



# LUND UNIVERSITY

## Improving the Specificity of Screening for Lethal Prostate Cancer Using Prostate-specific Antigen and a Panel of Kallikrein Markers: A Nested Case-Control Study.

Stattin, Pär; Vickers, Andrew J; Sjoberg, Daniel D; Johansson, Robert; Granfors, Torvald; Johansson, Mattias; Pettersson, Kim; Scardino, Peter T; Hallmans, Göran; Lilja, Hans

Published in:  
European Urology

DOI:  
[10.1016/j.eururo.2015.01.009](https://doi.org/10.1016/j.eururo.2015.01.009)

2015

[Link to publication](#)

### Citation for published version (APA):

Stattin, P., Vickers, A. J., Sjoberg, D. D., Johansson, R., Granfors, T., Johansson, M., Pettersson, K., Scardino, P. T., Hallmans, G., & Lilja, H. (2015). Improving the Specificity of Screening for Lethal Prostate Cancer Using Prostate-specific Antigen and a Panel of Kallikrein Markers: A Nested Case-Control Study. *European Urology*, 68(2), 207-213. <https://doi.org/10.1016/j.eururo.2015.01.009>

Total number of authors:  
10

### General rights

Unless other specific re-use rights are stated the following general rights apply:  
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117  
221 00 Lund  
+46 46-222 00 00

## Platinum Priority – Prostate Cancer

Editorial by Thorsten Schlomm on pp. 214–215 of this issue

# Improving the Specificity of Screening for Lethal Prostate Cancer Using Prostate-specific Antigen and a Panel of Kallikrein Markers: A Nested Case–Control Study

Pär Stattin<sup>a</sup>, Andrew J. Vickers<sup>b</sup>, Daniel D. Sjoberg<sup>b</sup>, Robert Johansson<sup>c</sup>, Torvald Granfors<sup>d</sup>, Mattias Johansson<sup>e</sup>, Kim Pettersson<sup>f</sup>, Peter T. Scardino<sup>g</sup>, Göran Hallmans<sup>h</sup>, Hans Lilja<sup>g,i,j,k,\*</sup>

<sup>a</sup> Department of Surgical and Perioperative Sciences, Urology and Andrology, Umeå University, Umeå, Sweden; <sup>b</sup> Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY, USA; <sup>c</sup> Regional Cancer Centre, Department of Radiation Sciences, Oncology, Umeå University, Umeå, Sweden; <sup>d</sup> Department of Urology, Sankt Görans Hospital, Stockholm, Sweden; <sup>e</sup> Section of Genetics, The International Agency for Research on Cancer, Lyon, France; <sup>f</sup> Division of Biotechnology, University of Turku, Turku, Finland; <sup>g</sup> Department of Surgery (Urology), Memorial Sloan Kettering Cancer Center, New York, NY, USA; <sup>h</sup> Department of Public Health and Clinical Medicine, Nutritional Research, Umeå University, Umeå, Sweden; <sup>i</sup> Departments of Laboratory Medicine and Medicine (Genitourinary Oncology), Memorial Sloan Kettering Cancer Center, New York, NY, USA; <sup>j</sup> Nuffield Department of Surgical Sciences, University of Oxford, John Radcliffe Hospital, Headington, Oxford, UK; <sup>k</sup> Department of Translational Medicine, Lund University, Skåne University Hospital, Malmö, Sweden

## Article info

### Article history:

Accepted January 9, 2015

### Keywords:

Prostate cancer  
Cancer metastasis  
Prostate-specific antigen  
Kallikrein-related peptidases  
Prostate biopsy

## Abstract

**Background:** A disadvantage of prostate-specific antigen (PSA) for the early detection of prostate cancer (PCa) is that many men must be screened, biopsied, and diagnosed to prevent one death.

**Objective:** To increase the specificity of screening for lethal PCa at an early stage.

**Design, setting, and participants:** We conducted a case–control study nested within a population-based cohort. PSA and three additional kallikreins were measured in cryopreserved blood from a population-based cohort in Västerbotten, Sweden. Of 40 379 men providing blood at ages 40, 50, and 60 yr from 1986 to 2009, 12 542 men were followed for >15 yr. From this cohort, the Swedish Cancer Registry identified 1423 incident PCa cases, 235 with distant metastasis.

**Outcome measurements and statistical analysis:** Risk of distant metastasis for different PSA levels and a prespecified statistical model based on the four kallikrein markers.

**Results and limitations:** Most metastatic cases occurred in men with PSA in the top quartile at age 50 yr (69%) or 60 yr (74%), whereas 20-yr risk of metastasis for men with PSA below median was low ( $\leq 0.6\%$ ). Among men with PSA >2 ng/ml, a prespecified model based on four kallikrein markers significantly enhanced the prediction of metastasis compared with PSA alone. About half of all men with PSA >2 ng/ml were defined as low risk by this model and had a  $\leq 1\%$  15-yr risk of metastasis.

**Conclusions:** Screening at ages 50–60 yr should focus on men with PSA in the top quartile. A marker panel can aid biopsy decision making.

**Patient summary:** For men in their fifties, screening should focus on those in the top 10% to 25% of PSA values because the majority of subsequent cases of distant metastasis are found among these men. Testing of four kallikrein markers in men with an elevated PSA could aid biopsy decision making.

© 2015 European Association of Urology. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

\* Corresponding author. Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA. Tel. +1 212 639 6982; Fax: +1 646 422 2379.  
E-mail address: [liljah@mskcc.org](mailto:liljah@mskcc.org) (H. Lilja).

## 1. Introduction

Recent evidence from randomized trials provides clear evidence that screening for prostate cancer (PCa) by testing serum levels of prostate-specific antigen (PSA) reduces cancer-specific mortality in men who would not otherwise be screened [1,2]. However, PSA is not specific to lethal PCa. As a result, many men need to be screened, biopsied, and diagnosed to prevent one death. One estimate is that 781 men need to be screened and 27 diagnosed per PCa death avoided at 13 yr [1].

One way to change the harm-to-benefit ratio would be to screen for cancers that are destined to become lethal. Metastatic PCa is associated with severe disease morbidity and a very high risk of PCa-specific death. We tested the hypothesis that PSA and a panel of PSA-related markers could predict the long-term risk of metastatic PCa in a large representative population-based longitudinal study of men providing cryopreserved blood at ages 40, 50, and 60 yr from 1986 to 2009.

## 2. Patients and methods

### 2.1. Study population

The Västerbotten Intervention Project (VIP) [3] is an ongoing population-based cohort study initiated in 1986 in which residents of Västerbotten County, Sweden, are invited to receive a health examination at ages 40, 50, and 60 yr with blood drawn for cryopreservation. By January 2009, VIP included data on 40 379 unique men with 50 557 blood draws, representing >57% of the total background population [4]. Initially the rate of PSA testing in this population was low, but it has increased over recent years: The proportion of PCa cases for which opportunistic screening was the cause for work-up leading to diagnosis increased from 9% in 2000 to 26% in 2005 and to 38% in 2011 [5]. However, these recent changes are likely to have little impact on metastasis rates in our current cohort due to the long lead time between diagnosis and metastasis.

### 2.2. Case identification and outcomes

In January 2009, the VIP cohort was linked to the Northern Sweden Regional Cancer Registry, part of the Swedish Cancer Registry, using the Swedish personal identity number. We identified 1423 incident PCa cases in the VIP cohort, 1377 of which had cryopreserved blood available for analysis. Clinical data were retrieved from the National Prostate Cancer Register. We reviewed hospital medical charts of men diagnosed with cancer to identify men who later had documented evidence of metastatic disease (ie, a positive bone scan) during the follow-up period. There were 126 patients with metastatic PCa diagnosed during follow-up who subsequently died from PCa, according to the Cause of Death Registry. Cause of death was assessed by medical chart review or, when charts were not available ( $n = 4$ ), the Swedish Cause of Death Registry. An additional 12 men who died of PCa but who had not had metastases diagnosed prior to death were considered to have had metastatic disease at the date of death.

### 2.3. Control selection

Separate case-control matches were conducted for each end point: PCa diagnosis, PCa metastases, and PCa-specific death. There were separate analyses for men aged 40, 50, and 60 yr at baseline. For each relevant end point, we randomly selected three controls who were alive and event

free at the date of the pertinent event for the index case, were within 3 mo of age of the index case, and had provided a blood sample within 3 mo of the blood draw for the index case. For the end point of PCa-specific death, all cases were matched successfully to three controls; for PCa metastases and PCa diagnosis, we expanded the window in 1-mo increments to 12 mo until three controls were identified.

All participants gave written informed consent at the time of recruitment, and the project was approved by the research ethics board at Umeå University (research authorization number 2009-1436-31).

### 2.4. Laboratory methods

We measured four kallikrein (KLK) markers—human kallikrein-related peptidase 2 (hK2) and total, free, and intact PSA—in cryopreserved blood samples from cases and controls. All laboratory analyses were conducted blind to outcome and case-control status. We measured total and free PSA with the dual-label DELFIA ProStatus assay (PerkinElmer, Turku, Finland) [6], calibrated against the World Health Organization (WHO) 96/670 (PSA-WHO) and WHO 68/668 (free PSA-WHO) standards, in previously unthawed cryopreserved heparin anticoagulated blood plasma. Intact PSA and hK2 were measured using F(ab')<sub>2</sub> fragments of the monoclonal capture antibodies to reduce the frequency of nonspecific assay interference [7].

### 2.5. Statistical methods

To estimate absolute risk, we used predictive mean matching to impute marker levels for men not selected as controls and for 16 men (1 man aged 50 yr and 15 men aged 60 yr) with metastasis who had missing samples. Statistical analyses were performed on the population level utilizing the measured and imputed values combined across 10 imputations using the Rubin rules. The four KLK markers were combined as previously described [8] into a statistical risk prediction model that gives

**Table 1 – Participant characteristics in the Västerbotten Intervention Project\***

	Age 40 yr <sup>†</sup> ( $n = 17\ 086$ )	Age 50 yr <sup>†</sup> ( $n = 17\ 837$ )	Age 60 yr <sup>†</sup> ( $n = 15\ 634$ )
PSA, ng/ml, at ages 40, 50, and 60 yr	Median (25th, 75th percentile)		
Subsequent prostate cancer diagnosis	$n = 77^{\ddagger}$ 1.3 (0.9, 2.1)	$n = 399^{\ddagger}$ 2.0 (1.3, 3.3)	$n = 947^{\ddagger}$ 3.6 (2.0, 6.0)
Subsequent distant metastasis	$n = 10^{\ddagger}$ 1.1 (0.7, 3.1)	$n = 52^{\ddagger}$ 1.7 (1.2, 3.4)	$n = 173^{\ddagger}$ 4.5 (2.1, 9.8)
Controls with PSA measurement	$n = 228$ 0.7 (0.5, 0.9)	$n = 1157$ 0.8 (0.6, 1.2)	$n = 2598$ 1.1 (0.7, 2.0)
Controls with imputed PSA measurement	$n = 16\ 781$ 0.7 (0.5, 0.9)	$n = 16\ 281$ 0.8 (0.6, 1.2)	$n = 12\ 089$ 1.1 (0.7, 2.0)
	No. of men		
Men at risk <sup>§</sup>			
10 yr	9172	9100	6725
15 yr	5115	4339	3088
20 yr	1117	645	422

PSA = prostate-specific antigen.

\* A total of 12 men died from prostate cancer without documented metastasis. These were recorded as having metastasis on the date of death.

<sup>†</sup> Age at baseline showing the number of men providing blood samples.

<sup>‡</sup> Number of men providing blood at ages 40, 50, and 60 yr later diagnosed with prostate cancer and with documented evidence of distant metastases.

<sup>§</sup> Number of men providing blood at ages 40, 50, and 60 followed for 10, 15, and 20 yr.

the risk of any grade (or Gleason score  $\geq 7$ ) cancer at prostate biopsy. However, because the current study used anticoagulated blood plasma rather than serum, the model was adjusted using biopsy data from the UK Prostate Testing for Cancer and Treatment ( ProtecT ) study [9] that used blood plasma samples anticoagulated with ethylenediaminetetraacetic acid to predict Gleason score  $\geq 7$  (high-grade) cancer at 10-core prostate biopsy. The model was completed before it was applied to the VIP cohort [10], making the current study an external validation of a prespecified model. All analyses were conducted in Stata v.12 software (StataCorp, College Station, TX, USA).

### 3. Results

Table 1 shows the number of men in the VIP cohort who were aged 40, 50, and 60 yr at the time of the blood draw and PSA concentrations at those ages in men subsequently diagnosed with PCa and in men with documented evidence of distant metastases and their matched controls. It also shows the number of living men at risk of a PCa diagnosis or metastatic PCa at different durations of follow-up and the number of men with documented metastases. The VIP cohort included 12 542 men followed for >15 yr, 1423 men diagnosed with incident PCa, and 235 cases with documented evidence of distant PCa metastasis. Details on patient and tumor characteristics (including PSA level at time of diagnosis) are described in Supplementary Table 1. PSA concentrations at ages 40, 50, and 60 yr in controls were similar to previous population estimates in Sweden [11,12], Ireland [13], and the United States [14–16].

There were very few metastatic cases ( $n = 10$ ) in men aged 40 yr at blood collection; further analyses are not presented in the primary text of this paper (Supplementary Table 2). For the older cohorts, as shown in Figure 1 and Table 2, there was a strong association between 15- to 20-yr risk of metastases and PSA, with a C-index of 0.816 (95% confidence interval [CI], 0.718–0.889) for PSA at age 50 and

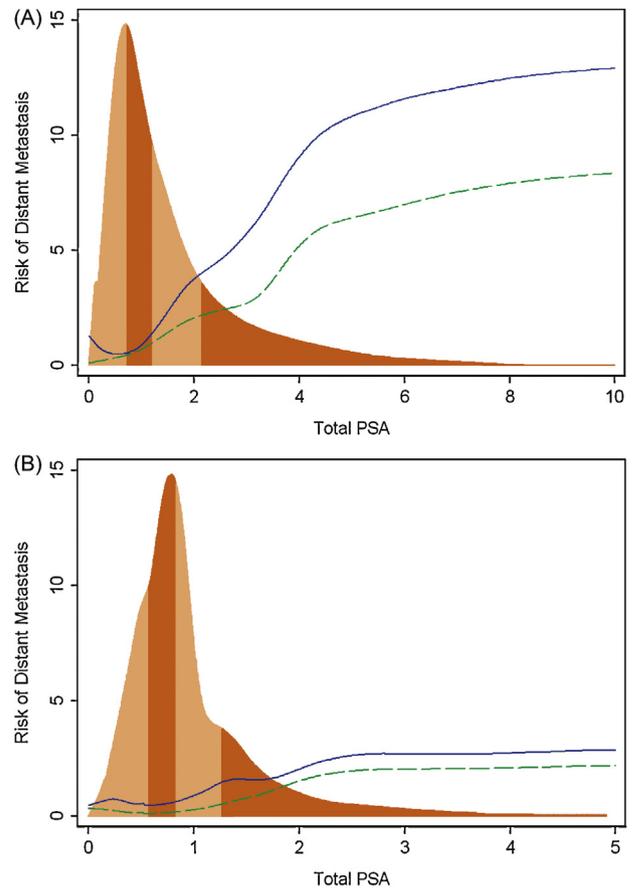


Fig. 1 – Risk of distant metastasis within 15 yr (dashed line) and 20 yr (solid line) by prostate-specific antigen (PSA). The four areas depicted in dark and light orange reflect the four quartiles of the population distribution of PSA levels. (A) Age 60 yr; (B) age 50 yr. PSA = prostate-specific antigen.

Table 2 – Risk of distant prostate cancer metastases by 15 and 20 yr of follow-up

	PSA level, ng/ml	Absolute risk of metastases (95% CI)		Cumulative proportion of metastases, % (95% CI)
		15-yr risk	20-yr risk	
<b>Age 50 yr</b>				
Top decile	>1.9	2.40 (1.47–3.70)	3.15 (1.94–4.82)	48 (34–61)
Top quartile	>1.3	1.48 (0.99–2.12)	2.14 (1.40–3.14)	69 (57–82)
Second quartile	0.8–1.3	0.25 (0.08–0.67)	0.89 (0.33–2.03)	85 (75–95)
Third quartile	0.6–0.8	0.13 (0.02–0.54)	0.56 (0.12–1.89)	92 (85–100)
Bottom quartile	<0.6	0.15 (0.03–0.47)	0.48 (0.07–1.98)	100
Below 63rd centile	<1.0	0.14 (0.05–0.31)	0.55 (0.23–1.17)	
Below median	<0.8	0.14 (0.05–0.35)	0.53 (0.18–1.34)	–
Overall		0.52 (0.37–0.72)	1.06 (0.72–1.51)	–
<b>Age 60 yr</b>				
Top decile	>3.8	9.33 (7.26–11.71)	14.29 (10.73–18.35)	56 (48–64)
Top quartile	>2.1	5.28 (4.25–6.46)	8.84 (6.97–10.99)	74 (67–81)
Second quartile	1.2–2.1	1.55 (0.95–2.41)	2.56 (1.53–4.02)	91 (86–95)
Third quartile	0.7–1.2	0.59 (0.27–1.18)	0.76 (0.34–1.52)	97 (95–100)
Bottom quartile	<0.7	0.26 (0.07–0.81)	0.48 (0.12–1.39)	100
Below median	<1.2	0.44 (0.23–0.79)	0.63 (0.32–1.14)	–
Below 41st centile	<1.0	0.43 (0.21–0.83)	0.56 (0.26–1.10)	–
Overall		2.00 (1.66–2.39)	3.31 (2.71–4.00)	–

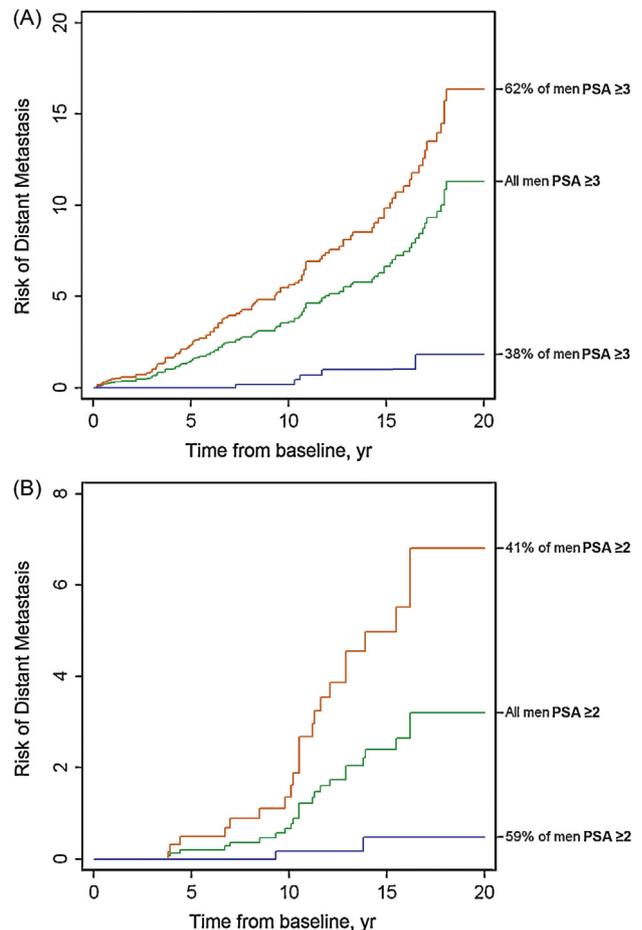
PSA = prostate-specific antigen.

\* Cumulative proportions are given by cumulative quantile. For example, 85% of documented metastases occurred in men who had PSA in the top or second quartile at age 50 yr; 56% of metastases occurred in men in the top decile of PSA levels at age 60 yr.

0.847 (95% CI, 0.807–0.886) for PSA at age 60 (Supplementary Table 3). Most cases with metastasis were found among men whose PSA was above median for a decade or more before metastases were diagnosed (Supplementary Fig. 1; Supplementary Table 4). PSA measured at age 50 or 60 provided a wide separation of risk, with a 4- to 14-fold higher relative risk of metastases in men with PSA concentrations in the top quartile compared with those below the median (Table 2). As shown in Table 2, 69% of metastatic cases were documented in men with PSA in the top quartile at age 50 ( $>1.3$  ng/ml); 74% of metastatic cases were documented in men with PSA in the top quartile at age 60 ( $>2.1$  ng/ml). However, PSA levels below the median at ages 50–60 yr were associated with a very low risk of distant metastases. The 20-yr risk of metastasis for men with PSA  $\leq 1.0$  ng/ml at age 60 (close to the median) was  $<0.6\%$  (Table 2). Despite the substantially elevated long-term risk of distant metastases associated with a PSA concentration in the top quartile or decile at age 50 or 60, the vast majority of these men remained metastasis free at 15–20 yr (Fig. 1; Supplementary Table 2 and 4).

We then examined whether a prespecified statistical model based on four KLK markers measured in the ProtecT study participants and developed to predict the risk of high-grade cancer at prostate biopsy [10] also could predict distant metastasis occurring 10–20 yr later (Supplementary Table 3). Although the model did not enhance discrimination of PSA in all men, it did so in men with PSA above the median at age 50 or 60. For instance, among men with PSA  $>2$  ng/ml at age 50, discrimination was 0.751 for total PSA alone compared with 0.863 for the model (increase of 0.112; 95% CI, 0.020–0.219); for men with PSA  $>2$  ng/ml at age 60, discrimination increased from 0.805 to 0.875 (0.070; 95% CI, 0.040–0.097).

Given these results, we conducted a decision analysis evaluating the hypothetical clinical outcomes had the KLK marker-based model data been used to aid decisions about biopsy in the VIP cohort. Biopsying all men with PSA  $\geq 3.0$  ng/ml, the approach used in the randomized trial supporting PSA screening [1,2] would have resulted in a biopsy performed on 15.6% of men aged 60 yr. This rate would have been reduced by 38% (Fig. 2A) if biopsy was recommended only to those with  $\geq 7.5\%$  risk of high-grade cancer according to the model (Table 3; Supplementary Table 5). Among men aged 60 yr with PSA  $\geq 3$  ng/ml and  $<7.5\%$  risk according to the model, the 15-yr risk of distant metastases remained low (0.99%) compared with the population average (2%). For men with PSA  $\geq 2$  ng/ml at age 50 (top decile of PSA levels at age 50), using  $\geq 5\%$  risk according to the model as the criterion for biopsy would lead to only 41% of these men undergoing a biopsy (Fig. 2B). Among all men aged 50 yr with PSA  $\geq 2$  ng/ml, those with  $<5\%$  risk according to the model had a very low 15-yr risk ( $\leq 0.5\%$ ) of distant metastasis (Table 3). Thus a substantial proportion of men with modestly increased PSA but low KLK risk scores identified by our screening strategy would be excluded from biopsy, and for these men, there is little risk of missing a lethal cancer even in the absence of frequent (e.g. biennial) follow-up.



**Fig. 2** – Risk of distant metastasis among men with elevated prostate-specific antigen (PSA) by four kallikrein (KLK) panel score. (A) Age 60 yr and PSA  $\geq 3$  ng/ml ( $n = 2432$ ). Green line: overall risk of distant metastasis; orange line: risk of distant metastasis among men with four KLK panel score  $\geq 7.5\%$  (62% of the men); blue line: risk of distant metastasis among men with four KLK panel score  $<7.5\%$  (38% of the men). (B) Age 50 yr and PSA  $\geq 2$  ng/ml ( $n = 1692$ ). Green line: overall risk of distant metastasis; orange line: risk of distant metastasis among men with four KLK panel score  $\geq 5\%$  (41% of the men); blue line: risk of distant metastasis among men with four KLK panel score  $<5\%$  (59% of the men). PSA = prostate-specific antigen.

#### 4. Discussion

In this large representative cohort from Sweden, with  $>12$  500 men followed for  $>15$  yr and initially low rates of opportunistic PSA testing, PSA measured in cryopreserved blood collected at age 50 or 60 predicted metastasis at 15- to 20-yr follow-up. In the subset of men with modestly elevated PSA, a prespecified model based on a panel of four KLK markers increased the predictive discrimination of metastasis.

Risk stratification contributed by PSA was far greater than that reported for other risk factors such as race or family history [17]. Among men with modestly elevated PSA at age 50 or 60, the four KLK panel yielded C-indexes from 0.82 to 0.88 for the prediction of documented distant metastasis. This can be compared with discrimination close

**Table 3 – Risk of distant metastasis by prostate-specific antigen and kallikrein risk\***

PSA group	n (%)	10 yr (95% CI)	15 yr (95% CI)	20 yr (95% CI)	Metastases by 10 yr per 10 000 men	Metastases by 20 yr per 10 000 men
<b>Age 50</b>						
PSA $\geq$ 0 ng/ml	17 837	0.09 (0.05–0.17)	0.52 (0.37–0.72)	1.06 (0.72–1.51)	9	106
PSA $\geq$ 2 ng/ml	1692	0.67 (0.30–1.36)	2.41 (1.46–3.73)	3.20 (1.96–4.93)	6	30
KLK risk $\geq$ 5%	696 (41)	1.37 (0.56–2.88)	4.97 (2.91–7.83)	6.80 (3.99–10.61)	5	27
<5%	996 (59)	0.18 (0.01–1.24)	0.49 (0.07–2.06)	0.49 (0.07–2.06)	1	3
KLK risk $\geq$ 7.5%	318 (19)	2.40 (0.88–5.31)	8.25 (4.41–13.62)	10.68 (5.74–17.38)	4	19
<7.5%	1374 (81)	0.26 (0.04–1.03)	0.90 (0.34–2.04)	1.25 (0.47–2.79)	2	11
KLK risk $\geq$ 10%	189 (11)	4.01 (1.41–8.83)	10.67 (5.10–18.58)	10.67 (5.10–18.58)	4	11
<10%	1502 (89)	0.23 (0.04–0.93)	1.26 (0.58–2.45)	2.17 (1.06–3.98)	2	19
PSA $\geq$ 3 ng/ml	549	1.51 (0.56–3.36)	4.66 (2.45–7.92)	6.13 (3.25–10.26)	5	19
<b>Age 60</b>						
PSA $\geq$ 0 ng/ml	15 634	0.88 (0.70–1.08)	2.00 (1.66–2.39)	3.31 (2.71–4.00)	88	331
PSA $\geq$ 2 ng/ml	4318	2.42 (1.89–3.06)	5.04 (4.06–6.17)	8.47 (6.69–10.50)	67	234
KLK risk $\geq$ 5%	3104 (72)	3.27 (2.54–4.13)	6.77 (5.44–8.29)	11.04 (8.71–13.69)	65	219
<5%	1214 (28)	0.19 (0.01–1.28)	0.38 (0.06–1.50)	1.42 (0.19–5.52)	2	15
KLK risk $\geq$ 7.5%	2005 (46)	4.81 (3.72–6.09)	9.05 (7.21–11.16)	14.17 (11.02–17.72)	62	182
<7.5%	2313 (54)	0.25 (0.06–0.76)	1.31 (0.64–2.44)	3.00 (1.48–5.40)	5	52
KLK risk $\geq$ 10%	1337 (31)	6.65 (5.09–8.47)	11.99 (9.45–14.86)	18.88 (14.51–23.69)	57	161
<10%	2982 (69)	0.42 (0.18–0.87)	1.70 (0.99–2.73)	3.22 (1.89–5.12)	10	73
PSA $\geq$ 3 ng/ml	2432	3.63 (2.77–4.65)	6.65 (5.22–8.29)	11.30 (8.65–14.35)	56	176
KLK risk $\geq$ 5%	1954 (80)	4.49 (3.43–5.74)	8.08 (6.34–10.08)	13.73 (10.50–17.38)	56	172
<5%	478 (20)	0 (NA)	0.48 (0.02–3.36)	0.48 (0.02–3.36)	0	4
KLK risk $\geq$ 7.5%	1510 (62)	5.62 (4.29–7.20)	9.85 (7.69–12.32)	16.36 (12.44–20.74)	54	158
<7.5%	922 (38)	0.18 (0.01–1.30)	0.99 (0.28–2.64)	1.82 (0.47–4.99)	2	18
KLK risk $\geq$ 10%	1180 (49)	7.01 (5.34–8.99)	12.35 (9.62–15.43)	19.88 (15.10–25.15)	53	150
<10%	1252 (51)	0.23 (0.04–0.94)	0.82 (0.27–2.01)	1.95 (0.64–4.66)	3	26

CI = confidence interval; KLK = kallikrein; PSA = prostate-specific antigen.

\* A previously developed model to predict risk of Gleason score  $\geq$ 7 (high-grade) prostate cancer at biopsy based on a panel of four KLK markers measured in the blood.

to 0.60 for the Gail model that is used clinically to determine eligibility for breast cancer chemoprevention [18].

Several long-term studies including a prospective observational study reported by Parkes et al [19] and others reviewed by Loeb et al [16] have found PSA to be highly prognostic of the long-term risk of aggressive PCa in unscreened men. The Malmö Preventive Project (MPP) [11,12] that involved blood samples taken from 1974 to 1986 with follow-up for metastasis and death through 2006 is most comparable with the current study. Although overall estimates of PCa risk are very similar between the two studies, the C-index of PSA for metastasis was somewhat lower in the current study: 0.86 at 25 yr in MPP versus 0.85 within <20 yr in VIP. This may be related to the advent of opportunistic PSA testing in Sweden. Some men with elevated PSA at age 60 destined to develop metastases would have their cancer detected early by PSA testing and be cured by early treatment.

Our results also replicated prior findings that the KLK markers measured in blood enhance the discrimination of malignant from benign PSA elevations [8,20]. Enormous efforts have been invested searching for biomarkers to be used with PSA to improve screening for PCa [21–25]. Although some markers are predictive of biopsy outcome, this is the first time markers have been shown to improve long-term prediction of distant metastasis, in this case with large increases in discrimination. These findings support the use of the KLK panel as a reflex test for biopsy in midlife among men with a modestly elevated PSA. For instance,

compared with biopsying all men with PSA  $\geq$ 3 ng/ml, biopsying men with PSA  $\geq$ 2 ng/ml and  $\geq$ 10% risk from the panel would reduce biopsy rates by 45% but detect a similar number of men who would develop distant metastases within 10 yr (56 vs 57 per 10 000 men). Men with a modest PSA elevation but a low risk of high-grade cancer according to the panel could be exempted from biopsy and reassured that they would be unlikely to develop metastatic disease, even if PSA and the other KLK markers were not monitored every year.

Our findings strongly support a risk-stratified approach to screening and biopsy. In men aged 50 yr, the 15-yr risk of metastasis among those in the top decile for PSA was 3.15%, sixfold higher than men with PSA below the median. The concentration of high-risk disease in this age group, with 48% of metastatic cases occurring in the men with PSA levels in the highest 10%, suggests that screening should focus on those men. In contrast, we were unable to identify a subgroup of men aged 40 yr at a substantially increased risk of distant metastasis within 15 yr, making it difficult to justify screening in this age group. The identification of a small subset of men with elevated PSA at ages 50–60 yr with a substantially increased risk of developing metastatic disease many years later has important implications for the development of novel preventive strategies. Use of the KLK markers as a reflex test may further refine stratification of risk.

The very low long-term risk for PCa [26,27] and metastases [11] in men with PSA <1 ng/ml was observed

in earlier studies. Our findings are consistent with prior research demonstrating that men with a low PSA at age 60 have no mortality reduction from PSA screening but are at considerable risk of overdiagnosis [28]. This supports the calls to limit screening in such men.

## 5. Conclusions

We found that blood levels of PSA at ages 50 and 60 yr are prognostic of the long-term risk of metastatic PCa and that a panel of KLK markers is strongly predictive of distant metastasis documented many years later in men with a modestly elevated PSA. Our study has the following clinical implications. First, widespread PSA testing at age 40 cannot be justified. Second, screening can stop in men with PSA below the median ( $<1$  ng/ml) at age  $\geq 60$  yr. Third, for men in their fifties, screening could focus mainly on those in the top decile of PSA ( $>1.9$  ng/ml) because close to half of the subsequent cases of distant metastasis are found in this group; men with lower PSAs should still be screened but less intensively. Finally, four KLK markers measured in the blood can be used as a reflex test to aid biopsy decisions.

**Author contributions:** Hans Lilja had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Stattin, Vickers, Lilja.

**Acquisition of data:** Stattin, Lilja, R. Johansson, Granfors, M. Johansson, Hallmans.

**Analysis and interpretation of data:** Stattin, Vickers, Sjöberg, R. Johansson, Granfors, M. Johansson, Hallmans, Lilja.

**Drafting of the manuscript:** Stattin, Lilja, Vickers.

**Critical revision of the manuscript for important intellectual content:** Stattin, Lilja, Vickers.

**Statistical analysis:** Vickers, Sjöberg.

**Obtaining funding:** Lilja, Vickers, Scardino, Stattin.

**Administrative, technical, or material support:** Lilja, Scardino, Pettersson.

**Supervision:** Stattin, Vickers, Lilja.

**Other (specify):** Scardino advised on the overall study concept and clinical interpretation.

**Financial disclosures:** Hans Lilja certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: Hans Lilja holds patents for free prostate-specific antigen, human kallikrein-related peptidase 2, and intact prostate-specific antigen assays and is named, along with Andrew J. Vickers, on a patent application for a statistical method to detect prostate cancer. The method was commercialized by OPKO. Hans Lilja, Andrew J. Vickers, Kim Pettersson, and Peter T. Scardino will receive royalties from any sales of the test.

**Funding/Support and role of the sponsor:** This work was supported in part by the National Cancer Institute at the National Institutes of Health (R33 CA127768 to Hans Lilja, R01 CA160816 to Hans Lilja and Andrew J. Vickers, P50 CA092629 to Peter R. Scardino and Andrew J. Vickers, and P30 CA008748 to Andrew J. Vickers); the Sidney Kimmel Center for Prostate and Urologic Cancers; David H. Koch through the Prostate Cancer Foundation to Peter T. Scardino, Andrew J. Vickers, and Hans Lilja; the National Institute for Health Research (NIHR) Oxford Biomedical

Research Centre Program to Hans Lilja; Swedish Cancer Society (projects no. 11-0624 and 14+0722 to Hans Lilja and 11-0471 to Pär Stattin); and Fundación Federico SA to Hans Lilja. The funding sources did not have any role in the design or conduct of the study; the collection, management, analysis, or interpretation of the data; or the preparation, review, or approval of the manuscript.

**Acknowledgments:** We thank Åsa Ågren, Västerbotten Intervention Project, Umeå, Sweden, for data management; Britt-Inger Dahlin, research nurse, Department of Urology, Umeå University Hospital (Sweden) for chart review; and the Västerbotten County (Sweden) for providing the blood samples. We also thank Gun-Britt Eriksson, Mona Hassan Al-Battat, and AnnaPeri Erlandsson for expert assistance with immunoassay measurements of the blood samples at the Wallenberg Research Laboratories at Lund University, Malmö, Sweden. All participants gave written informed consent at the time of recruitment, and the project was approved by the Research Ethics Board at Umeå University (research authorization number 2009-1436-31).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.eururo.2015.01.009>.

## References

- [1] Schroder FH, Hugosson J, Roobol MJ, et al. Screening and prostate cancer mortality: results of the European Randomised Study of Screening for Prostate Cancer (ERSPC) at 13 years of follow-up. *Lancet* 2014;384:2027–35.
- [2] Hugosson J, Carlsson S, Aus G, et al. Mortality results from the Göteborg randomised population-based prostate-cancer screening trial. *Lancet Oncol* 2010;11:725–32.
- [3] Hallmans G, Agren A, Johansson G, et al. Cardiovascular disease and diabetes in the Northern Sweden Health and Disease Study Cohort—evaluation of risk factors and their interactions. *Scand J Public Health Suppl* 2003;61:18–24.
- [4] Weinehall L, Hallgren CG, Westman G, Janlert U, Wall S. Reduction of selection bias in primary prevention of cardiovascular disease through involvement of primary health care. *Scand J Prim Health Care* 1998;16:171–6.
- [5] Pilebro B, Johansson R, Damber L, Damber JE, Stattin P. Population-based study of prostate-specific antigen testing and prostate cancer detection in clinical practice in northern Sweden. *Scand J Urol Nephrol* 2003;37:210–2.
- [6] Mitrunen K, Pettersson K, Piironen T, Bjork T, Lilja H, Lovgren T. Dual-label one-step immunoassay for simultaneous measurement of free and total prostate-specific antigen concentrations and ratios in serum. *Clin Chem* 1995;41:1115–20.
- [7] Vaisanen V, Peltola MT, Lilja H, Nurmi M, Pettersson K. Intact free prostate-specific antigen and free and total human glandular kallikrein 2. Elimination of assay interference by enzymatic digestion of antibodies to F(ab')<sub>2</sub> fragments. *Anal Chem* 2006;78:7809–15.
- [8] Vickers A, Cronin A, Roobol M, et al. Reducing unnecessary biopsy during prostate cancer screening using a four-kallikrein panel: an independent replication. *J Clin Oncol* 2010;28:2493–8.
- [9] Lane JA, Donovan JL, Davis M, et al. Active monitoring, radical prostatectomy, or radiotherapy for localised prostate cancer: study design and diagnostic and baseline results of the ProtecT randomised phase 3 trial. *Lancet Oncol* 2014;15:1109–18.
- [10] Bryant RJ, Sjöberg DD, Vickers AJ, et al. Predicting high-grade cancer at ten-core prostate biopsy using four kallikrein markers measured in blood in the ProtecT study. *J Natl Cancer Inst*. In press.

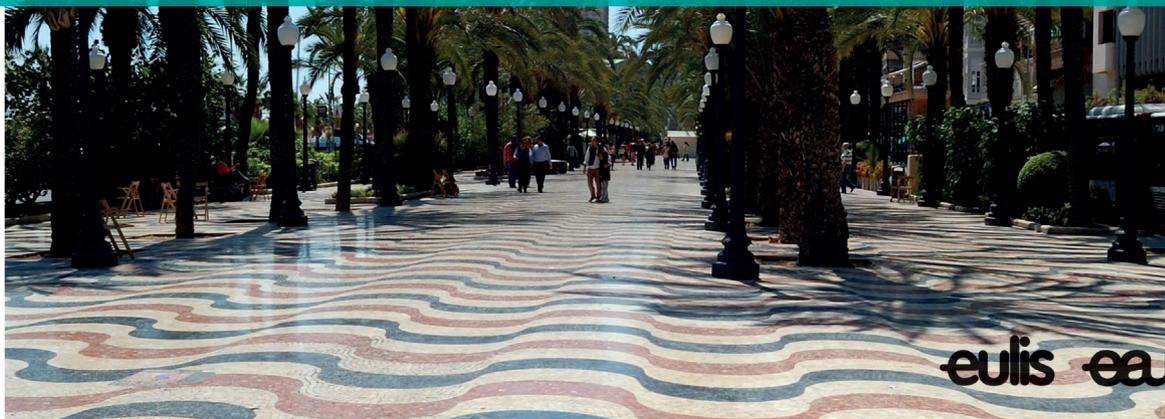
- [11] Vickers AJ, Cronin AM, Bjork T, et al. Prostate specific antigen concentration at age 60 and death or metastasis from prostate cancer: case-control study. *BMJ* 2010;341:c4521.
- [12] Vickers AJ, Ulmert D, Sjoberg DD, et al. Strategy for detection of prostate cancer based on relation between prostate specific antigen at age 40–55 and long term risk of metastasis: case-control study. *BMJ* 2013;346:f2023.
- [13] Casey RG, Hegarty PK, Conroy R, et al. The distribution of PSA age-specific profiles in healthy Irish men between 20 and 70. *ISRN Oncol* 2012;2012:832109.
- [14] Anderson JR, Strickland D, Corbin D, Byrnes JA, Zweiback E. Age-specific reference ranges for serum prostate-specific antigen. *Urology* 1995;46:54–7.
- [15] Kalish LA, McKinlay JB. Serum prostate-specific antigen levels (PSA) in men without clinical evidence of prostate cancer: age-specific reference ranges for total PSA, free PSA, and percent free PSA. *Urology* 1999;54:1022–7.
- [16] Loeb S, Carter HB, Catalona WJ, Moul JW, Schroder FH. Baseline prostate-specific antigen testing at a young age. *Eur Urol* 2012;61:1–7.
- [17] Vertosick EA, Poon BY, Vickers AJ. Relative value of race, family history and prostate specific antigen as indications for early initiation of prostate cancer screening. *J Urol* 2014;192:724–8.
- [18] Rockhill B, Spiegelman D, Byrne C, Hunter DJ, Colditz GA. Validation of the Gail et al. model of breast cancer risk prediction and implications for chemoprevention. *J Natl Cancer Inst* 2001;93:358–66.
- [19] Parkes C, Wald NJ, Murphy P, et al. Prospective observational study to assess value of prostate specific antigen as screening test for prostate cancer. *BMJ* 1995;311:1340–3.
- [20] Vickers AJ, Cronin AM, Aus G, et al. A panel of kallikrein markers can reduce unnecessary biopsy for prostate cancer: data from the European Randomized Study of Prostate Cancer Screening in Goteborg, Sweden. *BMC Med* 2008;6:19.
- [21] Bradley LA, Palomaki GE, Gutman S, Samson D, Aronson N. Comparative effectiveness review: prostate cancer antigen 3 testing for the diagnosis and management of prostate cancer. *J Urol* 2013;190:389–98.
- [22] Choudhury AD, Eeles R, Freedland SJ, et al. The role of genetic markers in the management of prostate cancer. *Eur Urol* 2012;62:577–87.
- [23] Hessels D, Schalken JA. The use of PCA3 in the diagnosis of prostate cancer. *Nat Rev Urol* 2009;6:255–61.
- [24] Johansson M, Holmstrom B, Hinchliffe SR, et al. Combining 33 genetic variants with prostate-specific antigen for prediction of prostate cancer: longitudinal study. *Int J Cancer* 2012;130:129–37.
- [25] Prensner JR, Chinnaiyan AM, Srivastava S. Systematic, evidence-based discovery of biomarkers at the NCI. *Clin Exp Metastasis* 2012;29:645–52.
- [26] Aus G, Damber JE, Khatami A, Lilja H, Stranne J, Hugosson J. Individualized screening interval for prostate cancer based on prostate-specific antigen level: results of a prospective, randomized, population-based study. *Arch Intern Med* 2005;165:1857–61.
- [27] Holmstrom B, Johansson M, Bergh A, Stenman UH, Hallmans G, Stattin P. Prostate specific antigen for early detection of prostate cancer: longitudinal study. *BMJ* 2009;339:b3537.
- [28] Carlsson S, Assel M, Sjoberg D, et al. Influence of blood prostate specific antigen levels at age 60 on benefits and harms of prostate cancer screening: population based cohort study. *BMJ* 2014;348:g2296.

[www.eulis15.org](http://www.eulis15.org)

**EULIS15**

3rd Meeting of the  
EAU Section of Urolithiasis

10-12 September 2015, Alicante, Spain



EAU Events are accredited by the EBU in compliance with the UEMS/EACME regulations

**eulis eau** European Association of Urology