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# Association between risk factors and testicular microlithiasis

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## Abstract

**Background:** Testicular microlithiasis and its clinical significance are not fully understood. Testicular microlithiasis and risk factors have been associated with testicular cancer. The role of testicular microlithiasis is investigated.

**Purpose:** To investigate the association between testicular microlithiasis and socioeconomic and other pre-diagnostic factors.

**Material and Methods:** All men who had a scrotal ultrasound examination at the Department of Radiology, Vejle Hospital, during 2001–2013 were included. They were categorized as patients with and without testicular microlithiasis and compared with pre-diagnostic data from a nationwide registry. A total of 2404 men (283 [11.8%] with testicular microlithiasis and 2121 [88.2%] without testicular microlithiasis) were included. The association between testicular microlithiasis and pre-diagnostic conditions was investigated with logistic regression.

**Results:** Overall, we found no statistically significant differences in demographics, socioeconomic characteristics, or testicular diseases in men with and without testicular microlithiasis. Men with testicular microlithiasis had more often been treated for infertility (odds ratio [OR] 2.09, 95% confidence interval [CI] 0.84–5.24) and testicular torsion (OR 1.58, 95% CI 0.34–7.36) compared to men without testicular microlithiasis. We found no association between sexually transmitted diseases and testicular microlithiasis.

**Conclusion:** Treatment for infertility and torsion was non-significantly associated with testicular microlithiasis and no other association was found. These data do not suggest early exposure is related to testicular microlithiasis.

## Keywords

Testicular microlithiasis, ultrasonography, registry, risk factors

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## Introduction

Testicular microlithiasis (TML) is a common finding in patients undergoing scrotal ultrasonography. In general, TML prevalence has been reported higher in symptomatic populations compared to asymptomatic populations. The prevalence of TML has been reported to be in the range of 0.6–9.0% (1–4) and 4.3–18.1% in asymptomatic and symptomatic populations, respectively (5–8). Denmark has a high prevalence of testicular cancer, where approximately 10 in 100,000 men develops testicular cancer (9), furthermore a Danish study has reported TML prevalence to be very high (12.8%) (5).

A few studies have investigated ultrasound follow-up surveillance in patients with TML. DeCastro et al. published a five-year follow-up study of 63 asymptomatic patients with TML, of whom 1 (1.6%) developed

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a germ cell tumor (10). Patel et al. studied a population with >20,000 patients where a total of 442 were diagnosed with TML; during the 14-year follow-up period, 2 (0.5%) patients develop a germ cell tumor (7). Richenberg and Brejt performed a literature study including 2656 patients referred for scrotal ultrasound examination and found 51 with TML (1.9%); none of the patients develop a germ cell tumor (11). In addition, a study investigated two-year scrotal ultrasound follow-up, including 103 patients with TML, and no cases of germ cell tumors were found (12).

The clinical significance of TML is not fully understood, but a link between TML and testicular cancer has been suggested (4,13,14). However, this connection is not fully apprehended. Risk factors for testicular cancer such as cryptorchidism (15–20), family history (21–24), and infertility (17,25–27) have also been separately associated with TML. Additionally, TML has been associated with other risk factors such as ethnicity (3,28), socioeconomic (28), and genetic disorders (29–32).

It is uncertain why some men develop TML and others do not; it remains unclear if previous scrotal diseases are associated with later development of TML.

In this study, a wide range of testicular conditions was investigated. Except for *Neisseria gonorrhoea* and *Chlamydia trachomatis*, all conditions have previously been associated with TML or other testicular conditions. Country of origin and ethnicity were investigated, since there is a variation in testicular cancer incidence between different parts of the world with highest rates of testicular cancer in European and North American countries (e.g. the incidence in Denmark was 10.1 per 100,000 men) and lowest in Asian and African countries (Africa with 0.3 per 100,000 men) (9). Also, Peterson et al. found that TML prevalence varied between ethnicities (3). Education, city population, personal gross income, and work were included to determine whether socioeconomic status was associated with TML. Some studies report a higher risk of testicular cancer in men with high income (33,34).

This study is a register-based investigation based on ultrasound investigations and focuses on risk factors and TML. The hypothesis was that if TML is considered a risk factor for testicular cancer, then men with TML may differ in socioeconomic and pre-diagnostic factors compared to men without TML. The aim of this study was to analyze the association of socioeconomic and pre-diagnostic factors with a later TML diagnosis.

## Material and Methods

### Design

The study was a register-based investigation in men with and without TML identified from the department's radiology information system (RIS) database, linked to national health registers.

### Clinical databases

The RIS database contains radiology images and radiology reports, including general practitioner and hospital referrals, for all patients examined at the Department of Radiology. All medical ultrasound reports were closely reviewed to classify men as having or not having TML.

We searched the laboratory information system at the Department of Clinical Microbiology for information on how many of the included men had been tested positive for microorganisms involved in sexually transmitted infections (*Neisseria gonorrhoea* and *Chlamydia trachomatis*).

### Study population

All men who had undergone scrotal ultrasonography at the Department of Radiology from January 2001 to December 2013 were included. We excluded men with a previous testis cancer and aged <18 years at the time of the ultrasound examination.

The patients had been referred to scrotal ultrasonography due to symptoms such as pain, discomfort, family history of germ cell tumor, swelling, or they had felt a scrotal lump. The inclusion criteria were: (i) ultrasound examination of the scrotum during the period 2001–2013; and (ii) valid unique Danish civil registration number (CPR) (35).

The study population was classified according to TML status and compared with pre-diagnostic data from a nationwide registry. A total of 2404 men (283 [11.8%] with TML and 2121 [88.2%] without TML) were included (Fig. 1). Figure 2 illustrate typically sonographic features of TML.

### National health registers

The Central Office of Civil Registration issues a unique 10-digit CPR to each Danish citizen at birth and to immigrants (35). The CPR number were used to link all participants to the National Patient Registry (NPR) (36) and data from Statistics Denmark (37). Anonymity and confidentiality were preserved by the use of unique pseudonyms before retrieval of data. Statistics Denmark collects yearly demographic and socioeconomic information from all residents in Denmark (37).

The NPR has collected all information of the individual patients' contact with the Danish hospital service since 1977. It also includes all recorded diagnoses classified according to the International Classification of Diseases (ICD-10) since 1994 (36).

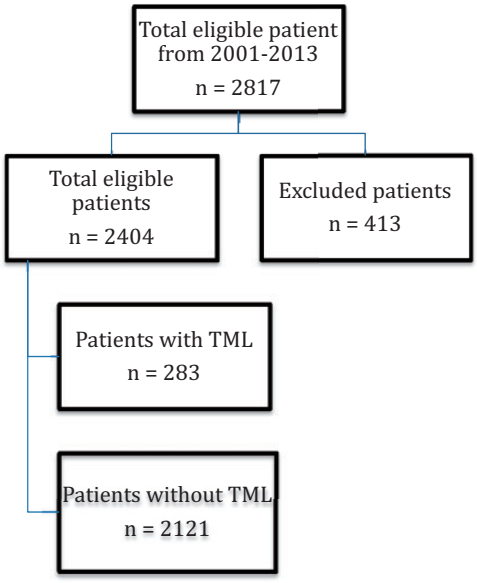


**Fig. 1.** Patient flow chart.

### *Variables*

All data were retrieved from the year before each man underwent scrotal ultrasound. Age was categorized into the following groups: 18–29 years; 30–39 years; 40–49 years; 50–59 years; 60–69 years; and >70 years. Marital status was divided into the following categories: married or cohabitating; living alone; or unknown. Cohabitating included couples regardless of their civil status. Country of origin was categorized into either Danish or Immigrant. Education was classified using the International Standard Classification of Education developed by UNESCO (38), as: basic (≤10 years), short (11–15 years); long (>15 years); or unknown. City population was categorized into the following groups: 0–4999; 5000–19,999; 20,000–99,999; ≥100,000; and unknown. The personal gross income of each patient was from the year before his ultrasound examination and divided as follows: DKK <199,999; 200,000–699,999; and ≥700,000. Labor market affiliation based on the main employment during the preceding 12 months was categorized as follows: Working; Unemployed; Retirement pension; and Other. "Other" included social welfare recipients, disability pensioners, and students.

Based on the Health Care Classification (SKS) system (36) building on the ICD-10 classification, we categorized all included men according to the event registered, i.e. treatment for infertility (DN46-DN469W [excluding DN469E]), vasectomy (KKFD46), cryptorchidism (DQ53-DQ539), varicocele (DN43-DN434), testicular torsion (DN44-DN449), or orchitis (DN450-DN450C). Only events dated before the ultrasound examination of the scrotum were included.

### *Statistical methods*

The association between TML and pre-diagnostic conditions was investigated with logistic regression estimating odds ratio (OR) with 95% confidence intervals (CI). All ORs were adjusted for age. $P \leq 0.05$ was considered statistically significant. All analyses and data management were performed using Stata Statistical Software (version 14.1, STATA Corporation, College Station, TX, USA).

### *Ethics*

The study was approved by the Danish Data Protection Agency (2008-58-0035/2009-41-3471). According to Danish law, the study did not require patients consent or approval from the Committee on Health Research Ethics of Southern Denmark since no biomedical intervention was performed.

## Results

The median age at time of ultrasound investigation was 43.4 years (age range = 18.1–85.4 years) for men with TML and 45.9 years for men without TML (age range = 18.0–90.0 years).

Patient characteristics, including demographic and socioeconomic status, stratified by TML are shown in Table 1. Patients aged >70 years have less frequently TML.

There were no statistically significantly difference in demographic, socioeconomic, or pre-diagnostic factors between men with and without TML. However, men with TML tended to have a longer education and live in cities with populations ≥100,000 compared to men without TML (OR = 1.13, 95% CI = 0.84–1.53, $P = 0.15$ and OR = 1.59, 95% CI = 0.73–3.46, $P = 0.46$, respectively).

A total of 22 (7.8%) men with TML and 152 (7.2%) without TML had a vasectomy (OR = 1.07, 95% CI = 0.67–1.70). Six men (2.1%) with TML and 21 (1.0%) without TML were infertile. Men with TML tended to have higher prevalence of treatment for infertility compared to men without TML (OR = 2.09, 95% CI = 0.84–5.24, $P = 0.08$). We found no significant difference in torsion, orchitis, or varicocele/hydrocele between the two groups ($P = 0.514$, $P = 0.982$, and $P = 0.827$, respectively).

A total of 17 men had previously been diagnosed with cryptorchidism; none had TML.

We found no association between sexually transmitted diseases and TML. A total of 53 (18.7%) men with

**Table 1.** Characteristics in men with and without testicular microlithiasis (TML) (n = 2404).

| | TML (n (%)) | No TML (n (%)) | *P* value* |
|---|---|---|---|
| Total | 283 (100) | 2121 (100) | |
| *Age (years)* | | | 0.018 |
| 18–29 | 37 (13.0) | 339 (16.0) | |
| 30–39 | 80 (28.3) | 421 (19.9) | |
| 40–49 | 65 (23.0) | 483 (22.8) | |
| 50–59 | 40 (14.1) | 353 (16.6) | |
| 60–69 | 41 (14.5) | 304 (14.3) | |
| 70+ | 20 (7.1) | 221 (10.4) | |
| *Marital status* | | | 0.676 |
| Married/cohabitating | 210 (74.2) | 1598 (75.3) | |
| Living alone | 69 (24.4) | 504 (23.8) | |
| Unknown | 4 (1.4) | 19 (0.9) | |
| *Education*† | | | 0.350 |
| Basic | 64 (22.6) | 569 (26.8) | |
| Short | 132 (46.7) | 968 (45.7) | |
| Long | 79 (27.9) | 514 (24.2) | |
| Unknown | 8 (2.8) | 70 (3.3) | |
| *City population* | | | 0.695 |
| 0–4999 | 9 (3.2) | 87 (4.1) | |
| 5000–19,999 | 64 (22.6) | 448 (21.1) | |
| 20,000–99,999 | 199 (70.3) | 1529 (72.1) | |
| ≥100,000 | 7 (2.5) | 38 (1.8) | |
| Unknown | 4 (1.4) | 19 (0.9) | |
| *Gross income (DKK)* | | | 0.543 |
| <199,000 | 74 (26.1) | 597 (28.1) | |
| 200,000–699,999 | 196 (69.3) | 1404 (66.2) | |
| ≥700,000 | 13 (4.6) | 120 (5.7) | |
| *Work* | | | 0.321 |
| Working | 214 (75.6) | 1491 (70.3) | |
| Unemployed | 10 (3.5) | 87 (4.1) | |
| Retirement pension | 49 (17.4) | 444 (20.9) | |
| Other‡ | 10 (3.5) | 99 (4.7) | |

*Chi-squared test.
†Education is defined according to the International Standard Classification of Education.
‡Includes students, social welfare recipients, and disability pensioners.

TML and 402 (19.0%) without TML had been tested for gonorrhea and/or chlamydia infection. Twenty men (7.1%) with TML and 189 (8.9%) men without TML were tested positive for *Neisseria gonorrhoea* or *Chlamydia trachomatis* (OR = 0.80, 95% CI = 0.50–1.29, $P = 0.33$).

## Discussion

### Main findings

This registry-based study is the first of its kind; no statistically significant association between TML and pre-diagnostic, demographic, or socioeconomic factors was found.

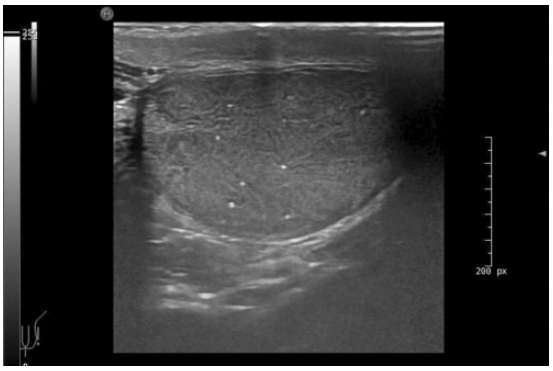


**Fig. 2.** The image shows a testicle with TML. In the study population, a total of 12% had TML; 88% had normal testicles.

### Strengths and weaknesses

The data from the Danish NPR has a high degree of completeness due to the mandatory electronic reporting, which ensures high external validity (39). Another strength is the availability of data on pre-diagnostic factors. The study is at risk of underreporting varicocele and hydrocele, since the NPR does not include diagnoses from radiology departments. Varicocele and hydrocele are often diagnosed during an ultrasound examination; if no further evaluation or treatment is needed, the information of varicocele or hydrocele will not be entered into the Danish NPR. However, the risk of underreporting is most likely equally distributed in men with and without TML. In addition, cryptorchidism could be at risk of underreporting. Some patients may have suffered from cryptorchidism and if their testicles descended spontaneously, no record would have been entered into the Danish NPR. Furthermore, there was no information in the Danish NPR or PACS on testicular volume, because testicular volume is not standard recorded. Selection bias may be present. However, to compensate for this, a large number of patients were included in this study and the patients were included over a long time interval (2001–2013).

### Comparison with other studies

Infertility has been suggested as a risk factor for testicular cancer (40,41). Several studies have investigated infertility, TML, and testicular malignancy and generally found a higher prevalence of TML in infertile men versus fertile men (26,42). A recent review also confirms this finding (43). A number of other studies found an association in infertility and TML (26,27,44,45).

We found no association between sexually transmitted diseases and TML. No other studies, to our knowledge, have previously investigated this.

Our findings showed no association between TML and cryptorchidism. The phenomenon of cryptorchidism has been suggested as a risk factor for cancer. Presently, only a few studies provide information on cryptorchidism and TML. Cooper et al. investigated 3370 children; nine were diagnosed with both cryptorchidism and TML (18). Patel et al. found 112 patients with undescended testes; eight had TML (19). Negri et al. found 232/2172 patients with a hormonally or surgically treated cryptorchidism; 22 of them had TML (17). Konstantinos et al. found 36/391 patients with ascending testes; six of them had TML (46). We found 17 patients with cryptorchidism and none of them had TML.

The role of demographic and socioeconomic factors in TML has not previously been investigated. Peterson et al. investigated a healthy asymptomatic population aged 18–35 years and found that black men had a higher TML prevalence (14.1%) compared to white men (4.0%) (3). Our study population included 15 men from countries of origin other than Denmark; the majority came from other northern European countries. One study investigated socioeconomic status in men with TML and found the most deprived socioeconomic groups to have a higher prevalence of TML (28). Overall, we did not find a difference in socioeconomic status between men with and without TML, except men with TML tended to have a higher education level compared to men without TML.

In conclusion, no significant difference in TML prevalence are seen between socioeconomic groups. Treatment for infertility or torsion was non-significantly associated with TML and no other associations are identified. Data have not suggested that exposure in testicular development is related to TML. However, this tendency between TML and treatment for infertility or testicular torsion needs to be confirmed in a larger population to rule out type 2 error. We did not find an association between sexually transmitted diseases and TML.

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