RESEARCH ARTICLE

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Sequential Application and Post-Test Probability for Screening of Bladder Cancer Using Urinary Proteomic Biomarkers: A Review based Probabilistic Analysis

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Abstract

Background: Bladder cancer is one of the most common cancers in the world, with men being affected more than women. Diagnosis by cystoscopy, cytology and biopsy is invasive. Urine cytology, a non-invasive modality is not sensitive. This study is undertaken to evaluate whether non- invasive urinary proteomic profiling is more sensitive, specific for bladder cancer. **Objective:** To evaluate the sensitivity and specificity of various urinary proteomic biomarkers as a screening tool for bladder cancer. **Methods:** PubMed database was searched from 4th December 2011 to 30th November 2021 using Mesh terms and n = 10,364 articles were found. PRISMA guidelines were followed and Review articles, animal studies, Urinary tract infections, non-bladder cancer and other irrelevant articles were excluded. All studies who have reported mean/median (SD/IQR), sensitivity, specificity, cut off values (ROC analysis) were included (n=5). Post-test probability of various biomarkers was calculated using sequential approach. Pooled analysis was depicted using Forest plot. **Results:** Analysis of diagnostic studies of bladder cancer showed the post-test probability of CYFRA21-1 was 36.6%. Using sequential approach, the panel of biomarkers CYFRA 21-1, CA-9, APE-1, COL13A1 has post-test probability of 95.10% to diagnose bladder cancer. Analysis of two observational studies with APOE (n= 447) showed non-significant increase of APO-E levels in bladder cancer cases (WMD: 66.41with 95% CI 52.70-185.51; p=0.27, I2 92.4%). **Conclusion:** In patients presenting with hematuria, a panel of CYFRA 21-1, CA-9, APE-1, COL13A1 markers can be considered for screening of bladder cancer.

Keywords: CYFRA 21-1- CA-9- APE-1- COL13A1- APO-E

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Introduction

Bladder cancer (BC) is the 10th most commonly diagnosed cancer worldwide, with approximately 573,000 new cases and 213,000 deaths in 2020 (Sung et al., 2021). With an incidence of 9.5 and a mortality rate of3.3 per 100,000 among men, BC is approximately four times more common in men than in women globally. It is the sixth most common cancer and the ninth leading cause of cancer deaths in men (Sung et al., 2021).

The risk of BC increases with age. Peak incidence is seen between ages 50 and 70. In general, among the different types of BC diagnosed, transitional cell carcinoma is the most common (90%), followed by squamous cell carcinoma (5%) and adenocarcinoma (<2%) (Kaufman et al., 2009). Among the diagnosed BC cases, approximately 70%–80% present with non-muscle–invasive carcinoma, 50%–70% will recur, and 10%–30% progress to muscle-invasive disease (Saad et al., 2002). Recurrence in most cases is seen within five years, and tumor progression is commonly seen in patients with higher-grade lesions (Jordan et al., 1987).

Cystoscopy and cytology are mainly relied upon for the diagnosis of BC. Most papillary and solid lesions are detected by cystoscopy, but it is invasive (Jordan et al., 1987). Urine cytology is non-invasive with reasonable specificity and sensitivity for the detection of high-grade BC; however, for detecting low-grade tumors, its sensitivity ranges from only 4% to 31% (Lotan and Roehrborn, 2003). Because of these limitations for clinical detection, there arises a need for the development of non-invasive urinary biomarkers for the diagnosis of BC.

Early detection remains one of the critical issues in BC research. The probability of successful patient treatment largely depends upon the stage of detection of BC. The development of a non-invasively obtained urine biomarker assay would be of great help not only for the diagnosis but also for screening asymptomatic populations at risk.

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So this review was undertaken to find better and more promising urinary proteomic markers for the screening of bladder cancer.

Materials and Methods

The literature search was done in the NCBI PubMed database using the MESH search strategies from 4th December 2011 to 30th November 2021. PRISMA guidelines were followed.

Search strategy

The following search string has been developed ((((((“urinary tract”[MeSH Terms] OR (“urinary”[All Fields] AND “tract”[All Fields]) OR “urinary tract”[All Fields] OR “urinary”[All Fields]) AND (“proteomics”[MeSH Terms] OR “proteomics”[All Fields]) AND (“neoplasms”[MeSH Terms] OR “neoplasms”[All Fields] OR “cancer”[All Fields])) NOT ((“review”[All Fields] OR “review literature as topic”[MeSH Terms] OR “review”[All Fields]) AND articles[All Fields])) NOT ((“animals”[MeSH Terms: noexp] OR animal[All Fields]) AND “studies”[All Fields])) NOT (“urinary tract infections”[MeSH Terms] OR (“urinary”[All Fields] AND “tract”[All Fields] AND “infections”[All Fields]) OR “urinary tract infections”[All Fields])) AND “2011/12/04”[PDat] : “2021/11/30”[PDat]) AND (“urinary bladder neoplasms”[MeSH Terms] OR (“urinary”[All Fields] AND “bladder”[All Fields] AND “neoplasms”[All Fields]) OR “urinary bladder neoplasms”[All Fields] OR (“bladder”[All Fields] AND “cancer”[All Fields]) OR “bladder cancer”[All Fields]) AND (“2011/12/04”[PDat] : “2021/11/30”[PDat]).

Inclusion criteria

a)Study subjects are humans b) articles written in English, c) studies where urinary proteomics in bladder cancer are compared with controls; d) provided the biomarker data on mean/median values, Standard deviation/Inter quartile range; e) provided method description for urinary proteomic markers estimation; f) All studies irrespective of the staging of bladder cancer.

Exclusion criteria

a) Animal studies b) Urinary tract infections c) studies with no control group e) Studies where proteomic analysis was done in serum and tissues; F) Reviews, meta-analysis, commentaries, and letter to the editor.

A total of 10,364 articles were obtained with a basic search using the key terms urinary proteomics and cancer in the PubMed database. Out of them, before the screening, 1226 review articles, 5040 animal studies, 442 articles on urinary tract infections, 451 articles published prior to 4th December 2011, and 2019 non-bladder cancer articles were excluded. All the authors have screened and extracted the data from the articles independently. Duplicate articles were examined by all the authors and consensus was taken to include the original data. Title screening was done for the remaining 1186 articles, out of which 1,148 articles were excluded. After the abstract screening of the remaining 38 articles, 16 were excluded. PDF screening was done for the remaining 22 articles, following which 17 articles were excluded. A total of 5 studies were included in the review (Figure 1).

Data extraction

The following data was noted from the studies; first author name, place of study, study type and year of publication, number of cases and control subjects, male and female subjects in each group, mean/median values of age, protein marker studied, mean and SD metrics of protein markers. Other study characteristics like sensitivity, specificity, and positive and negative predictive values were also retrieved from the studies (Tables 1, 2).

Statistical analysis

Positive likelihood ratio (LR+) and Post- test probability were calculated for different biomarkers (Tables 2, 3). Pre-test odds were calculated as Pre-test probability/ (1- Pre-test probability).Post-test odds were calculated as Pre-test odds × LR+. Post- test probability was calculated as Post-test odds/ (Post-test odds+1) (Garudadri et al., 2011).The studies on sensitivity and specificity of biomarkers have used single biomarkers for reporting the accuracy. However, the sequential (adding more than one biomarkers in the panel of tests sequentially and calculating the accuracy) or simultaneous (adding more than one biomarker at the same time for calculating accuracy) approach might have better accuracy compared to using single biomarkers. Hence we did as a post laboratory sequential approach for recalculating the accuracy of a panel of biomarkers. The prevalence of any type of bladder cancer reported among individuals with hematuria as in Khadra (2000) study being 12% , this was considered as pre-test probability. A marker with highest specificity was taken as the first test in the sequential panel, because, higher specificity ensures less false positive cases. The biomarker with highest specificity (cytokeratin 19 fragment (Cyfra21-1)) was considered as first biomarker for calculation of post-test probability. The biomarker with next highest specificity (Carbonic anhydrase 9 (CA-9)) was considered for calculation of post-test probability of screening for bladder cancer. We considered the post-test probability of Cyfra21-1 as pre-test probability of Cyfra21-1+CA-9. Similarly further calculation was done. Further, the levels of urinary APO-E (Apolipoprotein E) in patients with and without bladder cancer have been compared with WMD. Results were graphically depicted as forest plots.

Results

Majority of the subjects included in the studies are aged between 60-69 years and male preponderance was seen. Most of the included cases of bladder cancer were of high grade. A total of fifteen urinary proteomic biomarkers for bladder cancer were identified in five different studies (n=672 cases, n=575 controls). These include Interleukin-8 (IL-8), Matrix Metalloproteinase-9 (MMP-9), Syndecan-1(SDC1), Chemokine (C-C motif) ligand 18 (CCL-18), Alpha-1 antitrypsin (A1AT), Angiogenin (ANG), Carbonic anhydrase 9 (CA-9),

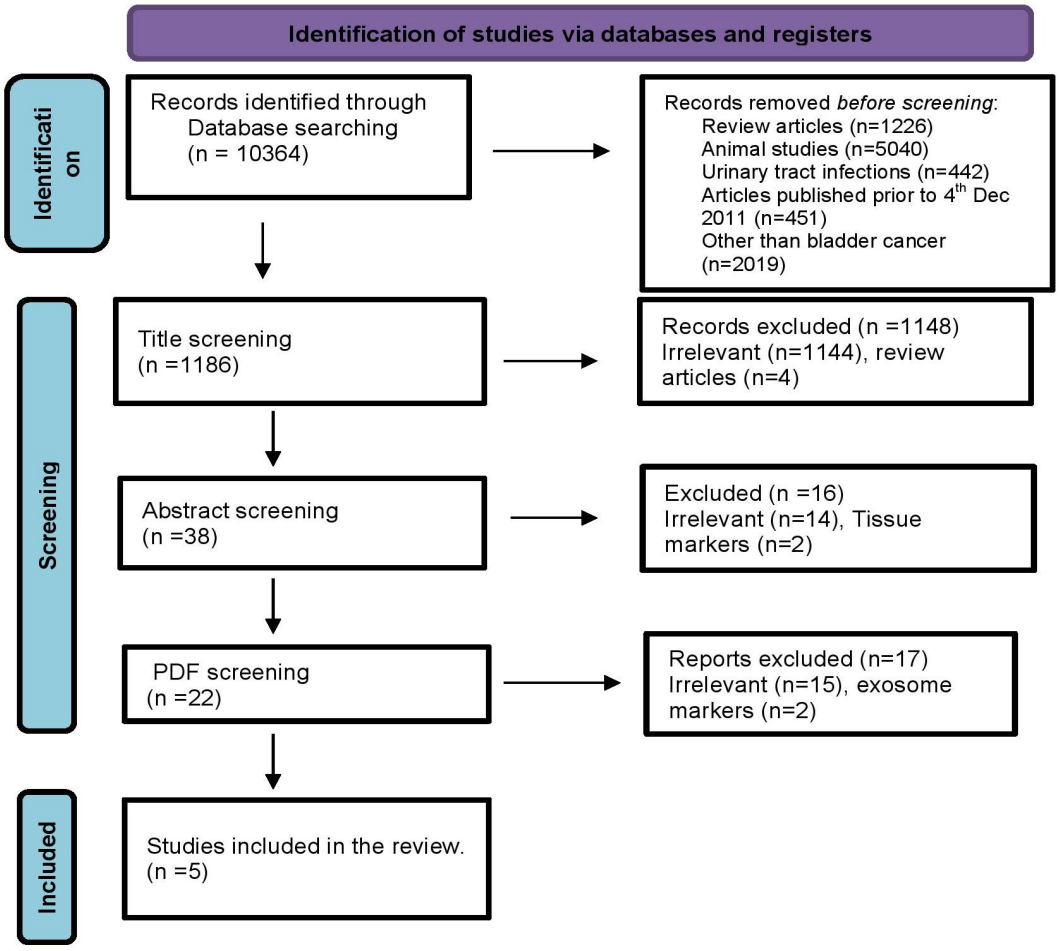

Figure 1. PRISMA Flow Diagram

Matrix metalloproteinase-10 (MMP-10), Apolipoprotein E (APO-E), Plasminogen activator inhibitor-1(PAI-1), Vascular Endothelial Growth Factor (VEGF), collagen 4A1 (COL4A1), collagen 13A1 (COL13A1), cytokeratin 19 fragment (CYFRA21-1), Apurinic/apyrimidinic endonuclease 1 (APE-1). An Eight biomarker panel (IL-8, MMP-9, PAI-1, VEGF, ANG, CA-9, APO-E, MMP-10) [9], a three biomarker panel (IL-8, VEGF, APO-E) (Goodison et al., 2012), and two biomarker panels [(VEGF, APO-E), (A1AT, APO-E)] (Goodison et al., 2012; Urquidi et al., 2012) were also reported in studies. All the markers reported in the studies were estimated by ELISA method.

Demographic and clinico-pathological characteristics of patients in different studies are illustrated in table 1 and the diagnostic specifications of different biomarkers in studies are illustrated in Table 2.

A combination of four biomarkers: CYFRA 21-1, CA-9, APE-1, COL13A1 have shown high post-test probability of 95.10% for screening of bladder cancer [Table 3]. However there was no much change in the post-test probability for the biomarkers IL-8, SDC-1, PAI-1, A1AT, COL4A1, CCL-18, MMP-10, VEGF, MMP-9, APO-E and ANG using sequential approach. Hence the panel of CYFRA 21-1, CA-9, APE-1/Ref-1, COL13A1 is a good model for screening of bladder carcinoma.

Analysis of two observational studies with APOE (n= 447) showed non-significant increase of APO-E levels in bladder cancer cases (WMD: 66.41with 95% CI 52.70-185.51;p=0.27, I^2 92.4%) (Figure 2).

Discussion

The study was undertaken mainly to identify non- invasive urinary proteomic biomarkers that are more sensitive and specific, for early detection of bladder cancer. Sequential analysis of biomarkers with highest specificity, yielded a four biomarker panel (CYFRA21-1, CA-9, APE-1, COL13A1) with high post-test probability of 95.10%. The included studies had patients from low grade to high grade cancer. Thus the four biomarker panel can be used for screening patients presenting with hematuria for early detection of bladder cancer.

CYFRA21-1 as a single marker is very promising as the degradation products of cytokeratins in serum could be used as surrogate markers in the diagnosis and follow-up of patients with solid tumors, including tumors of the bladder. Significantly higher levels of urinary CYFRA21-1 in bladder cancer patients were reported which also associated with higher stage and higher grade of bladder cancer (Miyake et al., 2017). Further it can clearly distinguish muscle-invasive ≥T2 tumors from Non Muscle invasive Bladder Cancer tumors. Among all the

Table 1. Demographic and Clinico-Pathological Characteristics of Patients in Different Studies

SNO	Author	Year of publication	Place of study	Total no of participants (n=)	Cases (n=)	Controls (n=)	Age(y)Mean(SD)/ Median (Range) cases	Age(y)Mean(SD)/Median (Range) controls	Males/Females cases	Males/Females controls	Stage and grade of Cancer (n=)
1	Steve Goodison et al	2012	Florida, USA	127	64	63	69.5 (22-90)	60 (30-81)	55/9	55/8	Tis: 6 Ta: 15 T1:9 T2: 31 T3: 4 T4: 2 N+: 3 Low grade: 9 High grade: 55
2	Li-Mei Chen et al	2014	USA	320	183	137	69 (33-92)	65 (21-96.5)	153/29	99/38	Tis high grade-4 Ta low grade: 59 Ta high grade:25 T1 low grade: 19 T1 high grade: 46 ≥ T2 high grade:23 Unknown: 7
3	Miyake et al	2013	Orlando, Florida	308	102	206	69 (20-93)	56 (18-89)	84/18	152/54	Tis: 6 Ta: 41 T1: 14 ≥ T2: 41 Low grade: 38 High grade: 64
4	Sunga Choi et al	2016	Korea	277	169	108	NMIBC(68.4±10.4) MIBC (66 ± 9.3)	60.9± 16.3	143/26	62/46	Ta: 108 T1: 49 T2: 10 T3-4: 2 Low grade: 98 High grade : 71
5	Makito Miyake et al	2017	Nara, Japan	215	154	61			133/21	36/25	Ta: 66 T1: 57 Tis: 7 T2-T4: 24 Low grade: 68 High grade: 86

SD, Standard deviation; MIBC, Muscle invasive bladder cancer; NMIBC, Non muscle invasive bladder cancer

Table 2. Sensitivity, Specificity, PPV, NPV, and Positive Likelihood Ratios of Different Biomarkers

SNO	Protein markers	Author	Cases (n=)	Controls (n=)	Sensitivity	Specificity	PPV	NPV	LR+
1	IL-8	Li-Mei Chen et al	183	137	77.1	75.2	69.2	82	3.11
		V.Urquidi et al	64	63	59.0	97.0	95.0	70	19.67
2	MMP9	Li-Mei Chen et al	183	137	82.3	60.9	60.3	82.7	2.1
		V.Urquidi et al	64	63	56.0	92.0	88.0	67.0	7.0
3	SDC1	Li-Mei Chen et al	183	137	78.1	74.4	68.8	82.5	3.05
		V.Urquidi et al	64	63	70.0	48.0	58.0	61.0	1.35
4	CCL18	Miyake et al	102	206	70.4	67.7	53.0	81.5	2.18
5	A1AT	Steve Goodison et al	64	63	87.0	84.0	-	-	5.44
		Miyake et al	102	206	70.6	71.8	55.4	83.2	2.5
6	ANG	Li-Mei Chen et al	183	137	88.5	51.9	57.0	86.2	1.84
7	CA9	Li-Mei Chen et al	183	137	68.8	80.5	71.7	78.1	3.53
8	MMP10	Li-Mei Chen et al	183	137	81.3	64.7	62.4	82.7	2.3
9	APOE	Li-Mei Chen et al	183	137	85.4	54.1	57.3	83.7	1.86
10	PAI-1	Li-Mei Chen et al	183	137	72.9	72.2	65.4	78.7	2.62
11	VEGF	Li-Mei Chen et al	183	137	84.8	62.8	63.6	84.3	2.28
12	COL4A1	Miyake et al	102	206	68.2	68.9	84.7	46.2	2.19
13	COL13A1	Miyake et al	102	206	54.6	77.1	85.7	40.2	2.38
14	COL4A1 + COL13A1	Miyake et al	102	206	72.1	65.6	84.1	48.2	2.1
15	CYFRA21-1	Miyake et al	102	206	74.6	82.4	90.7	58.3	4.24
16	Urinary APE-1/Ref-1	Sungachoi et al	169	108	81.7	79.6	86.3	73.5	4.0
17	Eight biomarker panel (IL-8, MMP-9, PAI-1, VEGF, ANG, CA-9,APOE, MMP-10	Steve Goodison et al	-	-	92.0	97	97.0	92	30.67
18	3 marker panel - IL-8 , VEGF, APOE	Steve Goodison et al	-	-	90.0	97.0	97.0	91	30
19	VEGF , APO-E	Steve Goodison et al	-	-	81.0	97.0	96.0	83	27
20	A1AT + APO-E	Virginia Urquidi et al	-	-	91.0	89.0	89.0	90	8.27

PPV, Positive predictive value; NPV, Negative predictive value; IL-8, Interleukin-8; MMP-9, Matrix Metalloproteinase-9; SDC1, Syndecan-1; CCL-18, Chemokine (C-C motif) ligand 18; A1AT, Alpha-1 antitrypsin; ANG, Angiogenin; CA-9, Carbonic anhydrase 9; MMP-10, Matrix metalloproteinase-10; APO-E, Apolipoprotein E; PAI-1, Plasminogen activator inhibitor-1; VEGF, Vascular Endothelial Growth Factor; COL4A1, Collagen 4A1; COL13A1, Collagen 13A1; CYFRA21-1, Cytokeratin 19 fragment; APE-1, Apurinic/Apyrimidinic endonuclease 1.

biomarkers, CYFRA21-1 had higher specificity of 82.4% when used as a single marker.

CA-9 is a tumor associated, cell-surface glycoprotein induced by hypoxia, involved in adaptation to acidosis, and implicated in cancer progression via its catalytic activity and non-catalytic functions. Individually, increased expression of Urinary CA-9 levels in bladder cancer were reported in these studies (Goodison et al., 2012; Chen et al., 2014). As a single marker it has specificity 80.5% and sensitivity 68.85%, whereas in panel reported by Goodison

Table 3. Post-test probability Calculation by Sequential Approach

SNO	Protein markers	pre-test probability %	pre-test probability	Positive Likelihood Ratio(LR+)	Pre- test odds	post-test odds	Post-test probability	Post- test probability %
1	Cyfra 21-1	12*	0.12	4.24	0.14	0.58	0.37	36.64
2	Cyfra 21-1, CA-9	36.6	0.37	3.53	0.58	2.04	0.67	67.08
3	Cyfra 21-1, CA-9, APE-1	67.08	0.67	4.00	2.04	8.15	0.89	89.07
4	Cyfra 21-1, CA-9, APE-1, COL13A1	89.07	0.89	2.38	8.15	19.39	0.95	95.10

*Pre-Test Probability, The prevalence of any type of bladder cancer reported among individuals with hematuria was 12% in Khadra et al study [8] and the same was considered for calculation of post-test probability. Positive likelihood ratio (LR+) is calculated as sensitivity/(100-specificity). Pre-test odds were calculated as Pre-test probability/(1- Pre-test probability). Post-test odds were calculated as Pre-test odds × LR+. Post- test probability was calculated as Post-test odds/ (Post-test odds+1).

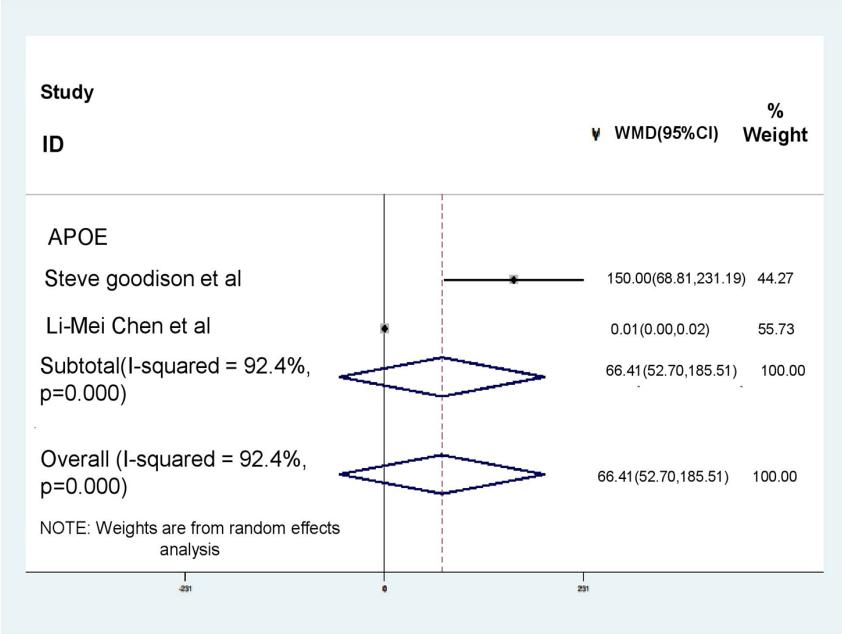

Figure 2. Meta-Analysis of Association between Levels of Urinary APO-E and Bladder Cancer. Forest plot detailing weighted mean difference (WMD) and 95% confidence interval for the association between levels of urinary APO-E and bladder cancer. Weights of the study are from random effect model. APO-E: Apolipoprotein E

(2012), specificity and sensitivity increased to 97 % and 92 % respectively.

Apurinic/apyrimidinic endonuclease 1 (APE-1) is a multifunctional redox signaling and DNA repair protein increased with unregulated cellular proliferation. Studies (Shin et al.,2015; Choi et al., 2016) reported an increased expression in the serum and significantly higher urinary levels of APE-1 of BC patients as compared to healthy controls. The levels not only correlated with tumor grade and stage but also were higher in patients with a history of recurrence. Used singly, it has a good combination of high specificity (79.6%), and high sensitivity (81.7%).

Collagen type 4A1 (COL4A1) is predominantly localized in the stroma around the tumor cells promoting angiogenesis and tumor progression. Collagen type 13A1 (COL13A1), a transmembrane protein expressed at cell-matrix junctions, supports vital oncogenic properties of tumor invasion and is strongly associated with poor clinical outcomes in human BC (Hagg et al., 1998; Miyake et al., 2017). A study reported significantly higher levels of COL4A1 and COL13A1 in bladder cancer cases as compared to healthy controls (Miyake et al., 2017). Among these two, COL13A1 had higher specificity (77.1%) than COL4A1 (68.9%).

Numerous biomarkers (IL-8, MMP9, SDC1, CCL-18, A1AT, ANG, MMP-10, APO-E, PAI-1, VEGF) have been reported by various studies with the varying specificity ranging from 51.9% to 75.2% and sensitivity from 68.2% to 88.5%. Using a panel with combination of markers will gives better specificity and sensitivity for screening as seen by Goodison (2012). We have analyzed a novel panel of four biomarkers (CYFRA 21-1, CA-9, APE-1, COL13A1) of which CYFRA 21-1, APE-1, and COL13A1 have never been studied as a panel. Inclusion of these biomarkers in analysis as a panel increased the post-test probability to 95.10%.

Significantly high levels of urinary Apolipoprotein E (APO-E) were reported in bladder cancer and it significantly differentiated high grade and low grade bladder cancer (Goodison et al., 2012; Chen et al., 2014). APO-E being a consistent marker in the panels studied by Goodison (2012), Urquidi (2012) and widely studied individually also, we did a pooled analysis which showed higher non-significant levels in bladder cancer cases. However, data should be interpreted with caution due to high heterogeneity between the studies. Though APO-E had good weight in the forest plot, it was not included in the panel of biomarkers because of its low specificity.

Using a panel of biomarkers is of greater utility than individual marker in screening of bladder cancer. The panel of four urinary biomarkers-CYFRA 21-1, CA-9, APE-1, COL13A1 with a high post-test probability of 95.10 % can be considered for screening of bladder cancer in patients presenting with hematuria. Our study has few limitations. There was high heterogeneity among different studies, due to differences in the study population, sample size and different number of patients in different stages of disease. Further validation has to be done in large sample size. The post-test probability using sequential panel approach was calculated using cumulative data available. However there may be differences in the post-test probabilities if the sequential panel approach is explored in laboratory settings.

Author Contribution Statement

Study concept and design: Aparna Varma Bhongir, Sangeetha Sampath, Gomathi Ramaswamy; Data acquisition: Rohit Kumar Bonthapally, Aparna Varma Bhongir, Sangeetha Sampath, Gomathi Ramaswamy,

Kiran Kumar Gudivada; Data analysis: Aparna Varma Bhongir, Sangeetha Sampath, Kiran Kumar Gudivada, Gomathi Ramaswamy; Drafting of manuscript: Rohit Kumar Bonthapally, Aparna Varma Bhongir, Sangeetha Sampath; Critical revision of the manuscript: Aparna Varma Bhongir, Sangeetha Sampath, Kiran Kumar Gudivada, Gomathi Ramaswamy.

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Ethical issue

Not applicable, as the study is a review/ meta-analysis.

Conflict of interest

The authors declare no conflict of interest.

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