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Original article

The role of the prostate cancer gene 3 urine test in addition to serum prostate-specific antigen level in prostate cancer screening among breast cancer, early-onset gene mutation carriers

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Abstract

Objective: To evaluate the additive value of the prostate cancer gene 3 (PCA3) urine test to serum prostate-specific antigen (PSA) in prostate cancer (PC) screening among breast cancer, early-onset gene (*BRCA*) mutation carriers. This study was performed among the Dutch participants of IMPACT, a large international study on the effectiveness of PSA screening among *BRCA* mutation carriers.

Materials and methods: Urinary PCA3 was measured in 191 *BRCA1* mutation carriers, 75 *BRCA2* mutation carriers, and 308 noncarriers. The physicians and participants were blinded for the results. Serum PSA level ≥3.0 ng/ml was used to indicate prostate biopsies. PCA3 was evaluated (1) as an independent indicator for prostate biopsies and (2) as an indicator for prostate biopsies among men with an elevated PSA level. PC detected up to the 2-year screening was used as gold standard as end-of-study biopsies were not performed.

Results: Overall, 23 PCs were diagnosed, 20 of which were in men who had an elevated PSA level in the initial screening round. (1) PCA3, successfully determined in 552 participants, was elevated in 188 (cutoff ≥25; 34%) or 134 (cutoff ≥35; 24%) participants, including 2 of the 3 PCs missed by PSA. PCA3 would have added 157 (≥25; 28%) or 109 (≥35; 20%) biopsy sessions to screening with PSA only. (2) Elevated PCA3 as a requirement for biopsies in addition to PSA would have saved 37 (cutoff ≥25) or 43 (cutoff ≥35) of the 68 biopsy sessions, and 7 or 11 PCs would have been missed, respectively, including multiple high-risk PCs. So far, PCA3 performed best among *BRCA2* mutation carriers, but the numbers are still small. Because PCA3 was not used to indicate prostate biopsies, its true diagnostic value cannot be calculated.

Conclusions: The results do not provide evidence for PCA3 as a useful additional indicator of prostate biopsies in *BRCA* mutation carriers, as many participants had an elevated PCA3 in the absence of PC. This must be interpreted with caution because PCA3 was not used to indicate biopsies. Many participants diagnosed with PC had low PCA3, making it invalid as a restrictive marker for prostate biopsies in

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[1] A complete list of the members of the IMPACT Steering Committee is available in Appendix A.

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men with elevated PSA levels.

Keywords: PSA; prostate cancer gene 3; PCA3; BRCA2; marker; diagnostic value

1. Introduction

Prostate cancer (PC) is the most frequently diagnosed cancer in men in the Western world [1]. Serum prostate-specific antigen (PSA) testing is the only commonly used biomarker for PC, but its low specificity has led to a consensus not to implement population-wide screening [2,3]. Possibly, the advantages of screening may outweigh the disadvantages for groups with an increased risk of PC. It has been suggested that carriers of a pathogenic mutation in one of the "breast cancer, early-onset" genes (*BRCA1* or *BRCA2*) have an increased risk of PC [4,5]. Particularly, *BRCA2* mutation carriers might present with PC at a younger age, more aggressive disease, and shorter survival [6–10]. To evaluate the effectiveness of PSA screening in *BRCA* mutation carriers, an international study was initiated, entitled "IMPACT: Identification of Men with a genetic predisposition to ProstAte Cancer: Targeted screening in BRCA1/2 mutation carriers and controls" (www.impact-study.co.uk). The results of IMPACT's first screening round have already been published. These preliminary results support PSA screening among *BRCA2* mutation carriers [11,12].

The large number of false-positive results on PSA tests, particularly in the range of 3 to 10 ng/ml, has prompted ongoing research into new (bio)markers to improve PC diagnosis. One of the promising biomarkers, prostate cancer gene 3 (*PCA3*), was discovered in 1999 as a gene that is strongly up-regulated in PC [13]. Based on the prostate-specific and cancer-associated expression of PCA3, the PROGENSA urine-based test was developed. Previous multicenter studies suggested that its specificity and sensitivity were significantly higher than those of evaluation of serum PSA levels [14–16]. PCA3 is currently used as a biomarker to determine the need for repeat biopsies when PSA level remains elevated after prostate biopsies with negative results. We aimed to determine the potential role of PCA3 in addition to PSA testing in this high-risk group by performing a substudy among the Dutch participants of IMPACT.

2. Materials and methods

Men who were eligible for IMPACT, i.e., *BRCA* mutation carriers and their relatives who were proven noncarriers, were identified and contacted by the 10 Dutch Clinical Genetic Centres. All eligible men received an invitation by mail, including a detailed patient information leaflet describing IMPACT and the Dutch substudy (IMPACT-NL). The IMPACT protocol has been described in detail elsewhere [17]. Owing to restrictions set by the Dutch Minister of Health (the Dutch "Law on Screening" requires all cancer screening projects to obtain ministerial approval), the IMPACT-NL protocol (Fig. 1) deviated from the IMPACT protocol: (1) the screening interval was once every 2 years instead of annually;

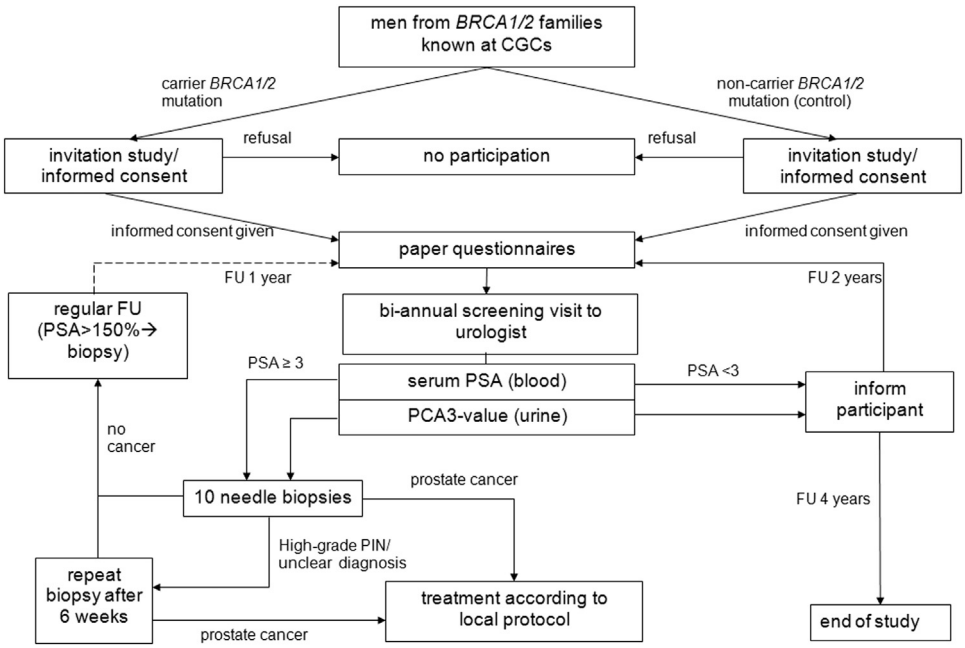

Fig. 1. The IMPACT screening protocol in the Netherlands adapted according to the ministerial approval. CGC = clinical genetic center; PIN = prostate intraepithelial neoplasia.

(2) men were eligible if they were between 45 and 69 years instead of 40 to 69 years of age; (3) only men who were not tested for PC before were eligible; and (4) end-of-study biopsies were not allowed. Informed consent was sent to the study coordinator (J.R.B.) at the Netherlands Foundation for the Detection of Hereditary Tumours.

Of the 2,181 men who were invited, 500 refused consent and 458 did not reply. Of the 1,223 men providing informed consent, 552 had undergone previous PC testing (including 42 men with PC) and were excluded (Fig. 2). Of the 671 remaining men, 634 completed a baseline questionnaire and were referred to a urologist. In IMPACT-NL, in addition to a serum sample for PSA analysis (analyzed at the screening site) all participants provided a urine sample after digital rectal examination (DRE). The urine samples were collected by the urologist at the screening site, immediately transferred to collection and transportation tubes, and sent to Novio-Gendix Servicelab (Nijmegen, the Netherlands). On arrival, the samples were frozen and stored. Samples were analyzed according to the manufacturer's protocol in regular runs together with clinical samples, using the PROGENSA PCA3 test. The initial screening round was completed by 574 participants (191 *BRCA1* mutation carriers, 75 *BRCA2* mutation carriers, and 309 noncarriers). To avoid interference with the IMPACT protocol, PCA3 scores were not used to indicate prostate biopsies. To ensure this, both urologists and participants were blinded toward the PCA3 scores.

Prostate biopsies were taken at the departments of urology of the hospitals of referral, all of which adhere to the guidelines of the Dutch Association of Urology. According to this guideline, at least 8 transrectal prostate biopsies are taken (the traditional "sextant biopsies" complemented with at least one extra biopsy on each side—preferably from the anterolateral peripheral zone). If PC was diagnosed, staging and treatment decisions were based on to the national guidelines at the discretion of the treating urologist.

If participants had an elevated PSA level and negative results on prostate biopsy, they were rescreened after 1 year (repeat screening round). Men with a normal PSA level on the initial screening were rescreened after 2 years. The results of the repeat and the 2-year screening round were also presented, as to enable the detection of PC up to and until the second screening (a 2-y period) to serve as gold standard for the diagnostic performance in the initial screening round.

The Netherlands Foundation for the Detection of Hereditary Tumours collected all data regarding PSA, prostate biopsies, PC diagnoses, staging, and initial therapy by standardized forms completed by the treating physicians. These data were used to stratify the PCs into low, intermediate, and high risk using the 2014 NICE-classification, which is identical to the d'Amico risk classification: high-risk included all PCs with lymph node or distant metastases and PC $\geq$pT2c, PSA level >20, or Gleason score ≥ 8; intermediate risk included PC without high-risk features and with pT2b, PSA 10 to 20 ng/ml, or Gleason score of 7; low risk included PC without high risk or intermediate-risk features [18,19]. If postsurgical TNM staging and Gleason score were not available, clinical stage and biopsy Gleason score were used. Data regarding carrier status and the PCA3 scores were sent directly to the study coordinator (J.R.B.), who coded and stored all data. The first author (R.C.) was provided with an anonymized version of the database to perform the data analyses.

2.1. Statistical analysis

PCA3 was evaluated (1) as a supplementary test, indicating prostate biopsies if either PSA level or PCA3 was increased or

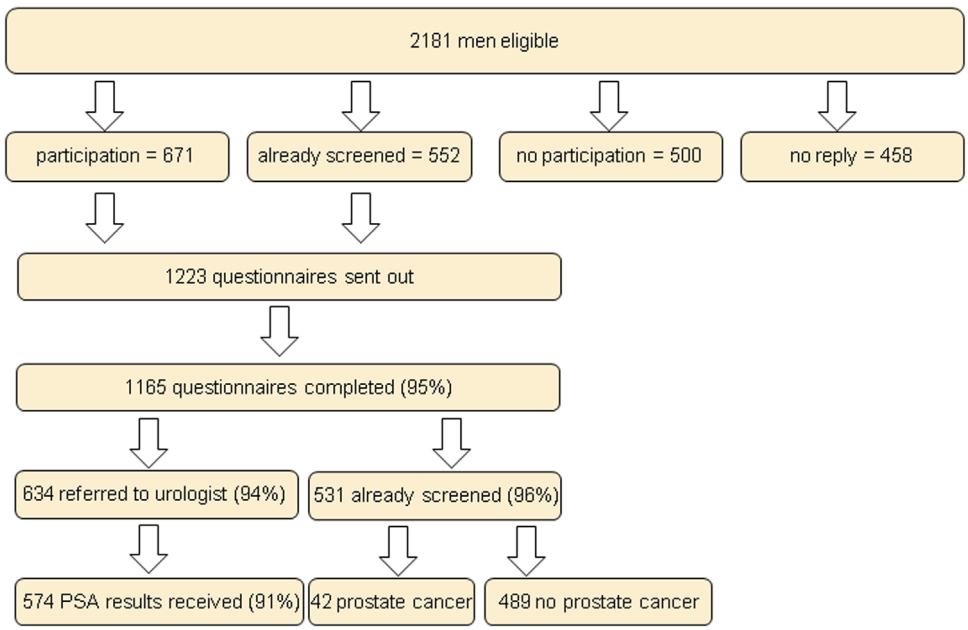

Fig. 2. Flowchart of the invitations and response rates for the Dutch part of the IMPACT study.

Table 1
Baseline characteristics of the participants, stratified by *BRCA* mutation status

	BRCA1 carriers, $n = 191$	*BRCA2* carriers, $n = 75$	Noncarriers, $n = 309$	Total, $n = 575$
Age group in years, *n* (%)				
45–49	38 (20%)	8 (11%)	44 (14%)	90 (16%)
50–59	73 (38%)	33 (44%)	139 (45%)	245 (43%)
60–69	80 (42%)	34 (45%)	126 (41%)	240 (42%)
Family history of prostate cancer				
Yes	25 (13%)	13 (17%)	51 (17%)	89 (15%)
No	99 (52%)	39 (52%)	147 (48%)	285 (50%)
Unknown	66 (35%)	23 (31%)	111 (36%)	200 (35%)
BMI (median, P5–P95)	26.5 (22.2–34.7)	26.2 (21.2–33.2)	26.5 (22.2–32.3)	
Comorbidity				
Cardiac disease	31 (17%)	15 (20%)	42 (14%)	88 (15%)
Renal disease	6 (3%)	3 (4%)	18 (6%)	27 (5%)
History of cancer	18 (10%)	13 (17%)	15 (5%)	46 (8%)
LUTS	37 (20%)	20 (27%)	69 (22%)	126 (22%)
Prostatitis	3 (2%)	2 (3%)	3 (1%)	8 (1%)
Urinary tract infections	12 (6%)	4 (5%)	14 (5%)	30 (5%)

BMI = body mass index; LUTS = lower urinary tract symptoms.

(2) as a restrictive test, indicating prostate biopsies only if PCA3 was elevated among men with an elevated PSA levels. Cutoff values ≥ 25 and ≥ 35 for PCA3 were evaluated and results were stratified by *BRCA* mutation status. We calculated detection rates as a percentage of diagnoses for all participants per *BRCA* mutation stratum and the positive predictive value (PPV) of the PSA test among the biopsied men per stratum. We calculated the Spearman rho correlation between PCA3 and PSA level and made cross-tables for the concordance between PCA3 and PSA level.

3. Results

The *BRCA* mutation carriers and the noncarriers were comparable with respect to age, BMI, and comorbidity. The *BRCA2* mutation carriers more often reported a medical history of neoplasms (Table 1), particularly nonmelanoma skin cancer (5 noncarriers, 2 *BRCA1*, and 2 *BRCA2* mutation carriers), colorectal cancer (2 noncarriers, 4 *BRCA1*, and 2 *BRCA2* mutation carriers) and breast cancer (1 noncarrier and 4 *BRCA2* mutation carriers).

In the initial screening round, PSA was elevated in 75 participants (Table 2). Additionally, 6 of these men also had abnormalities on DRE. Of the participants with an elevated PSA level, 69 underwent prostate biopsies, including all 6 men with abnormalities on DRE. In 14 of these men, PC was diagnosed (PPV 14/69 = 20%), none of whom had abnormalities on DRE. Of the men with a normal PSA level, 7 had abnormalities on DRE, 4 of whom underwent prostate biopsies. Additionally, 4 men with a normal PSA level and a normal findings on DRE underwent prostate biopsies. No PCs were found among these men. The detection rate in *BRCA2* mutation carriers was 4.0% vs. 1.6% in *BRCA1* carriers and 2.6% in noncarriers.

In the repeat screening round among 52 men with an elevated PSA level and negative findings on biopsies, 5 PCs were diagnosed.

In the 2-year screening round, 50 men had an elevated PSA level, including 4 with abnormalities on DRE. Of the, 25 men underwent prostate biopsies. Further, 4 PCs were detected in 435 screened men (78% of the 556 men at risk). One of these 4 men also had abnormalities on DRE in this round and 3 had PSA level < 3.0 ng/ml in the initial screening round. In the initial screening round, PSA level was elevated for all 13 intermediate- and high-risk PCs detected in this study (Table 3), 11 of which were detected in the first biopsy session. PSA performed best among *BRCA2* mutation carriers, i.e., in this group, all PCs were detected in the initial screening round. Using the gold standard of PC detected in the first 2 years, the diagnostic performance of PSA among *BRCA2* carriers was as follows: PPV = 25%, sensitivity = 100%, negative predictive value (NPV) = 100%, and specificity = 85%. DRE showed very poor results, with abnormalities in only 2 (1 low-risk and 1 intermediate-risk PC) of the 23 PCs.

The PCA3 score was successfully determined in 552 men (96%). The correlation between PSA level and PCA3 was 0.19 (Fig. 3). Use of PCA3 $\geq$ 25 as a supplementary marker would result in 157 additional biopsies, more than double the number of biopsies indicated by PSA alone (Table 4). Among these 157 men (28%), 2 men were diagnosed with PC during the 2-year screening round, based on an increased PSA level. A PCA3 score ≥ 35 would indicate prostate biopsies for 109 (20%) additional men, including the same 2 men with PC diagnosed after 2 years. The results for PCA3 $\geq$ 35 were best for the *BRCA2*

Table 2
Breakdown of serum PSA values and screening strategies at different time points, stratified by *BRCA* mutation status

	BRCA1 carriers	*BRCA2* carriers	Noncarriers	Total
Initial screening				
Participants with valid serum PSA level (*n*)	191	75	308	574
Serum PSA ≥3.0 ng/ml	19 (10%)	12 (16%)	44 (14%)	75 (13%)
Abnormalities on DRE	5 (3%)	3 (4%)	5 (2%)	13 (2%)
Prostate biopsies	18 (9%)	12 (16%)	47 (15%)	77 (13%)
Prostate cancer	3 (1.6%)	3 (4.0%)	8 (2.6%)	14 (2.4%)
1-year repeat screening (only for men with a known elevated PSA)				
Participants with valid serum PSA level (*n*)	17	7	28	52[a]
Serum PSA level≥3.0 ng/ml	14 (82%)	2 (29%)	19 (68%)	35 (67%)
Abnormalities on DRE	1 (6%)	0	1 (4%)	3 (6%)
Prostate biopsies	7 (41%)	0	15 (54%)	22 (42%)
Prostate cancer	2 (12%)	0	3 (11%)	5 (10%)
2-year screening				
Participants with valid serum PSA (*n*)	148 (out of 186)	62 (out of 72)	225 (out of 298)	435[a] (out of 556)
Serum PSA ≥3.0 ng/ml	18 (12%)	3 (5%)	29 (13%)	50 (12%)
Abnormalities on DRE	2 (1%)	0	6 (3%)	8 (2%)
Prostate biopsies	9 (6%)	2 (3%)	17 (8%)	28 (6%)
Prostate cancer	1 (0.7%)	0	3 (1.3%)	4 (0.9%)

[a]Reported reasons for nonparticipation in the subsequent screening rounds were mainly: personal reasons ($n = 19$), health insurance issues ($n = 28$), other illness ($n = 11$), second-year screening still to be performed ($n = 17$), and second-year screening results still to be received ($n = 20$).

mutation carriers (PPV = 13% in the absence of biopsies indicated by high PCA3; NPV = 98%), although the absolute numbers were small.

Use of PCA3 to withhold biopsies in men with an elevated PSA level would have reduced the number of biopsies in the initial screening round by more than half from 68 to 31 (Table 4). A PCA3 cutoff ≥35 would have decreased this further to 25 biopsy sessions. This would come at the cost of missing, respectively, 7 and 11 PCs of the 22 PCs in this substudy. Among these, multiple intermediate- and high-risk PCs would have been present.

4. Discussion

This study did not find a clear role for PCA3 in addition to PSA screening among *BRCA* mutation carriers. Use of PCA3 as a supplementary indicator of prostate biopsies seemed to result in a relatively large number of additional prostate biopsies to identify only a few PCs. However, in the absence of end-of-study biopsies, it is impossible to estimate the real PPV of PCA3 at this point. We can conclude that a restrictive use of PCA3, i.e., to diminish the number of biopsies among men with an elevated PSA level, is not a good strategy in this study population. Too many intermediate- and high-risk PCs would be missed, if prostate biopsies were to be withheld based on the PCA3 score.

PSA level in the initial screening round showed good results, appearing to miss only 3 PCs that were detected in the 2-year follow-up (FU), although not all PCs were found in the first biopsy session. All PCs that were detected during the 2-year screening round were low-risk PCs that revealed themselves by an increased PSA level during the 2-year screening round. These results might be an overestimation, as no end-of-study biopsies were performed. One may argue that end-of-study biopsies would give a more reliable estimate of the NPV of PSA than our somewhat arbitrary gold standard of PC detected in the first 2 years in this study. The counter argument is that end-of-study biopsies lead to a considerable overdiagnosis of PC, as was shown in the PCPT trial [20]. A better alternative for end-of-study biopsies is a much longer FU period to see which men will be diagnosed with PC. Another comment to the current screening strategy could be that, even though it does not seem likely that many high-risk PCs were missed, the *BRCA2* mutation carriers might require an even more stringent PSA cutoff, as discussed by Bancroft et al. [12]. In the absence of end-of-study biopsies or a longer FU period, we can only speculate about whether a different PSA cutoff should be used in *BRCA2* mutation carriers. Unfortunately, a limitation of the study is that the financial resources prohibited more screening rounds. For example, for a good evaluation of the risk of interval cancers, complete FU until the third screening round is necessary.

The results for PCA3 seem modest. For comparison purposes, the most important study is by Roobol et al. [21], which is to our knowledge the only study that used PCA3 (cutoff ≥10) as a supplementary indicator for prostate biopsies. Their biopsy protocol (biopsy indicated for either PSA level ≥3.0 ng/ml or PCA3 ≥ 10) resulted in 721 of the 965 men (75%) being biopsied. In these biopsy sessions, 122 PCs were diagnosed, only 19 of which were classified as "serious" PCs (defined as stage ≥cT2a or any Gleason

Table 3
Clinical and diagnostic characteristics of the Dutch patients with prostate cancer detected in different screening rounds, including family members diagnosed with PC before IMPACT

Patient	Detection	Mutation status	Age, y	Disease risk classification[a]	PSA level, ng/ml[b]	Abnormal DRE[b]	PCA3 score[b]	Gleason score[b]	pTNM/cTNM	Primary treatment
1	Initial screening	Noncarrier	61	High	4.80	N	>69	3 + 3 = 6	pT2cNxMxR0	RALP
2	Initial screening	Noncarrier	62	High	4.80	N	21	3 + 3 = 6	pT2cN0R0	RRP
3	Initial screening	Noncarrier	53	High	3.40	N	25	3 + 3 = 6	pT2cNxMxR0	RALP
4	Initial screening	Noncarrier	62	High	3.28	N	23	3 + 3 = 6	pT2c	RALP
5	Initial screening	Noncarrier	65	High	4.70	N	30	3 + 3 = 6	pT2cN0M0	RALP
6	Initial screening	*BRCA1* mutation	64	Low	6.20	N	13	3 + 3 = 6	cT1c	Active surveillance
7	Initial screening	*BRCA1* mutation	57	High	3.70	N	27	3 + 3 = 6	pT2cNxMxR0	RRP
8	Initial screening	*BRCA2* mutation	61	Low	3.60	N	38	3 + 3 = 6	cT1c	Brachytherapy
9	Initial screening	Noncarrier	62	High	9.90	N	–	3 + 3 = 6	pT2cN0MxR0	RALP
10	Initial screening	Noncarrier	60	Low	5.50	N	27	3 + 3 = 6	cT1c	Active surveillance
11	Initial screening	*BRCA2* mutation	55	High	4.50	N	14	4 + 3 = 7	pT3b	RRP
12	Initial screening	Non-carrier	57	High	4.50	N	17	3 + 4 = 7	pT2c	RALP
13	Initial screening	*BRCA2* mutation	61	High	6.30	N	61	8	pT2b	RRP
14	Initial screening	*BRCA1* mutation	61	Intermediate	9.70	N	22	4 + 3 = 7	pT2aR0	RRP
15	Repeat screening	Noncarrier	67	Low	4.40/2.50	N/N	40	NM/3 + 3 = 6	cT2NxM0	Active surveillance
16	Repeat screening	Noncarrier	62	Intermediate	3.70/–	N/N	144	NM/3 + 4 = 7	pT2aN0M0	RALP
17	Repeat screening	*BRCA1* mutation	67	Low	6.49/5.30	N/Y	56	NM/3 + 3 = 6	–	–
18	Repeat screening	*BRCA1* mutation	59	Low	9.00/4.40	N/N	103	NM/3 + 3 = 6	cT1cNxMx	Active surveillance
19	Repeat screening	Noncarrier	62	Low	4.20/11.0	N/N	16	NM/3 + 3 = 6	cT1a	–
20	Two-year screening	*BRCA1* mutation	57	Low	2.60/4.40	N/N	35/>41	3 + 3 = 6	cT1c	Active surveillance
21	Two-year screening	Noncarrier	60	Low	2.50/3.10	N/N	44/32	3 + 3 = 6	cT1cNxMx	Active surveillance
22	Two-year screening	Noncarrier	53	Low	2.30/3.20	N/N	<16/8	3 + 3 = 6	cT1c	Active surveillance
23	Two-year screening	Noncarrier	59	Intermediate	9.2/11.0/14.9	N/N/Y	47/–	NM/NM/3 + 3 = 6	cT1a	–
x1	Before IMPACT	Noncarrier	50	Low	5.10	–	–	5	pT2aNxMx	RRP
x2	Before IMPACT	Noncarrier	59	High	5.40	–	–	8	pT2cNxM0	Laparoscopic/RALP
x3	Before IMPACT	Unknown	65	High	–	–	–	6	pT3aNxM0	RRP
x4	Before IMPACT	*BRCA1* mutation	61	High	7.90	–	–	7	cT2cNxM0	TURP + thermo-ablation
x5	Before IMPACT	*BRCA2* mutation	56	High	6.00	–	–	7	pT2cNxMx	RRP
x6	Before IMPACT	Noncarrier	56	High	–	–	–	7	pT2cNxMx	RRP
x7	Before IMPACT	*BRCA1* mutation	58	High	6.20	–	–	6	pT2cNxM0	RRP
x8	Before IMPACT	*BRCA1* mutation	65	High	4.40	–	–	7	pT2cNx	Laparoscopic/RALP
x9	Before IMPACT	*BRCA2* mutation	61	High	12.3	–	–	9	cT1cN0M0	EBRT
x10	Before IMPACT	*BRCA1* mutation	59	Low	–	–	–	6	pT2NxMx	RRP
x11	Before IMPACT	*BRCA2* mutation	52	High	130	–	–	9	cT2NxM1B	HT (surgical)
x12	Before IMPACT	*BRCA2* mutation	63	Low	2.10	–	–	6	pT2aNx	Laparoscopic / RALP
x13	Before IMPACT	Unknown	47	High	–	–	–	9	cT3N1M1	HT (medicinal)
x14	Before IMPACT	Noncarrier	65	High	–	–	–	6	pT2cNxMx	RRP
x15	Before IMPACT	Noncarrier	59	High	7.70	–	–	–	pT3aN0M0	RRP
x16	Before IMPACT	*BRCA2* mutation	64	High	40.0	–	–	7	cT1cN1M0	HT (medicinal)
x17	Before IMPACT	Unknown	65	High	–	–	–	9	cT2cN1M0	HT (medicinal)
x18	Before IMPACT	*BRCA1* mutation	59	Intermediate	16.3	–	–	–	cT2bN0M0	EBRT + HT (medicinal)
x19	Before IMPACT	*BRCA1* mutation	59	Low	6.3	–	–	6	pT2aNxM0	laparoscopic / RALP
x20	Before IMPACT	*BRCA2* mutation	59	High	25.6	–	–	6	cT3aN0M1	HT (medicinal)
x21	Before IMPACT	*BRCA2* mutation	50	High	–	–	–	6	pT2cNxMx	RRP
x22	Before IMPACT	*BRCA1* mutation	59	Low	8.30	–	–	6	cT1cNxM0	active surveillance

x23	Before IMPACT	Noncarrier	54	Low	3.70	–	–	–	pT2aNxMx	RRP
x24	Before IMPACT	Unknown	63	Low	–	–	–	–	cT1cN0Mx	active surveillance
x25	Before IMPACT	*BRCA2* mutation	62	High	96.0	–	–	7	cT3aN1M0	EBRT + HT (medicinal)
x26	Before IMPACT	Non-carrier	60	High	18,1	–	–	6	cT2cN0M0	EBRT
x27	Before IMPACT	*BRCA2* mutation	57	High	20.1	–	–	7	pT2cN0M0	RRP
x28	Before IMPACT	Unknown	62	High	8.60	–	–	7	pT2cNxM0	laparoscopic/RALP
x29	Before IMPACT	*BRCA2* mutation	68	Intermediate	4.43	–	–	7	cT1cNxM0	active surveillance
x30	Before IMPACT	*BRCA2* mutation	51	High	29.5	–	–	6	cT3aN0M0	EBRT + HT (medicinal)
x31	Before IMPACT	*BRCA2* mutation	63	High	7.20	–	–	8	cT3aN1M0	HT (medicinal)
x32	Before IMPACT	*BRCA1* mutation	61	High	37.80	–	–	7	cT3aN1M0	HT (medicinal)
x33	Before IMPACT	*BRCA2* mutation	64	Intermediate	4.40	–	–	6	pT2bNxMx	RRP
x34	Before IMPACT	Noncarrier	60	Low	7.30	–	–	6	cT1cNxM0	Brachytherapy
x35	Before IMPACT	*BRCA1* mutation	66	High	8.40	–	–	6	pT2cN0M0	RRP
x36	Before IMPACT	*BRCA2* mutation	62	High	15.3	–	–	6	pT3aNxM0	RRP
x37	Before IMPACT	Non-carrier	58	Low	–	–	–	–	cT2aNxM0	EBRT
x38	Before IMPACT	Non-carrier	57	High	6.40	–	–	6	pT2cN0M0	Laparoscopic/RALP
x39	Before IMPACT	*BRCA2* mutation	63	Low	6.00	–	–	6	pT2NxMx	RRP
x40	Before IMPACT	*BRCA2* mutation	68	Low	–	–	–	6	cT1cNxM0	Active surveillance
x41	Before IMPACT	*BRCA1* mutation	63	Low	–	–	–	5	cT1aNxMx	TURP
x42	Before IMPACT	Noncarrier	54	High	134	–	–	7	pT3bN0M0	EBRT + HT (medicinal)

EBRT = external beam radiation therapy; HT = hormonal therapy; NM = no malignancy (on prostate biopsy); RALP = robot-assisted laparoscopic prostatectomy; RRP = radical retropubic prostatectomy; pTNM/cTNM = tumor-node-metastasis staging system (postsurgical pathological vs. clinical stage); TURP = transurethral resection of the prostate.

[a]Risk stratification was performed according to the 2014 NICE guidelines: high-risk PC: any PC with pT $\geq$ T2c, pN+, pM+, PSA level $>$ 20 ng/ml, or Gleason score $\geq$8; intermediate risk: any PC pT2b, PSA level = 10–20 ng/ml, or Gleason score = 7; low-risk: all PCs with pT1-pT2a, PSA level $<$10 ng/ml, or Gleason score 6. If pathological TNM staging and Gleason score were not available, clinical stage and biopsy Gleason score were used.

[b]For men with PC detected after the initial screening round, outcomes of the tests, as well as the outcomes of previous prostate biopsy sessions are denoted in consecutive order.

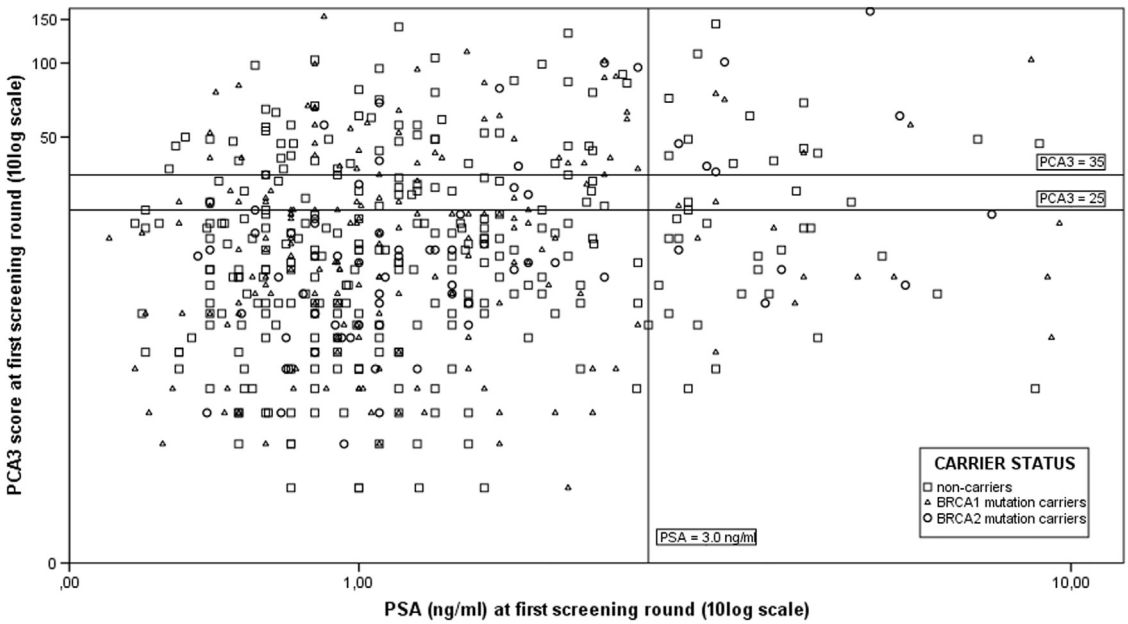

Fig. 3. Correlation (Spearman $\rho = 0.19$) between serum PSA level and urinary PCA3 in 552 participants with a valid *PCA3* score, stratified by *BRCA* mutation status.

score >6, i.e., all nonserious PCs would be low-risk PCs according to the NICE definition) [18]. If they had used the higher cutoff, as described in our substudy, to indicate prostate biopsies (PCA3 ≥ 35), this would still have resulted in biopsies in far too many men in their study (48% of their participants had a PCA3 ≥ 35 as compared with 24% in our study). The authors reported a higher PPV for PCA3 ≥ 35 (24%) when looking at all PCs. However, only 16% of the detected PCs were intermediate- or high-risk PCs. The Roobol study was different from ours in several ways: the participants were on an average older and had all undergone multiple previous screening sessions. The most important difference is that PCA3 was used to indicate prostate biopsies. When comparing the PPV in our study with yet other studies, the results in our study are still rather modest [15,22,23]. However, as discussed before, we cannot give a definitive statement about the PPV of PCA3 as the FU period is still too short. With respect to the NPV of PCA3, the performance was also modest than the other studies. A relatively large number of intermediate- and high-risk PCs would have been missed at PCA3 cutoff values that have performed significantly better in previous studies. A possible explanation for this could be that PC in *BRCA* families might have a different etiological pathway not involving (early) increased PCA3 expression. However, as no study investigated this hypothesis, we can only guess the correct explanation for the relatively modest performance.

Table 4
Concordance between PSA level and PCA3 at the initial screening and cancer detection, stratified by *BRCA* mutation status

	BRCA1 carriers		*BRCA2* carriers		Noncarriers		Total	
PSA level in the initial screening round								
Participants with valid PCA3 score (*n*)	181		73		298		552[a]	
	PCA3 ≥25	PCA3 <25	PCA3 ≥25	PCA3 <25	PCA3 ≥25	PCA3 <25	PCA3 ≥25	PCA3 <25
PSA level ≥3.0 ng/ml	7 (3 PC)	10 (2 PC)	6 (2 PC)	5 (1 PC)	18 (7 PC)	22 (4 PC)	31 (12 PC)	37 (7 PC)
PSA level <3.0 ng/ml[b]	60 (1 PC)	104 (0 PC)	14 (0 PC)	48 (0 PC)	83 (1 PC)	175 (1 PC)	157 (2 PC)	327 (1 PC)
	PCA3 ≥35	PCA3 <35	PCA3 ≥35	PCA3 <35	PCA3 ≥35	PCA3 <35	PCA3 ≥35	PCA3 <35
PSA level ≥3.0 ng/ml	5 (2 PC)	12 (3 PC)	6 (2 PC)	5 (1 PC)	14 (4 PC)	26 (7 PC)	25 (8 PC)	43 (11 PC)
PSA level <3.0 ng/ml	41 (1 PC)	123 (0 PC)	9 (0 PC)	53 (0 PC)	59 (1 PC)	199 (1 PC)	109 (2 PC)	375 (1 PC)

[a]Note that one of the men with PC (Table 2, patient 9) did not have a valid PCA3 score.

[b]Only men with PSA level ≥3.0 ng/ml underwent prostate biopsies. The 3 PCs in men with an initial PSA level <3.0 ng/ml were detected during the 2-year screening round; these participants underwent prostate biopsies because of a PSA level that had increased to ≥3.0 ng/ml in the time between the initial and the 2-year screening round. Of the PCs detected in men with a PSA level ≥3.0 ng/ml, 6 were detected in the repeat and second screening rounds after negative biopsy results in the previous screening round.

In our substudy, the *BRCA2* mutation carriers had an increased prevalence of PC in the first screening round. This difference diminished, as PCs in the consecutive screening rounds were only detected among *BRCA1* mutation carriers and noncarriers. The suggested association between *BRCA* mutations, particularly *BRCA2* mutations, and PC risk is more supported by the number of PCs detected before the study (Table 3). This may be the reflection of the genetically increased risk of PC that men with a *BRCA* mutation have been reported to have. An alternative explanation is ascertainment bias. It is imaginable that particularly men with a known *BRCA* mutation were more intensively screened for PC before IMPACT than noncarriers, as a relationship between *BRCA* mutations and PC has been reported for quite some time. Either way, assuming that a causal relationship exists between a germline *BRCA* mutation and PC, the exclusion of these family members from this study cannot affect the results among screening-naïve participants [24].

The highest PPV for PSA level was found for *BRCA2* mutation carriers, suggesting that this might be the most interesting group for further research. PCA3 also performed best among *BRCA2* mutation carriers. However, the absolute numbers (3 PCs were detected among 75 *BRCA2* mutation carriers) are small, warranting caution when drawing any conclusions.

5. Conclusions

PCA3 should not be used as a restrictive marker next to PSA level for PC screening in *BRCA* mutation carriers. PCA3 did not show sufficient additive value to be implemented as a supplementary indicator of prostate biopsies next to PSA level, although in the absence of prostate biopsies based on PCA3, its true diagnostic performance cannot be calculated. A longer FU period is needed to show whether PCA3 will be a valuable addition to any PC screening protocol.

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