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Abstract

Purpose:
To evaluate the influence of biofilms on morbidity associated with short-term ureteral stenting using contemporary methods of biofilm examination and validated assessment of symptoms.

Methods:
Patients undergoing temporary ureteral stenting for secondary ureterorenoscopy due to urinary calculi were prospectively included. The German Ureteral Stent Symptoms Questionnaire (USSQ) was used to assess stent-associated morbidity. Biofilms were removed from stents using ‘pinhole extraction’, a novel, validated, abrasion-based technique. Extracted biofilms were analyzed for total mass, bacterial load and mineral components. Correlation between total biofilm mass and USSQ total score was the primary outcome variable analyzed using Spearman correlation. Secondary outcomes included correlations between various biofilm characteristics and symptoms.

Results:
94 patients were included in the analysis. Extracted biofilm mass had a median of 37.0 mg (0–310.2 mg) per stent. No correlation between total biofilm mass and USSQ total score was found (Spearman $r = 0.012$; $p = 0.911$). Correlations between biofilm characteristics and morbidity were generally weak and not significant. Significant correlations could be found between biofilm mass and hematuria ($r = 0.280$; $p = 0.007$), and between the number of bacteria (qPCR) and the USSQ subscore for pain ($r = 0.243$; $p = 0.019$) and the intake of analgesics ($r = 0.259$; $p = 0.012$).

Conclusion:
Based on elaborated biofilm examination methods and validated self-reported outcome measures, our findings indicate that biofilms might aggravate some lower urinary tract symptoms but are not the main trigger for stent-associated morbidity in short-term ureteral stenting.

Keywords (separated by '-')	Ureteral stent - Biofilm - Morbidity - Ureteral Stent Symptoms Questionnaire - USSQ - Symptoms
Footnote Information	Patrick Betschart and Valentin Zumstein contributed equally to this work.

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ORIGINAL ARTICLE

Influence of biofilms on morbidity associated with short-term indwelling ureteral stents: a prospective observational study

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Abstract

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Conclusion Based on elaborated biofilm examination methods and validated self-reported outcome measures, our findings indicate that biofilms might aggravate some lower urinary tract symptoms but are not the main trigger for stent-associated morbidity in short-term ureteral stenting.

Keywords Ureteral stent · Biofilm · Morbidity · Ureteral Stent Symptoms Questionnaire · USSQ · Symptoms

Introduction

Temporary drainage of the upper urinary tract by internal ureteral stents is a standard procedure to assure renal function and to treat pain caused by ureteral obstruction. Although ureteral stenting is a simple and effective method of drainage and avoids external or visible devices, it is associated with a clear side effect profile. Irritative voiding symptoms have been reported in 78% of patients, and pain affecting daily activities in more than 80% [1, 2]. Possibilities of prevention and treatment of stent-related symptoms are limited [3, 4]; thus, ureteral stenting is associated with a considerable economic burden [5].

The pathophysiology of stent-associated symptoms and complications is poorly understood. On the one hand, lower urinary tract symptoms (LUTS) are thought to be caused by mechanical irritation of the urothelium and nerves,

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especially in the bladder trigone [6–8]. On the other hand, biofilm formation has been proposed as a further important etiological factor in stent-associated symptoms and complications. Thus, biofilms might cause infectious complications and stent dysfunction in patients with long-term indwelling stents [9]. Animal and in vitro studies have shown that biofilms and bacteria induce production of antibacterial substances and pro-inflammatory cytokines leading to local inflammation, which likely aggravate LUTS and pain [10–12].

Worsening of LUTS due to bacterial stent colonization has been suspected. Only one clinical study has, however, been published [13], which was limited by the non-validated questionnaires for the investigation of stent-related symptoms and assessment of biofilms using bacterial cultivation only. So far, no studies assessing the influence of cultivation-independent methods or analyses of non-viable bacterial and biofilm matrix components are available. In this study, we, therefore, evaluated the impact of biofilms on stent-related morbidity, considering the different biofilm components, and using validated, patient-reported assessment of stent-related symptoms.

Materials and methods

Study setting

The study was approved by the local ethics committee (EKSG 15/210) and registered at clinicaltrials.gov (NCT02845726). Patients who had transient ureteral stenting as part of the preparation for a secondary ureterorenoscopy (URS) due to urinary calculi between June 2016 and August 2017 were prospectively included in the study at the time of stent insertion after written informed consent. Exclusion criteria were bilateral stenting, ureteral obstruction due to malignancies, additional procedures or operations during stent indwelling time, cognitive impairment and urinary tract infection (UTI) at the time of stent insertion, defined as more than $> 10^2$ colony forming units (CFU) per mL.

The Percuflex® Plus Ureteral Stent (Boston Scientific, Natick, MA, USA) with a diameter of six French and a length of 26 cm or 30 cm according to the patient's height and the surgeon's estimation was used in all patients. A standardized prescription of drugs (i.e., alpha blocker and paracetamol) was made available for all patients and its use was assessed by item P8 of the Ureteral Stent Symptoms Questionnaire (USSQ).

Patients completed the German version of the USSQ [14] 1 week after insertion and on the day before surgery 4 weeks after stent insertion (permitted window 2–6 weeks). A single shot of Co-trimoxazole was given 1 h before stent insertion and for secondary URS, according to recent recommendations [15].

At the time of URS, stents were removed through the sheath of the cystoscope without guidewire intubation, cut in half, and stored in collection tubes humidified with a small amount of sterile saline solution at 4 °C. Biofilm examinations were performed by the Laboratory for Biointerfaces (Empa, Swiss Federal Laboratories for Materials Science and Technology, St. Gallen, Switzerland) within 6 h after removal according to a protocol described in detail elsewhere [16]. Bacterial cultivation was performed according to standard protocols at the clinical diagnostic microbiology laboratory (Center for Laboratory Medicine, St. Gallen, Switzerland).

The primary outcome variable was the degree of correlation between total biofilm mass on the stent surface and morbidity measured by the USSQ total score at the time of stent removal. Secondary outcome variables were the association between total biofilm mass, numbers of bacteria assessed by cultivation, and numbers of bacteria estimated by quantitative real-time PCR, and USSQ total score, subscores, and the most relevant single items, i.e., hematuria (U8), intake of analgesics (P8), antibiotics (A2), and need for hospitalization (A4).

Associations between mineral composition and stent-related symptoms, and changes in symptoms with increasing indwelling time were assessed as part of an unplanned analysis to further elucidate the findings of the main outcomes.

Biofilm—extraction and analyses

All analyses were performed as described previously by Buhmann et al. [16]. Stents were strictly handled in a sterile workbench using aseptic techniques during all laboratory procedures. Stent halves were passed through a tapered pinhole in a stainless-steel plate. Extracted biofilm was suspended in 2-mL saline solution, collected and balanced in 2-mL microcentrifuge tubes. After centrifugation for 5 min at 14,100×*g*, the supernatant was removed and the pellet wet weight was determined using an analytical balance.

Extracted biofilm pellets were each suspended in 500-µL physiological saline solution. Lysates were streaked onto Columbia agar ('sheep blood' agar) and a BD BBL™ CHROMagar™ Orientation agar (Becton–Dickinson, Franklin Lake, NJ, USA) and cultivated following established protocols in our routine laboratory (ISO/IEC 17025 accredited). Growth was recorded after 18–24 h of incubation. In case of no growth, the Columbia agar was incubated for another 18–24 h to ensure detection of slow growing bacteria. Identification was done by MALDI-TOF analysis, following standard protocols on a MALDI Biotyper instrument (Bruker Daltoniks, Bremen, Germany).

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For cultivation-independent assessment of the bacterial load, the content of bacterial 16S rRNA genes in DNA extracts prepared from an aliquot of the biofilm samples was estimated using a broad-range qPCR assay as described previously [16, 17].

In a post hoc analysis, mineral components of resuspended ureteral stent biofilm fractions were assessed by qualitative two-dimensional wide-angle X-ray diffraction (XRD) phase analysis, using a STOE IPDS-II instrument (Stoe and Cie GmbH, Germany). Diffraction patterns were recorded using Mo Kα radiation ($\lambda = 0.71073$ Å) at 40 mA and 50 kV, and analyzed qualitatively using the DIFFRAC. EVA software version V4.3 (Bruker, Germany) by comparison with the COD reference database [18]. Due to their dominance, only the three most common crystal phases (i.e., calcium oxalate monohydrate, calcium oxalate dihydrate and dicalcium phosphate dihydrate) were taken into account for comparative analyses. The following five groups were defined: calcium oxalate monohydrate, calcium oxalate dihydrate, combination of calcium oxalate monohydrate and dihydrate, combination of calcium oxalate dihydrate and dicalcium phosphate dihydrate, and no minerals detected.

Statistical analysis

The sample size was based on the calculation that 85 evaluable patients would provide a power of 80% to detect a Pearson correlation coefficient of ± 0.30 at a two-sided significance level of 5%.

Because most numeric variables had skewed or bimodal distribution, all associations between two numeric or ordinal variables were analyzed using Spearman rank correlation coefficients, together with 95% CI and two-sided tests of significance (null hypothesis: correlation equal to zero) based on the normal approximation obtained by Fisher's z transformation. Relationships between numeric or ordinal and categorical variables (e.g., questionnaire items A2: intake of antibiotics; and A4: need for hospitalization) were analyzed using the Kruskal–Wallis rank sum test, and those between two categorical variables using Fisher's exact test.

Results

Of the 101 patients enrolled, 11 had to be excluded from the analysis of the primary outcome variable. One patient had UTI at the time of stent insertion, one stent was stored too long between removal and biofilm assessment, and nine patients did not answer questionnaires appropriately or refused further study participation for personal reasons. Thus, the primary outcome variable was able to be analyzed for 90 patients, and the secondary outcome variables for up to 94 patients. Table 1 shows the demographics and clinical characteristics for all 94 patients who were included in the analysis. None of the patients had undergone ureteral stenting within the last 6 months prior to study inclusion. Twenty patients received an antibiotic therapy during stent indwelling time because of a suspected UTI; used antibiotics were Co-trimoxazole, Ciprofloxacin and Fosfomycin. Antibiotics

Table 1 Demographic and clinical characteristics of the study participants ($n = 94$)

	Median (range)
Age	54.0 (16–85)
BMI (kg/m^2)	26.7 (16.6–43.0)
Stent indwelling time (days)	28 (19–41)
	n (%)
Male/female	74 (78.7%)/20 (21.3%)
Diabetes mellitus	11 (11.7%)
Chronic renal insufficiency	7 (7.4%)
Permanent indwelling bladder catheter	2 (2.1%)
UTI in the month before stenting	1 (1.1%)
Stent indication	
Preparation for secondary URS	91 (96.9%)
After primary URS	2 (2.1%)
Stenting performed in emergency setting	74 (78.7%)
ASA score	
I	47 (50.0%)
II	40 (42.6%)
III	7 (7.4%)

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were prescribed in an outpatient setting by the general practitioner and urine cultures were not available. Five of the patients, who took antibiotics during stent indwelling time, showed a positive urine culture at the time of secondary intervention. None of the cultures showed a resistance against the previously taken antibiotics.

The frequencies and distributions of the most important outcomes are shown in Fig. 1. The USSQ total score and total biofilm mass (after square root transformation) had approximately normal distribution (Fig. 1a, b). There was no significant difference of USSQ total score medians for males and females (Wilcoxon test, $p = 0.305$). The median total extracted biofilm mass was 37.0 mg (range 0–310.2 mg) per stent.

16S qPCR detected a median of 193,573 bacteria (32,432–44,602.122) on 23 (24.7%) of the stents; the counts for the other 71 stents were below the limit of detection (LOD). The number of bacteria showed a strongly skewed distribution with many zeroes (i.e., below the LOD) (75.3%), and a small number of very large values (Fig. 1c), and was therefore analyzed log-transformed and as a categorical variable with three levels (0, 1–10^5, > 10^5).

Correlations between biofilm characteristics and morbidity were generally weak and not statistically significant. No correlation between total biofilm mass and USSQ total score (primary study outcome) was found [Spearman rank correlation $r = 0.012$ (95% CI −0.196 to 0.219; $p = 0.911$)]. No correlation between total biofilm mass and any of the USSQ subscores and the intake of analgesics was found (Table 2, Fig. 2). In contrast, a moderate but significant correlation was seen between total biofilm mass and the occurrence of hematuria (USSQ item U8) ($r = 0.280$; (95% CI 0.076–0.462; $p = 0.007$); Table 2, Fig. 2). The rank correlation (rs) of the occurrence of hematuria with the biofilm total mass was stronger in males than in females (males rs = +0.31, $p = 0.007$; females rs = +0.11, $p = 0.655$). However, the difference in slope of the two relationships was not significant ($p = 0.718$).

The number of bacteria assessed by qPCR did not correlate with the USSQ total score ($r = 0.154$; (95% CI −0.055 to 0.350; $p = 0.145$)), nor with most of the USSQ

Table 2 Correlation (Spearman rank correlation) between total biofilm mass and USSQ total score, subscores, and selected single items

Score	*N*	Correlation coefficient	95% CI	*p* value
USSQ total score (primary study outcome)	90	0.012	−0.196 to 0.219	0.911
Urinary score	90	0.011	−0.197 to 0.217	0.920
Pain score	92	0.073	−0.134 to 0.274	0.487
General health score	92	0.003	−0.202 to 0.207	0.979
Work performance score	92	−0.047	−0.250 to 0.159	0.655
Sexual matters score	92	0.025	−0.181 to 0.229	0.813
Hematuria (U8)	92	0.280	0.076 to 0.462	0.007
Use of analgesics (P8)	92	0.129	−0.079 to 0.326	0.221

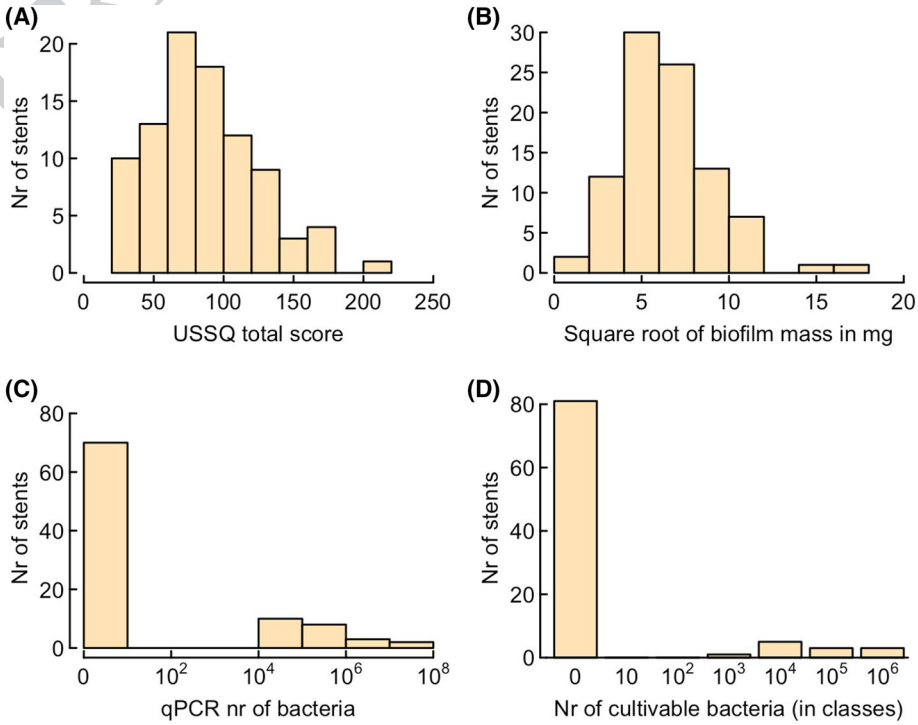

Fig. 1 Frequency distribution of outcomes among the 94 patients

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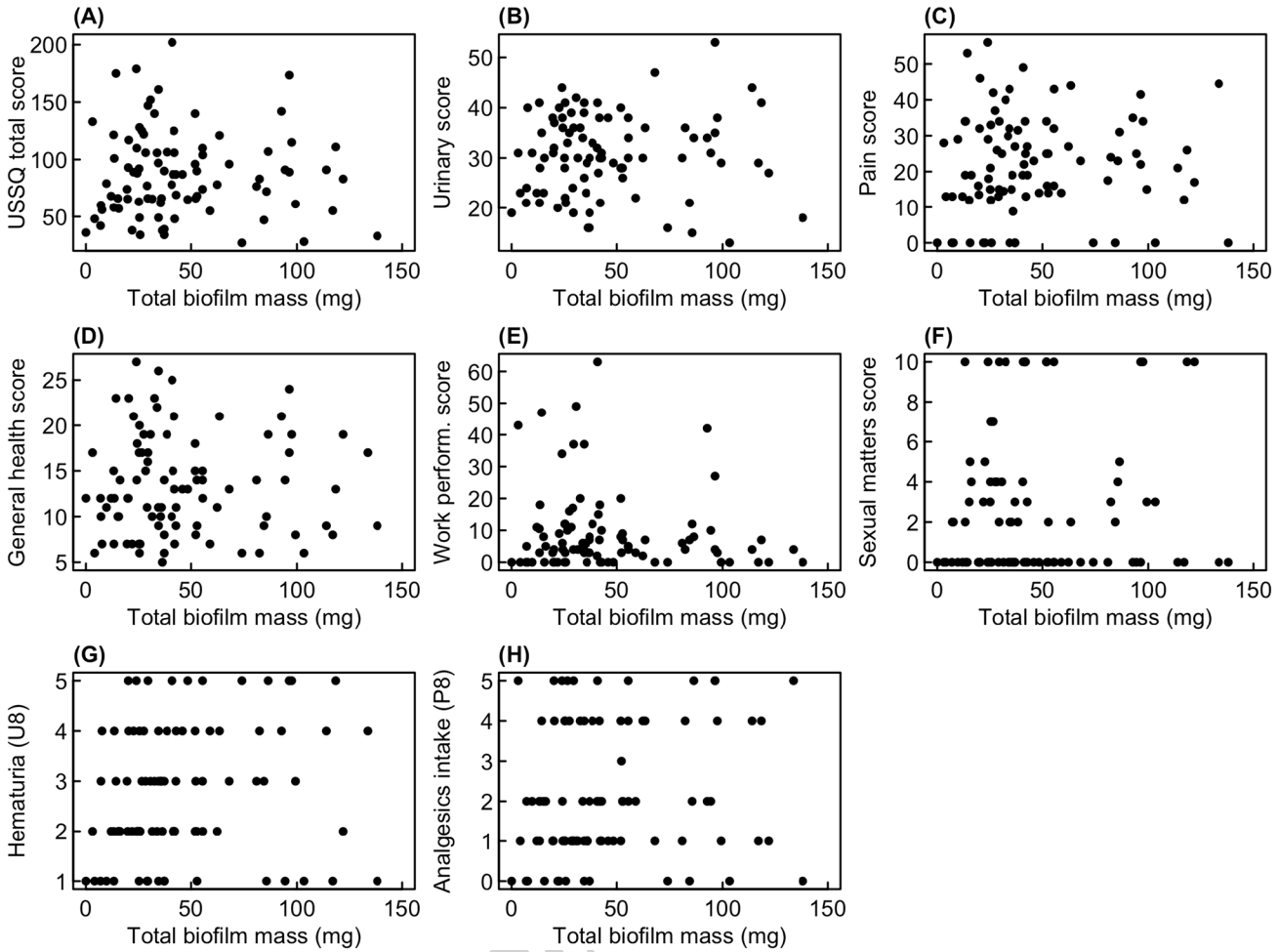

Fig. 2 Scatter plots illustrating correlations of total biofilm mass with USSQ total score, subscores, and selected single items. (See Table 2 for correlation statistics)

subscores (Table 3). However, weak but significant correlations were found for the USSQ subscore for pain ($r = 0.243$; (95% CI 0.038–0.428; $p = 0.019$)) and the intake of analgesics ($r = 0.259$; (95% CI 0.055–0.443; $p = 0.012$); Table 2, Fig. 2).

When stent colonization was assessed by urine cultures, no statistically significant relationships were found between the presence or absence of bacteriuria and stent-related symptoms evaluated as the USSQ total score, the tested USSQ subscores (urinary symptoms and pain subscore), or USSQ questions U8 and P8 (Kruskal–Wallis test $p > 0.05$, Table 3).

The use of antibiotics (USSQ item A2, at least occasionally vs. never) was not significantly related to total biofilm mass (median = 47.0 mg vs. 35.9 mg, Kruskal–Wallis test $p = 0.246$), the number of bacteria (qPCR, median = 0 in both groups, $p = 0.151$), nor the frequency of positive bacterial cultures (12.4% vs. 16.7%, Fisher's exact test $p = 0.651$). Moreover, the need for hospitalization (USSQ question A4, at least once vs. never) was not significantly related to total biofilm mass (median = 37.0 mg vs. 21.9 mg, $p = 0.108$), the number of bacteria (qPCR, median = 0 in both groups, $p = 0.930$), nor the frequency of positive bacterial cultures (0% vs. 13.5%, $p = 1$).

As crystalline components have been shown to substantially contribute to total biofilm mass [16], a post hoc analysis of the biofilm suspensions was performed to assess any potential influence of the different mineral components on the manifestation of symptoms. According to the results of XRD analysis, the following patient groups were formed: calcium oxalate monohydrate ($n = 29$), calcium oxalate dihydrate ($n = 13$), combination of calcium oxalate monohydrate and dihydrate ($n = 8$), combination of calcium oxalate dihydrate and dicalcium phosphate dihydrate ($n = 4$), and no minerals detected ($n = 38$). Associations between the USSQ total score, urinary symptoms and pain subscores, USSQ question P8 and the mineral composition groups were weak and not statistically significant. In contrast, the occurrence

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Table 3 Secondary outcomes: correlation between stent-associated symptoms and number of bacteria (qPCR), detection of bacteria by cultivation, and detection of minerals

Score/Item	*N*	Spearman rank correlation			Kruskal–Wallis test	
		Number of bacteria (qPCR)			Bacterial cultivation	Detection of minerals
		Correlation coefficient	95% CI	*p* value	*p* value	
USSQ total score	90	0.154	−0.055 to 0.350	0.145	0.515	0.871
Urinary score	91	0.093	−0.116 to 0.294	0.380	0.958	0.571
Pain score	92	0.243	0.038 to 0.428	0.019	0.696	0.559
General health score	93	0.158	−0.049 to 0.351	0.131		
Work performance score	93	0.186	−0.020 to 0.377	0.074		
Sexual matters score	93	−0.066	−0.266 to 0.140	0.533		
Hematuria (U8)	93	0.049	−0.156 to 0.250	0.64	0.170	0.007
Use of analgesics (P8)	93	0.259	0.055 to 0.443	0.012	0.298	0.161

Spearman rank correlations of the number of bacteria (qPCR) and USSQ total score, subscores, and selected single items. Significance (*p* values from Kruskal–Wallis tests) of correlation between questionnaire scores and results of bacterial cultivation (negative or positive culture) and mineral analysis (considering five groups of mineral composition)

of hematuria (USSQ question U8) showed a significant association with the detection of minerals (Kruskal–Wallis test $p=0.007$) (Table 3). Qualitative analysis (data not shown) showed that this association was rather caused by the presence or absence of minerals than by mineral type.

With increasing stent indwelling time, biofilms have been shown to occur more frequently and in increasing amounts [19]. Hence, any symptoms directly caused by biofilms should be expected to increase with stent indwelling time. Questionnaire scores recorded after 1 week were, therefore, compared to those recorded after 2–6 weeks after stent insertion as another post hoc analysis. As shown qualitatively in Fig. 3, the points representing the USSQ total score, subscores for pain and urinary symptoms, and severity of hematuria, respectively, are evenly scattered around the 1:1 line, showing that symptoms can increase or decrease with stent indwelling time, regardless of the initial symptoms and regardless of biofilm formation (biofilm biomass or bacterial numbers). The USSQ total score (median change −2.4, Wilcoxon signed-rank test, $p=0.089$) and the USSQ question for hematuria (U8 median change 0, $p=0.065$) did not change significantly, and the urinary symptoms score and the pain score even decreased (median change for both −1.0, $p=0.039$ and 0.002, respectively) during the time period.

Discussion

Using elaborated biofilm examination methods and validated self-reported outcome measures, our study suggests that biofilms (i.e., both total biofilm mass and bacterial colonization) on ureteral stents are not the main driver of stent-related symptoms. This finding is supported by our observation that stent-related symptoms generally decreased with increasing indwelling times in our study, while biofilm formation is well known to significantly increase over time [19].

Nevertheless, higher biofilm amounts were associated with the occurrence of hematuria. This finding is most likely explained by the sharp-edged microstructures of the crystalline components of biofilms, which have been demonstrated previously [8, 16]. As ureteral stents have been shown to move within ureter and bladder [20], more micro-trauma is likely to occur if the stent surface becomes less smooth. This hypothesis is also confirmed by the presented post hoc analysis assessing the influence of the different mineral components. Thus, occurrence and degree of hematuria showed a significant association with the detection of minerals and, moreover, were rather caused by the presence or absence of minerals than by a specific mineral type.

In addition, higher numbers of bacteria were associated with stent-related pain and consumption of analgesics in this study. Biofilms have been shown to cause a significant production of pro-inflammatory cytokines leading to local inflammation in animal and in vitro studies [10, 11], which might aggravate pain. However, although statistically significant, these correlations were weak.

Strengths of this study are its prospective design, the use of a validated questionnaire that was specifically developed to assess morbidity associated with ureteral stents, and assessments within clearly defined time frames. In contrast to previous studies, biofilms were assessed not only by cultivation, but also by a validated examination pipeline [16] allowing for assessment of total biofilm mass, cultivation-dependent, and cultivation-independent analysis of bacteria, and specification of crystalline components. Patients with UTI at the time of stent insertion were excluded as the UTI

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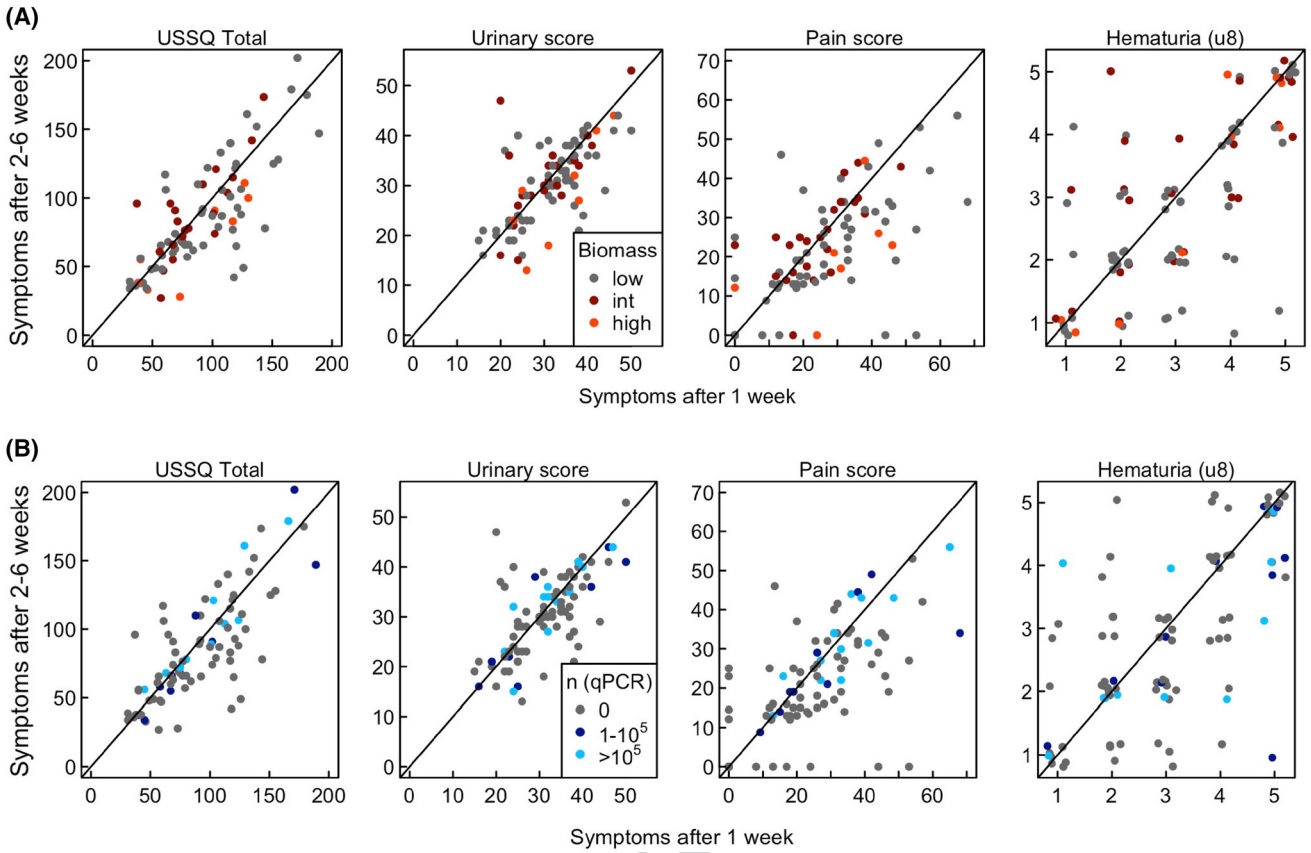

Fig. 3 Stent-related symptoms (questionnaire scores) 2–6 weeks after stent insertion versus those after 1 week, with different symbols indicating **a** biofilm mass, and **b** estimated number of bacteria (qPCR)

symptoms might have interfered with stent-related symptoms. Moreover, the same stent type was used in all of the patients.

The study has limitations that have to be addressed. Biofilm deposits in the stent lumen were not examined as standardized removal is hardly possible and is associated with a high risk of contamination. However, Laube et al. demonstrated that even most (75%) of the ureteral stents which seemed to be obstructed according to failure of the Seldinger technique did not show significant intraluminal deposits, and that biofilm formation in the inner part of the stent seems to be negligible [22]. Moreover, the luminal biofilm is not in direct contact with the urothelium and is rarely responsible for stent dysfunction as the main urine flow occurs outside the ureteral stent [23].

Intravesical stent positioning was not standardized in our study. Although the influence of length and position of the distal stent end has been discussed controversially [1, 20], this might have influenced the degree of stent-related symptoms. Instead of a prescribed medication, patients were instructed to take analgesics and alphablockers according to the severity of their symptoms, which might also have influenced the degree of symptoms in some patients. However, this allowed for treatment according to clinical routine and systematic assessment of drugs required. Moreover, a subanalysis of patients who took no analgesics ($n = 43$) also showed no significant correlations between stent-related symptoms (e.g., USSQ total score, urinary symptoms) and the biofilm mass or number of bacteria, respectively.

Twenty of the study patients underwent antibiotic therapy during stent indwelling time because of a suspected UTI. The lack of urine cultures of these patients before antibiotic treatment represents another limitation of the study. Thus, it remains unclear if patients were treated for UTI or stent-associated symptoms similar to UTI. Nevertheless, the use of antibiotics has been shown to have no significant influence on biofilm formation on ureteral stents elsewhere [9].

This study aimed to assess the influence of biofilms on stent-associated symptoms in short-term ureteral stenting and the median stent indwelling time was 4 weeks. Our study, therefore, does not provide information regarding the influence of biofilms in long-term ureteral stenting.

A previous study in the field found that microbial ureteral stent colonization was more common in patients with de novo or worsened storage LUTS [13]. In that study, sonication and vortexing were used for biofilm extraction, and stent

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colonization was assessed by bacterial cultures only. In our study, a correlation between urinary symptoms (as assessed by the corresponding USSQ subscore) with total biofilm mass was not found, nor with the presence and amount of bacteria in culture-dependent and culture-independent assessments. These conflicting results might be explained by differences in the study setting including different biofilm examination methods, stent indwelling times (stent indwelling time more than 30 days in 54% of the patients [13] vs. median of 28 days in our study) and the survey used to assess morbidity (the previous questionnaire contained only four "yes" or "no" questions about storage LUTS).

Previously, animal and in vitro studies suggested that biofilms on ureteral stents induce the production of various substances by urothelial cells, such as nitric oxide, cathelicidin, chemokines and pro-inflammatory cytokines. This was shown to lead to local inflammation, which activates afferent nerves and might result in LUTS and pain [10, 11]. Although this hypothesis seems plausible, the relevance of biofilms compared to the pure mechanical irritation caused by ureteral stents in vivo remains debatable. Although our study shows that there might be weak correlations between biofilms and stent-related symptoms, e.g., hematuria, pain and intake of analgesics, it clearly suggests that mechanical irritation by the stent itself is likely to represent the main reason for stent-associated symptoms.

Reducing biofilm formation on ureteral stents nevertheless seems to be worthwhile to reduce infectious complications and to facilitate longer indwelling times.

In summary, using elaborated biofilm examination methods and validated self-reported outcome measures, we showed that biofilms are not the main driver of stent-associated morbidity. Reduction of biofilm formation on ureteral stents seems to be worthwhile to reduce infectious complications and to facilitate longer indwelling times. For short-term ureteral stenting, however, other approaches have to be pursued to reduce its high morbidity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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