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TESIS DOCTORAL

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Barcelona, 2024

Empieza de una vez a ser quien eres, en vez de calcular quién serás.

Franz Kafka.

Agradecimientos

Al Prof. Morote por enseñarme a escribir e investigar, por no perder la paciencia conmigo y por darme las “collejas" necesarias para poder acabar este proyecto. Gracias por ser un ejemplo de tenacidad y “esforzarse en pescar en el Guadiana”.

Al Dr. Raventós por ser mi mentor, por enseñarme tantas cosas, por ser la persona más paciente del mundo.

Al Dr. Carrión por motivarme cada día y ser, además de compañero, un buen amigo.

A los residentes del servicio de Urología del hospital Vall d’Hebron, porque gracias a ellos no dejo de aprender cada día.

A Lali porque, sinceramente, sin ella este trabajo no habría sido posible.

A mis padres por enseñarme el valor de las cosas y darme oportunidades para llegar donde estoy.

A mi hermana por estar siempre ahí y entenderme, en las buenas y las malas situaciones.

A mi tía, porque me enseñaste a ser fuerte.

A Hugo y Eva por ser mi motivación diaria. Por sus sonrisas y por darme la energía necesaria para finalizar mi tesis.

A Irene e Isabel, por ayudarme en esos días de piscina y gimnasio.

A Maite, por ser todo en mi vida. Porque sin ella no sería lo que soy, porque cada día me enseña cómo ser un poco mejor. Porque me anima siempre que lo necesito, me riñe (aunque no siempre se me dé bien aceptarlo…) y me descubre nuevas cosas de la vida cada día. Gracias por estar siempre ahí y por compartir este proyecto vital conmigo.

Acrónimos

AG: Alto grado

AUA: *American Urological Association*

AUC: *Area under the curve*

BCG: Bacilo de Calmette-Guérin

BG: Bajo grado

Cis: Carcinoma *in situ*

CU: Carcinoma urotelial

CUETO: Club Urológico Español de Tratamiento Oncológico

EAU: *European Association of Urology*

EORTC: *European Organization for Research and Treatment of Cancer*

FDA: *Food and Drug Association*

LDA: *Linear Discriminant Analysis*

OMS: Organización Mundial de la Salud

RMNmp: Resonancia magnética multiparamétrica

RTU-V: Resección transuretral de vejiga

TC: Tomografía computarizada

TNM: *Tumor, Node and Metastasis*

TVNMI: Tumor vesical no músculo invasivo

US: Ultrasonidos

VI-RADS: *Vesical Imaging-Reporting and Data System*

VPN: Valor predictivo negativo

VPP: Valor predictivo positivo

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Resumen

1. Introducción

El carcinoma vesical es la séptima neoplasia más frecuente en humanos, y es causa de un importante consumo de recursos económicos. Su elevada tasa de recurrencias lo convierte en uno de los tumores más prevalentes. Tres de cada cuatro tumores vesicales son diagnosticados en estadio no músculo invasivo. En este contexto, las guías de la Asociación Europea de Urología (EAU) recomiendan un seguimiento riguroso y adaptado a grupos de riesgo de recurrencia y progresión específicos, utilizando cistoscopias y citologías de orina seriadas. No obstante, la cistoscopia es un método invasivo e incómodo para el paciente, que conlleva riesgos y una significativa afectación de su calidad de vida, siendo ésta una importante causa de pérdida de seguimiento. Por otro lado, la citología de orina es una prueba que presenta una baja sensibilidad y una importante variabilidad interobservador. Por lo tanto, en los últimos año años se ha producido un incremento exponencial en la investigación de alternativas más sensibles y menos invasivas en el seguimiento de estos tumores. En este sentido, los biomarcadores urinarios, especialmente aquellos basados en material genético, han surgido como una posible alternativa al *gold* estándar de seguimiento.

2. Hipótesis

La cuantificación en orina de micción espontanea de un biomarcador genético puede constituir una alternativa no invasiva al seguimiento convencional de seguimiento del tumor vesical no músculo invasivo (TVNMI), actualmente basado en la realización seriada de cistoscopia flexible y citología urinaria por lavado vesical.

3. Objetivos

Con la finalidad de contrastar la hipótesis previamente expuesta, se propuso el siguiente objetivo principal:

Establecer el papel de la determinación de un biomarcador genético en orina de micción espontanea en el contexto del seguimiento del TVNMI actualmente basado en la realización seriada de cistoscopia flexible y citología urinaria obtenida por lavado vesical.

Además de dicho objetivo principal, se establecieron los siguientes objetivos secundarios:

1. Realizar una revisión sistemática de la literatura para analizar el posicionamiento actual de los biomarcadores genéticos urinarios en el contexto del seguimiento del TVNMI y seleccionar el más apropiado entre ellos para realizar un estudio prospectivo.

2. Comparar la eficacia clínica del biomarcador genético seleccionado con el método estándar de seguimiento del TVNMI, basado en cistoscopia flexible y la citología urinaria por lavado vesical.

3. Evaluar puntos de corte alternativos al propuesto por el fabricante, que permitan adecuar su sensibilidad y especificidad al escenario clínico más seguro en el seguimiento de los TVNMI.

4. Evaluar el efecto anticipatorio del biomarcador genético seleccionado en el diagnóstico anticipado de las recurrencias del TVNMI.

4. Material y métodos

Este proyecto ha generado dos publicaciones y es presentado en forma de compendio.

La primera publicación, titulada "*Current status of genetic urinary biomarkers for surveillance of non-muscle invasive bladder cancer: a systematic review"*, tuvo como objetivo evaluar el papel de los biomarcadores genéticos en el seguimiento del TVNMI. Siguiendo los criterios PRISMA, identificamos 164 artículos originales, de los cuales seleccionamos finalmente 21, que recogen un total de 7.261 pacientes. La herramienta QUADAS-2 fue utilizada para analizar la calidad de los estudios incluidos.

En la segunda publicación, titulada "*Xpert Bladder Cancer Monitor for the early detection of non-muscle invasive bladder cancer recurrences. Could cystoscopy be substituted?"*, se realizó un estudio de cohortes, prospectivo, con 352 pacientes. A estos pacientes se les realizó el seguimiento convencional, que incluye cistoscopia flexible y citología por lavado. Se determinó el biomarcador en muestras de orina espontánea obtenida en el momento previo a la cistoscopia y se compararon los resultados con ambos métodos de seguimiento. Asimismo, se realizó seguimiento durante un año de todos los pacientes que fueron incluidos en el estudio con el objetivo de analizar el efecto anticipatorio de Xpert Bladder Monitor® (XBM).

5. Resultados

La revisión sistemática de la literatura reveló la existencia de diversos biomarcadores basados en DNA que analizaban diferentes mutaciones génicas, alteraciones de microsatélites y alteraciones epigenéticas. Por otro lado, se encontraron cinco artículos de biomarcadores basados en la detección de RNA. En términos generales, los biomarcadores actuales exhibieron valores predictivos negativos superiores al 90%, siendo incluso más elevados en el subgrupo de biomarcadores basados en RNA. Se evidenció que el marcador XBM presentaba un buen perfil de eficacia, aunque con un desarrollo clínico limitado.

En nuestro segundo artículo, obtuvimos una sensibilidad, especificidad y VPN de 69.4, 68.8 y 93.0%, respectivamente. En el caso de los tumores de alto grado (AG), dichos valores fueron del 63.6, 65.1 y 96.2%, respectivamente. En el análisis multivariante, XBM no pudo, por sí solo, predecir de manera significativa el riesgo de recurrencia. Durante el seguimiento anual después de la determinación de XBM, en los pacientes con resultado falso positivo (biomarcador positivo y resultados negativos de cistoscopia y citología) se evidenció un riesgo de recurrencia superior respecto a aquellos con biomarcador, cistoscopia y citología negativos. Finalmente, la modificación del punto de corte propuesto por el fabricante del biomarcador a 0.1294, logró obtener una sensibilidad de 96.3%, con un VPN de 92.1%, disminuyendo su especificidad a un 13.7%.

6. Conclusiones

1. Xpert Bladder Cancer Monitor® es un biomarcador genético que se cuantifica en orina obtenida en micción espontanea, candidato a ser comparado con el estándar de seguimiento actual del TVNMI, basado en cistoscopia y citología urinaria por lavado vesical.

2. A pesar de que Xpert Bladder Cancer Monitor® ofrece una elevada sensibilidad y valor predictivo negativo en el escenario de seguimiento de los TVNMI, su prometedor perfil de eficacia y seguridad oncológica no permite sustituir la cistoscopia y citología de orina como pruebas estándar de diagnóstico de recurrencias.

3. La modificación del punto de corte de Xpert Bladder Cancer Monitor® utilizando un valor inferior al prestablecido por el fabricante mejora la detección de recurrencias, equiparándose a la cistoscopia y citología, pero aumentando el número de exploraciones innecesarias por la pérdida de especificidad del test.

4. Xpert Bladder Cancer Monitor® presenta un efecto anticipatorio que permite identificar a los pacientes con un riesgo más elevado de desarrollar un carcinoma urotelial en el siguiente año de seguimiento y que, por tanto, precisan una vigilancia más exhaustiva.

5. En resumen, y para concluir nuestro objetivo principal, creemos que XBM no puede sustituir el método actual de seguimiento del TVNMI de manera integral. Sin embargo, presenta un efecto anticipatorio en el diagnóstico de la recurrencia de alto grado del TVNMI muy prometedor. Son necesarios estudios prospectivos y aleatorizados que permitan definir la complementariedad entre XBM y el método estándar de seguimiento para realizar un seguimiento menos invasivo, eficiente y oncológicamente seguro.

Abstract

1. Introduction

Bladder carcinoma is the seventh most common neoplasm in humans and is a significant driver of economic resources. Its high recurrence rate makes it one of the most prevalent tumors. Three out of every four bladder tumors are diagnosed at a non-muscle-invasive stage. In this context, the guidelines of the European Association of Urology (EAU) recommend rigorous follow-up tailored to specific risk groups, using cystoscopies and urine cytologies. However, cystoscopy is an invasive and uncomfortable method for the patient, involving a series of risks and a significant impact on their quality of life, which is a major cause of loss of follow-up. On the other hand, urine cytology is a test with low sensitivity and significant interobserver variability. Therefore, in recent years, there has been an exponential increase in the research of more sensitive and less invasive alternatives for monitoring these tumors. In this regard, urinary biomarkers, especially those based on genetic material, have emerged as a possible alternative to the gold standard of monitoring.

2. Hypothesis

The The quantification of a genetic biomarker in spontaneous urine may represent a non-invasive alternative to the conventional follow-up of non-muscle-invasive bladder tumor (NMIBC), currently based on serial flexible cystoscopy and bladder washing urinary cytology.

3. Objectives

In order to test the previously stated hypothesis, the following main objective was proposed:

To establish the role of determining a genetic biomarker in spontaneous urine in the context of the follow-up of non-muscle-invasive bladder tumors (NMIBC), currently based on serial flexible cystoscopy and urinary cytology obtained by bladder washings.

In addition to this main objective, the following secondary objectives were established:

1. Conduct a systematic literature review to analyze the current positioning of urinary genetic biomarkers in the context of NMIBC follow-up and select the most appropriate one for a prospective study.

2. Compare the clinical efficacy of the selected genetic biomarker with the standard method of NMIBC follow-up, based on flexible cystoscopy and urinary cytology with bladder washings.

3. Evaluate alternative cutoff points to those proposed by the manufacturer, allowing for the adjustment of sensitivity and specificity to the safest clinical scenario in the follow-up of NMIBC.

4. Assess the anticipatory effect of the selected genetic biomarker in the early diagnosis of NMIBC recurrences.

4. Materials and methods

This project has resulted in two publications and is presented in the form of a compendium.

The first publication, titled "Current status of genetic urinary biomarkers for surveillance of non-muscle invasive bladder cancer: a systematic review," aimed to assess the role of genetic biomarkers in the surveillance of non-muscle invasive bladder cancer (NMIBC). Following PRISMA guidelines, we identified 164 original articles, ultimately selecting 21 with 7,261 patients. The QUADAS-2 tool was used to analyze the quality of the included studies.

In the second publication, titled "Xpert Bladder Cancer Monitor for the early detection of non-muscle invasive bladder cancer recurrences. Could cystoscopy be substituted?" a prospective cohort study was conducted with 352 patients. These patients underwent conventional follow-up, including flexible cystoscopies and cytologies with bladder washings. The biomarker was determined in samples of spontaneous urine obtained just before cystoscopy, and the results were compared with both follow-up methods. Additionally, all patients were followed for one year to analyze the anticipatory effect of Xpert Bladder Cancer Monitor (XBM).

5. Results

The systematic review of the literature revealed the existence of various DNA-based biomarkers analyzing different gene mutations, microsatellite alterations, and epigenetic changes. On the other hand, five articles on biomarkers based on RNA detection were found. In general, current biomarkers exhibited negative predictive values exceeding 90%, with even higher values in the subgroup of RNA-based biomarkers. It

was evident that the Xpert Bladder Cancer Monitor (XBM) marker had a good efficacy profile, albeit with limited clinical development.

In our second article, we obtained a sensitivity, specificity, and negative predictive value (NPV) of 69.4%, 68.8%, and 93%, respectively. For high-grade tumors (HG), these values were 63.6%, 65.1%, and 96.2%, respectively. In the multivariate analysis, XBM alone could not significantly predict the risk of recurrence. During the annual follow-up after determining XBM, patients with false-positive results (positive biomarker and negative results in cystoscopy and cytology) showed a higher risk of recurrence in this group compared to those with negative biomarker, cystoscopy, and cytology results. Finally, by modifying the biomarker's cutoff point to 0.1294, we achieved a sensitivity of 96.3%, with an NPV of 92.1%, at the expense of reducing its specificity to 13.7%.

6. Conclusions

1. Xpert Bladder Cancer Monitor® is a genetic biomarker quantified in spontaneously voided urine, a candidate to be compared with the current follow-up standard for non-muscle-invasive bladder tumors (NMIBC), based on cystoscopy and urinary cytology with bladder washings.

2. Despite Xpert Bladder Cancer Monitor® offering high sensitivity and negative predictive value in the NMIBC follow-up scenario, its promising efficacy and oncological safety profile do not allow for the replacement of cystoscopy and urinary cytology as standard tests for diagnosing recurrences.

3. Modifying the cutoff point of Xpert Bladder Cancer Monitor® using a value lower than the manufacturer's preset improves recurrence detection, equating it to

cystoscopy and cytology, but increases the number of unnecessary examinations due to a loss of test specificity.

4. Xpert Bladder Cancer Monitor® exhibits an anticipatory effect that enables the identification of patients at higher risk of developing urothelial carcinoma in the following year of follow-up, requiring more comprehensive monitoring.

5. In summary, and to conclude our main objective, we believe that XBM cannot replace the current NMIBC follow-up method comprehensively. However, it shows a very promising anticipatory effect in diagnosing high-grade NMIBC recurrence. Prospective and randomized studies are needed to define the complementarity between XBM and the standard follow-up method for a less invasive, efficient, and oncologically safe monitoring approach.

1. Introducción

1.1 Epidemiología y etiología del cáncer de vejiga

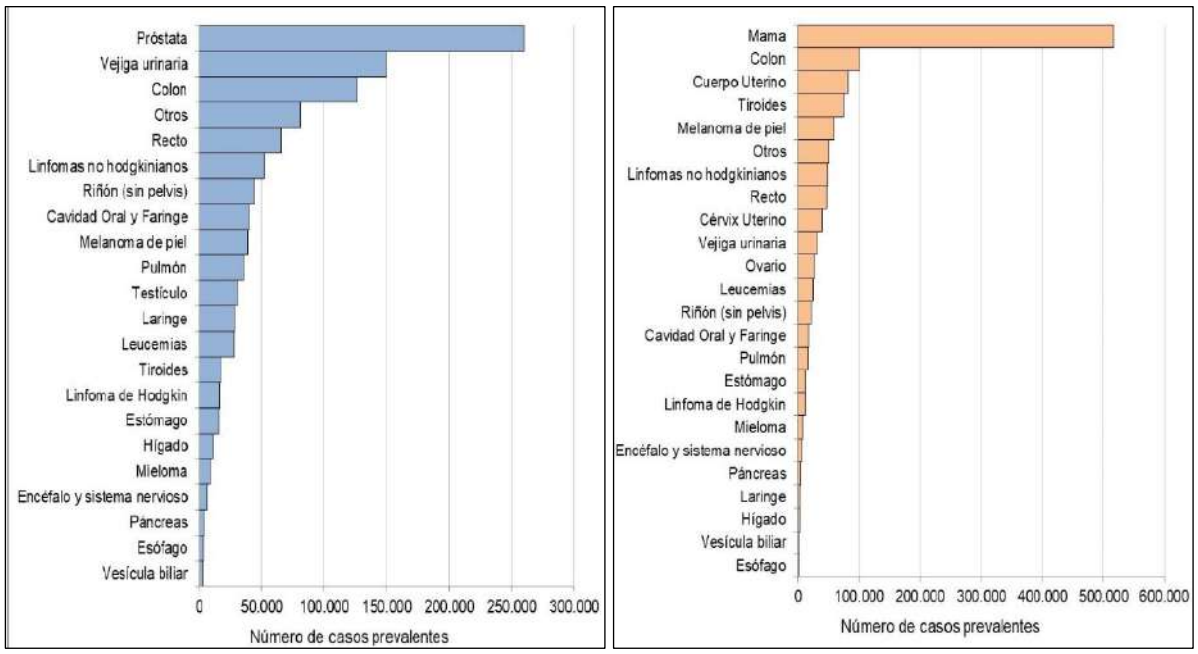
El tumor vesical (TV) es globalmente la sexta neoplasia más frecuente en el varón, la decimoséptima en mujeres y la décima si consideramos ambos géneros. Su incidencia anual es de 573.278 nuevos casos, resultando en 212.536 fallecimientos por esta causa en 2020 (1). Europa posee la incidencia más elevada a nivel mundial, con 11 casos por cada 100000 habitantes. En España, la incidencia estandarizada por edad es una de las más altas a nivel mundial, alcanzando los 39 casos por cada 100000 habitantes (2,3).

En el contexto español, la mortalidad atribuida al cáncer vesical es de 12 fallecimientos por cada 100.000 habitantes, con notables diferencias entre géneros. La mortalidad en varones alcanza una tasa de 8.1 defunciones por cada 100.000 varones con tumor vesical, situándose entre las más elevadas de Europa (4).

Aproximadamente el 80% de los pacientes diagnosticados con cáncer de vejiga presentan tumores no músculo invasivos (TVNMI), mientras que el resto tienen lesiones músculo invasivas. A pesar de no ser una neoplasia de alta incidencia, su singularidad radica en que es el segundo tumor más prevalente en el varón debido a la elevada tasa de recurrencias del TVNMI (1), que puede llegar hasta el 50% aún con adecuadas indicaciones de tratamiento radical (5).

Por esta razón, se recomienda un seguimiento intensivo con cistoscopias y citologías seriadas, adaptado al grado y el estadio patológico de la enfermedad. La figura 1 recoge la prevalencia de los diferentes cánceres en varones y mujeres en España, siendo el tumor vesical uno de los diez más prevalentes en ambos sexos.

Figura 1. Prevalencia total del cáncer (número de casos) en ambos géneros. España, diciembre de 2020, varones (gráfico izquierdo) y mujeres (gráfico derecho) (6)



El consumo de tabaco se erige como el factor de riesgo preeminente y ampliamente reconocido en el desarrollo del TV, contribuyendo en cerca del 60% de los casos en hombres y el 30% en mujeres (5,7). Diversos estudios han establecido la relación causal del tabaco en la carcinogénesis vesical, especialmente entre individuos expuestos de forma pasiva, incluso al considerar cuidadosamente posibles sesgos y factores de confusión (8). La literatura existente destaca que el riesgo asociado al desarrollo de un TV se ve exacerbado por la intensidad y la duración del hábito tabáquico (9).

En segundo lugar, la exposición laboral a aminas aromáticas, hidrocarburos policíclicos aromáticos y otros hidrocarburos emerge como el segundo factor de riesgo para el desarrollo de un TV, atribuyéndose a esta causa un 10% de los casos (7,10,11). Esta exposición ocupacional suele manifestarse en entornos industriales como plantas procesadoras de pinturas, tintes, metal y productos derivados del petróleo.

Adicionalmente, se han identificado otros factores de riesgo vinculados al desarrollo del carcinoma vesical. La infección por *Schistosoma haematobium* (12), se asocia de manera característica con el adenocarcinoma vesical, una histología poco frecuente de TV. Asimismo, la exposición vesical a radiaciones ionizantes (13) se perfila como un factor de riesgo relevante en este contexto.

1.2 Diagnóstico del tumor vesical

En nuestro entorno, más del 85% de los pacientes diagnosticados con un TV presentan algún signo o síntoma, siendo la hematuria el predominante, detectado hasta en un 90.8% de los casos (2).

Dentro de las exploraciones complementarias indicadas en el caso de sospecha de TV, destacan principalmente tres grandes grupos:

- Pruebas de imagen: Dentro de este grupo se incluyen los ultrasonidos (US), la tomografía computarizada (TC) y la resonancia magnética multiparamétrica (RMNmp). Los US convencionales desempeñan un papel útil en la valoración inicial de la vejiga debido a su inocuidad, fácil accesibilidad, reproductibilidad y rapidez, especialmente cuando se combinan con las nuevas versiones tridimensionales (14,15). Aunque su eficacia clínica es limitada, las nuevas técnicas, que incorporan el uso de contraste, parecen mejorar las tasas de detección de neoplasias vesicales, alcanzando aproximadamente el 95% en lesiones de más de 5mm (16). El TC se usa para detectar lesiones exofíticas en todo el tracto urinario, siendo identificadas como defectos de repleción y/o hidronefrosis (17). No obstante, la necesidad de realizar esta prueba de

forma basal a todos los pacientes es dudosa, dada la baja incidencia de hallazgos patológicos (18,19). Por su parte, la RMNmp ha experimentado un crecimiento exponencial como prueba de imagen para el diagnóstico del TV en los últimos años. Este progreso se atribuye a las mejoras en la precisión de la imagen y a la aparición de la clasificación VI-RADS (*Vesical Imaging-Reporting and Data System*) que permite estandarizar la metodología en la descripción de los hallazgos patológicos (20). De hecho, una revisión sistemática reciente indica que la clasificación VI-RADS presenta una buena correlación interobservador y un buen perfil de sensibilidad y especificidad para poder distinguir entre tumores músculo invasivos y no músculos invasivos (21).

- Citología de orina: La citología urinaria, tanto por micción espontánea como por lavado vesical, examina las células exfoliadas por el urotelio y tiene una sensibilidad del 44% y una especificidad del 96%, con un valor predictivo positivo (VPP) alrededor del 90% (22). Presenta una mayor sensibilidad para tumores de alto grado (AG), pero es más limitada en los tumores de bajo grado (BG), con valores entre el 4 y 31% (23). Para el carcinoma in situ (Cis), la sensibilidad es bastante variable en función de la publicación, pero se sitúa entre el 28 y 100% (23). La citología de orina es un buen complemento a la cistoscopia, y su combinación es superior a la cistoscopia aislada en la detección de tumores de AG y tumores del tracto urinario superior (24). No obstante, una citología positiva indica la probable presencia de un tumor urotelial en cualquier localización del tracto urinario, lo que requiere la elaboración de algoritmos diagnósticos para identificar el origen de dicha neoplasia (25). En cambio, una citología negativa no descarta la presencia de una neoplasia urotelial y resulta insuficiente por sí sola en el seguimiento de los TVNMI. La interpretación de la citología es dependiente del patólogo (26), y puede verse afectada por diversas condiciones “benignas” como la

presencia de litiasis, infecciones urinarias o tratamientos intravesicales (27). Recientemente, se ha desarrollado y actualizado una nueva clasificación con el objetivo de homogeneizar y estandarizar el informe citopatológico, llamado Sistema Paris (28), ver Figura 2. No obstante, su estandarización no ha logrado mejorar de manera significativa el perfil de la citología.

Figura 2: Categorías diagnósticas del Sistema Paris para reportar citología urinaria (29).

1	Nondiagnostic/unsatisfactory
2	Negative for high-grade urothelial carcinoma (NHGUC)
3	Atypical urothelial cells (AUC)
4	Suspicious for high-grade urothelial carcinoma (SHGUC)
5	High-grade urothelial carcinoma (HGUC)
6	Low-grade urothelial neoplasm (LGUN)
7	Other: primary and secondary malignancies and miscellaneous lesions

- Cistoscopia: Este procedimiento, generalmente realizado con instrumental flexible, es ambulatorio y se lleva a cabo con el uso de lubricante anestésico intrauretral para mejorar la tolerabilidad (30). A pesar de su naturaleza rutinaria, se trata de un procedimiento invasivo y puede acarrear una serie de efectos secundarios como disuria, hematuria, aumento en el riesgo de infecciones urinarias y la posibilidad de desarrollar estenosis uretral (31). De hecho, los pacientes informan que la sintomatología puede persistir más allá de siete días hasta en el 21% de los casos (32).

Una vez obtenido el diagnóstico clínico, el siguiente paso es la confirmación patológica mediante el tratamiento quirúrgico transuretral. La resección transuretral de vejiga (RTU-V) tiene como objetivo obtener un adecuado diagnóstico y un estadiaje patológico preciso, así como eliminar todas las lesiones visibles. Es un procedimiento crucial en el tratamiento del TVNMI, y la calidad de la resección tiene un impacto directo en la persistencia de enfermedad residual, recurrencia temprana e infraestadiaje tumoral (33). Durante la cirugía, una exhaustiva evaluación del número, la localización, la morfología, el tamaño y la calidad de la resección tumoral, así como la valoración de complicaciones, especialmente la perforación vesical, son fundamentales para el pronóstico de los pacientes (34).

A pesar de la estandarización de la técnica, se ha demostrado que el riesgo de tumor residual después de la cirugía es considerable (35). Revisiones sistemáticas indican que estas tasas de persistencia pueden llegar hasta el 51% e incluso mostrar un 8% de infraestadiaje en tumores T1 (36). Por lo tanto, en pacientes con una RTU-V inicial incompleta (debido a complicaciones intraoperatorias, complejidad de la resección o la extensión del tumor), en ausencia de músculo detrusor en el resultado patológico (salvo en Ta de bajo grado y Cis) y en todos los T1, está indicada una segunda resección transuretral en las siguientes 2 a 6 semanas posteriores a la primera intervención (37). Esta segunda resección ha demostrado mejorar la supervivencia libre de recurrencia, optimizar los resultados del tratamiento intravesical y proporcionar información sobre el pronóstico de los pacientes (38–40).

1.3. Estadiaje clínico-patológico y sistemas de clasificación

Los tumores confinados a la mucosa y aquellos que invaden la lámina propia se clasifican como Ta y T1, respectivamente, de acuerdo con la clasificación *Tumor, Node and Metastasis* (TNM) (Figura 3). Los tumores intraepiteliales de alto grado confinados a la mucosa

se clasifican como Cis. Este subgrupo de neoplasias vesicales se agrupa dentro de la categoría de TVNMI (Figura 4).

Figura 3: Clasificación TNM del carcinoma vesical (41).

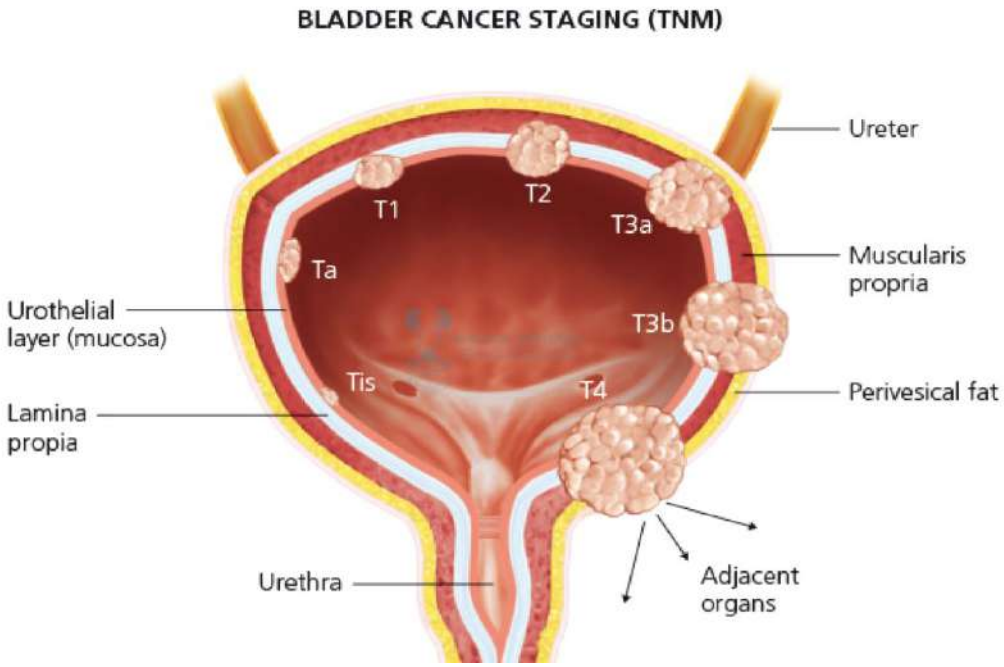
T - Primary tumour	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Ta	Non-invasive papillary carcinoma
Tis	Carcinoma *in situ*: 'flat tumour'
T1	Tumour invades subepithelial connective tissue
T2	Tumour invades muscle
	T2a Tumour invades superficial muscle (inner half)
	T2b Tumour invades deep muscle (outer half)
T3	Tumour invades perivesical tissue
	T3a Microscopically
	T3b Macroscopically (extravesical mass)
T4	Tumour invades any of the following: prostate stroma, seminal vesicles, uterus, vagina pelvic wall, abdominal wall
	T4a Tumour invades prostate stroma, seminal vesicles, uterus or vagina
	T4b Tumour invades pelvic wall or abdominal wall
N – Regional lymph nodes	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single lymph node in the true pelvis (hypogastric, obturator, external iliac, or presacral)
N2	Metastasis in multiple regional lymph nodes in the true pelvis (hypogastric, obturator, external iliac, or presacral)
N3	Metastasis in common iliac lymph node(s)
M - Distant metastasis	
M0	No distant metastasis
	M1a Non-regional lymph nodes
	M1b Other distant metastases

Los TVNMI constituyen aproximadamente el 75% de todos los tumores diagnosticados (4) y pueden ser tratados mediante RTU-V. Este grupo de neoplasias es heterogéneo, caracterizado por la ausencia de afectación de la capa muscular de la vejiga; no obstante, su pronóstico en cuanto a riesgo de recurrencia y progresión final viene determinado por una serie de variables que incluyen el estadio patológico, el grado celular y otros factores clínicos e

histopatológicos. El esquema de la afectación neoplásica de las diferentes capas de la vejiga se representa en la figura 4.

En función de estas variables, el manejo de la enfermedad puede incluir el uso de tratamiento intravesical adyuvante en función de la clasificación de grupos pronósticos de riesgo, con el objetivo de disminuir las tasas de recurrencia y progresión (42,43).

Figura 4: Representación del estadio patológico local en el carcinoma vesical. Los tumores Ta-Tis y T1 son considerados no músculos invasivos. El resto (T2-T4) pertenecen a la categoría de músculo invasivos (44).

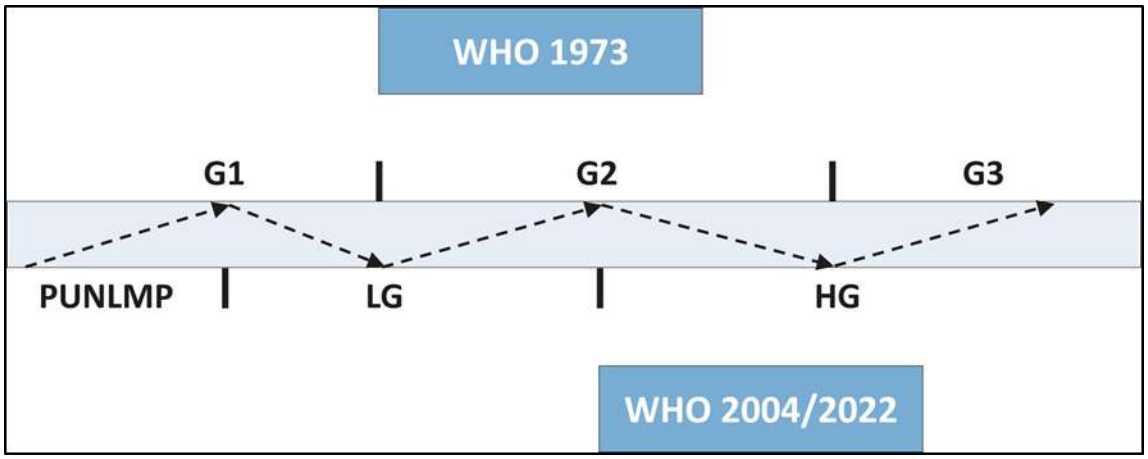


1.4 Grupos de riesgo del tumor vesical no músculo invasivo

En 2004, la Organización Mundial de la Salud (OMS) presentó una clasificación histológica de los carcinomas vesicales que diferenciaba entre bajo grado y alto grado. Este sistema fue actualizado en la clasificación 2016/2022 de la OMS (45,46), estratificando a los

pacientes más allá de las categorías clásicas de 1973, que distinguían entre grado 1 (G1), grado 2 (G2) y grado 3 (G3) (47,48), según se muestra en la Figura 5.

Figura 5: Estratificación de los tumores según ambas clasificaciones, OMS 1973 y OMS 2004/2022 (49)



Múltiples estudios, incluyendo una revisión sistemática y un metaanálisis, han demostrado que la clasificación de 2004/2016 mejora la capacidad de predicción de recurrencia y progresión en comparación con la de 1973 (50). A pesar de ello, un análisis realizado en más de 5000 pacientes con TVNMI evidenció que ambas clasificaciones eran capaces de predecir la progresión, pero no el riesgo de recurrencia. En este estudio, la clasificación de la OMS de 1973 mostró una mayor capacidad pronóstica de progresión que la clasificación de la OMS de 2004/2016 (51).

Por otro lado, se observa una importante variabilidad entre patólogos, especialmente en el diagnóstico de Cis, donde la concordancia entre ellos se limita al 70-78% en la mayoría de los estudios (52). También existe variabilidad en la clasificación de T1 y Ta, así como en el grado tumoral, con porcentajes de conformidad entre el 50-60% (53–55). No obstante, la clasificación de la OMS 2004/2016 parece ofrecer una mejor reproductibilidad que la de 1973

(56). Dada esta variabilidad, a nivel clínico, actualmente solo se utiliza la clasificación 2004/2022 de la OMS, quedando en desuso la de 1973. No obstante, en caso de ser posible, se recomienda reportar el grado según ambas clasificaciones.

Existen diversas clasificaciones predictivas, siendo las más utilizadas:

1.4.1 Modelo de puntuación basado en la clasificación 1973 de la OMS

- Modelo de la *European Organization for Research and Treatment of Cancer* (EORTC) (57): este sistema se fundamenta en seis factores clínico-patológicos significativos, que incluyen el número de tumores, el diámetro tumoral, la tasa de recurrencia previa, el estadio T, la presencia de Cis concurrente y el grado tumoral según la clasificación de la OMS de 1973. Existe una herramienta en línea que permite calcular la probabilidad de recurrencia y progresión a 1 y 5 años de manera individualizada (https://www.omnicalculator.com/health/eortc-bladder-cancer).

- Modelo para pacientes tratados con Bacilo Calmette-Guérin (BCG) del Club Urológico Español de Tratamiento Oncológico (CUETO): este modelo, basado en pacientes tratados con al menos 12 dosis de BCG intravesical, evalúa el riesgo de recurrencia y progresión a 1 y 5 años. Las variables incluidas son similares a las del modelo de la EORTC, como el género, la edad, el número de tumores, la tasa de recurrencia previa, el estadio T, el Cis concurrente y el grado tumoral según la clasificación de la OMS de 1973. Se destaca que la

probabilidad de progresión es menor en el grupo de alto riesgo por el probable efecto de la BCG (58,59).

- Modelo de la EORTC de 2016 para pacientes tratados con mantenimiento con BCG: este modelo se centra en pacientes tratados con BCG durante 1-3 años. Las principales variables pronósticas para la recurrencia son la tasa de recurrencia previa y el número de tumores. En cuanto al riesgo de progresión, el estadio patológico y el grado según la clasificación de la OMS de 1973 son determinantes, mientras que la edad y el grado nuevamente según la clasificación de la OMS de 1973 son relevantes para la supervivencia global. A través de estos datos, se obtuvieron nomogramas para pacientes tratados con BCG (60).

1.4.2 Modelo de puntuación basado en la clasificación 1973 de la OMS y la de 2004/2016

- Modelo de la EAU de tumores no músculo invasivos de 2021: a través del análisis de más de 3000 pacientes sin tratamiento con BCG, se identificaron variables independientes predictoras de progresión, que incluían el estadio tumoral, la clasificación de grado de la OMS de 1973, la clasificación de grado de la OMS de 2004/2022, la presencia de Cis, el número de tumores, l tamaño tumoral y la edad (61). Este modelo no es aplicable para calcular el riesgo de recurrencia tumoral.

Teniendo en cuenta todas estas variables, se han elaborado unas recomendaciones para

poder adaptar el tratamiento y seguimiento de los pacientes. Las guías de la EAU recomiendan estratificar los pacientes en grupos de riesgo en función de la probabilidad de progresión a enfermedad músculo invasiva (62). La clasificación ha experimentado modificaciones desde 2019 en respuesta a nueva evidencia científica. En la versión más reciente, se han incorporado tres criterios clínicos adicionales: edad ≥ 70 años, multiplicidad y tamaño ≥ 3 cm. Esto ha consolidado la categoría de "muy alto riesgo", donde la cistectomía radical se establece como el *gold* estándar (63).

Por ello, los grupos de riesgo de las guías de la EAU son cuatro, tal y como consta en la Figura 6.

Figura 6. Grupos de riesgo propuestos por las guías de la EAU de 2023 (64)

Risk group	
Low Risk	• A primary, single, TaT1 LG/G1 tumour < 3 cm in diameter without CIS in a patient ≤ 70 years
	• A primary Ta LG/G1 tumour without CIS with at most ONE of the additional clinical risk factors
Intermediate Risk	Patients without CIS who are not included in either the low-, high-, or very high-risk groups
High Risk	• All T1 HG/G3 without CIS, EXCEPT those included in the very high-risk group • All CIS patients, EXCEPT those included in the very high-risk group
	Stage, grade with additional clinical risk factors: • Ta LG/G2 or T1G1, no CIS with all 3 risk factors • Ta HG/G3 or T1 LG, no CIS with at least 2 risk factors • T1G2 no CIS with at least 1 risk factor
Very High Risk	**Stage, grade with additional clinical risk factors:** • Ta HG/G3 and CIS with all 3 risk factors • T1G2 and CIS with at least 2 risk factors • T1 HG/G3 and CIS with at least 1 risk factor • T1 HG/G3 no CIS with all 3 risk factors

Additional clinical risk factors are:
- age > 70;
- multiple papillary tumours;
- tumour diameter > 3 cm.

Es importante señalar que en esta clasificación no se tienen en cuenta ciertas variables clínico-patológicas relevantes. La ausencia de inclusión de factores como la presencia de Cis (primario), el número de recurrencias tumorales, variantes histológicas o la invasión

linfovascular pueden ser una limitación. Estos factores deberían ser considerados y valorados en la categoría de muy alto riesgo (65).

Las categorías de riesgo establecidas por la EAU son cruciales para evaluar el riesgo de recurrencia y progresión en pacientes con diagnóstico de tumor vesical. Sin embargo, es esencial destacar que dentro de estas categorías el porcentaje puede variar, abarcando un rango desde el 1% hasta el 40% en ambos casos. La probabilidad de progresión para los cuatro grupos de riesgo a 1, 5 y 10 años según esta nueva clasificación, se presenta en la Figura 7.

Figura 7. Probabilidad de progresión en función de los grupos de riesgo de la EAU (64).

Risk group	*Probability of Progression and 95% Confidence Interval (CI)*		
	1 Year	5 Years	10 Years
New Risk Groups with WHO 2004/2016			
Low	0.06% (CI: 0.01%–0.43%)	0.93% (CI: 0.49%–1.7%)	3.7% (CI: 2.3%–5.9%)
Intermediate	1.0% (CI: 0.50%–2.0%)	4.9% (CI: 3.4%–7.0%)	8.5% (CI: 5.6%–13%)
High	3.5% (CI: 2.4%–5.2%)	9.6% (CI: 7.4%–12%)	14% (CI: 11%–18%)
Very High	16% (CI: 10%–26%)	40% (CI: 29%–54%)	53% (CI: 36%–73%)
New Risk Groups with WHO 1973			
Low	0.12% (CI: 0.02%–0.82%)	0.57% (CI: 0.21%–1.5%)	3.0% (CI: 1.5%–6.3%)
Intermediate	0.65% (CI: 0.36%–1.2%)	3.6% (CI: 2.7%–4.9%)	7.4% (CI: 5.5%–10%)
High	3.8% (CI: 2.6%–5.7%)	11% (CI: 8.1%–14%)	14% (CI: 10%–19%)
Very High	20% (CI: 12%–32%)	44% (CI: 30%–61%)	59% (CI: 39%–79%)

Un estudio más reciente de Sylvester *et al*., realizado en una población de más de 500 pacientes de un único centro, validó los grupos de riesgo establecidos para tumores tratados con BCG intravesical. Como era esperable, los resultados del estudio indicaron que el riesgo de progresión en aquellos pacientes tratados con BCG, especialmente en los grupos de alto y muy alto riesgo, eran significativamente menores, probablemente debido a la eficacia del tratamiento intravesical (66).

1.5 Seguimiento en el tumor vesical no músculo invasivo y diagnóstico de la recidiva

Como se mencionó anteriormente, debido a los significativos riesgos de recurrencia y progresión, los pacientes con TVNMI requieren un seguimiento después del tratamiento. A pesar de la utilidad de ciertas pruebas de imagen, como la ecografía, que ofrece un examen rápido, económico, sin efectos secundarios, sin riesgo de radiación y con una sensibilidad moderada para el estudio del tracto urinario superior e inferior (67), el *gold* estándar en el seguimiento del tumor vesical se basa en dos pilares: la cistoscopia y la citología de orina y, ocasionalmente, pruebas de imagen del tracto urinario superior.

La cistoscopia flexible es un método ambulatorio, generalmente bien tolerado, aunque más del 45% de los pacientes experimentan síntomas limitantes en las primeras 24h y hasta un 27% durante la primera semana (68). Por lo general, se utiliza luz blanca para visualizar todas las paredes de la vejiga, presentado una sensibilidad y especificidad para lesiones papilares entre 62-84% y 43-98%, respectivamente. Sin embargo, la sensibilidad disminuye en lesiones de pequeño tamaño y en el carcinoma in situ (69).

Existen métodos para mejorar la imagen que permiten aumentar la tasa de detección de lesiones endovesicales. Se dividen en tres categorías (70):

-Tecnología macroscópica, como la cistoscopia de luz azul y el *Narrow-Band Imaging* (NBI)

-Tecnologías de imagen microscópica como la tomografía óptica y la endomicroscopia confocal láser. Estas tecnologías proporcionan información microscópica e incluso histológica.

-Imagen molecular utilizando hibridación fluorescente.

De todas las opciones disponibles, la tecnología que acumula más evidencia es la cistoscopia con luz azul, cuya aprobación en Europa fue en 2010. Su aplicación requiere de la instilación intravesical previa de ácido hexaminolevulinato, conocido como Cysview^{TM}. En un estudio prospectivo en Estados Unidos, se demostró que la cistoscopia con luz azul mejoró hasta un 20.6% la tasa de detección de tumores vesicales (71). No obstante, su utilización no se recomienda en la evaluación inicial de las lesiones vesicales, limitando sus indicaciones al seguimiento de los tumores con un alto riesgo de recidiva (72).

La citología de orina es un método adyuvante a la cistoscopia durante el seguimiento de los TVNMI. Su alta capacidad para detectar tumores de alto grado, incluyendo el carcinoma in situ y las lesiones de alto grado del tracto urinario superior, gracias a su elevada especificidad (<2% de falsos positivos) (73), la convierte en un método eficaz aunque con moderada variabilidad interobservador (74). La citología de orina por micción espontánea resulta menos sensible que la obtenida por lavado vesical (41% vs 60%) (75), lo cual hace más recomendable y práctico su obtención en el momento de la cistoscopia.

Respecto a la cadencia de dichas exploraciones, depende del riesgo individual del paciente de recurrencia y progresión del tumor (57,76). El uso de nomogramas, como la calculadora de la EAU para TVNMI, ayuda a establecer los grupos de riesgo y adaptar los esquemas de frecuencias de seguimiento (58,77). Sin embargo, la evidencia existente está basada en datos retrospectivos y de bajo nivel, lo que dificulta modificar los protocolos para reducir el número de maniobras invasivas anuales que los pacientes en seguimiento por TVNMI requieren. Además, estos porcentajes se basan en datos heterogéneos, incluyendo pacientes que no han recibido tratamiento endovesical en el caso de las tablas de la EORTC.

En el seguimiento de los pacientes con TVNMI, es crucial considerar varios aspectos fundamentales al planificar la frecuencia y la metodología de seguimiento:

- En los pacientes de alto riesgo, es esencial diagnosticar recurrencias o progresiones a tumores músculo invasivos lo más precozmente posible. La cistoscopia y la citología de orina siguen siendo primordiales en este grupo, y actualmente no hay evidencia científica que respalde la sustitución de este protocolo con la misma seguridad oncológica.

- En los pacientes de bajo riesgo, donde la detección precoz de recurrencias puede no impactar significativamente en la supervivencia global ni cáncer específica (78,79), se podría considerar una metodología menos invasiva basada en ecografía y citología (o, eventualmente, biomarcadores). Existe creciente evidencia científica que sugiere la opción de la vigilancia activa, postponiendo el tratamiento quirúrgico, en casos seleccionados (79–81). La consideración de esta alternativa de manejo de la enfermedad debe tener en cuenta factores como antecedentes de T1 de bajo grado, presencia de lesiones múltiples al inicio de la vigilancia y el número de resecciones transuretrales previas, puesto que estos factores predisponen a un posible fallo más precoz en esta estrategia (82,83).

- La primera exploración tras una RTU-V debe realizarse a los 3 meses tras la cirugía, ya que es un factor pronóstico independiente de recurrencia y progresión (84,85). Está indicada en todos los pacientes con TVNMI, independientemente del grupo de riesgo.

- En los tumores de bajo riesgo, la probabilidad de recurrencia después de 5 años de seguimiento es baja (77), lo que podría justificar la discontinuidad o el uso de exploraciones no invasivas después de este periodo.

- En los pacientes con tumores de riesgo intermedio, alto y muy alto riesgo, la probabilidad de recurrencia y progresión persiste incluso a los 10 años de seguimiento (85) por lo que una evaluación más prolongada sería óptima (86).

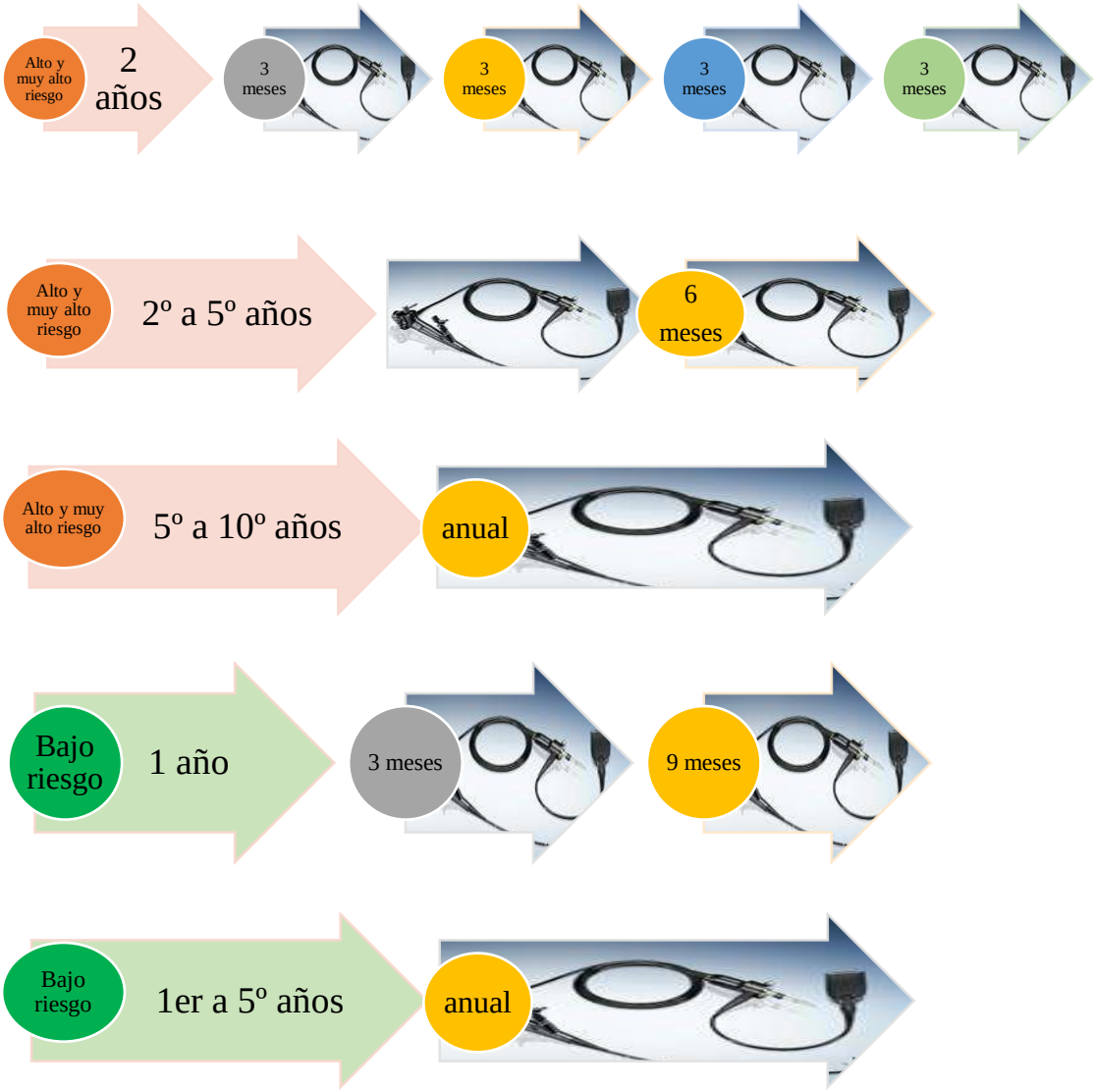
- Dada la posible asociación o riesgo de desarrollo de enfermedad panurotelial, la evaluación periódica de la uretra prostática y/o del tracto urinario superior debería reflejarse en la estrategia de seguimiento, aunque no existen unas pautas concretas y consensuadas.

Las guías actuales de la EAU para TVNMI proponen un esquema de seguimiento más laxo en casos de tumores de bajo riesgo, con evaluaciones a los 9 meses después de la cistoscopia inicial y, posteriormente anualmente durante 5 años. Por el contrario, en casos de pacientes con TVNMI de alto o muy alto riesgo, se aconseja un seguimiento trimestral durante los dos primeros años, semestral posteriormente hasta los 5 años y, finalmente, anual hasta completar al menos 10 años (63), tal y como se indica en la Figura 8.

En el caso de pacientes con tumores de riesgo intermedio, el enfoque de seguimiento se sitúa entre los grupos de alto y bajo riesgo. Este enfoque se caracteriza por una mayor individualización, adaptándose según el riesgo de recurrencia y progresión específico de cada paciente.

La cistoscopia flexible sigue siendo una herramienta esencial en este contexto. La estrategia de seguimiento debe considerar cuidadosamente factores como la historia clínica del paciente, características específicas del tumor, respuestas previas al tratamiento y otros elementos clínicos y patológicos relevantes. La frecuencia y la naturaleza de las evaluaciones, como la cistoscopia y la citología, se ajustarán en consecuencia a para optimizar la detección temprana de cualquier recurrencia o progresión. En general, la individualización del seguimiento es crucial en el manejo de los pacientes con tumores vesicales, permitiendo un equilibrio entre la vigilancia adecuada y la minimización de procedimientos invasivos innecesarios.

Figura 8. Esquema de seguimiento del TVNMI basado en la recomendación de las guías de la EAU (64).



La metodología de seguimiento invasiva que incluye cistoscopias y citologías frecuentes puede ser intensiva y asociarse a molestias para los pacientes. La baja sensibilidad de la citología, especialmente en casos de bajo grado, ha llevado a explorar alternativas, y los biomarcadores urinarios han surgido como un área de interés en la investigación del cáncer de vejiga. Estos biomarcadores se han evaluado con el objetivo de mejorar la detección, reducir la necesidad de procedimientos invasivos y proporcionar una alternativa menos molesta para los pacientes (87).

1.6. Biomarcadores urinarios en el seguimiento del tumor vesical

Los biomarcadores urinarios, desarrollados gracias al avance en las técnicas de estudio de material genético, han experimentado un crecimiento exponencial. Estos biomarcadores han sido explorados en las diversas fases de la enfermedad, abarcando desde el estudio de la hematuria hasta el proceso diagnóstico, el seguimiento del tumor vesical, e incluso como factores predictores de respuesta al tratamiento y progresión (88). Aunque estos biomarcadores, en particular los más actuales, presentan elevadas sensibilidades y valores predictivos negativos que superan en muchos casos a la citología, suelen exhibir por el contrario una especificidad y valor predictivo positivo relativamente bajos (89–91). Este hecho ha limitado su adopción generalizada en la práctica clínica diaria, y las guías clínicas aún no los han incorporado de manera sistemática en los protocolos de seguimiento. No obstante, biomarcadores clásicos como el UroVysion™ (FISH), el Nuclear Matrix Protein 22® (NMP22) y el *Fibroblast Growth Factor Receptor* 3 (FGFR3)/*Telomerase Reverse Transcriptase* (TERT) han sido evaluados en estudios con pacientes con citología positiva, pero cistoscopia y Uro-TC sin evidencia de neoplasia, con el fin de identificar aquellos pacientes con mayor riesgo de recurrencia y progresión (92–97).

Los biomarcadores en el seguimiento del TVNMI pueden ser de diversa índole, incluyendo marcadores proteicos, de origen genético (DNA, RNA, alteraciones epigenéticas o vesículas extracelulares) y pruebas celulares. Entre ellos, los biomarcadores genéticos están experimentando una notable evolución y se postulan como una alternativa a los métodos clásicos de seguimiento (98).

La orina, como fuente de biopsia líquida, presenta ventajas significativas en términos de obtención fácil, indolora, rápida y estabilidad del material genético (99). Sin embargo, el carcinoma vesical se caracteriza por una alta heterogeneidad genética, y su impacto en la

calidad de vida de los pacientes se asocia a la necesidad de un seguimiento invasivo riguroso debido a su propensión a la recurrencia y progresión (100). Aunque la *Food And Drug Association* (FDA) aprobó hace más de 10 años algunos biomarcadores con baja sensibilidad y especificidad en comparación con la citología, limitando su aplicabilidad y recomendación, la perspectiva clínica está cambiando (101). Las guías de la EAU y de la *American Urological Association* históricamente han sugerido que el seguimiento de los pacientes con TVNMI se base en la combinación de cistoscopia, citología y pruebas de imagen para valorar el tracto urinario superior (102,103). No obstante, en los últimos dos años, las recomendaciones de las guías europeas han abierto la posibilidad al uso de biomarcadores urinarios en grupos seleccionados de pacientes, especialmente aquellos con menor riesgo de progresión, es decir, los de bajo riesgo y riesgo intermedio (63,104). Este cambio en el paradigma ofrece la opción de utilizar biomarcadores en alternancia con la cistoscopia, con el objetivo de reducir el riesgo de procedimientos invasivos repetitivos sin comprometer la seguridad oncológica, especialmente en pacientes con bajo riesgo de progresión. Un ensayo clínico prospectivo randomizado concluyó que el conocimiento previo por parte del urólogo de los resultados de los biomarcadores mejora la sensibilidad de la cistoscopia y aumenta la tasa de detección de recidivas (105).

En la actualidad, los biomarcadores genéticos podrían considerarse como una opción prometedora dentro del espectro de biomarcadores urinarios utilizados en el seguimiento TVNMI. El número de publicaciones en este campo ha experimentado un crecimiento exponencial en los últimos cinco años, lo que ha permitido comparar diversos biomarcadores con la citología urinaria. Entre los biomarcadores genéticos disponibles en la actualidad, las guías europeas destacan cuatro que son comercialmente accesibles, aprobados por la FDA y con marca CE de la Unión Europea, y a los que se les ha realizado un mayor número de estudios con pacientes en seguimiento, así como un perfil mejorado en términos de sensibilidad,

especificidad, VPP y VPN. Estos biomarcadores son: Cx-Bladder™ (106,107), ADX-Bladder™ (108,109), Xpert Bladder® (110,111) y EpiCheck® (112).

Estos biomarcadores genéticos podrían ser empleados para postergar o incluso sustituir las cistoscopias en el seguimiento de los tumores de bajo riesgo y riesgo intermedio. Su uso presenta un perfil de seguridad óptimo para detectar las recidivas de alto grado, que son infrecuentes en estos subgrupos de pacientes.

En una revisión sistemática reciente que incluyó un metaanálisis y estudió datos de más de 7.000 pacientes, se obtuvo un perfil favorable para estos biomarcadores con una sensibilidad del 93%, una especificidad de hasta el 84%, un VPP de 67%, y un VPN cercano al 99% (113). De entre todos los biomarcadores analizados, Xpert Bladder Monitor® presenta un perfil oncológico destacado. Ha sido evaluado en catorce publicaciones hasta la fecha y en más de 3.000 pacientes, lo que refuerza su posible validez clínica. La sensibilidad y especificidad combinada del biomarcador fueron del 73% (95% intervalo de confianza 65%-80%) y 77% (95% intervalo de confianza 69%-84%) respectivamente (114). Se trata de un biomarcador basado en el análisis de 5 microRNAs (ABL1, ANXA10, UPK1B, CRH y IGF2) utilizando exclusivamente 4.5mililitros de orina. La principal ventaja es que se trata de un test que puede ser realizado en el mismo punto de visita del paciente, obteniendo un resultado cualitativo (positivo/negativo) en tan solo 90 minutos. Dicho resultado se obtiene a través de un algoritmo prestablecido por el fabricante a partir de un punto de corte denominado LDA (*linear discriminant analysis*), cuyo punto de corte se encuentra situado en 0.5.

Estos hallazgos respaldan la utilidad de Xpert Bladder Monitor® como una herramienta valiosa en el seguimiento del TVNMI, presentando un rendimiento prometedor en términos de sensibilidad y especificidad. Estos aspectos son cruciales para lograr una detección precisa de recidivas y respaldar la toma de decisiones clínicas.

2. Justificación del estudio

La justificación de nuestro proyecto se basa en la necesidad de mejorar el manejo del carcinoma vesical, especialmente en el seguimiento de los TVNMI. El cáncer de vejiga es una de las neoplasias más prevalentes (115). Tres de cada cuatro casos se presentan como TVNMI. De ellos, aproximadamente la mitad recurrirán en los siguientes 5 años de seguimiento (116). Es por ello por lo que requieren un seguimiento riguroso, según las guías europeas, mediante cistoscopia flexible y citología de orina seriadas. A pesar de su eficacia, este enfoque implica un coste elevado (117) y tiene un impacto relevante en la calidad de vida de estos pacientes. Aunque el riesgo de complicaciones de la cistoscopia flexible no supera el 3%, las molestias asociadas a dicha manipulación invasiva afectan a más del 40% de los casos, persistiendo la mayoría de ellas al menos 24h (31). La necesidad de repetir este procedimiento varias veces al año aumenta la ansiedad de los pacientes y afecta a su calidad de vida (118).

En respuesta a esta preocupación, se ha intensificado la investigación de biomarcadores que puedan reemplazar los procedimientos invasivos, por otros simples y con la misma fiabilidad oncológica. A pesar de los avances en la genética del carcinoma vesical y las mejoras en las técnicas de laboratorio, hasta ahora no se ha identificado un biomarcador con el mismo perfil de sensibilidad, especificidad y valores predictivos que la combinación de cistoscopia y citología (119).

Nuestro proyecto de tesis doctoral consiste en realizar una revisión sistemática de la literatura para identificar el biomarcador genético urinario comercializado más apropiado para sustituir a la cistoscopia y citología urinaria por lavado en el seguimiento del TVNMI.

En la siguiente fase de proyecto se propone evaluar el rendimiento clínico del biomarcador seleccionado en un estudio prospectivo de una cohorte propia de TVNMI, incluidos durante los dos primeros años de seguimiento tras la última RTU-V y tratados convencionalmente según las guías de la EAU, comparando con el seguimiento estándar actual basado en cistoscopia y citología por lavado vesical.

El objetivo final de este proyecto es validar la eficacia de un biomarcador genético urinario como una alternativa viable y menos invasiva para el seguimiento actualmente recomendado del TVNMI en el contexto de una serie representativa de la clínica diaria asistencial.

3. Hipótesis

La cuantificación en orina de micción espontánea de un biomarcador genético puede constituir una alternativa segura y no invasiva al seguimiento convencional del tumor vesical no músculo invasivo (TVNMI), actualmente basado en la realización seriada de cistoscopia flexible y citología urinaria por lavado vesical.

4. Objetivos

Con la finalidad de contrastar la hipótesis previamente expuesta, se propuso el siguiente objetivo principal:

Establecer el papel de la determinación de un biomarcador genético en orina de micción espontanea en el contexto del seguimiento del TVNMI actualmente basado en la realización seriada de cistoscopia flexible y citología urinaria obtenida por lavado vesical.

Además de dicho objetivo principal, se establecieron los siguientes objetivos secundarios:

1. Realizar una revisión sistemática de la literatura para analizar el posicionamiento actual de los biomarcadores genéticos urinarios en el contexto del seguimiento del TVNMI y seleccionar el más apropiado entre ellos para realizar un estudio prospectivo.

2. Comparar la eficacia clínica del biomarcador genético seleccionado con el método estándar de seguimiento del TVNMI, basado en cistoscopia flexible y la citología urinaria por lavado vesical.

3. Evaluar puntos de corte alternativos, al propuesto por el fabricante, que permitan adecuar su sensibilidad y especificidad al escenario clínico más seguro en el seguimiento de los TVNMI.

4. Evaluar el efecto anticipatorio del biomarcador genético seleccionado en el diagnóstico precoz de las recurrencias del TVNMI.

5. Compendio de publicaciones

La presente tesis doctoral se presenta como un compendio de publicaciones que se especifican a continuación.

5.1. Publicación 1

Título: *Current status of genetic urinary biomarkers for surveillance of non-muscle invasive bladder cancer: A systematic review*

Autores: Fernando Lozano; Carles X. Raventós; Albert Carrión; Juan Morote; Enrique Trilla.

Referencia: BMC Urol. 2020 Jul 14;20(1):99.

- PMID: 32664878 PMCID: PMC7362437

- DOI: https://doi.org/10.1186/s12894-020-00670-x

Impact Factor: 2.09

Lozano *et al. BMC Urology* (2020) 20:99
https://doi.org/10.1186/s12894-020-00670-x

BMC Urology

RESEARCH ARTICLE Open Access

Current status of genetic urinary biomarkers for surveillance of non-muscle invasive bladder cancer: a systematic review

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Abstract

Background: Genetic biomarkers are a promising and growing field in the management of bladder cancer in all stages. The aim of this paper is to understand the role of genetic urinary biomarkers in the follow up of patients with non muscle invasive bladder cancer where there is increasing evidence that they can play a role in avoiding invasive techniques.

Methods: Following PRISMA criteria, we have performed a systematic review. The search yielded 164 unique articles, of which 21 articles were included involving a total of 7261 patients. Sixteen of the articles were DNA based biomarkers, analyzing different methylations, microsatellite aberrations and gene mutations. Five articles studied the role of RNA based biomarkers, based on measuring levels of different combinations of mRNA. QUADAS2 critical evaluation of each paper has been reported.

Results: There are not randomized control trials comparing any biomarker with the gold standard follow-up, and the level of evidence is 2B in almost all the studies. Negative predictive value varies between 55 and 98.5%, being superior in RNA based biomarkers.

Conclusions: Although cystoscopy and cytology are the gold standard for non muscle invasive bladder cancer surveillance, genetic urinary biomarkers are a promising tool to avoid invasive explorations to the patients with a safe profile of similar sensitivity and negative predictive value. The accuracy that genetic biomarkers can offer should be taken into account to modify the paradigm of surveillance in non muscle invasive bladder cancer patients, especially in high-risk ones where many invasive explorations are recommended and biomarkers experiment better results.

Keywords: Biomarkers, Surveillance, Bladder, Genetic

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Background

Bladder cancer (BC) is the fifth most common neoplasm worldwide, with more than 54,000 new cases estimated per year in the United States alone [1] . BC is a heterogeneous tumour that is associated with very high economic costs and a substantial impact on patients' quality of life owing to its characteristically high risk of recurrence and the complexity of follow up [2]. Guidelines from the European Association of Urology (EAU) and the American Urological Association (AUA) suggest a combination of cystoscopy, cytology and imaging for the surveillance of patients with non-muscle invasive bladder cancer (NMIBC) [3, 4]. Cystoscopy is an invasive procedure that carries the risks of painful micturition, urinary frequency and macroscopic haematuria of 50, 37 and 19%, respectively [5], while cytology has a very low sensitivity, especially for low-grade tumours [6, 7] .

For this reason there has been an increase in research over the past years into urinary biomarkers for the three scenarios of haematuria, diagnosis and surveillance. The role of these new tests is to increase the sensitivity and the specificity of the available gold-standard techniques, while sparing the patient the discomfort of an invasive test and its potential complications. Although many types of urinary biomarkers have been investigated, biomarkers that use genetic materials such as DNA and RNA seem to be the most promising due to their potential to identify a genetic signature. Such a signature would not only prove useful in disease detection and follow-up but also in the facilitation of more precise treatment by avoiding unhelpful therapies that may delay the best oncological pathway.

The field of urinary biomarker research in BC is focused on balancing a non-invasive, safe method with a cost-effective strategy that can be used to improve the sensitivity of bladder tumour detection in the initial phase of the disease and during patient follow-up, compared with the current gold standard.

Selecting a biomarker must be based on the given scenario and follow the principles of the international guidelines [8, 9] . The current literature clearly differentiates between different biomarker tests and characteristics depending on whether the BC is low or high risk. For low-risk tumours, marker-guided testing of lesions is suggested to detect possible progression to high-risk tumours. For high-risk tumours, however, where early detection is the main objective, selection of high-sensitivity biomarkers is recommended [9].

The aim of this study is to analyse the current literature for the use of genetic urinary biomarkers in the surveillance of NMIBC.

Methods

FLP performed a bibliographic search of Medline (http://www.ncbi.nlm.nih.gov), Embase (http://www.embase.com) and the Cochrane library (http://www.cochrane.org) up to March 2020. MeSH terms used were Bladder cancer AND surveillance AND biomarkers AND DNA OR RNA OR methylation, yielding 2241 articles. After that, two authors (FLP and CXR) screened all published original articles appearing in the above search for eligibility. Studies using genetic urinary biomarkers for surveillance in non muscle invasive bladder cancer in humans were selected. Studies were excluded if they were not original research papers, used a language other than English, had less than 20 patients or did not report biomarker performance in terms of sensitivity, specificity, or area under the curve (AUC); or reported the performance of genetic markers only in combination with other factors (clinical data or non genetic biomarkers), yielding 164 articles.

After applying the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) criteria narrowed this down to 21 original articles (Fig. 1).

After reviewing each of the selected articles using the REMARK checklist [10], we designed a QUADAS-2 table to evaluate each study's risk of bias and quality (Fig. 2). The main bias observed was reference to the index test. Although we identified a 50% risk of bias, most of the articles reviewed met most of the QUADAS-2 criteria [11], using the four considerations (patient selection, index, reference and flux and timming) which suggested that the studies were of moderately high quality.

We have also used the SWIM (Synthesis without meta-analysis) reporting guideline [12] to clarify the reporting methodology of the article (Fig. 3). The evidence was reported in terms of sensitivity and specificity for each biomarker (for low grade and high grade tumors in case of studies that indicated that subestratification). Area under de curve has been informed in eleven of the seventeen studies. We have also reported negative and positive predictive value of the markers. Recurrence rate has been calculated using the positive cases (positive pathology) and the total number of samples. We have prioritized articles identified as low risk of bias based on QUADAS-2 table to draw the conclusions of this review. We have also performed exploratory analyses to determine whether different study characteristics varied the effects of the interventions. Almost all the studies accepted as confirmed positive case if there is a pathology report. Some studies generate artificial cohort. We examined whether this different type of targeted behaviour modified, on average, the effect of the interventions.

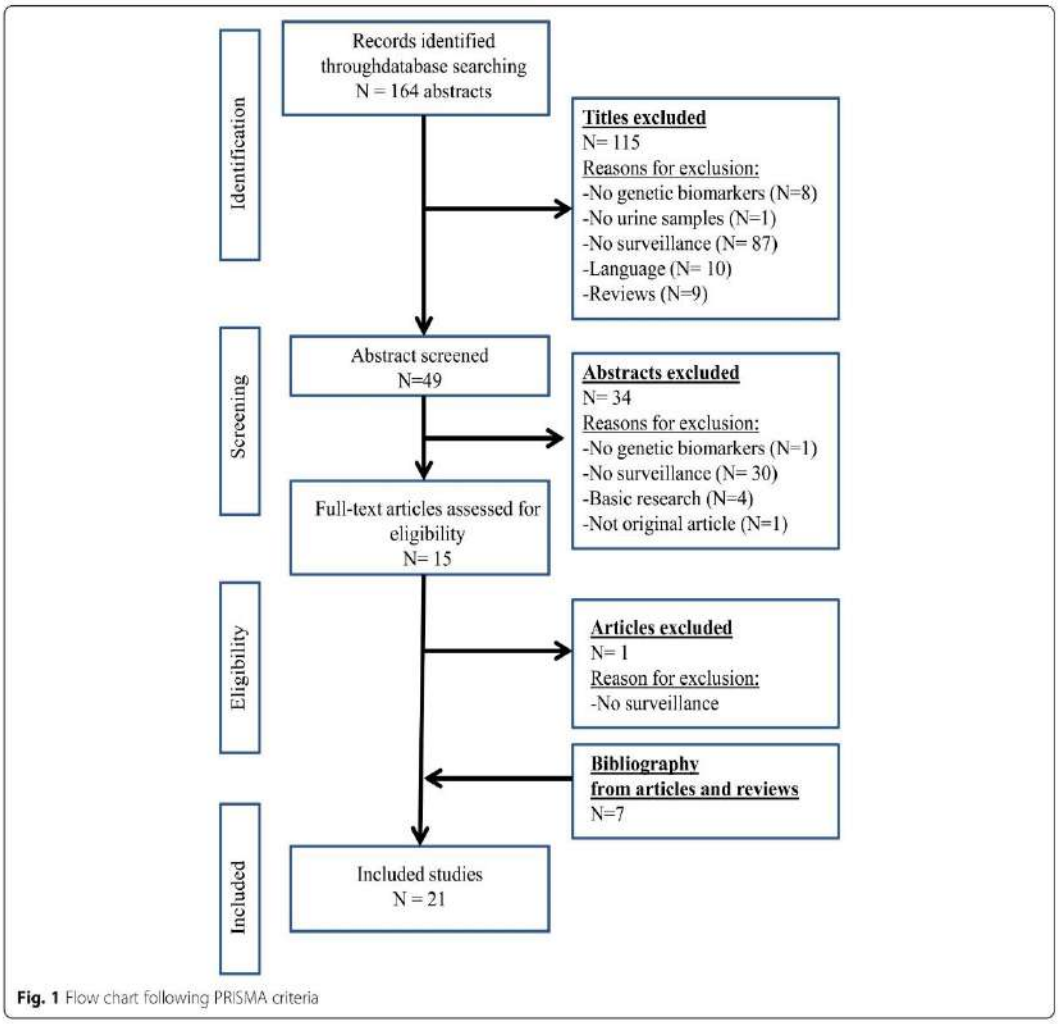

Fig. 1 Flow chart following PRISMA criteria

Results

In order to obtain a practical and visual description of the different studies, we divided the biomarkers by method into DNA-based (Table 1) and RNA-based (Table 2) tests.

DNA tests (Table 1)

DNA tests used for surveillance are based on microsatellite analysis (MA). They are employed to detect loss of heterozygosity, gene methylation levels and gene mutations in cells collected from urine.

Microsatellite markers are highly polymorphic tandem repeat DNA sequences distributed throughout the genome and easily amplifiable by standard polymerase chain reaction [34] . Rouprêt et al. [13] compared this biomarker with methylation biomarkers in a comparative cohort study of 40 patients. In this study, MA appeared to yield better results for detecting recurrences (AUC 0.81 vs 0.44). When a Bayesian network analysis was performed that combined variables and biomarkers, the panel of markers generated a sensitivity of 85% and a specificity of 86%. Van der Aa et al. [14] designed a multicentre study to evaluate the clinical utility of MA in low-grade tumours in combination with *FGFR3* mutations described previously [35]. The sensitivity in this study was 58% and the specificity 73%, with a negative predictive value of 94%.

DNA methylation has been recognized to be important in the developmental biology and cancer aetiology of many neoplasms [36–38]. DNA methylation is an epigenetic marker that mainly affects CpG dinucleotides. These dinucleotides are distributed throughout the genome and usually have a normal methylation status. Hypermethylation of CpG dinucleotides in the promoter

	Risk of bias				Concerns regarding applicability		
	Patient selection	Index test(s)	Reference standard	Flow and timing	Patient selection	Index test(s)	Reference standard
Roupret 2008	(-)	(-)	(+)	(+)	(+)	(+)	(+)
Van der Aa 2009	(+)	(+)	(-)	(+)	(+)	(+)	(+)
Zuuiverloon 2010	(-)	(+)	(+)	(+)	(+)	(+)	(+)
Reinert 2012	(+)	(-)	(+)	(-)	(+)	(+)	(+)
Zuiverloon 2011	(-)	(-)	(+)	(+)	(+)	(-)	(+)
Allory 2013	(+)	(-)	(+)	(+)	(+)	(+)	(+)
Abern 2014	(-)	(+)	(-)	(+)	(-)	(+)	(+)
Su 2014	(+)	(-)	(+)	(-)	(+)	(+)	(+)
Sapre 2015	(-)	(-)	(+)	(+)	(+)	(+)	(-)
Fantony 2015	(+)	(-)	(-)	(+)	(+)	(-)	(-)
Baukers 2016	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Kavalieris 2016	(+)	(-)	(+)	(+)	(+)	(+)	(+)
Roperch 2016	(-)	(+)	(+)	(-)	(+)	(+)	(+)
Lotan 2017	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Pilcher 2017	(+)	(-)	(+)	(+)	(+)	(+)	(+)
Van der Heijden 2018	(+)	(-)	(+)	(+)	(+)	(+)	(+)
Wallace 2018	(+)	(-)	(+)	(-)	(+)	(+)	(+)
Witjes 2018	(-)	(+)	(+)	(+)	(+)	(-)	(+)
Springer 2018	(-)	(+)	(+)	(+)	(-)	(+)	(+)
D'Andrea 2019	(-)	(+)	(-)	(+)	(-)	(+)	(+)
Batista 2019	(+)	(-)	(+)	(+)	(+)	(-)	(+)

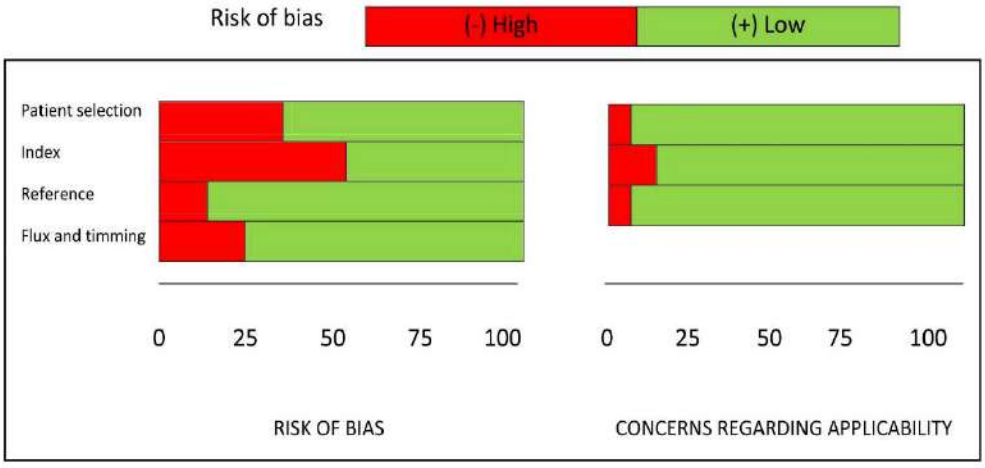

Fig. 2 QUADAS 2 table evaluating quality of the studies

Synthesis Without Meta-analysis (SWiM) reporting items

The citation for the Synthesis Without Meta-analysis explanation and elaboration article is: Campbell M, McKenzie JE, Sowden A, Katikireddi SV, Brennan SE, Ellis S, Hartmann-Boyce J, Ryan R, Shepperd S, Thomas J, Welch V, Thomson H. Synthesis without meta-analysis (SWiM) in systematic reviews: reporting guideline BMJ 2020;368:l6890 http://dx.doi.org/10.1136/bmj.l6890

SWiM is intended to complement and be used as an extension to PRISMA			
SWiM reporting item	**Item description**	**Page in manuscript where item is reported**	**Other***
Methods			
1 Grouping studies for synthesis	1a) Provide a description of, and rationale for, the groups used in the synthesis (e.g., groupings of populations, interventions, outcomes, study design)	3	
	1b) Detail and provide rationale for any changes made subsequent to the protocol in the groups used in the synthesis	-	
2 Describe the standardised metric and transformation methods used	Describe the standardised metric for each outcome. Explain why the metric(s) was chosen, and describe any methods used to transform the intervention effects, as reported in the study, to the standardised metric, citing any methodological guidance consulted	3	
3 Describe the synthesis methods	Describe and justify the methods used to synthesise the effects for each outcome when it was not possible to undertake a meta-analysis of effect estimates	-	
4 Criteria used to prioritise results for summary and synthesis	Where applicable, provide the criteria used, with supporting justification, to select the particular studies, or a particular study, for the main synthesis or to draw conclusions from the synthesis (e.g., based on study design, risk of bias assessments, directness in relation to the review question)	3	
SWiM reporting item	**Item description**	**Page in manuscript where item is reported**	**Other***
5 Investigation of heterogeneity in reported effects	State the method(s) used to examine heterogeneity in reported effects when it was not possible to undertake a meta-analysis of effect estimates and its extensions to investigate heterogeneity	3	
6 Certainty of evidence	Describe the methods used to assess certainty of the synthesis findings	3	
7 Data presentation methods	Describe the graphical and tabular methods used to present the effects (e.g., tables, forest plots, harvest plots). Specify key study characteristics (e.g., study design, risk of bias) used to order the studies, in the text and any tables or graphs, clearly referencing the studies included	4	
Results			
8 Reporting results	For each comparison and outcome, provide a description of the synthesised findings, and the certainty of the findings. Describe the result in language that is consistent with the question the synthesis addresses, and indicate which studies contribute to the synthesis	4-6	
Discussion			
9 Limitations of the synthesis	Report the limitations of the synthesis methods used and/or the groupings used in the synthesis, and how these affect the conclusions that can be drawn in relation to the original review question	6-7	

PRISMA=Preferred Reporting Items for Systematic Reviews and Meta-Analyses.
*If the information is not provided in the systematic review, give details of where this information is available (e.g., protocol, other published papers (provide citation details), or website (provide the URL)).

Fig. 3 Synthesis Without Meta-analysis (SWiM) reporting items

Table 1 DNA based biomarkers used in follow-up for non muscle invasive bladder cancer patients

Reference	Patients/ samples	Recurrence rate	Sensitivity(%)	Specificity(%)	NPV (%)	PPV (%)	AUC	Method	Markers
Roupret *et al* 2008 [13]	40/40	38%	80 (microsatellite) 86 (methylation) 85 (combination)	68 (microsat) 8 (methyl) 86 (combination)			0.81 (microsat) 0.44 (methyl)	DNA PCR	Microsatellite[a] vs methylation[b]
Van der Aa *et al* 2009 [14]	228/815	10.3%	58	73	94	61-77	NA	DNA PCR	Microsatellite + FGFR3 mutation
Zuiverloon *et al* 2010 [15]	134/463	9.7%	58	NA	89	25	NA	DNA PCR	FGFR3 mutations[c]
Reinert *et al* 2012 [16]	158/206	67.4%	87-94	28-47	55-78	72-78	0.68-0.78	DNA PCR	Methylation [d]
Zuiverloon *et al* 2012 [17]	NA/94	69.1%	72.3	55.2	NA	NA	NA	DNA PCR	Methylation genes APC_a , TERT_a , TER _b ,EDNRB
Allory *et al* 2013 [18]	194/395	44.8%	19(FGFR3) 42(TERT) 50(FGFR3+TERT)	73 (TERT) 90(FGFR3) 71(FGFR3+TERT)	NA	NA	NA	DNA PCR	Gene mutations (TERT and FGFR3)
Abern *et al* 2014 [19]	111/111	21.6%	75-79	63-71	92	37-42	0.74 (TWIST1) 0.68 (NID2)	DNA PCR	Methylation genes TWIST1, NID2
Su *et al* 2014 [20]	90/368	37.7%	80	97	NA	NA	0.95	DNA PCR	Hyper and hypomethylated genes (SOX1, IRAK3, L1-MET)
Fantony *et al* 2015 [21]	126/126	25%	58-67	61-69	83-85	36-38	0.66 (TWIST1) 0.63 (NID2)	DNA PCR	Methylation genes TWIST1, NID2
Beukers *et al* 2016 [22]	NA/2191	64%	57 (LG) 72 (HG)	59% LG	NA	NA	NA	DNA PCR	FGFR3 mutation, TERT mutation and OTX1 methylation
Roperch *et al* 2016 [23]	158/613	45.5%	94.5 96 (HG)	75.9	98.5	NA	0.82	DNA PCR	FGFR3 mutation +DNA methylation HS3ST2, SLIT2 and SEPTIN9
Van der Heijden *et al* 2018 [24]	NA/458	37.7%	90	31	82	50	0.74	DNA PCR	DNA gene Methylation (CFTR, SALL3, TWIST1)
Witjes *et al* 2018 [25]	353/353	13%	68.2 92.6 (HG)	88	95.1 99.3(HG)	44.8	0.82	DNA PCR	15 DNA methylation genes (Epicheck®)
Springer *et al* 2018 [26]	322/322	58%	68 71 (HG)	80	NA	NA	NA	DNA PCR	10 gen mutations[e] plus detection of aneuploidy (UroSEEK®)
D'Andrea *et al* 2019 [27]	357/357	13.7%	67.3 88.9 (HG)	88 88(HG)	94 99 (HG)	47 30 (HG)	85.9	DNA PCR	15 DNA methylation genes (Epicheck®)
Batista *et al* 2019 [28]	122/122	28%	73.5	73.2	NA	NA	NA	DNA PCR	TERT promoter and FGFR3 mutations (Uromonitor®)

LG low grade, *HG* high grade, *NA* not allowed

[a]FGA (4q28), D4S171(4q35)), 5 (ACTBP2(5q14)), 9 (D9S162 (9p), IFNA (9p21)), 14 (MJD52(14q32)), 16 (D16S310 (16q21)) and 18 (D18S51 (18q21), MBP (18qter).

[b](RASSF1a (3p21.3),E-cadherin (16q22.1), APC (5q21), DAPK (9q22.1), MGMT (10q26), BCL2 (18q21.33), h-TERT (5p15.33), EDNRB (13q22), WIF-1 (12q14.3), TNFRSF25 (1p36.31), IGFBP3 (7p13))

[c]R248C and S249C (exon 7); G372C,S373C, Y375C, G382R, and A393E (exon 10); and K652M, K652T, K652E, and K652Q (exon 15)

[d]EOMES, HOXA9, POU4F2, TWIST1, VIM, ZNF154

[e]FGFR3, TP53, CDKN2A, ERBB2, HRAS, KRAS, PIK3CA, MET, VHL, MLL and TERT promoter.

Table 2 RNA based biomarkers used in follow-up for non muscle invasive bladder cancer patients

Reference	Patients/samples	Recurrence rate	Sensitivity	Specificity	NPV	PPV	AUC	Method	Markers
Sapre *et al* 2016 [29]	131/131	NA	88	48	75	63	0.74	miRNA PCR	6 miRNA signature[a]
Kavalieris *et al* 2017 [30]	736/1036	15.1%	92	NA	96	NA	0.73	mRNA PCR	5 genes mRNA expression (Cx Bladder Monitor®)[b]
Lotan *et al* 2017 [31]	748/1016	14.8%	91 95 (HG)	NA	96	NA	NA	mRNA PCR	5 genes mRNA expression (Cx Bladder Monitor®)[b] vs NMP22 ELISA vs NMP22 BladderChek
Pilcher *et al* 2018 [32]	140/155	30.7%	84 100 (HG)	91	93	72	0.87	mRNA RT-PCR	ABL1, CRH, IGF2, UPK1B, ANXA10 (Xpert Bladder Cancer Monitor®)
Wallace *et al* 2018 [33]	370/370	13.2%	73 83 (HG)	77	92	44	0.87	mRNA RT-qPCR	ABL1, CRH, IGF2, ANXA10, UPK1B (Xpert Bladder Cancer Monitor®)

HG high grade, *NA* not allowed
[a]miR16, miR200c, miR205, miR21, miR221 and miR34a
[b]IGFBP5, HOXA13, MDK, CDK1, CXCR2

regions of tumour suppressor genes can repress their transcription in human cells [39, 40]. Methylation status is one of the most studied biomarkers in the follow-up scenario because it is both chemically stable and quantifiable [41]. Zuiverloon et al. [17] developed a retrospective four-step test, selecting methylation of the *APC_a*, *TERT_a*, *TER_b* and *EDNRB* genes as the combination providing a higher sensitivity and specificity (63.3 and 58.3%, respectively) than other combinations investigated in this study. Based on their previous study [42], Reinert et al. evaluated the methylation of *EOMES*, *HOXA9*, *POU4F2*, *TWIST1*, *VIM* and *ZNF154*. Their study consisted of a first step, validating the markers and establishing the cut-off levels, and a second step in the surveillance scenario excluding those patients who showed no aberrant methylation of their tumour marker genes. The authors reported a sensitivity of between 87 and 94% and a specificity ranging from 43 to 67%. Combining the different biomarkers did not improve the accuracy of the test [16]. Su et al. [20] tested six DNA methylation markers before building a model with *SOX1*, *IRAK3* and *L1-MET* as the best combination to detect recurrences. Using this model they obtaining a sensitivity of 80% and a very high specificity of 97%. Roperch et al. [23] combined four different *FGFR3* mutations and eighteen methylation markers based on the literature [43, 44]. Finally, they selected three of these markers (the genes *HS3ST2*, *SLIT2* and *SEPTIN9*) for combination with the *FGFR3* mutations in a logistic regression model, obtaining a sensitivity of 94.5% (96% in high-grade tumours) and a specificity of 75.9%. Van der Heijden et al. [24] evaluated seven selected genes that are found at significantly increased levels in the urine sediment from patients with BC. After testing a training set, they selected the *CFTR*, *SALL3* and *TWIST1* genes for validation in a large series (458 samples) and obtained a sensitivity of 90% (96% in combination with cytology). Witjes et al. [25] evaluated a combination of 15 methylated genes (Epicheck®), obtaining a sensitivity of 68.2% (92.6% for high-grade tumours) and a specificity of 88%. D'Andrea et al. [27] published another multicentric and independent study using the same test, supporting the sensitivity (67.3, 88.9% for high grade) and specificity (88%) described in the previous publication by Witjes. Abern [19] studied the role of two methylated genes, TWIST1 and NID2 based on Renard work [45] due to their high sensitivity and specificity for urothelial carcinoma. They observed that TWIST1 methylation had better AUA than NID2 or the combination of both genes. They also showed that adjusting the thresholds, the test had a sensitivity and specificity of 75 and 71%, respectively. Fantony et al. [21] published a more recent multi-institutional study using the same methylated genes, obtaining similar conclusions and results of sensitivity (58–67%) and specificity (61–69%). In this paper, prior BCG treatment for NMIBC reduced the accuracy of the test.

Many of the gene mutations investigated are related to the carcinogenesis of urothelial carcinomas, which are among the most heterogeneous tumours [46]. One of the most studied among these genes is fibroblast growth factor receptor 3 (*FGFR3*), mutations of which are found in almost 80% of the low-grade tumours and associated with a good prognosis [35, 47].

Zuiverloon et al. [15] evaluated this marker in non-high grade tumours, achieving a sensitivity of 58%. Beukers et al. [22] combined *FGFR3* mutation with *TERT* mutation and *OTX1* gene methylation in a large prospective European cohort study, obtaining a sensitivity of 57% for low-grade and 72% for high-grade BC. Allory investigated the role of telomerase reverse transcriptase (TERT) promoter mutations, frequently founded in many other non urothelial tumors [48] in combination with FGFR3 mutation [18]. This study showed that

combination of TERT and FGFR3 has higher sensitivity (50%) than TERT or FGFR3 individually. Moreover, FGFR3 had higher specificity than TERT mutation.

In a more recent multicentric study, Batista et al. [48] have developed a biomarker based on two TERT mutations (c. 1-124C > T and c.1-146C > T) plus FGFR3 (p.R248C and p.S249C) hotspot mutations. After a technical validation of the test, they achieved a73.5% of sensitivity and 93.2% of specificity. Springer et al. [26] have also analyzed mutations in TERT promoter, mutations in FGFR3 in combination with other nine gen mutations (TP53, CDKN2A, ERBB2, HRAS, KRAS, PIK3CA, MET, VHL, MLL) plus detection of aneuploidy,an abnormal chromosome number, that has been estimated to be present in > 90% of the cancer of most histopathologic types [49]. They found that this combination could detect recurrences with a sensitivity of 68% and a specificity of 80%.

RNA tests (Table 2)

RNA biomarkers are less well studied in the field of BC surveillance.

MicroRNAs (miRNAs) are B22-nucleotide long, single-stranded, non-coding RNAs that bind to complementary 'seed' regions found in the 30-untranslated region of particular messenger RNA (mRNA) species. MiRNAs can modulate the expression of their mRNA targets and are involved in many physiological processes, but also in carcinogenesis [50]. Sapre et al. [29] evaluated a 12-miRNA-panel test, with the aim of selecting the minimum number of miRNAs necessary to achieve an accurate prediction. They found that a selection of six miRNAs (miR16, miR200c, miR205, miR21, miR221 and miR34a) provided a sensitivity of 88% and a specificity of 48%.

Kavalieris et al. [30] and Lotan et al. [31] tested a combination of five mRNAs (*IGFBP5, HOXA13, MDK, CDK1, CXCR2*), commercially available under the brand name Cx Bladder Monitor®, and reported highly consistent results for the evaluation of the mRNA expression from the five genes. The studies included a scoring system, based on variables such as previous tumour status (primary or recurrent) and time since previous tumour in years, to classify the test as positive or negative. The authors reported sensitivities between 91 and 92% (95% in high-grade tumours) and a negative predictive value of 96%.

Wallace et al. [33] and Pichler et al. [32] tested the Xpert BC Monitor®, a commercial kit that measures five target mRNAs (*ABL1, CRH, IGF2, UPK1B, ANXA10*), in a population of 510 patients and obtained sensitivities between 73 and 84% (100% in high-grade tumours) and a negative predictive value of 92–93%. They also confirmed that cytology did not enhance diagnostic accuracy.

Discussion

Biomarker investigation is a growing field in the management of NMIBC. Many of the investigations are used in different scenarios: diagnosis, surveillance, and risk stratification of patients with NMIBC. Although many molecular marker tests have been developed to improve diagnostic and surveillance accuracy, with some having been approved by the US Food and Drug Administration, none of the currently available tests have been accepted or incorporated into the follow-up algorithms described in the guidelines [51].

Biomarkers can be divided into cellular, protein and genetic markers. The latter are the most recent and, in contrast to cytology, have the advantages of being reliable, easy to perform, and objective.

In fact, they perform significantly better in BC because thousands of genetic changes can be detected accurately and simultaneously compared with the lower-throughput protein-based biomarkers. As aberrant DNA methylation also occurs in non-malignant tissue it is not pathognomonic of malignancy and genetic methylation cannot be used to distinguish between cancer cells and other pre- or non-neoplastic cells [52]. However, this genetic biomarker has the benefits of always occurring in the same DNA location and chemical stability which make it easier to detect than gene mutations.

Protein-based and cell-based biomarkers are also more likely to be affected by benign conditions such as infection, inflammation and bladder treatments.

To date the gold standard for these cases, as outlined in the guidelines, is to use cystoscopy and cytology. Cystoscopy is an invasive procedure that may be associated with pain and discomfort [5]. Moreover, cystoscopy does not detect all lesions and is subject to the experience of the urologist or nurse [53]. Voided urine cytology needs trained cytopathologists and has the potential for inter-observer variability.

Researchers who are developing urinary biomarkers are looking for high sensitivity and a high negative predictive value. This profile is of special interest in the follow-up scenario because the aim of these tests is to reduce the number of cystoscopies by alternating the procedures, rather than avoiding cystoscopy altogether. Thus, cystoscopy will only be performed when the urine test is positive (urine-first strategy).

One of the major limitations of the use of DNA- or mRNA-based techniques is the difficulty in obtaining sufficiently large quantities of high-quality RNA from voided urine. In terms of monitoring, another limitation of non-invasive urine biomarkers is their low sensitivity,

particularly for early-stage and low-grade tumours that account for a significant proportion of recurrences.

Almost all the studies had a high percentage of 'false'-positive urine tests for the detection of concomitant recurrences, resulting in low specificity. In many articles, the authors justified these percentages with the well-known phenomenon of the anticipatory effect, i.e. the urine test detects recurrent tumours earlier than cystoscopy. It is accepted in the literature that anticipatory detection would include recurrences that occur within the next 18 months after a positive biomarker test [54]. In any case, performing a cystoscopy because of a false positive is more acceptable than missing a tumour because of a false negative.

Other limitations of the studies included in this review are the retrospective nature of some of the cohorts used for the outcome analysis, artificial oversampling of the recurrence rate by recruiting patients scheduled for transurethral resection of a proven bladder tumour, and using the same population for the training and the validation sets, which increases the possibility that the performance of the biomarker may be artificially inflated due to over-fitting.

In this review, most of the biomarker tests are dichotomous, providing either positive (tumour detected) or negative (no tumour) test results. However, giving a numerical prediction of the probability of a recurrent tumour may be more helpful to urologists in terms of their decision-making.

Moreover, there is a lack of uniformity in the design of the studies. Some of the works describe surveillance programs but they create the cohorts. Many of the biomarkers tested need clinical information to complete an algorithm and yield a positive or negative result, which increases subjectivity and decreases the homogeneity of results.

The main limitations were the lack of randomized control trials and the diverse study outcomes, which made meta-analysis impossible to perform. Comparison between sensitivity and specificity of different biomarkers may generate a bias due to the different incidence and different cohort.

Literature lacks of direct comparison between urinary biomarkers and gold standard maybe due to commercial interests.

Conclusion

BC is one of the most expensive tumours due to its high recurrence rate and the costs of the follow-up protocols.

This is the reason why there is an increased interest in biomarkers, in order to reduce the number of exploratory investigations and improve the quality of life of patients with BC. In this review, there are some genetic biomarkers with higher negative predictive value and sensitivity, especially for high-grade tumours, compared to the gold standard. European and US guidelines still recommend cystoscopy and cytology for follow-up. Genetic urinary biomarkers are a very heterogeneous group of test that nowadays cannot replace the standard pathway of surveillance with cystoscopy and cytology. Although there are some ongoing clinical trials comparing both options, there is no level 1 evidence to support their recommendation instead of the gold standard.

Abbreviations

PRISMA: Preferred reporting items for systematic reviews and meta-analyses; QUADAS: Quality Assessment of Diagnostic Accuracy Studies; BC: Bladder cancer; EAU: European Association of Urology; AUA: American Urological Association; NMIBC: Non-muscle invasive bladder cancer; MA: Microsatellite analysis; TERT: Telomerase reverse transcriptase; MiRNA: MicroRNAs; mRNA: Messenger RNA

Acknowledgements

Not applicable.

Authors' contributions

F. L and C.X.R. did the evidence acquisition and the tables. All authors (F.L., C.X.R., A.C., E.T., J.M.) contributed writing the manuscript, read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article (and its supplementary information files).

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 27 February 2020 Accepted: 8 July 2020
Published online: 14 July 2020

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Publisher's Note

5.2. Publicación 2

Título: *Xpert Bladder Cancer Monitor for the Early Detection of Non-Muscle Invasive Bladder Cancer Recurrences: Could Cystoscopy Be Substituted?*

Autores: Fernando Lozano; Carles X. Raventós; Albert Carrión; Carme Dinarés; Javier Hernández; Enrique Trilla; Juan Morote.

Referencia: BMC Urol. 2020 Jul 14;20(1):99

- PMID: 37509344 PMCID: PMC10378094

- DOI: https://doi.org/10.3390/cancers15143683

Impact Factor: 5.2

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Xpert Bladder Cancer Monitor for the Early Detection of Non-Muscle Invasive Bladder Cancer Recurrences: Could Cystoscopy Be Substituted?

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Simple Summary: Non-muscle invasive bladder cancer (NMIBC) accounts for three quarters of newly detected bladder tumors. NMIBC can be treated conservatively with a bladder transurethral resection (bTUR), although recurrences are common despite adjuvant treatments. High-risk recurrent NMIBC can progress to muscle invasive bladder cancer (MIBC) and decrease survival. Therefore, close invasive surveillance, based on cystoscopy and washing cytology, is currently recommended, especially in high-risk recurrent tumors. Urine biomarkers have been investigated unsuccessfully to avoid or postpone the invasive surveillance of NMIBC. Xpert Bladder Cancer Monitor® (XBM) is a new genetic urine biomarker that assesses the expression of five miRNA profiles. In the present study, XBM was not sensitive enough to detect all high-risk recurrences and avoid cystoscopy and washing cytology. However, false positive XBM results can predict early high-risk recurrences.

Abstract: XBM was prospectively assessed in spontaneous urine collected just before flexible cystoscopy and washing cytology carried out within the first 2 years follow-up of 337 patients with NMIBC. Recurrences were pathologically confirmed in 49 patients (14.5%), 22 of them being high-risk (6.5%). The XBM sensitivity for detecting any type of recurrence was 69.4% and 63.6% in the cases of high-risk NMIBC. Negative predictive value (NPV) for XBM was 93% for all recurrences and 96.2% for high-risk recurrences. XBM could have avoided 213 invasive controls but missed the detection of 15 recurrences (30.6%)–8 of them of high-risk (36.4%). XBM false positive elevations were detected in 90 patients (26.7%), whereas 10 patients with the invasive method had a false positive result (3%), p <0.001. However, early detection of recurrences during the first year's follow-up after an XBM false positive result was observed in 18 patients (20%). On the other hand, 19 recurrences were detected during this period among the rest of the patients (7.7%)—p = 0.003, and odds ratio (OR) 3.0 (95% CI 1.5–6.0). Regarding one-year follow-up recurrences, 10% were high-risk recurrences in the XBM false positive group and 3.2% in the rest of the patients—p = 0.021, and OR 3.3 (95% CI 1.2–8.9). Additionally, 11.3% of the patients without false positive results developed a recurrence, p = 0.897, for any recurrence, being 10% and 5.2%, respectively, and high-risk and low-risk recurrences, p = 0.506. After searching for the best XBM cutoff for detecting the 38 high-risk initial recurrences and the early high-risk recurrences after a one-year follow-up, a linear discriminant analysis (LDA) of 0.13 could have avoided 11.3% of cystoscopies and bladder wash cytologies, as this cutoff missed only 1 high-risk recurrence (2.6%). More extensive and well-designed studies will confirm if XBM can improve the surveillance of NMIBC.

Keywords: bladder cancer; biomarker; surveillance

Citation: Lozano, F.; Raventós, C.X.; Carrion, A.; Dinarés, C.; Hernández, J.; Trilla, E.; Morote, J. Xpert Bladder Cancer Monitor for the Early Detection of Non-Muscle Invasive Bladder Cancer Recurrences: Could Cystoscopy Be Substituted?. *Cancers* **2023**, *15*, 3683. https://doi.org/10.3390/cancers15143683

Academic Editors: Ke Chen and Young E. Whang

Received: 19 May 2023
Revised: 28 June 2023
Accepted: 15 July 2023
Published: 19 July 2023

1. Introduction

Bladder cancer is the sixth most common cancer in men, the seventeenth in women, and the tenth most frequent cancer in both sexes worldwide, with an estimated 573,278 new cases and 212,536 deaths in 2020 [1]. Europe has the highest incidence rate in the world, 11 cases per 100,000 persons per year, with the Spanish age-standardized incidence rate being one of the highest, 39 cases per 100,000 habitants in men [2,3]. In Spain, the crude mortality rate is 12/100,000, with significant differences in terms of gender comparison. In men, this mortality rate is 8.1 per 100,000, which is one of the highest in Europe [4,5].

Three quarters of newly diagnosed bladder tumors are non-muscle invasive bladder cancer (NMIBC), which are confined to the bladder mucosa (Ta stage and carcinoma in situ CIS) or the submucosa (T1 stage) [6]. NMIBC has a higher survival expectancy than muscle-invasive bladder cancer (MIBC) (T2-T4 stages), despite the treatment with radical cystectomy [7]. However, the overall recurrence rate of NMIBC is high, requiring frequent endoscopic controls with associated bothers and costs. Cystoscopy is an invasive procedure with a risk of side effects, such as painful micturition (50%), urinary frequency (37%), and macroscopic haematuria (19%) [8]. In addition, white light cystoscopy is not 100% sensitive to non-exophytic lesions or erythematous areas where CIS is suspected [9]. On the other hand, the cytological sensitivity is low, especially for low-grade tumors [10,11]. In addition, most biomarker studies use voided cytology rather than bladder washing, which is more sensitive and specific [12].

Cytology has a high interobserver variability and sometimes differentiates atypical changes, and inflammatory or infectious changes can be challenging for the pathologist [13]. The high NMIBC recurrence rate, usually defined by the European Organization for Research and Treatment of Cancer (EORTC) risk score, ranges from 31 to 78% [14]. It requires a precise surveillance program for early detection and treatment related to increased cancer-specific survival and overall survival [15]. The European Association of Urology (EAU) and the American Urological Association (AUA) guidelines recommend a combination of cystoscopy and cytology for the follow-up of patients with NMIBC [16,17], depending on its frequency on the EORTC risk group [14]. The Food and Drug Administration (FDA) approved urine biomarkers that have lower sensitivity and specificity [18], making their implementation in daily clinical practice challenging.

Research in new genetic urine biomarkers is increasing exponentially. However, most published studies compare voided cytology with the biomarker, which is not the real clinical gold standard. Furthermore, 2022 EAU Guidelines do not recommend using biomarkers in a surveillance protocol for high-risk NMIBC, because their performance cannot improve cystoscopy and cytology performance [19]. In the intermediate and low-risk groups, they suggest that, although there is no high-quality evidence, some of the newly available biomarkers could be used to replace or postpone cystoscopies. Recurrences in these groups are usually low grade, and biomarkers can identify with high sensitivity and negative predictive value the rare high-grade recurrence in this scenario [16].

Due to the lack of clinical alternatives to cystoscopy and cytology as surveillance methods for high-risk NMIBC, there is a trend towards developing new urinary biomarkers [20]. In fact, some of these available urine biomarkers have been recently approved by the FDA, but unfortunately, none have been incorporated into the clinical practice guidelines [21,22]. New modern biomarkers' sensitivity and negative predictive value for high-grade recurrences reach over 90%, but their specificity and positive predictive value are usually low [23–27].

In recent years, due to their reproducibility, reliability, effortless performance, and objectivity, genetic biomarkers have become a promising investigation field in NMIBC surveillance. Genetic material quantification (DNA, RNA, miRNA, and lncRNA) and epigenetic changes, such as DNA hyper- and hypomethylation and histone mutations, have been studied [28]. Genetic biomarkers in bladder cancer have constantly been evolving and encompassing other phases of the disease due to its multiple possibilities for surveillance,

screening, diagnosis, follow-up, treatment response, and prognosis. In addition, urine is an easy, harmless, fast liquid biopsy that contains stable genetic material.

The Xpert Bladder Cancer Monitor (XBM) test is a novel urinary biomarker that measures a panel of five micro-RNA targets (ANXA10, CRH y IGF2, ABL1, and UPK18) by qRT-PCR [29]. Micro RNAs (miRNA) are short, simple chains of 22 non-coding nu-cleotides that can induce posttranscriptional gene silencing by tethering an RNA-induced silencing complex to partly complementary sequence motifs in target mRNAs predominantly found within the 3′ untranslated regions [30]. MiRNAs are involved in multiple physiological and pathological events, including cell proliferation, survival, differentiation, growth, apoptosis, and immune activation [31]. miRNA expression in fluids like blood or urine is stable, allowing its quantification with qRT-PCR [32]. The first study exploring miRNA in bladder cancer was reported in 2007 by Gottardo et al. [33]; they identified the overexpression of ten miRNAs involved in the bladder carcinogenic pathway. Several pathological studies have suggested that low-grade and high-grade NMIBC have different molecular pathways activated, with low-grade tumors associated with the under-expression of some miRNAs. In contrast, the overexpression of miRNA is more common in high-grade tumors [34,35].

This study aimed to compare the urine genetic biomarker XBM with the gold standard methods of follow-up NMIBC based on white light flexible cystoscopy and urine cytology.

2. Materials and Methods

2.1. Design, Setting, and Participants

A prospective head-to-head comparison was made between XBM and the gold standard method of surveillance of NMIBC based on cystoscopy and washing bladder cytology [36] in 352 patients diagnosed between August 2018 and October 2020 in one academic institution. Follow-up evaluations were carried out for one year after XBM measurement to assess the early detection recurrences [37]. This project was approved by the institutional ethical committee (PRAG: 304/2018), and written consent was obtained from all participants.

2.2. Diagnostic Procedure

Bladder transurethral resection (bTUR) of the initial or recurrent tumor was performed. Randomized cold cup biopsies of the bladder and prostatic urethra were performed after bTUR in all initial bladder tumors as part of our hospital's protocol, and in those patients with suspected high-risk tumors to assess simultaneous CIS [19]. Specimens were sent under the protocol to the pathology department. An experienced uro-pathologist analyzed them according to the 2017 T classification of urinary bladder cancer and graded them according to the 1973 and 2004/2016 World Health Organization grade classification [38].

2.3. Adjuvant Preventive Treatment for Recurrences

Postoperative intravesical Mitomycin C (40 mg) was instilled in the recovery room within the first 60 min after surgery if there were clinical indications and no postoperative contraindications based on the guideline's recommendations [16]. Once NMIBC was diagnosed, intravesical recurrence prevention was scheduled according to the EORTC risk of recurrence and progression [39]. Nine patients received systemic immunotherapy in the context of a clinical trial (Table 1).

2.4. Follow-Up for Detection of Recurrences

The follow-up protocol included white light flexible cystoscopy under local anaesthesia in combination with bladder wash cytology obtaining 20 ccs of urine at the end of the procedure from the bladder neck with adequate fixation [16]. The frequency of follow-up cystoscopies and upper urinary tract imaging was based on the current EAU guidelines [16], with at least four cystoscopies and washing cytology per year in the first two years after bTUR in high-risk patients. In low-risk patients, cystoscopy and washing cytology were performed at three and nine months after bTUR and then yearly. In the intermediate-risk

group, cystoscopy and washing cytology were performed every four months within the first two years.

Table 1. Demographic and clinical characteristics of the study cohort.

Parameter	Value
Median age, years (IQR)	73 (65–80)
Gender, *n* (%)	
Male	274 (81.1)
Female	64 (18.9)
Smoke habit, *n* (%)	
Smoker/former smoker	261 (77.2)
Non- smoker	77 (22.8)
Type of tumour, *n* (%)	
Primary	231 (68.3)
Recurrence within one year follow-up	64 (18.9)
Recurrence later than one year follow-up	43 (12.7)
Previous number of recurrences	
One	63 (58.9)
Two or more	44 (41.1)
EORTC * risk of recurrence and progression, *n* (%)	
Low	84 (24.9)
Intermediate	67 (19.8)
High	187 (55.3)
Pathological stage, *n* (%)	
Ta	156 (47.1)
T1	115 (34)
CIS **	12 (3.6)
Tx	55 (16.3)
Pathological grade, *n* (%)	
Low	153 (45.3)
High	185 (54.7)
Adjuvant treatment, *n* (%)	
Postoperative Mytomicin C	128 (38)
No	152 (45)
BCG ***	159 (47)
Mytomicin C	18 (5.3)
Systemic immunotherapy	9 (2.7)
Recurrences diagnosed at the time of XBM assessment, *n* (%)	
Any recurrence	49 (14.5)
High-risk recurrence	22 (6.5)
No recurrence	266 (79)
Recurrences diagnosed within one year follow-up, *n* (%)	
Any recurrence	33 (9.8)
High-risk recurrence	16 (5.6)

* EORTC = European Organisation for Research and Treatment of Cancer; ** CIS = carcinoma in situ; *** BCG = Bacille Calmette-Guerin.

Cytology was evaluated by dedicated cytopathologists. Falcon tubes were centrifuged for 5 min at 2800 rpm. The resulting cell pellets were resuspended in ThinPrep vials (Hologic Inc., Santa Clara, CA, USA) containing a methanol-based PreservCyt solution (Hologic Inc.) and processed using the ThinPrep 5000 System (Hologic Inc.). Cytological specimens were stained in Papanicolaou staining (QCA Química Clinica Aplicada S.A., Amposta, Spain) according to the Papanicolaou staining procedure. Upper urinary tract imaging with a CT scan urography was performed yearly in high-risk tumors [16].

2.5. Recurrence Suspicion and Diagnosis

The recurrence suspect was based either on bladder lesions detected by flexible cystoscopy and/or positive bladder wash cytology. The diagnosis was confirmed after the pathological analysis of bTUR material and/or bladder biopsies. Disease-negative patients had negative cystoscopy and washing cytology or negative pathological biopsy in those with suspicious lesions detected with the cystoscopy in the bladder.

In patients with positive bladder wash cytology but no visible tumor in the cystoscopy, investigation of extravesical locations using CT urography was performed. If no upper urinary tract tumor was detected by imaging, mapping biopsies of the bladder and prostatic urethra biopsy were performed based on the EAU Guidelines algorithm [40].

Recurrences were classified using the 2006 EORTC scoring model and divided into low, intermediate, and high risk [14].

2.6. XBM Assessment

The XBM biomarker was assessed prior to cystoscopy in spontaneously voided urine. Urine was collected the same day of the scheduled cystoscopy. Patients were requested to avoid first void in the morning and asked to collect at least 10 to 20 mL of their spontaneous micturition, preferably of the middle of the voiding. The Xpert Bladder Cancer Monitor® (CE-IVD) was measured with the in vitro diagnostic Cepheid device (Sunnyvale Inc., Santa Clara, CA, USA). A 4.5 mL urine sample was added to the XBM urine transport reagent and mixed. Then, 4 mL of treated urine was transferred to the cartridge sample chamber, where cells in the urine sample were captured on a filter and lysed by sonication. The released nucleic acid was eluted and mixed with dry qRT-PCR reagents, and the solution was transferred to the reaction tube for RT-PCR and detection. The time to result was approximately 90 min. The XBM cartridges were preloaded with all reagents for the sample preparation, qRT-PCR analysis, and detection of five miRNA targets (ABL1, ANXA10, UPK1B, CRH, and IGF2). The cartridge also contained three controls: sample adequacy control (SAC), probe check control (PCC), and cepheid internal control (CIC) for sample-associated inhibition. The qualitative test provided a negative or positive result from the LDA algorithm, with a pre-set cutoff value at LDA $\geq$ 0.5 by the manufacturer, which used the cycle threshold results of these 5 miRNA targets.

Invalid XBM results were not considered, and missing data were not replaced. The XBM result was blinded for the urologist who performed the surveillance control and for the uropathologists.

2.7. XBM "False Positives" Follow-Up

All cohort participants were followed up for one year after the XBM assessment to evaluate early recurrences during this period and the possible anticipatory effect of the biomarker. All early recurrences and early high-risk recurrences were analyzed according to the XBM false positive, and incidents were compared to the rest of the cohort.

2.8. Statistical Analyses

Quantitative variables were expressed as the median and interquartile range (25 to 75 percentile). Qualitative variables were expressed as percentages. The association between quantitative variables was assessed with the Man Witney U test and between qualitative variables with the Chi-square test. The performance was analyzed with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy. Avoided diagnostic procedures, missed recurrences, and high-risk recurrences were also analyzed. Binary logistic regression analysis assessed the predictive value of XBM, cystoscopy, and washing cytology for recurrences and high-risk recurrence. Odd ratios (OR) and 95% confidence intervals (95% CI) were also estimated. Finally, a p-value of less than 0.05 (two-tailed) was considered significant. This analysis was carried out with the SPSS v.25 (IBM, Armonk, NY, USA).

3. Results

3.1. Characteristics of Analyzed Population

A total of 352 urine samples from patients with previous NMIBC were prospectively collected within the first 24 months of follow-up after their last NMIBC diagnostic performed by bTUR. Of these, eight patients were excluded due to invalid tests and five patients due to the absence of pathology. Another two patients were excluded due to the absence of histological confirmation of recurrence. Finally, 337 patients were included in the statistical analysis. The demographical and clinical characteristics of the patients are described in Table 1. A flowchart showing patient selection is described in Figure 1.

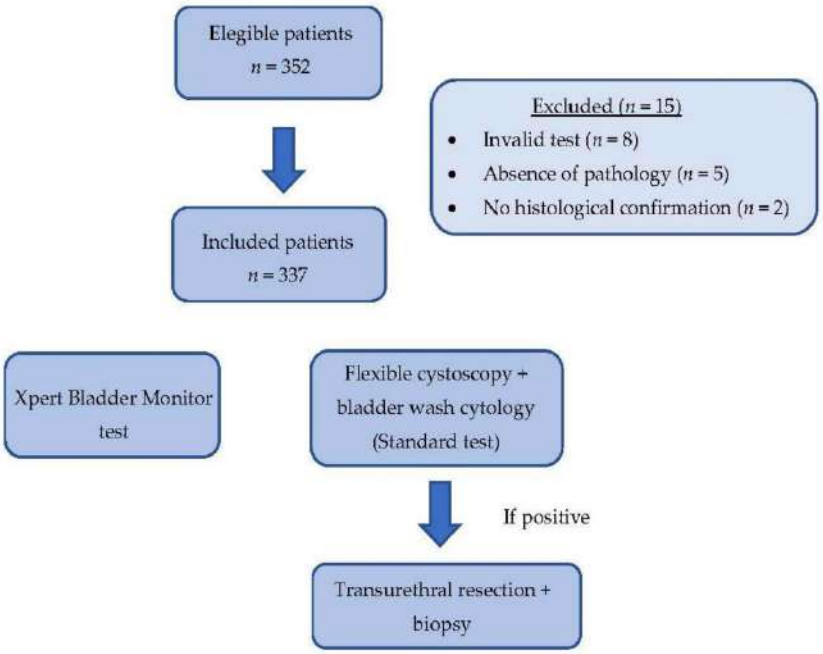

Figure 1. Flowchart of the patients' recruitment process.

The median age of the cohort was 73 years (interquartile range 65–80 years); 81.1% were males; 77.2% were smokers or former smokers; 68.3% were primary tumors, and 54.7% were high-grade tumors; 38% of the patients received postoperative mitomycin C; and 47% of the cohort received a full dose of BCG adjuvant therapy for one year.

During follow-up, 49 recurrences were detected (14.5% of the samples), with 5.5% being high-risk recurrences. During the one year follow-up, 33 recurrences were diagnosed—16 of them of high-risk. The median follow-up of the cohort was 13.5 months.

3.2. Performance of XBM

Sensitivity for any type of recurrences for XBM and cystoscopy plus bladder-wash cytology were 69.4% and 100%, respectively. Specificity for any type of recurrences of XBM and cystoscopy plus bladder-wash cytology were 68.8% and 96.5%, respectively. The NPV of XBM was 93%. Furthermore, the accuracy of XBM and cystoscopy plus bladder-wash cytology were 68.8% and 97%, respectively. In high-risk recurrences, the sensitivity of XBM and cystoscopy plus bladder-wash cytology were 63.6% and 100%, respectively. Specificity in the high-risk scenario for XBM and cystoscopy plus bladder-wash cytology were 65.1% and 88.3%, respectively. In high-risk, the NPV of XBM reached 96.2%. The accuracy of XBM

and cystoscopy plus bladder-wash cytology for high-risk recurrences was 65% and 89%, respectively. These results are given in Table 2.

Table 2. Incidence percentage of grade and stage diagnosed using the Xpert Bladder Monitor.

Variable	Xpert Bladder Monitor	
Grade	Positive	Negative
Low Grade, *n* (%)	20/27 (74.1)	7/27 (25.9)
High Grade, *n* (%)	14/22 (63.6)	8/22 (36.4)
Pathological stage		
Ta, *n* (%)	19/27 (70.4)	8/27 (29.6)
Tx, *n* (%)	6/7 (85.7)	1/7 (14.3)
Tis *, *n* (%)	4/4 (100)	0/4 (0)
T1, *n* (%)	3/9 (33.3)	6/9 (66.7)
T2, *n* (%)	2/2 (100)	0/2 (100)

* Tis: Cacinoma in situ.

3.3. Prediction of Risk of Recurrence

Univariate XBM analysis, bladder wash cytology and flexible cystoscopy showed statistical significance for detecting recurrences for the three suspicion methods. A logistic multivariant regression was performed with the same three methods. Cystoscopy showed statistical significance for all high-risk recurrences and washing cytology only for high-risk recurrences (Table 3).

Table 3. Performance of XBM compared with cystoscopy and washing cytology for the suspicion of any type of recurrence and high-risk recurrences at the time of XBM assessment.

Parameter	All Recurrences		High-Risk Recurrences	
	XBM	Cystoscopy and Washing Cytology	XBM	Cystoscopy and Washing Cytology
Sensitivity, *n* (%)	34/49 (69.4)	49/49 (100)	14/22 (63.6)	22/22 (100)
Specificity, *n* (%)	198/288 (68.8)	278/288 (96.5)	205/315 (65.1)	278/315 (88.3)
Positive predictive value, *n* (%)	34/124 (27.4)	49/59 (83.1)	14/124 (11.3)	22/59 (37.3)
Negative predictive value, *n* (%)	198/213 (93.0)	278/278 (100)	205/213 (96.2)	278/278 (100)
Accuracy, *n* (%)	232/337 (68.8)	327/337 (97.0)	219/337 (65.0)	300/337 (89)
Avoided diagnostic procedures, *n* (%)	213/337 (63.2)	0 (0)	213/337 (63.2)	0 (0)
Missed recurrences, *n* (%)	15/49 (30.6)	0 (0)	8/22 (36.4)	0 (0)

Univariate and multivariable analysis have been performed, selecting the three follow-up tests (Table 4).

Table 4. Univariate and multivariable analysis of XBM, cystoscopy, and washing cytology as suspicion methods for predicting any type of recurrence and high-risk recurrences diagnosed at the time of XBM assessment.

Method of Suspicion	Univariate Analysis		Multivariable Analysis	
	Odd Ratio (95% CI)	*p* Value	Odd Ratio (95% CI)	*p* Value
For any type of recurrence				
XBM	4.987 (2.586–9.616)	=0.001	3.585 (0.820–15.675)	=0.090
Cystoscopy	615.524 (153.624–2466.212)	<0.001	1517.105 (175.210–13136.239)	<0.001
Washing cytology	15.975 (4.700–54.296)	<0.001	100.409 (7.207–1398.817)	=0.110
For high-risk recurrences				
XBM	3.261 (1.327–8.014)	=0.007	0.723 (0.226–2.312)	=0.585
Cystoscopy	52.343 (14.720–186.124)	=0.001	53.712 (13.243–217.851)	<0.001
Washing cytology	24.033 (7.186–80.377)	=0.001	22.473 (3.530–143.048)	=0.001

3.4. Follow Up on False Positives of XBM

False positive patients (positive biomarker and negative cystoscopy plus washing cytology, as defined from protocol) were followed up for one year to evaluate the possibility of an anticipatory effect of the biomarker in detecting early recurrences. Of the 90 (23.7%) false positive patients, 18 (20%) developed a recurrence that year, including 8 low-grade, 8 high-grade, and 2 upper urinary tract tumors. Statistically significant differences ($p < 0.001$) were found with XBM-negative patients that presented a recurrence rate of 6.1%. The odds ratio of patients with positive biomarkers but negative cystoscopy and cytology was 3 (1.494–6.023) and 3.3 (1.239–8.890) for high-risk disease.

3.5. Searching for a Clinically Useful XBM Cutoff

The performance of XBM with the manufacturer LDA recommended a cutoff of 0.5 and exhibited a sensitivity of 63.6 for high-risk recurrences. Therefore, it was necessary to search for an XBM cutoff with higher sensitivity for high-risk recurrence discrimination at the time of its assessment and those early diagnosed high-risk recurrences within the first-year follow-up. The area under the curve (AUC) of XBM was 0.725 (95% CI: 0.620–0.829), Figure 2. Table 5 presents the LDA cutoff values of XBM of sensitivities between 100 and 75, its specificities, and the sensitivity and specificity corresponding to the 0.5 cutoff proposed by the manufacturer. We selected 0.1294 as the XBM cutoff with 95% sensitivity because it only missed 5% of high-risk recurrences, which is clinically reasonable for a biomarker that aspires to replace the gold standard surveillance protocol of NMIBC based on cystoscopy and cytology.

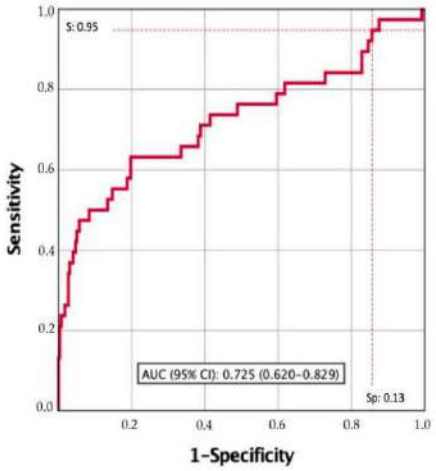

Figure 2. Discriminatory ability of XBM measurement to suspect high-risk recurrences at the time of its assessment and those diagnosed within the one-year follow-up. The 95% sensitivity of the XBM cutoff exhibits a 13% specificity, and the AUC is 0.725 (95% CI: 0.620–0.829).

The sensitivity of XBM increased to 96.3%, but the specificity decreased to 13.7%. NPV was 92.1% and PPV 26.4%. For high-risk recurrences, the sensitivity was 97.4%, specifically 12.4%, NPV 97.4% and PPV 12.4%. The accuracy for XBM was 33.8% and 22% for all recurrences and high-risk recurrences, respectively. What seems more important from a clinical point of view is that 11.3% of cystoscopies and bladder wash cytologies could be avoided, as this cutoff missed only 2.6% of high-risk recurrences (Table 6).

Table 5. Specificities corresponding to the cutoff sensitivities of 1.00 to 0.75 of XBM for the suspicion of high-risk recurrence at the time of its assessment and those diagnosed within the one-year follow-up. Sensitivity and specificity corresponding to the cutoff recommended by the manufacturer (LDA = 0.5) are 63% and 66.6%, respectively.

Sensitivity (%)	Specificity (%)	Cutoff
100	7.1	0.1117
95	13.4	0.1294
90	15.4	0.1459
85	17.1	0.6661
80	38.1	0.2950
75	51.2	0.3950
63	66.6	0.5000

Table 6. Performance of XBM (using the 0.1294 cutoff) compared with cystoscopy and washing cytology for the suspicion of any type of recurrence and high-risk recurrences at the time of XBM assessment and within one year follow-up.

Parameter	All Recurrences		High-Risk Recurrences	
	XBM	Cystoscopy and Washing Cytology	XBM	Cystoscopy and Washing Cytology
Sensitivity, *n* (%)	79/82 (96.3)	50/82 (61.0)	37/38 (97.4)	24/38 (63.2)
Specificity, *n* (%)	35/255 (13.7)	246/255 (96.5)	37/299 (12.4)	264/299 (88.3)
Positive predictive value, *n* (%)	79/299 (26.4)	50/59 (84.7)	37/299 (12.4)	24/59 (40.7)
Negative predictive value, *n* (%)	35/38 (92.1)	246/278 (88.5)	37/38 (97.4)	264/278 (95.0)
Accuracy, *n* (%)	114/337 (33.8)	296/337 (87.8)	74/337 (22.0)	288/337 (85.5)
Avoided diagnostic procedures, *n* (%)	38/337 (11.3)	278/337 (82.5)	38/337 (11.3)	278/337 (82.5)
Missed recurrences, *n* (%)	3/82 (3.7)	32/482 (39.0)	1/38 (2.6)	14/38 (36.8)

The univariate analysis for predicting high-risk recurrences showed a significant XBM, cystoscopy, and bladder wash-cytology value. However, XBM was not an independent predictor in the multivariable analysis, as described on Table 7.

Table 7. Univariate and multivariable analysis of XBM, cystoscopy, and washing cytology as suspicion methods to predict any type of recurrence, and high-risk recurrences diagnosed at the time of XBM assessment and those diagnosed within one year follow-up.

Method of Suspicion	Univariate Analysis		Multivariable Analysis	
	Odd Ratio (95% CI)	*p* Value	Odd Ratio (95% CI)	*p* Value
For any type of recurrence				
XBM	4.189 (1.253–14.004)	=0.090	2.178 (0.567–8.369)	=0.257
Cystoscopy	55.729 (22.200–139.897)	<0.001	49.818 (19.623–126.477)	<0.001
Washing cytology	11.667 (3.127–43.522)	<0.001	7.762 (1.597–37.711)	=0.110
For high-risk recurrences				
XBM	5.225 (0.96–39.227)	=0.052	2.644 (0.337–20.714)	=0.355
Cystoscopy	10.307 (4.932–21.540)	< 0.001	8.182 (3.766–17.773)	<0.001
Washing cytology	15.680 (4.824–59.968)	< 0.001	9.504 (2.515–35.919)	=0.001

4. Discussion

Although increasing evidence suggests that new urine biomarkers have good performance for NMIBC surveillance, none has been consolidated as an actual alternative to the gold standard of cystoscopy and washing bladder cytology [41]. EAU Guidelines of 2021 [42] confirm that urinary markers cannot replace cystoscopy during follow-up or re-

duce the cystoscopy frequency. However, for the first time, the possibility of using biomarkers or bladder ultrasounds in patients initially diagnosed with TaG1-2/LG bladder cancer for surveillance has been noted in the case where cystoscopy was not possible or refused by the patient [43]. In the EAU Guidelines of 2022 [36], the potential role of four promising and commercially available urine biomarkers, Cx-Bladder [25], ADX-Bladder [44], Xpert Bladder [45], and EpiCheck [46], have been highlighted [3]. These markers have not been tested in randomized trials, so this novel approach cannot routinely replace cystoscopy during follow-up or lower cystoscopy frequency. Nevertheless, their high sensitivities and negative predictive values in the referenced studies, mainly for high-grade tumors and diseases, make these biomarkers attractive in avoiding cystoscopies in the follow-up of low/intermediate NMIBC [47]. This new step for biomarkers opens an alternative to the classical follow-up. It points out the option to individualize the surveillance protocols, considering the tumor characteristics and the patient's age and performance status.

The first study of altered miRNA expression in bladder cancer was published in 2007 and detected the upregulation of 10 miRNAs [48] and miRNA as a urine biomarker for bladder cancer, as initially described by Weber et al. [49]. This genetic material expression in bladder cancer varies with intravesical treatment exposure and tumor grade. The profile of altered miRNAs differs between low- and high-grade tumors. In fact, high-grade NMIBCs share similar miRNA profiling to muscle-invasive tumors [32]. Since then, many miRNAs have been tested to detect and monitor bladder cancer patients. Although low-grade tumors usually have downregulation of many miRNAs, upregulation is more common in high-grade bladder cancer [31].

XBM was first validated in a multicentric study by Wallace et al. [50]. Since then, XBM has been tested in 10 studies [29,50–55] with more than 3000 patients. Overall sensitivity and specificity varied from 29.8 to 84% and 73.7 to 94.1%, respectively. The negative predictive value was between 83 and 96.5%, and the positive predictive value was between 44 and 90.9% [24]. One of the strengths of the XBM is that the test is automated. XBM can be assessed at the point of care and gives a fast result, with an easy and short hands-on sample preparation time of less than five minutes and single-use available disposable cartridges. It should, therefore, give the same result wherever patients are managed, whereas cytology results are pathologist-dependent [56]. All the previous studies made a direct comparison between both urine biomarkers, cytology, and XBM. However, none attempt to compare the whole follow-up protocol based on cytology plus cystoscopy was carried out [57].

This study was the first to compare XBM in a real clinical setting. The performance of XBM was compared to the gold standard follow-up of NMIBC, which included cystoscopy and bladder washing cytology. Considering this head-to-head comparison, XBM had a 68.8% sensitivity, 93% negative predictive value for all recurrences, and 96.2% for high-risk recurrences. This was in line with previous studies [51–53]. Nevertheless, compared to our daily clinical practice, XBM seemed unable to substitute the combination of cystoscopy and washing cytology by itself. Due to this and considering the biomarker's ROC curve, we had carried an ad hoc analysis to find a better cutoff for the biomarker that may help detect all the high-risk recurrences during the follow-up. The counterpart of modifying the XBM pre-set threshold was decreasing the test's specificity, which is associated with more negative cystoscopies, increasing the cost of the follow-up program and the patient's anxiety due to a higher risk of false positive results. With the LDA threshold of 0.1294, the sensitivity of XBM increased to 96.3%, with a negative predictive value of 92.1%. Using this new threshold, 11.3% of the cystoscopies could be avoided with only a 2.6% chance of missing a high-risk recurrence, which are parameters comparable to the gold standard follow-up. On the other hand, specificity for all recurrences decreased to 13.7%

Biomarkers' usefulness for the follow-up of NMIBC is based on four main criteria according to the ICUD-EAU International Consultation consensus [58]. First, they must be better—i.e., superior in clinical aspects to the standard tests (more sensitive, better NPV). Secondly, they must be simple, reproducible, and avoid complex infrastructures that complicate their standardization and dissemination. Thirdly, they should be faster

or, at least, the biomarkers' results should be available in a short period time. Lastly, they must be cheaper or economically similar to the gold standard combination. Cost-efficacy studies of biomarkers are complex and usually based on non-clinical models [59]. Due to the significant variability between countries' health care systems and the complexity of the evaluation of indirect costs of the cystoscopy (urologist time, nurse, material, and theatre time), the comparison between the standard protocol and biomarker follow-up had a high risk of bias. Nam et al. [60] demonstrated that a follow-up based exclusively on biomarkers was economically more efficient than the standard follow-up. However, their study did not take into account the costs and profiles of the most recent biomarkers. After evaluating the biomarker in a cohort study, a randomized control trial should be carried out comparing the gold standard method, cystoscopy plus cytology, and the urine biomarker in a real clinical scenario. If results confirm the non-inferiority performance, the next step to establish a new protocol based on biomarkers follow-up, subsidized by the National Health Care System, should include a cost-efficiency study.

Besides the four main criteria suggested by the ICUD-EAU International Consultation, another characteristic should be considered when changing the paradigm of NMIBC surveillance. Most of the biomarkers' studies presuppose that patients will agree to change their follow-up protocol because this new tool is essentially as sensitive as cystoscopy and cytology. Flexible cystoscopy continues to be an invasive procedure, costly, bothersome, and painful for the patients; it increases the risk of urinary tract infection, and over 60% of the patients experience adverse psychological effects related to the procedure [61,62]. Moreover, this method has limitations for detecting small and flat lesions (post-TURBT, CIS) [63]. Nevertheless, a study by van Osch et al. [64] confirmed that half of the patients would not replace cystoscopy unless the biomarker was 100% sensitive, and 85% of the patients would only change if the biomarker performance achieved 99% of sensitivity. Moreover, research by Shen Tan et al. [65] confirmed that although patients experienced bothersome symptoms after cystoscopy, with hematuria in 51% or dysuria in 69% of them, they are more confident with a visual diagnosis of the bladder and would only accept the change if the biomarker had at least the same sensitivity as cystoscopy. This situation may not reflect a complete understanding of the concept of patient sensitivity and their fear of the possibility of missing a recurrence. An actual clinical scenario should be transmitted to the patients and the differences in the profile of the biomarkers; in the low-risk group, missing a single, small bladder tumor does not impact the patient´s overall survival or cancer-specific survival. However, in the high-risk group, early detection is mandatory, and a biomarker will never substitute the gold standard if it cannot detect small high-grade recurrences.

A positive biomarker result had been demonstrated to increase the cystoscopies' detection rate [32]. When analyzing the longitudinal effect, definitions are contradictory. Some recent studies suggested that enhanced image cystoscopies may improve the detection of small or plain lesions, hence decreasing false positive biomarker results [66]. It has also been suggested that the Studer's algorithm should be applied to exclude extravesical recurrences in cases with negative cystoscopy but positive biomarkers, including cytology [67]. A previous XBM study by Cowan et al. [68] has explored this possible anticipatory effect. In their research, 131 patients were followed up for 1 year with negative cystoscopy, independently of the cytology result, comparing those with positive and negative biomarkers. It was found that the former had an increased risk of developing a high-grade recurrence.

As a secondary objective of this study, our patients were followed up for one year to evaluate if a false positive biomarker could have any anticipatory information about the risk of recurrence. Some positive urine biomarkers are associated with an increased risk of recurrence and progression, even if the patient had a negative cystoscopy at the time of the determination [69]. Gopalakrishna et al. [70] tried to define the positive anticipatory result for bladder cancer. They assumed a period of one year to define the possible anticipatory result. They demonstrated that a positive urine test does not always mean future tumor recurrence. Only 75% of the positive cytologies or 40% of the positive UroVysion FISH tests developed a tumor within one year.

It was unclear in the literature if this anticipatory effect should make us change our clinical practice protocol with these patients, such as a more intense cystoscopy follow-up, random biopsies, and upper urinary tract endoscopic exploration. In our cohort, false positive XBMs were followed for one year. The recurrence rate in the false positive XBM group was 20%, while only 6.1% of the patients with an XBM negative test experienced a recurrence. That means that when the biomarker was positive but gold standard methods were negative, the patient had a statistically increased odds ratio of 3 (1.5–6) with $p < 0.003$ for all recurrences, and an odds ratio of 3.3 (1.2–8.9) with $p < 0.02$ for high-risk recurrences within the following year. This study opened an option for a new interpretation of the genetic urine biomarkers. Until now, a negative or positive result was read transversally. Nevertheless, the biomarkers' field still has many open questions, and one of those is how a positive result without macroscopic translation must be read. The one year follow-up in our study demonstrated that patients with positive XBM had a higher risk of developing a recurrence, and this information should be taken into account by clinicians to adjust the follow-up scheme. However, this data should be interpreted carefully, and information given to the patient must be based on evidence-based follow-up protocols to avoid anxiety and changes in the follow-up protocol weighted due to the lack of clear perspective and high-level data. If that information should change our protocol is a question that cannot be answered nowadays. Moreover, we assumed that the definition of a false positive biomarker's result was based on a negative cystoscopy and cytology. Future studies may include a negative upper urinary tract study with a CT scan and/or bladder random biopsies with prostatic urethral biopsies as a confirmatory protocol.

This study had some limitations. On the one hand, negative and positive predictive values are parameters influenced by disease incidence prevalence. Selecting patients during the first two years of follow-up was not the actual clinical scenario and can increase the incidence prevalence of recurrences, hence overestimating both parameters. Moreover, the low recurrence rate in our study (14.5% of the cohort), may have had an impact on the difficulty of finding statistically significant differences. On the other hand, patients were monitored with cystoscopy, following the 2021 EAU Guidelines. The mean recurrence size was 0.8 cm (0.3–1.6 cm), which could be considered a low tumor burden detected by biomarkers. Different cytology specimens have also been used, such as spontaneous miction urine to avoid invasive follow-up methods for XBM and bladder wash cytology for cytopathologic study. That means that different samples were compared for urine biomarkers. Interestingly, although these are two different methods of obtaining the urine sample, sensitivity and NPV were still high for XBM.

Neither our study nor the previous papers had a real clinical design randomizing cystoscopy and XBM, which can complicate the implementation of daily clinical practice. Alternating biomarkers and cystoscopy plus cytology is another approach our study had not explored.

5. Conclusions

XBM had demonstrated an acceptable sensitivity and negative predictive value for high-risk recurrences of NMIBC. A change in the threshold proposed by the manufacturer increased its sensitivity and negative predictive value in our series, with a slight decrease of specificity. Although XBM did not guarantee the 100% prediction of high-risk recurrences, its positive result in absence of cystoscopic or cytologic confirmation of bladder tumors increased the probability of developing a tumor recurrence in the next year of its determination. Longitudinal and randomized studies are needed to identify the exact role of XBM in the surveillance of NIMBC.

Author Contributions: Conceptualization, F.L., J.M. and C.X.R.; methodology, F.L., J.M. and C.X.R.; software, F.L. and C.X.R.; validation, F.L., J.M. and C.X.R.; formal analysis, F.L., J.M. and C.X.R.; investigation, F.L.; resources, F.L.; data curation, F.L., J.M. and C.X.R.; writing—original draft preparation, F.L., J.M. and C.X.R.; writing—review and editing, F.L., J.M. and C.X.R.; visualization, all authors;

supervision, all authors; project administration, all authors; funding acquisition, F.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of VHIR (Vall Hebron Institut Recerca), protocol PR(AG)304/2018 approved 21 September 2018.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study is available on request from the corresponding author.

Acknowledgments: Thanks to Eulalia Barredo for her technical support. Thanks to Cepheid Ltd. for providing support to the study supplying the cartridges, the hardware, and the software to analyze the samples.

Conflicts of Interest: The authors of this article do not declare any conflict of interest. Cepheid had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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6. Resumen global de resultados

En la revisión sistemática de la literatura realizada en la primera publicación acerca de biomarcadores genéticos urinarios en el seguimiento del TVNMI, se seleccionaron 21 artículos siguiendo los criterios PRISMA (*Preferred Reporting Items for Systematic Reviews and Meta-analyses*) (120). A pesar de que el riesgo de sesgo alcanzó el 50% en el conjunto de los artículos, la mayoría de ellos lograron cumplir los objetivos establecidos por los criterios de la herramienta QUADAS-2 (121), indicando así una moderada-alta calidad de la evidencia. La revisión de los artículos seleccionados se llevó a cabo mediante el uso de *checklist* de REMARK (122) y las pautas para informar la metodología del estudio conforme los criterios SWIM (123).

Los resultados se resumieron en dos tablas, una para biomarcadores de DNA y otra para biomarcadores de RNA (Tablas 1-2, artículo 1) (98).

Table 1 DNA based biomarkers used in follow-up for non muscle invasive bladder cancer patients

Reference	Patients/ samples	Recurrence rate	Sensitivity(%)	Specificity(%)	NPV (%)	PPV (%)	AUC	Method	Markers
Roupret *et al* 2008 [13]	40/40	38%	80 (microsatellite) 86 (methylation) 85 (combination)	68 (microsat) 8 (methyl) 86 (combination)			0.81 (microsat) 0.44 (methyl)	DNA PCR	Microsatellite[a] vs methylation[b]
Van der Aa *et al* 2009 [14]	228/815	10.3%	58	73	94	61-77	NA	DNA PCR	Microsatellite + FGFR3 mutation
Zuiverloon *et al* 2010 [15]	134/463	9.7%	58	NA	89	25	NA	DNA PCR	FGFR3 mutations[c]
Reinert *et al* 2012 [16]	158/206	67.4%	87-94	28-47	55-78	72-78	0.68-0.78	DNA PCR	Methylation [d]
Zuiverloon *et al* 2012 [17]	NA/94	69.1%	72.3	55.2	NA	NA	NA	DNA PCR	Methylation genes APC_a , TERT_a , TER _b ,EDNRB
Allory *et al* 2013 [18]	194/395	44.8%	19(FGFR3) 42(TERT) 50(FGFR3+TERT)	73 (TERT) 90(FGFR3) 71(FGFR3+TERT)	NA	NA	NA	DNA PCR	Gene mutations (TERT and FGFR3)
Abern *et al* 2014 [19]	111/111	21.6%	75-79	63-71	92	37-42	0.74 (TWIST1) 0.68 (NID2)	DNA PCR	Methylation genes TWIST1, NID2
Su *et al* 2014 [20]	90/368	37.7%	80	97	NA	NA	0.95	DNA PCR	Hyper and hypomethylated genes (SOX1, IRAK3, L1-MET)
Fantony *et al* 2015 [21]	126/126	25%	58-67	61-69	83-85	36-38	0.66 (TWIST1) 0.63 (NID2)	DNA PCR	Methylation genes TWIST1, NID2
Beukers *et al* 2016 [22]	NA/2191	64%	57 (LG) 72 (HG)	59% LG	NA	NA	NA	DNA PCR	FGFR3 mutation, TERT mutation and OTX1 methylation
Roperch *et al* 2016 [23]	158/613	45.5%	94.5 96 (HG)	75.9	98.5	NA	0.82	DNA PCR	FGFR3 mutation +DNA methylation HS3ST2, SLIT2 and SEPTIN9
Van der Heijden *et al* 2018 [24]	NA/458	37.7%	90	31	82	50	0.74	DNA PCR	DNA gene Methylation (CFTR, SALL3, TWIST1)
Witjes *et al* 2018 [25]	353/353	13%	68.2 92.6 (HG)	88	95.1 99.3(HG)	44.8	0.82	DNA PCR	15 DNA methylation genes (Epicheck®)
Springer *et al* 2018 [26]	322/322	58%	68 71 (HG)	80	NA	NA	NA	DNA PCR	10 gen mutations[e] plus detection of aneuploidy (UroSEEK®)
D'Andrea *et al* 2019 [27]	357/357	13.7%	67.3 88.9 (HG)	88 88(HG)	94 99 (HG)	47 30 (HG)	85.9	DNA PCR	15 DNA methylation genes (Epicheck®)
Batista *et al* 2019 [28]	122/122	28%	73.5	73.2	NA	NA	NA	DNA PCR	TERT promoter and FGFR3 mutations (Uromonitor®)

LG low grade, *HG* high grade, *NA* not allowed

[a]FGA (4q28), D4S171(4q35)), 5 (ACTBP2(5q14)), 9 (D9S162 (9p), IFNA (9p21)), 14 (MJD52(14q32)), 16 (D16S310 (16q21)) and 18 (D18S51 (18q21), MBP (18qter).

[b](RASSF1a (3p21.3),E-cadherin (16q22.1), APC (5q21), DAPK (9q22.1), MGMT (10q26), BCL2 (18q21.33), h-TERT (5p15.33), EDNRB (13q22), WIF-1 (12q14.3), TNFRSF25 (1p36.31), IGFBP3 (7p13))

[c]R248C and S249C (exon 7); G372C,S373C, Y375C, G382R, and A393E (exon 10); and K652M, K652T, K652E, and K652Q (exon 15)

[d]EOMES, HOXA9, POU4F2, TWIST1, VIM, ZNF154

[e]FGFR3, TP53, CDKN2A, ERBB2, HRAS, KRAS, PIK3CA, MET, VHL, MLL and TERT promoter.

Table 2 RNA based biomarkers used in follow-up for non muscle invasive bladder cancer patients

Reference	Patients/samples	Recurrence rate	Sensitivity	Specificity	NPV	PPV	AUC	Method	Markers
Sapre *et al* 2016 [29]	131/131	NA	88	48	75	63	0.74	miRNA PCR	6 miRNA signature[a]
Kavalieris *et al* 2017 [30]	736/1036	15.1%	92	NA	96	NA	0.73	mRNA PCR	5 genes mRNA expression (Cx Bladder Monitor®)[b]
Lotan *et al* 2017 [31]	748/1016	14.8%	91 95 (HG)	NA	96	NA	NA	mRNA PCR	5 genes mRNA expression (Cx Bladder Monitor®)[b] vs NMP22 ELISA vs NMP22 BladderChek
Pilcher *et al* 2018 [32]	140/155	30.7%	84 100 (HG)	91	93	72	0.87	mRNA RT-PCR	ABL1, CRH, IGF2, UPK1B, ANXA10 (Xpert Bladder Cancer Monitor®)
Wallace *et al* 2018 [33]	370/370	13.2%	73 83 (HG)	77	92	44	0.87	mRNA RT-qPCR	ABL1, CRH, IGF2, ANXA10, UPK1B (Xpert Bladder Cancer Monitor®)

HG high grade, *NA* not allowed
[a]miR16, miR200c, miR205, miR21, miR221 and miR34a
[b]IGFBP5, HOXA13, MDK, CDK1, CXCR2

Los estudios iniciales de biomarcadores utilizando DNA se remontan a 2009, cuando se realizaron comparaciones entre las alteraciones en los microsatélites de los genes y los biomarcadores de metilación de DNA. En este primer estudio de Rouprêt *et al.* (94) se evaluó la eficacia en la detección de recidivas de las alteraciones de microsatélites en comparación con los biomarcadores de metilación de ADN en 40 pacientes, evidenciando mejores resultados en el primer grupo, mostrando unas áreas bajo la curva (AUC) de 0.81 y 0.44, respectivamente.

Las metilaciones en el DNA son alteraciones presentes en diversas neoplasias (124–126). Es un marcador epigenético, es decir, que es capaz de regular la expresión genética sin modificar la secuencia de DNA que lo compone (127). Afecta principalmente a los dinucleótidos de CpG, aunque también se puede encontrar de forma fisiológica en procesos de apoptosis celular. Estos dinucleótidos se encuentran distribuidos a lo largo del genoma y, en la mayoría de las ocasiones, presentan un estatus normal de metilación. La hipermetilación de los dinucleótidos de CpG a nivel de la región promotora de los genes supresores tumorales puede inhibir su transcripción en células humanas (128,129). Las metilaciones son biomarcadores eficaces en el seguimiento del tumor vesical debido a su estabilidad bioquímica, su fácil identificación en orina y capacidad de cuantificación (130). Además de las metilaciones de DNA, otra de las alteraciones estudiadas como biomarcador para el seguimiento de tumor vesical son las mutaciones genéticas, dada la gran heterogeneidad genética del

cáncer vesical (131). Una de las más estudiadas es la mutación en el *Fibroblast Growth Factor Receptor 3* (FGFR3), presente en hasta el 80% de los tumores de bajo grado y asociada a mejor pronóstico oncológico (132,133). Los estudios identificados en la revisión sistemática evidencian sensibilidades que oscilan entre el 19% y el 94.5% y VPN entre 89% y el 98.5% (134–137).

Existe menos evidencia sobre los biomarcadores basados en la identificación de RNA tumoral. Los biomarcadores desarrollados utilizan la detección de combinaciones de microRNA (miRNA) o RNA mensajeros (mRNA) para detectar las posibles recidivas. Tras una fase preclínica exploratoria, en la que se analizan diversas combinaciones de RNA, se seleccionan aquellas que presentaban una mejor correlación entre las muestras de orina de los pacientes y los tejidos tumorales. Los miRNA son moléculas de una sola cadena con una longitud de 22 nucleótidos que pertenecen al grupo de RNA no codificante y se unen a regiones complementarias de los mRNA. Este mecanismo les permite modular la expresión de los mRNA involucrados tanto en procesos fisiológicos como en procesos de carcinogénesis (138). Se identificaron cinco artículos en el seguimiento del TVNMI que analizaban diversas combinaciones de mRNA; la mayoría de estos estudios se centran en los biomarcadores comercializados en Europa: Cx Bladder Monitor™ y Xpert Bladder Cancer Monitor® (106,139–141).

En el segundo artículo, se realizó un estudio prospectivo con el objetivo de comparar el rendimiento del biomarcador urinario Xpert Bladder Monitor® (XBM) frente al seguimiento estándar en pacientes con TVNMI con cistoscopia flexible y citología urinaria por lavado. Se analizaron 337 pacientes con antecedente de TVNMI intervenido mediante RTU-V en los últimos 24 meses previos a la cistoscopia, con ambos métodos de seguimiento. Posteriormente, se realizó una evaluación de los pacientes durante un año de seguimiento, y RTU-V en aquellos en los que se detectó recidiva en la cistoscopia y/o pacientes con citología positiva para carcinoma urotelial sin lesiones exofíticas ni evidencia de tumor de tracto urinario superior por pruebas de imagen. La edad mediana de la cohorte fue de 73 años, el 77.2% de los pacientes eran fumadores o exfumadores, y el 81.1% varones. El

68.3% de los tumores en seguimiento eran primarios y el 54.7% eran de alto grado. Además, el 47% de los pacientes recibieron BCG a dosis plenas durante un año. A lo largo del seguimiento, se identificaron 49 recurrencias (14.5% de las muestras), siendo un 5.5% de alto grado. Durante el año posterior de seguimiento, se detectaron 33 recurrencias, de las cuales 16 fueron clasificadas de alto riesgo.

La sensibilidad de XBM para la detección de cualquier recidiva tumoral fue de 69.4%, en comparación con el 100% logrado mediante la combinación de cistoscopia y citología por lavado vesical. Respecto a la especificidad, XBM exhibió un valor de 68.8% en contraste con el 96.5% observado para la cistoscopia y la citología por lavado. El VPN para todas las recidivas de XBM fue del 93%. La exactitud diagnóstica de XBM fue del 68.8%, en comparación con el 97% obtenido mediante la realización de cistoscopia y citología por lavado. En el escenario de recurrencias de alto riesgo, la sensibilidad, especificidad y VPN de XBM fue 65.1%, 88.3% y 96.2%, respectivamente.

Con el objetivo de valorar la predicción del riesgo de recurrencia del biomarcador, se realizó un análisis univariante que demostró que XBM, la citología por lavado y la cistoscopia presentaban significación estadística para detectar recurrencias. Posteriormente, se llevó a cabo una regresión logística binaria que evidenció significación estadística para la cistoscopia flexible en la detección de todas las recurrencias (incluyendo las de alto riesgo) y la citología de orina en la detección de recurrencias alto riesgo. En cambio, XBM no obtuvo significación estadística en la regresión logística.

Los pacientes sin recidiva en la cistoscopia inicial fueron subdivididos en dos grupos: falsos positivos de XBM (biomarcador positivo, pero cistoscopia y citología negativas) y verdaderos negativos de XBM (biomarcador, cistoscopia y citología negativos). Se realizó un seguimiento de un año, observando que 18 (20%) pacientes del primer grupo desarrollaron una recurrencia (8 tumores de bajo grado, 8 de alto grado y dos tumores de vías), mientras que solo el 6.1% de los pacientes del segundo grupo tuvieron recurrencia, $p < 0.03$. La odds ratio de los pacientes con un biomarcador

positivo, pero cistoscopia y citología negativas fue de 3 (95% IC 1.494-6.023) y de 3.3 (95% IC 1.239-8.890) para la enfermedad de alto riesgo.

En la fase final del estudio, y con el objetivo de identificar un punto de corte con mejor perfil clínico del biomarcador y con mayor capacidad para detectar recurrencias de alto grado, se calculó el AUC de XBM para el valor preestablecido por el fabricante del biomarcador (*Linear Discriminant Analysis*, LDA = 0.5), siendo de 0.725 (95% IC 0.620–0.829). Se seleccionó el punto de corte de LDA = 0.1294 que presentó una sensibilidad del 96.3%, y una especificidad de 13.7%. El VPN fue de 92.1% y el VPP de 26.4%. En el subgrupo pacientes de alto riesgo, en el punto de corte seleccionado, mostró una sensibilidad del 97.4%, una especificidad del 12.4%, un VPN de 97.4% y un VPP de 12.4%. A nivel clínico, con este punto de corte, se lograrían evitar el 11.3% de las cistoscopias y citologías, dejando sin diagnosticar un 2.6% de las recurrencias de alto grado.

7. Resumen global de la discusión

7.1. Publicaciones

La investigación en biomarcadores es un campo en constante evolución en el manejo del TVNMI. Los avances en su aplicación abarcan los diferentes escenarios de la enfermedad: diagnóstico, seguimiento, predictores de respuesta a tratamiento endovesical y estratificación de riesgo de los pacientes. A pesar del desarrollo de multitud biomarcadores urinarios para el seguimiento del tumor vesical, habiendo sido algunos de ellos, como NMP22 o BTA stat, aprobados por la FDA (142–146), ninguno ha sido incorporado en los algoritmos recomendados por las guías de la EAU (64). A pesar de las indicaciones, la última versión de las guías sugiere la consideración de cuatro nuevos biomarcadores. Estos han sido seleccionados por su perfil oncológico y el soporte bibliográfico proporcionado por estudios prospectivos multicéntricos. Dichos marcadores incluyen el Bladder Epicheck™ (112,147), Xpert Bladder Monitor® (111,148) ADX-Bladder™ (108,109) y Cx-Bladder™ (106,107). Los biomarcadores genéticos han demostrado un perfil superior en el carcinoma vesical en comparación con la citología, sin embargo, el estándar actual en el seguimiento de los TVNMI sigue siendo la combinación de la cistoscopia y la citología de orina.

Si bien esta combinación ofrece una sensibilidad, especificidad y VPN muy elevados (149,150), tiene limitaciones significativas. La cistoscopia, a pesar de su precisión, es un procedimiento invasivo que puede causar dolor y malestar durante y después de su realización (151). Además, la capacidad de la cistoscopia para detectar todas las lesiones vesicales puede ser limitada y depende, en gran medida, de la experiencia del especialista que la lleva a cabo, ya sea un urólogo o una enfermera especializada (150). La citología es un procedimiento subjetivo que precisa de citopatólogos entrenados y que está expuesta a una importante variabilidad interobservador (52).

Las investigaciones actuales se centran en el desarrollo de nuevos biomarcadores urinarios con el objetivo de obtener pruebas que muestren una sensibilidad y un VPN elevados y comparables a los métodos establecidos. Este enfoque es crucial, especialmente en el contexto del seguimiento de TVNMI, ya que el propósito de estos nuevos biomarcadores es reducir la necesidad de realizar cistoscopias de manera rutinaria.

A pesar de los avances prometedores en biomarcadores genéticos, es importante destacar algunas limitaciones que deben abordarse para su implementación exitosa en el seguimiento del TVNMI. Las técnicas basadas en la detección de DNA o RNA enfrentan desafíos en la obtención de cantidad suficiente de material genético de calidad a partir de muestras de orina obtenidas por micción espontánea. La calidad y la cantidad del material genético pueden variar, lo que afecta a la precisión y a la fiabilidad de los resultados (152). Otra limitación de las pruebas no invasivas es su escasa sensibilidad, principalmente en pacientes con carga tumoral reducida y en tumores de bajo grado (153), que son una proporción significativa de las recurrencias (154).

La presencia de "falsos positivos" en los estudios con biomarcadores, donde el test indica positividad, pero no hay evidencia de lesiones en las pruebas estándar, es una preocupación relevante. Este fenómeno puede tener varias explicaciones y consideraciones importantes. La justificación para evitar el infradiagnóstico de las recidivas lleva a establecer puntos de corte bajos, que tengan una elevada sensibilidad. Sin embargo, es esencial que estos puntos de corte se definan cuidadosamente y se validen en diferentes cohortes de pacientes para garantizar su utilidad clínica y minimizar los "falsos positivos". La noción de que las alteraciones genéticas pueden preceder a las alteraciones morfológicas en el tumor vesical es un fenómeno interesante y poco estudiado. Sin embargo, es crucial comprender la duración y el alcance de este "efecto anticipatorio" para interpretar correctamente los resultados del biomarcador y evitar intervenciones innecesarias basadas solo en la positividad de la prueba.

La evidencia publicada al respecto consensúa que el efecto anticipatorio incluiría las recurrencias detectadas en los 18 meses posteriores a la determinación del biomarcador, basándose en la premisa de que las alteraciones genéticas pueden preceder en varios meses a las alteraciones morfológicas en el tumor vesical (155,156). Existe evidencia de que algunos de los falsos negativos son debidos a una baja carga tumoral (lesiones milimétricas, tumores únicos) cuyo pronóstico y retraso en el diagnóstico podría tener un escaso impacto en la supervivencia global del paciente (157). La interpretación de resultados de biomarcadores debe considerar el contexto clínico específico de cada paciente. La toma de decisiones clínicas no debe basarse únicamente en la positividad del biomarcador, sino que debe integrarse con otras pruebas y la evaluación clínica general.

La revisión sistemática de la literatura revela ciertas limitaciones y consideraciones importantes en los estudios con biomarcadores. Por un lado, se observa una alta proporción de estudios con diseño retrospectivo. Esta característica puede introducir sesgos y limitar la capacidad de establecer relaciones causales. La evidencia generada debe interpretarse, por tanto, con precaución, y cabe destacar la necesidad de estudios prospectivos para fortalecer la validez de los hallazgos.

La evaluación de los pacientes en los dos primeros años de seguimiento de los TVNMI, donde la tasa de recurrencias es más elevada que en la población general en seguimiento (158), podría conllevar a un sesgo de selección. La generalización de los resultados al resto de la población en seguimiento debe abordarse considerando esta limitación.

Por otro lado, la mayoría de los biomarcadores proporcionan resultados cualitativos dicotómicos (positivo/negativo). Sin embargo, ofrecer una predicción numérica de la probabilidad de recurrencia, por ejemplo, el *Episcore* de Bladder Epicheck® (159), podría ser de más utilidad para los urólogos en la toma de decisiones clínicas.

La falta de ensayos clínicos randomizados en el campo de los biomarcadores para el seguimiento del TVNMI limita la fuerza de la evidencia disponible. La realización de estudios controlados y aleatorizados sería crucial para establecer la eficacia real de estos biomarcadores (160). Asimismo, la falta de comparaciones directas entre los diferentes biomarcadores dificulta la identificación del más efectivo y limita la capacidad de establecer recomendaciones claras en la práctica clínica.

La presencia de algoritmos clínicos adyuvantes a las determinaciones de los biomarcadores puede complicar la estandarización y la validación externa (25). La ausencia de uniformidad en los métodos de evaluación y análisis puede afectar la comparabilidad entre biomarcadores (95,152,153).

La selección de biomarcadores para realizar el seguimiento de los pacientes con tumor vesical en la práctica clínica debería basarse en un escenario concreto (161) y siguiendo los criterios de las guías internacionales (162). Para ello se han de cumplir los tres principios básicos (163). El biomarcador seleccionado debe demostrar superioridad clínica en comparación con las pruebas habitualmente utilizadas. Esto implica mayor sensibilidad y VPN, lo que garantiza una capacidad efectiva para detectar recurrencias y reducir falsos negativos. Además, debe ser simple y reproducible, evitando la necesidad de infraestructuras complejas que dificulten su estandarización y adopción generalizada. La simplicidad en la aplicación contribuye a su integración eficiente en la práctica clínica diaria. Por otro lado, ha de ser económicamente equiparable, considerando los costos asociados a su implementación, en comparación con las pruebas estándar. Los estudios de costo-efectividad son esenciales, pero deben abordar la complejidad de los costos indirectos, como el personal, el material y el tiempo de quirófano, para proporcionar una evaluación precisa de su viabilidad económica. Los estudios económicos comparativos entre biomarcadores y cistoscopia a menudo son un desafío debido a la variabilidad en los costos de la cistoscopia según el país y a las dificultades para

calcular costes indirectos (154). A pesar de esta complejidad, la investigación de Nam *et al.* (164) sugiere que el seguimiento basado en biomarcadores puede ser económicamente más eficiente que el seguimiento clásico. Sin embargo, es crucial abordar posibles sesgos y considerar la evolución de los marcadores más recientes y sus perfiles de costos en futuras investigaciones.

Los dos modelos propuestos para la implementación de programas de seguimiento utilizando biomarcadores son los siguientes:

- Seguimiento alterno: en este modelo, se mantiene el estándar de seguimiento, que incluye cistoscopia y citología, pero se reduce el número de exploraciones invasivas alternándolas con biomarcadores con la misma periodicidad establecida por las guías. Se realiza una cistoscopia confirmativa en los casos en que el biomarcador proporcione un resultado positivo (158). Económicamente este seguimiento no implicaría un sobrecoste, incluso asumiendo la necesidad de cistoscopia y citología si el biomarcador es positivo. Dada la baja tasa de falsos negativos y el elevado VPN de la mayoría de los nuevos biomarcadores genéticos, este modelo es particularmente adecuado para el seguimiento en el escenario de los tumores de alto grado (165).

- Seguimiento basado exclusivamente en biomarcadores: en este modelo, debido a la elevada sensibilidad y VPN de los nuevos biomarcadores, el seguimiento es exclusivamente no invasivo. En caso de resultado negativo del biomarcador, los controles sucesivos se realizan con biomarcadores según la periodicidad sugerida por las guías de la EAU. Si el resultado es positivo, el paciente es sometido a una cistoscopia y a una cirugía transuretral si se confirma la recidiva. Este enfoque se

basa en la eficacia de los biomarcadores y su capacidad para reducir la necesidad de procedimientos invasivos y podría aplicarse en tumores de bajo grado, donde la demora en el diagnóstico de la recidiva no supone un riesgo oncológico significativo (166,167).

Las guías europeas plantean otro escenario para el uso de biomarcadores que analizaría cómo el resultado del biomarcador influiría en la tasa de detección. El estudio CEFUB (*Cost-Effectiveness of Follow-Up of Urinary Bladder Cancer*) de Van der Aa *et al.* (168) analiza cómo el conocimiento previo del resultado del biomarcador influye en la tasa de detección durante la cistoscopia. Los hallazgos indican que el uso del biomarcador antes de la cistoscopia proporciona una valiosa información adicional, mejorándose las tasas de detección en la cistoscopia.

En lo que respecta a la percepción de los pacientes sobre la aplicación de biomarcadores, Shen Tan *et al.* (169), reportan que a pesar de considerar la cistoscopia como un procedimiento invasivo, incómodo y asociado a efectos secundarios frecuentes, la sensibilidad mínima aceptable para que los pacientes consideren la sustitución de la cistoscopia por biomarcadores debería equivaler a la de la cistoscopia. Es esencial que los protocolos de seguimiento basados en biomarcadores comuniquen a los pacientes los diferentes escenarios y perfiles de las pruebas. En el grupo de bajo riesgo, retrasar el diagnóstico de una única y pequeña recidiva puede no tener un impacto relevante en la supervivencia global o en la supervivencia cáncer específica (170). Sin embargo, en el grupo de alto riesgo, donde la detección precoz es crucial, un biomarcador sólo será valioso si es capaz de detectar pequeñas recurrencias de alto grado.

Las guías de la EAU de 2021 ya mencionan cuatro biomarcadores urinarios (171), Bladder Epicheck®, Xpert Bladder Monitor®, ADX-Bladder™ y Cx-Bladder™, que

consideran como alternativas a la cistoscopia y a la citología de orina. Sin embargo, a pesar de la calidad y cantidad de publicaciones que respaldan estos biomarcadores, todavía no se han implantado en la práctica habitual. Esta falta de implementación puede deberse a la percepción por parte de los clínicos de un perfil bajo de sensibilidad y especificidad, lo que podría aumentar el riesgo para el paciente, teniendo en cuenta que los métodos estándar de seguimiento ofrecen actualmente una adecuada seguridad oncológica.

En nuestro estudio, hemos explorado la hipótesis de que Xpert Bladder Monitor® es un biomarcador que permite sustituir o posponer las cistoscopias debido a su elevada sensibilidad y VPN. XBM fue validado por primera vez por Wallace *et al* (141) en 2018, en un estudio multicéntrico que incluía solamente 49 pacientes. Desde este estudio, XBM ha sido evaluado en diez estudios más (111,148,172–176) que incluyen a más de 3.000 pacientes. Su sensibilidad y especificidad global varían entre 29.8 y 84%, y 73.7 y 94.1%, respectivamente. El VPN oscila entre el 83 y 96.5%, y el VPP entre 44 y 90.9% (114).

Una ventaja de XBM es la automatización de su determinación, gracias al suministro de los cartuchos precargados del dispositivo de análisis. Esto permite obtener el resultado en menos de dos horas en la propia consulta, con un tiempo de preparación de menos de 5 minutos (177). La tasa determinaciones no válidas en general no supera el 2%, y la causa más frecuente es un escaso volumen miccional.

Al analizar el efecto longitudinal de los biomarcadores, se observa que las definiciones son contradictorias en la literatura. Algunos estudios indican que, en el escenario de uso de biomarcadores, las cistoscopias con imagen mejorada podrían mejorar la detección de la prueba y minimizar los fasos positivos (178). De hecho, en estos casos, se recomendaría la aplicación del algoritmo de Studer para descartar enfermedad extravesical (179).

Como objetivo secundario de nuestro estudio, se llevó a cabo un seguimiento a los pacientes durante un período de un año para evaluar la posibilidad de que los resultados falsos

positivos de XBM pudieran tener un valor predictivo anticipatorio de recidiva. La tasa de recurrencia en este subgrupo de pacientes fue del 20% frente a sólo el 6.1% en pacientes con resultados negativos en todas las pruebas realizadas. Esto significa que cuando XBM es positivo pero la cistoscopia y la citología son negativas, los pacientes presentan una odds ratio de 3 (95% IC 1.5-6) para experimentar una recurrencia durante el año siguiente a la determinación, y una odds ratio de 3.3 (95% IC 1.2-8.9) de tener una recurrencia de alto grado en el mismo período de tiempo.

7.2. Limitaciones del proyecto

Nuestro proyecto presenta varias limitaciones. Respecto a la revisión sistemática, habría sido recomendable y más informativo realizar un metaanálisis de los datos. Sin embargo, la variabilidad entre estudios respecto a las cohortes incluidas y la metodología de seguimiento y la dificultad para obtener las "odds ratios" en los estudios analizados dificultaron esta alternativa.

Respecto al trabajo experimental, con el objetivo de incrementar el número de recurrencias centramos nuestro estudio en los dos primeros años de seguimiento tras la RTU-V, metodología similar al resto de estudios con biomarcadores. Este escenario impacta en el valor predictivo del biomarcador. La baja incidencia de recurrencias (14.5%) dificulta la obtención de diferencias estadísticamente significativas en la cohorte analizada. El tamaño medio de la recurrencia fue 8mm (3-16mm) lo que representa una baja carga tumoral para ser detectada a través del biomarcador.

Dado que XBM es un método no invasivo, su análisis se realizó en una muestra de micción espontánea, mientras que en la citología de orina por lavado se analiza en el líquido de aspiración endovesical. Ni nuestro trabajo ni ninguno de los estudios previos publicados

sobre biomarcadores presenta un diseño prospectivo y randomizado entre el test y el estándar de seguimiento, lo cual complica su implementación en la práctica clínica diaria.

8. Conclusiones

1. Xpert Bladder Cancer Monitor® es un biomarcador genético que se cuantifica en orina obtenida en micción espontanea, candidato a ser comparado con el estándar de seguimiento actual del TVNMI, basado en cistoscopia y citología urinaria por lavado vesical.

2. A pesar de que Xpert Bladder Cancer Monitor® ofrece una elevada sensibilidad y valor predictivo negativo en el escenario de seguimiento de los TVNMI, su prometedor perfil de eficacia y seguridad oncológica no permite sustituir la cistoscopia y citología de orina como pruebas estándar de diagnóstico de recurrencias.

3. La modificación del punto de corte de Xpert Bladder Cancer Monitor® utilizando un valor inferior al prestablecido por el fabricante mejora la detección de recurrencias, equiparándose a la cistoscopia y citología, pero aumentando el número de exploraciones innecesarias por la pérdida de especificidad del test.

4. Xpert Bladder Cancer Monitor® presenta un efecto anticipatorio que permite identificar a los pacientes con un riesgo más elevado de desarrollar un carcinoma urotelial en el siguiente año de seguimiento y que, por tanto, precisan un seguimiento más exhaustivo.

5. En resumen, y para concluir nuestro objetivo principal, creemos que Xpert Bladder Cancer Monitor® no puede sustituir el método actual de seguimiento del TVNMI de manera integral. Sin embargo, presenta un efecto anticipatorio en el diagnóstico de la recurrencia de alto grado del TVNMI muy prometedor. Son necesarios estudios prospectivos y aleatorizados que permitan definir la complementariedad entre XBM y el método estándar de seguimiento para realizar un seguimiento menos invasivo, eficiente y oncológicamente seguro.

9. Líneas de investigación futuras

Las líneas de investigaciones futuras en esta área deben incorporar diseños prospectivos, aleatorizados y multicéntricos, con un cálculo de tamaño muestral apropiado para demostrar igualdad entre la eficacia de los biomarcadores genéticos y el método estándar de seguimiento de los TVNMI.

Estos estudios futuros deberán incorporar análisis de coste-efectividad en un programa de seguimiento de TVNMI.

Los futuros diseños de ensayos clínicos sobre biomarcadores deberán explorar el perfil de mayor aplicabilidad y que más se ajuste a la seguridad y deseo de los pacientes, ya sea con un seguimiento exclusivo con biomarcadores o alternando con las pruebas estándar.

Explorar con estudios diseñados apropiadamente el efecto anticipatorio de los biomarcadores genéticos en el diagnóstico de las recurrencias de los TVNMI y su algoritmo diagnóstico.

Realizar estudios para identificar nuevos biomarcadores genéticos, basados en la utilización de las diversas técnicas ómicas actuales disponibles.

Explorar le eficacia de algoritmos basados en inteligencia artificial que incorporen variables clínicas, biológicas y quizás de imagen para detectar recurrencias del TVNMI.

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11. Anexos

11.1. Anexo 1. Informe de Comité de Ética.

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INFORME DEL COMITÉ DE ÉTICA DE INVESTIGACIÓN CON MEDICAMENTOS Y COMISIÓN DE PROYECTOS DE INVESTIGACIÓN DEL HOSPITAL UNIVERSITARI VALL D'HEBRON

Sra. Mireia Navarro Sebastián, Secretaria del COMITÉ DE ÉTICA DE INVESTIGACIÓN CON MEDICAMENTOS del Hospital Universitari Vall d'Hebron,

CERTIFICA

Que el Comité Ético de Investigación con Medicamentos del Hospital Universitario Vall d'Hebron, en el cual la Comisión de proyectos de investigación está integrada, se reunió en sesión ordinaria nº 354 el pasado 21/09/2018 y evaluó el proyecto de investigación PR(AG)304/2018 , presentado con fecha 01/07/2018, titulado "*Papel del Xpert Bladder Cancer en el seguimiento de los tumores vesicales no músculo infiltrantes*" que tiene como investigador principal al Dr. Fernando Lozano Palacio del Servicio de Urología de nuestro Centro.

Versión de documentos:

- Memoria V3 18/09/2018
- HIP/CI v2 10/Agosto/2018
- Solicitud informe CEIC 03/julio/2018

El resultado de la evaluación fue el siguiente:

DICTAMEN FAVORABLE

En el caso de que se evalúe algún proyecto del que un miembro sea investigador/colaborador, éste se ausentará de la reunión durante la discusión del proyecto.

Lo que firmo en Barcelona a 21 de septiembre de 2018

MIREIA NAVARRO SEBASTIAN

Firmado digitalmente por MIREIA NAVARRO SEBASTIAN
Nombre de reconocimiento (DN): c=ES, ou=Vegeu https://www.aoc.cat/CATCert/Regulacio, sn=NAVARRO SEBASTIAN, givenName=MIREIA, serialNumber=38121226Z, cn=MIREIA NAVARRO SEBASTIAN
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Sra. Mireia Navarro Sebastián

Secretaria del CEIm

11.2. Anexo 2. Acuerdo de colaboración científica con Cepheid.

Cepheid.

Amendment No. 1 Research Collaboration Agreement

This Amendment No. 1 (the "Amendment") to the Research Collaboration Agreement is made effective as of the date of last signature below (the "Amendment Effective Date") by and between **Cepheid**, a California corporation with its principal place of business at 904 Caribbean Drive, Sunnyvale, California 94089 ("Cepheid"), and the **Hospital Universitario Valle de Hebron** with an address at Passeig de la Vall d'Hebron, 119-129, 08035 Barcelona, Spain ("Institution") (Cepheid and Institution each a "Party" and together the "Parties").

WHEREAS, Cepheid and Institution entered into a Research Collaboration Agreement (the "Agreement") dated 1 June 2018, concerning a research project entitled **"Xpert Bladder Cancer Monitor Verification Study; Local Evaluation of Xpert Bladder Cancer Monitor in Barcelona, Spain"**; and

WHEREAS, the Parties now desire to amend the Agreement by means of this Amendment;

NOW, THEREFORE, the Agreement is amended as follows:

1. The Parties hereby agree to extend the Term from 31 December 2019 to 31 March 2020 or until prior termination by either party.

2. The Materials Exhibit II is hereby amended by:

   a. Substituting "54 kits" in lieu of "45 kits" for item Xpert Bladder Cancer Monitor CE-IVD Cartridge Kits (10 Cartridges/Kit) [GXBLAD-CM-CE-10]; and

   b. Substituting "18 kits" in lieu of "15 kits" for item Xpert Urine Transport Reagent Kit (CE-IVD) (30 UTR Tubes per kit) [GXUTR-CE-30].

All other provisions of the Agreement remain unchanged.

[Signatures on following page]