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Prospective validation of microseminoprotein-β added to the 4Kscore in predicting high-grade prostate cancer in an international multicentre cohort

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Abstract

Objectives—To prospectively evaluate the performance of a pre-specified statistical model based on four kallikrein markers in blood (total prostate-specific antigen [PSA], free PSA, intact PSA, and human kallikrein-related peptidase 2), commercially available as the 4Kscore, in predicting Gleason Grade Group (GG) ≥2 prostate cancer at biopsy in an international multicentre study at three academic medical centres, and whether microseminoprotein-β (MSP) adds predictive value.

Patients and Methods—A total of 984 men were prospectively enrolled at three academic centres. The primary outcome was GG ≥2 on prostate biopsy. Three pre-specified statistical models were used: a base model including PSA, age, digital rectal examination and prior negative biopsy; a model that added free PSA to the base model; and the 4Kscore.

Results—A total of 947 men were included in the final analysis and 273 (29%) had GG ≥2 on prostate biopsy. The base model area under the receiver operating characteristic curve of 0.775 increased to 0.802 with the addition of free PSA, and to 0.824 for the 4Kscore. Adding MSP to the

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Conflict of Interest

Dr Lilja holds a patent on assays to measure intact PSA and together with Dr Vickers is named on a patent for a statistical method to detect prostate cancer that has been commercialised as the 4Kscore test by OPKO Health. They receive royalties from sales of the test. Dr Vickers owns stock options and Dr Lilja owns stock in OPKO Health. Dr Sjoberg has received funds for consulting work from OPKO Health.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

4Kscore model yielded an increase (0.014–0.019) in discrimination. In decision-curve analysis of clinical utility, the 4Kscore showed a benefit starting at a 7.5% threshold.

Conclusion—A prospective multicentre evaluation of a pre-specified model based on four kallikrein markers (4Kscore) with the addition of MSP improves the predictive discrimination for GG ≥2 prostate cancer on biopsy and could be used to inform biopsy decision-making.

Keywords

Prostate-specific antigen; microseminoprotein-β; kallikreins; 4Kscore; prostate biopsy; #ProstateCancer; #PCSM; #uroonc

Introduction

The use of PSA for prostate cancer screening remains controversial, with the principle drawback being low specificity for clinically significant disease, particularly amongst older men [1]. Although a moderately elevated PSA is strongly associated with aggressive and lethal forms of prostate cancer, modest PSA elevations are much more common among men with low-risk cancer or a benign process. Many men with an elevated PSA undergo avoidable prostate biopsies, leading to the over detection of indolent disease and possibly overtreatment. As a result, better methods are needed to detect clinically significant prostate cancer (International Society of Urological Pathology [ISUP] Gleason Grade Group [GG] ≥2), to reduce the negative consequences of inappropriate PSA testing and also the risks of the biopsy itself.

PSA exists in a number of molecular sub-forms: complexed PSA vs free PSA and intact vs nicked PSA [2,3]. Human kallikrein-related peptidase 2 (hK2) is a related prostatic enzyme [4]. Specific measurements of distinct non-catalytic forms of these enzymes in blood improve the prediction of biopsy results in men with an elevated PSA level [5,6]. It has been shown that a statistical model including clinical parameters and a panel of four kallikrein markers in blood (total PSA, free PSA, intact PSA, and hK2) strongly predicts the risk of GG ≥2 cancer on biopsy [7]. This panel has been validated in multiple European and USA studies and is now commercially available as the ‘4Kscore’. The predictive accuracy of the 4Kscore has been evaluated in 11 independent clinical validation studies involving >10 000 patients with an area under the curve (AUC) >0.80 for the discrimination of GG ≥2 prostate cancer [7].

Microseminoprotein-β (MSP) is the second most abundantly secreted prostatic protein after PSA [8]. Unlike PSA, MSP is not directly regulated by androgens and is not affected by hormone manipulation. Several groups have assessed MSP expression in prostate tissue and have consistently found higher levels of MSP in benign tissue or serum compared to material from tumours [9–12]. However, there are conflicting results as to the potential value of MSP for aiding clinical decisions about prostate cancer biopsy. Two USA-based studies, the Multi-ethnic Cohort study and the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial, both reported an association between MSP and high-grade prostate cancer on biopsy [13,14]. However, two European-based studies found that adding MSP to the four

kallikrein model increased discrimination for GG ≥2 prostate cancer either very marginally [15] or not at all [16].

The aim of the present study was to prospectively evaluate the performance of a pre-specified 4Kscore model in predicting GG ≥2 cancer on biopsy in three large academic centres, as well as evaluating whether adding MSP to the prediction model improves discrimination. A secondary aim was to determine whether predictions based on these markers measured locally at each centre differed from those performed in a central laboratory.

Patients and Methods

Patients

Patients were prospectively enrolled at the Martini-Klinik (Hamburg, Germany) between April 2014 and October 2016; at Mayo Clinic (Rochester, MN, USA) between October 2014 and March 2016; and from 2006 to 2015 at the University of California, San Francisco (UCSF) (San Francisco, CA, USA) if they did not have a previous diagnosis of prostate cancer and met AUA or European Association of Urology guidelines for prostate biopsy. All prostate biopsies were standard systematic TRUS-guided 12-core biopsies. Blood was drawn before biopsy, and total PSA, free PSA, intact PSA and hK2 were measured on-site at the local laboratory for samples taken at the Martini-Klinik and Mayo Clinic. Cryopreserved sample aliquots from all patients enrolled at the Martini-Klinik, Mayo Clinic, and UCSF were also sent to Lund University in Sweden to obtain separate central laboratory measurements of total PSA, free PSA, intact PSA, hK2, and MSP conducted blind to outcome by H.L.'s laboratory as detailed below. Data were collected on patient age, patient race and prostate biopsy result, including GG group. Data were also collected on DRE at the time of biopsy, history of prior PSA tests, and history of prior negative prostate biopsy. Institutional Review Board approval was received from the Memorial Sloan Kettering Cancer Center (WA0307-12), Mayo Clinic (#11-007572), Martini-Klinik (PV3652-a), and UCSF (#11-05329).

Laboratory Methods

The four kallikrein marker panel was evaluated in cryopreserved samples using the AutoDelfia 1235 automatic immunoassay system (Perkin-Elmer, Turku, Finland) in H.L.'s laboratory at Lund University, Malmö, Sweden [14–16]. The panel was also validated for precision, concordance, and accuracy using Victor instruments (Perkin-Elmer) at all three local laboratories. The total and free PSA levels were measured using the dual-label DELFIA Prostatus total/free PSA-Assay (Perkin-Elmer) [17] calibrated against the WHO 96/670 (PSA-WHO) and WHO 68/668 (free PSA-WHO) standards. Intact PSA and hK2 were measured with a recombinant Fab fragment and a F(ab')2 fragment of the monoclonal capture antibodies, respectively, to reduce the frequency of nonspecific assay interference, as described previously [18]. The production and purification of the polyclonal rabbit anti-MSP antibody, protocols for biotinylation and Europium labelling of the anti-MSP antibody, and performance of the MSP immunoassay were carried out as previously reported [13].

Statistical Methods

Three models for the outcome of GG ≥2 on biopsy were used: (i) a base model, which included patient age and total PSA; (ii) a free PSA model, which included patient age, total PSA, and free PSA; and (iii) the 4Kscore, which included patient age, total PSA, free PSA, intact PSA, and hK2. Within each of these models, comparisons were performed between models that included patient age and biomarkers only; patient age, biomarkers, and DRE result; and patient age, biomarkers, DRE result, and history of prior negative prostate biopsy. All models were pre-specified and locked down before the data analysis. The 4Kscore model is the same pre-specified model as the commercially available 4Kscore test, which is used in routine clinical practice in the USA. The base model and free PSA model were built using data from the Prostate Testing for Cancer and Treatment (ProtecT) study [19]. These models were applied to both central laboratory measurements and measurements performed at the local laboratories of the Martini-Klinik and Mayo Clinic.

We then assessed model performance using central laboratory vs local laboratory measurements. Discrimination was compared across models using the AUC. Calibration was assessed using calibration plots, and clinical utility using decision-curve analysis. We also aimed to examine whether MSP added to the 4Kscore for prediction of GG ≥2 disease and report the change in discrimination when adding MSP to the 4Kscore model. All analyses were performed using Stata 15 (StataCorp, College Station, TX, USA). This study is reported in accordance with the REporting recommendations for tumour MARKer prognostic studies (REMARK) [20].

Results

Clinical data and central laboratory measurements were available for a total of 984 patients from the three centres. Six patients from UCSF were excluded from the analyses on the basis that the central laboratory measurements found that these patients had very high levels of both free and total PSA as well as free:total ratios >60%, strongly suggesting that these measurements were likely performed on blood drawn shortly after rather than before prostate biopsy. In all, 30 patients missing a DRE result and one patient missing GG on prostate biopsy were also excluded. Hence, a total of 947 patients were included in the final analysis. Patient characteristics are reported separately by institution in Table 1. The PSA levels across all institutions were typical of a screened cohort, with almost all PSAs in the 3–10 ng/mL range. A total of 273 patients (29%) had a GG ≥2 cancer on prostate biopsy. The concordance between 4Kscore calculated using central laboratory and local laboratory measurements is presented in Fig. 1.

Discrimination for all models was consistent between local and central laboratory measurements, indicating that the four kallikrein marker measurements performed in each of the local laboratories of the Martini-Klinik and Mayo Clinic were similarly predictive to those based on measurements done at the central laboratory (Table 2). Inclusion of DRE increased discrimination for all models and including the history of prior negative biopsy in addition to DRE result increased discrimination further. Discrimination was high for all 4Kscore-based models. The increase in AUC between the 4Kscore model and models based on total PSA or total and free PSA was very similar when analysed separately by prior

biopsy status (Table S1). The addition of MSP to all models increased discrimination, with an increase in AUC from 0.792 to 0.811 for the biomarker-only model, from 0.803 to 0.821 for the model including DRE, and from 0.824 to 0.838 for the model including both DRE and history of prior negative biopsy.

Calibration was reasonable for all 4Kscore models using either central laboratory measurements (Fig. 2) or local laboratory measurements (Fig. S1), although there was some evidence of under-prediction. To investigate this further, we examined calibration separately by centre. We found that calibration was similarly good for Mayo Clinic and Martini-Klinik (Figs S2a,b, respectively), but UCSF was miscalibrated with higher rates of GG ≥2 cancer than expected (Fig. S2c).

In decision-curve analysis of clinical utility, the 4Kscore showed a benefit starting at around a 7.5% threshold (Fig. 3), with findings of similar clinical utility when the 4Kscore was based on either local or central laboratory measurements among the men biopsied at Mayo Clinic and Martini-Klinik (Fig. S3). However, given the differences in calibration between sites, we also investigated clinical utility separately by site. Net benefit for the 4Kscore was seen in the Martini-Klinik cohort starting at approximately a 3% risk threshold (Fig. S4b) and for the Mayo Clinic and UCSF cohorts at a 10% threshold (Fig. S4a,c, respectively).

Clinical implications of basing biopsy decisions on 4Kscore thresholds are reported in Table 3. Among the 585 patients in the Mayo Clinic and Martini-Klinik cohorts, there were eight patients who had a 4Kscore <10% and GG ≥3 cancers. These patients included: three patients with GG3 cancer, two patients with GG4 cancer, and one patient with GG5 cancer (biopsy Gleason score 4 + 5), all with negative DRE. The PSA levels among patients with GG3 cancers ranged from 1 to 8.2 ng/mL. The PSA levels were 6.8 and 10.5 ng/mL in the two patients with GG4 disease, and 4.8 ng/mL in the patient with GG5 disease. The same analyses using central laboratory measurements in the UCSF cohort are presented in Table S2.

Discussion

In the present prospective, multicentre, international study of men who had an elevated PSA level and were subject to prostate biopsy, we found that a pre-specified 4Kscore model, identical to the commercially available test, based on the four kallikrein markers in blood improved the predictive discrimination for GG ≥2 prostate cancer at biopsy, compared to models based on age and total PSA, or those that also incorporated free PSA. Adding MSP to the 4Kscore model yielded an increase in discrimination. The model was well calibrated at two of the three centres, with decision-curve analysis demonstrating that use of the model in practice would improve biopsy-outcome predictions.

Our present data support previous studies that have found that the 4Kscore model improves the predictive discrimination for high-grade prostate cancer [16,19,21]. Bryant *et al.* [19] showed an increase in the AUC from 0.738 to 0.820 if levels of free PSA, intact PSA, and hK2 were added to a base model containing total PSA and patient age to predict high-grade prostate cancer in participants in the prospective ProtecT study. This pre-specified 4Kscore

model has also been validated in a USA cohort, in which almost all patients had prior screening, with an AUC of 0.82 and had a superior net benefit compared to the Prostate Cancer Prevention Trial Risk Calculator 2.0, which incorporates standard clinical variables (AUC 0.74) [21]. A recent meta-analysis found that the pooled AUC of the 4Kscore model for discrimination of high-grade prostate cancer is >0.80 across 11 clinical validation studies involving >10 000 men [7]. We report a similar AUC of 0.82 for our present 4Kscore model when including DRE and history of negative biopsy. Our present study furthers previous reports, primarily retrospective and in community cohorts by prospectively assessing the discriminative ability of the 4Kscore model in a multi-institutional academic cohort.

MSP is an abundantly secreted prostate protein and is thought to be a possible biomarker for prostate cancer. Immunohistochemical and *in situ* hybridisation studies from single centres and small cohorts have suggested that MSP is an independent prognostic factor for survival in men with prostate cancer [11,22–24]. The addition of MSP to the 4Kscore yields an improvement in the predictive discrimination for GG ≥2 prostate cancer, with an AUC ranging from 0.019 units greater in the 4Kscore model alone to 0.014 units greater in the 4Kscore model that included DRE and negative biopsy. Our present findings are similar to the results from the USA PLCO study [14], but different from studies of biopsy cohorts from Finland [15] and Sweden [16]. While our present data are encouraging, these conflicting findings, and possible differences between multi-ethnic USA populations and predominately White European cohorts, additional studies on MSP are warranted to determine its role in prostate cancer risk prediction.

We found that overall calibration was reasonable for the 4Kscore models when using either central laboratory measurements or local laboratory measurements. Determining calibration separately by centre, we found that Mayo Clinic and Martini-Klinik had similar and excellent calibration but UCSF was miscalibrated, with higher rates of GG2 cancer than expected. This difference between cohorts was not unexpected, as other studies have shown a lower rate of upgrading in UCSF patients undergoing surgery and on active surveillance, suggesting a more extensive or otherwise sensitive biopsy technique, and/or a greater propensity to describe tumours as high grade [25].

A number of limitations are inherent to our present analyses that need to be acknowledged. The use of multiparametric MRI (mpMRI) or transperineal prostate biopsy was not evaluated and may have influenced sampling of the prostate and possibly detected clinically significant cancers not detected by TRUS. While a prior study has demonstrated that the addition of mpMRI to the 4Kscore improves the detection of GG ≥2 cancer, it was limited by its retrospective design and that only half the cohort were biopsied [26]. Although the cohort included few men of African ancestry, the 4Kscore has been validated elsewhere across multiple ethnicities [27] including African Americans [14,28]. Prostate volume combined with PSA may improve the discriminatory ability of PSA for detecting clinically significant tumours; however, prior studies have found that adding prostate volume to a multivariate model with the four kallikrein panel did not improve prediction of progression at follow-up biopsy in a surveillance cohort [29]. Currently, blood drawn for a 4Kscore test is processed in the USA. The Medicare list price of a 4Kscore test is US$760 and the maximum out-of-pocket cost is US$395.

Conclusions

In three international, academic centres, we prospectively validated that MSP added to the 4Kscore improves the predictive discrimination for GG ≥2 prostate cancer at biopsy. Our present data provide further evidence that 4Kscore models can be used in practice to improve biopsy decision-making. This model helps reduce unnecessary biopsies without missing an undue number of high-grade cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations:

AUC	area under the curve
GG	Grade Group
hK2	human kallikrein-related peptidase 2
ISUP	International Society of Urological Pathology
4Kscore	four kallikrein markers in blood (total PSA, free PSA, intact PSA, and hK2)
MSP	microseminoprotein-β
mpMRI	multiparametric MRI
PLCO	Prostate, Lung, Colorectal and Ovarian
rotecT	Prostate Testing for Cancer and Treatment

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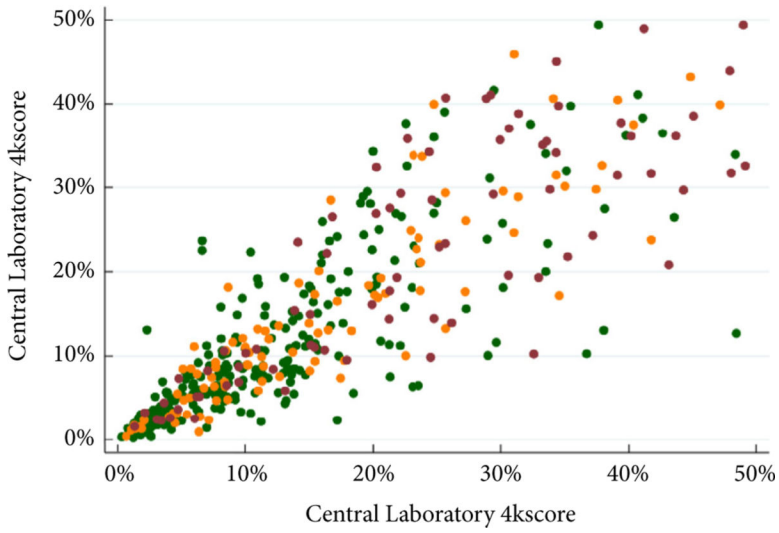

Fig. 1.

Concordance between 4Kscore calculated using central laboratory measurements and local laboratory measurements performed on-site for men biopsied at the Martini-Klinik and Mayo Clinic, by biopsy result. Green indicates negative prostate biopsy, yellow indicates ISUP GG1 disease on biopsy, and red indicates ISUP GG ≥2 prostate cancer.

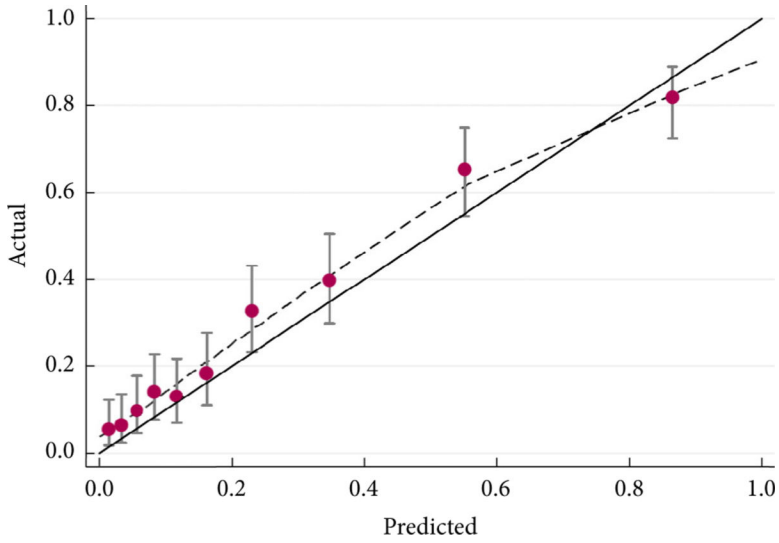

Fig. 2.

Calibration of the 4Kscore model based on central laboratory measurements of cryopreserved samples from all three sites and including DRE result and history of prior negative biopsy.

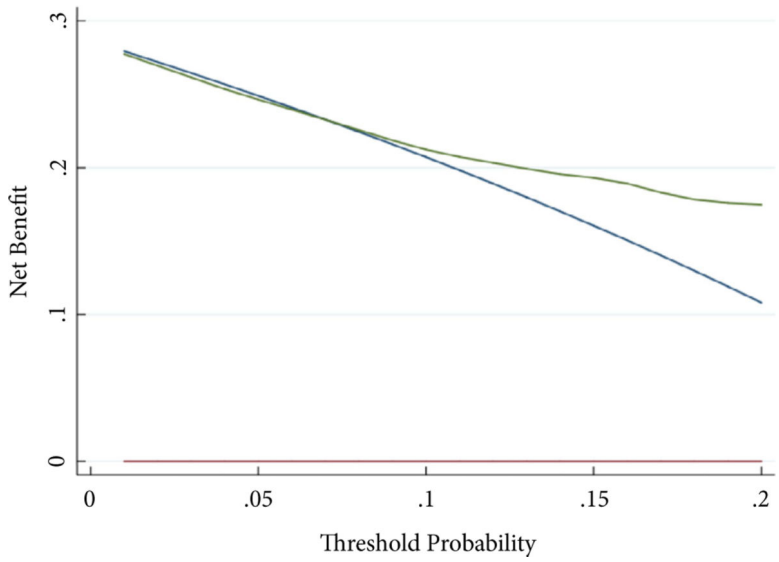

Fig. 3.

Decision-curve analysis for the 4Kscore including DRE and prior negative biopsy, based on central laboratory measurements of cryopreserved samples from all three sites. The blue line represents biopsying all patients, while the red line represents biopsying no patients.

Table 1

Patient and disease characteristics by institution ($N = 947$). Data are reported as frequency (%) or median (quartiles).

Characteristic	Martini-Klinik ($N = 266$)	Mayo Clinic ($N = 319$)	UCSF ($N = 362$)	*P*
Age at biopsy, years, median (quartiles)	65 (58, 70)	64 (58, 70)	64 (60, 69)	0.6
Total PSA, ng/mL, median (quartiles)	6.0 (4.3, 9.3)	5.0 (3.5, 7.4)	5.8 (3.9, 8.7)	<0.001
African American/Black race, *n* (%) ($N = 914$)	2 (0.8)	2 (0.6)	8 (2.4)	0.13
Any prior negative biopsy, *n* (%)				
No	222 (83)	265 (83)	198 (55)	<0.001
Yes	44 (17)	53 (17)	143 (40)	
Unknown	0 (0)	1 (0.3)	21 (5.8)	
Any prior PSA screening, *n* (%)				
No	96 (36)	11 (3.4)	0 (0)	<0.001
Yes	170 (64)	250 (78)	0 (0)	
Unknown	0 (0)	58 (18)	362 (100)	
Abnormal DRE, *n* (%)	37 (14)	122 (38)	99 (27)	<0.001
Any Grade cancer on biopsy, *n* (%)	117 (44)	170 (53)	181 (50)	0.079
ISUP GG ≥2 cancer on biopsy	71 (27)	103 (32)	99 (27)	0.2

Kruskal–Wallis tests and exact tests were used to test for differences in patient and disease characteristics by institution.

Table 2

Discrimination with 95% CI for the base model, free PSA model, and pre-specified 4Kscore panel risk score model, with and without DRE result and history of prior negative biopsy, based on central laboratory and local laboratory measurements.

Model	Central laboratory (All sites)		Central laboratory (Martini-Klinik and Mayo Clinic)		Local laboratory (Martini-Klinik and Mayo Clinic)	
	N	AUC (95% CI)	*N*	AUC (95% CI)	*N*	AUC (95% CI)
Base model	947	0.711 (0.674, 0.748)	585	0.728 (0.682, 0.774)	585	0.729 (0.683, 0.774)
With DRE	947	0.731 (0.695, 0.767)	585	0.755 (0.712, 0.799)	585	0.755 (0.711, 0.799)
With DRE and prior negative biopsy	925	0.775 (0.741, 0.809)	584	0.778 (0.736, 0.820)	584	0.779 (0.737, 0.821)
Free PSA model	947	0.764 (0.729, 0.798)	585	0.782 (0.740, 0.823)	585	0.785 (0.744, 0.826)
With DRE	947	0.776 (0.742, 0.810)	585	0.798 (0.758, 0.839)	585	0.802 (0.762, 0.842)
With DRE and prior negative biopsy	925	0.802 (0.769, 0.834)	584	0.812 (0.773, 0.852)	584	0.815 (0.777, 0.854)
4Kscore model	947	0.792 (0.759, 0.824)	585	0.816 (0.777, 0.855)	585	0.811 (0.772, 0.850)
With DRE	947	0.803 (0.772, 0.835)	585	0.830 (0.793, 0.868)	585	0.828 (0.790, 0.865)
With DRE and prior negative biopsy	925	0.824 (0.793, 0.855)	584	0.842 (0.805, 0.879)	584	0.836 (0.799, 0.873)

Results are presented for central measurements from all three sites; central measurements from two sites also providing local laboratory measurements (Martini-Klinik and Mayo Clinic); and local laboratory measurements (Martini-Klinik and Mayo Clinic).

Table 3

Clinical implications of basing biopsy decisions 4Kscore thresholds, with and without the addition of MSP, per 1000 biopsied men, calculated from central laboratory measurements or local laboratory measurements performed on-site at the Martini-Klinik and Mayo Clinic.

Threshold	Biopsies avoided	ISUP GG1 cancers detected	ISUP GG1 cancers not detected	ISUP GG2 cancers detected	ISUP GG2 cancers not detected	ISUP GG ≥3 cancers detected	ISUP GG ≥3 cancers not detected
4Kscore measured at local laboratories							
≥7.5%	312	145	48	100	14	174	10
≥10%	392	122	71	96	17	170	14
4Kscore measured at central laboratory							
≥7.5%	318	154	54	103	12	159	12
≥10%	403	136	73	95	20	155	16
4Kscore + MSP measured at central laboratory							
≥7.5%	247	171	37	106	6	163	6
≥10%	328	154	54	102	10	159	10

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