REVIEW PAPER

Urine miRNA as a potential biomarker for bladder cancer detection – a meta-analysis

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Introduction White light cystoscopy (WLC), often supported by urine cytology, is considered the 'goldstandard' in the diagnosis and follow-up of bladder cancer (BCa). In recent years, urine microRNA (miRNA) tests have been performed for the detection of bladder cancer.
Material and methods A systematic review of the PubMed platform was performed by searching for articles in which miRNA in the urine was used for the detection of BCa.
Results The greatest sensitivity (86.6%) in BCa detection was achieved for multi-miRNA in urine sediment. The greatest specificity (85.3%) was achieved for multi-miRNA from voided urine. There were significant differences (p <0.01) between single-miRNA (OR 8.96; CI 6.37–12.59) and the multi-miRNA group (OR 19.95; CI 13.35–29.81). There were no differences among the specimens (voided urine, supernatant, sediment) used for the test.
Conclusions Urine miRNAs have the potential to be a valid marker for bladder cancer detection. They can successfully compete with other non-invasive diagnostic tests.

Key Words: bladder cancer ‹› miRNA ‹› microRNA ‹› biomarkers ‹› urine

INTRODUCTION

Bladder cancer (BCa) is the ninth most commonly diagnosed cancer worldwide and the 13th most frequent cause of death [1]. At the time of presentation, most patients have non-muscle invasive bladder cancer (NMIBC), which is characterized by high recurrence rates of up to 70% within five years of initial endoscopic treatment [2]. Gross, painless hematuria is the main symptom in 82.4% of patients with first-time BCa. Although 18.9% of patients with macroscopic hematuria will eventually be diagnosed with urinary tract malignancy, another 8.8% will have a benign disease (e.g. stone, inflammation) [3]. According to urological guidelines, all patients presenting with painless hematuria should undergo a full diagnostic evaluation to identify any potential malignancy of the urinary tract [4]. Currently, white

light cystoscopy (WLC), often supported by urine cytology, is considered the 'gold-standard' in the diagnosis and follow-up of BCa [5]. However, cystoscopy is a painful and expensive invasive procedure which is not free of complications, and one that requires a doctor, usually a specialist in urology. Despite its non-invasiveness and high specificity, urine cytology cannot be used as a sole test because it has low sensitivity, especially in detecting low grade tumors [6]. Cystoscopy and cytology have yet to be replaced in clinical practice, as the biomarkers investigated thus far offer insufficient sensitivity and specificity in detecting bladder malignancies [7]. BCa is characterized by a long survival period, and it has the highest cost per patient of all cancers from diagnosis to death due to the need for lifelong routine monitoring and treatment; much of these costs are spent on diagnosis and usually lifelong follow-up treatment [8].

The discovery of a comprehensive marker for BCa could not only lead to a reduction in the costs associated with the management of the disease, but also to an improvement in the care of patients.

MicroRNAs (miRNAs) are short (20–24 nucleotides), non-coding, single chain RNAs that occur in eukaryotes [9]. By binding to complementary sequences of mRNAs they play a role in the post-transcription regulation of gene expression [10]. MicroRNAs take part in many physiological and pathological processes like angiogenic signaling, cell proliferation and differentiation, apoptosis and tumorigenesis [11–14]. Some of them function as oncogenes, and others as tumor suppressor genes in cancers [15]. Both the pro- and anti-tumorigenic functions of miRNAs have also been observed in bladder cancer cells [16, 17, 18]. MicroRNA can be found in the tumor tissue itself and in body fluids like urine, blood, saliva and peritoneal fluid [19]. The acquisition of urine offers two key advantages for the patient: urine is easier and less invasive to obtain than blood, and its miRNA content may better represent the local stage of the disease due to it having direct contact with the tumor tissue in the urinary tract [20]. Due to their short length, miRNAs are less vulnerable to degradation than mRNA chains and can be stored for up to 48 hours at room temperature [21, 22].

The aim of the present article is to compare current studies in which the levels of miRNA in urine were assessed for their value in the detection of BCa and to determine their utility as a diagnostic tool.

MATERIAL AND METHODS

A systematic review of the PubMed platform was performed with the use of following terms: 'microRNA + bladder cancer + urine'. We included to the analysis original papers which assessed the utility of miRNA extracted from urine for BCa detection. The search was restricted to data published between 01.03.2010 and 28.02.2017. A total of 64 articles met the search criteria. Of those, the following articles were excluded: review articles, those whose methodology did not indicate which part of the urine was used for RNA extraction, those whose results did not detail the sensitivity or specificity for test assessment, and those in which miRNAs were not used for diagnostic purposes. This filtering stage rejected 48 articles. The references of the remaining 16 articles were searched for further relevant publications, resulting in the addition of one more study for the meta-analysis (Figure 1).

The meta-analysis and groups comparison was carried out using the statistical program PQStat version 1.6.4.121. The test probability was assumed to be significant at p <0.05 and test probability at p <0.01 was considered to be highly significant.

RESULTS

Table 1. summarizes the main characteristics (including the name of the first author, year of publication, number of patients and control group, specimen, evaluated miRNA, test sensitivity and specificity) of studies included in the meta-analysis. Of the 17 publications, seven used urine supernatant for DNA extraction, five used urine sediment and five used voided urine. In 17 articles, 29 studies evaluated the usefulness of single-miRNA profiling while another 9 used tests based on combinations of various miRNA (multi-miRNA).

The results of published studies were divided depending on the type of specimen used for miRNA extraction (voided urine, urine sediment or supernatant) and amount of profiled miRNAs (single vs. multi). For each subgroup, sensitivity and specificity was evaluated. The results are presented in Table 2 and Table 3. Sensitivity for BCa detection of single-miRNA testing was lower than the multi-miRNA test, 71.3% vs. 80.9% (p <0.05). Specificity also was in favor of multi-miRNA tests; however, the results were not statistically significant. miRNA tests performed from urine supernatant have greater (78.4%) sensitivity than voided urine (74.3%) and urine sediment (75.6%). At the same time urine supernatant has the highest specificity among all tests: 79.4% compared to 76.5% and 79.3%. An analysis of all subgroups revealed that greatest sensitivity (86.6%) was reached when only multi-miRNA in urine sediment was tested. The greatest speci-
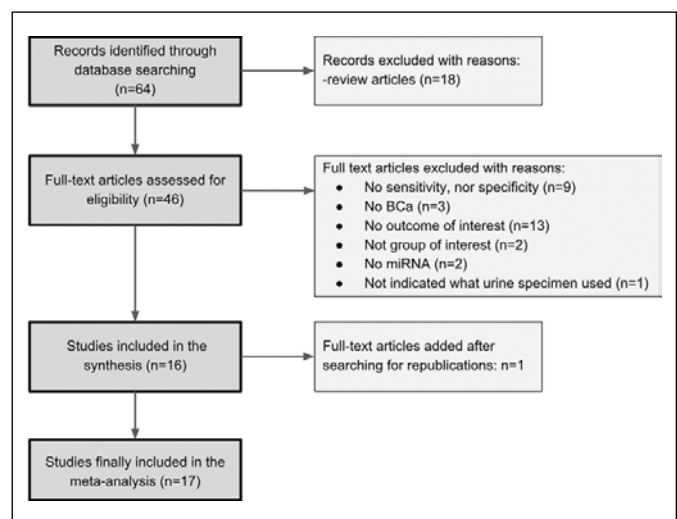
Figure 1. *Flow diagram outlining search results, final included and excluded studies.*

ficity (85.3%) was achieved for multi-miRNA from voided urine. A further meta-analysis comparing all 6 groups revealed that the odds ratio for BCa detection was highly significant (p <0.01; OR 5.87–28.66) (Figure 2) for all the groups; however, there were no statistically significant differences (p >0.05) among them.

When the influence of number of miRNAs used for the tests and kind of specimen were analyzed separately it revealed that there were significant differ-

Table 1. *Main characteristics of the studies included in the meta-analysis*

| Ref.	Author		Year	Specimen	No. patients	No. controls	miRNA	SN (%)	SP (%)
[23]	Zhou		2014	Supernatant	112	78	106-b	76.8	72.4
[24]	Pospisilova	1	2016	Supernatant	46	13	125	59.3	95.7
		2			46	13	99-a	74.1	82.6
[25]	Yun	1	2012	Supernatant	138	144	145	77.8	61.1
		2			69	144	145	84.1	61.1
[26]	Zhang X		2015	Supernatant	162	152	155	80.2	84.6
[27]	Wang		2015	Supernatant	192	169	214	90.5	65.6
[28]	Zhang D	1	2014	Supernatant	50	21	125-b	84.8	76.2
		2			50	21	99-a	78	85.7
		3			50	21	99-a/125-b	86.7	81.1
[29]	Long		2015	Supernatant	85	45	26-a/93/191/940	70	84
[30]	Yamada	1	2011	Sediment	100	74	96	71	89.2
		2			100	74	183	74	77.3
[31]	Eissa		2014	Sediment	94	90	96	72.3	88.9
[32]	Miah	1	2012	Sediment	68	53	135-b/15-b/1224-3p	94.1	51
		2			68	53	15a	51.7	72
					68	53	15b	67.8	81.3
					68	53	4-1	60	58.5
					68	53	27b	60.3	81.1
					68	53	100	60.4	78.7
					68	53	135b	71.2	74.4
					68	53	203	66.1	66
					68	53	212	54.2	64
					68	53	328	55.4	86.8
					68	53	1224-3p	75.9	82.4
[33]	Urquidi		2016	Sediment	61	60	combination of 25miRNA	87	100
[34]	Shimizu		2012	Sediment	86	20	137/124-2/124-3/9-3	81	89
[35]	Mengual		2013	Sediment	151	126	187/18a/25/142-3p/ 140-5p/204	84.4	86.5
[36]	Snowdon		2012	Voided urine	8	5	125-b/126	80	100
[37]	Tolle	1	2013	Voided urine	36	19	520e	70	63.2
		2			36	19	618	70	68.4
		3			36	19	1255b-5p	85	68.4
[38]	Dudziec	1	2010	Voided urine	68	53	152/212/328/1224-3p	81	75
		2			68	53	152	88	58
		3			68	53	212	40	66
		4			68	53	328	59	91
		5			68	53	1224-3p	84	58
[39]	Hanke		2010	Voided urine	29	11	126/152	72	82
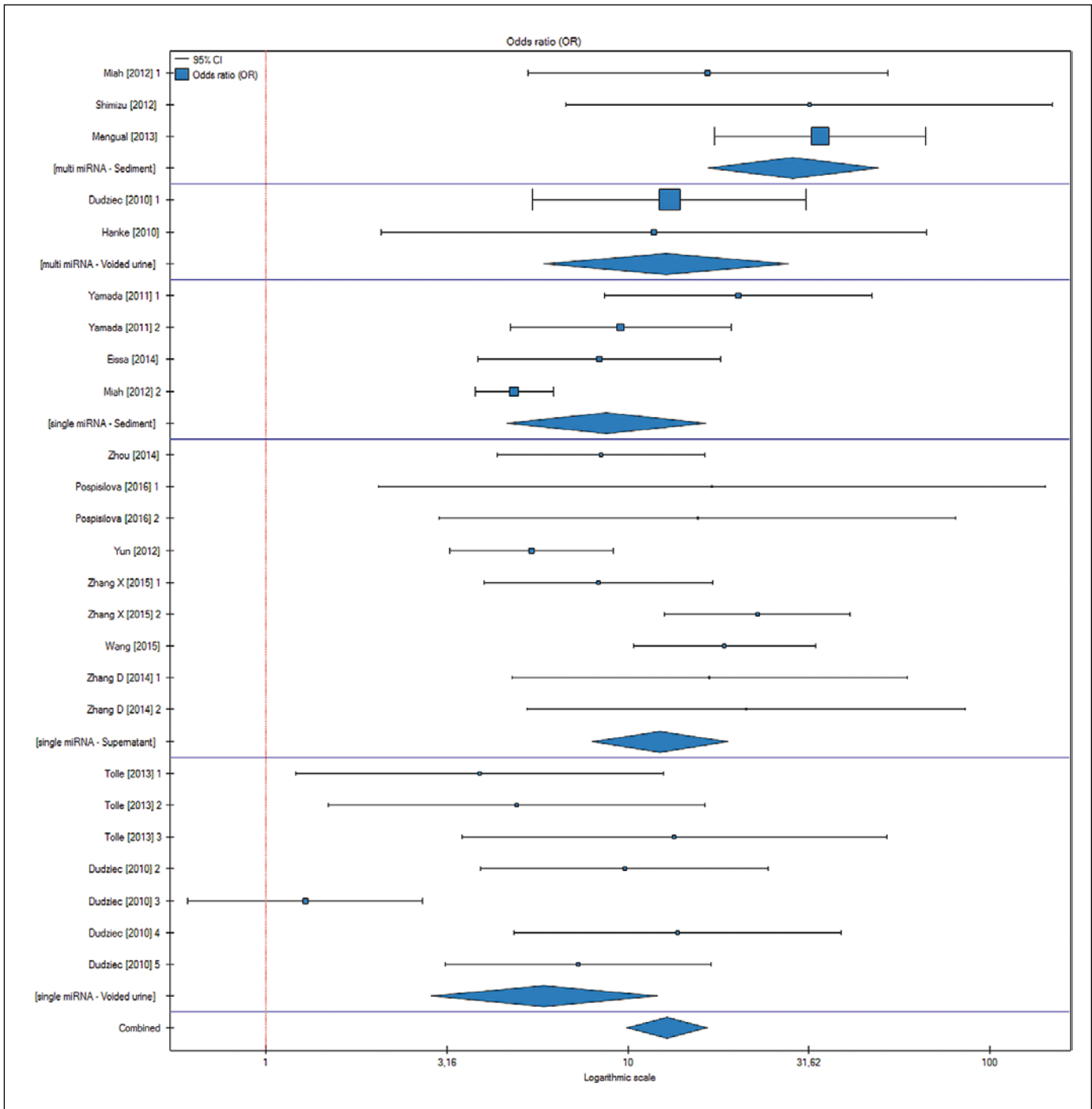
Figure 2. *Forrest plot comparing 6 groups of markers (each consisting of paired: number of miRNA used for the test and kind of urine specimen used) for risk of bladder cancer detection. See Table 1 for more information about the studies qualified for the analysis.*

ences (p <0.01) between single-miRNA (OR 8.96; CI 6.37–12.59) and multi-miRNA group (OR 19.95; CI 13.35–29.81) (Figure 3) for confirming the diagnosis of BCa. There were no differences in terms of the specimen (voided urine, supernatant, sediment) used for the test.

DISCUSSION

Although many biomarkers have been assessed as potential diagnostic tools of BCa, none of them have reached sufficient accuracy to replace cystoscopy and cytology [40]. As there is still a need to find a new
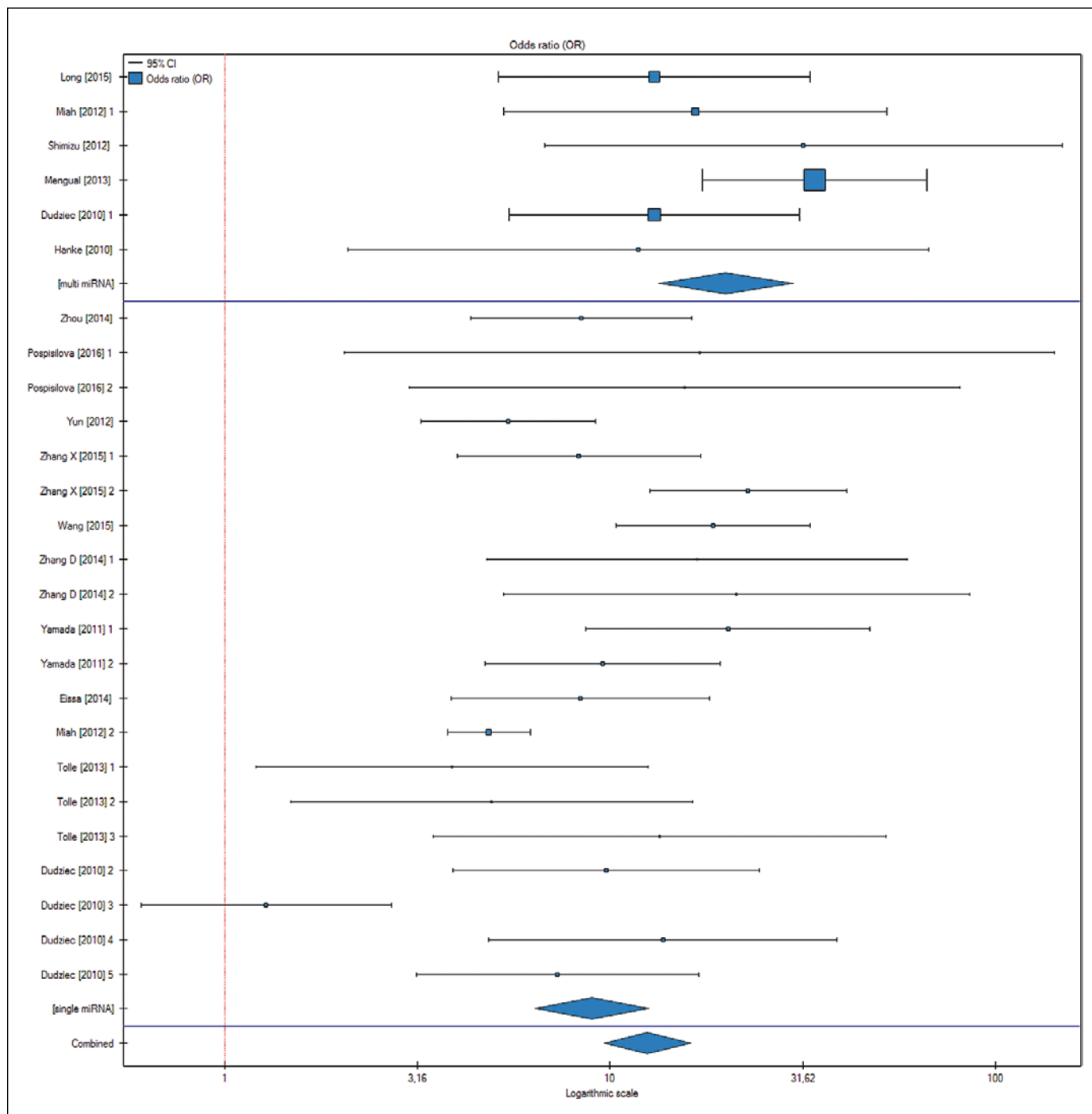
Figure 3. *Forrest plot comparing 2 groups of markers (single vs. multiple microRNA tests) for risk of bladder cancer detection. See Table 1 for more information about the studies qualified for the analysis.*

marker of tumorigenesis for BCa, further studies focusing on miRNA profiling have been conducted. The first report about altered miRNA expression in bladder cancer was published in 2007 [41]. The lack of invasiveness and stability of miRNA in body fluids (eg. urine) are the main advantages of using it as a potential biomarker [42].

The miRNA profiling in urine specimens has evolved over time. Since 2014, no original study has been performed using voided urine for miRNA detection, and recent years have seen growing interest in tests based on profiling miRNA from urine supernatant. This trend has arguably arisen in response to studies by Wang et al., which found that many cell types

are present in urine sediment, including renal tubular cells, urothelial cells, lymphocytes, erythrocytes and tumor cells, which may influence the received test results. As these cells are not present in urine supernatant, their miRNA is therefore free of such contamination [43, 44]. Despite the attempts to increase sensitivity and specificity by trying different specimens used for the test, it seems that this action is of less impact than number and kind of miRNA used for the analysis.

Our meta-analysis confirms that miRNA profiling performed from the three specimens does not differ between the groups (p = 0.23). The highest specificity (86.6%) was established for multi-miRNA from voided urine. The specificity levels may be even higher as miRNA alterations occur before the histological and morphological onset of malignancy, and these results in turn may be interpreted as false positive when only cystoscopy is used to check the obtained results [45].

The simultaneous increase of both sensitivity and specificity when single-miRNA tests are compared to multi-miRNA (Tables 2 and 3) may not be intuitive. A large proportion of the examined studies profile only an individual miRNA or a small combination thereof. In addition, most studies use different miRNAs for the profiling. Indeed, only three articles [28, 32, 38] evaluated the sensitivity and specificity of a few single miRNAs and then calculated how the results changed when a combination of previously-assessed single miRNAs was used for bladder cancer detection (a creation of multi-miRNA test from several single-miRNA tests). The direct comparison of these three studies revealed that sensitivity significantly increased from 71.4% for the single test to 87.1% for the multi-miRNA test, while specificity declined from 87.1% for the single-miRNA test to 66.1% for multi-miRNA test.

In the period from 2010 to 2013, the median sensitivity of elaborated miRNA tests for BCa detection was 71.6%. In more recent studies, dated 2014 to 2016, sensitivity was found to increase to 77.6%, and specificity also improved from 72.3% to 80.2%. This growing increase in the accuracy of miRNA tests is a good sign, as most of newly introduced biomarkers often lose their initially promising results in following studies [46].

It seems reasonable to compare the current sensitivity and specificity of miRNA tests with those demonstrated by some previously assessed markers. To this end, miRNA has been added to a ranking of bladder cancer biomarkers devised by Rhijn [46] (Tables 4 and 5). Cytology, with a median specificity of 94%, offers better specificity than the performed miRNA tests, as do microsatellite and UBC. The highest specificity obtained in multi-miRNA from voided urine ranks it in fourth place out of the 18 ranked tests. Tests based on a combination of different miRNAs extracted from urine sediment offer greater sensitivity for BCa detection than many other tests (86.6%). As noted in Table 5, CYFRA21-1 and Cytokeratin20 have only slightly worse results.

The high sensitivity and moderate specificity of miRNA tests indicate that they may be especially useful in the primary diagnosis of patients with a suspicion of urinary tract malignancy. When

Table 2. *Median sensitivity (SN) and specificity (SP) in single and multiple miRNA tests – overall and in 3 different specimens used for the test (voided urine, urine supernatant, and sediment)*

	Single miRNA			Multi miRNA		
	Voided urine	Supernatant	Sediment	Voided urine	Supernatant	Sediment
SN (%)	70.9	78.4	64.6	77.6	78.4	86.6
		Overall: 71.3			Overall: 80.9	
	Voided urine	Supernatant	Sediment	Voided Urine	Supernatant	Sediment
SP (%)	67.6	76.1	77	85.3	82.6	81.6
		Overall: 73.6			Overall: 83.1	

Table 3. *Median sensitivity (SN) and specificity (SP) in different urine specimens overall and when only single or multiple miRNAs were used for the study*

	Voided urine			Supernatant			Sediment		
	Single miRNA	Multi miRNA	Overall	Single miRNA	Multi miRNA	Overall	Single miRNA	Multi miRNA	Overall
SN (%)	70.9	77.6	74.3	78.4	78.4	78.4	64.6	86.6	75.6
SP (%)	67.6	85.3	76.5	76.1	82.6	79.4	77	81.6	79.3

Table 4. *The median sensitivity (SN) of bladder cancer (BCa) diagnostic tests. The numbers in white fields borrowed from Rhijn's systematic review [46]*

Marker	Median SN (%)	Range (min–max)
miRNA multi-sediment	86.6	81.4-94.1
CYFRA21-1	85	75–88
Cytokeratin20	85	79–87
Microsatellite	82	75–92
miRNA multi	80.9	70-94.1
FISH	79	70–86
miRNA supernatant	78.4	70–90.5
miRNA sediment	75.6	51.7–94.1
LewisX	75	68–79
miRNA voided urine	74.3	40-88
miRNA single	71.3	40–90.5
NMP22	71	47–100
BTA trak	71	60–83
ImmunoCyt	67	52–100
UBC	60	21–80
Cytometry	60	45–85
BTA stat	58	29–74
Cytology	35	13–75

BTA – bladder tumor antigen; FISH – fluorescence in situ hybridization; NMP22 – nuclear matrix protein 22; UBC – urinary bladder cancer antigen; CYFRA21-1 – cytokeratin 19 fragments

Table 5. *The median specificity (SP) of bladder cancer (BCa) diagnostic tests. The numbers in white fields borrowed from Rhijn's systematic review [46]*

Marker	Median SP (%)	Range (min–max)
Cytology	94	85–100
Microsatellite	89	79–100
UBC	87	72–95
miRNA multi-voided urine	85.3	75–100
LewisX	85	67–86
miRNA multi	83.1	51–100
CYFRA21-1	82	73–95
Cytometry	82	50–92
miRNA supernatant	79.4	61.1–95.7
miRNA sediment	79.3	51–100
miRNA voided urine	76.5	58–100
Cytokeratin20	76	76
ImmunoCyt	75	62–82
miRNA single	73.6	58–95.7
NMP22	73	55–98
BTA stat	73	56–86
FISH	70	66–93
BTA trak	66	60–79

BTA – bladder tumor antigen; FISH – fluorescence in situ hybridization; NMP22 – nuclear matrix protein 22; UBC – urinary bladder cancer antigen; CYFRA21-1 – cytokeratin 19 fragments

attempting to rule out the disease, credible negative results are necessary because they provide few false negatives. However, if the aim is cost reduction, a high positive predictive value (PPV) is desirable. To increase the PPV of a diagnostic tool, it is necessary to choose tests which provide greater specificity [47]. This in turn may lead to emerging two different kind of tests, one for evaluation of gross painless hematuria, and the second used in follow-up of BCa. While some of the markers (e.g. BTA stat, UBC) provide instant results, those which use miRNA require additional laboratory tests. With currently-available technology, such measurements can prove to be expensive, time-consuming and demanding; however, these limitations will slacken in the near future. For example, by analogy with human genome sequencing, the costs may be reduced by as much as 99.8% over the next 13 years [48]. However, even if these disadvantages are resolved, miRNA tests still need to undergo many validation steps (including the standardization of urine collection or RNA sequencing) before they can be used in everyday clinical practice. There are several limitations related to the methodology. Firstly, it should be taken into consideration that mainly positive results are being published. Studies that do not show the positive impact of the studied marker on disease diagnosis at initial phases of research are of less interest, which may be a cause of a shift in results. Secondly, the study only includes articles that state both the sensitivity and specificity of miRNAs on BCa detection, which may result in studies with both positive and negative effects on the results being omitted. Finally, each original study was performed by the use of a different miRNA or panel of miRNAs. Thus, a meta-analysis of all the articles gives only a general view of the topic, even though it may show future directions for research. What is considered to be one of the limitations of meta-analyses of miRNA studies is at the same time a great advantage of miRNA tests over the other markers (e.g. cytology), as researchers may add, remove or replace the miRNAs used in the diagnostic panel to optimize the test.

CONCLUSIONS

The results of our analysis show that urine miRNAs have the potential to act as a proper marker for bladder cancer detection. They can successfully compete with other non-invasive diagnostic tests. The chance of choosing many different miRNAs used in the panel,

adding the next ones to the existing tests to improve their sensitivity and specificity (which is not possible in 'traditional' tests) should be used to create a biomarker that can finally be accepted by the Urological society. Although many up-to-date studies have been conducted, there is still a need to perform clinical trials to validate the aforementioned data before miRNA use can be adopted in everyday clinical practice.

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