still not recommended in the clinical practice, although recent studies show the presence of HPV-DNA in the semen of 2–31% of male general population and in 10–35% of men who undergo assisted reproductive techniques for infertility (Foresta *et al.*, 2015).

Didymo-epididymal and prostato-vesicular ultrasound scan allows a more accurate classification of MAGI by identifying the site and the extension of the inflammatory process. Therefore, according to the number of involved glands, MAGI may be divided into uncomplicated (prostatitis) and complicated (prostato-vesiculitis and prostato-vesciculo-epididymitis), and, depending on their extension, into unilateral or bilateral forms. Complicated and bilateral forms have the worst impact on sperm parameters (La Vignera *et al.*, 2012b) and worse scores in all three domains of SI-MAGI questionnaire, indicating a greater clinical severity (La Vignera, 2012a,b). The ultrasound criteria for the diagnosis of MAGI are shown in Table 2.

**Table 2** Conventional and additional ultrasound criteria for MAGI diagnosis (La Vignera *et al.*, 2012a,b,c,d)

| The classification of MAGI | Ultrasound criteria for the diagnosis |
|---|---|
| *Prostatitis* (>2 criteria simultaneously present among the following) | a. Asymmetry of the gland volume<br>b. Areas of low echogenicity<br>c. Areas of high echogenicity<br>d. Dilatation of the peri-prostatic venous plexus<br>e. Single or multiple internal similar cystic areas<br>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<br>b. Asymmetry >2.5 mm compared with the contralateral vesicle<br>c. Reduced (<7 mm) anteroposterior diameter, mono- or bilateral<br>d. Thickened and/or calcified glandular epithelium<br>e. Polycyclic areas separated by hyperechoic septa in one or both vesicles<br>f. Fundus-to-body ratio >2.5<br>g. Fundus-to-body ratio <1<br>h. Anteroposterior diameter unchanged after recent 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)<br>b. Presence of multiple microcystis in the head and/or tail (finding single or bilateral)<br>c. Low echogenicity or high echogenicity, mono- or bilateral<br>d. Large hydrocele, mono- or bilateral<br>e. Enlargement of the superior part of the cephalic tract and a superior-to-inferior part ratio >1<br>f. Unchanged anteroposterior diameter of tail just after ejaculation |

*Prostatitis*: a–d = conventional ultrasound criteria; e–f = additional ultrasound criteria. *Vesiculitis*: a–e = conventional ultrasound criteria; f–h = additional ultrasound criteria. *Epididymitis*: a–d = conventional ultrasound criteria; e–f = additional ultrasound criteria.

Depending on the different combinations of these ultrasound criteria, it is also possible to identify two different ultrasound subforms of MAGI, called 'hypertrophic-congestive' (HCUF) and 'fibro-sclerotic' forms (FSUF) (Table 3). Patients with the fibro-sclerotic form have worse sperm parameters than patients with hypertrophic-congestive form, while the latter exhibit higher leukocyte and radical oxygen species (ROS) concentrations (La Vignera *et al.*, 2011a,b,c). The hypertrophic-congestive form is, in fact, a less severe clinical form than the fibro-sclerotic one but with higher levels of oxidative stress due to the presence of congestive and stagnant areas in prostate and seminal vesicles, expression of higher levels of flogosis.

## MAGI AND INFERTILITY

Over the years, a long debate about the potentially negative impact of MAGI on sperm parameters has been going on. This debate has recently been settled by the guidelines of the European Association of Urology, which have included MAGI among the causes of male infertility (Jungwirth *et al.*, 2015).

**Table 3** Ultrasound features of hypertrophic-congestive and fibro-sclerotic variants of MAGI (La Vignera *et al.*, 2011a,b,c)

| Hypertrophic-congestive ultrasound form (HCUF) | Fibro-sclerotic ultrasound (FSUF) |
|---|---|
| *Prostate* | *Prostate* |
| • increase of volume | • asymmetry of the gland volume |
| • areas of hypoechogenicity | • areas of hyperechogenicity |
| • single or multiple internal similar cystis areas | |
| • dilatation of peri-prostatic venous plexus | |
| *Seminal vesicles* | *Seminal vesicles* |
| • mono- or bilateral increased (>14 mm) ADP | • reduced (<7 mm) mono- or bilateral ADP |
| • polycystic areas separated by hyperechoic septa | • thickened and/or calcified glandular epithelium |
| • fundus/body ratio >2.5 | • fundus/body ratio <1 |
| *Epididymis* | *Epididymis* |
| • increased (>6 mm) tail craniocaudal diameter | • bilateral head and tail areas of hyperechogenicity |
| • bilateral head and tail areas of hypoechogenicity | |
| • unchanged tail APD just after ejaculation | |

The frequency of MAGI among infertile patients ranges from 2% to 18% according to the diagnostic criteria used (La Vignera *et al.*, 2011a,b,c). MAGI can compromise male fertility through four main mechanisms: production of ROS and/or inflammatory cytokines, impaired secretory capacity of the accessory glands, anatomical obstruction or subobstruction of the seminal tract, direct effect of microorganisms on spermatozoa (La Vignera *et al.*, 2011a,b,c).

In a simplified model, the dynamics of the inflammatory processes of the urogenital tract can be subdivided into several phases (Fraczek & Kurpisz, 2007). The presence of bacteria and/or leukocytes in the seminal fluid represents the initial element. Subsequently, ROS overproduction causes an oxidative imbalance, accumulation of leukocytes, and the onset of phagocytosis. The subsequent activation of specific signal transduction pathways generate biological substances (inflammatory cytokines) that modulate the activation of pro- and antioxidant systems and induce a further significant increase in ROS (oxidative burst). The next stage is the peroxidative damage of spermatozoa. Finally, remnants of the oxidative stress response may persist in the seminal fluid for a long time after eradication of the infectious agent, further damaging the spermatozoa.

It has been shown that increased ROS production and/or decreased scavenger activity causes several sperm abnormalities, including decreased motility, acrosin activity, and penetration rate (Aitken *et al.*, 1989), hyperviscosity (Aydemir *et al.*, 2008), and sperm DNA alteration (DNA fragmentation, single- and double-strand breaks, increased DNA–proteins cross-linking) (Aitken *et al.*, 1998; Twigg *et al.*, 1998; Barroso *et al.*, 2000; Cocuzza *et al.*, 2007).

The secretory dysfunction of male accessory glands, triggered by the microorganisms or by the inflammatory response, is the most important cause of MAGI-related negative impact on sperm conventional and biofunctional parameters. Epididymal, prostate, and/or seminal vesicles damage caused by infection/inflammation results in a functional impairment, sometimes detectable by measuring the secretory products of these gland in the seminal fluid, which play a relevant role in spermatozoon function. Under normal conditions, substances produced and secreted by the epididymis (L-carnitine, neutral α-glucosidase) are involved in spermatozoon maturation. Seminal vesicles produce fructose, ascorbic acid, ergothioneine, prostaglandin, and bicarbonate; these factors act as reducing agents and prevent sperm agglutination (Okamura *et al.*, 1986). Seminal pH, citric acid, γ-glutamyl transpeptidase, and zinc have been proposed as prostate exocrine function markers. It has been showed that concentrations of neutral α-glucosidase, fructose, and zinc in seminal plasma are significantly lower in patients with urogenital infection than healthy controls, in the absence of clear signs of seminal tract obstruction at any level. A decreased concentration of these factors is associated with a lower sperm volume and higher pH values, indicating that patients with urogenital infection have an impaired function of the accessory glands. However, none of these parameters was sufficiently accurate in the statistic analysis to discriminate between infected and non-infected men (Marconi *et al.*, 2009). The glandular chemical markers alone have limited utility and must be correctly interpreted along with the other seminal characteristics.

The presence of secretory alterations in the absence of evident obstructions suggests that obstruction of the seminal tract does not represent the major cause of functional alteration of the male accessory glands during infection. Recent studies confirm that the obstruction of the seminal tracts, which in the past was considered a frequent consequence of urogenital infections, rarely occurs in reality (Weidner *et al.*, 2008).

Some microorganisms are able to directly alter spermatozoon function without the intermediation of ROS and inflammatory cytokines. This can be due to two main mechanisms: the direct adhesion of the microorganism to the spermatozoon (*Escherichia coli, mycoplasmas, Candida albicans, Trichomonas vaginalis,* HPV) or the production of soluble factors capable of altering sperm motility and/or inducing apoptosis (e.g., SIF—sperm immobilization factor—produced by *Escherichia Coli*, *Chlamydia trachomatis* lipopolysaccharide, farnesol produced by *Candida albicans*) (La Vignera *et al.*, 2011a,b,c).

It is now undoubted that MAGI represents a pathological condition capable of altering both conventional and biofunctional sperm parameters. We have shown that patients with MAGI have lower seminal fluid volume, concentration and total sperm count, percentage of normal forms, and progressive motility as well as higher concentrations of seminal leukocytes than controls. In addition, patients with MAGI have higher percentages of spermatozoa with low mitochondrial membrane potential, with phosphatidylserine externalization (a sign of early apoptosis), with higher abnormal chromatin compactness and fragmented DNA than controls, while they show a decreased percentage of alive spermatozoa evaluated by the annessin V/PI assay (La Vignera *et al.*, 2012a,b,c,d). DNA fragmentation is closely associated with several reproductive outcomes. In particular, it is negatively related to fertility rates and embryo quality, while it is positively related to miscarriages and neonatal diseases (Lewis & Simon, 2010).

The eradication of infection does not always coincide with the improvement of seminal quality, probably due to the persistence of a chronic inflammatory state. In fact, the effects of flogosis may persist even in the absence of microorganisms due to overproduction of ROS and pro-inflammatory cytokines (Moretti *et al.*, 2005).

 

A meta-analysis of 27 studies, including a total of 3241 participants (381 with chronic bacterial prostatitis, 1670 with chronic prostatitis/CPPS and 1190 controls), was recently performed to evaluate the association between chronic prostatitis and alteration of sperm parameters. The meta-analysis showed that chronic bacterial prostatitis is associated with reduction in sperm concentration, vitality, total and progressive motility, while chronic prostatitis/CPPS is related to the reduction in semen volume, sperm concentration, progressive motility, and normal morphology. Furthermore, chronic prostatitis was significantly associated with reduced zinc concentration and with the risk of developing antisemen antibodies (ASA) on seminal plasma (Condorelli *et al.*, 2017).

The progressive involvement of a higher number of glands is associated with worst sperm parameters and a more severe symptomatology (La Vignera *et al.*, 2011a,b,c). Indeed, although all patients with MAGI are at higher risk for infertility, both bilateral prostato-vesciculo-epididymitis and the fibro-sclerotic variant represent the MAGI categories with the worst sperm quality and the worst response to pharmacological treatment (La Vignera *et al.*, 2011a,b,c, 2012a,b,c,d).

## MAGI AND SEXUAL DYSFUNCTIONS

Patients with MAGI have a high prevalence of sexual dysfunction, identifiable through dedicated questionnaires.

The current scientific literature clearly shows the link between prostatitis and sexual dysfunction. Nickel suggested that the pathogenesis may involve several interconnected pathways. These may begin with an initial stimulus (infection, high pressure dysfunctional urinary flow, trauma, etc.) and may lead to a neuropathic state of pelvic nerves and muscles and/or of the prostatic tissue (Nickel, 2002). Inflammation is initially circumscribed to the prostate and peri-prostatic area, but it can progress because of the persistence of the initial factor and promote a neurogenic reaction that results in a state of chronic neuropathy. Recently, we have found that in patients with varicocele there may be concomitant dilation of the peri-prostatic venous plexus with possible repercussions on persistent worsening of the seminal viscosity which is a parameter associated with chronic inflammation (Condorelli *et al.*, 2016a). The upregulation of the pelvic neural circuit perpetuates the neuropathic state. This results in perineal, pelvic, and genital pain, abnormal urinary flow parameters, and, due to the anatomical proximity of the structures that regulate the erectile mechanism, various degrees of sexual dysfunction.

Several studies reported a high prevalence of erectile dysfunction and premature ejaculation in patients with MAGI compared with the general population (Screponi *et al.*, 2001; Liang *et al.*, 2004). We have shown that the prevalence of sexual dysfunctions (erectile dysfunction, premature ejaculation, and decreased libido) was 42% in patients with prostatitis, 52% in patients with prostato-vesiculitis, and 60% in patients with prostato-vesciculo-epididymitis (La Vignera *et al.*, 2012a,b,c,d).

Patients with MAGI and acquired premature ejaculation have a significantly higher number of MAGI ultrasonographic criteria and, in particular, a greater frequency of ultrasound alterations suggesting chronic epididymitis and vesiculitis than patients with premature ejaculation but without MAGI (La Vignera *et al.*, 2016). This obvious and preliminary consideration is functional to explain that through the ultrasound evaluation it has been demonstrated a positive correlation between premature ejaculation and anteroposterior diameter (APD) of the caudal tract of epididymis and APD of the seminal vesicles may suggest a particular ultrasound phenotype associated with premature ejaculation (La Vignera *et al.*, 2016). These ultrasonographic features may reflect an acceleration of the ejaculatory latency due to the increased seminal fluid volume. Therefore, the extension of the inflammation to the epididymis and seminal vesicles could be a risk factor for developing premature acquired ejaculation.

On the other hand, some authors suggest that prostatic syndrome and associated sexual dysfunctions may have a psychosomatic origin (Keltikangas-Järvinen *et al.*, 1981). Indeed, it has been reported that chronic prostatitis decreases the frequency of sexual intercourse, that depression and psychological disorders are common in patients with chronic prostatitis, and that patients with prostatitis tend to be more nervous and meticulous than healthy men (Berghuis *et al.*, 1996; Mehik *et al.*, 2001).

## EMERGING RISK FACTORS FOR MAGI

Chronic prostatitis affects about 1/22 men (Clemens *et al.*, 2007) and may cause, through various mechanisms (increased urinary flow pressure, intraprostatic urine reflux, sclerosis or obstruction of bladder's neck, urethral stenosis, sphincterial dyssynergia), the backscatter of pathogenic agents to the seminal tract, resulting in chronic/recurrent vesiculitis or epididymitis (Kaplan *et al.*, 1997; Nickel, 1999). To prevent post-infectious complications of chronic prostatitis and its evolution in prostato-vesiculitis and prostato-vesiculo-epididymitis, attention should be paid to the risk factors exhibited by the patient (anatomical anomalies of the urogenital tract, history of sexually transmitted diseases, sexual habits, previous pelvic traumas).

Chronic prostatitis has also been reported frequently associated with non-urological conditions such as gastrointestinal disorders, immune deficiency, and psychiatric pathologies (anxiety and mood disorders) (Rodríguez *et al.*, 2009). Among these comorbidities, a growing role is attributed to the irritable bowel syndrome (IBS), a complex of symptoms in the absence of any biochemical or structural anomaly diagnosed on the basis of the Rome III criteria (Drossman *et al.*, 2002). We, recently, reported the simultaneous presence of IBS and chronic prostatitis (mainly bacterial) in approximately 30% of patients evaluated by two different clinical settings (e.g., andrological and gastroenterological). Patients with chronic bacterial prostatitis and IBS had more severe symptoms, higher concentrations of leukocytes in the seminal fluid, and higher bacteriospermia than patients with prostatitis alone. In addition, patients with chronic bacterial prostatitis and IBS were more likely to have inflammation extended to epididymis and seminal vesicles (82%) compared to patients with chronic bacterial prostatitis without IBS (Vicari *et al.*, 2012). These evidences suggest that treatment of IBS may reduce the evolution of prostatitis into complicated forms of MAGI. Intestinal imbalance between commensal bacterial flora and pathogenic bacteria, low-grade local inflammation, abnormal immune function and intestinal motility, and alteration of the intraluminal environment characteristic of IBS could favor the retro-canalicular spreading of microorganisms to one or more male accessory sexual glands. In addition, the afferent sensitivity of a pelvic organ can affect and sensitize the innervation of other structures through direct or reflex neural connections. A generalized and prolonged sensitization of the colon caused by

 

infectious, inflammatory, neurogenic, or metabolic mechanisms could promote the cross-sensitization of male accessory glands (Ustinova *et al.*, 2010). The consequences of this sensitization include chronic pelvic pain and aberrant contractility of smooth muscle cells. Therefore, IBS could favor the onset of chronic bacterial prostatitis and its progression in complicated forms of MAGI, with greater negative impact on sperm parameters.

Recently, we reported an increased frequency of MAGI in patients with type 2 diabetes mellitus (43%) and we hypothesized that MAGI may represent an underdiagnosed complication of diabetic patients (Condorelli *et al.*, 2013). This evidence in our opinion is important, as type 2 diabetes and obesity are very common and in obese patients there may be an increase in leptin involved in mechanisms that favor the onset of prostate cancer (Alshaker *et al.*, 2015). We also described the different ultrasound characteristics of the seminal vesicles in patients with diabetes mellitus, particularly those with diabetic neuropathy (La Vignera *et al.*, 2011a,b,c). Patients with diabetic neuropathy have a higher MAGI frequency and more ultrasound criteria evocative of complicated forms than non-neuropathic diabetic patients. From the seminal point of view, they also show worse sperm parameters and increased lymphocytes concentration in semen (detected by flow cytometry) (Condorelli *et al.*, 2014a). This reflects the greater involvement of seminal vesicles in patients with neuropathy, as also shown by the decreased fructose concentrations in the seminal plasma.

In recent years, our group has shown that patients with HPV infection have a higher frequency of complicated forms of MAGI (prostato-vesiculitis and prostato-vesciculo-epididymitis) and that these patients present more frequently the fibro-sclerotic form and ultrasound abnormalities predominantly localized in the periurethral region of the prostate. This peculiar localization, possibly caused by the canalicular backscatter of urethral infection, could represent an ultrasound sign useful in the differential diagnosis of microbial vs. amicrobial (viral) forms of MAGI (Condorelli *et al.*, 2016a,b,c). Patients with MAGI and HPV infection show significantly lower percentage of spermatozoa with progressive motility and a higher concentration of leukocytes. This latter is not always detectable. In fact, in the presence of viral infections the measurement of leukocytes in the seminal fluid with the method of peroxidation—reference methodology indicated by the WHO 2010 manual—has many limitations. The technique only detects the presence of neutrophil granulocytes, and it is unable to detect activated granulocytes that already released their granules and other types of peroxidase-negative leukocytes (lymphocytes, macrophages, and monocytes). On the contrary, the evaluation of seminal leukocytes concentration using the CD45 pan-leukocyte antigen as a marker in flow cytometry is capable of detecting the presence of leucocytospermia also in viral infection and chronic inflammation (La Vignera *et al.*, 2015).

Recently, our team found that patients with testosterone deficiency have a greater extension of the inflammatory process (Condorelli *et al.*, 2014a,b), in agreement with other experimental evidence showing a modulatory action of testosterone on the inflammatory response in the male accessory sex glands (Meng *et al.*, 2011) and that prostate inflammation is associated with altered concentrations of circulating androgens and estrogens (Jia *et al.*, 2015). Male hypogonadism could therefore represent a risk factor for complicated forms of MAGI (prostato-vesiculitis and prostato-vesciculo-epididymitis) and, in particular, for bilateral and fibro-sclerotic forms that have a worse therapeutic response. Several mechanisms have been proposed to explain the potential anti-inflammatory effect of testosterone on the prostate tissue. It has been hypothesized that testosterone suppresses pro-inflammatory cytokines and T-lymphocytes production and antagonizes macrophage and neutrophil activation in prostatic tissue, preventing fibrosis progression. On addition, testosterone positively modulates the expression of prostate epithelium junctional proteins and negatively activates the immune response (Yatkin *et al.*, 2009). The alteration of prostate epithelium junctional structures induced by testosterone deficiency would cause inflammatory tissue degeneration and trigger the immune response (Condorelli *et al.*, 2014a,b). Finally, other potential mechanisms of testosterone anti-inflammatory action are the anti-estrogenic effect and the modulation of Toll-like receptor 4 expression (Quintar *et al.*, 2006; Bernoulli *et al.*, 2008).

In our recent studies, patients with MAGI showed serum 17β-estradiol (E2) concentrations significantly higher than controls, as well as a lower total testosterone (TT)/E2 ratio and higher prevalence of hypogonadism. Furthermore, patients with MAGI and testosterone deficiency or with high serum E2 concentrations showed significant abnormalities of the main sperm parameters and higher concentrations of leukocytes compared to patients with MAGI and normal hormonal serum concentrations (Condorelli *et al.*, 2016a,b,c). The hypothesis is that a decreased serum TT/E2 ratio must be a risk factor for chronic inflammation. Estrogens, in fact, regulate their peripheral effects on target cells interacting with two types of receptors: ER-α and ER-β. ER-α in prostate is predominantly localized in stromal cells, and its activation is associated with hyperplasia and inflammation; ER-β is localized in epithelial cells and mediates antiproliferative effects, as shown by ER-β knockout mice who develop stromal cell hyperplasia (Yang *et al.*, 2009). According to this evidence, Naslund and colleagues in the 1980s showed that exogenous administration of E2 worsens the incidence and the severity of inflammatory prostatitis in rats (Naslund *et al.*, 1988), while Bernoulli has recently documented that a decreased TT/E2 ratio is associated with the development of prostatitis without urodynamic alterations in animal models with hypoandrogenism and hyperestrogenism. On the other hand, obstructive disorders appear in hyperandrogenic animals with low TT/E2 ratio (Bernoulli *et al.*, 2008). In another experimental model, chronic estrogen treatment triggered the activation of metalloproteinases 2, 7, and 9 and increased the inflammatory infiltrate within the prostatic tissue (Wilson *et al.*, 2004).

## THERAPEUTIC STRATEGIES

The poor capability of antibiotics to penetrate within the prostate tissue, the altered pharmacokinetics of the inflamed gland, the antibiotic resistance of uropathogenic microorganisms, the adverse effects related to the antibiotic treatment, the presence of prostate calcification, the formation of microcolonies, and biofilm in the ductal and acinar inflamed walls are factors which contribute to the low eradication rate in patients with chronic bacterial prostatitis (Nickel *et al.*, 1995; Letkiewicz *et al.*, 2010).

Fluoroquinolones represent the first-choice therapy for chronic bacterial prostatitis because to their favorable

 

pharmacokinetic properties at the site of infection (Wagenlehner & Naber, 2006). In our study carried out on 100 infertile patients with *E. Coli* infection treated for 28 days with levofloxacin (500 mg once daily), 52% of patients eradicated the infection, 20% showed eradication with superinfection, 16% showed persistent infection, and 12% persistent infection with superinfection. Only patients with infection eradicated had sperm parameter improvement compared to patients with persistent infection, with or without superinfection. Finally, patients with persistent infection after antibiotic therapy (alone or with superinfection) showed a greater prevalence of complicated forms of MAGI (prostato-vesiculitis and prostato-vesciculo-epididymitis) than patients who obtained eradication (La Vignera *et al.*, 2012a, b,c,d). In fact, the eradication rate is inversely related to the extension of the inflammatory process, being very high (92.5%) after three cycles of antibiotic therapy in patients with prostatitis alone, intermediate (70.4%) in patients with prostato-vesiculitis, and low (52%) in patients with prostato-vesciculo-epididymitis (Vicari, 2000). Other ultrasound features associated with antibiotic therapy failure in patients with MAGI are the bilateral extension and the presence of unilateral or bilateral ejaculatory ducts subobstruction (La Vignera *et al.*, 2008). Patients with persistent infection after six antibiotic cycles are defined as non-responders (Vicari, 2000). Administration of α-blockers in combination with antibiotics in patients with chronic prostatitis and low urinary tract symptoms (LUTS) seems to decrease symptoms and prostatitis recurrence rates, probably by decreasing the degree of urinary obstruction caused by the prostatic enlargement and the inflammatory congestion (Anothaisintawee *et al.*, 2011).

Antibiotics play an important therapeutic role in bacterial prostatitis, while in non-microbial forms, the treatment of choice is based on the use of anti-inflammatory compounds. The main anti-inflammatory compounds include non-steroidal anti-inflammatory drugs (salicylates, fenamic acids, profens, Cox-2 inhibitors, arylacetics, sulfonanilides, oxicams), steroidal anti-inflammatory drugs, and fibrinolytic treatment (serratiopeptidase, bromelain, escin). In inflammatory MAGI, long-term (at least 3 months) non-steroidal anti-inflammatory treatment should be prescribed (Vicari, 1999; Vicari *et al.*, 2001; Everaert *et al.*, 2003).

In patients with prostato-vesciculo-epididymitis, antibiotic and anti-inflammatory therapy can achieve a full antimicrobial response, but may not be able to restore the oxidative balance. Therefore, antioxidant therapy should be also administered to these patients. The main antioxidant substances commercially available are shown in Table 4. However, it should be emphasized that antioxidant treatment lacks significant effects on sperm parameters when administered simultaneously with antibiotics or in the presence of bacteriospermia and leucocytospermia. This is probably due to the persistence in the seminal tract of pro-oxidant agents (bacteria or leukocytes) that generate a hostile testicular or epididymal micro-environment (Potts *et al.*, 1999; Vicari *et al.*, 2001). Therefore, in patients with leukocytospermia, the antioxidant treatment should be preceded by an anti-inflammatory therapy. Accordingly, we showed that the administration of carnitine (which exerts an antioxidant effect by correcting high intracellular acetyl-CoA concentrations and restoring the plasma membrane phospholipids) has been fully effective in lowering ROS production in patients with prostato-vesciculo-epididymitis if administered after 3 months of treatment with non-steroidal anti-inflammatory agents, while it was less effective when administered concomitantly with anti-inflammatory therapy. These findings support the hypothesis that the best therapeutic strategy in these patients is a sequential treatment with antibiotics (when needed) and anti-inflammatory drugs followed by antioxidant treatment (Vicari *et al.*, 2002). The improvement of sperm parameters obtained with this regimen might be partly explained by the complete restoration of the seminal oxidative balance resulting from the removal of pro-oxidant factors (inflammatory cytokines) both during spermatogenesis and during sperm storage in the epididymis. In addition, as sperm damage caused by a hostile epididymal environment is also related to epididymal transit, slower in oligozoospermic patients, the improvement in some sperm parameters could be due to increased sperm energetic metabolism, which accelerated spermatozoon epididymal transit. However, it is worth remembering that certain amounts of ROS are critical to perform physiologic reactions (such as acrosome reaction), and additionally, over-treatment of antioxidants can push patients toward reductive stress. It would therefore be appropriate to evaluate the oxidative stress of these patients by appropriate methods (e.g., lipid peroxidation, 8-hydroxylguanosine) (Lanzafame *et al.*, 2009; La Vignera *et al.*, 2013).

**Table 4** Main antioxidant substances commercially available (Calogero *et al.*, 2017)

| Category | Molecules |
|---|---|
| Micronutrients | Selenium, zinc, magnesium, copper |
| Amino acids and peptides | Glutathione, N-acetylcysteine, carnitine, arginine, taurine, ornithine, citrulline |
| Vitamins | Vitamin A, vitamin C, vitamin E, vitamins of group B complex, niacin (vitamin PP), pantothenic acid, folic acid |
| Omega-3 fatty acids | Docosanoic acid (DHA), eicosanoid acid (EPA) |
| Others | Inositol, coenzyme Q10, flavonoids, superoxide dismutase, *Serenoa repens, Astaxantina, Curcuma longa, Camellia sinensis, Urtica dioica, Lepidium meyenii Walp.*, muira puama *(Ptychopetalum olacoides Benth), Ginkgo biloba, Scutellaria baicalensis Georgi* and *Radix, Pinus massoniana, Cucurbita maxima, Aesculus hippocastanum, Crocus sativus, Epilobium (angustifolium and parvifl orum), Citrus bergamia, Ortosiphon*, etc. |

As seen, patients with MAGI often have sexual dysfunction, primarily erectile dysfunction, and therefore more and more patients with MAGI take type 5 phosphodiesterase inhibitors (PDE5i). This allowed to accumulate scientific data about the functional effects of PDE5i on seminal vesicles. In 2012, we carried out a study on 80 infertile patients with MAGI and erectile dysfunction: 40 of them with hypertrophic-congestive ultrasound form and 40 with fibro-sclerotic form of vesiculitis, treated with tadalafil 5 mg daily for 3 months or placebo. Prolonged treatment with tadalafil was associated with an improvement in ultrasound abnormalities in infertile patients with hypertrophic-congestive variant of vesiculitis and, particularly, an increase in the ejaculation fraction of seminal vesicles. At the same time, an improvement in seminal parameters was obtained, and in particular an increased sperm concentration and total count, seminal fluid volume, and seminal fructose concentrations (La Vignera, 2012b). Phosphodiesterase isoenzymes are present in the seminal vesicles in both smooth muscle cells (PDE3A) and

glandular epithelium (PDE3A, PDE4A, PDE5A, and PDE11A) and are involved in the control of secretory activity and efferent neurotransmission (Ückert *et al.*, 2011). Probably, sperm motility improvement in infertile patients with hypertrophic-congestive form of MAGI was due to increased levels of fructose and the raise of sperm count was associated with increased seminal volume. These findings suggest another potential therapeutic indication of tadalafil, which is particularly effective in patients with erectile dysfunction and hypertrophic-congestive form of MAGI and, because of this positive effects on sperm parameters, might also be prescribed to patients with vesiculitis and infertility.

In the presence of risk factors, it is mandatory to treat the condition responsible for the triggering and amplification of the inflammatory process. In patients with IBS, rifaximin treatment is effective in decreasing the symptoms of both IBS and chronic prostatitis (Weinstock *et al.*, 2011), while probiotics are able to suppress the low degree of inflammation associated with IBS and to restore the normal local immune function, to re-establish the intraluminal milieu, and to regularize intestinal motility. After the eradication of the microorganism responsible for prostatitis, a rifaximin and probiotic treatment should be prescribed to prevent prostate reinfection and extension of the infection to the seminal vesicles and epididymis (Hoesl & Altwein, 2005). This is crucial especially in patients who look for fertility to prevent sperm parameters worsening associated with the extension of the inflammatory process.

In clinical practice, off-label pharmacological therapy with aromatase inhibitors (Schlegel, 2012) is recommended for infertile patients with TT/E2 < 10 ratio. Finally, in diabetic patients, it is important to achieve the best possible metabolic control, avoiding hyperglycemia to prevent urogenital infections and the onset of diabetic neuropathy.

## CONCLUSIONS

Many clinical variables can affect sperm parameters and the clinical features of patients with MAGI. These include factor that trigger the inflammatory process, inflammation duration, seminal antioxidant system efficiency, anatomical extension of the inflammatory process, and seminal fluid leukocyte subpopulations. Among men with MAGI, those with prostato-vesiculo-epididymitis exhibit the worst sperm parameters and the most severe symptomatology, including sexual dysfunction. Ultrasound is an indispensable tool for evaluating the extension of the inflammatory process and for stratifying the patients, even from a prognostic point of view.

Particular attention should be paid to the risk factors that can trigger and enhance the inflammation of the male accessory sexual glands. The Roma III questionnaire should be administered to infertile patients with chronic or recurrent bacterial prostatitis in order to identify a concomitant IBS; the evaluation of testosterone and TT/E2 ratio may be helpful for the endocrine evaluation of patients with MAGI, as well as focus on glycemic control in patients with MAGI and diabetes mellitus.

Leukocytospermia, one of the parameters that—according to WHO—is used to diagnose MAGI, is not always evident using the peroxidation method. The identification of leukocytes in the seminal fluid by flow cytometry might be more helpful in detecting the presence of chronic inflammatory response cells (lymphocytes, macrophages, monocytes), and it could identify cases of MAGI (such as the viral ones) that would otherwise remain undiagnosed.

Finally, a correct diagnosis of the MAGI form and extension allows the prescription of an appropriate therapeutical strategy patient-tailored to achieve the best benefits.

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