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TITLE: Deployable Interstitial Cystitis Urine Diagnostic Technology Development

PRINCIPAL INVESTIGATOR: Bernadette Zwaans, PhD

CONTRACTING ORGANIZATION: William Beaumont Hospital Research Institute

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14. ABSTRACT This proposal directly focuses on the PRMRP Topic Area of Interstitial Cystitis (IC), including the Area of Encouragement for the identification of biological markers for making a definitive diagnosis of IC. This is not a clinical trial as there is no intervention. The <i>objective</i> of this grant is to develop a new diagnostic tool, the Interstitial Cystitis Risk Score (IC-RS), to identify and classify IC patients. In year 3, we continued online and clinical collection with all sites; we currently have 1817 samples collected. In addition, 3 manuscripts related to this study were published, and our work was presented at the 2022 annual meeting of the American Urological Association. During year 3, this grant got transitioned to a new PI, Dr. Bernadette Zwaans, as Dr. Lamb pursued other career opportunities. Dr. Zwaans is dedicated to completing the aims of this grant. Although we encountered delays in our research due to the COVID-19 pandemic, we are on track to complete all the aims of this grant. A request for NCE was approved in spring of 2022.					
15. SUBJECT TERMS Interstitial Cystitis; Bladder Pain Syndrome; Diagnostic Test; Machine Learning; Cystitis, Interstitial; Pelvic pain; Lower Urinary Tract Symptoms; Pelvic Floor Disorders; Biomarkers					
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1. INTRODUCTION:

The subject of this research is to improve diagnosis of Interstitial Cystitis (IC), a devastating disease of pain and altered urinary function that is challenging to diagnose. The purpose of this research is to develop a simple test for diagnosing IC based on urine and symptom scores called the Interstitial Cystitis Risk Score (IC-RS). Our scope of this research includes: 1) Refining and evaluating IC-RS in a large, crowdsourced collection of urine samples from IC patients and normal controls; 2) Comparing patient collected IC-RS from similar collected urine samples and demographic from three academic urology practices that will test civilian and military patients with and without IC and also cohorts of patients with UTI, bladder cancer, and overactive bladder with incontinence to assess specificity of the IC-RS; and 3) Urine kit optimization.

2. KEYWORDS:

Interstitial Cystitis; Bladder Pain Syndrome; Diagnostic Test; Machine Learning; Cystitis, Interstitial; Pelvic pain; Lower Urinary Tract Symptoms; Pelvic Floor Disorders; Biomarkers

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: Refine and evaluate IC-RS in a large, crowdsourced collection of urine samples from IC patients and normal controls.

Major Task 1: Study Start Up

- Target Date: 6 months
- Milestones Achieved: Local IRB and HRPO Approval; Trained research team; Study logistics determined; Online tools and urine collection kits ready
- Completed, all milestones achieved by 11 months

Major Task 2: Strategy for Participant recruitment, shipping & returning of collection kits

- Target Date: 28 months
- Milestones: Study begins; 1000 IC, 1000 non-IC human urine samples recruited and processed; Evaluation of shipping kits and recruitment strategies
- Year 3 actual: We have shipped 1192 IC and 2384 non-IC human urine sample collection kits 564 IC and 701 non-IC human urine samples have been collected and processed. Manuscript on description of crowdsource methodology has been published.
- 86% completed

Aim 1b. Refine and evaluate IC-RS in urine samples from men and women with IC and from normal controls within the military.

Major Task 3: Participant recruitment, shipping & returning of collection kits targeting military personnel.

- Target Date: 28 months
- Milestones: Study begins; 100 urine samples recruited and evaluated; Evaluation and adjustment of shipping kits and recruitment strategies for military personnel (including veterans).
- Year 3 actual: 126 urine samples collected and processed
- Completed, all milestones completed by 28 months

Major Task 4: Data Acquisition and Analysis

- Target Date: 34 months
- Milestone: Report and review results from data analyses
- Year 3 Actual: Data acquisition is completed on 100% of collected samples; data analysis is under way
- 80% completed

Specific Aim 2: Compare patient collected IC-RS from similar collected urine samples and demographic from three academic urology practices that will test civilian and military patients with and without IC to assess specificity of the IC-RS.

Major Task 1: Study start-up

- Target Date: 6 months
- Milestones: Local IRBs and HRPO Approvals; Trained research teams; Study logistics determined.
- Completed, all milestones achieved by 10 months

Major Task 2: Recruitment and Collection

- Target Date: 28 months
- Milestones: Study begins; Completed urine sample and corresponding survey collection; 75 IC and 150 non-IC human urine samples recruited and evaluated per site (total: 225 IC and 450 non-IC)
- Year 3 actual: 79 IC and 140 non-IC urine samples collected at Baylor. 33 IC and 159 non-IC urine samples have been collected at Einstein. 15 non-IC urine samples have been collected at Beaumont. Total 112 IC and 314 non-IC samples are collected.
- 60% completed. This is expected due to COVID-19 restrictions.

Major Task 3: Data Acquisition and Analysis

- Target Date: 36 months
- Milestones: Report and review results from data analyses; manuscript and abstracts submissions
- Year 3 actual: Data analysis under way. Manuscripts on 1. cost comparison between clinical and crowdsource urine collections, and 2. urinary cytokine level comparison between bladder conditions with similar symptoms were published, and several accepted meeting abstracts (2022 annual meeting for American Urological Association) are included in appendix.
- 70% completed

Specific Aim 3: Urine kit optimization

Major Task 1: Evaluation of kits and online support tools (e.g. videos, documents, etc.)

- Target Date: 28 months
- Milestones: Optimized urine collection kit ready-for market
- Year 3 actual: No change from prior year. Milestone had been completed.
- Completed, all milestones achieved at 24 months

Major Task 2: As needed, redesign kits to reflect suggested improvements from Task 1 above

- Target Date: 28 months
- Milestones: Optimized urine collection kit ready-for market
- Year 3 actual: Redesign of kit completed; kit manufacturing delays due to COVID-19; need to finish additional analysis for survey design.
- 50% completed

Aim 3b: Advance the technology to perform IC-RS in a small table-top system that can be run in any basic clinical laboratories regardless of location and without specialized expertise.

Major Task 1: Develop and optimize assay on new platform

- Target Date: 28 months
- Milestones: IC-RS work-flow includes a small table-top system; verified to work at three different sites; manuscript and abstract submission
- Year 3 actual: We have obtained and setup the ELLA system (Protein Simple) and begin validation testing for initial Quality Controls, a critical step to ensure robust and meaningful results. We have determined that there was cross-reactivity for one of the analytes, GRO/CXCL1, in human urine samples and we are working to resolving this issue.
- 30% completed

Major Task 2: Verify results across platforms at two other geographical locations

- Target Date: 36 months
- Milestones: IC-RS work-flow includes a small table-top system; verified to work at three different sites; manuscript and abstract submission
- 0% completed

Major Task 3: Translation of final product for commercialization with Beaumont Technology Transfer Mi-KickStart

- Target Date: 36 months
- Milestones: Patent issued.
- 20% completed

What was accomplished under these goals?

To date, we have completed most of our participant recruitment both online and in the clinic, sample processing, and data acquisition using the Luminex system. We maintained regular research study communication through virtual, online meetings in addition to email and phone calls. Some data analysis has been completed, which has resulted in 2 publications in 2022. Full data analysis using computer learning is ongoing. Feedback on the urine at-home collection kit and online support tools was completed and optimization is completed. Despite significant work, we have so far been unsuccessful in transferring the IC-RS to work on a smaller platform due to issues around quality controls and specificity which we will continue to work on addressing. We have also published a paper describing our online recruitment. We have also had several oral presentations and abstracts presented on our work at the 2022 annual meeting of the American Urological Association in New Orleans, LA.

What opportunities for training and professional development has the project provided?

Study training for all study investigators was done at a meeting in person in Washington DC in September 2019. Due to COVID-19 and travel restrictions, additional study training for study staff has occurred by video conferencing (Microsoft Teams). Webinars that covered laboratory techniques related to this grant were shared with laboratory staff and attended by Sarah Bartolone and Elijah Ward. Dr. Lamb attended several Rapid Acceleration of Diagnostics (RADx) seminars and met with staff regarding the development and commercialization of innovative technologies for diagnostic testing. This project was not intended to provide training and professional development opportunities outside of what was necessary for the research study. However, we have provided an opportunity for Prasun Sharma, a promising medical student who is a veteran, to gain research experience. In the past year, Prasun has published 1 manuscript, co-authored another manuscript, and has presented his work at an international conference.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

Our main focus will be finalizing data analysis, optimization of the technology to perform IC-RS in a small table-top system, and translation of final product for commercialization. Data analysis is already underway and we do not anticipate any issues. We are currently trouble-shooting the ELLA system to be able to run the IC-RS as a small table-top system. We are doing this in partnership with the vendor. Lastly, we will work with Beaumont's Commercialization Center to develop a final product package ready for commercialization in partnership with a vendor who has extensive expertise in this area. We plan to apply for Mi-KickStart program early 2023.

4. IMPACT:**What was the impact on the development of the principal discipline(s) of the project?**

We have published and presented our crowdsourcing methodology allowing for other research groups to apply our methods to other research projects. In addition, we have analyzed and published the cost effectiveness of online sample collection to clinical sample collection and have found that online sample collection is more cost effective.

What was the impact on other disciplines?

Because of our work on this DoD project, we have an intimate knowledge of how to design good, clinically meaningful diagnostic tests including how to ensure they are sensitive, specific, reliable, and validated. We are also knowledgeable as to the regulatory requirements for FDA approval. Because of our work on this DoD, we were well positioned to be the first globally to observe that some COVID-19 patients suffer from severe bladder dysfunction, including inflammation in their urine.

What was the impact on technology transfer?

We were issued the following patent.

Chancellor MB, Lamb LE, Janicki JJ. Methods for Detecting, Diagnosing, and Treating Ulcerative Interstitial Cystitis. United States Patent US 10,859,579 (filed March 31, 2016, and issued December 18, 2020).

What was the impact on society beyond science and technology?

Given the extensive disruption of COVID-19 on society, and the letters we get from patients and doctors from across the globe, we know there has been an incredible impact on global public health.

5. CHANGES/PROBLEMS:

Due to an initial delay to obtain HRPO approval and then COVID-19, we experienced delays to study start in year 1. However, we have overcome this and are now on track to accomplish our aims without changes required to our approach or SOW. In addition, there was a change in PI at the beginning of year. Dr. Zwaans has taken over as PI of this grant and is dedicated to successfully complete the aims of this study.

Actual or anticipated problems or delays and actions or plans to resolve them

1. COVID-19 pandemic completely closed some clinics for a while and has reduced some clinical staff to only 1 day per week. Like any current research study, it is difficult to fully anticipate how the COVID-19 pandemic will continue to impact this study. *If anything, this validates that there is a strong need for research studies that can be completed outside the clinic and instead use alternative strategies like online crowdsourcing and at-home sample collection, as will be done in Aim 1 of this grant.* As such, we have been very successful so far with our crowdsourcing and online recruitment of patients. We will ensure that as our crowdsourcing collection progresses, that we will pay special attention to both effective and ineffective online recruitment strategies, and make sure to disseminate these findings with the larger scientific and medical research communities. We have already shared with the larger urology community our plans for effective crowdsourcing strategies at the annual American Urological Association meeting and a prepared manuscript submitted to PLoS Digital Health (see section 6).
2. We have obtained and setup the ELLA system (Protein Simple) and begin validation testing for initial Quality Controls, a critical step to ensure robust and meaningful results. We have determined that there was cross-reactivity for one of the analytes, GRO/CXCL1, in human urine samples and we are working to resolving this issue in cooperation with the vendor's tech support team.
3. Change in PI took place in fall of 2021. Dr. Zwaans now serves as PI of the study. Dr. Lamb still contributes 5% of her time to the study as consultant.

Changes that had a significant impact on expenditures

As stated previously, enrollment was initially limited by the COVID-19 pandemic. Subsequently, the rate of enrollment has been slower than anticipated. Therefore, cumulative expenses are less than expected. There is a cost saving in salary, travel, subcontract and other miscellaneous costs. Budgeted funds will be needed to achieve the aims of the study.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

None

Significant changes in use or care of human subjects

None

Significant changes in use or care of vertebrate animals

Not applicable

Significant changes in use of biohazards and/or select agents

Not applicable

- 6. PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”*

Publications, conference papers, and presentations

Lamb LE. 2020. Translational Research in Benign Bladder Disorders. 17th Annual Inside Urology, Great Lakes SUNA Chapter 2020 Conference. Livonia, MI, March 13, 2020. *canceled due to COVID-19

Lamb LE, Bartolone SN, Janicki JJ, Peters KM, ICA, Abraham N, Laudano M, Smith CP, Chancellor MB. 2020. National Collaborative Crowdsourcing Method of Diagnostic Biomarker Development for Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS). American Urological Association’s 2020 Annual Meeting, Washington DC, May 15. *virtual due to COVID-19; published in journal

Lamb LE. 2020. Developing a Point-of-Risk Molecular Test for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). European Society for Translational Medicine (EUSTM), Vienna, Austria, September 25, 2020. *virtual due to COVID-19

Lamb LE. 2020. Developing a Point-of-Risk Test for novel Coronavirus 2019. Frontiers in Bioengineering Research, Rice University. Houston, TX, April 30, 2020. *virtual due to COVID-19

Lamb LE. 2021. Improving Diagnostic Testing for Interstitial Cystitis, Radiation Cystitis, and COVID-19. Oakland University William Beaumont School of Medicine Medical Education Week. Rochester, MI, May 20, 2021. *virtual due to COVID-19

Lamb LE. 2021. Interstitial Cystitis Diagnostic Score Development using Crowdsourcing and Machine Learning Algorithm. American Urological Association Annual Meeting/Society of Basic Urological Science Breakout Session. Las Vegas, NV, September 11, 2021. *published in AUA journal

Lamb LE and Chancellor MB. 2021. Improving Patient Outcomes through Research in Benign Urology. Neurourology & Pelvic Reconstructive Surgery (NPR) Joint Urogyn Conference. University of Michigan, Ann Arbor, MI. February 15, 2021. *virtual due to COVID-19

Lamb LE. 2020. Developing a Point-of-Risk Test for COVID-19 and Interstitial Cystitis. University College Cork's Translational Medicine Conference, University College Cork. Cork, Ireland, January 28, 2020. *virtual due to COVID-19

Ward E, Bartolone S, Carabulea A, Sharma P, Janicki J, Laudano M, Abraham N, Smith C, Zwaans B, *Chancellor M, Lamb L. Comparing Concentration of Inflammatory Cytokines in IC, OAB, UTI, and Bladder Cancer Urine Samples. 2022 American Urological Association annual meeting, New Orleans, LA, USA. May 13-16, 2022

*Sharma P, Bartolone S, Ward E, Carabulea A, Peters K, Janicki J, Abraham N, Laudano M, Smith C, Zwaans B, Chancellor M, Lamb L. Conducting Urology Research during COVID-19: Social Media-Based Crowdsourcing of Urine Samples for Biomarker Development. 2022 American Urological Association annual meeting, New Orleans, LA, USA. May 13-16, 2022

Sharma P*, Bartolone S, Ward E, Carabulea A, Peters K, Janicki J, Abraham N, Laudano M, Smith C, Zwaans B, Chancellor M, Lamb L. A Cost and Time Analysis of Participant Recruitment and Urine Sample Collection through Social Media Optimization. 2022 American Urological Association annual meeting, New Orleans, LA, USA. May 13-16, 2022.

Journal publications

Lamb LE, Dhar N, Timar R, Wills M, Dhar S, Chancellor MB. COVID-19 inflammation results in urine cytokine elevation and causes COVID-19 associated cystitis (CAC). *Med Hypotheses*. 2020 Nov 5:110375. doi: 10.1016/j.mehy.2020.110375. Epub ahead of print. PMID: 33213997; PMCID: PMC7644255.

Dhar N, Dhar S, Timar R, Lucas S, Lamb LE, Chancellor MB. De Novo Urinary Symptoms Associated With COVID-19: COVID-19-Associated Cystitis. *J Clin Med Res*. 2020 Oct;12(10):681-682. doi: 10.14740/jocmr4294. Epub 2020 Sep 21. PMID: 33029276; PMCID: PMC7524562.

Ward EP, Bartolone SN, Chancellor MB, Peters KM, Lamb LE. Proteomic analysis of bladder biopsies from interstitial cystitis/bladder pain syndrome patients with and without Hunner's lesions reveals differences in expression of inflammatory and structural proteins. *BMC Urol*. 2020 Nov 7;20(1):180. doi: 10.1186/s12894-020-00751-x. PMID: 33160333; PMCID: PMC7648270.

Lamb LE, Bartolone SN, Ward E, Chancellor MB. Rapid detection of novel coronavirus/Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by reverse transcription-loop-mediated isothermal amplification. *PLoS One*. 2020;15(6):e0234682. doi:

10.1371/journal.pone.0234682. eCollection 2020. PubMed PMID: 32530929; PubMed Central PMCID: PMC7292379.

Preprints on medRxiv:2020.2002.2019.20025155. doi:10.1101/2020.02.19.20025155 and on SSRN: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3539654

Appeared in Top Ten of All Time Lancet Papers on SSRN

Appeared in Top Ten of All Time MedRN: Respiratory Tract Infections on SSRN

Top 10% SSRN Authors by total new downloads for 2020 and 2021

Top Article Downloaded from Institution according to ResearchGate in 2020

Ward EP, Bartolone SN, Carabulea AL, Sharma P, Chancellor MB, Lamb LE. Using Social Media to Crowdfund Collection of Urine Samples during a National Pandemic. *Int Urol Nephrol.* 2022 Mar;54(3):493-498. doi: 10.1007/s11255-022-03108-5. Epub 2022 Jan 26. PMID: 35080680

Sharma P, Lamb LE, Bartolone SN, Ward EP, Janicki JJ, Peters KM, Abraham N, Laudano M, Smith CP, Zwaans BMM, Chancellor MB. A Cost, Time, and Demographic Analysis of Participant Recruitment and Urine Sample Collection through Social Media Optimization. *Urology Practice*, doi: 10.1097/UPJ.0000000000000339

Chancellor MB, Lamb LE, Ward EP, Bartolone SN, Sharma P, Janicki JJ, Smith CP, Laudano M, Abraham N, Zwaans BMM. Comparing Concentration of Urinary Inflammatory Cytokines in Interstitial Cystitis, Overactive Bladder, Urinary Tract Infection and Bladder Cancer. *Urological Science*, Mar 2022. doi: 10.4103/UROS.UROS_26_22

Books or other non-periodical, one-time publications

Nothing to Report

Other publications, conference papers and presentations.

Nothing to Report

Website(s) or other Internet site(s)

<https://www.icstudy.org/>

This website is dedicated to this study and when active will have all study information available on it, study questions and answers, videos on the study, sample collection, and shipping, contact information, and additional study material. There is an English (default) and Spanish version of the website that triggers back and forth with a clickable button.

<https://www.Beaumont.org/urology-research>

Website focused on our translational research lab at Beaumont. This includes a description of our research studies including a link to the above study website, publications, funding sources including DoD, study personnel, and contact information.

Technologies or techniques

Nothing to Report

Inventions, patent applications, and/or licenses

Chancellor MB, Lamb LE, Janicki JJ. Methods for Detecting, Diagnosing, and Treating Ulcerative Interstitial Cystitis. United States Patent US 10,859,579 (filed March 31, 2016; issued December 18, 2020).

Other Products

Dr. Lamb was awarded the 2021 COVID Pandemic-related Outstanding Research Accomplishment (Individual) at the Military Health System Research Symposium. This award “recognizes outstanding research contributions by an individual research scientist with the focus on significant accomplishment(s) of high impact related to the COVID-19 pandemic. This individual has demonstrated outstanding leadership in pursuit of excellence for their country and service.”

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**What individuals have worked on the project?**

Name:	Bernadette Zwaans
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0002-4717-5751
Nearest person month worked:	2
Contribution to Project:	No Change
Funding Support:	No Change

Name:	Laura Lamb
Project Role:	Previous PI
Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0003-0386-4165
Nearest person month worked:	2
Contribution to Project:	No Change
Funding Support:	No Change

Name:	Michael Chancellor
Project Role:	co-I
Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0001-9480-8972
Nearest person month worked:	1
Contribution to Project:	No Change
Funding Support:	No Change

Name:	Sarah Bartolone
Project Role:	Assistant Manager, Research (promoted from Research Coordinator)
Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0001-9040-7055

Nearest person month worked:	9
Contribution to Project:	No Change
Funding Support:	No Change
Name:	Elijah Ward
Project Role:	Research Coordinator I (promoted from Research Assistant II)
Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0002-9405-6763
Nearest person month worked:	10
Contribution to Project:	No Change
Funding Support:	No Change
Name:	Nitya Abraham, MD
Project Role:	Investigator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	No Change
Funding Support:	No Change
Name:	Melissa Laudano, MD
Project Role:	Sub-I
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	No Change
Funding Support:	No Change
Name:	Rutul Patel
Project Role:	Research Coordinator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	Coordinated regulatory and study processes
Funding Support:	No Change
Name:	Christopher Smith MD
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	No Change
Funding Support:	No Change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report for: Nitya Abraham, Melissa Laudano, Christopher Smith

Dr. Zwaans and Dr. Chancellor obtained P20 funding from the NIH/NIDDK for an Exploratory Center for Interdisciplinary Research in Benign Urology, that was termed MI-CURE (Michigan interdisciplinary center for benign urology research and education). Dr. Zwaans dedicates 2.4 calendar months and Dr. Chancellor dedicates 1.8 calendar months to MI-CURE. There is no overlap or conflict between the P20 funding and this award.

Title: Michigan Interdisciplinary Center for Urology Research and Education (MI-CURE)

Major Goals: To understand the effect of changes in mechanical characteristics of urinary bladder tissue, due to fibrosis, on the development of bladder dysfunction.

Status of Support: Awarded

Name of PD/PI: Dr. Bernadette Zwaans

Role: PI

Source of Support: National Institutes of Health/NIDDK

Primary Place of Performance: Beaumont Hospital, Royal Oak

Project/Proposal Start and End Date: 9/22/2021 – 9/21/2023

Total Award Amount (including Indirect Costs): \$615,071.00

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	B. Zwaans	M. Chancellor
1. 2021	0.8 Calendar months	0.6 Calendar months
2. 2022	2.4 Calendar months	1.8 Calendar months
2. 2023	1.6 Calendar months	1.2 Calendar months

What other organizations were involved as partners?

Baylor College of Medicine and Michael E. DeBakey VA Medical Center
Houston, Texas

Contribution: Dr. Smith actively participates in all online meetings and email/phone correspondence along with other key study personnel. He assists in protocol review and the contributes to the development of the patient recruitment plan. Dr. Smith is actively recruiting study participants. Dr. Smith also helps to design strategies to troubleshoot anticipated and unanticipated issues as the study progresses. Finally, he remains engaged with military and Veteran's Affair communities in anticipation of eventual transition of study findings to their IC/BPS patient populations.

Einstein College of Medicine
Bronx, New York

Contribution: Drs. Abraham and Laudano continue to actively participate in all online meetings and email/phone correspondence along with other key study personnel. They assist in protocol review and the contributes to the development of the patient recruitment plan. They are actively recruiting study participants. They also helped to design strategies to troubleshoot anticipated and unanticipated issues as the study progresses.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Not Applicable

QUAD CHARTS:

Not Applicable

9. APPENDICES:

Appendix 1_Ward et al_crowdsource methodology

Appendix 2_Chancellor et al_urinary cytokines bladder conditions

Appendix 3_Sharma et al_cost comparison

Appendix 4_AUA 2022 abstracts



Using social media to crowdsource collection of urine samples during a national pandemic

Elijah P. Ward¹ · Sarah N. Bartolone¹ · Prasun Sharma² · Michael B. Chancellor^{1,2} · Laura E. Lamb^{1,2}

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Abstract

The COVID-19 pandemic and subsequent lockdown had a substantial impact on normal research operations. Researchers needed to adapt their methods to engage at-home participants. One method is crowdsourcing, in which researchers use social media to recruit participants, gather data, and collect samples. We utilized this method to develop a diagnostic test for Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS). Participants were recruited via posts on popular social-media platforms, and enrolled via a website. Participants received and returned a mail kit containing bladder symptom surveys and a urine sample cup containing room-temperature preservative. Using this method, we collected 1254 IC/BPS and control samples in 3 months from all 50 United States. Our data demonstrate that crowdsourcing is a viable alternative to traditional research, with the ability to reach a broad patient population rapidly. Crowdsourcing is a powerful tool for at-home participation in research, particularly during the lockdown caused by the COVID-19 pandemic.

Introduction

The COVID-19 pandemic has massively impacted everyday life, shutting down nonessential services and forcing people to shelter in place for weeks at a time. One of the many effects of the global lockdown was the decrease in clinical research with the closing of research labs. With non-COVID-19 research activities suspended through a significant portion of 2020, nonessential researchers and scientists had no laboratory access [1]. A survey of 881 life scientists revealed that 57% reported some loss of work, stemming from decreased laboratory access [2]. This represents a financial problem, as costly experiments, such as those involving animal models, may need to be repeated. In a survey of postdoctoral fellows, 80% reported difficulty in performing experiments and discussing ideas with colleagues [3]. The National Institute of Health's survey of principal investigators and research leaders revealed extreme concerns with institutional financial stability and productivity of research [4]. Recruiting for a clinical trial

during the pandemic also represents an ethical dilemma, as researchers and study participants could be potentially exposed to SARS-CoV-2 during study visits. Because traditional research recruitment and participation methods were hampered by the COVID-19 pandemic, different approaches were necessary. Crowdsourcing, where researchers employ modern communication methods to recruit participants and gather data, is one such approach.

Crowdsourcing as a research method has gained traction in the medical community. Increasing ease of communication and data collection via the internet and social media has resulted in innovations in public health, education, communication, and ethics [5, 6]. By utilizing social media, researchers are able to advertise their studies, recruit and engage with participants, and gather data. Mail-based collection of samples allows for participation from the safety of home. It also has the potential to significantly lower costs while increasing sample size, compared to traditional research [7]. Crowdsourcing also has the benefit of improved study completion. We believe that these benefits make crowdsourcing an ideal tool for reaching groups suffering from rare diseases that are poorly understood or restrict travel, particularly during the COVID-19 pandemic, when participants were not able to leave their homes. Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is one such disease that benefits greatly from an at-home, social media-based crowdsourcing approach.

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IC/BPS is a debilitating chronic disease that affects roughly 3–8 million women and 1–4 million men in the United States [8, 9]. Approximately 10% of IC/BPS cases present with Hunner's lesions, which are visible via hydrodystension and cystoscopy; however, the majority of those suffering with IC/BPS do not present with lesions [10]. Diagnosis of IC/BPS is based on patient reported symptoms and exclusion of other diseases with overlapping clinical presentation. Therefore, a non-invasive, rapid, diagnostic test is required. We sought to develop a urine-based biomarker test by crowdsourcing urine from both IC/BPS patients and asymptomatic control participants, utilizing social media and other web-based recruitment tools to reach a large population. We then sought to determine the usefulness and efficacy of these recruitment methods during a nation-wide lockdown and pandemic.

Methods

Study recruitment and enrollment

All study materials were approved by the Beaumont Health Institutional Review Board (IRB# 2019–266). A study website was created using Wix, a site development tool. This website contained study background videos, a link to the enrollment survey, shipping information videos, and frequently asked questions. A form for contacting the research team via email was also included. The enrollment survey was created using SurveyMonkey, a Health Insurance Portability and Accountability Act (HIPAA)-compliant survey website. The enrollment survey included demographic information, health history, and IC/BPS diagnosis information. Participants were asked to provide a name and address to which we sent a collection kit.

We partnered with the Interstitial Cystitis Association (ICA), an IC/BPS patient advocacy group, to reach a large audience of people suffering from IC/BPS. The patient advocacy group sent out emails informing members of the study, with links to the recruitment website. The study was also advertised via Google Ads, appearing under the search terms “IC”, “IC/BPS”, “interstitial cystitis”, and “urology study”. Social media posts on websites and phone software applications Instagram, Facebook, and TikTok were used to advertise the study and recruit participants. Once a potential IC/BPS participant had completed the enrollment survey and met enrollment criteria, and consented to participate, an at-home collection kit was prepared and shipped to them. IC/BPS participants were asked to find a male and female of similar age with no bladder health history willing to participate as control samples; inability to find control participants did not exclude the person with

IC from participating in the study. Control participants who discovered the study independently were allowed to enroll via the study website.

At-home collection kit and mailing

At-home collection kits were sent to the address provided by the study participant via FedEx. The mailing kits contained 3 boxes for the IC/BPS participant, male control, and female control. Each box was a self-contained independent sample return mailing kit. All three boxes contained study information sheets, bladder symptom surveys, a urine collection cup containing a room-temperature urine preservative (Norgen Biotek, Thorold, Ontario, Canada), and a pre-paid United States Postal Service (USPS) return mailing envelope. The urine preservative stabilizes proteins and nucleic acids in samples and allows them to be shipped at ambient temperatures, while also inactivating bacteria and viruses in the sample. The male and female control boxes also included demographic survey for the control participants, mimicking the information collected from IC participants during their enrollment survey. All return boxes contained a non-reversible temperature recording label, which indicated how long the package was exposed to temperatures exceeding 30° C. At this temperature, the preservative no longer protects the biomarker proteins of interest from degradation. Bladder symptom surveys included the Interstitial Cystitis Symptom/Problem Index (ICSI/PI), the Overactive Bladder questionnaire (OABq), the Pelvic Pain, Urgency, and Frequency questionnaire (PUF), and the Visual Analog Scale (VAS).

Urine sample collection and return shipping

Participants were asked to provide a urine sample via mid-stream catch and place it in the biohazard bag containing absorbent material. They were then asked to fill out the included surveys, place the sample and survey in a pre-labeled USPS envelope, and place the envelope in their mailbox.

Instructions for collecting and returning the urine samples were provided in the kit. The paper instructions included directions to the study website, where videos showed how to collect and ship the samples. The study website also contained a frequently-asked-questions section, which provided answers to common questions, and a “Contact Us” form for questions or concerns about the study.

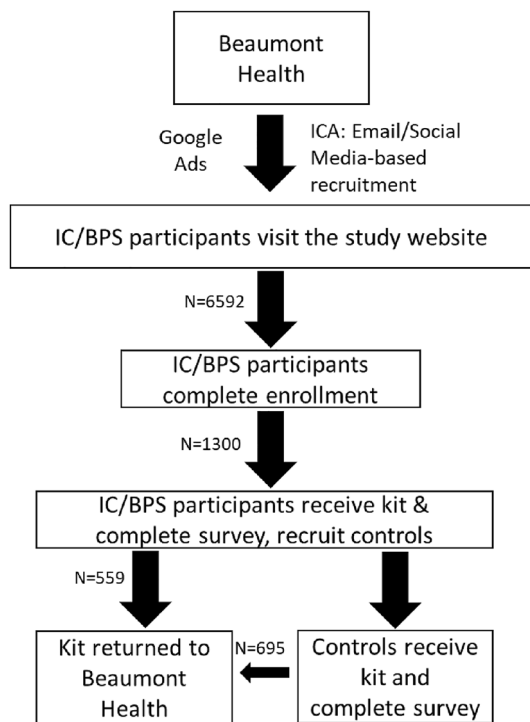


Fig. 1 Xxxx

Fig. 2 Yyy

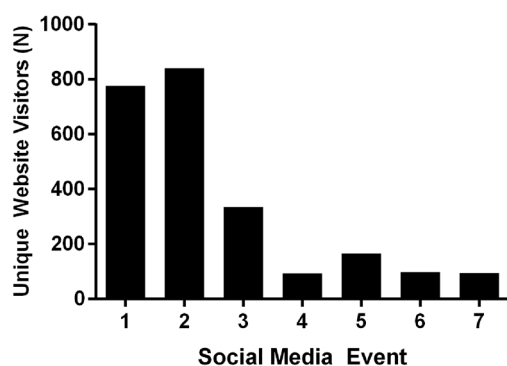
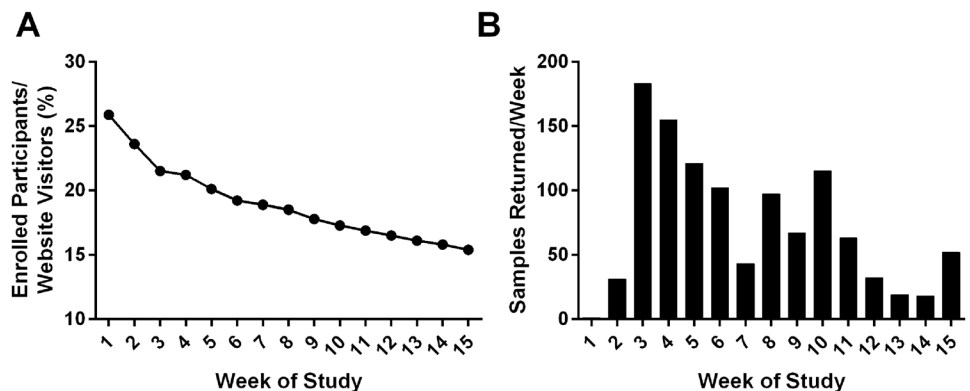


Fig. 3 Zzzz

Results

Study enrollment goal was met three months after launch

The study was launched September 14, 2020 and closed December 26, 2020. The study recruited 1,254 IC/BPS and control samples from all 50 United States (Fig. 1). The first week of study enrollment had the highest percentage of enrolled participants versus unique website visitors (for the purposes of this study, a “unique website visitor” is only counted once for website visitation, regardless of return visits); this percentage slowly decreased through the duration of the study (Fig. 2A). Sample return rate was highest at week 3 of the study. There was a 2 week average turnaround time from initial enrollment to sample return. Rate-of-return fell slowly over time but rose slightly at weeks 8 and 10, when the patient advocacy group sent reminder emails to participants to return their kits (Fig. 2B).

Event	Social Media Post	Methods Used
1	Study Launch	Facebook, Instagram
2	Recruitment Video- Dance	TikTok, Facebook, Instagram
3	Reminder of Study Launch	Email, Facebook
4	Interview with Researchers (ICA Walk for a Cure)	YouTube
5	IC Participant- Kit Content Video	Facebook, Instagram
6	Bladder Health Month Challenge	Facebook, Instagram
7	Shipping Reminder Video	Facebook

Social media posts using video attracted the most website visitors

Seven major social-media posts were made during the enrollment period (Fig. 3). The posts that were the most effective were posted on multiple social-media sites. The social-media post with the largest impact on unique website visitors was an 11-s TikTok video advertising the study; this video was also re-posted to Facebook and Instagram.

Majority of participants used mobile devices to enroll and visit website

80% of unique website visitors used their mobile devices to visit the study website (Fig. 4A). Mobile devices are responsible for 61% of US website visits, though social networks are predominantly accessed via personal computers [11].

Google and Facebook are effective tools for study recruitment

Visitors discovered the study website in multiple ways (Fig. 4B). Of these methods, the most common was a direct link, found in recruitment emails. Other methods included Google links (through organic searches or Google Advertisements) and Facebook posts by the ICA and other interested individuals. Finally, the ICA's website and Beaumont Health's website resulted in moderate traffic over the study period.

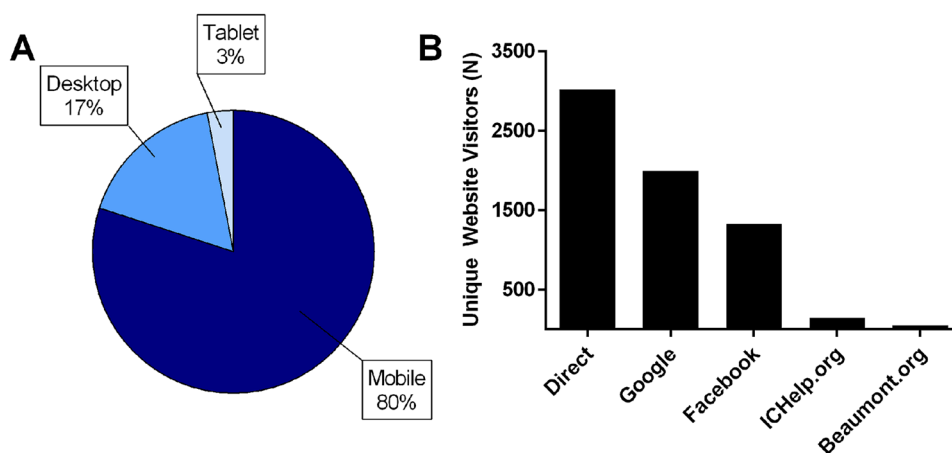
Discussion

This study confirms the versatility and usefulness of social media-based recruitment and crowdsourcing for clinical sample collection. Social media-based recruitment and crowdsourcing have significant benefits over traditional

recruitment methods. This is especially evident as this study was completed during the fall of 2020, in the middle of the COVID-19 pandemic. Social media-based recruitment gathers participants at a significantly faster rate than traditional clinical sample collection. In this study, both clinical sites recruited ten times fewer participants than the at-home, social media-based recruitment in the same three-month period. This may be due to the increased number of people at home and the ease of at-home recruitment and participation during the COVID-19 pandemic lockdown. Online recruitment and crowdsourcing of samples also bypass the geographical limitations of traditional studies; where a normal clinical study may recruit a population in the immediate geographic area around the site(s), a crowdsourcing study with online recruitment methods can recruit participants from all over the country. Another benefit of social-media/web-based recruitment is the ability to tailor to specific target populations. By tailoring recruitment advertisements and partnering with specialized patient advocacy groups, it is possible to target a subset of the normal population, enhancing recruitment goals. Finally, online recruitment and at-home participation is safer than traditional recruitment methods during a pandemic. As SARS-CoV-2 is not highly transmitted on surfaces, it is advantageous to receive a sample kit containing survey responses and biological samples by mail, rather than in-person, where the risk of viral transmission is significantly higher [12].

Online recruitment and crowdsourcing are not without their disadvantages. The biggest disadvantage of crowdsourcing studies is the absence of a patient medical record. For studies such as this that gather both diseased and control samples, researchers are trusting that the participant answers questions honestly, rather than relying on a clinician-confirmed diagnosis. Studies utilizing these methods also frequently lack a personal component. For example, this study had no face-to-face or video-based interaction with participants; while there were email and phone hotline

Fig. 4 XXXX



for participant questions and concerns, the lack of personal interaction may have dissuaded some people from participating. In this study, this could not be avoided, as we sought to eliminate participant-physician interaction due to the COVID-19 pandemic. Lack of control participation was also challenging. Control participants who were not recruited by an IC/BPS participant rarely returned their surveys and urine sample. We have observed this in a previous study [13], and tried to address it by including paper surveys for control participants, rather than directing them to a website to complete surveys.

One of the challenges in completing a successful social media-based research study is preparing user-friendly study materials, such as advertisements and websites. In this study, 80% of unique website visitors accessed the study website from their mobile devices. This may be due to users accessing social media more frequently from their phones versus personal computers, as these are app-based platforms. It is also important to have multiple advertisement methods to reach the largest number of potential participants. In this study, we partnered with a patient advocacy group for primary recruitment, created a Google Ad, and optimized the search engine results for the study website. The patient advocacy group advertised the study via direct emails and social-media posts on Facebook, Instagram, and TikTok. The posts that were linked to multiple social-media platforms resulted in the largest spike in unique website visitors. Social media-based recruitment studies can also grow on their own once they are large enough. In this study, a post advertising the study was made on an IC/BPS forum by a user that was not affiliated with our research group nor the patient advocacy group. In another instance, a clinic in South Dakota discovered the study via a social-media post and began to advertise locally for recruitment. We are currently analyzing the large data set from the crowdsourcing collection via machine learning, a method we recently described [14]. Optimizing the algorithm with big data takes time, but we believe it will provide fruitful results towards developing an IC/BPS diagnostic test.

The final consideration for an online recruitment/crowdsourcing study is the timing of study launch, especially during a national lockdown due to a spreading pandemic. This study was initially prepared for launch in the first quarter of 2020; however, there were multiple problems that we attempted to address, resulting in delay of initial study launch. Firstly, we wanted to ensure that the mail kits and urine samples could not transmit SARS-CoV-2. We waited until publication of research studies that declared surface transmission risk via mail was minimal. We also wanted to confirm that the room-temperature urine preservative prevented transmission of the SARS-CoV-2 virus. The state of the US Postal Service was also considered at the planned time of launch in early 2020. The mail system became

Table 1 Crowdsourcing IC/BPS and control demographics

	IC/BPS	Control
No. of participants	559	695
Age (mean $\pm$ SD, range)	49.8 $\pm$ 16.4 (19–91)	48.0 $\pm$ 16.8 (19–95)
No. of males (%)	30 (5%)	401 (58%)
No. of females (%)	529 (95%)	294 (42%)
ICSI/PI (mean $\pm$ SD)	23.2 $\pm$ 8.1	5.1 $\pm$ 5.3
OABq (mean $\pm$ SD)	68.9 $\pm$ 24.6	25.4 $\pm$ 12.1
PUF (mean $\pm$ SD)	19.4 $\pm$ 6.5	3.3 $\pm$ 4.0
VAS (mean $\pm$ SD)	5.1 $\pm$ 2.3	1.0 $\pm$ 0.7

backlogged due to the nation-wide lockdown. Finally, full laboratory staff was not allowed in the research facility initially during the pandemic; we were required to stagger our on-site presence, which would have made processing the rapid influx of samples much more difficult. Therefore, the study was postponed until sufficient research staff was available to process incoming samples.

This novel method is potentially limitless in application; though focused on IC/BPS in this study, crowdsourcing and social media-based recruitment could be applied to many urological diseases including overactive bladder, BPH, prostate cancer and bladder cancer, by utilizing existing social-media groups and disease interest groups to spread awareness of the study. While collecting biological specimens can be a challenge, the existence of room-temperature urine preservatives allows researchers to analyze a large volume of samples that are traditionally difficult to acquire due to geographic limitations.

Conclusion: Social media is a powerful untapped resource for researchers. By advertising via social media, it is possible to rapidly gather data from a larger population and geographical region. These new recruitment and participation methods are even more useful during the COVID-19 pandemic, which hampered and sometimes halted normal research operations. Using these methods, we were successful in gathering over 1254 control and IC/BPS urine samples in 3 months from across the country, aiding us in our goal of developing a urine-based diagnostic test for IC/BPS (see Table 1).

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Author contributions LEL and MC conceived and designed this study. EPW and SNB curated study data. EPW, SNB, LEL, and PS analyzed data and prepared figures. LEL and MC provided project administration and supervision. EPW wrote the manuscript. SNB, PS, LEL, and MC reviewed and edited the manuscript. All authors approved the manuscript for submission.

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Data availability Data available upon reasonable request from corresponding author.

Declarations

Conflict of interests LEL and MBC have intellectual property associated with methods for diagnosing interstitial cystitis. All other authors declare no competing interests.

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Original Research

Comparing Concentration of Urinary Inflammatory Cytokines in Interstitial Cystitis, Overactive Bladder, Urinary Tract Infection, and Bladder Cancer

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Abstract

Purpose: We sought to determine if urinary cytokine concentration profiles were different between various bladder conditions. **Methods:** Participants at three clinical sites completed a demographics survey and provided a urine sample in a collection cup containing a room-temperature urine preservative. Participants were divided into the following categories based on physician-documented diagnosis: asymptomatic control, nonulcerative interstitial cystitis (IC), overactive bladder with incontinence (OAB wet), urinary tract infection (UTI), and bladder cancer. Urinary cytokines were measured through Luminex multiplex assay. **Results:** Two hundred and seventy-seven urine samples were collected from three clinical sites. Urinary pro-inflammatory cytokines had an increasing trend in bladder disease versus control, with a significant increase for chemokine (C-X-C) ligand 1 growth-regulated protein alpha CXCL1 (GRO). Further analyses demonstrated that patients with UTI had significantly higher levels of GRO and interleukin-8 (IL-8) in comparison to control, nonulcerative IC, OAB wet, and bladder cancer. Both are chemokines that stimulate chemotaxis resulting in the rapid accumulation of immune cells such as neutrophils. IL-6 levels overall were at the lower limit of assay range but were significantly increased in urine of UTI patients versus IC patients. MCP-1 (CCL2) had the least separation among the control group and the various bladder diseases. **Conclusion:** Urinary concentrations of GRO were higher in disease state compared to control. Specifically, levels of GRO and IL-8 were higher in urine samples from patients with UTI compared to controls and other bladder conditions. Comparing and contrasting urinary cytokines may help improve our understanding of these important bladder diseases with great unmet needs.

Keywords: Bladder cancer, interstitial cystitis, overactive bladder, urinary tract infection

INTRODUCTION

Interstitial cystitis (IC)/bladder pain syndrome (BPS) is a debilitating chronic disease that affects roughly 3–8 million women and 1–4 million men in the United States.^[1,2] Patients

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with IC/BPS are identified based on the presence of Hunner's lesions, though only an estimated 10% of IC/BPS cases present with these bladder lesions.^[3] Diagnosis of IC/BPS is based on patient-reported symptoms and exclusion of other diseases with overlapping clinical presentation, such as overactive bladder with incontinence (OAB wet), urinary tract infection (UTI), and bladder cancer. Bladder cancer and IC patients, especially those IC with Hunner's lesion (HIC), are most often diagnosed by cystoscopy with hydrodistension.^[4] However, this can be painful and requires anesthesia. Some patients may experience painful urination, hematuria, UTI, and rare risk of bladder wall perforation. Identifying these patients presents a therapeutic opportunity as bladder-directed therapies such as electrocautery, resection, or injection of these lesions with steroids can provide temporary symptom relief. Given the invasiveness and the side effects of cystoscopy with hydrodistension, there is a strong need for a noninvasive, rapid, diagnostic test to easily identify and diagnose bladder diseases, and to measure patients' response to therapy.

Urine cytokines have been of high interest as potential biomarkers for IC, and there is a strong suggestion that urinary cytokines are elevated with IC.^[5] A valid biomarker for diseases with similar symptomatology such as IC, bladder cancer, UTI, or OAB wet would be advantageous for both clinicians and patients. Our research group has previously identified three urinary cytokines, interleukin-6 (IL-6), IL-8, and GRO, to be elevated in IC/BPS patients with Hunner's lesions in comparison to patients without Hunner's lesions or healthy controls.^[6] In addition, IL-8 and IL-6 were found to be significantly higher in urine from nonulcerative IC/BPS patients versus healthy controls.^[6] However, bladder inflammation is also common in other bladder conditions. In a study by Kumari *et al.*, 12 urinary cytokines, including IL-8, were identified to be more abundant in patients with bladder cancer versus healthy controls.^[7] Likewise, increased urinary cytokines have been identified during active UTI and in patients with OAB.^[8-10] Thus, we saw a need for a study to directly compare urinary cytokines in several confusable bladder conditions.

The objective of this study was to compare the levels of four urinary cytokines that we previously identified as being elevated in IC/BPS to determine if they can be used to differentiate different types of bladder diseases.^[6] We collected urine samples from patients with clinically diagnosed nonulcerative IC/BPS, bladder cancer, OAB wet, or UTI, and healthy controls. We sought to understand if there were differences in urinary cytokine concentration between these conditions.

METHODS

Study population

Urine samples were collected with local Internal Review Board approval from three academic urology centers from different regions of the United States, Montefiore Medical Center Albert Einstein College of Medicine in Bronx, New York (IRB #2019-10949), Baylor College of

Medicine/Michael E. DeBakey VA Medical Center in Houston, Texas (IRB #H-49346), and Beaumont Health, Royal Oak, Michigan (IRB #2019-266). This study was also approved by the Department of Defense Human Resource Protect Office (E00749) and aimed at enrolling participants with IC and without IC including asymptomatic/normal controls, bladder cancer, current UTI, and OAB wet (OABw) patients. All participants had confirmed physician diagnosis for nonulcerative IC, bladder cancer, UTI, and/or OAB wet. Urine collection was carried out by trained clinical research staff. A urine preservative (Norgen Biotek, Thorold, ON, Canada) was added to all urine samples immediately after collection to allow for storage of urine samples at room temperature up to 1 year. Urine samples and corresponding surveys were deidentified and marked with a unique study identification number before shipping to Beaumont through FedEx.

Patients meeting study inclusion and exclusion criteria as described in Table 1 were recruited by the study physicians at their respective clinic. Candidates with IC were given the diagnosis based on NIDDK criteria and satisfied the inclusion and exclusion criteria. All physicians involved in this study are fellowship trained and have years of experience evaluating women with IC. Patients with OABw, UTI, and bladder cancer were recruited in the clinic if they met all inclusion and exclusion criteria [Table 1].

VISUAL ANALOG SCALE

A 10-point VAS for maximum daily pain was utilized where 0 is no pain and 10 is maximum pain. Extensive research supports the validity of the VAS for assessment of pain intensity.^[11]

Multiplex Luminex assay

Samples were aliquoted and stored at room temperature and ten 1.0 mL aliquots were stored at -80°C . Samples were randomized and assigned an experimental ID number to run and analyze samples in a blinded fashion. Expression of a panel of cytokines (GRO, IL-6, IL-8, and MCP-1) was determined using the MILLIPLEX MAP Human Cytokine/Chemokine Multiplex Immunoassay (Millipore) following manufacturer's protocol and as previously described.^[6]

Statistical analysis

Two hundred and seventy-seven urine samples were collected from three clinical sites. To compare cytokine levels between control and disease groups, a two-tailed nonparametric independent *t*-test was performed using Mann-Whitney U-test. To determine statistical difference between control and different disease states, we performed a Kruskal-Wallis nonparametric test followed by Dunn's multiple comparison test. $P < 0.05$ was considered statistically significant.

RESULTS

Patient population

Patient demographics among the groups are listed in Table 2. Among the three sites, a total of 277 urine samples were

Table 1: Inclusion and exclusion criteria

Interstitial cystitis
Inclusion criteria
Male or female at least 18 years of age
IC/BPS diagnosed by a health-care provider based on the following criteria
Complaint of suprapubic pain related to bladder filling, accompanied by other symptoms such as increased daytime and nighttime frequency, in the absence of proven urinary infection or other obvious pathologies
Have had IC symptoms for at least 6 months
Urinary frequency >10 times a day
Have IC that in investigator's judgment has been stable in the previous 30 days
IC-related pain defined as a score of >3 and <9 on the pain VAS
Exclusion criteria
Prior intravesical therapy or bladder hydrodistention within 30 days
Participated in IC research trial within 90 days prior
Previous augmentation cystoplasty, cystectomy, neurectomy, or bladder botulinum toxin injections
Sacral and/or tibial nerve neuromodulation within the last 6 months
Any condition that in the judgment of the investigator would interfere with the patient's ability to provide informed consent, comply with study instructions, place the patient at increased risk, or might confound the interpretation of the study results
Urinary tract or prostatic infection in the past 90 days before study entry
Active genital herpes or vaginitis
Urethral diverticulum
Pelvic malignancy within the past 5 years
History of cyclophosphamide or chemical cystitis, tuberculosis, or pelvic radiation
Urinary tract infection
InCr
Male or female at least 18 years of age
Symptomatic bacteria, positive urine culture with >10 ⁵ colony-forming units/mL
ExCr
History of IC, OAB, or bladder cancer
Urine culture with no growth in 48 h
Overactive bladder wet
InCr
Male or female at least 18 years of age ≥6-month history of symptoms of OAB including frequency, urgency, and urgency or mixed urinary incontinence
ExCr
History of IC, UTI, or bladder cancer
History of pelvic or bladder disease, for example, uterine prolapse, malignancy, prior surgery, or treatment with botulinum toxin
Diabetes insipidus or poorly controlled Type 1 or Type 2 diabetes mellitus
Bladder cancer
InCr
Male or female at least 18 years of age
Pathologic diagnosis of any stage tumor grade of urothelial carcinoma of the bladder from biopsy or resection specimens at the time of urine collection
ExCr
History of IC, OAB, or UTI
History of other pelvic or bladder diseases, for example, uterine prolapse and prostate cancer.

Contd...

Table 1: Contd...

Normal control
InCr
Male or female at least 18 years of age
ExCr
Urologic symptoms including pain or incontinence
History of urologic cancer, infection, or stone disease

InCr=Inclusion criteria; ExCr=Exclusion criteria; IC: Interstitial cystitis; VAS=Visual Analog Scale; OAB=Overactive bladder; UTI=Urinary tract infection; BPS=Bladder pain syndrome

collected: 31 urine samples were collected of healthy controls and 246 of patients with OAB wet ($n = 84$), nonulcerative IC/BPS ($n = 83$), bladder cancer ($n = 61$), and UTI ($n = 18$). For all conditions, more women were enrolled in the study, except for bladder cancer. The age ranges of study participants were similar among the five groups, though the average age for bladder cancer was the highest followed by UTI (73.4 and 69.3, respectively). The VAS score indicated that the patients with IC/BPS and UTI reported higher pain scores than control, OAB wet, and bladder cancer groups.

Increase in cytokine levels in bladder disease

Urinary cytokine levels were measured through Luminex multiplex assay. Urinary concentrations of IL-6, IL-8, MCP-1, and GRO were measured. Initially, cytokine levels were compared between control and disease treatment groups [Figure 1]. Of the four cytokines investigated, all cytokines were higher in disease state versus control. However, only the increase in GRO (CXCL1) was statistically significant. We further divided the disease states into nonulcerative IC, OABw, UTI, and bladder cancer to identify potential disease-specific changes in cytokine levels [Table 3 and Figure 2]. Overall, average cytokine levels had a higher trend in all four diseases states studied, but only urine samples from patients with UTI had significantly higher levels of GRO and IL-8 compared to control samples and other bladder conditions studied. IL-6 levels were also significantly higher in UTI samples versus IC samples, though in most samples, IL-6 concentrations were below the lower level of detection of the assay (3.2 pg/ml). All samples were adjusted for creatinine.

DISCUSSION

In this study, we sought to determine if urinary GRO, IL-6, IL-8, and MCP-1 were increased in patients with bladder disease in comparison to healthy control samples, and if there was a distinction in their urinary levels between different bladder conditions (nonulcerative IC, OABw, UTI, and bladder cancer). These four cytokines were specifically chosen as our group has previously identified these cytokines as potential biomarkers for the diagnosis of IC.^[6,12]

Chemokines are divided into four families (CXC, CC, C, and CX3C) based on the relative position and the number of conserved N-terminal cysteine residues as well as the absence (CC) or presence of intervening amino acid(s) between

Table 2: Clinical interstitial cystitis/BPS and control demographics

	Control	IC	OABw	UTI	Bladder cancer
Number of participants, <i>n</i> (%)	31	83	84	18	61
Male	15 (48.4)	7 (8.4)	12 (14.3)	4 (22.2)	42 (68.9)
Female	16 (51.6)	76 (91.6)	72 (85.7)	14 (77.8)	19 (31.1)
Age (range)	53.7±18.5 (27-80)	57.1±14.8 (26-91)	62.3±17.1 (26-95)	69.3±12.8 (49-89)	73.4±10.9 (29-93)
VAS	1.01±0.24	6.00±2.74	2.25±2.14	3.47±3.01	1.27±0.84

Values reported as mean±SD. IC=Interstitial cystitis; SD=Standard deviation; VAS=Visual Analog Scale; OABw=Overactive bladder wet; UTI=Urinary tract infection; BPS=Bladder pain syndrome

Table 3: Concentration of urine inflammatory cytokines in study groups

	Control	IC	OABw	UTI	Bladder cancer
Number of participants	31	83	84	18	61
GRO* (pg/mL)	20.94 (26.76)	52.67 (85.34)	61.31 (137.4)	276.2 (253.7)	95.91 (139.3)
IL-6 [†] (pg/mL)	0.52 (0.65)	1.16 (3.36)	0.76 (1.38)	28.91 (103.3)	3.89 (12.86)
IL-8* (pg/mL)	15.75 (28.32)	31.27 (105.9)	21.64 (63.09)	309.3 (512.9)	102.4 (244.9)
MCP-1 [‡] (pg/mL)	340.3 (287.6)	396.2 (468.6)	476.9 (576.1)	612.0 (555.4)	502.0 (614.6)

*Significant differences are summarized in Figure 2; [†]UTI significantly different from IC group $P=0.0442$; [‡]No statistical differences found between groups; Values reported in pg/mL±SD; Multiplex Luminex assay detection range for all analytes is 3.2 – 10,000 pg/ml. IC=Interstitial cystitis; SD=Standard deviation; OABw=Overactive bladder wet; UTI=Urinary tract infection; IL=Interleukin; GRO=Growth-regulated protein alpha CXCL1

the cysteine residues (CXC). The CC class of chemokines, such as MCP-1, provokes mast cell activation and has chemotactic activity for monocytes but not for neutrophils.^[13] Bacterial infection is reported to induce higher levels of chemokines from the CXC family versus the CC family.^[14]

Previous *in vitro* studies using detrusor muscle cells have shown that low levels of chemokines and cytokines are secreted in a healthy bladder state. It has been further demonstrated that basal levels of cytokines and chemokines are responsible for the autocrine, paracrine, and endocrine signals in the detrusor, and that these chemokines are produced at higher levels during inflammation.^[13,15] In the urine samples of asymptomatic control study participants, we have detected low levels of GRO, IL-8, and MCP-1, suggesting that these cytokine levels reflect the amount needed to maintain basal immune surveillance [Table 3].

When comparing each of the cytokines in disease versus healthy state, an increase in cytokines appears to be a common characteristic among different bladder conditions. GRO (CXCL1) specifically is elevated in the urine of patients with IC, OABw, UTI, and bladder cancer. Preclinical studies have shown that increased urine levels of Th2-polarizing factor MCP-1 and GRO accompany bladder inflammation.^[16] GRO is a chemokine that acts as a chemoattractant, hereby playing an important role in inflammation and attracting neutrophils to the site of infection. Neutrophils subsequently produce cytokines and inflammatory factors themselves that help regulate the immune response and aid in the resolution of inflammation.^[17] Initially, it was thought that the role of neutrophils was limited to an antimicrobial function, but this role has been widely expanded.^[17] The elevated levels of GRO in bladder conditions combined suggest that neutrophils may play a role in each of these bladder conditions.

When analyzing the bladder conditions separately, GRO was elevated significantly in urine samples of UTI and OABw patients, though GRO levels for OABw were significantly lower than those seen in UTI. Bacterial infection is reported to induce higher levels of chemokines from the CXC family (e.g., GRO) versus the CC (e.g., MCP-1) family, which we also observe in this study.^[14] The presence of increased levels of neutrophil-specific chemokine GRO in urine from UTI and OABw patients, therefore, suggests an infiltration of neutrophils in the bladder tissue. Given the role of neutrophils in fighting bacterial infections, the high levels of GRO in UTI are not surprising. A recent study identified that the neutrophil-to-lymphocyte ratio is increased in the serum of patients with OAB.^[18] Thus, the increased levels of GRO in OABw could indicate that neutrophils play an important role in mediating OAB symptoms. The finding that UTI and OABw have different cytokine profiles suggests that different inflammatory pathways are activated following UTI versus OAB. The quantitative differences in the elevation of specific chemokines in UTI and OAB may assist in differentiating two disease categories.

Like GRO, IL-8 levels are significantly higher in patients with UTI versus control, nonulcerative IC, OABw, and bladder cancer [Table 3 and Figure 2]. Elevated seminal plasma levels of IL-8 have been linked to the symptoms of chronic prostatitis/chronic pelvic pain syndrome and nonspecific urethritis.^[19] IL-8 plays an important role in bladder inflammation and is secreted by infiltrating immune cells and resident immune cells present in both the urothelium and detrusor.^[13,15,20] Elevated levels of IL-8 have also been reported in bladder cancer, where neoplastic cells are capable of secreting IL-8.^[21,22]

A weakness of the study is that the stage of disease was not taken into consideration. As observed in Table 3, the standard

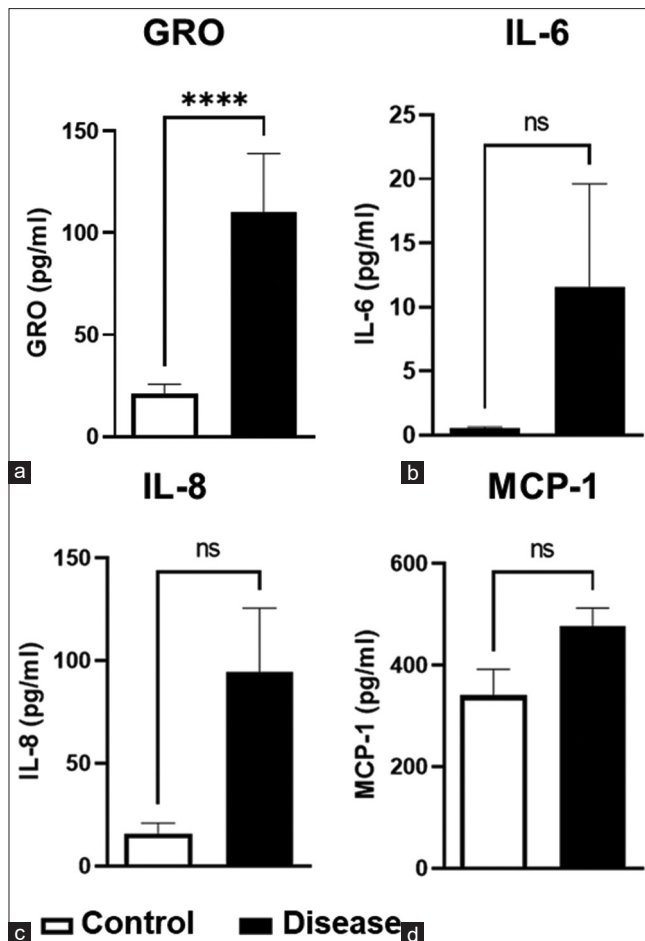


Figure 1: Urinary chemokine GRO is significantly increased in bladder disease versus normal control. Protein concentrations of (a) GRO, (b) interleukin-6, (c) interleukin-8, and (d) MCP-1 were measured in urine samples of patients with bladder disease and healthy controls using multiplex Luminex assay. Statistical analysis was carried out using Mann-Whitney U-test. **** $P < 0.0001$

deviations vary greatly between patients, which could be explained by disease stage. For IC, it was previously shown that IL-6, IL-8, and GRO were increased in IC patients with Hunner's lesions versus those without lesions.^[6] Our IC cohort included urine samples of both types of IC, though the number of patients with Hunner's lesions enrolled ($n = 10$) was too low to draw any conclusions. In addition, there may be differences between patients going through an IC flare-up versus those who are not. In bladder cancer, higher urinary levels of IL-8 are associated with increased risk of recurrence.^[22] However, our analysis did not take stage of bladder cancer or ongoing treatment into consideration.

CONCLUSION

Urinary concentrations of GRO were higher in disease state compared to control. Specifically, levels of GRO and IL-8 were higher in urine samples from patients with UTI compared to controls and other bladder conditions. These findings provide preliminary data for further studies. Comparing and contrasting urinary cytokines may help improve our understanding of these important bladder diseases with great unmet needs.

Acknowledgments

The authors gratefully acknowledge the IC community that contributed to this study and the Interstitial Cystitis Association (ICA; <http://www.ichelp.org/>) for assistance with participant recruitment and supporting IC research.

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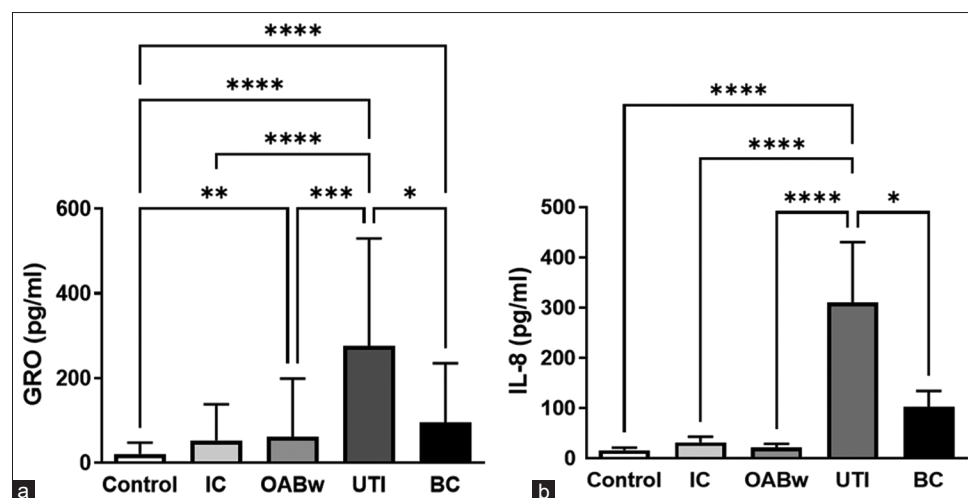


Figure 2: Urinary tract infections result in increased cytokines that are distinct from other bladder conditions. Protein concentrations of (a) GRO and (b) interleukin-8 were measured in urine samples of healthy controls and patients with IC, OABw, UTI, or bladder cancer using multiplex Luminex assay. Statistical analysis was carried out using Kruskal-Wallis nonparametric test followed by Dunn's multiple comparison test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$

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Conflicts of interest

LEL, JJ, and MBC have intellectual property associated with methods for diagnosing IC. All other authors declare no competing interests.

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Original Research

Comparing Concentration of Urinary Inflammatory Cytokines in Interstitial Cystitis, Overactive Bladder, Urinary Tract Infection, and Bladder Cancer

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Abstract

Purpose: We sought to determine if urinary cytokine concentration profiles were different between various bladder conditions. **Methods:** Participants at three clinical sites completed a demographics survey and provided a urine sample in a collection cup containing a room-temperature urine preservative. Participants were divided into the following categories based on physician-documented diagnosis: asymptomatic control, nonulcerative interstitial cystitis (IC), overactive bladder with incontinence (OAB wet), urinary tract infection (UTI), and bladder cancer. Urinary cytokines were measured through Luminex multiplex assay. **Results:** Two hundred and seventy-seven urine samples were collected from three clinical sites. Urinary pro-inflammatory cytokines had an increasing trend in bladder disease versus control, with a significant increase for chemokine (C-X-C) ligand 1 growth-regulated protein alpha CXCL1 (GRO). Further analyses demonstrated that patients with UTI had significantly higher levels of GRO and interleukin-8 (IL-8) in comparison to control, nonulcerative IC, OAB wet, and bladder cancer. Both are chemokines that stimulate chemotaxis resulting in the rapid accumulation of immune cells such as neutrophils. IL-6 levels overall were at the lower limit of assay range but were significantly increased in urine of UTI patients versus IC patients. MCP-1 (CCL2) had the least separation among the control group and the various bladder diseases. **Conclusion:** Urinary concentrations of GRO were higher in disease state compared to control. Specifically, levels of GRO and IL-8 were higher in urine samples from patients with UTI compared to controls and other bladder conditions. Comparing and contrasting urinary cytokines may help improve our understanding of these important bladder diseases with great unmet needs.

Keywords: Bladder cancer, interstitial cystitis, overactive bladder, urinary tract infection

INTRODUCTION

Interstitial cystitis (IC)/bladder pain syndrome (BPS) is a debilitating chronic disease that affects roughly 3–8 million women and 1–4 million men in the United States.^[1,2] Patients

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with IC/BPS are identified based on the presence of Hunner's lesions, though only an estimated 10% of IC/BPS cases present with these bladder lesions.^[3] Diagnosis of IC/BPS is based on patient-reported symptoms and exclusion of other diseases with overlapping clinical presentation, such as overactive bladder with incontinence (OAB wet), urinary tract infection (UTI), and bladder cancer. Bladder cancer and IC patients, especially those IC with Hunner's lesion (HIC), are most often diagnosed by cystoscopy with hydrodistension.^[4] However, this can be painful and requires anesthesia. Some patients may experience painful urination, hematuria, UTI, and rare risk of bladder wall perforation. Identifying these patients presents a therapeutic opportunity as bladder-directed therapies such as electrocautery, resection, or injection of these lesions with steroids can provide temporary symptom relief. Given the invasiveness and the side effects of cystoscopy with hydrodistension, there is a strong need for a noninvasive, rapid, diagnostic test to easily identify and diagnose bladder diseases, and to measure patients' response to therapy.

Urine cytokines have been of high interest as potential biomarkers for IC, and there is a strong suggestion that urinary cytokines are elevated with IC.^[5] A valid biomarker for diseases with similar symptomatology such as IC, bladder cancer, UTI, or OAB wet would be advantageous for both clinicians and patients. Our research group has previously identified three urinary cytokines, interleukin-6 (IL-6), IL-8, and GRO, to be elevated in IC/BPS patients with Hunner's lesions in comparison to patients without Hunner's lesions or healthy controls.^[6] In addition, IL-8 and IL-6 were found to be significantly higher in urine from nonulcerative IC/BPS patients versus healthy controls.^[6] However, bladder inflammation is also common in other bladder conditions. In a study by Kumari *et al.*, 12 urinary cytokines, including IL-8, were identified to be more abundant in patients with bladder cancer versus healthy controls.^[7] Likewise, increased urinary cytokines have been identified during active UTI and in patients with OAB.^[8-10] Thus, we saw a need for a study to directly compare urinary cytokines in several confusable bladder conditions.

The objective of this study was to compare the levels of four urinary cytokines that we previously identified as being elevated in IC/BPS to determine if they can be used to differentiate different types of bladder diseases.^[6] We collected urine samples from patients with clinically diagnosed nonulcerative IC/BPS, bladder cancer, OAB wet, or UTI, and healthy controls. We sought to understand if there were differences in urinary cytokine concentration between these conditions.

METHODS

Study population

Urine samples were collected with local Internal Review Board approval from three academic urology centers from different regions of the United States, Montefiore Medical Center Albert Einstein College of Medicine in Bronx, New York (IRB #2019-10949), Baylor College of

Medicine/Michael E. DeBakey VA Medical Center in Houston, Texas (IRB #H-49346), and Beaumont Health, Royal Oak, Michigan (IRB #2019-266). This study was also approved by the Department of Defense Human Resource Protect Office (E00749) and aimed at enrolling participants with IC and without IC including asymptomatic/normal controls, bladder cancer, current UTI, and OAB wet (OABw) patients. All participants had confirmed physician diagnosis for nonulcerative IC, bladder cancer, UTI, and/or OAB wet. Urine collection was carried out by trained clinical research staff. A urine preservative (Norgen Biotek, Thorold, ON, Canada) was added to all urine samples immediately after collection to allow for storage of urine samples at room temperature up to 1 year. Urine samples and corresponding surveys were deidentified and marked with a unique study identification number before shipping to Beaumont through FedEx.

Patients meeting study inclusion and exclusion criteria as described in Table 1 were recruited by the study physicians at their respective clinic. Candidates with IC were given the diagnosis based on NIDDK criteria and satisfied the inclusion and exclusion criteria. All physicians involved in this study are fellowship trained and have years of experience evaluating women with IC. Patients with OABw, UTI, and bladder cancer were recruited in the clinic if they met all inclusion and exclusion criteria [Table 1].

VISUAL ANALOG SCALE

A 10-point VAS for maximum daily pain was utilized where 0 is no pain and 10 is maximum pain. Extensive research supports the validity of the VAS for assessment of pain intensity.^[11]

Multiplex Luminex assay

Samples were aliquoted and stored at room temperature and ten 1.0 mL aliquots were stored at -80°C . Samples were randomized and assigned an experimental ID number to run and analyze samples in a blinded fashion. Expression of a panel of cytokines (GRO, IL-6, IL-8, and MCP-1) was determined using the MILLIPLEX MAP Human Cytokine/Chemokine Multiplex Immunoassay (Millipore) following manufacturer's protocol and as previously described.^[6]

Statistical analysis

Two hundred and seventy-seven urine samples were collected from three clinical sites. To compare cytokine levels between control and disease groups, a two-tailed nonparametric independent *t*-test was performed using Mann-Whitney U-test. To determine statistical difference between control and different disease states, we performed a Kruskal-Wallis nonparametric test followed by Dunn's multiple comparison test. $P < 0.05$ was considered statistically significant.

RESULTS

Patient population

Patient demographics among the groups are listed in Table 2. Among the three sites, a total of 277 urine samples were

Table 1: Inclusion and exclusion criteria

Interstitial cystitis
Inclusion criteria
Male or female at least 18 years of age
IC/BPS diagnosed by a health-care provider based on the following criteria
Complaint of suprapubic pain related to bladder filling, accompanied by other symptoms such as increased daytime and nighttime frequency, in the absence of proven urinary infection or other obvious pathologies
Have had IC symptoms for at least 6 months
Urinary frequency >10 times a day
Have IC that in investigator's judgment has been stable in the previous 30 days
IC-related pain defined as a score of >3 and <9 on the pain VAS
Exclusion criteria
Prior intravesical therapy or bladder hydrodistention within 30 days
Participated in IC research trial within 90 days prior
Previous augmentation cystoplasty, cystectomy, neurectomy, or bladder botulinum toxin injections
Sacral and/or tibial nerve neuromodulation within the last 6 months
Any condition that in the judgment of the investigator would interfere with the patient's ability to provide informed consent, comply with study instructions, place the patient at increased risk, or might confound the interpretation of the study results
Urinary tract or prostatic infection in the past 90 days before study entry
Active genital herpes or vaginitis
Urethral diverticulum
Pelvic malignancy within the past 5 years
History of cyclophosphamide or chemical cystitis, tuberculosis, or pelvic radiation
Urinary tract infection
InCr
Male or female at least 18 years of age
Symptomatic bacteria, positive urine culture with >10 ⁵ colony-forming units/mL
ExCr
History of IC, OAB, or bladder cancer
Urine culture with no growth in 48 h
Overactive bladder wet
InCr
Male or female at least 18 years of age ≥6-month history of symptoms of OAB including frequency, urgency, and urgency or mixed urinary incontinence
ExCr
History of IC, UTI, or bladder cancer
History of pelvic or bladder disease, for example, uterine prolapse, malignancy, prior surgery, or treatment with botulinum toxin
Diabetes insipidus or poorly controlled Type 1 or Type 2 diabetes mellitus
Bladder cancer
InCr
Male or female at least 18 years of age
Pathologic diagnosis of any stage tumor grade of urothelial carcinoma of the bladder from biopsy or resection specimens at the time of urine collection
ExCr
History of IC, OAB, or UTI
History of other pelvic or bladder diseases, for example, uterine prolapse and prostate cancer.

Contd...

Table 1: Contd...

Normal control
InCr
Male or female at least 18 years of age
ExCr
Urologic symptoms including pain or incontinence
History of urologic cancer, infection, or stone disease

InCr=Inclusion criteria; ExCr=Exclusion criteria; IC: Interstitial cystitis; VAS=Visual Analog Scale; OAB=Overactive bladder; UTI=Urinary tract infection; BPS=Bladder pain syndrome

collected: 31 urine samples were collected of healthy controls and 246 of patients with OAB wet ($n = 84$), nonulcerative IC/BPS ($n = 83$), bladder cancer ($n = 61$), and UTI ($n = 18$). For all conditions, more women were enrolled in the study, except for bladder cancer. The age ranges of study participants were similar among the five groups, though the average age for bladder cancer was the highest followed by UTI (73.4 and 69.3, respectively). The VAS score indicated that the patients with IC/BPS and UTI reported higher pain scores than control, OAB wet, and bladder cancer groups.

Increase in cytokine levels in bladder disease

Urinary cytokine levels were measured through Luminex multiplex assay. Urinary concentrations of IL-6, IL-8, MCP-1, and GRO were measured. Initially, cytokine levels were compared between control and disease treatment groups [Figure 1]. Of the four cytokines investigated, all cytokines were higher in disease state versus control. However, only the increase in GRO (CXCL1) was statistically significant. We further divided the disease states into nonulcerative IC, OABw, UTI, and bladder cancer to identify potential disease-specific changes in cytokine levels [Table 3 and Figure 2]. Overall, average cytokine levels had a higher trend in all four diseases states studied, but only urine samples from patients with UTI had significantly higher levels of GRO and IL-8 compared to control samples and other bladder conditions studied. IL-6 levels were also significantly higher in UTI samples versus IC samples, though in most samples, IL-6 concentrations were below the lower level of detection of the assay (3.2 pg/ml). All samples were adjusted for creatinine.

DISCUSSION

In this study, we sought to determine if urinary GRO, IL-6, IL-8, and MCP-1 were increased in patients with bladder disease in comparison to healthy control samples, and if there was a distinction in their urinary levels between different bladder conditions (nonulcerative IC, OABw, UTI, and bladder cancer). These four cytokines were specifically chosen as our group has previously identified these cytokines as potential biomarkers for the diagnosis of IC.^[6,12]

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	Control	IC	OABw	UTI	Bladder cancer
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Female	16 (51.6)	76 (91.6)	72 (85.7)	14 (77.8)	19 (31.1)
Age (range)	53.7±18.5 (27-80)	57.1±14.8 (26-91)	62.3±17.1 (26-95)	69.3±12.8 (49-89)	73.4±10.9 (29-93)
VAS	1.01±0.24	6.00±2.74	2.25±2.14	3.47±3.01	1.27±0.84

Values reported as mean±SD. IC=Interstitial cystitis; SD=Standard deviation; VAS=Visual Analog Scale; OABw=Overactive bladder wet; UTI=Urinary tract infection; BPS=Bladder pain syndrome

Table 3: Concentration of urine inflammatory cytokines in study groups

	Control	IC	OABw	UTI	Bladder cancer
Number of participants	31	83	84	18	61
GRO* (pg/mL)	20.94 (26.76)	52.67 (85.34)	61.31 (137.4)	276.2 (253.7)	95.91 (139.3)
IL-6 [†] (pg/mL)	0.52 (0.65)	1.16 (3.36)	0.76 (1.38)	28.91 (103.3)	3.89 (12.86)
IL-8* (pg/mL)	15.75 (28.32)	31.27 (105.9)	21.64 (63.09)	309.3 (512.9)	102.4 (244.9)
MCP-1 [‡] (pg/mL)	340.3 (287.6)	396.2 (468.6)	476.9 (576.1)	612.0 (555.4)	502.0 (614.6)

*Significant differences are summarized in Figure 2; [†]UTI significantly different from IC group $P=0.0442$; [‡]No statistical differences found between groups; Values reported in pg/mL±SD; Multiplex Luminex assay detection range for all analytes is 3.2 – 10,000 pg/ml. IC=Interstitial cystitis; SD=Standard deviation; OABw=Overactive bladder wet; UTI=Urinary tract infection; IL=Interleukin; GRO=Growth-regulated protein alpha CXCL1

the cysteine residues (CXC). The CC class of chemokines, such as MCP-1, provokes mast cell activation and has chemotactic activity for monocytes but not for neutrophils.^[13] Bacterial infection is reported to induce higher levels of chemokines from the CXC family versus the CC family.^[14]

Previous *in vitro* studies using detrusor muscle cells have shown that low levels of chemokines and cytokines are secreted in a healthy bladder state. It has been further demonstrated that basal levels of cytokines and chemokines are responsible for the autocrine, paracrine, and endocrine signals in the detrusor, and that these chemokines are produced at higher levels during inflammation.^[13,15] In the urine samples of asymptomatic control study participants, we have detected low levels of GRO, IL-8, and MCP-1, suggesting that these cytokine levels reflect the amount needed to maintain basal immune surveillance [Table 3].

When comparing each of the cytokines in disease versus healthy state, an increase in cytokines appears to be a common characteristic among different bladder conditions. GRO (CXCL1) specifically is elevated in the urine of patients with IC, OABw, UTI, and bladder cancer. Preclinical studies have shown that increased urine levels of Th2-polarizing factor MCP-1 and GRO accompany bladder inflammation.^[16] GRO is a chemokine that acts as a chemoattractant, hereby playing an important role in inflammation and attracting neutrophils to the site of infection. Neutrophils subsequently produce cytokines and inflammatory factors themselves that help regulate the immune response and aid in the resolution of inflammation.^[17] Initially, it was thought that the role of neutrophils was limited to an antimicrobial function, but this role has been widely expanded.^[17] The elevated levels of GRO in bladder conditions combined suggest that neutrophils may play a role in each of these bladder conditions.

When analyzing the bladder conditions separately, GRO was elevated significantly in urine samples of UTI and OABw patients, though GRO levels for OABw were significantly lower than those seen in UTI. Bacterial infection is reported to induce higher levels of chemokines from the CXC family (e.g., GRO) versus the CC (e.g., MCP-1) family, which we also observe in this study.^[14] The presence of increased levels of neutrophil-specific chemokine GRO in urine from UTI and OABw patients, therefore, suggests an infiltration of neutrophils in the bladder tissue. Given the role of neutrophils in fighting bacterial infections, the high levels of GRO in UTI are not surprising. A recent study identified that the neutrophil-to-lymphocyte ratio is increased in the serum of patients with OAB.^[18] Thus, the increased levels of GRO in OABw could indicate that neutrophils play an important role in mediating OAB symptoms. The finding that UTI and OABw have different cytokine profiles suggests that different inflammatory pathways are activated following UTI versus OAB. The quantitative differences in the elevation of specific chemokines in UTI and OAB may assist in differentiating two disease categories.

Like GRO, IL-8 levels are significantly higher in patients with UTI versus control, nonulcerative IC, OABw, and bladder cancer [Table 3 and Figure 2]. Elevated seminal plasma levels of IL-8 have been linked to the symptoms of chronic prostatitis/chronic pelvic pain syndrome and nonspecific urethritis.^[19] IL-8 plays an important role in bladder inflammation and is secreted by infiltrating immune cells and resident immune cells present in both the urothelium and detrusor.^[13,15,20] Elevated levels of IL-8 have also been reported in bladder cancer, where neoplastic cells are capable of secreting IL-8.^[21,22]

A weakness of the study is that the stage of disease was not taken into consideration. As observed in Table 3, the standard

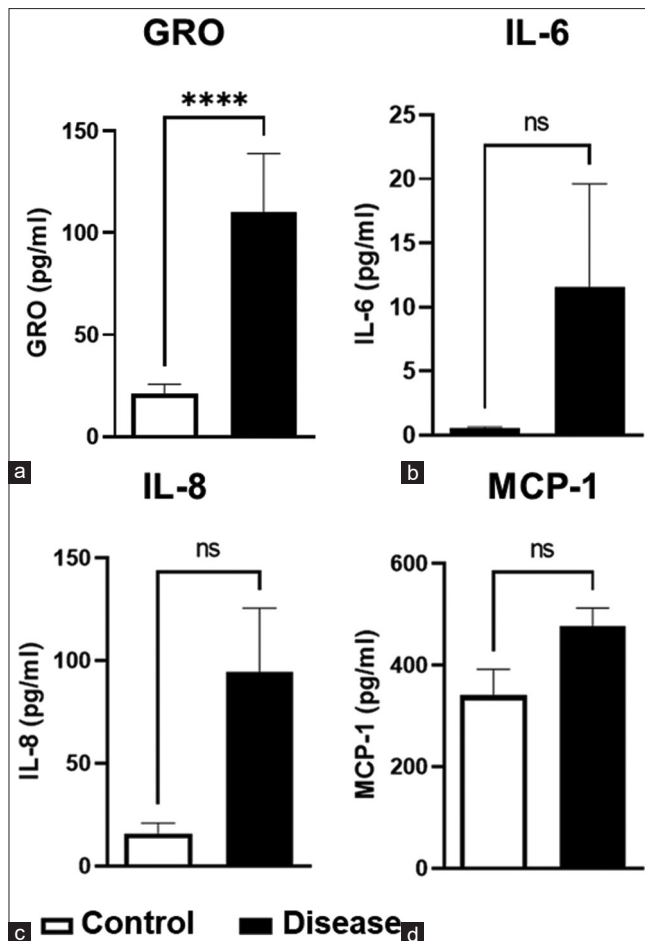


Figure 1: Urinary chemokine GRO is significantly increased in bladder disease versus normal control. Protein concentrations of (a) GRO, (b) interleukin-6, (c) interleukin-8, and (d) MCP-1 were measured in urine samples of patients with bladder disease and healthy controls using multiplex Luminex assay. Statistical analysis was carried out using Mann-Whitney U-test. **** $P < 0.0001$

deviations vary greatly between patients, which could be explained by disease stage. For IC, it was previously shown that IL-6, IL-8, and GRO were increased in IC patients with Hunner's lesions versus those without lesions.^[6] Our IC cohort included urine samples of both types of IC, though the number of patients with Hunner's lesions enrolled ($n = 10$) was too low to draw any conclusions. In addition, there may be differences between patients going through an IC flare-up versus those who are not. In bladder cancer, higher urinary levels of IL-8 are associated with increased risk of recurrence.^[22] However, our analysis did not take stage of bladder cancer or ongoing treatment into consideration.

CONCLUSION

Urinary concentrations of GRO were higher in disease state compared to control. Specifically, levels of GRO and IL-8 were higher in urine samples from patients with UTI compared to controls and other bladder conditions. These findings provide preliminary data for further studies. Comparing and contrasting urinary cytokines may help improve our understanding of these important bladder diseases with great unmet needs.

Acknowledgments

The authors gratefully acknowledge the IC community that contributed to this study and the Interstitial Cystitis Association (ICA; <http://www.ichelp.org/>) for assistance with participant recruitment and supporting IC research.

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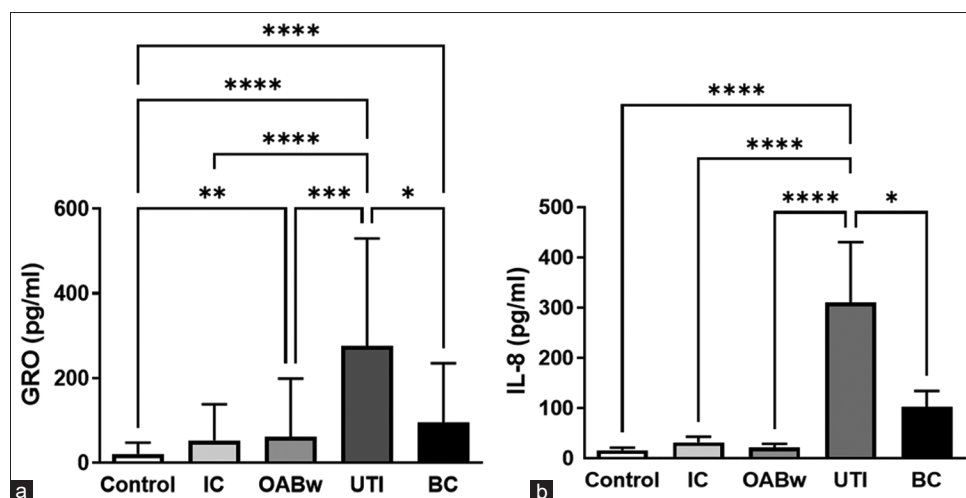


Figure 2: Urinary tract infections result in increased cytokines that are distinct from other bladder conditions. Protein concentrations of (a) GRO and (b) interleukin-8 were measured in urine samples of healthy controls and patients with IC, OABw, UTI, or bladder cancer using multiplex Luminex assay. Statistical analysis was carried out using Kruskal-Wallis nonparametric test followed by Dunn's multiple comparison test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$

of Defense. This work was also supported by Oakland University William Beaumont School of Medicine's EMBARK capstone program. The American Urological Association also supported this work through 2021 Summer Medical Student Fellowship Award.

Conflicts of interest

LEL, JJ, and MBC have intellectual property associated with methods for diagnosing IC. All other authors declare no competing interests.

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INTRODUCTION AND OBJECTIVE: Interstitial Cystitis (IC) has significant unmet clinical need, including the lack of an objective diagnostic laboratory test, such as a urine-based biomarker. We have previously reported our initial development of a machine-learning-based diagnostic score using 4 urinary cytokines: GRO, IL-6, IL-8, & MCP-1. As part of our development of a diagnostic tool for IC, we collected urine samples from confusable diseases, such as Overactive Bladder (OAB), Urinary Tract Infection (UTI), and Bladder Cancer. We sought to understand if there were differences in urinary cytokine concentration between diseases.

METHODS: Participants at two clinical sites completed a demographics survey, a bladder survey, and provided a urine sample in a collection cup containing a room-temperature urine preservative. Participants were divided into the following categories based on physician-documented diagnosis: control, IC, OAB with incontinence), UTI, and Bladder Cancer. Urinary cytokine concentrations were measured via Luminex multiplex assay.

RESULTS: 266 urine samples were collected from both clinical sites. ANOVA with multiple comparisons test revealed no significant difference in urinary cytokine concentration between the 5 groups.

CONCLUSIONS: Our study demonstrated urinary proinflammatory cytokines are elevated in IC, OAB, UTI and bladder cancer versus normal control. The UTI group has the highest concentration of GRO (CXCL1), a chemokine that acts as a chemoattractant, especially neutrophils to the site of infection. The bladder cancer group had the highest concentration of IL-8, which plays an important role in inflammation and has also been implicated in bladder cancer via secretion by neoplastic cells. MCP-1 (CCL2) had the least separation among the control group and the various bladder diseases. Comparing and contrasting urinary cytokines may help improve our understanding these important bladder diseases with great unmet needs.

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Table 1. Concentration of urine inflammatory cytokines in study groups

Sample Type	N	GRO (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	MCP-1 (pg/mL)
Control	16	16.7 ± 12.8	0.5 ± 0.7	11.3 ± 14.5	348.9 ± 282.1
IC	80	76.6 ± 133.3	2.7 ± 9.9	58.9 ± 172.0	420.7 ± 442.3
OABw	89	69.9 ± 140.5	1.4 ± 3.8	39.3 ± 98.1	505.5 ± 586.0
UTI	18	143.9 ± 208.1	0.6 ± 1.0	56.0 ± 106.7	567.8 ± 637.1
Cancer	63	74.4 ± 106.5	2 ± 7.3	77.5 ± 208.3	425.1 ± 570.4

A Cost and Time Analysis of Participant Recruitment and Urine Sample Collection through Social Media Optimization

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INTRODUCTION AND OBJECTIVE: Clinical research study can be expensive and time consuming due to high cost and/or long duration of a study. We hypothesized that research studies using online recruitment and engaging patients via social medial channels have the potential to reach a large population in a small timeframe, at a reasonable cost. This is an especially appropriate adaptation during COVID-19, with limited hospital and clinic access. To our knowledge, no prior study has performed a detailed cost analysis of the use of online recruitment of clinical sample collection. We sought to address this knowledge gap by comparing cost and efficiency of the recruitment of urine samples online compared to recruitment of urine samples at clinical research sites.

METHODS: We performed a retrospective cost analysis of a cohort study comparing cost per sample and time per sample for both online and clinically recruited participants. The retrospective review was conducted from August 2020 to September 2021. During this time, cost data was collected based on patient surveys, urine sample analyses, invoices, and budget spreadsheets. The data was subsequently subjected to statistical analysis. This study was performed with full institutional IRB approval.

RESULTS: Each sample collection kit contained 3 urine cups, 1 for the IC sample and 2 for control samples. Out of the 3576 (1192 IC + 2384 control) total sample cups mailed, 1,211 (677 control) samples were returned, with a return rate of 44.8% for IC samples and 28.4% for controls. Comparatively, the two clinical sites collected 305 samples in the same period. Men and women of all age groups (18+) and ethnicities enrolled in the study, representing all 50 states. Although the initial startup cost of online recruitment was higher, cost per sample for online recruited was found to be \$147.06 compared to \$398.14 for clinic sample.

CONCLUSIONS: We conducted a nationwide, contactless, urine sample drive through online participant recruitment, in the midst of the COVID-19 pandemic. Results were compared with the samples collected in traditional clinical setting. Online patient recruitment can be utilized to collect urine sample rapidly, efficiently and at a cost per sample that was 37% of in an in-person clinic and without risk of COVID-19 exposure.

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	Online Recruited (\$)	Clinic Recruited (\$)
Total Samples	1211	305
Total Cost	178,084.62	121,432.00
Cost Per Sample	147.06	398.14
Supplies	30,864.17	409.58
Salary & Wages	93,455.92	96,025.56
Fringe Benefits	26,166.83	24,996.84
Web Development	196.70	0
Google Ads	2,325	0
Social Media Promotion	6,500	0
Shipping	18,576	180



Figure: Study cost comparison and demographics data. A) Total and cost per sample comparison between online recruited against clinic recruited urine samples. B) Geographical distribution of urine samples received from 50 states.

Conducting Urology Research during COVID-19: Social Media-Based Crowdsourcing of Urine Samples for Biomarker Development

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INTRODUCTION AND OBJECTIVE: Conducting research during the COVID-19 pandemic remains a challenge for the entire urologic community. We hereby report our effort to use social media-based crowdsourcing methodology to recruit participants from the entire United States, including Alaska and Hawaii. We developed a novel research model that can engage multiple stakeholders and allows for fast and broad participation for urine biomarker development towards a diagnostic tool for Interstitial Cystitis (IC).

METHODS: We partnered with a patient advocacy group, the Interstitial Cystitis Association (ICA), to aid in recruitment efforts. A website with study information and links to the HIPAA-compliant enrollment questionnaire was created. The study was advertised through Google, and Beaumont's and ICA's social media using Twitter, emails, YouTube videos, Facebook posts, websites, and TikTok. Enrolled participants were shipped at-home collection kits containing urine cups with preservative, a prepaid return mailing envelope, instructions, and survey materials.

RESULTS: National public participation was strong, and enrollment was closed within three months. Men and women of all age groups and ethnicities enrolled in the study, representing all 50 United States. Currently, 1,211 crowdsourced samples have been returned, including 677 asymptomatic control samples. Surprisingly, most participants accessed the website via mobile devices, as opposed to via desktop computer or tablet. Potential participants were referred to the website primarily by direct links (i.e. email links), with other referring sites being Facebook, Google Advertisements, the ICA's website, and Beaumont Hospital's website.

CONCLUSIONS: Following COVID-19 safety guidelines, we conducted a nationwide crowdsourced collection of urine samples. Social media and web-based recruitment tools were used to recruit participants. Having a website user-friendly on mobile devices was essential. Nontraditional recruitment methods, such as TikTok videos, encouraged study participation. Utilizing various social media platforms, it is possible to crowdsourcing urine samples from across the United States quickly, economically, and most importantly during the COVID-19 pandemic, safely.

Source of Funding: This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs through the Technology/Therapeutic Development Research Program under Award No. W81XWH-19-1-0288. The opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the Department of Defense.

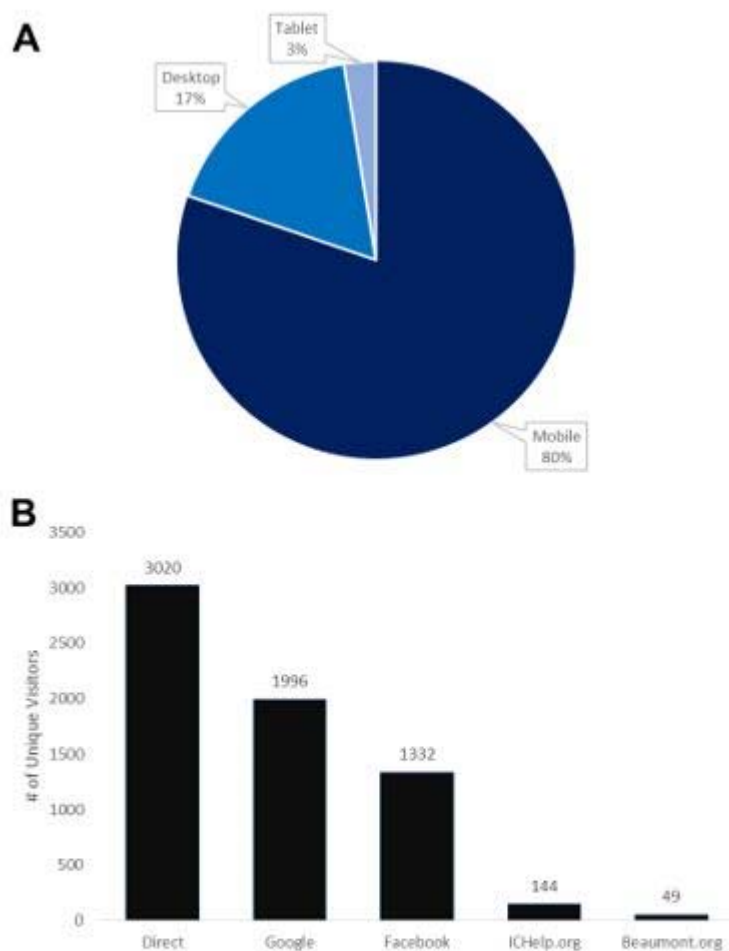


Figure 1. Study Participant recruitment by device type and referral method. A) Devices used by study participants to register for study. **B)** Number of participants registered through study website referral by direct link, Google advertising, Facebook posts, ICA website, or study institution website.