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Monocyte Chemotactic Protein-1 (MCP-1) as a Predictor of Prolonged Urinary Incontinence After Radical Prostatectomy

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Abstract:

Objectives:

To investigate monocyte chemotactic protein-1 (MCP-1) as a novel urinary biomarker to predict prolonged post prostatectomy incontinence.

Methods:

Men submitted urine samples prior to robotic radical prostatectomy. MCP-1 values were derived using an ELISA test. Pad usage at 7, 30, and 60 days were documented by patient post cards mailed when zero pads was reached. The primary outcome was defined as no incontinence pad usage at 30 days at prostatectomy.

Results:

After exclusions, 76 patients were included in analyses. Continence was reached by 29% (22/76), 56% (42/76), and (75/76) 98% at 7, 30, and 60 days, respectively. The average MCP-1 ($p=0.258$) was not different between the continent and incontinent groups. Highest quartile of MCP-1 (MCP > 166 pg/mL) and normalized MCP-1 (MCP-1/TV >0.53) noted a significant delay in continence at 30 days ($p=0.050$ and $p=0.003$). Only 26% (5/19) in the highest MCP1/TV quartile were continent, whereas 65% (37/57) of men in the 3 lower quartiles reached zero pad continence ($p=0.003$). In a logistic regression model the highest quartile of MCP1/TV had a significant chance of being incontinent at 30 days (OR 0.22; 95% CI 0.058-0.80; $p=0.022$).

Conclusion:

MCP-1/TV is a urinary biomarker that may predict prolonged urinary incontinence after radical prostatectomy.

Keywords: Incontinence, overactive bladder, prostate cancer, prostatectomy, quality of life.

INTRODUCTION

Prostate cancer, the most common non-cutaneous malignancy prevalent in males, is the 2nd leading cause of death compared to other cancers in men [1]. Radical prostatectomy is one of the treatment options available for organ-confined disease. Postoperative incontinence is a common side effect after prostatectomy that can severely impact quality of life [2 - 6]. The pathophysiology of incontinence is largely due to extrinsic sphincter insufficiency; however

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may be exacerbated by various other factors [7].

As divulged by a recent review of the literature, other factors such as detrusor overactivity, reduced bladder capacity, and impaired detrusor contractility can be pivotal for developing bladder dysfunction after prostatectomy [8]. Moreover, *de novo* detrusor instability after prostatectomy can lead to overactive bladder (OAB) symptoms, which may be detectable by urodynamic evaluation [9]. Currently, lack of the knowledge on the preoperative biomarkers alluding to postoperative incontinence is a limiting factor in the counseling process because the information may potentially be used as an additional component at the time of counseling. To this end, a previous study using a proteomics chip based analysis of urinary cytokine expression demonstrated that OAB group expressed Monocyte Chemotactic Protein-1 (MCP-1) two-fold higher than UTI population and controls, thus suggesting MCP-1 to be a potential target for an OAB biomarker [10]. Based on these premises, we conducted a clinical study to assess whether urinary MCP-1 can be a potential preoperative biomarker associated with the outcome of 30-day urinary continence after robotic radical prostatectomy (RRP).

METHODS

Patient Population and Study Design

After institutional review board approval, we enrolled biopsy proven prostate cancer patients who were undergoing pre-operative evaluation for robotic assisted radical prostatectomy. One hundred twenty men volunteered to provide urine samples after going through informed consent. For all these patients, serum laboratory studies, urine analysis, uroflowmetry results, and post-void residual were recorded. In order to minimize differences in surgical technique, we only enrolled patients in which the robotic radical prostatectomy was performed by a single surgeon (TA). The sparing technique was decided by the primary surgeon on a case-by-case basis and the reasons for such decision were documented. Patients were sequentially enrolled to evaluate both cancer specific and incontinence specific pre-operative urinary biomarkers. Therefore, patients were excluded from final analysis if they had previous radiation therapy (n=2), pT4 disease (n=1), chronic inflammation on final pathology (n=11), active urinary tract infection, or were taking anticholinergic medication preoperatively (n=25). Patients from non-white ethnicity were excluded because of very limited numbers (n=4).

Questionnaires

The patient also completed the International Index of Erectile Function (IIEF-5) and the International Prostate Symptom Score (IPSS) questionnaires, including the urinary quality of life (QOL) question. By and large, the primary components to urinary incontinence recovery after radical prostatectomy are age, sexual function (IIEF-5) and obesity [11]. Specifically the inquiries involving IIEF-5 score focus on the confidence of an erection, erection firmness, maintenance of an erection, and sexual satisfaction. On the other hand, the IPSS score evaluates preoperative obstructive or urgency symptoms that may contribute to postoperative incontinence.

Urine Sample Collection and Processing

The urine samples were collected when the participants had a strong desire to void with “extremely full” bladders. After the patients voided, the data on uroflowmetry characteristics such as the volume of void (VV), peak flow rate (PFR) and postvoid residual (PVR) were collected. The total volume in the bladder was calculated by adding VV to PVR. The urine samples were placed on ice immediately after collection and transferred to the laboratory for the preparation to store them appropriately. Routinely, the urine samples were centrifuged at 3000g for 10 min at 4°C to collect the supernatant and 1.5 mL aliquots were preserved at -80°C.

Determination of Monocyte Chemotactic Protein - 1 level

The Quantikine Human MCP-1 Immunoassay (R&D Systems, Minneapolis, MN) is a 3.5-4.5 hour solid phase ELISA designed to measure MCP-1 in cell culture, serum, plasma, and urine. All reagents were prepared as specified by the manufacturer’s instructions, which include well washing of the samples followed by incubation for 30 minutes at room temperature by addition of 200 µL of substrate into each well. Finally, a stop solution was added to the wells and then optical density of each well was assessed by using a microplate reader set at 450 nm. All measurements were performed in duplicate and the average was used to subtract from the zero standard optical density. From the manufacturer the initial evaluation on 37 healthy volunteers demonstrated an average of 211 pg/mL (range 42-410) MCP-1 protein. However no further information regarding age, gender, or urinary symptoms are provided. Previous

studies have normalized urinary biomarkers with urinary creatinine and/or specific gravity [12]. We include the total urinary volume as dilution factor of the protein that can potentially vary and can be used to normalize the results.

Follow-Up

We asked participants to return pre-stamped postcards when they achieved pad free status date and/or keep a daily log of their progress, which is standard of care in our department. Patients also kept continence logs for the first week after the catheter was removed. Patients who did not reach continent status or did not return logs, were contacted by phone for updates. At the end of the study period (3 months post-operatively) patients with missing data were contacted via telephone.

Table 1. Demographics of 30 Day continence populations.

Demographic	Continence at 30 Days		P value	Statistical Test
	Yes	No		
	N=42	N=34		
Demographic	Mean (SD)	Mean (SD)	P value	Statistical Test
Age	60.8 (7.1)	61.8 (7.1)	0.528	<i>t</i> test
Prostate Specific Antigen	5.4 (2.3)	6.7 (5.5)	0.164	<i>t</i> test
American Urologic Association Symptom Score	6.1 (6.2)	7.7 (6.2)	0.237	<i>t</i> test
Sexual health inventory for Men (SHIM)	22.8 (3.5)	18.9 (8.0)	0.008	<i>t</i> test
Body Mass Index (BMI)	27.3 (3.6)	27.3 (2.6)	0.996	<i>t</i> test
Voided Volume (mL)	396 (227)	312 (195)	0.092	<i>t</i> test
Post Void Residual (mL)	96 (89)	85 (64)	0.562	<i>t</i> test
Total bladder volume (TV) (mL)	482 (260)	397 (224)	0.098	<i>t</i> test
Prostate Weight (gm)	49.1 (41.4)	55.5 (39.5)	0.105	<i>t</i> test
Urinary Creatinine	34.2 (98.7)	43 (39.5)	0.171	Mann-Whitney
Monocyte Chemotactic Protein 1 (ng/mL)	164.7 (177.1)	108.4 (98.7)	0.208	Mann-Whitney
Monocyte Chemotactic Protein 1 (ng/mL) / Total bladder volume	0.342 (0.450)	0.724 (1.13)	0.085	Mann-Whitney
	Number (%)	Number (%)		
Bilateral Nerve Sparing	27 (79.4)	40 (95.2)	0.070	Fishers Exact
Smoker	2 (5.9)	2 (4.8)	1.0	Fishers Exact
Diabetes	1 (2.9)	0 (0)	0.447	Fishers Exact
Statins	10 (29.4)	20 (47.6)	0.157	Fishers Exact
Pathologic Stage			0.433	Fishers Exact
<i>p</i> T2	13 (31.0)	7 (20.6)		
<i>p</i> T3	29 (69)	27 (79.4)		

Statistical Analysis

For purposes of this study, continence was defined as no pads. Our primary outcome was 30 day continence. The primary predictor variable was Monocyte chemotactic protein 1 (MCP-1) which was compared using the *t*-test between continent vs. incontinent men at specific time points. The cut off values were not known preoperatively; therefore, quartiles are used to identify differences at extremes. The association between MCP-1 and other variables were explored with the Spearman correlation coefficient or Mann-Whitney test. Specifically we performed correlation of MCP-1 to the normalization variables of total urinary volume or urinary creatinine. As secondary outcomes, we also investigated continence at day 7 (early) and day 60 (delayed) associated with MCP-1. In general, continence was evaluated using the Chi-squared test at 7 day, 30 day, and 60 day time points. The univariate analysis was done with Chi-Squared tests and the multivariate analyses were performed with logistic regression.

RESULTS

After exclusions, 76 patients were included in analyses. Demographics of the group are presented in Table 1. Approximately in 80% (61/76) of cases, patients followed the log or sent post cards of their zero pad date, while remaining 20% (15/76) patients were contacted by either telephone or email. The primary outcome of continence consisting of using zero pads was reached by 29% (22/76), 56% (42/76), and (75/76) 98% at 7, 30, and 60 days, respectively. Comparing incontinent and continent men at 30 days after prostatectomy, the IPSS score (7.7 vs. 6.1; $p=0.237$), urgency question (1.5 vs. 1.1; $p=0.283$), or quality of life question (1.0 vs. 0.7; $p=0.317$) were not associated

with urinary continence. The urgency question (none=0 vs. any =1) of the IPSS score did not demonstrate any association with time to urinary continence (36 vs. 34 days; $p=0.858$). Furthermore, the correlations between MCP1 and other variables were investigated. Of note, the urinary creatinine ($r=0.21$; $p<0.001$) and the total bladder volume ($r=-0.56$; $p<0.001$) showed the highest correlation with MCP-1. Because creatinine excretion may vary due to diurnal creatinine production, physical activity, emotional stress, diet, muscle mass (hence age, sex, and body weight variation), and disease state, we chose to correct the MCP-1 value using the better correlative value of total urine volume, and then accounted for creatinine in multivariable analysis [13]. Other variables significantly correlated with MCP-1 include the sexual health inventory for men (IIEF-5; $r=-0.24$; $p=0.037$) and the American Urological Association symptom score (IPSS; $r=0.25$, $p=0.030$). No significant correlations were identified between MCP-1 and other demographic variables.

We investigated the associations with 30 day continence in univariate analysis (Table 1). We identified no significant difference in 30-day continence between the average MCP-1 ($p=0.258$) or MCP after adjusting for bladder volume (MCP1/TV; $p=0.14$). Additionally, bladder volume was larger in those who were continent at 30 days but was not statistically significant (482 vs. 397, $p=0.098$). We then analyzed MCP by quartiles and comparing the highest quartile of MCP-1 (MCP > 166 pg/mL) to all other quartiles, we noted a delay in continence ($p=0.050$). Subsequently, we normalized MCP-1 values by dividing them by the total bladder volume (TV, mL) and obtained the highest quartile (MCP-1 >0.53). Comparing MCP-1/TV to all other quartiles, we again noted a significant delay in continence among patients with the highest quartile. At 30 days only 26% (5/19) in the highest MCP1/TV quartile were continent, whereas 65% (37/57) of men in the 3 lower quartile reached zero pad continence ($p=0.003$) (Fig 1). Additionally, men in the highest MCP-1/TV quartile were also noted to have significantly higher incontinence at 7 days (89.5% vs. 64.9%; $p=0.041$) compared to all other quartiles combined. At 60 days, the difference in continence rates for highest quartile of MCP1/TV vs. lower quartiles (68.4% vs. 78.9%; $p=0.350$) was no longer statistically significant.

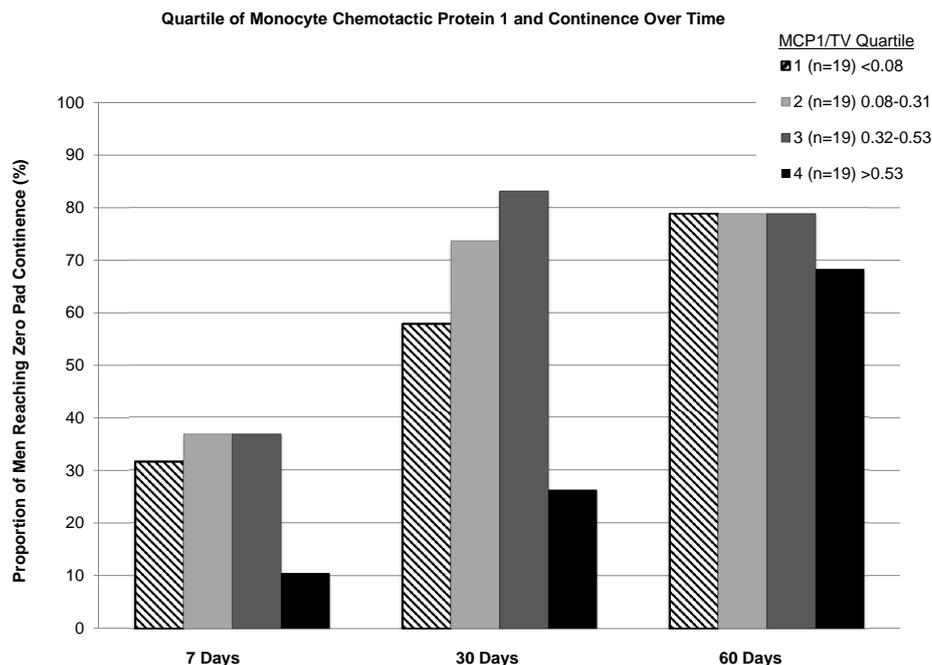


Fig. (1). Bar graph representing zero pad continence at selected time points for the highest quartile MCP-1/TV.

Next, a logistic regression model was developed including MPC1/TV (highest quartile vs. lower quartiles), and IIEF-5 as independent variables in the model. In addition, urinary creatinine was included in the model to account for urine concentration and bilateral nerve sparing was included as it trended to significance in univariate analysis. We noted that patients in the highest quartile of MCP-1/TV bear a significant risk of being incontinence at 30 days from robotic prostatectomy which is controlling other factors (OR 0.22; 95% CI 0.058-0.80; $p=0.022$) (Table 2).

DISCUSSION

To summarize, our studies indicate that high preoperative levels of Monocyte chemotatic protein-1 corrected for total bladder volume may play a role in developing incontinence at 30 day. These findings further suggest that a

preconditioned state of high inflammation may be present in some patients causing bladder pathology thus contributing to prolonged recovery of continence. Indeed our previous study leading to this investigation postulated that elevated expression of MCP-1 in patients with overactive bladder could contribute to incontinence [10]. Another important aspect of our investigation is the pinning down MCP-1/TV as a potential biomarker to identify subjects that can start medications early in the recovery process. It is expected that such patients may benefit more from OAB therapy prior to prostatectomy .

Table 2. Univariate and multivariate analysis achieving 30 day continence.

Multivariant	OR (95% CI)	P Value
MCP1/TV >0.53	0.22 (0.058-0.80)	0.022
Urinary Creatinine	1.00 (0.99-1.02)	0.785
Bilateral Nerve Sparing	3.45 (0.29-40.04)	0.322
SHIM	1.08 (0.99-1.21)	0.076

At present, studies are indeed limited regarding anti-inflammatory therapy to prevent post-operative incontinence. However, rising interest in MCP-1 as a diagnostic or therapeutic target in other diseases may provide such opportunity. It will be quite prudent to undertake studies to test whether the ablation of MCP-1 prior to radical prostatectomy will diminish the extent of urinary incontinence. It is worth mentioning that CCR2 (chemokine receptor 2), which is the primary MCP-1 receptor, has been considered to be an important therapeutic target in treating the deleterious effects of myocardial infarction and acute kidney injury [14, 15]. Likewise, the same mechanism may be tested in a pre-prostatectomy cohort investigating urinary outcomes. Another evolving concept regarding reducing inflammation is hypothermia of the prostate *via* a rectal balloon at the time of surgery [16]. Our previous study is quite remarkable because all patients who received hypothermia during prostatectomy, had a significant reduction in post prostatectomy incontinence [17]. However, in this pilot study, we were unable to determine the true effect of hypothermia, specifically on MCP-1 because we did not measure intraoperative or postoperative MCP-1 levels.

Currently, surgeons are compelled to assess the bladder symptoms of the patients by using demographics and questionnaires prior to prostatectomy due to the lack of any specific biomarkers. In line with this, a systematic review and meta-analysis of post-prostatectomy incontinence reported associations with age, BMI >30, comorbidities, severity of lower urinary tract symptoms, and preoperative erectile function (IIEF-5) [18]. We also investigated these variables in our cohort and noted preoperative sexual function (IIEF-5) to be an exclusive albeit significant determinant of incontinence. To the contrary, our studies do not conform to the notion that one of the most significant factors for incontinence is the age of the patient. Our study enrolled many men of similar age group and revealed that age difference exerts a negligible effect on the incontinence in this study population [19]. Other preoperative indicators have been examined including ultrasound of pelvic muscles and MRI of membranous urethral length, however these would add to the current cost and may rely on other specialists [20, 21]. In all, a urinary biomarker, such as MCP-1 would be valuable because this could provide uniform, objective risk profile that may be used across various sites. At this stage, a larger study potentially in the context of a clinical trial to confirm the results of MCP-1 as a urinary biomarker to predict post-operative continence is further warranted. The information obtained from such a biomarker could weigh heavily in the discussion regarding radical prostatectomy expectations and an individualized treatment plan, which may include pre-operative pelvic therapy and early use of medications.

We are aware of the limitations of this study because of the small sample size and homogeneous nature of our population especially in the context of age and race/ethnicity. We specifically chose to use strict entry criteria for this pilot study to show proof of concept and obtain preliminary data to justify inclusion into a clinical trial. Additionally, urinary biomarkers can indicate substantial variability from day to day, hydration status, and a multitude of other factors [22]. We limited the evaluation of urinary biomarkers for post prostatectomy solely to MCP-1. However, we plan to further investigate other urinary biomarker such as nerve growth factor that has been demonstrated to be promising for detrusor over-activity [23]. Further study would need to seriously test preoperative MCP-1 at various time points prior to surgery and changes noted post operatively. Of note, the origin and causality of MCP-1 are beyond the scope of this study. In this pilot study, we did not deploy urodynamics peri-operatively to identify exact type of urinary incontinence. Overall, we feel the prospective nature and the addition of bladder volume are novel in post-prostatectomy incontinence.

CONCLUSION

In addition to other factors, high levels of Monocyte chemotactic protein - 1 may be associated with delayed recovery of continence after radical prostatectomy. Comprehensively, additional investigations are absolutely critical to establish MCP-1 as a urinary biomarker to predict delayed post-prostatectomy continence preoperatively. Based on these premises we infer that further studies with larger and broader populations are pivotal for confirmation of the findings presented in this report.

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CONFLICT OF INTEREST

Dr. Ahlering is a consultant for Astellas and Phillips Healthcare Dr. Gohniem is a speaker for Astellas and Allergan, consultant for Uroplasty.

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