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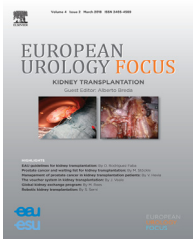

From Lab to Clinic

Urine Gene Expression Profiles in Bladder Pain Syndrome Patients Treated with Triamcinolone

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Abstract

Background: The pathogenesis of bladder pain syndrome (BPS) remains incompletely defined, and there is no standard treatment for BPS as yet.
Objective: To gain detailed insight into the disease pathobiology of BPS through comparative gene expression analysis of urine from BPS patients versus control individuals and, furthermore, to determine the efficacy of triamcinolone treatment in BPS patients in terms of the gene expression profiles in urine.
Design, setting, and participants: A prospective pilot study including 21 urine samples from patients with Hunner's lesions ($n = 6$) and controls ($n = 9$) between January and August 2017.
Intervention: Triamcinolone treatment of BPS patients.
Outcome measurements and statistical analysis: Urine samples from BPS patients were collected before (pretreatment group) and 2 wk after triamcinolone treatment (post-treatment group). Gene expression of urine sediment was analyzed using RNA sequencing. Pathways and biological processes in which differentially expressed genes are involved were analyzed.
Results and limitations: A total of 3745 genes were found to be differentially expressed between the three groups tested. Gene expression differences between controls and BPS samples (630 differentially expressed genes) were more pronounced than the differences between pre- and post-treatment BPS samples (197 differentially expressed genes). Gen Set Enrichment Analysis showed that differentially expressed genes in BPS patients (pretreatment), compared with controls, were enriched for some functional gene networks associated with several metabolic processes and ribosome biogenesis. The limited number of patients included may not accurately represent the BPS population.
Conclusions: Gene expression profiles of urine sediment are able to discriminate between BPS and control patients. Moreover, we show that triamcinolone induces changes in urine gene expression profiles.
patient summary: In this report, we looked at gene expression profiles of urine sediment from patients with Hunner's lesions, before and after triamcinolone treatment, and control individuals. We found that urine gene expression profiles are able to discriminate Hunner's lesions patients from controls. Furthermore, we report, for the

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first time, that triamcinolone treatment of patients with Hunner's lesions induces changes in bladder gene expression profiles that can be observed in urine samples.

1. Introduction

Chronic pelvic pain syndrome consists of the presence of pain perceived in the pelvic structures, frequently associated with negative cognitive and emotional consequences and symptoms suggestive of urinary, sexual, bowel, or pelvic floor dysfunction, once infection or other local pathology have been ruled out. When pain is perceived in the bladder, the term bladder pain syndrome (BPS) is used [1]. The prevalence of BPS is variable mainly due to the multiple definitions and diagnostic criteria used, although it may be as high as 30% [2,3], affecting mostly women in any age range [4]. BPS is a debilitating condition, with negative consequences on mental health, quality of life, and social and sexual life patterns of patients [5,6].

The pathogenesis of BPS remains incompletely defined. Several pathophysiological mechanisms such as bladder urothelial dysfunction, epithelial disruption, and mast cell and vascular abnormalities have been proposed. However, evidence of multifactorial etiology is emerging [7,8].

There is no standard treatment for BPS as yet. The therapeutic response to conservative treatments such as dietary changes or intravesical instillation is often brief, relapse is common, and patients may develop refractory symptoms requiring invasive management [9]. It has been suggested that patients who present with Hunner's lesions (HLs) may represent a subgroup with different inflammatory characteristics [10] and with good response to local treatment of the lesion [9].

Triamcinolone is a synthetic corticosteroid used to treat inflammatory pain disorders, and its use in managing BPS is presumed to control inflammatory reaction in the bladder [11]. We recently reported the improvement in bladder pain assessed with a visual analog scale after infiltration of triamcinolone in HLs [11]. However, it is still an empirical and symptomatic treatment, as most patients required retreatment during follow-up. Currently, treatment response to triamcinolone in terms of gene expression has not been described yet. Here, we have applied RNA sequencing (RNA-Seq) techniques to assess gene expression differences in urine samples from BPS patients compared with control individuals in order to gain better insight into disease pathobiology. Moreover, we have determined gene expression changes in urine from patients with BPS and HLs after triamcinolone treatment. Such scopes could contribute to improving BPS diagnosis at earlier stages, assessing therapeutic response, and guiding toward specific treatment.

2. Patients and methods

2.1. Patient population

We conducted a prospective study of selected BPS cases ($n = 6$) treated between January and August 2017 at the Urology Department of the Hospital Clinic of Barcelona (Spain) with corticoid injection in HLs causing pelvic pain. For the diagnosis, all patients had undergone a urine culture to elucidate an infection and a flexible cystoscopy to explore bladder walls. Apart from white light technology, narrow-band imaging technology had been used to better define the presence of HLs. During the procedure, urinary cytology was carried out to rule out carcinoma in situ. Bladder biopsies were taken as needed because of doubt on cystoscopic imaging. Nine urological patients treated at our institution without bladder disease (four with ureteral lithiasis, three with cystocele, one living donor, and one with incisional hernia) were also prospectively included as controls. All patients and controls were females. Institutional Review Board approval and informed consent from all patients and controls were obtained.

2.2. Triamcinolone injection procedure

Corticoid injections were performed as an outpatient procedure under sedation with target-controlled infusion of propofol. The corticoid used was triamcinolone at a dose of 40 mg in 1 ml diluted with 9 ml of 5% sugar solution (CN 606072 EC; Fresenius KABI). Triamcinolone injections were guided by a 16Ch digital flexible cystoscope. A 27-gauge single-use needle was used to deposit the drug into the ulcer. Every ulcer was treated with three to five injections of 1 ml of the solution depending on ulcer extent. All injections were performed by the same experienced urologist.

2.3. Data and sample collection

A complete medical history from all patients was gathered, including previous treatment for pelvic pain and symptoms. All patients underwent an exhaustive physical examination, with pelvic floor muscle palpation (levator ani, internal obturator, sciatic spine, Alcock's canal) via vaginal examination to detect the presence of myofascial pain.

Urine samples from each BPS patient were collected prior to (BPS pretreatment [preT] group) and 2 wk after (BPS post-treatment [postT] group) the corticoid injection procedure. This procedure was performed as an outpatient technique with the patient under sedation with target-controlled infusion of propofol and remifentanil. Urine samples from controls were collected prior to their surgical treatment.

2.4. RNA extraction

Around 50–100 ml of urine was collected from all patients and controls. Urine samples were processed as previously described [12], except that collected urine samples were stored at 4 °C and processed within the next 24 h instead of ice cooled. RNAs from the urinary cell pellets were extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instructions and quantified with NanoDrop 1000. RNA integrity was assessed with an Agilent Bioanalyzer by using Eukaryote Total RNA Nano kit (Agilent Technologies; mean RIN number 4.5, range 2.1–8.9).

2.5. Library preparation and sequencing method

The Ion AmpliSeq Transcriptome Human Gene Expression Kit (Thermo Fisher Scientific) was used for the library preparation. Briefly, cDNA was synthesized from total RNA using the SuperScript VILO cDNA synthesis kit from 10 ng of RNA. Then, cDNA was amplified using the Ion AmpliSeq technology. Finally, after partial digestion of the primer sequence with FUPA reagent, ligation of the barcoded adapters, and purification by

Agencourt AMPure XP Reagent of the amplified cDNA, the library was quantified with Ion Library TaqMan Quantitation Kit (Thermo Fisher Scientific).

An input concentration of eight pooled library copies/ion sphere particles (ISPs) was added to the emulsion polymerase chain reaction master mix, and the emulsion was generated using the Ion Chef Instrument using the Ion PI Hi-Q Chef Kit (Thermo Fisher Scientific). Template-positive ISPs were enriched, and sequencing was undertaken using an Ion PI v3 on the Ion Proton sequencer using the Ion PI Hi-Q Sequencing 200 Kit (Thermo Fisher Scientific).

2.6. Read alignment and differential gene expression analysis

Partek Flow 6.0 (http://www.partek.com/) was used to analyze AmpliSeq transcriptome data. Briefly, primary read alignment for AmpliSeq sequencing data of all samples was performed using the Torrent Mapping Alignment Program. After quantification, features with a minimum of $\leq$1.0 were excluded. Between-sample normalization at gene level was performed using the trimmed mean method followed by quantile normalization. Gene-specific analysis was used to identify the best statistical model for a specific transcript, and then the best model was used to test for differential expression.

The differentially expressed gene set between BPS preT and control samples was entered into the Ingenuity Pathway Analysis (IPA) package to list the associated canonical pathways and diseases. A p value of 0.05 was used as a cut-off to determine significant enrichment of a pathway. Gene Set Enrichment Analysis (GSEA) software was also used to enrich gene sets or groups from these differentially expressed genes.

3. Results

3.1. Characteristics of the dataset

A total of 21 urinary samples from six BPS patients and nine urological patients without bladder disease were included. Median age of the series was 70 yr (26–85) and median time from diagnosis to treatment of BPS patients was 7 yr (3–12). Two of the six patients had a history of depression, and none of them had fibromyalgia. One patient had undergone a pelvic surgery (hysterectomy). At the time of treatment, we observed frequency and nocturia in all six cases, urgency in five, and urge urinary incontinence in one. In all cases, other treatments for pelvic pain had failed before treatment with corticoid injection. Two cases had received treatment with nonsteroidal anti-inflammatory drugs and anticholinergics, two patients with hyaluronic acid instillation, one patient with Botox injection (Allergan Company), and one patient with bladder hydrodistention and Botox injection. Three patients had already received corticoid injections during the course of the disease. All these therapies were performed 6 mo before the present treatment.

3.2. Overall assessment of transcriptomic differences across all three groups tested: BPS preT, BPS postT, and control

Initial exploratory assessment of the RNASeq dataset was performed using principal component analysis (PCA) based on all the genes. Fig. 1A shows separation of urine samples from controls and BPS patients (preT and postT groups). Urine samples from BPS preT and postT patients separated also, but less distinctly and with notable overlap between these two groups.

A total of 3745 genes were found to be differentially expressed (1875 upregulated; 1870 downregulated) across the three groups tested (false discovery rate $<$0.05). Unsupervised clustering of urine samples using these 3745 differentially expressed genes revealed distinct subsets (Fig. 1B). Of note, samples from controls clustered separately from BPS samples (preT and postT). BPS preT and postT samples also clustered separately, although less distinctly, with one of the BPS preT samples clustering together with BPS postT samples and one of the BPS postT samples clustering together with BPS preT samples. Such clustering indicated that differences between controls and BPS samples were more pronounced than differences between BPS preT and postT samples, thus echoing the observations gained from PCA analysis.

To further explore the validity of this notion, comparisons of BPS versus control and BPS preT versus postT were performed (Fig. 2). These analyses revealed large numbers of differentially expressed genes with more pronounced changes between control and BPS groups than between BPS preT and postT groups. These combined observations indicate that BPS patients manifested substantial transcriptomic abnormalities.

3.3. Transcriptomic changes in BPS (preT and postT) and control urine samples

A total of 3475 genes were found to be differentially expressed between BPS preT and control urine samples: 1780 and 1695 genes with, respectively, significantly elevated and reduced expression in BPS preT compared with control urine samples. A much lower number of differentially expressed genes were found between BPS postT and control samples (n = 691): 215 and 476 genes with, respectively, significantly elevated and reduced expression in BPS postT compared with control urine samples (Supplementary Fig. 1 and 2). An overlap gene set that contained genes significantly different from control in both BPS preT and postT urine samples was found (n = 630; 194 upregulated and 436 downregulated).

IPA software showed the correlation between differentially expressed genes in patients with BPS preT and several canonical pathways and the biofunctions in which the genes are involved (Table 1). Additionally, GSEA results showed that this differentially expressed gene set was enriched for some functional gene networks that are clearly associated with several metabolic processes and ribosome biogenesis (Fig. 3).

3.4. Transcriptomic differences between BPS preT and postT urine samples

A total of 197 genes were found to be differentially expressed between BPS preT and postT urine samples: 45 upregulated and 152 downregulated genes in BPS postT compared with BPS preT urine samples (Fig. 2B and Supplementary Fig. 1).

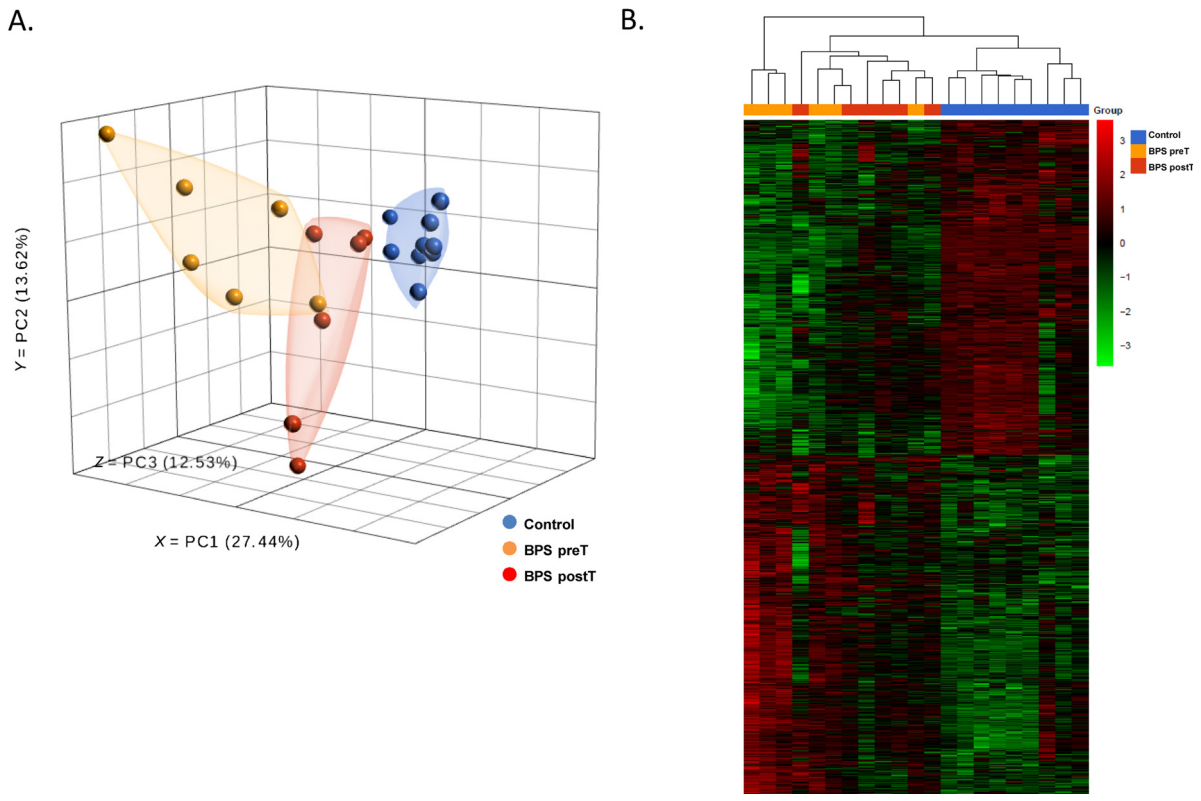

Fig. 1 – Overview of the differential expression of genes between urine samples from BPS patients and controls. (A) Three-dimensional principal component analyses (PCA) of the RNASeq transcriptome dataset for urine BPS samples in this study reveals a clear separation between control and BPS patient samples. The separation of BPS samples from pre- and post-treatment groups is less pronounced, with some overlap present. (B) Unsupervised clustering of log 2-transformed normalized counts of urine samples based on 3745 differentially expressed genes. Note that the control samples clustered separately from BPS samples, whereas one BPS preT sample clustered together with BPS postT samples and one BPS postT sample clustered together with BPS preT samples. BPS = bladder pain syndrome; postT = post-treatment; preT = pretreatment; RNASeq = RNA sequencing.

IPA software showed the correlation between this set of differentially expressed genes and several canonical pathways and the biofunctions in which the genes are involved (Table 2).

4. Discussion

Currently, the tendency in the field of BPS is the search for biomarkers to improve diagnosis, assess the therapeutic

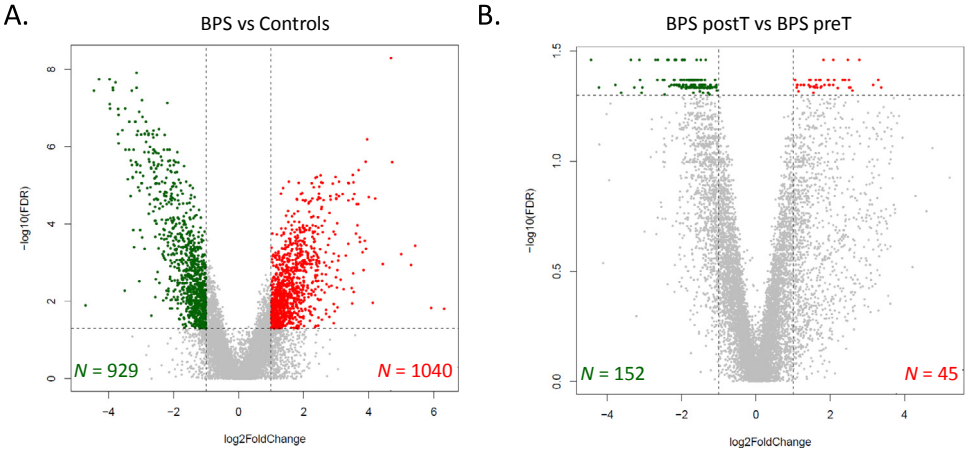

Fig. 2 – Volcano plots of genes in (A) BPS versus control and (B) BPS postT versus preT comparisons. The total number of genes meeting the selection criteria for elevated (red) or reduced (green) expression is indicated in each panel. BPS = bladder pain syndrome; FDR = false discovery rate; postT = post-treatment; preT = pretreatment.

Table 1 – Canonical pathways and biofunctions in which genes differentially expressed between BPS preT patients and controls are involved

Top canonical pathways		
Name	*p* value	Overlap
EIF2 signaling	4.28E-27	48.6% (103/212)
Regulation of eIF4 and p70S6K signaling	2.50E-13	41.6% (64/154)
mTOR signaling	3.37E-10	35.0% (69/197)
Sirtuin signaling pathway	3.50E-09	30.7% (87/283)
Estrogen receptor signaling	4.28E-09	38.3% (49/128)
Top diseases and biofunctions		
Name	*p* value	# Molecules
Infectious diseases	1.60E-03–1.18E-28	535
Cancer	1.94E-03–1.56E-11	3071
Organismal injury and abnormalities	1.94E-03–1.56E-11	3118
Immunological disease	1.88E-03–5.28E-11	655
Gastrointestinal disease	1.87E-03–6.20E-11	2807

6 BPS = bladder pain syndrome; mTOR = mammalian target of rapamycin; preT = pretreatment.

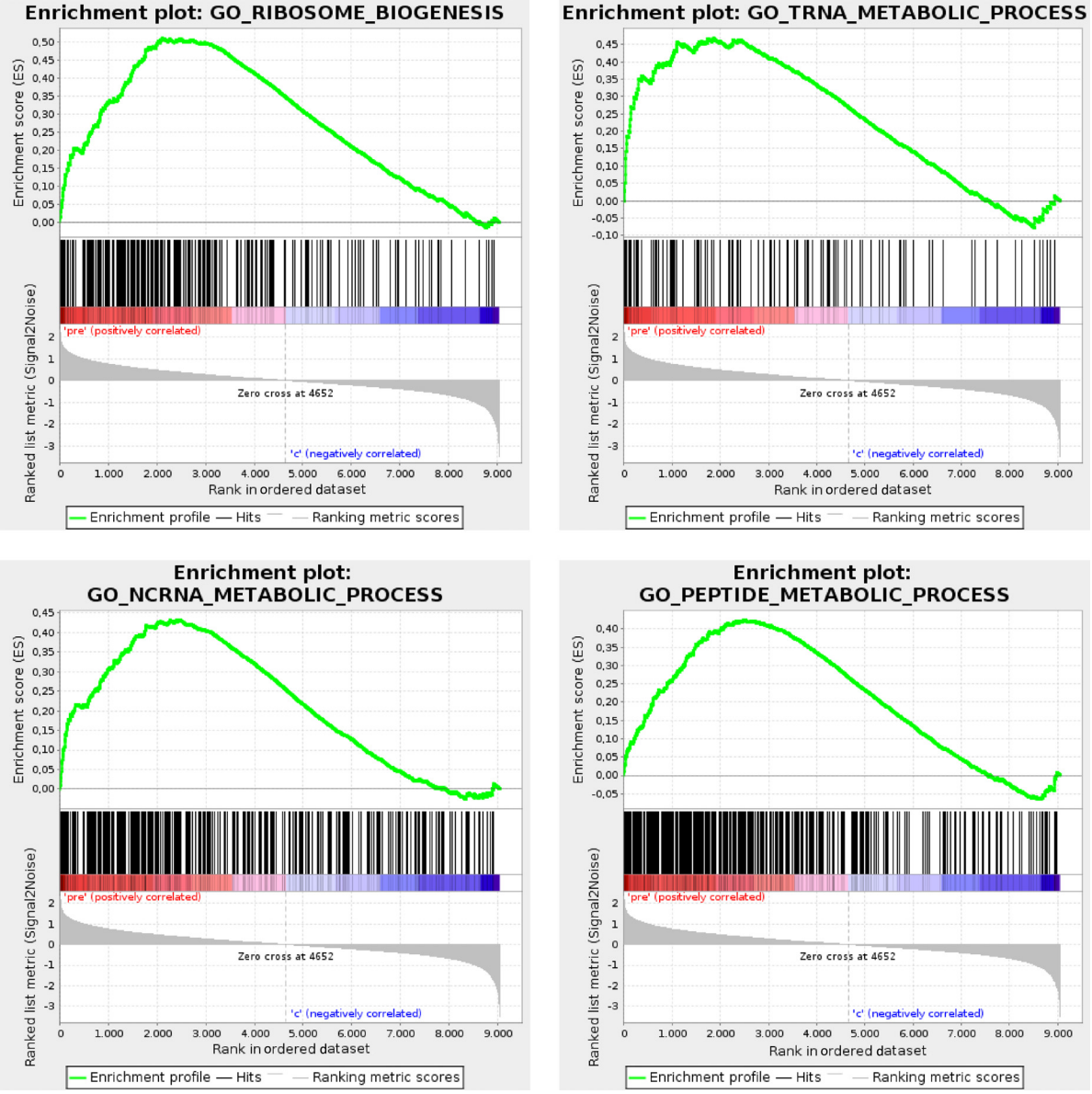

Fig. 3 – Gene Set Enrichment Analysis enriches differentially expressed genes in BPS preT urine samples into multiple functional gene networks. BPS = bladder pain syndrome; preT = pretreatment.

Table 2 – Canonical pathways and biofunctions in which genes differentially expressed between BPS preT and postT patients are involved

Top canonical pathways		
Name	*p* value	Overlap
Prolactin signaling	1.58E-04	7.2% (6/83)
PDGF signaling	2.46E-04	6.7% (6/90)
Insulin receptor signaling	3.90E-04	5.1% (7/137)
Role of NFAT in regulation of the immune response	4.01E-04	4.4% (8/181)
Glutaryl-CoA degradation	4.61E-04	18.8% (3/16)

Top diseases and biofunctions		
Name	*p* value	# Molecules
Cancer	3.83E-02–7.19E-04	181
Organismal injury and abnormalities	3.83E-02–7.19E-04	185
Gastrointestinal disease	3.83E-02–9.50E-04	170
Infectious diseases	2.89E-02–1.82E-03	17
Hereditary disorder	3.83E-02–2.53E-03	47

BPS = bladder pain syndrome; PDGF = platelet-derived growth factor; postT = post-treatment; preT = pretreatment.

response, and guide toward a specific treatment. However, identification of BPS biomarkers is challenging as such disorders are often based on subjective symptoms rather than on objective clinical measurements and are multifactorial in origin.

The recently developed RNASeq technology is suitable for compiling gene expression signatures and thus providing insight into the extent to which gene expression alterations are present in BPS. Since we described previously several gene expression signatures in urine samples from bladder cancer patients [13,14], we adapted our urine gene expression analysis experience to characterize the molecular pathogenesis of BPS and determine whether specific gene expression alterations in BPS cells reverse after triamcinolone treatment. The use of cells shed into the urine as a indirect way to evaluate bladder urothelium for such objectives has the advantage of being noninvasive, but it has also some drawbacks such as the low quantity and quality of the RNA obtained. To overcome these limitations, we have used a targeted gene RNASeq technology that generates small amplicon size, and thus the total number of raw reads needed for differential expression gene analysis is much smaller than typical whole-transcriptome RNASeq.

In accordance with the work of Blalock et al. [15], we found that urine from women with HLs has a differential gene expression profile compared with controls. Additionally, they showed that these patients had an increased proinflammatory gene expression profile in urine sediment. We have identified several pathways in which our differentially expressed genes in patients with BPS (preT) were involved. The EIF2 pathway is involved in the response to a wide range of different cellular stresses, while facilitating programs of stress-induced gene expression [16]. One of the pathophysiological mechanisms proposed for BPS is the altered permeability of bladder epithelial cells due to changes in the glycosaminoglycans and glycoproteins of the bladder surface, which allows the penetration of allergens, toxins, and other insults into the bladder wall leading to inflammation [17]. The implication of the EIF2 signaling pathway may support this mechanism, as cellular stress seems to be involved in the pathogenesis of BPS.

A downstream effector of the mammalian target of the rapamycin complex 1 (mTORC1) pathway is p70S6 K; mTOR and its downstream effectors contribute to the transmission and modulation of pain. It has recently been shown that, in a rat model of bladder pain with cystitis, mTOR and its effectors are upregulated, which results in mechanical pain and bladder hyperactivity. Moreover, intrathecal administration of rapamycin has a significant analgesic effect in this model [18]. Thus, attending to our results, these findings could open a new path in the treatment of patients with BPS.

Sirtuin signaling pathway is involved in anti-inflammatory effects [19]. also In addition, it has gained enormous attention in other smooth muscle cell research, including differentiation, apoptosis, and proliferation [20].

One of the most unexpected findings of our study is the implication of the sex hormone signaling pathway in BPS. There is evidence supporting a direct effect of estrogens on bladder nociception. Actually, symptoms associated with BPS are more common in females and fluctuate during the menstrual cycle [21], and it has previously been reported that local estrogen therapy may improve pain in patients with BPS [22].

To the best of our knowledge, this is the first study to provide information about gene expression changes in urine samples after treatment for BPS. According to our results, urine gene expression profiles of BPS patients become more similar to those of the control group after treatment with triamcinolone. Although the mechanism of action of triamcinolone has not been elucidated completely, we have previously shown the clinical benefit of corticoid treatment in these patients [11]. Altogether these data support the use of triamcinolone in clinical practice.

We acknowledge that our study has some drawbacks. First of all, the limited number of patients included may not represent the BPS population accurately. This could lead to a misdetection of other relevant genetic pathways involved in BPS and reported by other authors, such as genes related to the major histocompatibility complex [23] or to other inflammatory or immune responses [24]. However, the number of samples assessed was enough to detect significant differences in gene expression when present. Second, it is possible that we have missed some genes differentially expressed in the urine of BPS patients because of the use of a targeted gene RNASeq technology. Third, two patients were on antidepressants, and many of them such as selective serotonin reuptake inhibitors cause noradrenaline reuptake inhibition at the spinal and brain levels, modifying pain perception. This, in turn, could putatively lead to attenuation of the effectors of the mTOR pathway, which contributes to pain modulation and would impact the results. Finally, this can be considered a pilot study, and our results should be validated in a larger series.

5. Conclusions

In this work, we show that gene expression profiles of urine sediment are able to discriminate HLs from control patients. Furthermore, we report, for the first time, that triamcinolone treatment of HL patients induces changes in bladder gene expression profiles that can be observed in urine samples. Validation of these data in a larger series of patients could lead to the development of a noninvasive tool for the diagnosis and treatment monitoring of BPS patients, which would have a huge impact in clinical practice.

Author contributions: Lourdes Mengual had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Izquierdo, Mateu, Mengual, Franco, Alcaraz.
Acquisition of data: Izquierdo, Mateu, Montalbo, Ingelmo-Torres, Gómez, Peri.
Analysis and interpretation of data: Izquierdo, Mateu, Lozano, Peri, Mengual, Franco, Alcaraz.
Drafting of the manuscript: Izquierdo, Mateu, Mengual.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Lozano.
Obtaining funding: Franco, Alcaraz.
Administrative, technical, or material support: None.
Supervision: Izquierdo, Mengual, Franco, Alcaraz.
Other: None.***Financial disclosures:*** Lourdes Mengual certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.euf.2018.10.001.

References

[1] Fall M, Baranowski AP, Fowler CJ, et al. EAU guidelines on chronic pelvic pain. Eur Urol 2004;46:681–9.
[2] Temml C, Wehrberger C, Riedl C, Ponholzer A, Marszalek M, Madersbacher S. Prevalence and correlates for interstitial cystitis symptoms in women participating in a health screening project. Eur Urol 2007;51:803–8.
[3] Parsons CL, Tatsis V. Prevalence of interstitial cystitis in young women. Urology 2004;64:866–70.
[4] McLennan MT. Interstitial cystitis: epidemiology, pathophysiology, and clinical presentation. Obstet Gynecol Clin North Am 2014;41:385–95.
[5] Chrysanthopoulou EL, Doumouchtsis SK. Challenges and current evidence on the management of bladder pain syndrome. Neurourol Urodyn 2014;33:1193–201.
[6] Gafni-Kane A, Botros SM, Du H, Sand RI, Sand PK. Measuring the success of combined intravesical dimethyl sulfoxide and triamcinolone for treatment of bladder pain syndrome/interstitial cystitis. Int Urogynecol J 2013;24:303–11.
[7] Grigorescu B, Powers K, Lazarou G. Update on urinary tract markers in interstitial cystitis/bladder pain syndrome. Female Pelvic Med Reconstr Surg 2016;22:16–23.
[8] Offiah I, Didangelos A, Dawes J, et al. The expression of inflammatory mediators in bladder pain syndrome. Eur Urol 2016;70:283–90.
[9] Nordling J, Fall M, Hanno P. Global concepts of bladder pain syndrome (interstitial cystitis). World J Urol 2012;30:457–64.
[10] Logadottir Y, Delbro D, Lindholm C, Fall M, Peeker R. Inflammation characteristics in bladder pain syndrome ESSIC type 3C/classic interstitial cystitis. Int J Urol 2014;21(Suppl 1):75–8.
[11] Mateu L, Izquierdo L, Franco A, Costa M, Lawrentschuk N, Alcaraz A. Pain relief after triamcinolone infiltration in patients with bladder pain syndrome with Hunner's ulcers. Int Urogynecol J 2017;28:1027–31.
[12] Mengual L, Burset M, Marin-Aguilera M, Ribal MJ, Alcaraz A. Multiplex preamplification of specific cDNA targets prior to gene expression analysis by TaqMan arrays. BMC Res Notes 2008;1:21.
[13] Mengual L, Ribal MJ, Lozano JJ, et al. Validation study of a noninvasive urine test for diagnosis and prognosis assessment of bladder cancer: evidence for improved models. J Urol 2014;191:261–9.
[14] Ribal MJ, Mengual L, Lozano JJ, et al. Gene expression test for the noninvasive diagnosis of bladder cancer: a prospective, blinded, international and multicenter validation study. Eur J Cancer 2016;54:131–8.
[15] Blalock EM, Korrect GS, Stromberg AJ, Erickson DR. Gene expression analysis of urine sediment: evaluation for potential noninvasive markers of interstitial cystitis/bladder pain syndrome. J Urol 2012;187:725–32.
[16] Donnelly N, Gorman AM, Gupta S, Samali A. The eIF2alpha kinases: their structures and functions. Cell Mol Life Sci 2013;70:3493–511.
[17] Bouchelouche K, Nordling J. Recent developments in the management of interstitial cystitis. Curr Opin Urol 2003;13:309–13.
[18] Liang S, Li J, Gou X, Chen D. Blocking mammalian target of rapamycin alleviates bladder hyperactivity and pain in rats with cystitis. Mol Pain 2016:12.
[19] Csiszar A. Anti-inflammatory effects of resveratrol: possible role in prevention of age-related cardiovascular disease. Ann N Y Acad Sci 2011;1215:117–22.
[20] He Y, Zeng H, Yu Y, et al. Resveratrol improves smooth muscle carcinogenesis in the progression of chronic prostatitis via the downregulation of c-kit/SCF by activating Sirt1. Biomed Pharmacother 2017;95:161–6.
[21] Cheng Y, Keast JR. Effects of estrogens and bladder inflammation on mitogen-activated protein kinases in lumbosacral dorsal root ganglia from adult female rats. BMC Neurosci 2009;10:156.
[22] Gardella B, Iacobone AD, Porru D, et al. Effect of local estrogen therapy (LET) on urinary and sexual symptoms in premenopausal women with interstitial cystitis/bladder pain syndrome (IC/BPS). Gynecol Endocrinol 2015;31:828–32.
[23] Tseng LH, Chen I, Wang CN, Lin YH, Lloyd LK, Lee CL. Genome-based expression profiling study of Hunner's ulcer type interstitial cystitis: an array of 40-gene model. Int Urogynecol J 2010;21:911–8.
[24] Gamper M, Viereck V, Geissbuhler V, et al. Gene expression profile of bladder tissue of patients with ulcerative interstitial cystitis. BMC Genomics 2009;10:199.