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Can one blood draw replace transrectal ultrasonography-estimated prostate volume to predict prostate cancer risk?

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Abstract

- To explore whether a panel of kallikrein markers in blood: total, free and intact prostate-specific antigen (PSA) and kallikrein-related peptidase 2, could be used as a non-invasive alternative for predicting prostate cancer on biopsy in a screening setting.
- The study cohort comprised previously unscreened men who underwent sextant biopsy owing to elevated PSA (≥ 3 ng/mL) in two different centres of the European Randomized Study of Screening for Prostate Cancer, Rotterdam ($n=2914$) and Göteborg ($n=740$).
- A statistical model, based on kallikrein markers, was compared with one based on established clinical factors for the prediction of biopsy outcome.
- The clinical tests were found to be no better than blood markers, with an area under the curve in favour of the blood measurements of 0.766 vs. 0.763 in Rotterdam and 0.809 vs. 0.774 in Göteborg.
- Adding digital rectal examination (DRE) or DRE plus transrectal ultrasonography (TRUS) volume to the markers improved discrimination, although the increases were small. Results were similar for predicting high grade cancer.
- There was a strong correlation between the blood measurements and TRUS-estimated prostate volume (Spearman's correlation 0.60 in Rotterdam and 0.57 in Göteborg).
- In previously unscreened men, each with indication for biopsy, a statistical model based on kallikrein levels was similar to a clinical model in predicting prostate cancer in a screening setting, outside the day-to-day clinical practice.

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CONFLICT OF INTEREST

H.L. holds patents for free PSA, intact PSA, and hK2 assays, and K.P. is named as co-inventor on the patent for intact/nicked PSA assays.

- Whether a clinical approach can be replaced by laboratory analyses or used in combination with decision models (nomograms) is a clinical judgment that may vary from clinician to clinician depending on how they weigh the different advantages and disadvantages (harms, costs, time, invasiveness) of both approaches.

Keywords

prostate cancer; transrectal ultrasonography; kallikreins; prostate-specific antigen

INTRODUCTION

Elevated PSA levels can arise from both malignant and benign prostate disease, which lowers the specificity of PSA level in screening for prostate cancer. The positive predictive value of PSA in the European Randomized Study of Screening for Prostate Cancer (ERSPC) is close to 25%, which means that three out of four men underwent biopsy unnecessarily [1]. Many studies aim to increase the diagnostic specificity of PSA and reduce the number of unnecessary prostate biopsies by searching for new molecular markers or by combining isoforms of markers.

The most common benign cause of elevated PSA levels is BPH, a condition affecting close to half of men >60 years [2]. Serum PSA level increases with the size of the prostate and age; thus, serum PSA level is strongly related to volume in men with BPH and no evidence of prostate cancer. It is known that TRUS-estimated prostate volume is predictive of prostate cancer in men with elevated PSA levels [3–5]. Previous studies have recommended volume, and derivatives such as PSA density (PSA/volume), as markers for prostate cancer risk.[6–8] In addition, the prostate cancer detection rate is higher in smaller prostates [5]. This suggests that men should have prostate volume measured by TRUS before considering prostate biopsy. Yet, although TRUS has high patient acceptance [9], it is not without discomfort for the patient, and may be time- and resource-consuming. Although TRUS may avoid an even more uncomfortable and risky biopsy [10, 11], a non-invasive test of volume would clearly be of considerable clinical benefit.

We have previously developed a statistical model, based on the concentrations of four kallikreins in blood, which predicts the biopsy outcome in men with elevated PSA levels [12, 13] and which is as good as a clinical model (age, PSA and DRE) in reducing the number of unnecessary biopsies without missing the diagnosis of an undue number of high grade cancers [12].

The multikallikrein panel consists of total PSA, free PSA, intact PSA and kallikrein-related peptidase 2 (hK2). Intact PSA is an isoform of free PSA which does not contain an internal cleavage at Lys145 and Lys146 [14]. By subtracting the intact PSA concentration from the free PSA concentration, the concentration of internally nicked PSA can be calculated. Increased concentrations of intact PSA have been associated with prostate cancer more frequently than with benign diseases of the prostate, while the situation is inversed with nicked PSA [15, 16].

Of the markers in the multikallikrein panel, free and nicked PSA have been associated with prostate volume, while intact PSA and hK2 seem to have a greater association with tumour volume [17, 18]. To explain the prostate volume dependency of circulating PSA it has been proposed that BPH tissue has more epithelial cells to produce PSA, whereas the PSA secretion of cancerous tissue may actually decrease [19]. The different PSA forms have been explained by differences in exposure to proteases in the extracellular matrix of BPH or cancer tissue before diffusion into the circulation [20].

The aim of the present study was to determine whether a non-invasive blood test could replace invasive measurement of prostate volume for assessment of prostate cancer risk. We compared the discrimination of a statistical model based only on blood markers to that of a 'clinical' model that included DRE and TRUS-estimated prostate volume. We also examined how adding information about prostate volume to the multikallikrein model would change the predictive accuracy of the model.

SUBJECTS AND METHODS

Patient cohort

The study populations consisted of two independent populations from the ERSPC, one sample from Rotterdam, the Netherlands, and one from Göteborg, Sweden. Owing to a change in immunoassays [13], data from each centre were analysed separately.

The ERSPC in Rotterdam recruited 42 176 men (aged 55–74 years) and randomized these to an intervention and a control arm. Of the 21 210 men randomized to the intervention arm, 19 970 men participated in the first screening round during 1993–2000. Since our scientific question relates to an elevated blood PSA level, the present study included men with an indication for biopsy on the basis of an elevated PSA level. Among these men, abnormal findings on DRE and/or TRUS were recorded (Table 1). The study design of the Rotterdam protocol, reported in detail previously [13], therefore excluded 1090 men with PSA <3 ng/mL who were biopsied for other reasons, i.e. if they only had an abnormal DRE and/or an abnormal TRUS. Men with an elevated PSA level and also, but not only, positive DRE and/or TRUS were included. Thus, the study included 2914 previously unscreened men enrolled in the first screening round in 1993–1999, biopsied because of elevated PSA level, i.e. ≥ 3 ng/mL (range 3–260 ng/mL), and for which kallikrein concentrations were available (114 men were excluded because of insufficient blood samples) [13]. Men with a biopsy indication underwent DRE followed by biplanar TRUS using a Bruel & Kjaer model 1846 mainframe and a 7-MHz biplanar endorectal transducer (B&K Medical Systems, Marlborough, MA, USA), in the left lateral decubitus position. The TRUS prostate volume was measured by planimetry in 0.5-cm step sections. Lateralized sextant biopsy was performed with an additional core for hypoechoic lesions on TRUS.

The design of the Göteborg cohort has been described elsewhere [12, 21]. In brief, 20 000 men among all men born during 1930–1944, living in the city of Göteborg on 31 December 1994 were randomly allocated to either biannual PSA screening or to a control group in a 1:1 ratio. After the first screening round (1995–1996), the men were re-invited every second year up to 2005 until they reached 70 years or unless they were diagnosed with prostate cancer or died. Men with a serum-PSA ≥ 3 ng/mL were referred for further clinical evaluation, including laterally directed TRUS-guided sextant prostate biopsy. The cohort in the present study comprised 740 men with an indication for biopsy, i.e. a serum PSA ≥ 3 ng/mL only, undergoing biopsy after their first PSA test.

Laboratory methods

The laboratory methods have been described previously [12, 13]. In Rotterdam, the archival serum samples, which were processed within 3 h from venipuncture and stored at -80°C in a serum bank in Rotterdam, were shipped on dry ice to Malmö, Sweden in 2005–2007. The Göteborg cohort archival serum samples were processed within 3 h from venipuncture and stored at -20°C for up to 2 years and after aliquoting at -70°C until analysis. Immunoassay measurements were performed in Dr Lilja's laboratory at the Wallenberg Research Laboratories, Department of Laboratory Medicine, Lund University, Skåne University Hospital in Malmö, Sweden during 2005–2007 for the Rotterdam cohort and 1995–2006 for

the Göteborg cohort. The total and free PSA concentrations were measured using the dual-label DELFIA Prostatus Total/Free PSA-assay (Perkin Elmer, Turku, Finland). The intact PSA and hK2 concentration measurements in the Rotterdam cohort were done using F(ab')₂-based research immunoassays as described previously [22] and in the Göteborg cohort using the older Mab-based assays [12, 15, 23]. All analyses were conducted blinded to the biopsy results. In Göteborg, the analysis was based on measurement of fresh samples for total and free PSA but frozen, stored and thawed samples for intact PSA and hK2 [12]. Free, total and intact PSA assays were calibrated against the WHO PSA calibration standards; 96/670 (PSA-WHO), and WHO 68/668 (free PSA-WHO). For measurements done before the implementation of WHO calibration in 2004, a correction factor provided by the assay manufacturer was applied.

Statistics

In Rotterdam, the cohort was randomly divided into a training and validation set in a 1:3 ratio, including a total of 728 and 2186 men, respectively. The random allocation was performed by means of stratification on cancer diagnosis (any and high grade), but no other variables. We applied the model built on the training set to the validation set. In Göteborg the cohort of 740 men was split 2:1 (494 in training vs 246 in validation), a ratio reflecting the number of events.

We assessed whether the clinical measurements, DRE (abnormal vs not suspicious for prostate cancer) and TRUS-estimated prostate volume results, could be replaced by the kallikrein panel in a prognostic model without a loss in discrimination by comparing the performance characteristics between these two models. Additionally, we assessed whether DRE alone would improve the discrimination of a model including age and the kallikrein panel. Lastly, we assessed the change in discrimination that both DRE and TRUS-estimated prostate volume make when added to a model with age and the kallikrein panel alone. For Göteborg and Rotterdam separately, each of the four models was generated on the training set and then applied to the validation set. We calculated the area under the receiver-operator characteristic curve (AUC) for each model on the validation set, comparing AUCs between models according to DeLong et al. [24]. We hypothesized that the kallikrein panel would not be inferior to the clinical model (PSA, age, TRUS-estimated prostate volume and DRE). We furthermore hypothesized that DRE would add some additional discrimination to the kallikrein panel but TRUS-estimated prostate volume would not add substantially to the panel plus DRE. Total PSA and free PSA were entered into the logistic models using restricted cubic splines to account for nonlinearity.

As an additional set of analyses, we sought to determine whether the models could also be useful in predicting high grade cancer on biopsy, defined as Gleason score ≥ 7 cancer. The AUC for high grade cancer was calculated from the predicted probabilities of any cancer and compared with negative biopsy and Gleason score ≤ 6 . In Rotterdam, pathological grade was defined as the Anderson grade in a small number of patients ($n=7$), with high grade defined as Anderson grade ≥ 2 [13].

Sensitivity analyses, restricted to the cohort of men with PSA > 4 ng/mL as well as 3–10 ng/mL, were performed.

We assessed the correlations between prostate volume and the full panel or the individual isoforms by the non-parametric Spearman's rank correlation coefficient. Nicked PSA was calculated as free PSA subtracted from intact PSA. All statistical analyses were performed using Stata 11.0 (StataCorp, College Station, TX, USA).

RESULTS

Of the first-time screened men with an indication for biopsy, 807 (28%) were diagnosed with prostate cancer on biopsy in Rotterdam and 192 (26%) in Göteborg. Table 1 shows the clinical characteristics for the men in the training and validation sets for Rotterdam (Table 1A) and Göteborg (Table 1B). In Rotterdam, 45 vs. 46% of the patients with prostate cancer on biopsy in the training and in the validation set had an abnormal DRE, respectively. The corresponding figures for Göteborg were 36 vs. 47%.

The predictive accuracy of the laboratory models and the clinical models are shown in Table 2 for both cohorts. The differences in AUCs among the models are shown in Table 3. For the outcome of any cancer, the model based on blood markers (age and kallikrein panel) had an AUC of 0.766 and 0.809 and for the high grade cancers 0.847 and 0.786 in Rotterdam and Göteborg, respectively. There was no evidence that using invasive clinical tests led to greater discrimination (model B: age, PSA, DRE and TRUS-estimated prostate volume) with changes in AUCs by 0.003, $P=0.8$ in Rotterdam and 0.035, $P=0.3$ in Göteborg. Adding DRE (model C) or DRE plus TRUS-estimated prostate volume (model D) to the kallikrein panel (model A) did slightly increase discrimination for any cancer, although this was not significant in the Göteborg cohort. Results were similar for predicting high grade cancer.

Our secondary aim was to quantify the relationship between TRUS-estimated prostate volume and the kallikrein panel as well as the individual isoforms (Table 4). The full panel showed a correlation of 0.60 in Rotterdam and 0.57 in Göteborg. The correlations with TRUS-estimated prostate volume varied from 0.19 to 0.55 for individual isoforms. Nicked PSA showed this highest correlation with TRUS-estimated prostate volume; 0.55 in Rotterdam and 0.41 in Göteborg.

DISCUSSION

In the present study, we applied our previously developed statistical method, useful in predicting biopsy outcome and based on four kallikreins [12, 13], and studied how information about prostate volume changed the predictive accuracy of the model. In summary, our data suggest that one single blood draw sent for kallikrein panel analysis could replace TRUS-estimated prostate volume because of its similar discriminatory ability for any and high grade cancer. DRE and TRUS did increase the discrimination added to the four kallikrein panel but the effect size was small.

Sensitivity analyses restricted to the Rotterdam cohort of men with PSA > 4 ng/mL resulted in no major changes in the differences in AUCs as compared to the analyses presented in Table 3 (data not shown). Neither did analyses restricted to men within the PSA range 3–10 ng/mL in both Rotterdam and Göteborg (data not shown). Thus, the interpretation and conclusion of the present study were not affected by these additional analyses.

Given that DRE has relatively high patient acceptability, it may still be a useful tool in clinical screening practice; conversely, the more invasive TRUS-estimated prostate volume would not appear to be indicated given its limited benefit; however, whether a clinical approach can be replaced by laboratory analyses, or used in combination with decision models (nomograms), is a clinical judgment varying from clinician to clinician depending on how they weigh the different advantages and disadvantages (harms, cost, time, invasiveness) of both approaches.

Prostate volume combined with serum PSA level, or PSA density, has been associated with prostate cancer risk in many studies [3–5]. Although the volume measurement by TRUS is subject to intra-observer variation, it is an important tool in patient management [25].

Although TRUS is acceptable to most men [9], some men find TRUS uncomfortable. The disadvantage of DRE and TRUS is also that, if the initial blood draw was to be performed outside a urology clinic, for example during a GP appointment, a man would then need an additional clinical examination performed by a urologist. Sending the blood for further analyses of the kallikrein panel may be beneficial and cost-effective[12] as compared with scheduling several clinical visits for example.

Roobol et al. [7] suggested that TRUS-based volume measurement could be replaced by DRE-based estimation of the volume in the prostate cancer risk calculator without a significant change in predictive accuracy. By replacing the prostate volume measurement with immunoassays done from a blood sample, the clinical practice of estimating a patient's cancer risk in a screening setting would, in theory, be even more simplified.

There was a moderately high correlation between the kallikrein panel and volume (Spearman's correlation coefficient 0.60 in Rotterdam and 0.57 in Göteborg). Nicked PSA was the single isoform with the highest independent correlation (0.55) in Rotterdam, and free PSA in Göteborg (0.51). This is consistent with previous findings that free PSA and nicked PSA were the most important predictors for prostate transition zone volume, which is enlarged in BPH [17]. The switch to using the more precise fPSA-I assay may have affected the differences seen between the Rotterdam and Göteborg cohorts. To study the independent role of nicked PSA in the present model, a direct immunoassay for nicked PSA is needed instead of the calculated value. A previously published nicked PSA immunoassay needs improvements to suit routine clinical use [26].

The cancer-associated intact PSA was not expected to correlate strongly with the volume itself [15, 16]. Our intact PSA assay also measures different proPSA forms [27] of which [-2]proPSA has been included in the prostate health index, *phi* [28]. [-2]proPSA and its derivatives have been suggested to aid predicting prostate cancer pathology [29].

A possible limitation of the present study is the fact that fresh samples were not available and the kallikreins were measured from the frozen and re-thawed samples. The freezing and thawing cycles as well as the storage conditions of the sample are known to affect the stability of the free PSA in serum [30] and may thus affect the predictive accuracy of the model. We cannot exclude this despite the recent finding that [-2]proPSA was not affected by two freezing and thawing cycles [31]; however, any effects of freezing and thawing on the stability of the blood markers would be to reduce predictive value.

An additional limitation is that the panel is not currently widely available in clinical practice, and another is that sextant biopsies were the standard in this screening trial during the first round, whereas 10–12 core biopsies or more are recommended today [32], which may alter the applicability of our findings to contemporary patient series.

In conclusion, a statistical model using only the measurements of a panel of blood markers predicts the outcome of prostate biopsy equally well when compared with a model including DRE and TRUS-estimated prostate volume. As such, non-invasive blood measures may replace invasive clinical tests as part of the screening procedures evaluating the indication for biopsy.

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Abbreviations

ERSPC	European Randomized Study of Screening for Prostate Cancer
hK2	kallikrein-related peptidase 2
AUC	area under the receiver–operator characteristic curve

What is known on the subject?

Previous studies have shown that a statistical model based on a panel of kallikrein markers in blood (total, free and intact PSA and kallikrein-related peptidase 2) can predict prostate cancer on biopsy.

What does the study add?

The current study explores the relationship between the above-mentioned panel and prostate volume, and whether this panel could be an alternative for clinical measures such as DRE and TRUS in predicting prostate cancer on biopsy.

Table 1

A. Clinical characteristics: Rotterdam cohort				
	Training set, N=728		Validation set N=2186	
	No cancer, n=526	Cancer, n=202	No cancer, n=1581	Cancer, n=605
Median (IQR) age	67 (62, 70)	67 (62, 70)	66 (62, 70)	67 (63, 71)
Abnormal DRE, n (%)	94 (18)	91 (45)	299 (19)	281 (46)
Median (IQR) volume, cc	48 (37, 62)	39 (30, 52)	48 (38, 63)	38 (30, 51)
Median (IQR) total PSA, ng/mL	4.8 (3.9, 6.4)	6.5 (4.4, 11.5)	4.8 (3.8, 6.4)	6.1 (4.3, 10.5)
Median (IQR) free PSA, ng/mL	1.0 (0.74, 1.45)	1.1 (0.7, 1.52)	1.1 (0.77, 1.46)	1.0 (0.69, 1.52)
Median (IQR) intact PSA, ng/mL	0.49 (0.34, 0.66)	0.56 (0.38, 0.85)	0.49 (0.35, 0.68)	0.53 (0.38, 0.83)
Median (IQR) nicked PSA, ng/mL	0.53 (0.37, 0.76)	0.44 (0.27, 0.71)	0.54 (0.38, 0.80)	0.43 (0.27, 0.73)
Median (IQR) hK2, ng/mL	0.067 (0.044, 0.092)	0.085 (0.055, 0.13)	0.066 (0.046, 0.096)	0.083 (0.057, 0.122)
Biopsy Gleason score, n (%)				
6		128 (63)		384 (63)
7		57 (28)		178 (29)
8		15 (7)		38 (6)
Missing*		2 (1)		5 (2)

B. Clinical characteristics: Göteborg cohort				
	Training set, N=494		Validation set, N=246	
	No cancer n=366	Cancer n=128	No cancer n=182	Cancer n=64
Median (IQR) age	61 (58, 64)	62 (59, 64)	61 (57, 64)	61 (57, 64)
Abnormal DRE, n (%)	32 (9)	46 (36)	18 (10)	30 (47)
Median (IQR) volume, cc	43 (32, 54)*	32 (27, 40) [†]	43 (34, 52) [‡]	32 (24, 40) [‡]
Median (IQR) total PSA, ng/mL	4.2 (3.4, 5.6)	6.1 (4.1, 11.2)	4.2 (3.4, 5.6)	5.4 (3.9, 8.8)
Median (IQR) free PSA, ng/mL	0.84 (0.64, 1.25)	0.86 (0.61, 1.39)	0.92 (0.70, 1.27)	0.82 (0.55, 1.28)
Median (IQR) intact PSA, ng/mL	0.36 (0.27, 0.52)	0.47 (0.28, 0.80)	0.39 (0.30, 0.52)	0.41 (0.29, 0.68)
Median (IQR) nicked PSA, ng/mL	0.35 (0.24, 0.56)	0.41 (0.24, 0.65)	0.39 (0.27, 0.61)	0.33 (0.25, 0.55)
hK2, ng/mL	0.045 (0.03, 0.069)	0.076 (0.048, 0.136)	0.047 (0.03, 0.067)	0.075 (0.052, 0.11)
Biopsy Gleason score, n (%)				
6		101 (79)		51 (80)
7		24 (19)		9 (14)
8		3 (2)		4 (6)

IQR, interquartile range.

* Missing Gleason score: not available for seven participants diagnosed with cancer before Gleason grading was used (one participant in 1993 and six participants in 1994). Anderson grade was available for these participants: in the training set, two patients were grade 2; in the validation set, two patients were grade 1, and three patients were grade 2.

IQR, interquartile range.

* Three missing;

[†] one missing;

[‡] two missing.

Table 2

Predictive accuracy of models built on the training set and applied to the validation set

Model	Rotterdam		Göteborg	
	AUC Any cancer	AUC High grade cancer	AUC Any cancer	AUC High grade cancer
A: Kallikrein panel (age, PSA, intact PSA, free PSA, hk2)	0.766	0.847	0.809	0.786
B: Clinical model (age, PSA, DRE, TRUS-estimated prostate volume)	0.763	0.846	0.774	0.681
C: Full laboratory model: Kallikrein panel (age, PSA, intact PSA, free PSA, hk2) + DRE	0.778	0.856	0.810	0.802
D: Laboratory plus clinical model: Kallikrein panel (age, PSA, intact PSA, free PSA, hk2) + DRE + TRUS- estimated prostate volume	0.792	0.860	0.826	0.802

Table 3

Comparison of the difference in predictive accuracy between the models

Comparison	Rotterdam		Göteborg	
	Any cancer	High grade cancer	Any cancer	High grade cancer
A vs B	0.003 ($P=0.8$)	0.001 ($P=1$)	0.035 ($P=0.3$)	0.105 ($P=0.2$)
C vs A	0.012 ($P=0.033$)	0.009 ($P=0.17$)	0.001 ($P=0.9$)	0.016 ($P=0.3$)
C vs B	0.015 ($P=0.15$)	0.010 ($P=0.075$)	0.036 ($P=0.3$)	0.121 ($P=0.3$)
D vs C	0.014 ($P=0.002$)	0.004 ($P=0.2$)	0.016 ($P=0.14$)	0.000 ($P=0.9$)

Data are given as difference in AUCs with P values for the difference.

Table 4

Correlation between TRUS-estimated prostate volume and kallikrein isoforms

	Rotterdam	Göteborg
Measure	Spearman's correlation*	Spearman's correlation*
Kallikrein panel	0.60	0.57
hK2	0.33	0.19
Nicked PSA	0.55	0.41
Intact PSA	0.44	0.36
Total PSA	0.21	0.15
Free PSA	0.53	0.51
Free to total PSA ratio	0.43	0.48
Nicked to total PSA ratio	0.46	0.39

* All correlations were statistically significant, $P < 0.05$; analyses performed on the total cohorts.