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Testicular Microlithiasis Is Associated with Impaired Spermatogenesis in Patients with Unexplained Infertility

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Testicular microlithiasis · Unexplained male infertility · Spermatogenesis

Abstract
Introduction: Testicular microlithiasis (TML) was shown to be associated with an increased risk of infertility. However, the association of TML with spermatogenesis in patients with unexplained infertility is still unknown. In this study, we therefore investigated the effect of TML on hormones and sperm parameters in a large cohort of infertile men without major factors for impaired fertility and azoospermic men serving for comparison. ***Methods:*** Over a period of 10 years, we retrospectively analyzed 2,914 patients who attended our centre with the diagnosis of unexplained infertility and sperm count >1 million/ejaculate, as well as 281 patients with unexplained azoospermia. From the 2,914 patients, we identified 218 patients with TML as revealed by ultrasound imaging. Further, 26 out of 281 azoospermic patients showed TML. Subsequently, we performed a thorough analysis of reproductive parameters and their association with TML. ***Results:*** The overall incidence of TML in patients with unexplained infertility and in unexplained azoospermic men was 7.5 and 9.3%, respectively. Patients with unexplained infertility and TML showed significantly smaller testicular volume, elevated FSH level, and lower sperm count and motility. Impaired spermatogenesis was not associated with the amount of microlithiasis, considered after classification into subgroups (<5 vs. ≥5 microliths/testis), and instead was associated with presence or absence of TML. TML in unexplained infertile azoospermic patients was not significantly associated neither with andrological reproductive parameters nor with sperm retrieval rate in microsurgical testicular sperm extraction. ***Discussion/Conclusion:*** TML itself, and not the number of microliths, is associated with impaired spermatogenesis in patients with unexplained infertility. The parameter TML alone is not sufficient to predict spermatogenic impairment in azoospermic patients. This study highlights the importance of ultrasound imaging in the clinical evaluation of infertile men, taking into account that TML is a negative co-factor for male fertility.

Introduction

Testicular microlithiasis (TML) was first described in 1987 as multiple 1- to 3-mm small echogenic foci in testicular ultrasound imaging of a young patient scheduled

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for orchidopexy due to cryptorchidism [1]. In later years, further analyses of microliths with optical or electron microscopy and Raman spectroscopy revealed mineralized structures that are located in the seminiferous tubules of the testis and are composed of hydroxyapatite, regularly surrounded with glycogen layers [2, 3]. The formation of these microliths is believed to be derived from small eosinophilic bodies in the tunica propria (basal lamina) of the seminal tubules [4].

In andrological patients, testicular inhomogeneities including TML are frequent sonographic finding in 8.8% of cases [5]. Several previous studies showed an association not only between TML and testicular germ cell neoplasia but also with increased risk of infertility [6, 7]. Van Casteren et al. [8] showed an increase in the prevalence of TML from 1.5 to 5.6% in a normal population (n = 1,702) to 0.8–20% in men having impaired fertility (n = 5,899) [8]. However, all studies performed were heterogeneous regarding the definition of TML, number of patients included, and selection criteria. Two recent studies compared the presence of TML and outcome of semen analyses and showed no differences in number, motility, and morphology of sperms; however, study cohorts were small with 97 and 60 men, respectively [6, 9]. In a larger cohort of infertile patients, a slight association of TML with testicular volume and sperm count was shown recently; however, major factors of infertility were not excluded and thus the effect of TML on male infertility was not considered independently [10]. In the case of azoospermia, Fedder [11] described a prevalence of microlithiasis testis of 13.4%, but data on clinical andrological status and sperm retrieval rate were not included [11].

Previously, it was suggested to categorize TML by ultrasound imaging into 2 subgroups: (i) <5 and (ii) ≥5 microliths in the whole testis or per transducer field [12, 13]. The main reason for such categorization was to investigate the prevalence of testicular cancer in association with TML. However, Middleton et al. [14] showed in a prospective analysis of 1,079 patients that there is no difference in the prevalence of coexisting testicular tumours and the number of TML in ultrasound imaging after categorizing patients in <5 microliths/image versus ≥5 microliths/image [14]. This was further corroborated by Sanli et al. [15], who sub-classified TML into 3 groups (grade I 5–10, grade II 10–20, and grade III >20 microliths/image) [15].

No such studies have been undertaken in patients with unexplained infertility (no major obvious cause for their infertility) although they resemble the major fraction within the group of infertile men. At our centre, about 72% fell into this category, giving the demand to identify further factors impacting spermatogenesis in these patients [16]. The aim of this study was therefore to evaluate the association of TML with spermatogenesis in a large cohort of men with unexplained infertility and compare this on a cohort of azoospermic men with microsurgical testicular sperm extraction ([m]-TESE).

Patients and Methods

We retrospectively screened, over a 10-year period (2008–2018), our patient database Androbase© [17] to select patients diagnosed with unexplained infertility. We investigated 2 patient groups, azoospermia versus >1 million sperm/ejaculate, separately. This allowed us to analyze sperm retrieval rate in (m)-TESE independent from sperm parameters in the ejaculate.

Inclusion and exclusion criteria were set to rule out other factors causing male infertility. Inclusion criteria were as follows: inability to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse, age older than 18 years; and ejaculate volume ≥1.5 mL (to minimize chances for so far undetected obstruction of seminal duct causing infertility). Further inclusion criteria were testosterone >8 nmol/L and FSH >1 U/L, to exclude androgen pituitary deficiency. Patients with known major factors causing infertility (genetic disorders [chromosomal anomalies such as 47,XXY, and microdeletions of the Y chromosome], congenital bilateral absence of the vas deferens, oncological diseases, testicular atrophy [testicular volume <8 mL], hyperprolactinemia, and varicocele III) were excluded from our study cohort. Patient with germ cell tumour including germ cell neoplasia in situ were also excluded, as this may negatively affect spermatogenesis. Furthermore, patients who had undergone/received systemic or hormonal treatment (e.g., tamoxifen or gonadotropins) or gonadotoxic treatment (i.e., chemotherapy and radiotherapy) causing infertility were also excluded. Finally, if a major female factor (i.e., oophorectomy, tubal occlusion, polycystic ovary syndrome, and endometriosis) was present, these patients were excluded as well.

Through strict selection criteria, we achieved a highly selected patient group without known major factors causing infertility. This allowed us to investigate TML as independent effect seen in infertile men. To achieve adequate evaluation and minimize confounding factors, we divided unexplained infertile men depending on their sperm count into patients with azoospermia versus >1 million sperm/ejaculate. Subsequently, we analyzed the andrological status of these men and performed association analyses with respect to TML. In addition, we investigated sperm retrieval rate in (m)-TESE in patients with azoospermia.

TML was detected by ultrasound imaging as hyperechogenic foci (Fig. 1). Testicular ultrasound was performed with BK Medical Pro Focus by using a linear array transducer (type 8670, 12–4 MHZ). To measure testicular volume in ellipsoid form as described by Behre et al. [18], we used a BK Medical Pro Focus convex array transducer (type 8820e, 2–6 MHZ). Bi-testicular volume was determined by summated volumes of both testes. The measurements were performed by senior clinicians with experience in andrology and ultrasound.

Patients matching the criteria were further sub-classified into 2 groups, 1st: ≥5 microliths/testis and 2nd: <5 microliths/testis, according to a categorization previously suggested (Bennett et al.

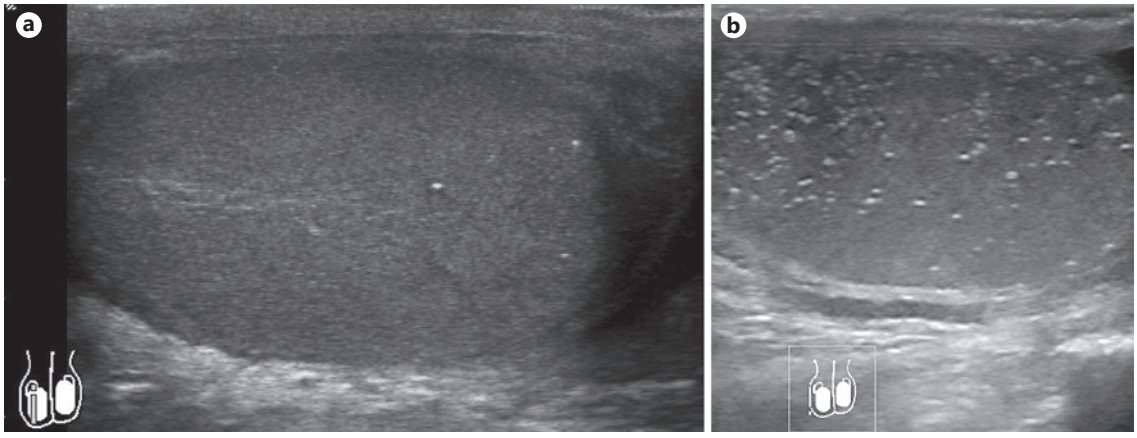

Fig. 1. Ultrasound imaging of TML of infertile patients. **a** TML <5 microliths/transducer field. **b** TML ≥5 microliths/transducer field. TML, testicular microlithiasis.

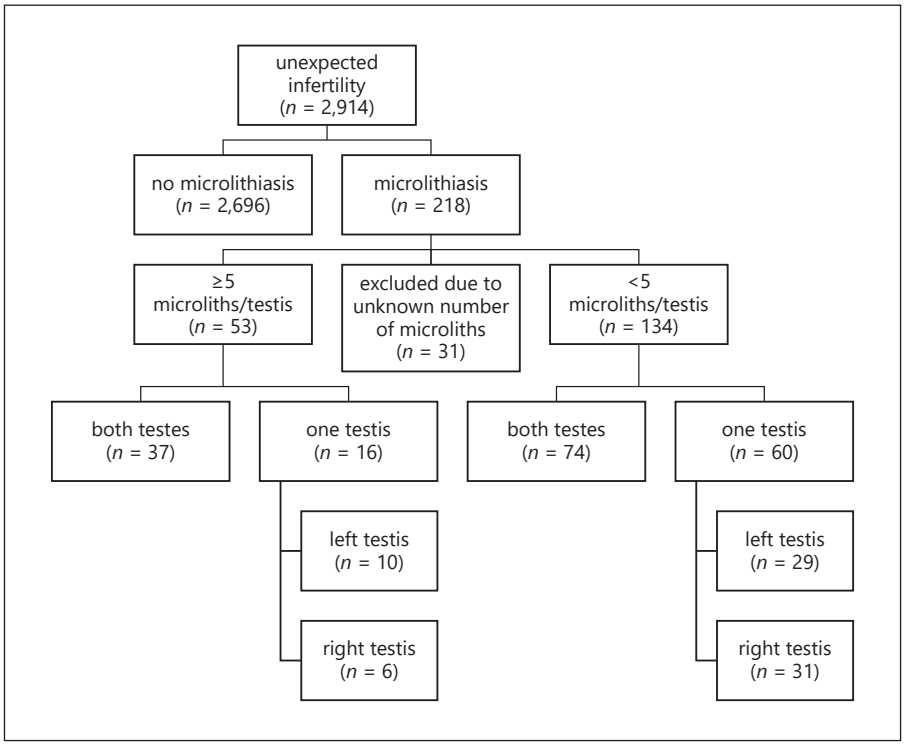

Fig. 2. Cohort of patients with unexplained infertility, building of subgroups by microlithiasis and the number of microliths.

[13]). Thirty-one patients in whom the number of microliths was not available were removed from the cohort. The 2 groups were further sub-classified depending on if one or both testes showed echogenic foci for TML. If both testes showed echogenic foci, distribution between the left and the right testis was given.

Hormone measurements of FSH, LH, and testosterone were performed by venous blood sampling between 8 a.m. and 12 p.m. Between 2008 and 2014, serum testosterone was measured using the immunoassay by a commercial ELISA kit (DRG Instruments GmbH, Marburg, Germany) (normal adult range: ≥12 nmol/L). Serum concentrations of LH and FSH were determined using highly specific time-resolved fluoro-immunoassays (Autodelfia, Freiburg, Germany) [19, 20]. Since 2014, serum testosterone, LH, and FSH were measured using a chemiluminescent microparticle immunoassay (CMIA) (ARCHITECT i1000; Abbott, IL, USA).

Semen analyses were performed according to the World Health Organization guidelines (WHO 1999 and 2010) [21, 22]. Further, for additional differentiation of sperm grade a-motility (rapid) and b-motility (slow), progressive motility was analyzed and documented. To evaluate seminal tract obstruction, neutral α-glucosidase, fructose, and zinc were measured by multi-well spectrophotometric assays [20].

Table 1. Biometric, hormonal, and semen parameters of patients without TML ($n \geq 2{,}540$) and patients with TML ($n \geq 205$)

	No microlithiasis		Microlithiasis		*p* value
	n	median (25–75 quart.)	*n*	median (25–75 quart.)	
Age, year	2,696	35 (31–39)	218	35 (31–38)	0.149
BMI	2,573	26 (24–28.7)	210	26.3 (24–28.4)	0.942
Bi-test vol., mL	2,696	40 (31–50)	218	38 (29–48)	**0.034**
FSH, U/L	2,694	4 (2.7–6)	218	4.6 (2.8–6.7)	**0.029**
LH, U/L	2,696	3.1 (2.3–4.2)	218	3.2 (2.3–4.5)	0.204
Testosterone, nmol/L	2,696	15.9 (12.4–20)	218	15.7 (12.5–20.5)	0.881
Sperm conc., mill/mL	2,696	19.5 (6.2–45.1)	218	11.5 (4.2–37.7)	**0.001**
Total sperm count, mill/ejac.	2,696	70.75 (21–164)	218	52.1 (15.7–139.4)	**0.004**
Sperm a-motility, %	2,695	33 (20–40)	218	31 (13–38)	**0.005**
Sperm ab-motility, %	2,696	47 (38–53)	218	45 (35–52)	0.051
Sperm normal morphology, %	2,540	4 (2–6)	205	4 (2-5)	**0.001**
Glucosidase, mU/ejac.	2,625	78.2 (49.5–119)	213	76.6 (47.8–127.8)	0.952
Fructose, µmol/ejac.	2,632	55.3 (34.2–86.5)	213	59.5 (37.8–82.4)	0.383
Zinc, µmol/ejac.	2,625	6.7 (4.1–10.2)	213	7 (4.4–11.2)	0.146

Missing values in the respective parameters were <5%.

(m)-TESE was performed by senior clinicians in our centre, according to the method established by Kliesch [23]. For statistical analyses, we used the non-parametric Mann-Whitney *U* test provided in IBM SPSS Statistics Version 25 (Chicago, IL, USA) to evaluate clinical datasets; *p* level (2-sided) was set as 0.05.

Results

The first study cohort consisted of 2,914 patients with unexplained non-azoospermic infertility fulfilling the given inclusion and exclusion criteria, from which 218 (7.5%) were diagnosed with TML. After sub-classification by the number of microliths, we identified 53 patients with ≥5 microliths/testis (24.3%) and 134 patients with <5 microliths/testis (61.5%) (Fig. 2). The 2 groups were further subdivided according to presence of TML in one or both testes. In both groups, the number of patients with bilateral microliths was higher than those with unilateral microlithiasis (group 1: $n = 37$ [70%]; group 2: $n = 74$ [55%]). For unilateral TML, the distribution between the left and the right testis was similar in group 2 (<5 microliths/testis) (left = 29 [48%], right = 31 [52%]), while for group 1, more patients showed microliths in the left testis (left testis = 10 [61%] vs. right testis = 6 [37%]).

There was no significant association between microlithiasis and age ($p = 0.149$) or BMI ($p = 0.942$) (Table 1). However, we observed a significant decrease in bi-testicular volume of patients having TML ($p = 0.034$) and a significant increase in FSH serum level compared to patients without microlithiasis ($p = 0.029$), which was not apparent for LH or testosterone (Table 1).

Concerning spermatogenic parameters, we observed a significantly lower sperm concentration ($p = 0.001$) as well as total sperm count ($p = 0.004$) in patients with TML (Table 1). Concerning sperm motility, progressive sperm motility (WHO 2010), a + b motility (WHO 1999), was lower in patients with TML, although not significant ($p = 0.051$); however, a significant association was seen for sperm grade a-motility (WHO 1999) ($p = 0.005$). Patients without microlithiasis had a significantly higher percentage of sperms with normal morphology ($p = 0.001$) (Table 1). For all other parameters such as seminal tract obstructions, pH, ejaculate volume, non-progressive motility, and non-motile sperm, no significant changes could be observed between groups (data not shown).

We further analyzed the microliths group with respect to the number of microliths <5 versus ≥5 microliths/testis (Fig. 1). However, for all parameters tested, we could not observe any significant association with the number of microliths (Table 2).

To investigate a putative effect of TML on the most severe form of male infertility, we evaluated a cohort of

Table 2. Biometric, hormonal, and semen parameters of patients with TML, <5 microliths/testis ($n \geq 126$) and ≥5 microliths/testis ($n \geq 49$)

	<5 microlithiasis/testis		≥5 microlithiasis/testis		*p* value
	n	median (25–75 quart.)	*n*	median (25–75 quart.)	
Age, year	134	35 (31–38)	53	33 (29–37)	0.081
BMI	129	26.3 (24–28.4)	50	26.3 (24.3–28.9)	0.739
Bi-test vol., mL	134	39 (30–46.3)	53	33 (27–47)	0.131
FSH, U/L	134	4.4 (2.8–6.7)	53	5.5 (3.1–8.6)	0.078
LH, U/L	134	3.3 (2.3–4.5)	53	3.1 (2.6–4.8)	0.416
Testosterone, nmol/L	134	15.1 (12.4–20.7)	53	14.3 (12.1–19.6)	0.552
Sperm conc., mill/mL	134	13.7 (3.6–38.7)	53	7.5 (4.1–21.4)	0.171
Total sperm count, mill/ejac.	134	59.1 (14.1–140.6)	53	29.3 (12.2–92.4)	0.181
Sperm a-motility, %	134	32 (13–38.3)	53	25 (14–36.5)	0.349
Sperm ab-motility, %	134	46 (35–52)	53	43 (32–52.5)	0.531
Sperm normal morphology, %	126	3 (2–5)	49	3 (1.5–4)	0.318
Glucosidase, mU/ejac.	130	73.1 (49.1–127.33)	53	72.4 (40.8–118)	0.449
Fructose, µmol/ejac.	130	58.5 (36–83.7)	53	62.1 (41.2–84.6)	0.637
Zinc, µmol/ejac.	130	6.7 (4.3–9.7)	53	6.9 (4–11.7)	0.843

Missing values in the respective parameters were <5%.

Table 3. Biometric and hormonal parameters and sperm retrieval of all azoospermic patients who received (m)-TESE, patients with no TML ($n \geq 232$) and patients with TML ($n \geq 24$)

	No microlithiasis		Microlithiasis		*p* value
	n	median (25–75 quart.)	*n*	median (25–75 quart.)	
Age, year	255	33 (30–37)	26	32.5 (29.8–36)	0.407
Bi-test vol., mL	255	28 (22–39)	26	29.5 (22.8–41.3)	0.426
FSH, U/L	255	13.4 (5.7–21.1)	26	13.3 (3.6–19.7)	0.504
LH, U/L	255	4.6 (3.2–6.7)	26	4.7 (2.9–7)	0.749
Testosterone, nmol/L	255	14.9 (11.4–18.9)	26	15.1 (13–19.3)	0.430
Glucosidase, mU/ejac.	232	39.7 (19.2–69)	24	28.2 (9.7–51.1)	0.098
Fructose, µmol/ejac.	232	52.6 (34–81.5)	24	53.7 (29.9–100.9)	0.831
Zinc, µmol/ejac.	232	6.4 (3.8–10.6)	24	5.4 (3.6–11.3)	0.707
SRR right (*n* sperm/all aliquots)	254	0 (0–127.5)	26	0 (0–57)	0.761
SRR left (*n* sperm/all aliquots)	254	0 (0–110.5)	26	0 (0–57.8)	0.887

Missing values in the respective parameters were <5%. (m)-TESE, microsurgical testicular sperm extraction; SRR, sperm retrieval rate in all testicular tissue aliquots taken from (m)-TESE.

281 patients with unexplained azoospermia and found a prevalence of 9.3% of TML ($n = 26$) from which 13 patients had <5 microliths/testis and 9 patients had ≥5 microliths/testis, and 4 patients had an unknown number of microliths. Interestingly in all patients with ≥5 microliths/testis, both sides were affected. Unlike the first cohort of patients with unexplained infertility we evaluated, in patients with azoospermia we did not find significant differences in any andrological parameter being tested (age, bi-testicular volume, hormones [FSH, LH, and testosterone] as well as in the sperm retrieval rate after (m)-TESE in association with TML) (Table 3).

Discussion/Conclusion

TML is a common and often neglected phenotype in ultrasound imaging during clinical workup of infertile men. This is mainly due to the fact that the knowledge of the association of TML with spermatogenesis is scant and the literature is inconsistent. A recent meta-analysis confirmed the association between testicular cancer, infertility, and TML with an OR of 18.11 (95% CI: 8.09–40.55), which marks the importance of identifying TML during clinical workup [24].

In our study, we used a large patient cohort consisting of more than 3,000 patients with unexplained non-azoospermia and unexplained azoospermia. The incidence of TML with 7.5 versus 9.3% was not significantly different between groups. Incidence of TML is in the range given for a general infertile population (0.8–20%) [8]. In line with previous studies on testicular cancer [12, 13], we categorized our patients depending on the number of microliths shown in ultrasound imaging and set a cut-off at 5 microliths/testis (Fig. 2). We noticed in both patient cohorts a 2-fold over-representation of patients with only few microliths compared to patients with ≥5 microliths/testis. Furthermore, bilateral TML was more frequent than unilateral TML (Fig. 2).

In our study cohort with sperm number >1 million/ejaculate, we noticed a significant association of TML with reduced bi-testicular volume and sperm count. These patients also displayed significantly elevated FSH level reflecting impaired spermatogenesis. This effect was not associated with the number of microliths present in each testis (Table 2). This suggests that TML per se is associated with impaired spermatogenesis and highlights the importance of ultrasound imaging in the clinical evaluation of infertile men. However, further classification into subgroups depending on the number of microliths found in ultrasound imaging seems to be clinically not relevant as reproductive parameters did not vary significantly.

Furthermore, TML alone is not sufficient to predict spermatogenic impairment in men with unexplained azoospermia, as reproductive parameters and sperm retrieval rate in (m)-TESE were not significantly altered (Table 3). It is very likely that other underlying factors and the severeness of spermatogenic impairment in azoospermia are masking the relatively mild sign of TML.

The pathophysiological mechanism of TML is still not well understood. To which extent the mineralized structures correspond to hyperechogenic foci seen in ultrasound imaging in testes is still questionable. Fedder [11] recently showed an absence of micro-calcification in all testicular biopsies of men with azoospermia and ultrasonographically detected hyperechogenic foci that participated in the study.

Moreover, it remains to be clarified if TML impairs function of seminiferous tubules and maybe therefore contributes to the impairment of spermatogenesis, or whether TML only reflects disturbed testicular function. Taken together, TML is clearly associated with spermatogenic impairment in patients with unexplained infertility and resembles as an indicator for impaired spermatogenesis, irrespective of microlith number, which should be clinically addressed by routine ultrasound examination.

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Statement of Ethics

All patients gave written informed consent for the evaluation of their clinical data according approved protocols by the Ethics Committee of the Medical Faculty and the state medical board (Az4INie; 2012-555-f-S; No. 2011-520-f-S).

Conflict of Interest Statement

All authors have nothing to disclose.

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Author Contributions

Yousif Rassam designed the study, analyzed the data, and wrote the manuscript. Jörg Gromoll and Maria Schubert designed and supervised the study and wrote the manuscript. Sabine Kliesch designed the study, supervised clinical data management, and revised the manuscript.

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