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Bladder Cancer

Prognostic Impact of a 12-gene Progression Score in Non–muscle-invasive Bladder Cancer: A Prospective Multicentre Validation Study

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Abstract

Background: Progression of non–muscle-invasive bladder cancer (NMIBC) to muscle-invasive bladder cancer (MIBC) is life-threatening and cannot be accurately predicted using clinical and pathological risk factors. Biomarkers for stratifying patients to treatment and surveillance are greatly needed.
Objective: To validate a previously developed 12-gene progression score to predict progression to MIBC in a large, multicentre, prospective study.
Design, setting, and participants: We enrolled 1224 patients in ten European centres between 2008 and 2012. A total of 750 patients (851 tumours) fulfilled the inclusion and sample quality criteria for testing. Patients were followed for an average of 28 mo (range 0–76). A 12-gene real-time qualitative polymerase chain reaction assay was performed for all tumours and progression scores were calculated using a predefined formula and cut-off values.
Outcome measurements and statistical analysis: We measured progression to MIBC using Cox regression analysis and log-rank tests for comparing survival distributions.

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Results and limitations: The progression score was significantly ($p < 0.001$) associated with age, stage, grade, carcinoma in situ, bacillus Calmette-Guérin treatment, European Organisation for Research and Treatment of Cancer risk score, and disease progression. Univariate Cox regression analysis showed that patients molecularly classified as high risk experienced more frequent disease progression (hazard ratio 5.08, 95% confidence interval 2.2–11.6; $p < 0.001$). Multivariable Cox regression models showed that the progression score added independent prognostic information beyond clinical and histopathological risk factors ($p < 0.001$), with an increase in concordance statistic from 0.82 to 0.86. The progression score showed high correlation ($R^2 = 0.85$) between paired fresh-frozen and formalin-fixed paraffin-embedded tumour specimens, supporting translation potential in the standard clinical setting. A limitation was the relatively low progression rate (5%, 37/750 patients).
Conclusions: The 12-gene progression score had independent prognostic power beyond clinical and histopathological risk factors, and may help in stratifying NMIBC patients to optimise treatment and follow-up regimens.
Patient summary: Clinical use of a 12-gene molecular test for disease aggressiveness may help in stratifying patients with non–muscle-invasive bladder cancer to optimal treatment regimens.

1. Introduction

Bladder cancer is a common malignancy, with 429 000 new cases and 165 000 deaths annually [1]. Approximately 75% of patients are initially diagnosed with non–muscle-invasive bladder cancer (NMIBC) [2]. For NMIBC the 5-yr recurrence rate is high (50–70%) and progression to muscle-invasive bladder cancer (MIBC) is observed in 10–15% of cases [3,4]. Treatment of patients with NMIBC includes a surveillance regimen of varying intensity for at least 5 yr, depending on risk stratification for recurrence and progression [2]. The need for frequent and long-term surveillance makes bladder cancer the most expensive cancer to treat [5,6]. Clinical and histopathological risk factors for disease progression to MIBC include stage, grade, size, concomitant carcinoma in situ (CIS), tumour multiplicity, and recurrence rate [3]. Risk factors are incorporated into European Organisation for Research and Treatment of Cancer (EORTC) risk tables [3,7], which are widely used in the clinic. Patients with intermediate- and high-risk NMIBC are treated with adjuvant intravesical instillations of bacillus Calmette-Guérin (BCG) or mitomycin C for 1–3 yr [2]. Adjuvant instillations reduce the risk of recurrence [8] and delay or prevent progression to MIBC [9]. However, tumours with similar histopathological characteristics can have widely different molecular features and may belong to distinct molecular subgroups with different disease aggressiveness. Such subgroups, representing different clinical risks, have been identified in NMIBC by us and others [10–15]. Consequently, clinically useful molecular tests for stratifying patients to treatment and follow-up regimens beyond well-established clinical risk factors are greatly needed. We previously validated a microarray-based gene expression signature for disease progression in an independent cohort of 294 patients with NMIBC [16]. This signature was transferred to a 12-gene real-time qualitative polymerase chain reaction (RT-qPCR) assay for calculating a progression score [17]. Our aim here was to validate the assay in a multicentre prospective study.

2. Patients and methods

2.1. Patients, follow-up, and biological material

All patients gave their written informed consent, and the study was approved by the scientific ethics committee in each country (for details see [15]). The inclusion criteria were: (1) patients diagnosed with NMIBC (primary and recurrent); and (2) patients not previously diagnosed with MIBC. Patients were included from year 2008 to 2012 and followed according to national guidelines. Follow-up was registered online from each clinical centre and censored at the time of the most recent cystoscopy. Follow-up included evaluation of progression to MIBC. Progression to MIBC and/or metastatic disease was verified by pathological examination and measured from the time of surgery for the tumour analysed to the time of the event. Patients were censored for cystectomy with no verified progression to MIBC, death, or discontinuation of follow-up. Representative diagnostic tumour sections were re-evaluated by one expert uropathologist (F.A.) using the American Joint Committee on Cancer recommendations from 2002 and graded according to the WHO 2004 guidelines. Pathology review was performed for 89% (760/851) of tumours, with a concordance rate of 78% when staging was possible based on the single reviewed tumour section (508/654). When only the original grade (1973 system) was available ($n = 43$) this information was translated into the WHO 2004 grading system (G1 = low, G3 = high, and G2 [$n = 17$] were included as unknown grade). The highest stage (original or reviewed) and re-evaluated grade information were used in the study where possible. Tissue material from primary and recurrent tumours was collected fresh from resection in each clinical centre, embedded in Tissue-Tek O.C.T. and snap frozen in liquid nitrogen before storage at −80 °C. In all centres, standardised procedures for sampling, freezing, and shipment of samples were applied. All subsequent analytical procedures were performed at the Department of Molecular Medicine, Aarhus University Hospital.

2.2. RNA extraction and quality assessment

Two sections stained with haematoxylin and eosin (top and bottom) were evaluated for the presence of carcinoma cells for each tumour. Only tumours with a carcinoma cell percentage >10% (average for the two sections) were used. Total RNA was extracted from serial cryosections using an RNeasy Mini Kit (Qiagen); no trimming or microdissection was performed. All samples were quantified using an Infinite 200 PRO