Title page:

Role of the 4Kscore Test as a Predictor of Reclassification in Prostate Cancer Active Surveillance

Authors:

Ángel Borque-Fernando[1] (MD, PhD)*, José Rubio-Briones[2] (MD, PhD), Luis M. Esteban[3] (PhD), Yan Dong[20], Ana Calatrava[4], Álvaro Gómez-Ferrer[5], Enrique Gómez-Gómez[6], Jesús M. Gil Fabra[7], Nuria Rodríguez-García[8], Pedro Á. López González[9], Jorge García-Rodríguez[10], Miguel Rodrigo-Aliaga[11], Bernardo Herrera-Imbroda[12], Juan Soto-Villalba[13], Sara Martínez-Breijo[14], Virginia Hernández-Cañas[15], Ana M. Soto-Poveda[16], Carlos Sánchez-Rodríguez[17], Carlos Carrillo-George[18], Yumaira E. Hernández-Martínez[19], David Okrongly[21]

[1] Department of Urology, Hospital Universitario Miguel Servet, Zaragoza (Spain). IIS Aragón. aborque@comz.org

[2] Department of Urology, Instituto Valenciano de Oncología, Valencia (Spain). jrubio@fivo.org

[3] Escuela Universitaria Politécnica La Almunia, Universidad de Zaragoza, Zaragoza (Spain). lmeste@unizar.es

[4] Department of Pathology, Instituto Valenciano de Oncología, Valencia (Spain). acalatrava@fivo.org

[5] Department of Urology, Instituto Valenciano de Oncología, Valencia (Spain). alvarogomezferrer@gmail.com

[6] Department of Urology, Hospital Reina Sofía, Córdoba (Spain). IMIBIC. enriquegomezgomez@yahoo.es

[7] Department of Urology, Hospital Universitario Miguel Servet, Zaragoza (Spain). IIS Aragón. gilfabra@yahoo.es

[8] Department of Urology, Hospital Son Llàtzer, Palma de Mallorca (Spain). nuriaroga8@yahoo.es

[9] Department of Urology, Hospital Universitario Virgen de la Arrixaca, Murcia (Spain). pedroa.lopezgonzalez@gmail.com

[10] Department of Urology, Hospital Universitario Central de Asturias, Oviedo (Spain). jgrmed@hotmail.com

[11] Department of Urology, Hospital General Universitario de Castellón, Castellón de La Plana (Spain). m-rodrigo@hotmail.com

[12] Department of Urology, Hospital Universitario Virgen de la Victoria, Málaga (Spain). ber.urologia@gmail.com

[13] Department of Urology, Hospital Universitario Puerta del Mar, Cádiz (Spain). juansotovi@hotmail.com

[14] Department of Urology, Complejo Hospitalario Universitario de A Coruña, A Coruña (Spain). smarbre@hotmail.com

[15] Department of Urology, Hospital Universitario Fundación Alcorcón, Madrid (Spain). vhernandez@fhalcorcon.es

[16] Department of Urology, Hospital de Manises, Valencia (Spain). ana82s@hotmail.com

[17] Department of Urology, Hospital Universitario Príncipe de Asturias, Alcalá de Henares (Spain). walki99@gmail.com

[18] Department of Urology, Hospital General Universitario Morales Meseguer, Murcia (Spain). :carlos_carrillo_87@hotmail.com

[19] Department of Urology, Hospital de Manacor, Manacor (Spain). yumairah@yahoo.com

[20] OPKO Diagnostics, Woburn MA (ydong2@opko.com)

[21] OPKO Diagnostics, Miami, FL (dokrongly@opko.com)

***Corresponding autor:**
Ángel Borque-Fernando (MD, PhD)
Department of Urology, Hospital Universitario Miguel Servet
Paseo Isabel la Católica 1 y 3
50009, Zaragoza (Spain)
Phone number: +34 976765599
Fax number: +34 976765614
E-mail: aborque@comz.org

Key words:

prostatic neoplasms, serum markers, biopsy, watchful waiting, 4Kscore Test, active surveillance

Abstract:

Background: Management of active surveillance (AS) in low-risk prostate cancer (PCa) patients could be improved with new biomarkers such as the 4Kscore Test. We analyze its ability to predict tumor reclassification by upgrading at the confirmatory biopsy at 6 months.

Methods: Observational, prospective, blinded, and non-randomized study, within the Spanish National Registry on AS (AEU/PIEM/2014/0001; NCT02865330) with 181 patients included after initial Bx and inclusion criteria: PSA ≤10 ng/mL, cT1c-T2a, Grade Group 1, ≤2 cores and ≤5 mm/50% length core involved. Central pathological review of initial and confirmatory Bx was performed on all biopsy specimens. Plasma was collected 6 months after initial Bx and just before confirmatory Bx to determine 4Kscore result. In order to predict reclassification defined as Grade Group ≥2, we analyzed 4Kscore, percent free to total (%f/t) PSA ratio, prostate volume, PSA density, family history, body mass index, initial Bx, total cores, initial Bx positive cores, initial Bx % of positive cores, initial Bx maximum cancer core length and initial Bx cancer % involvement. Wilcoxon rank sum test, non-parametric trend test or Fisher's exact test, as appropriate established differences between groups of reclassification.

Results: One hundred thirty-seven patients met inclusion criteria. Eighteen patients (13.1%) were reclassified at confirmatory Bx. The %f/t PSA ratio and 4Kscore showed differences between the groups of reclassification (Yes/No). Using 7.5% as cut-off for the 4Kscore, we found a sensitivity of 89% and a specificity of 29%, with no reclassifications to Grade Group 3 for patients with 4Kscore below 7.5% and 2 (6%) missed Grade Group 2 reclassified patients. Using this threshold value there is a biopsy reduction of 27%. Additionally, 4Kscore was also associated with changes in tumor volume.

Conclusions: Our preliminary findings suggest that the 4Kscore may be a useful tool in the decision-making process to perform a confirmatory Bx in active surveillance management.

INTRODUCTION

Active surveillance (AS) an accepted strategy for patients with low-risk prostate cancer (PCa)[1,2], but its implementation is quite heterogeneous among urologists. Many collaborative groups are investigating different strategies to improve selection criteria, analyze meaningful reclassification criteria, and optimize follow-up protocols[3,4,5]. New

imaging tools such as multiparametric magnetic resonance image (mpMRI) have revolutionized the field[6,7], but its lack of reproducibility, costs, heterogeneity in reporting and availability are recognized drawbacks that have to be resolved, as recommendations derived from reference centers[8,9,10] may not be applicable in every radiology setting. This was recently pointed out in a survey among a diverse group of Spanish urologists performing AS in different hospital settings. When asked to rate their confidence in their radiologist's specialization, training and experience for reporting mpMRI in prostate cancer, only 27% of them believed their radiologist had a high level of expertise[3].

This finding suggests that biomarkers may hold more immediate promise for better PCa characterization. In particular, the newest class of multiparametric biomarker tests such as 4Kscore[11] or SelectMDx[12] hold promise, as they combine proteins, nucleic acids, and clinical variables into a statistical models that predict with high accuracy the chance of harboring high grade prostate cancer (HGPCa).

Due to standard transrrectal ultrasound (TRUS)-guided 10-12 cores biopsy (Bx) deficiencies to characterize PCa, most AS protocols rely on the results of the so-called confirmatory Bx, usually performed during the first year after PCa diagnoses, to pick up reclassified tumors and offer active treatment or definitely enroll a patient in AS. But the time elapsed from initial Bx to confirmatory Bx and the confirmatory Bx itself, are not free of side effects[8] as the time lag confers stress to the patient and their families. It would be helpful to use risk calculators[10], biomarker tests, or mpMRI, or their combination [5,13] to better understand the risk of reclassification to HGPCa.

4Kscore is one of the biomarkers accepted by Guidelines to avoid Bx in the PSA grey zone[14,15], and decrease overdiagnosis of Grade Group 1 PCa[11,16], which is the group very often directed towards AS. Recognizing that the 4Kscore was not developed to optimize an AS program, we nonetheless hypothesized that perhaps it could provide additional value in the AS confirmatory Bx setting. In July 2014, a National Registry (AEU/PIEM/2014/0001, www.piem.aeu.es, ClinicalTrials.gov Identifier: NCT02865330), supported by the Spanish Urological Association (AEU), was opened online[17]. Within it, we designed a non-randomized, observational, prospective, blinded study with the primary objective to analyze the ability of the 4Kscore to prospectively

predict tumoral reclassification by upgrading at the confirmatory Bx at 6 months from initial Bx. If the 4Kscore showed sufficient predictive power, it could refine the necessity of performing the confirmatory Bx or the need for additional tools such mpMRI to reinforce or alter the decision of performing confirmatory Bx.

METHODS

The protocol was opened to all associate investigators (AI) recruiting for AEU/PIEM/2014/0001. It was approved by Hospital 12 de Octubre (Madrid) Ethics Committee (ref. number 14/242). Patients enrolled in this project had a PSA ≤10 ng/mL, although PSA >10 ng/mL was permitted if PSA density was <0.20 in cases with a prostate size >60mL. The clinical stage of the disease had to be cT1c or cT2a. The initial Bx had to be performed with ≥10 cores. PCa had to be Grade Group 1 in ≤2 cores, and not exceed 5 mm or 50% of the core length. The local pathological report had to be confirmed by central review of an expert uropathologist (A.C.), in an attempt to select a pure low-risk cohort. All participating patients had a life expectancy of 10 years or more by Charlson score, were less than 80 years of age, and signed a specific written consent.

Patients with previous atypical small acinar proliferation or high-grade prostatic intraepithelial neoplasia were excluded, as were patients taking finasteride or dutasteride. Any endo-urological procedure, including urethral catheterization or urinary infections within 6 months after the initial Bx were excluded. The 4Kscore was done just before the confirmatory Bx. Blood samples were sent to OPKO Health Europe S.L.U, following the manufacturer's recommendations. The 4Kscore results were blinded to the investigators up to the completion of the study. Clinical records were uploaded online on AEU/PIEM/2014/0001, and were blinded to the biomarker company.

The confirmatory Bx could be either TRUS guided with 18 cores or transperineally guided by a brachytherapy template, allowing in this case 24 to 32 cores. In either scenario, the patient may have also received a 1.5T mpMRI, but this was not obligatory as some centers did not have this capability.

The primary objective of the study was to analyze the ability of the 4Kscore for predicting tumoral reclassification at the confirmatory Bx. Criteria for reclassification at the confirmatory Bx was detection of HGPCa defined as Grade Group ≥2.[16] As a

secondary objective we evaluated surrogate variables of progression in tumor volume, such as length/percentage of maximum core involvement or number/percentage of positive cores.

For the statistical analysis, we performed a descriptive study of the variable including: total PSA, intact PSA, free PSA, hK2, age, digital rectal examination (DRE), and prior negative biopsies.

As the variable "negative prior biopsy" is integral to the 4Kscore algorithm and confers it less risk of harboring HGPCa, we decided, together with the company, to consider this variable in the 4Kscore calculation. If the patient had a prior negative biopsy before the diagnostic initial Bx the 4Kscore reflected this fact. The rest of patients with only the positive initial Bx were considered as “no prior negative biopsy” for 4Kscore calculation. In addition, for comparison purposes, we analyzed %f/t PSA ratio, prostate volume, PSA density, family history, body mass index (BMI), initial Bx total cores, initial Bx positive cores, initial Bx % of positive cores, initial Bx maximum cancer core length (mm) and initial Bx cancer % involvement.

We analyzed differences between the groups of patients that had reclassification or not, using the Wilcoxon rank sum test, non-parametric trend test or Fisher’s exact test, as appropriate.

The data were analyzed using R language programming version 3.3.2 (The R Foundation for Statistical Computing, Vienna, Austria)

RESULTS

Between February 2015 and December 2015, 181 patients were enrolled. After exclusions (Figure 1) 137 patients (75.7%) were finally analyzed. The confirmatory Bx was performed at least 6 months after the initial Bx (median 6.5; IQR: 6.2, 7.6 months). After local and central pathological review, 18 patients (13.1%) were reclassified in grade at the confirmatory Bx. Their different characteristics regarding this fact are shown at Table 1. We found significant differences between the groups with or without reclassification for 4Kscore and %f/t PSA.

The 4Kscore value was associated with the likelihood of more severe cancer at confirmatory Bx, while %f/t PSA ratio did not show this association at different cut-off points. No patient with 4Kscore <7.5% showed upgrade to Grade Group 3[16] (Table 2). The sensitivity and specificity for finding Grade Group ≥2 at confirmatory Bx for a cut-

off point of 4Kscore ≥7.5% were 89% (95% CI: 65-99%) and 29% (21-38%), respectively; positive predictive value, 16% (CI 9-25%) and negative predictive value (NPV): 95% (CI 82-99%). That implies a 27% biopsy reduction (37/137) with no missed Grade Group 3, if we accepted a 4Kscore <7.5% cut-off to avoid confirmatory Bx. An extrapolation of our findings to 1000 hypothetical patients exposed to confirmatory Bx and 4Kscore are shown in Table 3. The 4Kscore was also associated with changes in tumor volume estimation at confirmatory Bx (Table 4).

A logistic regression analyses showed association between 4Kscore (on logit scale) and Grade Group upgrade, Odds Ratio (OR): 2.14 (95% CI: 1.19-4.24; p=0.018).

DISCUSSION

Of all the clinical variables and diagnostic initial Bx pathology variables, 4Kscore and %f/t PSA ratio are significantly associated with the Grade Group upgrade at the confirmatory Bx; prostate volume and PSA density are borderline but not associated with the reclassification outcome (Table 1).

A 4Kscore cut-off point of 7.5% have been proved to detect HGPCa before prostate Bx in a common clinical practice, with barely any delayed diagnosis of cancers that may lead to metastasis[18, 25]. This cut-off point can reduce costs and avoid Bx[19]. It has also demonstrated to predict distant metastasis within 20 years[20]. Our results showed how a 4Kscore cut-off point of less than 7.5% previous to confirmatory Bx, significantly identify patients at very low risk of harboring HGPCa instead of patients with 4Kscore over 7.5% with a risk of aggressive PCa of 16% (Table 2), whereas %f/t PSA did not (Table 2).

Using a cutoff of 7.5%, 4Kscore could predict Grade Group upgrade at confirmatory Bx with a sensitivity of 89%, specificity of 29%, NPV of 95%, few delayed diagnoses of Grade Group 3 or higher cancer; and potentially avoid prostate Bx in 27% of patients. These results for 4Kscore in the AS scenario are close to those found with prostate Bx before diagnosis[11].

The high NPV of 95% observed in a cohort with a reclassification risk of 13%, combined with a 27% potential Bx reduction for those with a 4Kscore ≤7.5%, make 4Kscore a reliable and cost-effective biomarker to identify non-reclassifying patients at confirmatory Bx in AS management.

Although 4Kscore was created and validated to identify HGPCa before cancer diagnosis, it has been explored to identify tumor volume on conventional prostate Bx. It was significantly associated with the number of positive cores, percent of cores positive, and the mm of cancer found on biopsy, with higher scores denoting more cancer[21,22]. In our study on AS scenario higher 4Kscore was significantly associated with higher tumor involvement, larger tumor size and more positive cores at the confirmatory Bx (Table 4).

In a recent study, a 4Kpanel was evaluated to predict reclassification to Grade Group ≥2 at the first surveillance Bx, occurring in approximately 20% similarly to our 18% findings. The 4Kpanel is not the commonly available marker for clinical use, but it is used in the 4Kscore in combination with clinical variables. The 4Kpanel in a multivariable model with BMI, core ratio, previous biopsies, and prostate volume improved accuracy from base clinical models for predicting reclassification (AUC 0.78 vs 0.74) at the confirmatory Bx, but both models performed comparably for the prediction of progression at follow-up Bx (AUC 0.75 vs 0.76)[23], arguing that the impact of other Bx information, primarily volume of core involvement in previous Bx and the number of previous negative Bx, carry significant statistical weight in predicting grade progression, and the impact of the four kallicrein panel is decreased[23].

We propose for consideration in clinical implementation a cut-off point of 7.5% of 4Kscore to identify men with a very low risk of reclassification at confirmatory Bx. This management would drive us to avoid a 27% of confirmatory Bx missing only 2 (6%) Grade Group 2 cancer and no Grade Group ≥3 cancer (0%). We accept as a limitation that our sample size and our inclusion criteria of AS patients resulted in a very low proportion of adverse Grade Group ≥3 (2 cases in 137). They have been correctly classified by 4Kscore, but in order to gaining confidence in correct identification of this relevant subgroup and to validate 4Kscore at confirmatory Bx, an external validation of our preliminary results must be done.

Our study confirms the proportion of reclassifications found in a previous study in the confirmatory Bx setting[23], and the ability of the 4Kscore alone, as opposed to the 4Kpanel in a more complex model, to predict patient reclassification at confirmatory Bx. The main limitation of this study is the relatively small number of patients and the low number of only 18 reclassification events. While exploratory in nature, this study does support the utility of the 4Kscore as a predictor of adverse pathological findings at confirmatory Bx.

Our study was blind and prospective in design, but our model has to be validated in another series of patients and also be studied in conjunction with other tools such as mpMRI. Better selection of AS candidates and establishment of different follow-up strategies depending on a patient's risk of progression holds great promise to close the narrow gap of achieving excellent long-term outcomes already seen in AS series where biomarkers or mpMRI were not used.[29]

This study must also be placed in context of newer approaches for selecting patients for AS with favorable intermediate risk (higher volumes of Grade Group 1 or low Gleason grade pattern 4). Some guidelines continue to recommend conservative inclusion and follow-up criteria as proposed in our study,[14] but our findings should not be extrapolated to those AS selection guidelines proposing extended criteria without a specific validation.

For HGPCa prediction at the confirmatory Bx, the 4Kscore has been shown to aid individual decision-making as to whether or not to perform a confirmatory Bx, or when to follow up with mpMRI to better define risk for adverse pathology. External validation of our findings could confirm their combined use in AS protocols.

CONFLICT OF INTEREST

Funding support: OPKO Health Europe S.L.U. support for design of study, logistic of samples, honoraria for patient recruitment, and analysis of data. Employment: Yan Dong and David Okrongly are employed at OPKO Diagnostics. Personal financial interest: Dr. Borque-Fernando, Rubio-Briones and Esteban, have received compensation as members of scientific advisory board and by lectures in scientific meetings. The remaining author declares no conflict of interest.

REFERENCES

1 Wilt TJ, Jones KM, Barry MJ, Andriole GL, Culkin D, Wheeler T *et al.* Follow-up of Prostatectomy versus Observation for Early Prostate Cancer. *N Engl J Med* 2017; **377**: 132–142.

2 Hamdy FC, Donovan JL, Lane JA, Mason M, Metcalfe C, Holding P *et al.* 10-Year Outcomes after Monitoring, Surgery, or Radiotherapy for Localized Prostate Cancer. *N Engl J Med* 2016; **375**: 1415–1424.

3 Rubio-Briones J, Borque-Fernando A, Esteban-Escaño LM, Martínez-Breijo S, Medina-López R, Hernández V *et al.* Variability in the multicentre National Registry in Active Surveillance; a questionnaire for urologists. *Actas Urológicas Españolas* 2018. doi:10.1016/j.acuro.2018.01.007.

4 Bruinsma SM, Zhang L, Roobol MJ, Bangma CH, Steyerberg EW, Nieboer D *et al.* The Movember Foundation's GAP3 cohort: a profile of the largest global prostate cancer active surveillance database to date. *BJU Int* 2018; : 737–744.

5 Drost F-JH, Rannikko A, Valdagni R, Pickles T, Kakehi Y, Remmers S *et al.* Can active surveillance really reduce the harms of overdiagnosing prostate cancer? A reflection of real life clinical practice in the PRIAS study. *Transl Androl Urol* 2018; **7**: 98–105.

6 Schoots IG, Petrides N, Giganti F, Bokhorst LP, Rannikko A, Klotz L *et al.* Magnetic resonance imaging in active surveillance of prostate cancer: a systematic review. *Eur Urol* 2015; **67**: 627–636.

7 Barrett T, Haider MA. The emerging role of MRI in prostate cancer active surveillance and ongoing challenges. *Am J Roentgenol* 2017; **208**: 131–139.

8 Siddiqui MM, Rais-Bahrami S, Truong H, Stamatakis L, Vourganti S, Nix J *et al.* Magnetic resonance imaging/ultrasound-fusion biopsy significantly upgrades prostate cancer versus systematic 12-core transrectal ultrasound biopsy. *Eur Urol* 2013; **64**: 713–9.

9 Ahmed HU, El-Shater Bosaily A, Brown LC, Gabe R, Kaplan R, Parmar MK *et al.* Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confirmatory study. *Lancet* 2017; **389**: 815–822.

10 Kasivisvanathan V, Rannikko AS, Borghi M, Panebianco V, Mynderse LA, Vaarala MH *et al.* MRI-Targeted or Standard Biopsy for Prostate-Cancer Diagnosis. *N Engl J Med* 2018; : NEJMoa1801993.

11 Parekh DJ, Punnen S, Sjoberg DD, Asroff SW, Bailen JL, Cochran JS *et al.* A Multi-institutional Prospective Trial in the USA Confirms that the 4Kscore Accurately Identifies Men with High-grade Prostate Cancer. *Eur Urol* 2015; **68**: 464–470.

12 Van Neste L, Hendriks RJ, Dijkstra S, Trooskens G, Cornel EB, Jannink SA *et al.* Detection of High-grade Prostate Cancer Using a Urinary Molecular Biomarker–Based Risk Score. *Eur Urol* 2016; **70**: 740–748.

13 Hendriks RJ, van der Leest MMG, Dijkstra S, Barentsz JO, Van Criekinge W, Hulsbergen-van de Kaa CA *et al.* A urinary biomarker-based risk score correlates with multiparametric MRI for prostate cancer detection. *Prostate* 2017; **77**: 1401–1407.

14 Mottet N, Bellmunt J, Briers E, Bolla M, Bourke L, Cornford P *et al. EAU - ESTRO - ESUR - SIOG Guidelines on Prostate Cancer 2018*. 2018http://uroweb.org/guideline/prostate-cancer/.

15 Mohler JL, Lee RJ, Antonarakis ES, Armstrong AJ, D'Amico AV, Davis BJ *et al. Prostate cancer v2.2018 (NCCN Guidelines)*. 2018https://www.nccn.org/professionals/physician_gls/pdf/prostate.pdf.

16 Epstein JI, Zelefsky MJ, Sjoberg DD, Nelson JB, Egevad L, Magi-Galluzzi C *et al.* A Contemporary Prostate Cancer Grading System: A Validated Alternative to the Gleason Score. *Eur Urol* 2016; **69**: 428–435.

17 Rubio-Briones J, Borque A, Esteban LM, Iborra I, López PA, Gil JM *et al.* Preliminary results of the Spanish Association of Urology National Registry in Active Surveillance for prostate cancer. *Actas Urol Esp*; **40**: 3–10.

18 Punnen S, Pavan N, Parekh DJ. Finding the Wolf in Sheep's Clothing: The 4Kscore Is a Novel Blood Test That Can Accurately Identify the Risk of Aggressive Prostate Cancer. *Rev Urol* 2015; **17**: 3–13.

19 Voigt JD, Dong Y, Linder V, Zappala S. Use of the 4Kscore Test to Predict the Risk of Aggressive Prostate Cancer Prior to Prostate Biopsy: Overall Cost Savings and Improved Quality of Care to the US Healthcare System. *Rev Urol* 2017; **19**.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5434830/pdf/RIU019001_0001.pdf (accessed 9 Jan2018).

20 Stattin P, Vickers AJ, Sjoberg DD, Johansson R, Granfors T, Johansson M *et al.* Improving the Specificity of Screening for Lethal Prostate Cancer Using Prostate-specific Antigen and a Panel of Kallikrein Markers: A Nested Case-Control Study. *Eur Urol* 2015; **68**: 207–13.

21 Punnen S., Zappala S., Palou J, Sjoberg D., Mathur V., Roberts R., Vincent V., Reeve M., O'Krongly D., Newmark J., Sant G., Steiner M., Morote J. PD. Among men with low-grade prostate cancer on prostate biopsy, the 4Kscore predicts the presence of more aggressive prostate cancer. In: *30th Annual EAU Congress Madrid*. Eur Urol suppl: Madrid, 2015, p 433.

22 Punnen S, Zappala S, Mathur V, Reeve M, Parekh D. MP77-20 AMONG MEN WITH LOW-GRADE PROSTATE CANCER ON PROSTATE BIOPSY, THE 4KSCORE PREDICTS MORE AGGRESSIVE PROSTATE CANCER AT PROSTATECTOMY. *J Urol suppl* 2015; **193**: e1001.

23 Lin DW, Newcomb LF, Brown MD, Sjoberg DD, Dong Y, Brooks JD *et al.* Evaluating the Four Kallikrein Panel of the 4Kscore for Prediction of High-grade Prostate Cancer in Men in the Canary Prostate Active Surveillance Study. *Eur Urol* 2017; **72**: 448–454.

24 Bul M, Zhu X, Valdagni R, Pickles T, Kakehi Y, Rannikko A *et al.* Active Surveillance for Low-Risk Prostate Cancer Worldwide: The PRIAS Study. *Eur Urol* 2013; **63**: 597–603.

25 Iremashvili V, Burdick-Will J, Soloway MS. Improving risk stratification in patients with prostate cancer managed by active surveillance: a nomogram predicting the risk of biopsy progression. *BJU Int* 2013; **112**: 39–44.

26 Ankerst DP, Xia J, Thompson IM, Hoefler J, Newcomb LF, Brooks JD *et al.* Precision Medicine in Active Surveillance for Prostate Cancer: Development of the Canary-Early Detection Research Network Active Surveillance Biopsy Risk Calculator. *Eur Urol* 2015; **68**: 1083–8.

27 Giagulli VA, Guastamacchia E, Licchelli B, Triggiani V. Serum Testosterone and Cognitive

Function in Ageing Male: Updating the Evidence. *Recent Pat Endocr Metab Immune Drug Discov* 2016; **10**: 22–30.

28 M. Mamawala, K. Rao, P. Landis, J. Epstein, B. Trock, J. Tosoian, K. Pienta. Risk prediction tool for grade reclassification in favorable-risk men on active surveillance. *J Urol* 2016; **195**: e230.

29 Klotz L, Vesprini D, Sethukavalan P, Jethava V, Zhang L, Jain S *et al.* Long-term follow-up of a large active surveillance cohort of patients with prostate cancer. *J Clin Oncol* 2015; **33**: 272–277.

FIGURE AND TABLE LEGENDS

Figure 1: REMARK diagram detailing study cohort.

Table 1: Patient demographics, split by reclassification to Grade Group ≥2 at confirmatory Bx, (N=137).

Table 2: Distribution of patients by reclassification at confirmatory Bx, by 4Kscore Test prediction and %f/t PSA ratio. All p values are from non-parametric trend test, exploring classifying variables as dichotomized ones as we use then in clinical practice.

Table 3: Extrapolation of our findings to a hypothetical scenario of 1000 patients in active surveillance exposed to confirmatory Bx 6 months after initial Bx, and how 4Kscore could stratify them.

Table 4: Distribution of patients by changes in tumour volume at confirmatory Bx by 4Kscore Test prediction.

Figure 1: REMARK diagram detailing study cohort

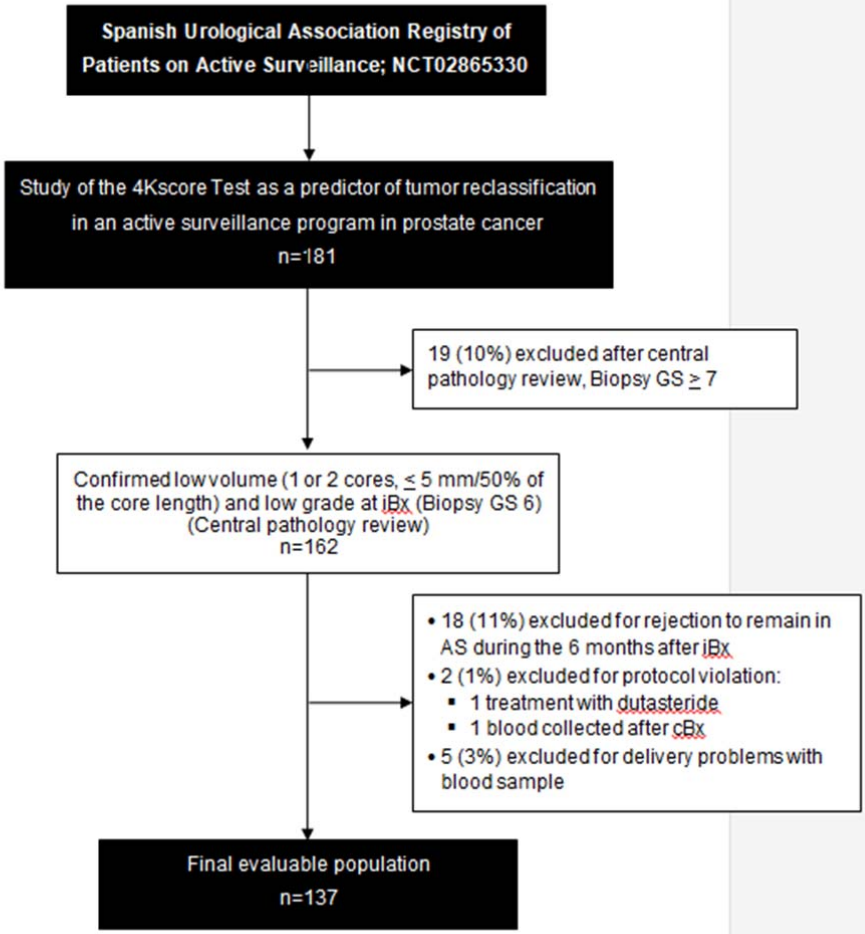

Table 1: Patient demographics, split by reclassification to Grade Group ≥2 at confirmatory Bx, (N=137).

	Statistic	No Reclassification (N=119; 87%)	Reclassified Grade Group ≥2 (N=18; 13%)	p value
Age (years)	Median (IQR)	64 (60, 70)	67 (62, 72)	0.4
4Kscore Test (%)	Median (IQR)	13% (7%, 26%)	25% (15%, 41%)	0.011
Total PSA	Median (IQR)	5.24 (3.82, 7.14)	5.93 (4.82, 7.04)	0.3
%f/t PSA ratio	Median (IQR)	0.16 (0.11, 0.21)	0.13 (0.10, 0.15)	0.019
Prostate volume (cc)	Median (IQR)	40 (31, 55)	35 (25, 40)	0.070
PSA density	Median (IQR)	0.12 (0.09, 0.17)	0.14 (0.12, 0.19)	0.089
Abnormal DRE	N (%)	7 (5.9%)	0 (0%)	0.6
Prior negative biopsy before initial biopsy	N (%)	21 (18%)	1 (5.6%)	0.3
Family history				
Yes	N (%)	13 (10.9%)	3 (16.7%)	0.7
No	N (%)	102 (85.7%)	14 (77.8%)	
N/A	N (%)	4 (3.4%)	1 (5.5%)	
Body Mass Index	Median (IQR)	27.0 (25.5,28.9)	27.3 (25.9,28.9)	0.8
Initial biopsy clinical stage				
T1a	N (%)	1 (0.8%)	0 (0%)	0.2
T1b	N (%)	0 (0%)	1 (5.6%)	
T1c	N (%)	111 (93.3%)	17 (94.4%)	
T2a	N (%)	7 (5.9%)	0 (0%)	
Initial biopsy total cores (N)	Median (IQR)	12 (12, 14)	12 (12, 12)	0.8
Initial biopsy positive cores (N)	Median (IQR)	1 (1, 2)	1 (1, 2)	0.8
Initial biopsy % positive cores	Median (IQR)	8% (8%, 13%)	8% (8%, 17%)	0.6
Initial biopsy max cancer core length (mm)	Median (IQR)	1.4 (0.8, 3)	1.4 (0.7, 2)	0.5
Initial biopsy max cancer % involvement (%)	Median (IQR)	12% (6%, 24%)	11% (7%, 15%)	0.5

Table 2: Distribution of patients by reclassification at confirmatory Bx, by 4Kscore Test prediction and %f/t PSA ratio. All p values are from non-parametric trend test, exploring classifying variables as dichotomized ones as we use then in clinical practice.

	Total	**Confirmatory Bx No Cancer**	**Confirmatory Bx Grade Group 1**	**Confirmatory Bx Grade Group 2**	**Confirmatory Bx Grade Group 3**	**p value**
All Patients N (%)	137	44 (32%)	75 (55%)	15 (11%)	3 (2%)	
4Kscore						
4Kscore <7.5% N (%)	37	22 (59%)	13 (35%)	2 (6%)	0	p <0.001
4Kscore ≥7.5% N (%)	100	22 (22%)	62 (62%)	13 (13%)	3 (3%)	
%f/t PSA ratio						
<0.10 N (%)	25	4 (16%)	17 (68%)	3 (12%)	1 (4%)	p =0.102
≥0.10 N (%)	112	40 (36%)	58 (52%)	12 (11%)	2 (2%)	
<0.25 N (%)	123	37 (30%)	69 (56%)	14 (11%)	3 (2%)	p =0.143
≥0.25 N (%)	14	7 (50%)	6 (43%)	1 (7%)	0	

Table 3: Extrapolation of our findings to a hypothetical scenario of 1000 patients in active surveillance exposed to confirmatory Bx 6 months after initial Bx, and how 4Kscore could stratify them.

	Total	Findings at confirmatory Bx			
		No Cancer	Grade Group 1	Grade Group 2	Grade Group 3
All Patients N	1002	322	548	110	22
4Kscore					
4Kscore <7.5% N	271	161	95	15	0
4Kscore ≥7.5% N	731	161	453	95	22

Table 4: Distribution of patients by changes in tumour volume at confirmatory Bx by 4Kscore Test prediction.

4Kscore	**Confirmatory Bx max tumor length (mm) Median (IQR)***	**Confirmatory Bx max tumor % involvement Median (IQR)***	**Confirmatory Bx % positive cores Median (IQR)***	**Positive cores≥3 Nº patients (%)****
<7.5% (n=37)	0 (0, 1.5)	0% (0%, 11.5%)	0% (0%, 7%)	3 (8.1%)
≥7.5% (n=100)	1.6 (0.38, 4.0)	13% (2.9%, 32.0%)	10% (5%, 18%)	39 (39%)
p value	p <0.0001	p <0.0001	p <0.0001	p <0.001

*p value from Wilcoxon rank sum test.

**p value from Fisher's exact test.