Research article

Semaphorin-3A levels in urine demonstrate promising sensitivity for detection of upper-tract urothelial carcinoma – a preliminary case series

Itamar Getzler[1], Zahava Vadasz[2], Jacob Rubinstein[3], Sarel Halachmi MD[1,*]

[1] *The department of Urology, Bnai Zion Medical Center, Haifa, 31048, Israel*
[2] *The division of clinical immunology, Bnai Zion Medical Center, Haifa, 31048, Israel*
[3] *The Department of Mathematics, Technion, Israeli Institute of Technology, Haifa, 32000, Israel*

*__Correspondence__: sarel.halachmi@b-zion.org.il (Sarel Halachmi MD)

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Abstract

The purpose of this study was to evaluate the utility of Semaphorin-3A (Sema-3A) as a biomarker for diagnosis and management of upper tract urothelial carcinoma, independently and in conjunction to cytology. Upper tract noninvasive urothelial carcinoma is a lifelong chronic non-curable disease. It is infamously difficult both to diagnose and to follow-up, and current non-invasive methods are commonly inadequate. Sema-3A protein levels can be measured from a simple urine test and can aid in the diagnosis and follow-up and early non-invasive tumor recurrence detection. Assigned cases for this series were those with pathologically verified upper tract neoplastic lesions. Urine samples for cytology and Sema-3A were taken on admission from all recruited patients. Sema-3A protein levels were determined using ELISA in every sample, and the tumor was graded and staged according to the 2004 WHO grading system. Descriptive and statistical analysis was performed to evaluate the performance of Sema-3A and the cytology exam. This case series included 10 patients with pathologically proven upper tract urothelial carcinoma. Sensitivity for recognizing disease was calculated as 80% for cytology, and 89% for a predetermined Sema-3A cutoff. Combining the strengths of both urine tests to a single criterion (Sema-3A $>$ 5 or positive cytology) resulted in 100% sensitivity. In conclusion, in the current preliminary study, high levels of Sema-3A correlated with upper-tract urothelial cancer stage and displayed higher sensitivity than cytology. Combined analysis of both positive Sema-3A and cytology demonstrated 100% sensitivity. Thus, Sema-3A levels can potentially serve as a reliable biomarker for diagnosis and management for the disease, given that further dedicated studies with larger patient cohorts are undertaken.

Keywords

Urothelial cancer; Noninvasive detection; Urine; Semaphoring-3A; Overexpression

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1. Introduction

Upper urinary tract carcinoma is a highly challenging disease to diagnose and follow. The urothelium is lining the whole urinary collecting system from the calyces via the renal pelvis, ureters, the bladder and urethra. Malignancy can appear in each part, however it is more frequent in the bladder and upper tract urothelial carcinoma is less frequent comprising 5-10% of all urothelial tumors [1]. Although upper tract tumors share similarities with urothelial cancers of the bladder, there are innate differences between these two neoplastic processes (anatomic and molecular) that warrant consideration regarding their management [2]. In contrast to bladder cancer, upper tract tumors are more difficult to diagnose and treat, due to the anatomical considerations and the special medical equipment that is required for the diagnosis and treatment of these tumors. The frequency of upper tract urothelial tumors has been increasing in recent years [3]. The disease prognosis is heavily influenced by the stage and grade of the disease, which makes early detection of a new and recurrent lesion a major task for the following physician. As in bladder cancer, upper tract urothelial malignancy is a chronic non-curable disease with a chance of 70% recurrence lifelong, mandating close follow-up for early detection of recurrent tumors.

Cancer detection in urine samples can be the most convenient method for the patient and physician. Although several urine markers are available, none of them is specific and sensitive enough to be used regularly [4]. As a result, most of the physicians worldwide still use urine cytology as the main non-invasive and low risk method for urothelial cancer detection [5]. Using urine cytology generally yields high specificity, but its sensitivity is usually inadequate-accuracy estimates have ranged from about 20% for grade 1 tumors to 45% and 75% for grade 2 and grade 3 tumors, respectively [6]. Therefore, all urothelial cancer patients will have lifelong, multiple invasive cystoscopies and ionizing radiation imaging, which imply severe side effects, morbidity and even mortality [7]. In case of upper tract cancer endoscopic procedure is much more complex and can it is usually done under anesthesia. Hence, a highly specific and sensitive non-invasive marker would alleviate the need for some of the invasive procedures.

Semaphorins are a family of membrane-bound, soluble proteins classified into sub-classes based on their structural domains [8].

Semaphorins mainly regulate focal adhesion assembly/disassembly and induce cytoskeletal remodeling, thus affecting cell shape, cell attachment to the extracellular matrix, cell motility, and cell migration. Semaphorins were originally identified as affecting axon guidance during development of the nervous system, they are thought to fulfill diverse physiological roles including organogenesis, vascularization, angiogenesis, neuronal apoptosis, neoplastic transformation and metastases [9, 10]. Specifically, Semaphorin-3A (Sema-3A) was found to be associated with several malignancies, including breast cancer, where sema3A impedes tumor cell migration [11]; lymph node metastasis in patients with prostate cancer [12]; and overexpression in lung cancer [13]. Semaphorins were also studied as druggable targets [14]. Regarding the urinary system, our group found in a previous study that Sema-3A is overexpressed in the presence of urothelial cancer, both in urine and in bladder tissue, and that it statistically improved cancer detection sensitivity along with the cytology exam [15].

The aim of the current study was to assess the utility of monitoring Sema-3A protein levels in upper tract malignancies, because of the greater risks both in the diagnosis and follow-up relative to bladder cancer. These risks include multiple cystoscopies, ureteroscopies with general anesthesia, and multiple CT-Urography radiation exposure.

2. Materials & Methods

2.1. Participants

The current study was approved by the Helsinki committee of Bnai Zion hospital. participants received a comprehensive explanation and signed an informed consent form before recruitment. Recruited patients were those who had pathologically verified urothelial tumors; were hospitalized for transurethral resection of lesions; had a history of urothelial cancer and admitted for endoscopic follow up; had other non-malignant urological conditions such as prostatic hyperplasia, stress incontinence, urethral stricture, ureteral and kidney stones. Out of this inclusive cohort-all patients with upper tract neoplastic lesions were assigned to this study.

2.2. Procedures

Urine samples for cytology and Sema-3A protein levels were taken on admission from all recruited patients. Sema-3A protein level was determined using ELISA in every sample, as described in the next paragraph. All included patients were pathologically confirmed to have upper tract urothelial carcinoma, which was graded and staged according to the 2004 WHO grading system [16].

2.3. Sema-3A values in urine:

Fifty milliliters of fresh urine samples were collected from recruited patients who were scheduled for cystoscopy at the Urology ward at Bnai Zion hospital. The samples were blinded and stored at -80°C until analysis. Samples were then thawed and centrifuged at 2,500 rpm for 20 minutes, to rid of residual cells or cell debris. The supernatant was then concentrated up to 50-fold the initial concentration by a membrane pore cut-off of 7.5 kD (“Vivapore”, Startorius stedim biotechnologies, UK). Later, the concentrated samples were subjected to specific human Sema-3A ELISA kit for the quantification of Sema-3A levels (MBS732622, San Diego, CA, USA) according to the manufacturer instructions.

2.4. Analysis

The aim of this quantification was to evaluate the performance of Sema-3A when compared to the current standard diagnostic urine test – cytology. Sema-3A values divided to groups according to a previously established cut off: Semaphorin value > 5, which was found indicative of a urinary neoplastic disease [15]. Cytology was marked either positive or negative. Multiple chi-square or gamma tests were performed between the pathological tumor features and the urine-based markers. An ordinal regression was performed for statistically significant correlations to examine predictive capabilities for stage and grade.

3. Results

This case series included 10 patients with pathologically confirmed upper tract urothelial carcinoma, or with a past upper tract malignancy. The mean age was 75.5 years, and most patients were expectedly males. The average Semaphorin value in ng/ml was over 40 for the whole series, and over 50 for those above the > 5 cut off. It has a rather large range, with a minimum of 3.3 ng/ml and a maximum value of 165.7 ng/ml, resulting in a median of 12.7 and a SD of 58.5. All other patients’ characteristics are shown in Table. 1.

Concerning sensitivity, urine cytology was available in 9 patients and despite bearing a malignant lesion in the upper tract, the test was negative for malignant cells in 4 out the 9 patients (44%). Hence, the gold standard noninvasive test available today for patient’s detection and follow up misses almost half of the patients with tumors. In contrast, Semaphorin levels were above the cutoff in 8 out of the 10 patients tested. In the 2 patients with lower levels of Semaphorin both had a single lesion with a small diameter, one showed also low pathological grade and stage and in the other the lesion was so small and superficial that it could be vaporized with laser energy without taking a sample for pathology.

Concerning specificity, in our current study, cytology had no true negatives (only false ones), while Semaphorin had no false positives (only true ones). Sensitivity for recognizing disease was calculated as 80% for cytology and 89% for Sema-3A alone. Applying the strengths of both urine tests to a single criterion, i.e. Sema-3A > 5 or positive cytology, results in 100% sensitivity. In this respect, this result is consistent with our previous study [15].

Stage, Grade and Semaphorin were regarded as ordinal variables, as there is meaning to a higher value. Using the appropriate Gamma test, Semaphorin correlated significantly with disease stage ($p = 0.05$) and showed a promising result regarding disease grade ($p = 0.065$). Cytology and the ‘past Urothelial Carcinoma’ characteristic were not close to significance in any of the tests.

In an ordinal regression for Stage and Grade, Sema-3A was found significant: $p = 0.019$ and $p = 0.025$, respectively. While definite conclusions cannot be inferred from a 10 patient case series, a promising trend is certainly observed.

4. Discussion

Our current finding showed that Sema-3A is a novel potential marker to detect and follow up upper tract urothelial carcinoma. In 8 out of 10 (80%) patients with upper tract tumor Sema-3A level was higher than a cutoff level. In conjunction with cytological results, we could identify upper tract malignancy in all 10 patients. In 2 patients, Sema-3A levels were lower than 5 units in both patients of which,

Table 1. Patients' Characteristics

	Age	Sex	Hx of urothelial cancer	Urine Semaphorin level *ng\ml*	Semaphorin group	Urine cytology	Tumor stage	Tumor grade	Lesion number	Max. lesion size cm.
1	82	M	+	8.052	>5	−	Ta	1	Multiple	<3
2	88	M	−	25.346	>5	+	Ta	3	1	3
3	71	M	+	3.354	<5	+	Ta	1	1	<3
4	73	M	−	13.669	>5	+	T2	3	1	3
5	70	F	+	3.714	<5	+	N.A.	N.A.	1	<3
6	82	M	−	65.04	>5	−	Ta	R 1	Multiple	<3
7	78	F	+	165.71	>5	−	T3	1	1	<3
8	78	M	+	130.402	>5	N.A.	T3	3	1	<3
9	64	M	−	11.782	>5	+	Ta	1	N.A.	N.A.
10	69	M	−	8.278	>5	−	T3	3	1	3

N.A = Not Available.

the lesions were small; in one lesion, the histology showed also low stage and grade and in the other the lesion was so small that it was vaporized without histology. One of the patients with lower levels of Sema-3A was a female and we already shown in our previous study that females have in general lower levels of Sema-3A in the urine when compared to males [15].

Upper tract urothelial carcinoma is a technically challenging disease to diagnose and follow up, requiring lifelong multiple invasive procedures. In the latest EORTC guidelines for upper tract urothelial carcinoma, the authors admit that "follow-up after kidney-sparing management is difficult, and frequent repeated endoscopic procedures are necessary". Furthermore, even with surgical intervention, a stringent follow-up regime is mandated [16]. These high intensity follow-up regimes are very likely to cause a marked increase in discomfort, morbidity, and even mortality from various complications like ureteral perforation or avulsion [17]. It is crucial to find a better modality to diagnose and follow up patients with a urothelial neoplasm, with higher sensitivity and specificity than current methods. A highly specific and sanative marker may reduce the need of some of the invasive procedures reducing morbidity mortality patients discomfort and economical costs.

Sema-3A is known to be an important factor in many physiological processes such as immune regulation [18, 19], however it has also been involved in neoplastic processes. It was argued that Sema-3A is an antitumor agent since it inhibits angiogenesis by competing with VEGF for the same receptors on the surface of endothelial cells. There is also cumulative evidence that a systemic delivery of Sema-3A *in vivo* inhibited tumor growth and progression [20, 21], and even that low levels of Sema-3A is associated with non-small cell lung carcinoma and melanoma [21, 22]. In contrast, several studies suggest that an immunosuppressive activity of Sema-3A might explain reports where high levels of Sema-3A are associated with poor prognosis in pancreatic cancer [23], glioblastoma multiforme [24], and liver cancer [25]. These conflicting expression levels of Sema-3A in tumors are indeed intriguing, and a possible explanation might be that the role played by Sema-3A depends on its source [26, 27].

While Sema-3A levels in urine was previously examined in relation to kidney failure [28], it was not correlated so far with urothelial cancer. Our group was the first to discover a correlation between Sema-3A and urothelial cancer [15]. We showed that Sema-3A is a potential marker for bladder urothelial carcinoma alone and in conjunction to urine cytology in a larger scale study comprising 183 urine samples, including healthy volunteers and patients with no urothelial malignancy that represented the control group. The current cases reported herein highlight the potential of Sema-3A as a tumor marker in upper-tract urothelial cancer, with the limitation of being a preliminary small case series study without a control group.

It is however important to point out that this is a retrospective case series that deals with already diagnosed patients, most of whom presented with urological symptoms. It remains to be seen whether Sema-3A can successfully detect cancer in asymptomatic patients in very early stages of the disease, although some initial promising results are already published [15]. These preliminary results hopefully open the door for more research in this important matter.

5. Conclusion

In the current preliminary study, high levels of Sema-3A correlated with upper-tract urothelial cancer stage and displayed higher sensitivity than cytology. Combined analysis of both positive Sema-3A and cytology demonstrated 100% sensitivity. Thus, Sema-3A levels can potentially serve as a reliable biomarker for diagnosis and management of the disease, given that further dedicated studies with larger patient cohorts are undertaken.

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Conflict of Interest

All authors have no conflict of interest.

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