ISRCTN37551129 https://doi.org/10.1186/ISRCTN37551129

Exploring DNA methylation biomarkers associated with bladder cancer in patients with neurogenic bladder

Submission date
11/06/2023

Recruitment status
No longer recruiting

Registration date
14/06/2023

Overall study status
Completed

Last Edited
30/07/2025

Condition category
Cancer

[_] Prospectively registered
[_] Protocol
[_] Statistical analysis plan
[X] Results
[_] Individual participant data

Plain English summary of protocol

Background and study aims
The term neurogenic lower urinary tract dysfunction (NLUTD, also known as neurogenic bladder) refers to abnormal function of the bladder and urinary system. Previous studies have shown that NLUTD patients might have an increased risk of bladder cancer (BCa), which is diagnosed at a younger age and at a more advanced stage in comparison to the general population. Also, there is a higher risk of dying from BCa among NLUTD patients compared to the general population. For these reasons, early diagnosis and definitive treatment of BCa are more crucial in this patient group. However, there is currently scarce research on the most appropriate screening method and follow-up for these patients. Risk factors and possible biomarkers for BCa in patients with NLUTD have to be identified, with future research carried out to provide guidance for screening.
BCa shows frequent alterations in DNA methylation. Nowadays, with the application of modern methods DNA methylation can be recognized in body fluids with high sensitivity and specificity. Several studies support the use of DNA methylation in urine for the early detection of BCa.
This study will evaluate the presence of DNA methylation of a panel of five BCa biomarkers in the urine of patients with NLUTD in comparison with healthy controls, and their association with the patients' clinical characteristics/demographics as well as with histopathological findings and DNA methylation of the panel of genes from bladder tissue samples from the same patients.

Who can participate?
1. Adults aged 18 years or older with NLUTD (or neurogenic bladder) for at least 5 years who have been scheduled for cystoscopy with cold cup biopsy and wash cytology for bladder cancer screening
2. Adults aged 18 years or older who do not have urinary symptoms or disease

What does the study involve?
Patients with NLUTD provide urine and tissue samples for DNA methylation analysis. Volunteers without lower urinary tract symptoms provide a urine sample for DNA methylation analysis.

What are the possible benefits and risks of participating?
Participants are contributing to an effort to investigate new early non-invasive methods of diagnosing bladder cancer in NLUTD patients. There is no risk associated with participating in this study.

Where is the study run from?
Papageorgiou Hospital of Thessaloniki (Greece)

When is the study starting and how long is it expected to run for?
August 2019 to December 2023

Who is funding the study?
Investigator initiated and funded

Who is the main contact?
Dr Periklis Koukourikis, perikliskoukourikis@gmail.com

Contact information

Type(s)
Scientific

Contact name
Dr Periklis Koukourikis

ORCID ID
https://orcid.org/0000-0003-4688-8343

Contact details
Ethnikis Aminis 20
Thessaloniki
Greece
54621
+30 (0)2310224444
pkoukour@auth.gr

Additional identifiers

EudraCT/CTIS number
Nil known

IRAS number

ClinicalTrials.gov number
Nil known

Secondary identifying numbers
PhD/28/08/2019

Study information

Scientific Title
A study of DNA methylation of bladder cancer markers in urine and bladder tissue of patients with neurogenic lower urinary tract dysfunction

Study objectives
The aim of the study is to evaluate the presence of DNA methylation of a panel of five gene promoters, previously associated with bladder cancer, in the urine and bladder tissue of patients with neurogenic lower urinary tract dysfunction and in comparison with controls, their association with the patients' clinical characteristics/demographics and their potential as urine biomarkers for bladder cancer screening and diagnosis.

Ethics approval required
Ethics approval required

Ethics approval(s)
Approved 29/07/2020, Aristotle University of Thessaloniki, Greece, Faculty of Health Sciences, School of Medicine, Ethics Committee (Administration Building (opposite AHEPA Hospital), University Campus, Thessaloniki, 54124, Greece; +30 (0)2310 999338; sakkageor@auth.gr), ref: 6665

Study design
Single-center case-control study

Primary study design
Observational

Secondary study design
Case-control study

Study setting(s)
Hospital, Laboratory

Study type(s)
Diagnostic, Screening

Participant information sheet
Not available in web format, please use contact details to request a participant information sheet

Health condition(s) or problem(s) studied
Neurogenic lower urinary tract dysfunction (neurogenic bladder), screening for bladder cancer

Interventions
This study will recruit two groups of participants.

1. NLUTD Group: patients suffering from neurogenic lower urinary tract dysfunction (NLUTD) for at least 5 years, who have been scheduled for cystoscopy with cold cup biopsy and wash cytology for bladder cancer screening. Patients will provide a urine sample via spontaneous voiding, clean intermittent catheterization or indwelling catheterization, depending on their voiding habits, and part of their bladder biopsy for DNA methylation analysis.

2. Control Group: volunteers without lower urinary tract symptoms. Patients' attendants and hospital staff members will be asked to participate in the study as controls. Volunteers will provide a free-voiding midstream urine sample for DNA methylation analysis and will be assessed with a transabdominal ultrasonography of the urinary system.

Demographics from all participants will be recorded.
The following additional clinical data will be recorded from the NLUTD group:
1. Neurological disease and duration
2. Duration of NLUTD
3. Method of bladder voiding (spontaneously, clean intermittent catheterization [CIC], indwelling catheter [IDC])
4. Years of IDC or CIC usage
5. History of bladder stone(s)
6. History of hematuria
7. History of recurrent UTIs
8. Histopathology results
9. Urine wash cytology results
10. Cystoscopy results

In all urine samples the content of the Urine Preservative Single Dose (Cat#18124, NORGEN BIOTEK CORP., Canada) will be added, which stabilizes DNA, RNA and proteins at room temperature for over 2 years without the need for immediate freezing of the samples. The tissue samples from the NLUTD group that will be subjected to molecular processing will be placed in microvials and immediately cooled in liquid nitrogen, which will then be stored in a deep freeze (-80 oC) until their processing. The samples will be processed, in a blinded fashion, at the Laboratory of Biological Chemistry of the School of Medicine of the Aristotle University of Thessaloniki (Au.Th.). DNA will be extracted from urine and bladder tissue samples using the Cells and Tissue DNA Isolation Kit (Norgen Biotek Corp) and will be modified with sodium bisulfite employing the EZ DNA Methylation-GoldKit (Zymo Research). Quantitative Methylation Specific PCR (qMSP) will be performed to detect bisulfite-induced changes at the promoters of the investigated genes. qMSP will be performed with Luna Universal Probe qPCR Master Mix (New England Biolabs, USA), using the Applied Biosystems StepOnePlus Real Time PCR System (Thermo Fisher Scientific, Inc.).

Intervention Type
Other

Primary outcome measure
DNA methylation of a panel of five genes’ promoters (Ras-Association domain Family member 1 [RASSF1], Retinoid Acid Receptor beta [RARβ], Death-Associated Protein Kinase [DAPK], Telomerase Reverse Transcriptase [TERT] and Adenomatous Polyposis Coli [APC]) in urine measured using Quantitative Methylation Specific PCR at a single timepoint

Secondary outcome measures
DNA hypermethylation of the panel of five genes’ promoters (RASSF1, RARβ, DAPK, TERT and APC) in the bladder tissue of patients suffering from neurogenic lower urinary tract dysfunction (NLUTD) measured using Quantitative Methylation Specific PCR at a single timepoint

Overall study start date
28/08/2019

Completion date
31/12/2023

Eligibility

Key inclusion criteria
Neurogenic lower urinary tract dysfunction group:
1. Neurogenic lower urinary tract dysfunction duration for at least 5 years scheduled for cystoscopy, wash cytology and bladder biopsy
2. Age 18 years or older
3. Consent to provide urine sample and bladder tissue

Control group (healthy volunteers):
1. Age 18 years or older
2. Consent to provide urine sample

Participant type(s)
Healthy volunteer, Patient

Age group
Adult

Lower age limit
18 Years

Upper age limit
90 Years

Sex
Both

Target number of participants
35 participants in each group

Total final enrolment
76

Key exclusion criteria
Neurogenic lower urinary tract dysfunction group:
1. Prior diagnosis and treatment of bladder cancer
2. History of other urinary tract malignancy

Control group (healthy volunteers):
1. Lower urinary tract symptoms
2. History of urinary tract malignancy

Date of first enrolment
01/08/2020

Date of final enrolment

31/12/2023

Locations

Countries of recruitment
Greece

Study participating centre
2nd Department of Urology Aristotle University of Thessaloniki, Papageorgiou University Hospital of Thessaloniki
Ring Road
Nea Efkarpia
Thessaloniki
Greece
54603

Sponsor information

Organisation
Aristotle University of Thessaloniki

Sponsor details
University Campus
Thessaloniki
Greece
541 24
+30 (0)2310991476
apoapo@auth.gr

Sponsor type
University/education

Website
https://www.med.auth.gr/

ROR
https://ror.org/02j61yw88

Funder(s)

Funder type
Other

Funder Name
Investigator initiated and funded

Results and Publications

Publication and dissemination plan
Planned publication in a high-impact peer reviewed journal.

Intention to publish date
01/07/2023

Individual participant data (IPD) sharing plan
The datasets generated during and/or analysed during the current study are/will be available upon request from Dr Periklis Koukourikis MD, FEBU (perikliskoukourikis@gmail.com). Raw data in Excel sheets of demographics, clinical characteristics and results of methylation analysis would be available upon request after the completion of the study. All the participants of the study receive a number during recruitment and participation for anonymization reasons. Signed consent for data availability in an anonymous way was received from the participants.

IPD sharing plan summary
Available on request

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Results article	DNA hypermethylation results	23/05/2024	30/07/2025	Yes	No
Results article	Results	12/08/2023	30/07/2025	Yes	No