European Journal of Cancer 54 (2016) 131–138

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

Gene expression test for the non-invasive diagnosis of bladder cancer: A prospective, blinded, international and multicenter validation study

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Received 15 May 2015; received in revised form 15 October 2015; accepted 5 November 2015

KEYWORDS
Bladder cancer;
Biomarkers;
Gene expression;
Molecular test;
Non-invasive

Abstract ***Objective:*** This study aimed to validate, in a prospective, blinded, international and multicenter cohort, our previously reported four non-invasive tests for bladder cancer (BC) diagnosis based on the gene expression patterns of urine.
Methods: Consecutive voided urine samples from BC patients and controls were prospectively collected in five European centres (n = 789). Finally, 525 samples were successfully analysed. Gene expression values were quantified using TaqMan Arrays and previously reported

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http://dx.doi.org/10.1016/j.ejca.2015.11.003

diagnosis;
Sensitivity and specificity;
Urine

diagnostic algorithms were applied to gene expression data. Results from the most accurate gene signature for BC diagnosis were associated with clinical parameters using analysis of variance test.

Results: High diagnostic accuracy for the four gene signatures was found in the independent validation set (area under curve [AUC]=0.903–0.918), with the signature composed of two genes (GS_D2) having the best performance (sensitivity: 81.48%; specificity: 91.26%; AUC: 0.918). The diagnostic accuracy of GS_D2 was not affected by the number of tumours ($p = 0.58$) but was statistically associated with tumour size ($p = 0.008$). Also, GS_D2 diagnostic accuracy increases with increasing BC tumour risk. We found no differences in the performance of the GS_D2 test among the populations and centres in detecting tumours ($p = 0.7$) and controls ($p = 0.2$).

Conclusions: Our GS_D2 test is non-invasive, non-observer dependent and non-labour-intensive, and has demonstrated diagnostic accuracy in an independent, international and multicenter study, equal or superior to the current gold standard (cystoscopy combined with cytology). Additionally, it has higher sensitivity than cytology while maintaining its specificity. Consequently, it meets the requirements for consideration as a molecular test applicable to clinical practice in the management of BC.

1. Introduction

Current guidelines define cystoscopy and cytology as the standard of care in bladder cancer (BC) diagnosis and follow-up [1]. Cystoscopy is an invasive technique with patients' discomfort and possible complications such as urinary tract infections or haematuria and it has been estimated than it can overlook 10–20% of the papillary lesions and 50% of flat bladder lesions, and could also be inconclusive in cases of gross haematuria or patients with abnormal bladder mucosa due to an inflammation process [2,3]. For this reason, cystoscopy is always associated with cytology in the BC diagnostic and follow-up schedules as its specificity (SP) reaches 98% [4]. Cytology is a non-invasive methodology but it has some limitations, such as low sensitivity (SN), inter-observer variability, subjective evaluation and low-accuracy in low grade (LG) tumours [5].

Finding a reliable non-invasive marker of BC would be enormously useful in clinical practice. In fact, several urinary biomarkers have been reported in the literature, but although they improve the overall SN of cytology, they do not reach the desired SP [6] which is why they are not incorporated into routine practice [7].

We have previously reported and validated a 12 gene expression signature (GS_D12) in urine for the non-invasive diagnosis of BC. Although the signature performed well in the validation cohort, it did not achieve the diagnostic accuracy of the current gold standard. Thus, we developed three new urinary gene expression signatures composed of two, five and ten genes respectively (GS_D2, GS_D5 and GS_D10) and an improved GS_D12. These gene panel sets have demonstrated a high accuracy for BC diagnosis (area under curve [AUC] 0.913–0.949) in voided urine samples [8].

However, to demonstrate the potential clinical utility of these gene signatures, they should be externally validated. In this paper we present the results of a prospective, blinded, international and multicenter study to establish the true accuracy of our gene set panels in BC diagnosis.

2. Materials and methods

2.1. Patients and samples

Prospective, multicentre and international clinical trial performed according to the Standards for Reporting of Diagnostic Accuracy (STARD) guidelines [9]. The protocol was approved by an institutional review committee at the Hospital Clinic of Barcelona (Spain), and by local committees of the four external participating institutions. External centres were asked to collect and prepare the urine samples for final processing at the Hospital Clinic.

BC patients submitted to transurethral resection of the bladder (TURB) and controls with non-neoplasic urological diseases were consecutively enrolled between February 2009 and July 2010 in the different centres. The inclusion and exclusion criteria for BC patients and controls, as well as sample processing details, are reported in the supplementary material.

2.2. Gene expression quantification and data analysis

RNA extraction, complementary DNA synthesis and gene expression quantification were performed in the Hospital Clínic as described in the supplementary material [8,10]. All the 45 target genes and two endogenous controls analysed in our previously reported studies were also analysed in the present study [8,11].