uro

MDPI

Protocol

Analysis of Multi-Cavity (Bladder, Intestinal and Vaginal) Microbiome in Bladder Cancer Patients: Protocol for a Systematic Review

Marie Semmler [1,†], Uwe Bieri [1,†], Andres Affentranger [1], Dominik Enderlin [1], Luca Truscello [2], Thomas Scherer [1], Silvan Sigg [1], Ernest Kaufmann [1], Michael Scharl [2], Daniel Eberli [1] and Cédric Poyet [1,*]

[1] Department of Urology, University Hospital Zurich, 8091 Zurich, Switzerland; marie.semmler@med.uni-muenchen.de (M.S.); uwe.bieri@usz.ch (U.B.); andres.affentranger@uzh.ch (A.A.); dominik.enderlin@uzh.ch (D.E.); thomas.scherer@usz.ch (T.S.); silvan.sigg@usz.ch (S.S.); ernest.kaufmann@usz.ch (E.K.); daniel.eberli@usz.ch (D.E.)

[2] Department of Gastroenterology and Hepatology, University Hospital Zurich, 8091 Zurich, Switzerland; luca.truscello@usz.ch (L.T.); michael.scharl@usz.ch (M.S.)

* Correspondence: cedric.poyet@usz.ch; Tel.: +41-44-255-54-40

† These authors contributed equally to this work.

Abstract: The overall pathogenesis of bladder cancer is still unknown. The microbiota has been shown to play a critical role in the development of different types of cancer. Nevertheless, the role of the microbiota in the development of bladder cancer is still not fully discovered. This review aims to assess the urinary, vaginal, and intestinal microbiota analyzed from the bacterial, viral, and fungal compartments of bladder cancer patients compared with the microbiota of controls to reveal possible differences. A systematic review according to the PRISMA guidelines will be performed. The findings will be presented in narrative form as well as in tables and graphs.

Keywords: bladder cancer; microbiome; bladder microbiota; intestinal microbiota; vaginal microbiota; bacteria; viruses; fungi

Citation: Semmler, M.; Bieri, U.; Affentranger, A.; Enderlin, D.; Truscello, L.; Scherer, T.; Sigg, S.; Kaufmann, E.; Scharl, M.; Eberli, D.; et al. Analysis of Multi-Cavity (Bladder, Intestinal and Vaginal) Microbiome in Bladder Cancer Patients: Protocol for a Systematic Review. *Uro* **2022**, *2*, 151–156. https://doi.org/10.3390/uro2030018

Academic Editor: Tommaso Cai

Received: 17 May 2022
Accepted: 28 June 2022
Published: 1 July 2022

1. Introduction

1.1. Rationale

In 2020, around 570,000 people were diagnosed with bladder cancer worldwide [1], leading to approximately 210,000 deaths. Generally speaking, many risk factors for the development of bladder cancer are well known. The most prominent factor accounting for around half of all cases is smoking [2]. Another risk factor is chronic inflammation triggered by urinary tract infections or urothelial irritants [3]. Although these risk factors have been identified and studied in detail, comprehensive information to explain the overall pathophysiology of bladder cancer is still lacking.

Recent investigations support the hypothesis that the bladder has its own local microbiome [4], a term that encompasses the combined genetic material of the microorganisms in this particular environment [5,6]. Due to new technologies, bacterial markers can be found in voided urine, which was previously thought to be sterile [7,8]. Since it has been shown that the human microbiota (i.e., the collection of microorganisms in a particular environment [9,10]) has a vast influence on the development of cancer (e.g., colorectal carcinoma, oropharyngeal carcinoma, and cervix carcinoma [7,8]), it seems reasonable to assume that this is also the case for the "Urobiome" (i.e., "microbial communities in the urinary tract" [11]) and the development of bladder cancer. Nevertheless, the question remains to be answered whether the urinary or, in particular, the bladder microbiota is one of the missing pieces in the etiology of bladder cancer.

Research on the urinary and bladder microbiota has mainly focused on bacteria; however, it is well known that the microbial community consists not only of bacteria but also

viruses and eukaryotic microorganisms (such as archaea and fungi) [10]. Moreover, it has been discovered that the human microbiota across different body cavities communicate with or influence each other [10]. Supporting evidence comes from data on the gut microbiota playing a role in urological diseases [12], thus raising the question of whether the gut microbiota or the vaginal microbiota influence the development of bladder cancer, presumably due to their anatomical proximity.

Despite considerable research on bacterial colonization of the urinary tract, different sampling and analysis methods lead to inconsistent results. This is even aggravated in terms of viruses and fungi sampling and cultivation, which is why a clear link between the microbiota and bladder cancer remains elusive [10].

To the best of our knowledge, several (systematic) reviews analyzing the urinary or explicitly the bladder microbiota and its correlation with bladder cancer have been conducted [10,13–16]. Nevertheless, no systematic review of the bacterial, viral, and fungal microbiota of the bladder, intestine, and vagina in patients with bladder cancer has been performed.

1.2. Objective

This systematic review aims to summarize the evidence of existing studies on the composition of the bladder, vaginal, and intestinal microbiota in patients with bladder cancer compared with controls. Since the microbiota does not only consist of bacteria, we intend to include viral and fungal components in our investigation as well. Therefore, this systematic review will answer the following question: Are there differences in the composition of the bladder, vaginal, and intestinal microbiota analyzed from the bacterial, viral, and fungal compartments between patients with bladder cancer and controls?

2. Materials and Methods

This systematic review protocol was developed and will be conducted according to the PRISMA [17,18] and PRISMA-P [19] guidelines, respectively, with additional guidance from the Cochrane Handbook of Systematic Reviews [20] and the Systematic Reviews guidance from the Centre for Reviews and Dissemination [21]. The PRISMA-P checklist [19] is attached in the Supplementary Materials. The eligibility criteria for studies included in this systematic review are presented below.

2.1. Inclusion Criteria

2.1.1. Study Characteristics

In accordance with Yacouba et al., the following types of studies will be included in this systematic review: "(1) studies using bladder cancer and control groups (case-controlled studies); (2) studies that provided information on the presence or abundance of microbial taxa; and (3) studies that provided information on increased or decreased taxa in bladder cancer and/or control groups" [15]. Additionally, and in line with Yacouba et al., we will exclude review articles and animal studies [15]. However, in contrast to most systematic reviews, we will not limit our analysis to the bacterial taxa in the bladder but rather consider the bacterial, viral, and fungal taxa in the vaginal, bladder, and intestinal microbiomes in this systematic review. Studies published until April 2022 will be included without restrictions on the year of publication, publication status, or the minimum number of participants. Only studies in English will be considered.

2.1.2. Participants/Population

Studies that refer to patients with diagnosed bladder cancer of both sexes and a minimum age of 18 years will be included. There will be no restrictions for bladder cancer stages or grades. Possible differences will be analyzed.

2.1.3. Intervention

Intervention is defined as any analysis of the bladder, intestinal, and vaginal microbiota of patients with bladder cancer.

2.1.4. Comparator(s)/Control

The microbiota of control patients, e.g., patients with absent bladder cancer and other genitourinary cancer, will be used as reference microbiota for bladder cancer patients. No specific exclusion criteria for controls will be defined.

2.1.5. Exclusion Criteria

In order to achieve an in-depth analysis of the microbiota of bladder cancer patients, the search will not be restricted, and no exclusion of specific subgroups beforehand will be performed.

2.1.6. Outcome

The difference in three microbiota systems between bladder cancer patients and control patients will be analyzed based on the bacterial, viral, and fungal composition. These three microbiota systems are the bladder, vaginal, and intestinal microbiota. First, the differences in the composition (presence or absence) (if any) of the bacterial, viral, and fungal microbiota will be evaluated concerning taxonomic ranks such as family, genus, or species. Second, the relative numerical ratio (more or less) will be evaluated. Finally, diversity (i.e., alpha- and beta-diversity) will be evaluated as applicable. Each of these results is equivalent to the others, with none prioritized or higher ranked higher.

2.2. Information Sources and Search Strategy

The following databases will be searched up until April 2022: PubMed, Scopus, Embase, and Web of Science. Medical Subject Headings (MeSH), Emtree or their equivalents (if available), keywords, truncations, and Boolean operators will be used in the search strategy. To optimize the search (higher sensitivity), different search queries will be conducted on each database. Broadly, search terms such as the following will be included in our search: "bladder cancer", "neoplasia", "microbiota", "urinary", "intestinal", "gut", "vaginal", "bacteria", "fungi", "virus", "mycobiome", and "virome". Aside from that, to identify additional relevant studies that have not been identified during the initial search, the reference lists of the included studies will be screened. The literature search will be performed by three authors (A.A., D.En., and E.K.).

2.3. Selection Process

After searching the different databases, duplicates in each database and duplicates between the databases will be removed. The screening will be performed in a two-step procedure: in line with the PRISMA-guidelines, two authors (L.T. and S.S.) will independently screen the titles and abstracts. If there is no exclusion of a study through the title and abstract screening, the publication will be moved onto the next step. In the second step, full texts will be screened for eligibility. If agreement cannot be established, a senior author (C.P.) will be consulted. If information is missing, the author of the study is contacted. The two-step screening process will be performed using an excel spreadsheet and, if applicable, additionally available AI-tools. In addition, documentation of the screening will be carried out by applying the PRISMA flow diagram.

2.4. Data Extraction

After the studies have been evaluated, they will be analyzed for relevant information. The data collection process will be performed using a standardized form including the following elements: the first author's name; the year of publication; the country of publication; the type of study; the origin of the study; and the sample sizes, average age and age range, information about sex, BMI, smoking status, ethnicity, catheterization, antibiotic use

and other interventions (such as BCG therapy, chemotherapy, radiotherapy), information about the bladder cancer state, definition of the control group, the analyzed material, the sampling method, the analysis method, the sequencing data, bacterial/viral/fungal taxa, differences in the composition, alpha- and beta-diversity for case and control group, and sources of funding for the study (if available). If information is missing, the authors will be contacted to complete the search as far as possible. Nevertheless, studies will not be excluded if part of the information was not available. Data extraction will be performed by two authors (M.Se. and U.B.).

2.5. Risk of Bias in Individual Studies

Since no statistical analysis will be conducted, there will be no impact of a possible risk of bias on the outcome. If it becomes apparent during the process that an assessment of the risk of bias would be appropriate, risk of bias will be assessed and reported independently by two authors (T.S. and E.K.) applying the Newcastle–Ottawa Scale (NOS) to assess the quality of nonrandomized studies in meta-analyses [22].

2.6. Data Synthesis

Since this systematic review is primarily of a descriptive nature, including different study types, the results of this review will be given in narrative form and illustrated in the form of tables and graphs, and no statistical analysis of the results of the selected studies will be conducted.

A subgroup analysis will be performed if there is evidence for one of the following elements to identify possible heterogeneity of the overall results:

1. Gender;
2. Age range;
3. Origin of study, defined as the country in which the study was conducted;
4. Bladder cancer grading and staging;
5. Definitions of controls (healthy controls, nonneoplastic conditions, etc.);
6. Sample analyzed (urine, bladder tissue, stool, etc.) and sampling method (e.g., for urine: midscream urine, transurethral catheterization, etc.);
7. Analysis method (e.g., for urine: standard urine culture, enhanced quantitative urine culture, 16S rRNA sequencing, etc.);
8. Catheterization prior to assessment (binary yes/no), in which if more granular information is available, then additional reporting of time frame is provided (<1 month, $\geq$1 month, $\geq$6 months, and permanently inserted urinary catheter);
9. Treatments prior to assessment:
    a. Antibiotic treatment (for a period of one month before analysis);
    b. Immuno-/chemotherapy (for a period of 6 months before analysis);
    c. Immunosuppressive therapy present (yes/no);
    d. Diagnosis known to affect the bladder, gut, or vaginal microbiome (e.g., inflammatory bowel disease, bacterial vaginitis, and neuropathic bladder);
    e. Previous intestinal surgery, such as a special case of the creation of a neobladder and bladder augmentation surgery;
    f. Gastrointestinal symptoms (e.g., diarrhea, rectal bleeding, or blood in the stool);
    g. Vaginal symptoms (e.g., abnormal uterine bleeding).

However, we are aware that the treatments prior to assessment, in particular, may have led to exclusion in the studies themselves, so the opportunity to analyze them will not be available. If data for subgroup formation are not provided, the authors will not be contacted, and studies will not be included in subgroup analyses. In the case that no information about treatments prior to assessment is given, the studies will be scored as if they had already excluded patients with these treatments so as not to restrict data analysis due to a lack of information.

2.7. Meta-Bias(es)

Not applicable due to the descriptive nature of the review.

2.8. Confidence in Cumulative Evidence

Not applicable due to the descriptive nature of the review.

2.9. Ethics and Dissemination

Ethical approval is not necessary for this systematic review since no individual patient data will be included. The findings of this review will be published in a peer-reviewed journal and presented at national and international conferences.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/uro2030018/s1. PRISMA-P checklist.

Author Contributions: Conceptualization, M.S. (Marie Semmler) and U.B.; writing—original draft preparation, M.S. (Marie Semmler); writing—review and editing, U.B., D.E. (Dominik Enderlin), L.T., T.S., S.S., E.K., A.A., M.S. (Michael Scharl), D.E. (Daniel Eberli) and C.P.; supervision, U.B. and C.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Registration Details: This protocol has been registered in the International Prospective Register of Systematic Reviews (PROSPERO) (registration ID: CRD42022327475).

References

1. Global Cancer Oberservatory. Available online: https://gco.iarc.fr/today/data/factsheets/cancers/30-Bladder-fact-sheet.pdf (accessed on 5 April 2022).
2. Freedman, N.D.; Silverman, D.T.; Hollenbeck, A.R.; Schatzkin, A.; Abnet, C.C. Association between smoking and risk of bladder cancer among men and women. *JAMA J. Am. Med. Assoc.* **2011**, *306*, 737–745. [CrossRef] [PubMed]
3. Vermeulen, S.H.; Hanum, N.; Grotenhuis, A.J.; Castaño-Vinyals, G.; Van Der Heijden, A.G.; Aben, K.K.; Mysorekar, I.U.; Kiemeney, L. Recurrent urinary tract infection and risk of bladder cancer in the Nijmegen bladder cancer study. *Br. J. Cancer* **2015**, *112*, 594–600. [CrossRef] [PubMed]
4. Thomas-White, K.; Brady, M.; Wolfe, A.J.; Mueller, E.R. The bladder is not sterile: History and current discoveries on the urinary microbiome. *Curr. Bl. Dysfunct. Rep.* **2016**, *11*, 18–24. [CrossRef] [PubMed]
5. Turnbaugh, P.J.; Ley, R.E.; Hamady, M.; Fraser-Liggett, C.M.; Knight, R.; Gordon, J.I. The human microbiome project. *Nature* **2007**, *449*, 804–810. [CrossRef] [PubMed]
6. Wolfe, A.J.; Brubaker, L. "Sterile Urine" and the Presence of Bacteria. *Eur. Urol.* **2015**, *68*, 173–174. [CrossRef] [PubMed]
7. Brubaker, L.; Wolfe, A.J. The new world of the urinary microbiota in women. *Am. J. Obstet. Gynecol.* **2015**, *213*, 644–649. [CrossRef] [PubMed]
8. Lewis, D.A.; Brown, R.; Williams, J.; White, P.; Jacobson, S.K.; Marchesi, J.R.; Drake, M.J. The human urinary microbiome; bacterial DNA in voided urine of asymptomatic adults. *Front. Cell. Infect. Microbiol.* **2013**, *3*, 41. [CrossRef] [PubMed]
9. Drake, M.J.; Morris, N.; Apostolidis, A.; Rahnama'I, M.S.; Marchesi, J.R. The urinary microbiome and its contribution to lower urinary tract symptoms; ICI-RS 2015. *Neurourol. Urodyn.* **2017**, *36*, 850–853. [CrossRef] [PubMed]
10. Javan Balegh Marand, A.; Van Koeveringe, G.A.; Janssen, D.; Vahed, N.; Vögeli, T.A.; Heesakkers, J.P.; Hajebrahimi, S.; Rahnama'I, M.S. Urinary Microbiome and its Correlation with Disorders of the Genitourinary System. *Urol. J.* **2021**, *18*, 259–270. [PubMed]
11. Wolfe, A.J.; Brubaker, L. Urobiome updates: Advances in urinary microbiome research. *Nat. Rev. Urol.* **2019**, *16*, 73–74. [CrossRef] [PubMed]
12. Lee, K.W.; Song, H.Y.; Kim, Y.H. The microbiome in urological diseases. *Investig. Clin. Urol.* **2020**, *61*, 338–348. [CrossRef] [PubMed]
13. Karam, A.; Mjaess, G.; Albisinni, S.; El Daccache, Y.; Farah, M.; Daou, S.; Kazzi, H.; Hassoun, R.; Bou Kheir, G.; Aoun, F.; et al. Uncovering the role of urinary microbiota in urological tumors: A systematic review of literature. *World J. Urol.* **2022**, *40*, 951–964. [CrossRef] [PubMed]

14. Markowski, M.C.; Boorjian, S.A.; Burton, J.P.; Hahn, N.M.; Ingersoll, M.A.; Maleki Vareki, S.; Pal, S.K.; Sfanos, K.S. The Microbiome and Genitourinary Cancer: A Collaborative Review. *Eur. Urol.* **2019**, *75*, 637–646. [CrossRef] [PubMed]
15. Yacouba, A.; Tidjani Alou, M.; Lagier, J.C.; Dubourg, G.; Raoult, D. Urinary microbiota and bladder cancer: A systematic review and a focus on uropathogens. *Semin Cancer Biol.* 2022; in press. [CrossRef] [PubMed]
16. Li, J.K.M.; Chiu, P.K.F.; Ng, C.F. The impact of microbiome in urological diseases: A systematic review. *Int. Urol. Nephrol.* **2019**, *51*, 1677–1697. [CrossRef] [PubMed]
17. Liberati, A.; Altman, D.G.; Tetzlaff, J.; Mulrow, C.; Gøtzsche, P.C.; Ioannidis, J.P.; Clarke, M.; Devereaux, P.J.; Kleijnen, J.; Moher, D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: Explanation and elaboration. *BMJ* **2009**, *339*, b2700. [CrossRef] [PubMed]
18. Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ* **2021**, *372*, n71. [CrossRef] [PubMed]
19. Shamseer, L.; Moher, D.; Clarke, M.; Ghersi, D.; Liberati, A.; Petticrew, M.; Shekelle, P.; Stewart, L.A. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015, elaboration and explanation. *BMJ* **2015**, *350*, g7647. [CrossRef] [PubMed]
20. Cochrane. *Cochrane Handbook for Systematic Reviews of Interventions Version 6.3 (Updated February 2022)*; Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M.J., Welch, V.A., Eds.; Cochrane: London, UK, 2022; Available online: www.training.cochrane.org/handbook (accessed on 11 April 2022).
21. Centre for Reviews and Dissemination; Akers, J. *2009 Systematic Reviews: CRD's Guidance for Undertaking Reviews in Health Care*; CRD; University of York: York, UK, 2009. Available online: https://www.york.ac.uk/crd/SysRev/!SSL!/WebHelp/SysRev3.htm (accessed on 11 April 2022).
22. Wells, G.; Shea, B.; O'Connell, D.; Peterson, J.; Welch, V.; Losos, M.; Tugwell, P. The Newcastle-Ottawa Scale (NOS) for Assessing the Quality if Nonrandomized Studies in Meta-Analyses 2012. Available online: https://www.ohri.ca//programs/clinical_epidemiology/oxford.Asp (accessed on 22 June 2022).