Received: 29 April 2023 | Accepted: 8 July 2023

DOI: 10.1002/jmv.28970

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Acute orchitis deciphered: Coxsackievirus B strains are the main etiology and their presence in semen is associated with acute inflammation and risk of persistent oligozoospermia

Adrian Pilatz[1] | Borros Arneth[2] | Rolf Kaiser[3] | Eva Heger[3] | Martin Pirkl[3] | Sindy Böttcher[4] | Moritz Fritzenwanker[5] | Harald Renz[2] | Annette Mankertz[6] | Hans-Christian Schuppe[1] | Florian Wagenlehner[1]

[1]Department of Urology, Pediatric Urology and Andrology, Justus Liebig University Giessen, Giessen, Germany

[2]Institute of Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics, Justus Liebig University Giessen, Giessen, Germany

[3]Institute of Virology, University of Cologne, Cologne, Germany

[4]National Reference Centre for Poliomyelitis and Enteroviruses, Robert Koch-Institute, Berlin, Germany

[5]Institute for Medical Microbiology, Justus Liebig University Giessen, Giessen, Germany

[6]National Reference Center Measles, Mumps, Rubella, Robert Koch-Institute, Berlin, Germany

Correspondence

Adrian Pilatz, Department of Urology, Pediatric Urology and Andrology, Justus Liebig University Giessen, Rudolf-Buchheim-Strasse 7, 35392 Giessen, Germany.
Email: adrian.pilatz@chiru.med.uni-giessen.de

Funding information

This study was partially financed by a start-up funding from the Faculty of Medicine at Justus Liebig University (A.P.) and by the German Research Foundation (Project PI 1247/1-1).

Abstract

Although various viruses are considered to be the clinical cause for acute orchitis, it is completely unclear to what extent and which viruses are etiologically involved in acute orchitis and what the clinic and course of these patients are like. Therefore, a prospective study was set up to decipher acute isolated orchitis. Between July 2007 and February 2023, a total of 26 patients with isolated orchitis were recruited and compared with 530 patients with acute epididymitis. We were able to show for isolated orchitis, that (1) orchitis is usually of viral origin (20/26, 77%) and enteroviruses with coxsackievirus B strains (16/26, 62%) are predominant, (2) virus isolates could be received from semen indicating the presence of replication-competent virus particles, (3) a polymerase chain reaction (PCR) for enteroviruses should be conducted using semen provided at the onset of disease, because the virus is not detectable in serum/urine, (4) there is a circannual occurrence with the maximum in summer, (5) orchitis is associated with a characteristic inflammatory cytokine panel in the semen and systemic inflammation, (6) orchitis is usually rapidly self-limiting, and (7) about 30% of patients (6/20) suffer ongoing oligozoospermia. These seven emerging aspects are likely to fundamentally change thinking and clinical practice regarding acute isolated orchitis.

KEYWORDS

coxsackievirus, enterovirus, epididymitis, fertility, mumps, Orchitis

Adrian Pilatz and Borros Arneth are co-first authors.

1 | INTRODUCTION

Orchitis is defined as inflammation of one or both testicles. A distinction is made between acute, symptomatic courses and chronic, asymptomatic orchitis. While chronic orchitis is typically diagnosed histologically in the course of a testicular biopsy in cases of infertility,[1] the characteristic feature of acute orchitis is painful swelling of the testicle. Acute orchitis can occur in two different forms: (1) as concomitant orchitis together with epididymitis or (2) as isolated orchitis. Concomitant orchitis is the more common form and occurs in up to 90% of patients with epididymitis, which is usually caused by bacterial ascension along the urogenital tract.[2] In contrast, isolated orchitis is not associated with urinary tract infections and typically manifests in the setting of systemic viral infections.[3–10] Based on epidemiological and clinical findings, a wide variety of viruses have been proposed to be etiologically relevant for isolated orchitis.[11]

The available literature reports predominantly on mumps virus.[12–14] Historical data before the availability of mumps vaccination showed that isolated orchitis manifests in about 18% of cases (1468/8153) and usually about 5–10 days after parotitis.[8] Since the introduction of the mumps–measles–rubella vaccine, the incidence of mumps orchitis has drastically decreased. Nevertheless, mumps orchitis still occurs despite mumps vaccination, although the prevalence of orchitis (30% vs. 6%) and the clinic seem to be less pronounced.[7]

In addition to mumps, which has been known for many centuries, epidemic pleurodynia/myalgia in association with orchitis was first described in 1930 on the Danish island of Bornholm.[15] In a Swedish epidemic in 1931, 6% of those affected (50/884) suffered also isolated orchitis.[16] Although a viral etiology was suspected, it was not until 1948 that a coxsackievirus strain was isolated from a diseased patient and thus identified as the cause of epidemic pleurodynia/myalgia, or Bornholm disease.[17]

In the following decades, several case series were published describing large outbreaks of mumps and Bornholm disease and also reporting cases with orchitis as a complication.[18–21] In addition, there are individual case reports of patients with isolated orchitis whose underlying etiology could be attributed to distinct other viruses.[10,22,23] Testicular involvement in terms of orchitis has also been described for severe acute respiratory syndrome coronavirus 2 (SARS-COV-2).[24] Nevertheless, systematic cohort studies on patients with isolated orchitis involving molecular biological diagnostics do not exist.

The male genital tract is a reservoir for a variety of viruses that can have a marked pathogenic potential.[11] In this context, the possibility of viral transmission and infection with important viral pathogens such as human immunodeficiency virus (HIV), hepatitis B virus, and Zika virus must be considered in terms of sexuality and reproduction.[11]

In addition, local inflammation is feared, since it can be associated with destruction of the testicular architecture leading to testicular atrophy and thus to infertility. In this regard, publications cover exclusively mumps virus infection and describe a testicular atrophy rate of 53% and azoospermia of 15% after unilateral and 60% after bilateral orchitis.[12,25] Conversely, because of these detrimental effects, many studies have investigated patients presenting with infertility for possible causal viral pathogens.[26,27]

Although various viruses are considered to be causally relevant for acute orchitis, it is completely unclear to what extent the etiological agent may be of viral nature and which viruses are actually involved in the initiation of acute orchitis. Moreover, descriptions of the respective clinical and course of these patients are lacking. Therefore, the present prospective study was set up to decipher the etiology, clinic, course, and impact on semen quality of isolated orchitis.

2 | MATERIALS AND METHODS

2.1 | Study population

After receiving approval from the institutional review board (Ref. No 100/7 and 45/11), we conducted a prospective study on patients with acute isolated orchitis at the Department of Urology, Pediatric Urology and Andrology, Giessen, Germany (German clinical trials register: DRKS00003325) in the period from July 2007 to February 2023. The inclusion criterion was acute isolated orchitis, defined as onset within the last 2 weeks, enlarged testis on palpation typically associated with pain, and testicular hyperemia in ultrasound.[4,28–30] The exclusion criteria were involvement of the epididymis, post vasectomy pain, and age younger than 18 years. Altogether, 26 patients with isolated orchitis were recruited (Figure 1). For comparison, 530 patients with acute epididymitis were recruited in the same time period as controls. Acute epididymitis was defined as onset within the last 2 weeks, enlarged epididymis on palpation typically associated with pain, and epididymal hyperemia in ultrasound.[2] Concomitant orchitis was explicitly allowed in patients with epididymitis. Exclusion criteria were postvasectomy pain, age younger than 18 years, or other scrotal pathology. Finally, 39 patients were recruited as controls without urogenital tract inflammation who presented before scheduled vasectomy.[31] The characteristics of the group of patients with epididymitis and the control cohort in whom vasectomy was scheduled are provided in the Supporting Information: Tables 1 and 2. Written informed consent was obtained from all participants.

2.2 | Medical history, physical, and ultrasound examination

A structured general and sexual history was taken from all patients. Because vaccination cards were not available and recall of mumps vaccination is unreliable, vaccination status was not evaluated. By means of palpation and ultrasound, the scrotal contents were evaluated as described in detail elsewhere.[2,32] Transrectal ultrasound was performed to assess the morphology and volume of the prostate. Body temperature was measured in the ear and recorded in degree Celsius.

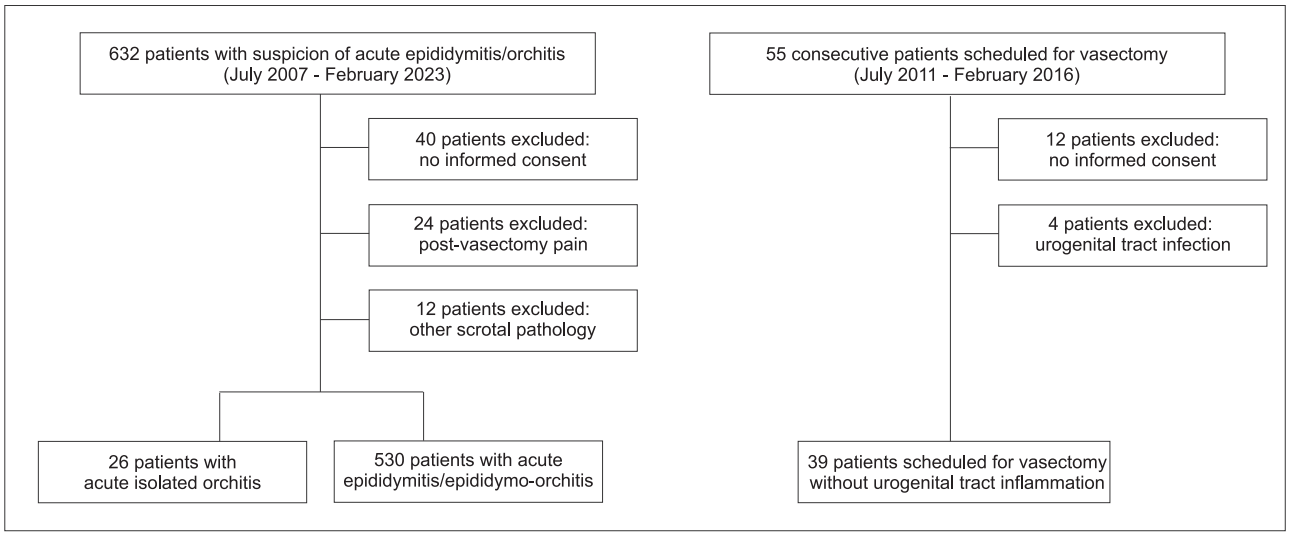

FIGURE 1 Flowchart of patient inclusion.

2.3 | Laboratory methods

Routine blood samples were taken in all patients to determine white blood cell count (WBC), C-reactive protein (CRP), and serum prostate-specific antigen (PSA). In patients with semen analysis, a parallel evaluation of the sex hormones was always carried out including follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, testosterone, estradiol, sex hormone-binding globulin (SHBG), and albumin by routine laboratory methods in the central laboratory of our university hospital (ADVIA and ADVIA Centaur, Siemens Health Care). Leukocyturia was determined by urine dipstick analysis with an automated quantitative urine particle analyzer (cobas u 411, Roche Diagnostics GmbH). Assays were performed by a technician blinded to the sources of the samples.

2.4 | Bacteriological diagnostics

All patients were screened in urine and semen for sexually transmitted infections (STIs) (*Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis*) and received bacterial cultures. All cases without a bacterial pathogen in culture and negative STI polymerase chain reaction (PCR) received 16 S rDNA analysis from midstream urine, as described.[33] A detailed description is provided in the Supporting Information Material and the diagnostic algorithm is provided in Figure 2.

2.5 | Virological diagnostics

Extensive virological diagnostics was performed in all patients with isolated orchitis by PCR from serum, urine, and semen samples stored at −80°C. Virologic diagnostic workup was also performed in patients with acute epididymitis when bacteriologic diagnostic workup was negative. Control patients scheduled for vasectomy received virological screening for enteroviruses only. The diagnostic algorithm is shown in Figure 2. A total of 19 respiratory viruses (influenza A, B, H1N1 pdv 2009; parainfluenza 1, 2, 3; coronavirus 229E, OC43, NL63; HKU1, SARS-CoV-2 (since 2020), human metapneumovirus A, B; human respiratory syncytial virus A, B; rhinovirus/enterovirus, bocavirus, adenovirus) were tested using a commercial real-time multiplex assay (Luminex) and in case of a positive reaction for rhinovirus/enterovirus, reflex testing was performed with the enterovirus specific test (Quidel). Assays for BK virus (BKV), parvovirus B 19 (PB19) were from Altona diagnostics (Altona Diagnostics), herpes simplex virus 1 and 2 (HSV 1/2), varicella zoster virus (VZV), Epstein–Barr virus (EBV), and cytomegalovirus (CMV) were commercial assays from a different supplier (Medac Diagnostika). All samples for virus analyses were prepared with ROCHE MagNA Pure 96 (Roche Diagnostics GmbH), while mumps PCR was performed as in-house test as described previously.[34] For molecular typing of the enterovirus strains identified with screening PCR, the VP1 region was amplified using enterovirus species-specific reverse-transcription polymerase chain reaction (RT-PCR) assays.[35] Sanger sequencing was conducted with primers used for amplification and resulting partial VP1 sequences were aligned to reference sequences available in Genbank® using BLAST algorithm. Left-over residues of three original semen samples (coxsackievirus strains B2, B4, and B5) were inoculated into RD-A, Vero, and CaCo-2 cells and incubated at 37°C with 5% CO_2 for 7 days and passaging for a maximum of two times to fresh cells was conducted until a cytopathic effect (CPE) was visible. In addition, all patients with isolated orchitis underwent serological analysis for mumps-specific immunoglobulin M (IgM) and immunoglobulin G (IgG) using DiaSorin assays. From 2013, PCR diagnostics for mumps was also performed from gingival swabs in the National Reference Center Measles, Mumps, Rubella (NRC MMR).

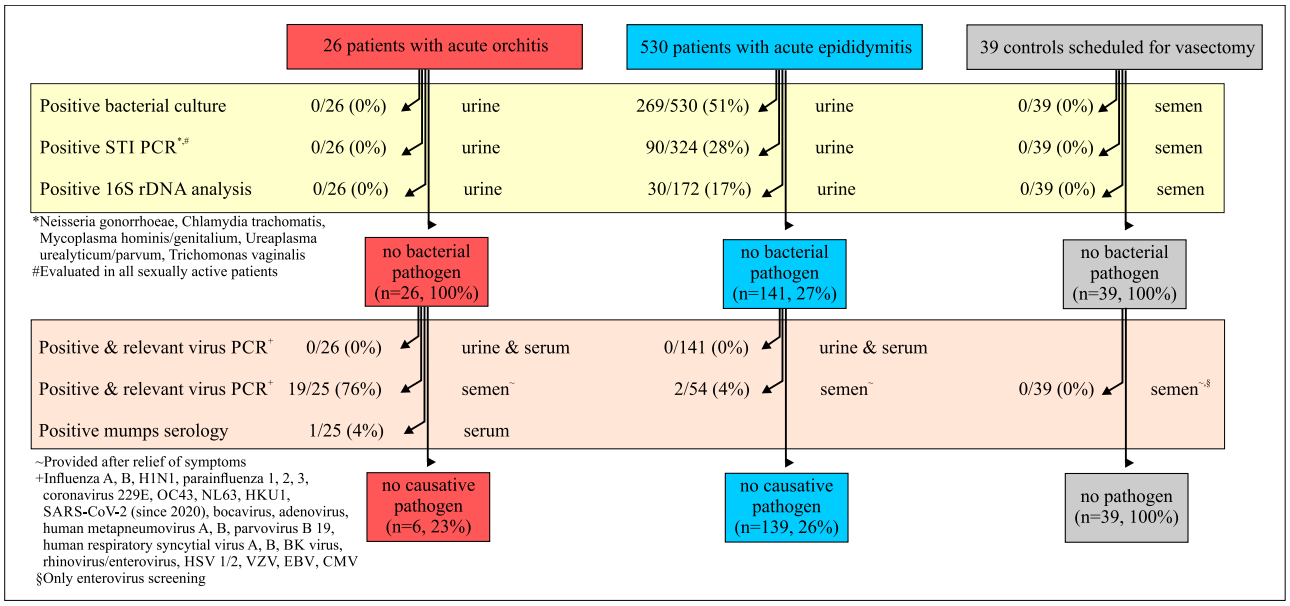

FIGURE 2 Bacteriological and virological diagnostic algorithm of patients with acute orchitis and control groups with acute epididymitis and men scheduled for vasectomy. Bacteriology was always conducted first, followed by virological diagnosis in patients without an etiologically relevant pathogen. The diagnostics were performed from the body fluids obtained at the initial presentation of the patients. Solely, the semen samples of patients with acute inflammation could only be obtained after the pain symptoms had subsided (typically a few days). STI polymerase chain reaction (PCR) was performed exclusively in sexually active patients (= activity within the last 6 months). In control patients for scheduled vasectomy, viral analysis was done in the semen samples exclusively for enteroviruses.

2.6 | Semen analysis

Semen samples were collected by masturbation into a sterile container at the clinic. Men had been asked to adhere to sexual abstinence of 2–7 days. Analysis was performed within 1 h of collection in a blinded fashion, according to World Health Organization (WHO) recommendations (WHO, 2010). As part of standard processing, the concentration of peroxidase-positive leukocytes was determined (Leucoscreen, FertiPro). In addition, polymorphonuclear (PMN) elastase reflecting local inflammation was measured in cell-free seminal plasma by means of an enzyme-linked immunoassay in each semen sample (Demeditec Diagnostics GmbH). Levels of neutral α-glucosidase and fructose (total enzymatic activity) were determined by spectrophotometrical methods. Zinc was assessed using a commercially available kit (Zinc Kit). From each semen sample, 100 µL were used for the comprehensive microbiological work-up, and a further 100 µL seminal plasma was stored at −80°C for cytokine analysis while the seminal pellet was used for virological diagnostics and stored at −80°C until en bloc analysis.

2.7 | Cytokine analysis

Measurement of cytokine concentrations in human samples was performed in a blinded fashion using the cytometric bead array (CBA) (BD Biosciences), as previously published.[31] The analyzed cytokines included the proinflammatory interleukin-1α (IL-1α), IL-1β, IL-6, IL-12p70, and tumor necrosis factor-α (TNF-α), T-cell-associated cytokines interferon-γ (IFN-γ), IL-2, IL-4, IL-5, IL-9, IL-10, IL-13, IL-17A, growth factors vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), granulocyte colony-stimulating factor (GCSF), Granulocyte-macrophage colony-stimulating factor (GMCSF), Granzyme A, and chemokines monocyte chemoattractant protein-1 (MCP-1, CCL2), macrophage inflammatory protein-1 alpha (MIP-1α) (CCL3), RANTES (CCL5), Eotaxin (CCL11), IL-8 (CXCL8), IP-10 (CXCL10), I-TAC (CXCL11), and Fractalkine (CX3CL1). In brief, after gentle thawing, 50 µL sample volumes were transferred to a 96-well plate and measured together with the appropriate cytokine standards in duplicates (0–5.000 pg/mL) and two blank wells according to the manufacturer's instructions. Parameters exceeding the range of measurable concentration were diluted individually before transfer. Finally, samples were analyzed using the BD FACS Canto II Flow Cytometer together with the FCAP v 3.0 software (BD Bioscience and SoftFlow).

2.8 | Therapy and follow-up

Patients were managed on an outpatient basis or hospitalized as medically indicated. As no causal therapy has been established for patients with isolated orchitis, symptomatic analgetic therapy was primarily used. Close sonographic control was essential to recognize possible differential diagnoses at an early stage. In contrast, the control group with epididymitis received empiric antibiotic therapy until after receipt of the microbiology could be switched to test-appropriate therapy if necessary.

After initial management, an early follow-up was scheduled after 2 weeks to assess the immediate response and a further follow-up

after 12 weeks, and if possible a late follow-up after several years to assess clinical outcomes.

2.9 | Statistical analysis

Data are expressed as the median and interquartile range (IQR) in the case of metric variables and number (%) when having nominal/categorical variables. Statistical analysis was done to investigate possible differences between the affected and the healthy side, to assess changes after the acute infection in the clinical course as well as to compare different parameters of patients with isolated orchitis to those with epididymitis. Nonparametric tests were utilized for metric variables comparing two or more groups in related (Wilcoxon test, Friedman test), or unrelated samples (Mann–Whitney *U* test, the Kruskal–Wallis test), as indicated. The cytokine characteristics of isolated orchitis patients and controls were subjected to a two-way hierarchical cluster analysis, using Morpheus, https://software.broadinstitute.org/morpheus. A value of $p < 0.05$ was considered statistically significant. Statistical analyses were performed by means of IBM SPSS Statistics 27 for Windows (IBM GmbH).

3 | RESULTS

3.1 | Demographics and clinical characteristics of patients with isolated orchitis

Twenty-six patients with isolated orchitis were prospectively recruited over a 16-year period. The patients typically presented during the summer months (Figure 3). Baseline characteristics are provided in Table 1. It is striking that, at a median age of 35 years, these were young men who reported a previous respiratory tract infection in the last 10 days in 54% of the cases and did not show any symptoms suggestive of a urinary tract infection (dysuria, urethritis). A systemic inflammation with borderline elevated white blood cells (WBCs) and a clearly elevated c-reactive protein (CRP) level of 36 mg/L at the median was evident. The testicular size of the affected testis was significantly larger at 18.7 mL than the opposite side at 13.9 mL ($p < 0.001$), likewise the PSV of the testicular artery was significantly increased ($p < 0.001$), while the epididymal head diameter was comparable ($p = 0.339$).

3.2 | Bacteriological and virological findings in patients with isolated orchitis

Despite extensive bacterial diagnostics from urine and semen examination by means of culture, STI PCR, and 16 S rDNA analysis, no etiologically relevant bacterial pathogen was found in any patient with isolated orchitis. This is in clear contrast to the group of patients with acute epididymitis without antimicrobial pretreatment, where bacterial pathogens could be found in 85% (Figure 2, Supporting Information: Table 3).

Mumps serology was available for 25/26 patients. Only one patient with bilateral orchitis and myositis showed a positive IgM titer, while 13 patients had IgG-positive titers after immunization/disease, and 11 others did not demonstrate IgG antibodies. Unfortunately, the patient with the positive IgM titer did not show

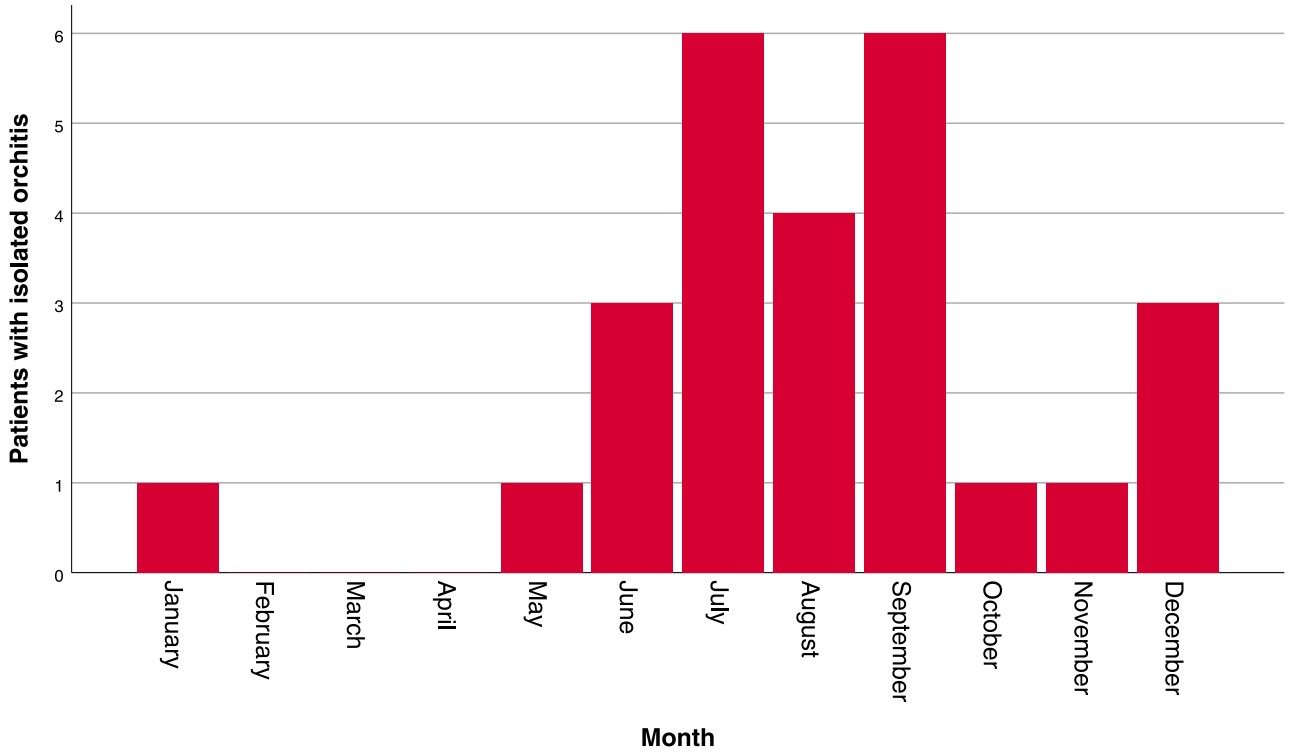

FIGURE 3 Circannual presentation of the patients ($n = 26$).

TABLE 1 Baseline characteristics of patients with isolated orchitis.

Parameter	Median (IQR) or n (%)	*n*
Patient demographics		
Age, year	35 (28–39)	26
Weight, kg	83 (73–91)	26
Patient history		
Respiratory tract symptoms within last 10 days	14 (54)	26
History of vasectomy	0 (0)	26
Endourological surgery within last 20 days	0 (0)	26
History of previous orchitis	1 (4)	26
Sexually active within last 6 months	23 (89)	26
Sexual history suggestive of STIs	0 (0)	26
Last ejaculation before presentation, days	5.5 (2.6–10.0)	26
Hematospermia at last ejaculation	0 (0)	26
Antibiotic premedication	5 (19)	26
Analgetic premedication	12 (46)	26
Clinical characteristics		
Fever >38°C	6 (23)	26
Side (right/left/bilateral)	14/10/2 (54/38/8)	26
Pain severity score, 0–10	7 (4–8)	26
Onset of symptoms, days	2 (1–3)	26
Indwelling catheter	0 (0)	26
Urethritis	0 (0)	26
Dysuria	0 (0)	26
Number of patients hospitalized	13 (50)	26
Hospitalization, days	3 (2–4)	13
Laboratory findings		
WBC, giga/L	8.8 (7.3–11.5)	26
CRP, mg/L	36.1 (17.4–49.6)	26
PSA, ng/mL	0.7 (0.4–0.8)	25
Leukocytes,/µL urine	0 (0–0)	26
Ultrasound findings		
Testicular volume healthy testis, mL[a]	13.9 (9.8–17.4)	22
Testicular volume affected testis, mL[a]	18.7 (14.9–23.7)	22
Epididymal head width healthy side, mm[a]	8.1 (6.9–9.9)	22
Epididymal head width affected side, mm[a]	8.8 (6.7–11.2)	22
Peak systolic velocity testicular artery healthy side, cm/sec[a]	9.1 (6.7–14.3)	22
Peak systolic velocity testicular artery affected side, cm/sec[a]	20.9 (17.2–27.6)	22
Prostate volume, mL	14.7 (13.6–16.9)	21

Abbreviations: CRP, C-reactive protein; PSA, prostate-specific antigen; WBC, white blood cell.

[a]Patients with bilateral orchitis excluded.

up for follow-up and the mumps PCR in the semen taken during the acute disease gave a negative result.

No viral pathogen was detected in the serum at the first presentation of any of the patients with isolated orchitis. BK virus was detected in the urine provided at first presentation in four patients and CMV in three patients. At the 12-week follow-up, BK virus was still present in two of the three available urine samples (Table 2).

After improvement of the acute pain symptoms, 25 patients with isolated orchitis were able to provide a semen sample for diagnostic issues a median of 3.5 days after initial presentation. In these semen samples, the enterovirus PCR was positive in 19/25 patients. Subtyping using the VP1 sequence revealed coxsackievirus B2 (CVB2) ($n = 1$), CVB3 ($n = 1$), CVB4 ($n = 9$), CVB5 ($n = 5$), and enterovirus 71 C2 (EV-A71). In two patients, further subtyping was not possible. To test whether the viruses were replication-competent and not just genome fragments, semen samples from three patients (CVB2, CVB4, and CVB5) were inoculated into cell culture. All three virus strains were successfully isolated from cell culture. It should be emphasized that the enterovirus PCR was always negative in all available ejaculates in the follow-up after 12 weeks (Table 2).

In contrast, virological diagnostics in semen was positive (1× CVB3, 1× CVB4) only in 2/54 patients in the control group of patients with acute epididymitis, where bacteriological analyses were negative (Supporting Information: Table 3).

While usually one to three patients per year presented with acute isolated orchitis, there was no single case of isolated orchitis during the SARS-CoV-2 pandemic in 2020 and 2021. It was not until 2022 that two patients again presented with isolated orchitis (Table 2). In contrast, the yearly number of cases in the control group with acute epididymitis remained stable, regardless of the SARS-CoV-2 pandemic.

3.3 | Assessment of seminal inflammation in patients with isolated orchitis

In 19 of the 26 patients with isolated orchitis, a comprehensive cytokine panel from the seminal plasma in the acute phase could be evaluated and compared with the cytokines in 39 control patients who presented for vasectomy. Various cytokines were

TABLE 2 Detailed overview of virological diagnostics.

Patient	Age	Year	History of resp. infect.	Baseline urine Virus, copies/mL	Early semen[a] Virus, copies/mL	Mumps IgG Baseline	Mumps IgM Baseline	Follow-up urine[b] Virus, copies/mL	Follow-up semen[c] Virus, copies/mL
74	25	2009	Yes	BKV, 738	Negative	<5.0	<0.5	BKV, 1730	ND
77	38	2009	Yes	Negative	CVB4	23.9	<0.5	ND	Negative
80	35	2009	No	BKV, 65	CVB5	<5.0	<0.5	BKV, 1690	Negative
128	32	2010	No	Negative	EV-A71	<5.0	<0.5	ND	Negative
130	36	2010	No	Negative	Negative	<5.0	<0.5	NA	NA
200	47	2011	No	Negative	NA	<5.0	<0.5	NA	NA
215	41	2012	Yes	Negative	Negative	<5.0	5.4	NA	NA
216	38	2012	yes	Negative	CVB5	<5.0	<0.5	ND	Negative
257	35	2013	Yes	Negative	CVB3	150	<0.5	ND	Negative
268	39	2013	No	Negative	Negative	127	<0.5	NA	NA
298	23	2014	Yes	Negative	CVB4	25.0	<0.5	ND	Negative
335	29	2015	Yes	Negative	CVB5	<5.0	<0.5	ND	Negative
346	41	2016	No	Negative	CVB2	104	<0.5	ND	Negative
383	28	2016	No	Negative	CVB4	145	<0.5	ND	Negative
412	27	2017	No	BKV, 1140	BKV, 42	12.7	<0.5	NA	NA
417	34	2017	No	Negative	negative	79.7	<0.5	ND	Negative
419	39	2017	Yes	Negative	CVB4	<5.0	<0.5	ND	Negative
424	37	2017	Yes	Negative	CVB4	<5.0	<0.5	ND	Negative
441	39	2017	No	Negative	CVB4	295	<0.5	ND	Negative
468	23	2018	Yes	CMV, 1710; BKV, 204	CVB4	54.8	<0.5	Negative	Negative
477	21	2018	Yes	CMV, 4460	CVB5	<5.0	<0.5	NA	NA
481	45	2018	Yes	CMV, 3380	CVB5	<5.0	<0.5	NA	NA
517	34	2019	Yes	NA	Enterovirus pos, typing not successful	NA	NA	NA	NA
523	20	2019	Yes	Negative	CVB4	75.1	<0.5	NA	NA
628	36	2022	Yes	Negative	CVB4	57.9	<0.5	NA	NA
631	50	2022	Yes	Negative	Enterovirus pos, typing not successful	188	<0.5	NA	NA

Abbreviations: BKV, BK virus; CMV, cytomegalovirus; CV, coxsackievirus; enterovirus pos, typing not successful: enterovirus screening PCR positive, but VP1 sequencing failed; NA, sample not available; ND, not determined.

[a]Semen analysis after a median of 3.5 days after initial presentation.

[b]12 weeks after first presentation, analysis only performed when baseline was positive.

[c]Only performed, when previous semen analysis was positive.

significantly increased in patients with isolated orchitis. The two-way hierarchical cluster analysis provides a clear categorization of the individual samples into patients with isolated orchitis and controls. In the case of the patients with isolated orchitis, there is also no difference as to whether the viral analysis was positive in semen or not (Figure 4).

3.4 | Management and clinical course of patients with isolated orchitis

Thirteen out of 26 patients with isolated orchitis were monitored as inpatients for a median of 3 days due to pronounced pain symptoms. No patient was misdiagnosed as having an overlooked testicular

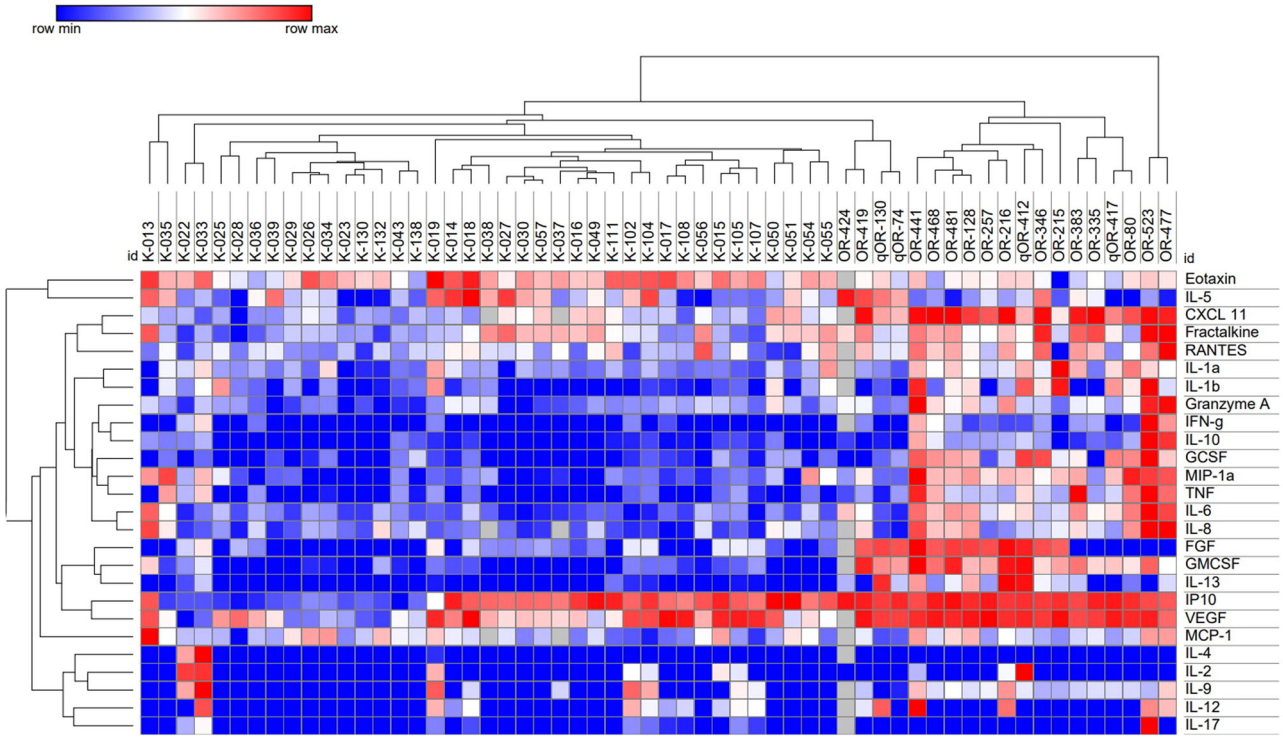

FIGURE 4 Heat map of the seminal cytokines in orchitis patients (OR) and controls (K). The hierarchical two-way cluster analysis shows a clear categorization into patients with isolated orchitis and controls based on the cytokine profile in the semen. The "q" in front of the orchitis number symbolizes that no virus was detected by polymerase chain reaction (PCR) in semen. Linkage method: average; metric: one minus Pearson correlation; data adjusted by log2 (1 + x). Gray color is used for missing values, the colors from blue to red symbolize per cytokine the values from minimum (blue) to maximum (red).

torsion. A total of 85% (22/26) of the patients received symptomatic therapy with nonsteroidal anti-inflammatory drugs for a median duration of 5 days. Antibiotic therapy was omitted in 12 of 26 patients (46%). These were especially the most recently recruited patients. The other patients received either levofloxacin ($n = 12$), doxycycline ($n = 1$), or azithromycin ($n = 1$) for a median duration of 8 days.

Eighteen patients with isolated orchitis received follow-up investigations after 2 weeks (median 13 days), 14 patients another follow-up 12 weeks (median 92 days) after initial presentation, and finally six patients a late follow-up after a couple of years (median 214 weeks). As the disease is usually self-limiting, the pain subsided rapidly during the course ($p < 0.001$, Friedman test, Supporting Information: Figure 1).

When looking at the systemic inflammatory parameters in the course, a normalization of the white blood cell counts as well as of CRP was evident (in both cases $p < 0.001$), while the PSA value was stable in patients with isolated orchitis ($p = 0.718$). This is in clear contrast to the patients with acute epididymitis where PSA was elevated in the acute phase and dropped significantly in the follow-up ($p < 0.001$) (Supporting Information: Figure 2).

The local cytokine milieu in semen was determined in 12 paired patients in the acute phase of isolated orchitis and again after 12 weeks. This showed a significant decrease in the sense of a normalization of the cytokine concentrations of fractalkine, IFN-γ, IL-6, IL-8, IL-10, and MIP-1α (all $p < 0.05$), although the levels of several cytokines were still lower in the control group of patients without urogenital inflammation (Figure 5).

3.5 | Impact of isolated orchitis on testicular function and semen quality

Testicular volumetry during the course shows that the initially strong swelling on the affected side normalizes and the testicular volume then continues to decrease toward testicular atrophy in the long-term follow-up. However, only six patients could be recruited for a late follow-up after a median of 214 weeks, so that there is no statistically significant difference between affected and contralateral sides, although the healthy testicle had a median volume of 15.7 mL compared with 10.0 mL on the initially diseased side ($p = 0.075$, Wilcoxon test). Comparable to testicular size, the peak systolic velocity (PSV) of the testicular artery was significantly increased in the acute phase and normalized rapidly. In contrast, the diameter of the head of the epididymis was comparable over time and independent of the side (always $p > 0.05$) (Supporting Information: Figure 3).

The semen parameters and the corresponding sex hormones can be found in Table 3. Here, all median values were within the normal

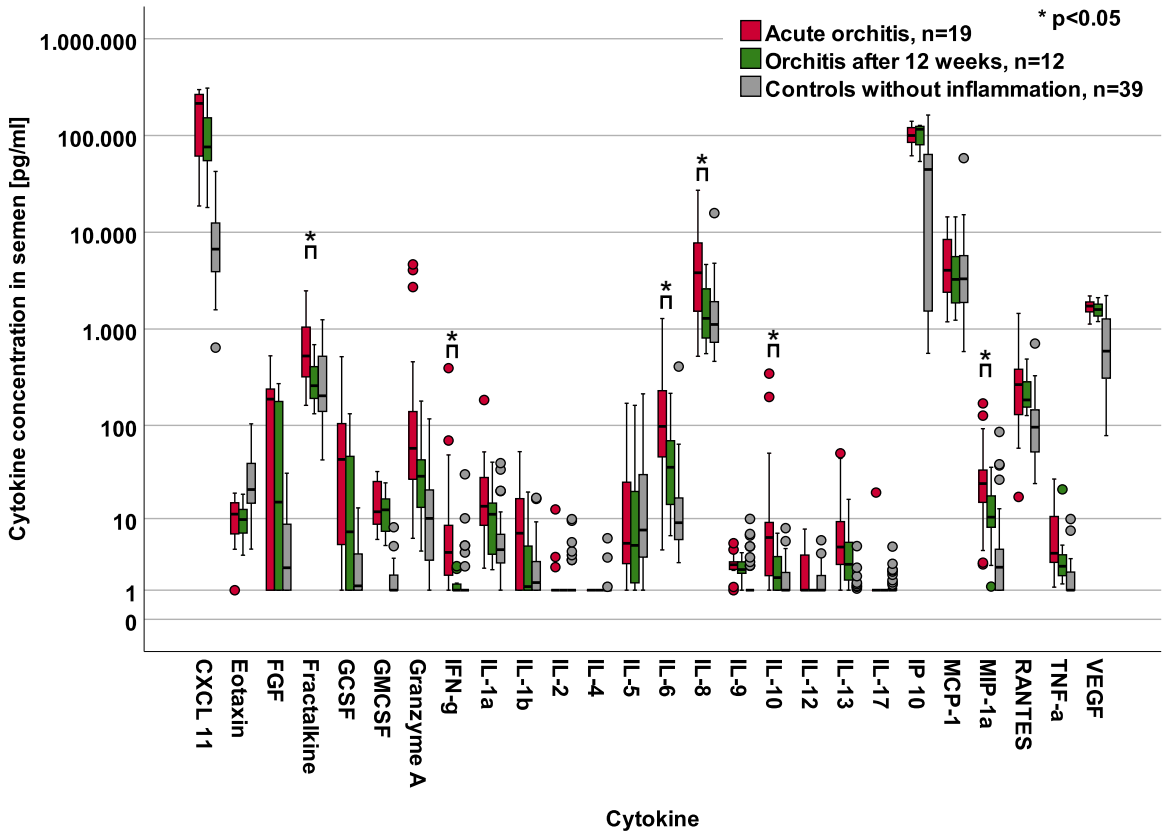

FIGURE 5 Graphical illustration of cytokine concentrations in seminal plasma during acute orchitis ($n = 19$) and 12 weeks later ($n = 12$) compared with controls without inflammation ($n = 39$). Significant decrease of fractalkine, interferon-γ (IFN-γ), interleukin-6 (IL-6), IL-8, IL-10, and MIP-1α in 12 paired samples (all $p < 0.05$). Boxplots indicating median and interquartile range (IQR) on a logarithmic scale.

range. Nevertheless, one patient had azoospermia (5%) and 10 patients had oligozoospermia (42%) according to WHO 2010 lower reference limits in the acute episode shortly after the pain symptoms had subsided. Semen quality and sex hormones were reanalyzed at 12 and 214 weeks in 14 and 6 patients, respectively. There was no significant difference in any of these parameters measured (in all cases $p > 0.05$) except glucosidase, which increased from 14.2 to 26.4 mU/mL (Table 3). At follow-up, no patient had azoospermia, but oligozoospermia was found in 4/14 (29%) and 2/6 (33%) patients at 12 and 214 weeks, respectively. One patient with two children before acute isolated orchitis reported fertility problems in late follow-up and the use of IVF/ICSI twice before the third child could be conceived naturally.

4 | DISCUSSION

In this prospective study recruiting 26 patients from 2007 to 2023, we were able to comprehensively decipher acute isolated orchitis from etiology to clinical course and effects on fertility.

In contrast to the common acute epididymitis, in which concomitant orchitis may occur in up to 90% of cases as part of the bacterial ascension,[32] acute isolated orchitis is rare, accounting for 5% of all cases of epididymitis/orchitis in this study. While an incidence of approximately 300 per 100,000 men per year has been reported for acute epididymitis,[36] this results in an estimated incidence of approximately 15 per 100,000 men per year.

Acute orchitis has been described predominantly as a complication of systemic viral diseases (e.g., mumps, pleurodynia epidemica, SARS-CoV-2),[3–10,37] although a wide variety of viruses have been reported as possible etiology of acute isolated orchitis.[10,11,22,23] However, no study has ever been conducted that focuses primarily on acute isolated orchitis and its etiology. This is likely due to the fact that patients are usually admitted as emergencies outside of regular working hours.

Essential for our study was the focus on pure acute isolated orchitis. In the available studies on orchitis, the assessment of whether the testis, epididymis, or both are involved is often unclear.[9,23,30,38] Whereas the initial descriptions of orchitis caused by mumps and enteroviruses relied on palpation alone,[8,15,39] scrotal sonography is essential for a precise evaluation. Interestingly, only a few studies reported ultrasound parameters in more than three patients ($n = 11–23$). Here, the epididymis was found to be involved in mumps orchitis in 33%, 56%, and 100%, respectively.[28–30] To obtain a homogeneous group and not to achieve an admixture with incidences of bacterial epididymitis, major inclusion criteria in the

TABLE 3 Semen and sex hormone analysis.

Parameter	Acute Median (IQR)	n	12 weeks Median (IQR)	n	214 weeks Median (IQR)	n	p^a
Semen							
Volume, mL	2.7 (1.9–3.5)	24	2.7 (1.9–3.9)	14	2.1 (1.2–3.1)	6	0.555
pH value	7.8 (7.6–8.1)	24	7.6 (7.4–8.1)	14	8.4 (7.7–8.7)	6	0.100
Sperm concentration, 10^6/mL	23.5 (4.7–54.3)	24	28.0 (6.7–73.5)	14	66.7 (3.5–94.6)	6	0.524
Total sperm count, 10^6	47.5 (7.7–177.1)	24	72.6 (22.3–177.4)	14	150.9 (6.5–250.9)	6	0.789
Progressive motility, %	49 (32–57)	22	49 (38–61)	12	57 (49–75)	6	0.214
Normal sperm morphology, %	7 (2–11)	22	9 (2–16)	12	11 (6–12)	6	0.529
Peroxidase-positive Leukocytes, 10^6/mL	0.2 (0–0.7)	24	0.1 (0–0.5)	14	0.2 (0–0.3)	6	0.720
Fructose, µmol/mL	13.6 (8.3–17.0)	18	16.1 (11.0–19.6)	10	12.9 (10.1–18.8)	6	0.684
Glucosidase, mU/mL	14.2 (12.8–23.7)	19	12.9 (11.5–25.7)	10	26.4 (19.1–34.6)	6	**0.042**
Zinc, µmol/mL	3.7 (2.6–7.1)	17	4.6 (2.3–6.3)	10	7.6 (2.1–9.0)	5	0.865
Elastase, ng/mL	63 (23–241)	23	56 (16–366)	12	74 (21–112)	6	0.741
Hormones							
FSH, mU/mL	6.9 (4.8–9.5)	25	6.1 (5.0–11.8)	14	6.6 (4.7–19.6)	6	0.915
LH, mU/mL	4.7 (3.4–8.2)	25	3.9 (2.8–5.6)	14	4.0 (3.6–5.8)	6	0.202
Testosterone, nmol/L	347 (190–443)	25	422 (309–559)	14	449 (304–534)	6	0.145
Free testosterone, pmol/L	7.1 (4.4–8.9)	25	8.4 (6.9–10.4)	14	8.8 (6.4–12.3)	6	0.079
Prolactin, uIU/mL	175 (124–204)	25	173 (135–276)	14	138 (87–228)	6	0.633
Estradiol, pmol/L	29 (22–32)	25	24 (22–28)	14	26 (22–38)	6	0.376
Albumin, g/L	46.4 (44.5–49.0)	25	45.8 (44.1–47.7)	14	47.4 (46.0–48.4)	6	0.540
SHBG, nmol/L	32 (21–37)	25	33 (22–39)	14	27 (25–34)	6	0.895

Note: Bold values indicate $p < 0.05$.

Abbreviation: SHBG, sex hormone-binding globulin.

[a]Statistical analysis performed with Kruskal–Wallis test.

present study were isolated testicular pain, enlargement, and hyperemia of the affected testis. The fact that 54% of the patients with isolated orchitis reported a respiratory infection in the last 2 weeks before the onset of orchitis, shows the precision of the inclusion criteria and already gives the suspicion of a viral etiology.[39] In addition to a blank urine analysis, a negative urine culture, and negative STI PCR from urine/semen, we were also able to show with the stable PSA course that there was no concomitant prostatitis, which is common with bacterial epididymitis and urinary tract infections.[32,40] Unfortunately, STI PCR was not regularly performed in a few case reports from 2000 and later, so the evidence for viral orchitis in these reports is limited.[9,13,41]

An important point in the discussion of etiology is, of course, whether the virus is found directly in the testis during the acute phase or whether orchitis is an inflammatory postinfectious phenomenon.[42] In mumps, two small case series ($n = 3–5$) reported successful direct cultivation from testicular tissue[43,44] and two single case reports succeeded in detecting coxsackievirus A6 in hydrocele fluid[38] or coxsackievirus B5 in testicular tissue obtained by testicular biopsy.[5] In contrast, acute epididymitis in young boys was thought to be a postinfectious inflammatory phenomenon on the basis of the finding that the patients had symptoms of upper respiratory infections preceding scrotal symptoms.[42] Since we detected coxsackievirus B strains in the semen of 2/54 patients with acute epididymitis as well as 16/25 patients with orchitis, our data point toward a direct virus-induced etiology. Presumably, the coxsackie and adenovirus receptor (CAR) plays a crucial role in organ tropism.[45] This may explain why the testis was more frequently clinically infected than the epididymis.

Since performing diagnostic testicular biopsies in patients with acute orchitis is not ethically justified nowadays, our approach was to perform viral diagnostics in blood, urine, and semen. We were able to show by molecular diagnostics, that acute isolated orchitis is usually viral in origin (77%) and coxsackievirus B strains being predominant, accounting for 62% of all cases.

This is likely to fundamentally change thinking about acute isolated orchitis. The observed circannual occurrence of isolated orchitis with a maximum in the late summer months is consistent with

the seasonal occurrence of epidemic pleurodynia described in the literature.[15,16,21,39,46,47] Of interest, none of the patients reported the typical symptoms of pleurodynia. Regarding viral etiology, we were particularly surprised by the lack of patients with mumps-associated orchitis.[14] Interestingly, the only patient in our cohort with a positive mumps IgM serology displayed a negative result for mumps-PCR in semen. Moreover, he did not have parotitis upfront but had myositis. All of this underlines that mumps virus is not the leading cause for isolated orchitis and this is probably due to mumps vaccination in childhood, although 44% were seronegative in our cohort.[7] Actually, we had expected a variety of other respiratory viruses as causative agents of orchitis.[11] In particular, at the onset of the SARS-CoV-2 pandemic, there was repeated speculation of testicular involvement in up to 23% of patients. However, most of those reports were based on medical history alone (= testicular discomfort).[24] Nevertheless, there are also some histologic and electron microscopic studies that have demonstrated the presence of SARS-CoV-2 in the testis with subsequent testicular tissue destruction. However, these examinations have typically been performed on patients deceased by SARS-CoV-2.[48] In our center, no patients with isolated orchitis presented at all during the SARS-CoV-2 pandemic, nor were we consulted as urologists for orchitis in infectious wards. In contrast, the presentation of patients with bacterial epididymitis did not decrease in 2020 and 2021. This aspect might be explained by a recent meta-analysis on SARS-CoV-2 showing that the virus was detected in the semen in only about 7% of those affected.[24] Therefore, whether SARS-CoV-2 is actually capable of causing acute orchitis must be questioned based on the available data.[24,48]

Of note, enteroviruses were exclusively detectable in semen during the acute phase, whereas other samples (urine, blood) did not contain the virus, as has been reported.[5,13] Because 54% of patients with orchitis reported respiratory infection in the 10 days before acute orchitis, viremia with seeding in the testes should be assumed, even if viremia was no longer detected at the time of presentation with acute orchitis. In addition, the viruses were not detected in the 3-month follow-up in semen, underscoring their relevance to acute infection rather than mere random detection of enteroviruses. The data show that isolated orchitis is associated with local (comprehensive cytokine panel in seminal plasma) and systemic inflammation (median CRP 36 mg/L) at the acute phase. Furthermore, it was even possible to isolate replication-competent viruses from cryopreserved semen samples, demonstrating that infectious pathogens are present and not just genomic remainders after virus clearance. Thus, the data suggest a direct virus-induced phenomenon resulting in painful orchitis. In summary, our findings should initiate a change in diagnostics because early masturbation and semen collection for virologic diagnosis will be required after symptoms subside (usually 2–4 days). This is fundamental and will change clinical practice because semen analysis in bacterial epididymitis has no clinical relevance in the acute situation, but only in the follow-up for the assessment of fertility.

Orchitis is usually rapidly self-limiting even without any medical therapy[39,46] and close serial sonographic controls clearly demonstrated restitution.[29] However, the long-term effects on fertility are most feared. Testicular histology in patients with acute viral orchitis (mumps or coxsackie virus) showed pictures of lymphocytic infiltrates, tubules with neutrophils, edema, and mixed atrophy.[5,49] A comprehensive review demonstrated that azoospermia occurs in about 5% and oligozoospermia in 45% of those affected more than 3 months after mumps orchitis.[50] In the present work, we demonstrated for the first time that in acute orchitis typically caused by coxsackievirus B strains, 30% of patients suffered from persistent oligozoospermia as defined by WHO 2010 after 3 months and after 4 years. An assessment of semen parameters in relation to sex hormones is desirable, but only one study reported sex hormones in mumps orchitis. Here, testosterone ($n = 19$) was significantly reduced in the acute phase compared with healthy individuals and patients with mumps without orchitis, while FSH ($n = 11$) and LH ($n = 10$) were significantly increased. The authors attributed the results to the high number of cases with bilateral orchitis (16/27).[3] Using systematic follow-up data, we were able to show for the first time that the sex hormones were all stable over time, so that a relevant persistent endocrine testicular dysfunction cannot be assumed in the majority of patients with isolated orchitis. However, further long-term data are necessary to clarify in particular the question of persistent low-grade testicular inflammation and atrophy, which cannot be adequately answered with $n = 6$ patients on average 4 years after acute viral orchitis.

5 | LIMITATIONS

A limiting factor is that the disease is rare and, despite a long recruitment period of 16 years, the cohort of 26 patients is naturally not large, but comparable to one report on mumps orchitis with 12 cases in 8 years.[29] Furthermore, no viruses were isolated from the stool. The reason for this was that many patients were only seen as outpatients and the length of stay of patients who were hospitalized was short, so that a systematic stool collection could not have been carried out.[21,47]

6 | CONCLUSION

Isolated acute orchitis is usually of viral in origin (77%) and enteroviruses were predominantly detected, with coxsackievirus B strains accounting for 62% of all cases. If clinical suspicion exists, early semen analysis with PCR testing for enteroviruses is advised to confirm the diagnosis. Acute orchitis is usually self-limiting, associated with a characteristic inflammatory cytokine panel in the semen, and about 30% of patients suffer ongoing oligozoospermia.

AUTHOR CONTRIBUTIONS

Adrian Pilatz: Conceptualization; formal analysis; investigation; data curation; writing—original draft; visualization; project administration; funding acquisition. **Borros Arneth:** Investigation; writing—review &

editing. **Rolf Kaiser:** Formal analysis; investigation; data curation; writing—original draft. **Eva Heger:** Investigation; writing—review & editing. **Martin Pirkl:** Formal analysis; investigation; data curation; writing—original draft. **Sindy Böttcher:** Formal analysis; investigation; data curation; writing—original Draft. **Moritz Fritzenwanker:** Formal analysis; investigation; data curation; writing—original draft. **Harald Renz:** Investigation; writing—review & editing. **Annette Mankertz:** Investigation; writing—review & editing. **Hans-Christian Schuppe:** Conceptualization; investigation; data curation; writing—review & editing. **Florian Wagenlehner:** Conceptualization; writing—review & editing; supervision.

ACKNOWLEDGMENTS

This study was partially financed by a start-up funding from the Faculty of Medicine at Justus Liebig University (A.P.) and by the German Research Foundation (Project PI 1247/1-1). Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ORCID

Adrian Pilatz http://orcid.org/0000-0001-8072-1841
Martin Pirkl http://orcid.org/0000-0002-6986-0813

REFERENCES

1. Schuppe HC, Meinhardt A, Allam JP, Bergmann M, Weidner W, Haidl G. Chronic orchitis: a neglected cause of male infertility? *Andrologia*. 2008;40(2):84-91. doi:10.1111/j.1439-0272.2008.00837.x
2. Pilatz A, Wagenlehner F, Bschleipfer T, et al. Acute epididymitis in ultrasound: results of a prospective study with baseline and follow-up investigations in 134 patients. *Eur J Radiol*. 2013;82(12):e762-e768. doi:10.1016/j.ejrad.2013.08.050
3. Adamopoulos DA, Lawrence DM, Vassilopoulos P, Contoyiannis PA, Swyer GIM. Pituitary-testicular interrelationships in mumps orchitis and other viral infections. *BMJ*. 1978;1(6121):1177-1180. doi:10.1136/bmj.1.6121.1177
4. Artul S, Abu Rahmah Y, Abu Shkara H, Yamini A. Inferno: colour Doppler ultrasound sign of orchitis. *Case Reports*. 2014;2014:bcr2014203613. doi:10.1136/bcr-2014-203613
5. Craighead JE, Mahoney EM, Carver DH, Nafic K, Fremont-Smith P. Orchitis due to coxsackie virus group B, type 5: report of a case with isolation of virus from the testis. *N Engl J Med*. 1962;267:498-500. doi:10.1056/nejm196209062671008
6. Morrison RJG, Baird JP. Orchitis in bornholm disease. *BMJ*. 1952;1(4751):198-199. doi:10.1136/bmj.1.4751.198
7. Tae BS, Ham BK, Kim JH, Park JY, Bae JH. Clinical features of mumps orchitis in vaccinated postpubertal males: a single-center series of 62 patients. *Korean J Urol*. 2012;53(12):865-869. doi:10.4111/kju.2012.53.12.865
8. Wesselhoeft C. Orchitis in mumps. *Boston Med Surg J*. 1920;183(17):491-494. doi:10.1056/NEJM192010211831704
9. Wharton IP, Chaudhry AH, French ME. A case of mumps epididymitis. *Lancet*. 2006;367(9511):702. doi:10.1016/S0140-6736(06)68274-3
10. Willems W, Hornig C, Bauer H, Klingmüller V. Orchitis caused by coxsackie A9. *Lancet*. 1977;310(8052-8053):1350.
11. Le Tortorec A, Matusali G, Mahé D, et al. From ancient to emerging infections: the odyssey of viruses in the male genital tract. *Physiol Rev*. 2020;100(3):1349-1414. doi:10.1152/physrev.00021.2019
12. Bertschat FL, Alexander M. [Infertility after mumps orchitis (author's transl)]. *MMW Munch Med Wochenschr*. 1981;123(15):606-608.
13. Jalal H, Bahadur G, Knowles W, Jin L, Brink N. Mumps epididymo-orchitis with prolonged detection of virus in semen and the development of anti-sperm antibodies. *J Med Virol*. 2004;73(1):147-150. doi:10.1002/jmv.10544
14. Emerson C, Dinsmore WW, Quah SP. Are we missing mumps epididymo-orchitis? *Int J STD AIDS*. 2007;18(5):341-342. doi:10.1258/095646207780749754
15. Sylvest E. A Bornholm epidemic-myositis epidemica. *Ugeskr Lag*. 1930;92:798-801.
16. Huss R. La myalgie epidemique en suede. *Bull office internat d'hyg pub*. 1934;26:1083-1098.
17. Curnen EC. Disease resembling nonparalytic poliomyelitis associated with a virus pathogenic for infant mice. *JAMA*. 1949;141(13):894-901. doi:10.1001/jama.1949.02910130008003
18. Patel LN, Arciuolo RJ, Fu J, et al. Mumps outbreak among a highly vaccinated university community-New York city, January-April 2014. *Clin Infect Dis*. 2016;64(4):ciw762. doi:10.1093/cid/ciw762
19. Willocks LJ, Guerendiain D, Austin HI, et al. An outbreak of mumps with genetic strain variation in a highly vaccinated student population in Scotland. *Epidemiol Infect*. 2017;145(15):3219-3225. doi:10.1017/s0950268817002102
20. Veneti L, Borgen K, Borge KS, et al. Large outbreak of mumps virus genotype G among vaccinated students in Norway, 2015 to 2016. *Euro Surveill*. 2018;23(38):1700642. doi:10.2807/1560-7917.es.2018.23.38.1700642
21. Artenstein MS. Epidemic coxsackie virus infection with mixed clinical manifestations. *Ann Intern Med*. 1964;60:196-203. doi:10.7326/0003-4819-60-2-196
22. Piantadosi A, Mukerji SS, Chitneni P, et al. Metagenomic sequencing of an echovirus 30 genome from cerebrospinal fluid of a patient with aseptic meningitis and orchitis. *Open Forum Infect Dis*. 2017;4(3):ofx138. doi:10.1093/ofid/ofx138
23. Kerget F, Demirdöğen ŞO, Kerget B. Case report: a rare case of Crimean-Congo hemorrhagic fever associated with Epididymo-Orchitis. *Am J Trop Med Hyg*. 2021;104(3):1055-1057. doi:10.4269/ajtmh.20-1143
24. Kloping YP, Hidayatullah F, Rahman ZA, Chung E, Hakim L. Male reproductive tract involvement and sperm parameters in SARS-CoV-2 patients: a systematic review and meta-analysis.*World J. Men's Health*. 2023;41:538. doi:10.5534/wjmh.220019
25. Gazibera B, Gojak R, Drnda A, et al. Spermiogram part of population with the manifest orchtis during an ongoing epidemic of mumps. *Med Arch*. 2012;66(3 suppl 1):27-29. doi:10.5455/medarh.2012.66.s27-s29
26. Lyu Z, Feng X, Li N, et al. Human papillomavirus in semen and the risk for Male infertility: a systematic review and meta-analysis. *BMC Infect Dis*. 2017;17(1):714. doi:10.1186/s12879-017-2812-z
27. Mohseni M, Mollaei HR, Arabzadeh SA, Mirshekari TR, Ghorbani P. Frequency of cytomegalovirus in fertile and infertile men, referring to Afzalipour hospital IVF research center, kerman, Iran: a case-control study. *Int J Reprod Biomed*. Jul 2018;16(7):443-446.
28. Park SJ, Kim HC, Lim JW, Moon SK, Ahn SE. Distribution of epididymal involvement in mumps epididymo-orchitis. *J Ultrasound Med*. 2015;34(6):1083-1089. doi:10.7863/ultra.34.6.1083

29. Tarantino L, Giorgio A, de Stefano G, Farella N. Echo color Doppler findings in postpubertal mumps epididymo-orchitis. *J Ultrasound Med*. 2001;20(11):1189-1195. doi:10.7863/jum.2001.20.11.1189
30. Başekim CÇ, Kizilkaya E, Pekkafali Z, Baykal KV, Karsli AF. Mumps epididymo-orchitis: sonography and color Doppler sonographic findings. *Abdom Imaging*. 2000;25(3):322-325. doi:10.1007/s002610000039
31. Pilatz A, Hudemann C, Wolf J, et al. Metabolic syndrome and the seminal cytokine network in morbidly obese males. *Andrology*. 2017;5(1):23-30. doi:10.1111/andr.12296
32. Pilatz A, Hossain H, Kaiser R, et al. Acute epididymitis revisited: impact of molecular diagnostics on etiology and contemporary guideline recommendations. *Eur Urol*. 2015;68(3):428-435. doi:10.1016/j.eururo.2014.12.005
33. Domann E, Hong G, Imirzalioglu C, et al. Culture-independent identification of pathogenic bacteria and polymicrobial infections in the genitourinary tract of renal transplant recipients. *J Clin Microbiol*. 2003;41(12):5500-5510. doi:10.1128/JCM.41.12.5500-5510.2003
34. Otto W, Mankertz A, Santibanez S, et al. Ongoing outbreak of mumps affecting adolescents and young adults in Bavaria, Germany, August to October 2010. *Euro Surveill*. 2010;15(50):19748.
35. Keeren K, Böttcher S, Diedrich S. Enterovirus surveillance (EVSurv) in Germany. *Microorganisms*. 2021;9(10):2005. doi:10.3390/microorganisms9102005
36. Bohm MK, Gift TL, Tao G. Patterns of single and multiple claims of epididymitis among young privately-insured males in the United States, 2001 to 2004. *Sex Transm Dis*. 2009;36(8):490-492. doi:10.1097/OLQ.0b013e3181a396d8
37. Chen L, Huang X, Yi Z, et al. Ultrasound imaging findings of acute testicular infection in patients with coronavirus disease 2019: a single-center-based study in Wuhan, China. *J Ultrasound Med*. 2021;40(9):1787-1794. doi:10.1002/jum.15558
38. Vuorinen T, Österback R, Kuisma J, Ylipalosaari P. Epididymitis caused by coxsackievirus A6 in association with hand, foot, and mouth disease. *J Clin Microbiol*. 2014;52(12):4412-4413. doi:10.1128/JCM.02441-14
39. Warin JF, Davies JBM, Sanders FK, Vizoso AD. Oxford epidemic of bornholm disease, 1951. *BMJ*. 1953;1(4824):1345-1351. doi:10.1136/bmj.1.4824.1345
40. Townes CL, Ali A, Gross N, et al. Prostate specific antigen enhances the innate defence of prostatic epithelium against *Escherichia coli* infection. *Prostate*. 2013;73(14):1529-1537. doi:10.1002/pros.22700
41. Di Lella E, Angelini F, Campagnano S, Messineo D, Drudi FM. An unusual location of hand, foot and mouth disease. *J Ultrasound*. 2022;25(2):361-364. doi:10.1007/s40477-021-00558-y
42. Somekh E, Gorenstein A, Serour F. Acute epididymitis in boys: evidence of a post-infectious etiology. *J Urol*. 2004;171(1):391-394. doi:10.1097/01.ju.0000102160.55494.1f
43. Bjorvatn B. Mumps virus recovered from testicles by fine-needle aspiration biopsy in cases of mumps orchitis. *Scand J Infect Dis*. 1973;5(1):3-5. doi:10.3109/inf.1973.5.issue-1.02
44. Hook, Jr. EW, Poole SO, Friedewald WF. Virus isolation and serologic studies on patients with clinical mumps. *J Infect Dis*. 1949;84(3):230-234. doi:10.1093/infdis/84.3.230
45. Mirza M, Petersen C, Nordqvist K, Sollerbrant K. Coxsackievirus and adenovirus receptor is up-regulated in migratory germ cells during passage of the blood-testis barrier. *Endocrinology*. 2007;148(11):5459-5469. doi:10.1210/en.2007-0359
46. Jamieson WM, Prinsley DM. Bornholm disease in the tropics. *BMJ*. Jul 12 1947;2(4514):47-50. doi:10.1136/bmj.2.4514.47
47. Huebner RJ, Risser JA, Bell JA, Beeman EA, Beigelman PM, Strong JC. Epidemic pleurodynia in Texas; a study of 22 cases. *N Engl J Med*. 1953;248(7):267-274. doi:10.1056/nejm195302122480701
48. Torge D, Bernardi S, Arcangeli M, Bianchi S. Histopathological features of SARS-CoV-2 in extrapulmonary organ infection: a systematic review of literature. *Pathogens*. 2022;11(8):867. doi:10.3390/pathogens11080867
49. Gall EA. The histopathology of acute mumps orchitis. *Am J Pathol*. 1947;23(4):637-651.
50. Pilatz A, Boecker M, Schuppe HC, Diemer T, Wagenlehner F. Infektionen und infertilität. *Urologe*. 2016;55(7):883-889. doi:10.1007/s00120-016-0151-0

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Pilatz A, Arneth B, Kaiser R, et al. Acute orchitis deciphered: coxsackievirus B strains are the main etiology and their presence in semen is associated with acute inflammation and risk of persistent oligozoospermia. *J Med Virol*. 2023;95:e28970. doi:10.1002/jmv.28970