On the diagnosis of early stage Prostate Cancer with an emphasis on Prostate Cancer Gene 3 (PCA3) and Real-Time Elastography (RTE)

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A dream doesn't become reality through magic; it takes sweat, determination and hard work.

Colin Powell, US-General and Secretary of State 2001-2005.

The challenges of science in modern society might be expressed by these brilliant words by sir Winston Churchill:

"A lie gets halfway around the world before the truth has a chance to get its pants on."

Contents

Scientific Environment

The work in this thesis was carried out at the Department of Urology at Haukeland University Hospital in the period from 2009 to 2016. The thesis is a part of the PhD programme at the Department of Clinical Medicine, University of Bergen, Norway.

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Bergen, April 2016 Yngve Nygård

List of Publications

This thesis is based on four original papers. Papers I, II and III are published, whereas Paper IV was submitted for publication, with a decision still yet pending.

I. Nygård Y, Haukaas SA, Waage JE, Halvorsen OJ, Gravdal K, Frugård J, Akslen LA, Beisland C. Combination of real-time elastography and urine prostate cancer gene 3 (PCA3) detects more than 97% of significant prostate cancers. *Scand J Urol.* 2013 Jun;47(3):211-216.

II. Nygård Y, Haukaas SA, Halvorsen OJ, Gravdal K, Frugård J, Akslen LA, Beisland C. A positive real-time elastography is an independent marker for detection of high-risk prostate cancers in the primary biopsy setting. *BJU Int.* 2014 May;113(5b):E90-97.

III. Nygård Y, Haukaas SA, Eide GE, Halvorsen OJ, Gravdal K, Frugård J, Akslen LA, Beisland C. Prostate cancer antigen-3 (PCA3) and PCA3-based nomograms in the diagnosis of prostate cancer: An external validation of Hansen's nomogram on a Norwegian cohort. Scand J Urol. 2015 Feb;49(1):8-15.

IV. Nygård Y, Haukaas SA, Halvorsen OJ, Gravdal K, Frugård J, Akslen LA, Beisland C. A positive Real-Time Elastograpy (RTE) combined with a Prostate Cancer Gene 3 (PCA3) score above 35 convey a high probability of intermediate- or high-risk prostate cancer in patient admitted for primary prostate biopsy. BMC Urol 2016 Jul 8;16(1):39

Abbreviations

ADT	-Androgen deprivation therapy
AS	-Active surveillance
AUC	-Area under the curve
BCR	-Biochemical recurrence
BPH	-Benign prostatic hyperplasia
CRF	-Clinical report form
CZ	-Central zone
DRE	-Digital rectal examination
DVC	-Dorsal vein complex
EAU	-European Association of Urology
EBRT	-External beam radiation therapy
EPE	-Extraprostatic extension
ePLND	-Extended Pelvic lymph node dissection
ERSPC	-The European Randomized Study of Screening for Prostate Cancer
ICC	-Intraclass correlation
ISUP	-International Society of Urological Pathology
LHRH	-Luteinizing-hormone-releasing hormone
LN	-Lymph node
LRP	-Laparoscopic radical prostatectomy
mp-MRI	-Multiparametric magnetic resonance imaging
NCCN	-The National Comprehensive Cancer Network
NPV	-Negative predictive value
NVB	-Neurovascular bundle
PC	-Prostate cancer
PCA3	-Prostate Cancer Gene 3
PET	-Positron emission tomograph
PI-RADS	-Prostate Imaging Reporting and Data System
PLCO	-The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial
PPV	-Positive predictive value
PSA	-Prostate specific antigen
PZ	-Peripheral zone
RARP	-Robotic-assisted radical prostatectomy
ROC	-Receiver operating curves
ROI	-Region of interest
RP	-Radical prostatectomy
RRP	-Retropubic radical prostatectomy
RT	-Radiation therapy
RTE	-Real-time elastography
SVI	-Seminal vesicle invasion
TNM	-Tumour, Node, Metastasis Classification
TRUS	-Transrectal ultrasound
TZ	-Transitional zone
US	-Ultrasound
WHO	-World Health Organization

Abstract

Aims:

To investigate real-time elastography (RTE) and prostate cancer gene 3 (PCA3) to see if these emerging markers/methods could contribute to a faster and more precise diagnosis and also to investigate the possibility of a better selection of patients in need of prostate biopsies.

Material and methods:

Paper I:

40 consecutive patients planned for radical prostatectomy (RP) was investigated with RTE and PCA3 prior of RP. The results were compared to the whole-mount section pathology of the RP specimen.

Paper II-IV:

127 consecutive patients planned for initial prostate biopsies were included. They were first examined with DRE for determination of clinical stage and for the prostatic massage needed before PCA3 analysis. Then they were examined with RTE and RTE targeted biopsies were obtained from hard lesions. Then a transrectal ultrasound guided systematic 10-core biopsy was performed in all patients.

Results:

Paper I:

Using PCA3 score with cut off of 35, 26 patients had a positive PCA3 test and 11 patients had a false negative test. The largest tumour with correct location was found in 73% and at least one tumour was found in 89%. Only one patient had both a negative RTE and PCA3 score leading to a total detection rate of 97%.

Paper II:

64 patients were diagnosed with PC in the initial biopsy setting. The RTE targeted biopsies had a higher frequency of positive cores and also a trend towards a higher fraction of PC in the targeted biopsy cores than in the cores from the systematic biopsies (42% vs. 33%). RTE was found to be an independent marker for the detection of high-risk PC. Per region of

interest (ROI) a negative predictive value (NPV) of 97% was found for high grade PC with Gleason grade 4+3, score 7 and higher.

Paper III:

The systematic initial biopsies were used for the analyses. PC was diagnosed in 59 patients. PCA3 was tested for the cut-off values 21 and 35. The sensitivity/specificity was 71%/ 72% using 35 as cut-off and 81%/55% using 21 as cut-off respectively. Hansen´s nomogram was valid for our cohort. PCA3 contributed significantly to the performance of the nomogram, a threshold value of 20 as biopsy decision seems to be safe.

Paper IV:

This paper also includes a follow-up period with a mean observation time of 46.7 (range 41–55) months. Included are the results from the initial biopsies and also the results of eventual repeat biopsies. If both RTE and PCA3 are negative there is a low probability of detecting aggressive PC. The combined use of RTE and PCA3 lead to a NPV of 90% for the group of intermediate- and high-risk PC together, for the high-risk group NPV was 100%.

Conclusions:

RTE has the ability of detecting PC and can be used for detection and also for targeted biopsies. PCA3 can be used in an initial biopsy setting and it contributes significantly to the area under the curve when applied to a nomogram. The combined use of PCA3 and RTE is better than the methods used alone.

Introduction

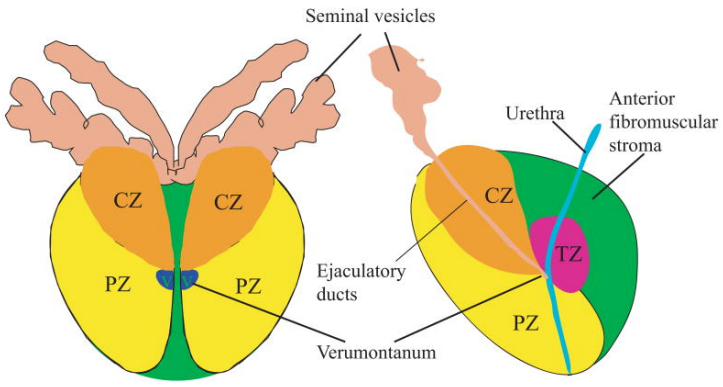
Prostate anatomy and function

The prostate is located between the bladder and the urethra; its dorsal limitation is the rectum and its anterior aspect is directly retropubic. The prostate lies within the small pelvis and is dorsally covered from the Denonvillier´s facia, while on its lateral aspects it is covered by the lateral pelvic facia. On the anterior part is the dorsal vein complex (DVC) draining the blood from the penis. In the posterolateral aspect on both sides, the main part of the neurovascular bundles (NVB) is found. The major part of the nerves and vessels supporting erectile function are in this position, but many smaller nerves and vessels are spread along the dorsal and lateral aspect of the prostate as well. This must be considered when performing a nerve-sparing radical prostatectomy, as the results are better if as much as possible of the NVBs are spared.

Anatomically, the prostate is divided into three zones: the peripheral zone (PZ), the transitional zone (TZ) and the central zone (CZ); the anterior part is described as the anterior fibromuscular stroma, see figure below.

Figure 1:

Figure below shows the zonal distribution of the prostate described by McNeal 1981 [1]. Copyright has been obtained.



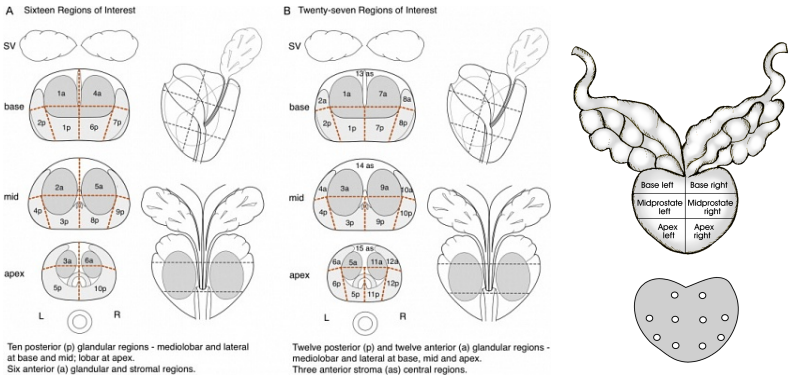
Prostate cancer (PC) is most frequent in the PZ, benign prostatic hyperplasia develops in the TZ and the central zone is the tissue in the area surrounding the ejaculatory ducts from the colliculus to the base of the seminal vesicles. For the purposes of imaging, several zone distributions have been developed. For the use of magnetic resonance imaging (MRI), a 16- and 27-zone distribution has been developed to be able to exactly locate suspect foci [2]. For the evaluation of real-time examinations like in the ultrasound (US) a six-zone distribution has been developed, see Figure 2. In this case, the prostate is divided into three regions, the base, mid and apex of the prostate on each side.

The prostate has several functions. It produces the transport fluid for the semen, as approximately 70% of the ejaculate is fluid produced in the prostate. The prostate also has a part in the urethral sphincter function as an internal sphincter; it supports the function of the external urethral sphincter in maintaining urinary control, and is therefore one of the reasons that men rarely have urinary stress incontinence as long as they have not had prostatic surgery. This function is clearly demonstrated in patients after a radical prostatectomy, in which urinary stress incontinence is a common complication of the surgery [3]. The internal sphincter also plays an important role during ejaculation, as it contracts during ejaculation and blocks the ejaculate from passing to the bladder.

Figure 2:

Figure to the left below shows the minimum 16-zone (A) and the recommended 27-zone (B) distribution used in MRI for PC localization. Figure after Dickinson et al. [2].

Figure to the right shows the 6-zone distribution often used in real-time examinations as real-time elastography (RTE) and a standard biopsy grid used in transrectal ultrasound-guided 10-core biopsies.



Prostate diseases

In the prostate, many benign diseases can occur. Benign prostatic hyperplasia (BPH) is a very common disease, with an increasing incidence with an increasing age, which can result in bladder outlet obstruction with lower urinary tract symptoms (LUTS). Other benign conditions of the prostate are acute and chronic prostatitis. An acute prostatitis is an acute bacterial infection associated with prostatic swelling, pain and fever, which can lead to urinary retention. A chronic prostatitis is a more diffuse disease. It is only in a subset of patients that there is an actual bacterial infection, as in most cases it is a part of an idiopathic chronic pelvic pain syndrome.

The prostate cells are regulated by androgen, of which dihydrotestosterone is the intracellular specific receptor binding hormone [4].

PC is most commonly an adenocarcinoma (>95%) developing from the normal glands of the prostate. Other types are neuroendocrine differentiation and small cell carcinoma. The adenocarcinomas are usually dependent of androgens for growth and proliferation, in that androgen deprivation leads to growth arrest and cell death. Charles Huggins and

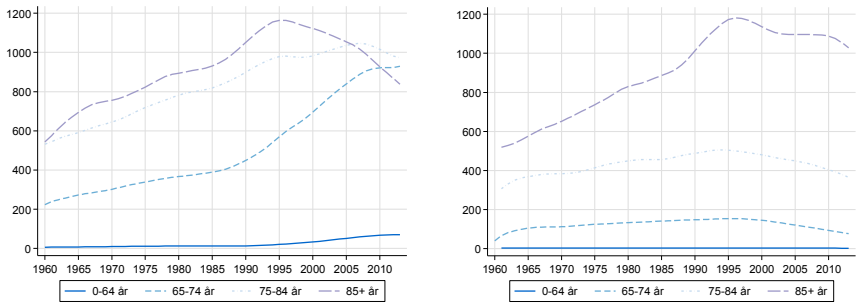
Clarence Hodges first established the concept of androgen deprivation in the treatment of PC in 1941; their research resulted in Charles Huggins being awarded the1966 Nobel Prize for Physiology or Medicine [5].

Prostate cancer epidemiology

PC is a common cancer among men. Worldwide, there is a different incidence of PC, with the highest incidence in North America and in Scandinavia, where the incidence rate is >200 per 100,000 [6, 7]. In Norway, the latest complete set of data from the Norwegian Cancer Registry are from 2014: the number of newly diagnosed patients was 4,836, and the number of men who died from PC was 979 [8]. This yields an incidence rate of 203 per 100,000. Another publication from the Cancer Registry in Norway shows an increasing incidence of PC in Norway in the 10-year period from 2004–2013. In 2004, the incidence was 3,854 and the mortality was 1,051. The increase is most likely the result of an increase in PSA screening [9], with approximately 50% of the patients diagnosed solely because of an elevated PSA level [10]. The mortality rates are more stable, but there is a trend towards a lower mortality rate. As the curves clearly demonstrate, PC is a disease most common in elderly men.

Figure 3:

The figure below shows the development of the incidence (left) and PC mortality (right) in Norway expressed for different age groups [10].



"The study has used data from the Cancer Registry of Norway. The interpretation and reporting of these data are the sole responsibility of the authors, and no endorsement by the Cancer Registry of Norway is intended nor should be inferred."

Staging of Prostate Cancer

In PC, the Tumour Node Metastasis (TNM) classification from the International Union Against Cancer (UICC) is used. Table 1 shows the 2009 TNM classification [11].

Table 1:

TNM classification from 2009

T – Primary tumour	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
T1	Clinically inapparent tumour not palpable or visible by imaging
	T1a Tumour incidental histological finding in 5% or less of tissue resected
	T1b Tumour incidental histological finding in more than 5% of tissue resected
	T1c Tumour identified by needle biopsy e.g. because of elevated prostate-specific antigen (PSA level)
T2	Tumour confined within the prostate[1]
	T2a Tumour involves one half of one lobe or less
	T2b Tumour involves more than half of one lobe, but not both lobes
	T2c Tumour involves both lobes
T3	Tumour extends through the prostatic capsule[2]
	T3a Extracapsular extension (unilateral or bilateral), including microscopic bladder neck involvement
	T3b Tumour invades seminal vesicle(s)
T4	Tumour is fixed or invades adjacent structures other than seminal vesicles: external sphincter, rectum,
	levator muscles and/or pelvic wall
N - Regional lymph nodes[3]	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis[4]
M - Distant metastasis[5]	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis
	M1a Non-regional lymph node(s)
	M1b Bone(s)
	M1c Other site(s)

[1] Tumour found in one or both lobes by needle biopsy, but not palpable or visible by imaging, is classified as T1c.

[2] Invasion into the prostatic apex, or into (but not beyond) the prostate capsule, is not classified as pathological T3, but as pathological T2.

[3] The regional lymph nodes are the nodes of the true pelvis, which essentially are the pelvic nodes below the bifurcation of the common iliac arteries.

[4] Laterality does not affect the N-classification

[5] When more than one site of metastasis is present, the most advanced category should be used.

Comments to staging

T-stage

The T-stage was previously only determined by the digital rectal examination (DRE) findings. Stage T1c included all non-palpable tumours found through biopsy. Because imaging, especially multiparametric MRI (mp-MRI), is becoming more commonly used, stage T1c is defined as tumours not palpable or visible on imaging. When considering the limitations previously described on mp-MRI in staging, this raises new questions. Most nomograms currently used in PC treatment and risk assessment have been validated on DRE-based T-stage [12-14]. An upstaging from clinical stadium T1c to the stage determined by MRI might overestimate the individual patient risk for lymph-node involvement and for extraprostatic extension.

N-stage

For determining the N-stage, extended pelvic lymph node dissection is the gold standard, as no imaging techniques have been able to rule out lymph node metastases [15, 16]. In clinical practice, nomograms can be used to determine the individual risk of lymph node involvement, which can be useful as patient counselling on treatment decisions, as well as for preoperative planning in cases where a radical prostatectomy is performed [14]. When external beam radiation therapy (EBRT) is planned, nomograms are used to determine the indication for whole-pelvic radiation. Some newer studies on positron emission tomography (PET) indicates that PET/CT can be more accurate then nomograms, and can be used to better identify the individual risk of lymph node (LN) involvement [17].

M-stage

For M-staging, the standard method is still a bone scan. Choline- and Fluor-PET have been shown to perform better than a bone scan. The same is true for a whole body MRI with diffusion-weighted images [18, 19]. Due to availability and the lack of cost-benefit analyses, bone scans are still considered the gold standard of care in detecting bone metastases, even if newer methods have proven their superiority.

Grading of Prostate Cancer: Gleason grade, score and grade groups

Since PC is a highly diverse disease, in which both aggressive and less aggressive cancers exist, grading systems have been introduced. The pathologist Donald Gleason introduced the Gleason grade and score as a grading system for PC in the 1960s [20]. It has been modified, with the International Society of Urological Pathology (ISUP) presenting a consensus on Gleason grading in 2005 [21].

The Gleason grade is on a scale from 1-5, and the Gleason score is 2-10. The Gleason score is calculated from the most common patterns in the histopathology specimen, and if two patterns are present the score reflects the most common pattern first and then the second most common pattern. If just one pattern is present the grade is doubled in the score, and if more than two grades are present the most common grade is first and the highest grade is second. That means that if the most common pattern is grade 4 and the second most common pattern is 3, the score will be 4+3=7. The Gleason grade and score has been shown to be of prognostic value for the patients, providing information on progression, recurrence after radical treatment and cancer-specific mortality. Criteria based on the Gleason score have been introduced, the so-called "Epstein criteria", which are commonly used to distinguish between clinically insignificant PC and PC with a higher risk of progression and PC mortality [22].

The Gleason score has an impact on the PC outcome. One weakness of the grading is that tumours with a very different outcome receive the same score. There is a difference in outcome between a Gleason grade 3+4 and grade 4+3, even though they both have the same score of 7. Currently, the lowest Gleason score reported on prostate biopsies is 6 [23]. Consequently, it is sometimes challenging to inform patients that they most likely have an insignificant cancer when they have Gleason score of 6 on a scale ranging from 2-10.

In 2014, there was a new ISUP consensus conference on the grading of PC. A new grading system was proposed from Epstein based on the modified Gleason grading [23], and there was a consensus to implement the new grading system. The World Health Organization (WHO) has accepted this system for the 2016 edition of Pathology and

Genetics: Tumours of the Urinary System and Male Genital Organs, which is based on five Grade Groups:

Grade Group 1: Gleason scores ≤ 6

Grade Group 2: Gleason grade 3+4, score of 7

Grade Group 3: Gleason grade 4+3, score of 7

Grade Group 4: Gleason score of 8

Grade Group 5: Gleason score of 9-10

Biomarkers for Prostate Cancer

Prostate-specific antigen

Prostate-specific antigen **(**PSA) is a glycoprotein produced in the prostate; it is a part of the human kallikrein family, and is found in both semen and plasma. The concentrations of PSA in semen are approximately a million times higher than the levels measured in serum. Its function is most likely to lyse the clot in the ejaculate to keep the ejaculate liquid. It is prostate-specific, though not specific for PC. It is used to monitor PC, and is tested and used as a marker for PC [24].

In serum, PSA is found in both bound and unbound forms, as PC cells do not make more PSA than normal prostate cells [24]. Different diseases can lead to a disruption of cellular architecture within the prostate gland, and a loss of barrier probably leads to an increase in serum PSA. For the use of PSA as a screening marker, see later chapters discussing PSA-screening in the thesis.

Free to total PSA

Since PSA is not a very good marker for PC, there is a continuous search for new biomarkers and for a better use of PSA measurement. The free to total (f/t) PSA ratio is used to differentiate PC from benign prostatic disease. In patients with a PSA of 4-10 ng/ml and a negative DRE, there is only an 8% risk of having PC diagnosed on prostate biopsies if the f/t PSA >0.25, in contrast to a 56% risk of PC in patients with an f/t PSA <0.1. The f/t PSA can only be used in the PSA range of <10, as it has no diagnostic value if the PSA >10. It is also to be used with caution because of several pre-analytical and

clinical factors [25-27]. It can be used to reduce the need for rebiopsies and performs well in this setting [28].

PSA density

PSA density is defined as a PSA level divided by prostate volume (Pvol), which reflects that the PSA increases in benign prostatic hyperplasia (BPH) and not just in PC. The higher the PSA density, the larger the risk of finding clinically significant PC on prostate biopsies [22].

PSA velocity/doubling time

PSA velocity is defined as the annual increase in PSA level, while the PSA doubling time is an expression of the exponential increase in PSA over time. For diagnostic purposes, it has not been established that it has value over a PSA alone [29, 30].

Prostate Cancer Gene 3

The Progensa™ prostate cancer gene 3 (PCA3) test is a urine marker; at first a three-stroke prostatic massage is performed and the first catch urine is obtained and transferred to a transport medium. The Progensa PCA3 test measures PCA3, which is a non-coding messenger ribonucleic acid (mRNA). PCA3-mRNA is overexpressed in cancerous tissue compared to benign tissue; it is described to be a 10-100 fold overexpression[31]. This level of overexpression was found to be lower in a later publication[32]. The result is expressed as a score: PCA3 mRNA/PSA mRNA x 1,000, though an undisputable cut-off or threshold value has not been established. PCA3 performs better than PSA and f/t PSA in predicting the outcome of biopsies, and PCA3 has shown to increase the diagnostic performance of nomograms [33, 34]. It also increases the predictive values when used together with mp-MRI [35-37]. The US Food and Drug Administration (FDA) approved the Progensa™ PCA3 test for the indication to aid in the decision for a rebiopsy in men older than the age of 50 [38]. It is indicated to be used together with other clinical parameters (such as PSA, DRE and nomograms) in men in whom a repeat biopsy would be recommended based on standard care; the cut-off is set to be a PCA3 score of 25 in this setting, as a higher score is considered positive and a lower score considered negative. A negative score indicates a low probability of finding clinically significant PC.

PCA 3 in the initial biopsy setting is described in Papers III and IV of this thesis.

In one study, PCA 3 has exhibited superiority to other markers in the first rebiopsy setting, but not in the second and third rebiopsy [28]. It is an established and commercially available biomarker used in the rebiopsy setting according to FDA approval, although many aspects regarding the role of this biomarker have yet to be defined, and further research is needed.

Transmembrane Protease, Serine 2 and v-ets erythroblastosis virus E26 oncogene homolog (TMPRSS2-ERG)

As PCA3, TMPRSS2:ERG is a novel urinary marker measuring mRNA from oncogenes [39]. The two different markers are tested as a combination, and several studies have shown that it adds information to the patient regarding clinically significant PC in both a biopsy setting and in a radical prostatectomy [40]. It can be combined with PCA3, and together the performance is better than for the markers alone. It can also be used in risk calculators before biopsy and the performance is better, especially when used together with PCA3 [41].

Prostate Health Index (PHI) test

A PHI is a blood test that combines a PSA, f/t PSA and the pro PSA isoform p2PSA. It has shown a better accuracy in predicting pathological features such as pT3 tumours and Gleason score ≥7 than PSA, and has been shown to be able to better identify patients at risk of having PC before biopsy. Its clinical use is still yet to be established [42, 43].

Stockholm 3 (STHLM3)

This study was conducted to validate a combination of different markers in PC: a combination of several plasma protein biomarkers, genetic polymorphisms and clinical variables. The results are promising regarding a more personal approach in risk assessment before biopsy, as it demonstrated an ability to reduce the number of biopsies without reducing the ability to detect clinically significant PC. It also detected high-risk PC in patients with a PSA in the range of 1-3 ng/ml, who are patients being missed by PSA screening [44].

PSA screening

A PSA is a marker highly specific for prostate but unfortunately not for PC, since there is a substantial overlap in values between benign and malignant prostate diseases [45]. The PSA has been widely adopted as a screening/early detection marker, at first in the USA and then increasingly in Europe. Many studies have investigated the effect of PSA screening; however the results from these studies are diverse.

The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) did not show any reduction in PC cancer-specific survival. A major bias in this study is the large amount of men in the control group having at least one PSA test during the study period, so at the end of the study a total of 54% of the patients had their PSA measured at least once. In this study, the authors concluded there was no significant difference in mortality rates between organized annual screenings and opportunistic screenings [7].

The European Randomized Study of Screening for Prostate Cancer (ERSPC) trial did show a reduction in PC-specific mortality. The results have been evaluated after the nine-, 11- and 13-year follow-up, and the number needed to screen (NNS) and the numbers needed to treat (NNT) are decreasing. After nine years of follow-up the NNS was 1410 and the NNT was 48, while after 13 years the NNS was 781 and NNT was 27 [46, 47]. The greatest benefit was found in the Gothenburg randomized population-based prostate cancer screening trial, in which the PC-specific mortality was reduced by half after 14 years [47]. This study started as a separate study, but has later been implemented in the ERSPC trial.

A Cochrane review from 2013 investigated the role of PSA screening, concluding that PSA screening did lead to an increase in PC incidence, and that PC was diagnosed at an earlier stage, though screening did not reduce the risk of PC-specific mortality rates [48]. They also stated that overdiagnosis and overtreatment are common, and associated with treatment-related harm to the patients. As a result of this evidence, the US Preventive Services Task Force recommended against routine PSA screening [49, 50]. In the 2014 European Association of Urology (EAU) guidelines, a population-based screening is not recommended, but a risk-adapted strategy for early detection can be offered to a well-informed man [51]. The current Norwegian guidelines on PC are the same as the EAU guidelines [52], and for risk groups the screening of asymptomatic patients is recommended [53].

As risk factors, the following criteria are used:

- Three or more first degree relatives with PC;
- Two or more first degree relatives when both were diagnosed at age < 60 years;
- In patients with a known or suspected BRCA2-mutation.

For this group, an annual measurement of PSA is recommended starting at the age of 40, whereas a further diagnostic work-up is recommended for PSA levels of >3ng/ml.

Early detection

Early detection is a patient-individualized approach in which the goal is to find PC in a curative stage. It is not population-based, but instead based on the individual patient. In early detection, it is recommended that the patients should not only be well informed about the possible benefits, but also about the risks associated with diagnosis and treatment, as well as the risks of overdiagnosis and overtreatment. This approach is recommended by the EAU [51].

Diagnostic procedures and imaging

Digital rectal examination

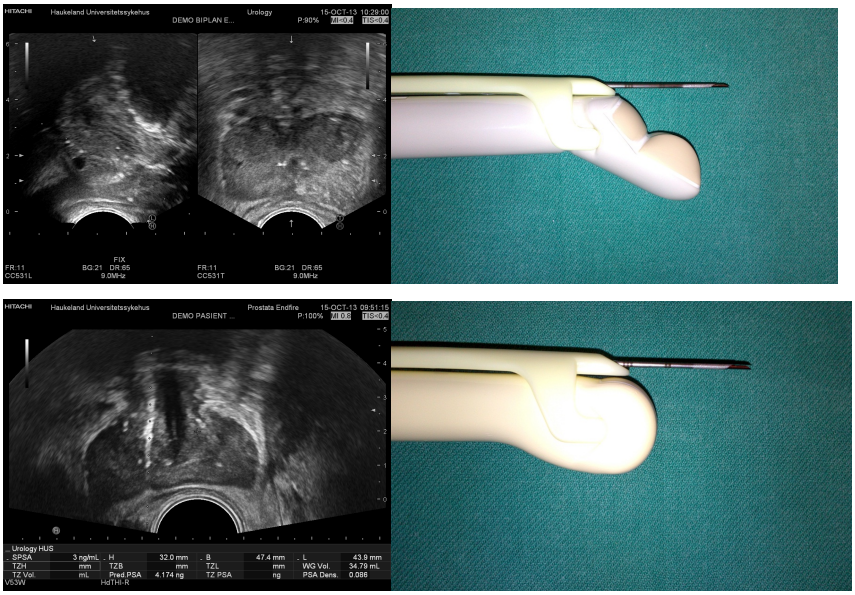
A DRE has a low sensitivity for the detection of early stage PC. On the other hand, if a malignancy is suspected as a result of a DRE, it is an indication for a prostate biopsy, even if the PSA is low. If the DRE is abnormal, there is an increased risk of higher Gleason score [54, 55], as a DRE alone has not proven to be sensitive enough as a screening test [56].

Transrectal Ultrasound

A standard transrectal ultrasound (TRUS) uses B-mode, and produces standard grey-scale US pictures. For TRUS, two different probes are in use: a side-fire biplane probe, which in most cases it has the ability to simultaneously produce pictures in both planes, in addition to an end-fire probe with one plane projection. Recently, most urologists have used a side-fire transrectal probe, see pictures below for a demonstration of the different probes.

Figure 4:

The pictures below show the B-mode TRUS pictures to the left, with the corresponding probes with a biopsy needle to the right



These two different probes have some advantages and disadvantages:

<u>Simultaneous biplane probe</u>

This probe projects both the sagittal and transversal planes at the same time, and shows the real-time pictures on a split screen monitor, which many urologists have seen as an advantage. To get to the different sides of the prostate, one just has to make a small rotation of the probe. The right and left side of the prostate is always projected on the same side on the screen, which makes it easier to be sure that biopsies are being sampled from the correct side of the prostate. One disadvantage of this is that the biopsy line is not exactly in any of the planes, thus making it more difficult when used in targeted biopsies.

End-fire probe

This probe produces one real-time plane at a time. To get from the sagittal to the transversal plane, the probe must be rotated 90 degrees, while the rotation must be 180 degrees to access the contralateral side. By rotating the probe 180 degrees, the picture on the monitor becomes adverse, so the examiner has to manually flip the image on the machine to have the same projection of both the left and right side. This allows some margin for error in an untrained examiner. The end-fire probe also has some advantages. The biopsy line is in the same projection as the picture, and the complete line is visualized during the real-time examination. Because of the direction of the probe and the biopsy line, it is easier to obtain biopsies from the anterior aspect of the prostate. The biopsy can also be better documented since the line is visible in the pictures. It is also used for other applications, such as for example real-time elastography (RTE), in which a biplane probe would produce an insufficient quality of the strain images. When targeted biopsies are performed, most publications focus on end-fire probes; moreover, the fusion tools for MRI-TRUS fusion also use end-fire probes [57-59]. There is also evidence supporting that end-fire probes might be superior when performing standard systematic biopsies [58, 60, 61].

At the beginning of the TRUS era, there was quite a bit of enthusiasm regarding B-mode TRUS. Typically, a cancer lesion would appear as a hypo-echoic lesion, but unfortunately this was not proven, and many lesions can be iso-echoic and even hyper-echoic. This means that many lesions cannot be visualized, and that many lesions that appear abnormal are in fact benign lesions. Because of this, standard B-mode TRUS is not sufficient for the screening or staging of PC [62, 63].

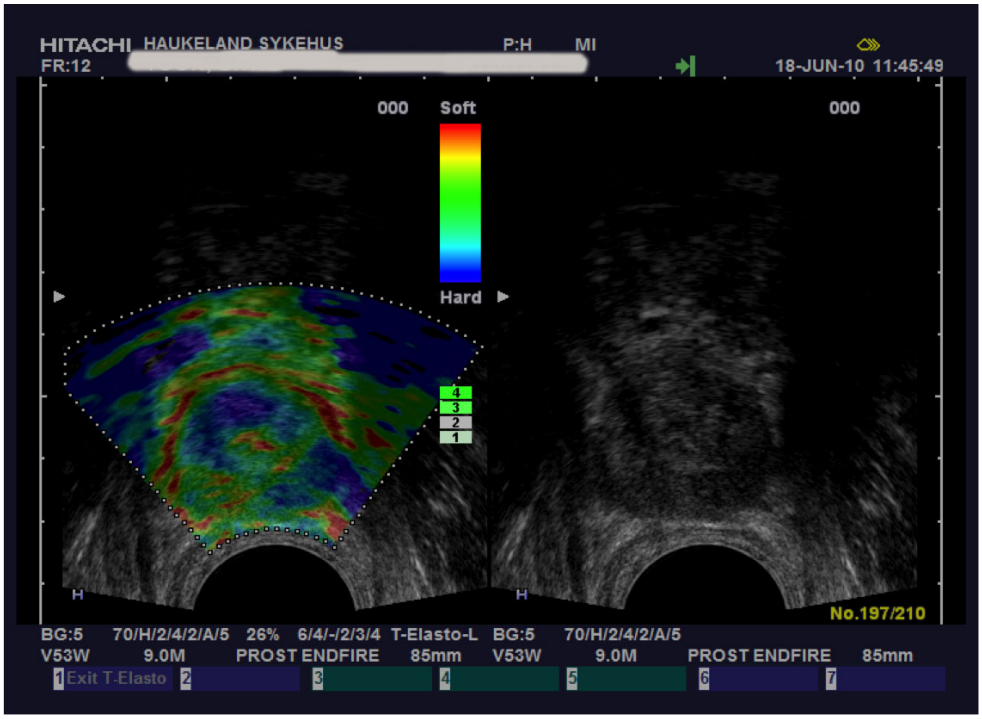
Real-time elastography

Real-time elastography (RTE) is a software-based ultrasound modality that visualizes the strain of tissue, which is achieved by performing compression and decompression cycles. The US computer then calculates the strain using the extended combined autocorrelation method, and produces a colour-coded elastogram that is superimposed on the B-mode image. These images are presented simultaneously as B-mode images on a split-screen monitor. The colour code used displays soft lesions as red, intermediate

lesions as green and hard lesions are blue. RTE has been shown to be valuable in the diagnosing of several cancers, e.g. breast cancer and rectal cancer [64, 65].

Figure 5:

The picture below shows a tumour in the left peripheral zone of the apex identified by RTE before radical prostatectomy (Paper 1)



Five to ten years ago, some studies on RTE in PC diagnostics were already published with promising results, mainly for preoperative patients planned for radical prostatectomy [66-69]. For the RTE of the prostate, an end-fire probe is used. RTE has been shown to be able to visualize PC foci in a preoperative setting with a good sensitivity. Of course this is a group of patients where the examiner is in a biased position, as he/she knows that the patient has PC. On the other hand, it is the best way to examine and evaluate whether a method is worth further examination, as the findings can be correlated to the gold standard: The whole-mount section pathology of the prostate. In this way, the true sensitivity, specificity, NPV and PPV can be estimated for all lesions at the time of the examination.

Later on, more studies on the role of RTE in an ordinary biopsy setting have been performed [70-72]. In most studies, the sensitivity of RTE and targeted biopsies is too low to replace systematic biopsies, but RTE with targeted biopsies increases the rate of PC diagnosed in the initial biopsy setting; the cores have a larger amount of tumour tissue, and it also improves the correlation of Gleason grade and score between biopsies and the final pathology after RP, thus implicating that targeted biopsies are more representative [73]. RTE is further elucidated in the papers and later in the discussion section of the thesis.

Computed tomography

Computed tomography (CT) of the prostate has no place in the detection or local staging of PC [74].

Magnetic resonance imaging

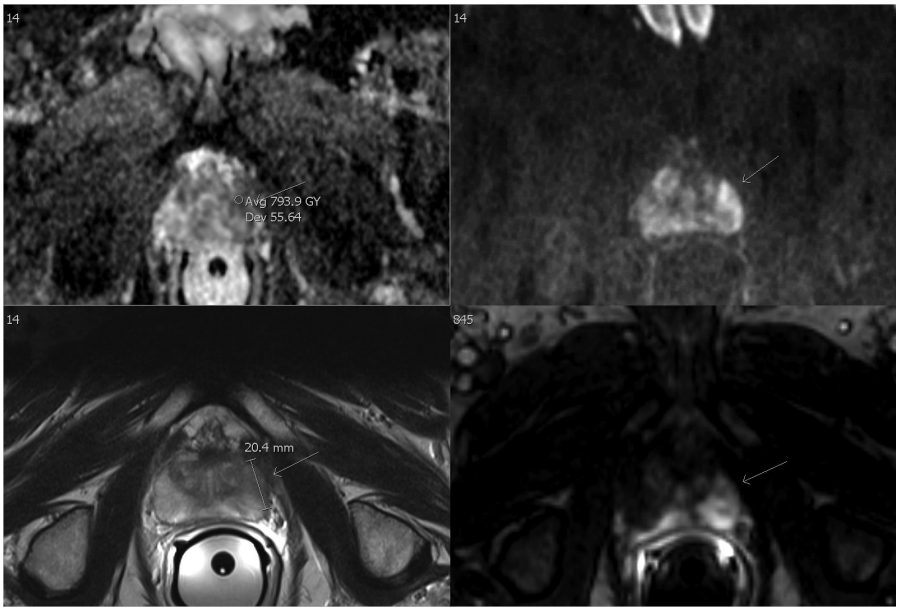
When we initiated these studies, an MRI of the prostate consisted of only T2-weighted anatomical images. MRI was then used in some patients with a persistent suspicion of PC despite negative prostate biopsies. It was used as a tool for a repeat biopsy decision, but only patients with at least two previous biopsy sessions would be considered for MRI. When finding suspect lesions, an attempt at targeted biopsies was performed; in this case, targeted biopsies were mostly limited to taking several biopsies from the suspected region of interest, unlike now when targeted biopsies are more specifically aimed against lesions. If the MRI was negative, these patients with at least two negative biopsy sessions would not undergo a new repeat biopsy. At Haukeland University Hospital this approach was in use for several years, resulting in limiting the number of biopsy sessions and in-hospital follow-ups for each patient.

Multiparametric magnetic resonance imaging

mp-MRI includes T2-weighted images in different planes and diffusion-weighted images, including high and low B-values and apparent diffusion coefficient (ADC). In addition, digital contrast enhancement (DCE) using intravenous (iv) contrast agents visualizing the blood-flow pattern within the prostate can be performed. A spectroscopy

with an estimation of the Choline/Citrate ratio can also be performed, although the latter is mostly used in study settings.

Figure 6:
The picture below shows a typical suspected cancer lesion in the left peripheral zone on the ADC picture (top left), high B-values (top right), on the T2-weighted images (bottom left) and DCE (bottom right; in this case it was a Gleason grade 4+3, score 7 tumour).



mp-MRI was first tested on patients planned for a radical prostatectomy, and it has shown the dual ability to detect PC foci within the prostate and the largest tumour [2, 75-78]. The first studies on MRI in a biopsy setting were performed on patients with previously negative biopsies and a persistent suspicion of PC [79, 80]. In these patients, mp-MRI as a method is well-established, as it has the ability to better detect anterior tumours and tumours at the apex; these are tumours that are more often missed at initial systematic biopsies, as these biopsies are directed towards the rear peripheral zone of the prostate [81, 82]. The current EAU guidelines recommend mp-MRI after initial negative biopsies as the next step in the diagnostic procedures [51].

mp-MRI can be useful in the preoperative setting before RP. It can be used as a preoperative planning tool and be helpful in the decision if a nerve-sparing procedure can be performed, it is also useful for the surgeon to be better prepared regarding the size of the bladder neck, and the location of the tumour in relation to other structures such as the bladder, rectum, DVC and NVB [83, 84]. It must be used with caution since mp-MRI seems to underestimate the total tumour volume [76]. mp-MRI can also be helpful in the staging of PC and in the T-staging precautions must be undertaken because MRI as a staging tool is not very accurate. It also plays a role in the detection of both lymph node metastases and distant metastases. Nevertheless, it has a too low sensitivity and specificity to be of great value in detecting lymph node (LN) metastases, and cannot rule out such metastases [16]. What it can do is to visualize lymph nodes being removed if an extended pelvic lymph node dissection is performed. In the staging of distant metastases, MRI has been shown to be of great value, as it is better than a bone scan for the detection of metastases to the axial skeleton and to the bony structures of the pelvis, and can be used as a tool for detecting bone metastases [18].
A standardized system for the reporting of mp-MRI findings has been developed, which is called the Prostate Imaging Reporting and Data System (PI-RADS). It is recognized as a helpful tool, but there is still some inter-observer variability [75, 85].
In January 2015, new national pathways in PC diagnostics were established[86]. In these pathways, the time frames within which the patients must be diagnosed and treated are defined[87]. As a part of these standards, an mp-MRI is recommended before a first biopsy. Some evidence supports this approach, but only when applied with caution since a negative mp-MRI does not rule out PC[57, 88, 89]. The evidence for an mp-MRI pre-biopsy is still not considered to be sufficient to recommend it for all patients, particularly regarding the cost-benefit and availability of mp-MRI[90].

Bone scan

A bone scan is a method using a small amount of radioactively marked tracers, which are injected intravenously. After approximately three-four hours, a gamma camera is used to measure the level of radioactivity. In PC diagnostics, the test is used for the

detection of bone metastases [91, 92]. It is recommended as a tool for the staging and determination of M-stadium in patients at risk for having PC cancer metastases, especially in high-risk patients [93]. The advantage of this method is that it is a whole body examination, so it gives the possibility of determining the grade of the dissemination of the disease. Unfortunately, it has a weakness in detecting small foci with a more normal metabolism, and it can also be difficult to differentiate between benign and malignant disease. Moreover, it can be combined with a CT or MRI, and this gives a better performance.

Positron emission tomography

PET is a nuclear medicine, functional imaging using radioactive tracers. PET is mostly combined with a CT or MRI, which makes it possible to obtain anatomical and functional pictures at the same time. Different tracers are used. The most common tracer is fluorodeoxyglucose (FDG), which is of limited value because of its low uptake in PC. Other tracers such as 11C- or 18F-Choline and 11C-Acetate are used with promising results in PC [94]. The most recent tracer in use is 68 Ga-PSMA (prostate-specific membrane antigen), which shows very promising results in regard to detection rates and specificity [95, 96].

The main role of PET-CT is the detection of metastases. It has been shown to perform better than a bone scan in detecting skeletal metastases, and it also can detect soft tissue metastases.

Some studies have evaluated PET-CT in the role of lymph node metastases in patients with a recurrence after radical prostatectomy. The reference gold standard was extended pelvic lymph node dissection, and a meta-analysis revealed a pooled sensitivity of 62% and a specificity of 92% [97].

The major role for PET-CT is in the diagnostic work-up of patients with biochemical recurrence (BCR). A weakness of the method is that it has a low sensitivity for detecting recurrent PC in patients with PSA levels <0.5 ng/ml. This is a weakness because the salvage radiotherapy must be applied as early as possible to achieve the best results. For that reason, and due to its high costs, PET-CT is not recommended for all patients with

BCR. In selected patients with higher PSA levels and/or a short PSA doubling time, PET-CT can be helpful in determining the stage of the disease.

Prostate biopsy

Systematic prostate biopsies

The gold standard of PC diagnostics is still considered to be systematic prostate biopsies, whose indication depends on many factors. Since the procedure has several complications, a biopsy should only be performed if the results have influenced treatment decisions [98]. In early detection, the patients should have a life expectancy of at least 10-15 years, there should be at least two measurements of PSA, both with elevated levels, before a decision to perform biopsies is taken. If there is a suspicion of locally advanced or metastatic PC, biopsies are then performed in patients with a shorter life expectancy. A prostate biopsy should also be performed if there is an abnormal DRE.

The standard of care is TRUS-guided prostate systematic biopsies; the prostate is visualized using a standard TRUS B-mode, using either a simultaneous biplane or an end-fire probe, and periprostatic local anaesthesia is recommended. The current recommendation for initial or baseline biopsies is a 10-12-core systematic biopsy from the prostate. In the initial setting, a further increase in the number of biopsies is not beneficial [99]. The biopsies are directed towards the PZ, as this is the area where cancer is most common. A biopsy of the TZ or the seminal vesicles is not recommended in the initial setting. The rate of positive biopsies also varies a lot, and is dependent on several factors such as the degree of screening in the population, and perhaps also which type of procedure is performed [57, 61, 71, 72].

The follow-up after a negative initial biopsy is diverse. Previously, a new biopsy session would be performed if there was a persistent suspicion of PC. In this setting, at least a 12-core biopsy should be performed and the TZ should be biopsied as well. In some cases, a saturation biopsy of at least 20 cores was performed, with some doing this with

a trans-perineal approach. Both these methods have the disadvantage that the patients need regional or general anaesthesia.

In the last 5-10 years mp-MRI has emerged as a very helpful tool in these patients, and several studies have investigated this method [57, 59, 100]. mp-MRI detects many of the tumours missed by systematic biopsies, including the anterior tumours and tumours at the apex of the prostate [81]. In addition, a negative baseline biopsy, together with a negative mp-MRI, reduces the risk of having undiagnosed PC. This has led to the conclusion that with a persistent suspicion of PC after a negative baseline biopsy, an mp-MRI should be performed.

Targeted biopsies

Targeted biopsies are defined as biopsies obtained from a lesion visible on imaging. The different US modalities (RTE, contrast-enhanced US, different Doppler examinations) have the advantage of being cheap to use, and they are real-time examinations that can be performed in the same setting as the initial examination of each individual patient. The disadvantage is that they must be interpreted instantly, and it is not possible to do a reinterpretation without performing a new examination. Another possibility is to perform targeted biopsies in patients with lesions detected on mp-MRI. In these cases, MRI-guided targeted biopsies can be performed, though this is time consuming since MRI is not a real-time examination. More recently, in this case there has been some kind of image fusion between MRI and US, with the biopsies in fact carried out as TRUS with image fusion. Several fusion tools have been developed over the past years. It is also possible to perform targeted biopsies of mp-MRI lesions using cognitive fusion, also called *"fusion in mind"*. Evidence exists that in this setting an end-fire probe should be used, and the results from cognitive fusion are in line with the results using software-based fusion [58, 101]. In our institution we have a good experience using this method, as the success criteria for this approach is a close collaboration between the radiologist describing the MRIs and the urologist performing the targeted biopsies. The person performing the TRUS should be experienced in the method, and a good understanding of prostate anatomy is essential.

Combined approach

In a combined approach the patients have targeted biopsies in the same setting as the systematic biopsies. This has shown to increase sensitivity in the initial biopsy setting when compared to targeted biopsies or systematic biopsies alone, which is valid for both mp-MRI and RTE [57, 71, 72].

Complications to prostate biopsies

There are several complications to prostate biopsies, see Table 2 for frequencies.

Table 2:

The table below shows complications after prostate biopsies (after EAU guidelines on Prostate Cancer [51])

Complications	Percentage of Patients Affected
Haematospermia	37.4
Haematuria > 1 day	14.5
Rectal bleeding < 2 days	2.2
Prostatitis	1.0
Fever > 38.5°C	0.8
Epididymitis	0.7
Rectal bleeding < 2 days ± surgical intervention	0.7
Urinary retention	0.2
Other complications requiring hospitalization	0.3

Procedure-related infections are an increasing issue that needs to be addressed. Antibiotic prophylaxis is recommended, but even with prophylaxis some patients experience severe infections with bacteraemia and clinical septicaemia presenting a life-threatening clinical picture. Infections are becoming more frequent, with the frequency of severe infections in the EAU guidelines reported to be approximately 1%, although newer publications indicate a higher incidence of up to about 2-4% [102-104]. This is probably because of the increase in antibiotic resistant bacteria, as the antibiotic resistance also makes the treatment of these patients more difficult.

Histopathology

Needle biopsies

Needle biopsies are the basis of PC diagnostics. There is also a consensus on the reporting of needle prostate biopsies made at the ISUP conference in 2005. Each core should be reported separately. For each core, the length of the core and cancer length, the Gleason grade and score, as well as any extraprostatic extension, lymphovascular invasion, atypic small acinar proliferation (ASAP), inflammation (active or granulomatous) and high-grade prostatic intraepithelial neoplasia (PIN), should all be reported. Grade Groups are expected to replace Gleason grade and score, or at least be reported in addition to Gleason grade and score [21, 23].

Radical prostatectomy specimen

The evaluation of radical prostatectomy specimen is based on a whole-mount pathology of the prostate. The prostate is embedded and transverse sections of 3-5 mm perpendicular to the urethra are made. At the apex and at the base, sagittal sections are performed to better evaluate the extension of the disease, as well as the surgical margins. The whole-mount sectioning is very helpful when evaluating new imaging modalities, as the pathology serves as the gold standard. The different sections can then be assigned to the same zone distribution as the images, and the true sensitivity, specificity and predictive values of the imaging techniques can be calculated.

In the pathology report of a prostatectomy specimen, several factors should be reported.

The histopathological type should be assigned, whereas the most common type is adenocarcinoma (>95%). Other types are neuroendocrine differentiation and small cell carcinoma. For adenocarcinoma, the Gleason grade and score and/or the Grade Group should be reported. The index tumour should be assigned, which is the tumour with the highest Gleason score, in most cases this is also the largest tumour. The sizes in millimetres of the largest- and of the index tumour should also be reported. The extension of the tumour must be evaluated regarding extraprostatic extension (EPE), seminal vesicle invasion (SVI) and invasion of the bladder neck. A pathological stage is determined and described using the TNM classification, with a p- before the T- and N-

stage. If a lymph node dissection is performed, the number of extracted lymph nodes, the presence and extension of lymph node metastasis and the size of the metastasis should be reported [21, 23].

Treatment

This thesis focuses on the diagnostic of early PC amenable for radical treatment. Recurrence after radical treatment, castration-resistant PC and palliative care is therefore beyond the scope of the thesis.

For treatment decision, there has to be discrimination between PC with metastatic disease outside the pelvis (M1 patients) and patients with M0 disease. In the M1 group, the treatment is palliative and without curative intent. In M0 patients, a curative intended treatment must be discussed. Since such major diversity between these groups exists, the treatment is being presented in separate chapters.

Non-metastatic prostate cancer

This is a large, heterogeneous group, ranging from patients with minimal disease with a low risk of progression and patients with large, aggressive tumours with lymph node metastases within the pelvis, with the latter group having a high risk for progression and even death from PC. To do a better risk assessment of these patients, several clinical tools have been developed. The most used classification is the D´Amico classification, in which patients are being split into three groups: low-risk, intermediate-risk and high-risk [105], which has been slightly modified by the EAU [51]. A weakness of this stratification is the large heterogeneity of the patients within the intermediate-risk group. Some of the intermediate group patients have more or less the same prognosis as low-risk patients, whereas some have the same prognosis as high-risk patients. Because of this, a suggestion has been made to split this group into two different groups, favourable and non-favourable intermediate risk [106]. Unfavourable intermediate-risk patients are those with more than one intermediate-risk criteria and all patients with a

predominantly Gleason grade 4 at biopsy. Patients with one intermediate-risk criterion are those classified as favourable.
It is possible to use different tables, in which the Partin´s table has been widely adopted [12]. These tables are based on data from patients treated with RP.

Another possibility is to use different nomograms. A nomogram incorporates different patient specific criteria, and calculates the individual risk for disease progression and recurrence after treatment, and also provides information on the probability of lymph-node involvement, EPE and SVI. These nomograms exist as pre-treatment tools used in patient counselling before treatment and as post-prostatectomy tools to more accurately define the individual risk of progression and recurrence. Some are available as online calculators, such as for example on Memorial Sloan-Kettering Cancer Centre's website [14]. Since the nomogram is based on series with a limited amount of patients with high-risk PC, the nomograms might not be as reliable in these patients.

Scoring systems, such as the CAPRA score developed at the University of California San Francisco, are also being used [107].

There is no absolute agreement upon which risk assessment tools should be preferred; in the literature, the D´Amico, standard or modified is most widely adapted, and by using this it is easier to compare results from different institutions.

Radical prostatectomy

Radical prostatectomy (RP) is a procedure in which the whole prostate and the seminal vesicles are surgically removed. The procedure can be performed with an open surgical technique, then mostly as a retropubic radical prostatectomy (RRP), as a laparoscopic radical prostatectomy (LRP) or as a robotic-assisted radical prostatectomy (RARP). No procedure has proven to be better than the other regarding cancer control or functional outcome. There are some differences, as in RARP and LRP a laparoscopic procedure is performed, and on average these have less bleeding and a shorter hospital stay. LRP has a longer learning curve and a longer operating time than the two other methods, and many centres have converted from LRP to RARP [108]. In total, the use of robotic

surgery has been steadily increasing over the last few years. A disadvantage of robotic surgery is the increased costs, especially compared to open surgery.

In randomized controlled trials, RP has shown a reduction in PC-specific mortality, but still there is a major risk of overtreatment, particularly in the low-risk group and in patients > 70 years [109-111].
RP is considered a safe procedure when performed by a skilled surgeon. Possible complications are bleeding, infections, deep-vein thrombosis, anastomotic leakage, ileus and damage to other organs, e.g. bowel injury. The frequency of these complications is not high. Common problems after RP are erectile dysfunction and urinary incontinence. To help reduce the frequency of side effects, a nerve-sparing procedure can be performed in many cases. By using a nerve-sparing procedure, the surgeon attempts to keep as much of the NVBs intact as possible. Tumour stage is a limiting factor; if a T3a tumour is suspected it is not recommended to perform a nerve-sparing procedure, at least not on the side where EPE is expected. To achieve the best possible functional results regarding erectile function and urinary incontinence, it is recommended to perform nerve-sparing procedures if it is considered to be safe regarding cancer control [112, 113].

Pelvic lymph node dissection

The gold standard for lymph node staging is still pelvic lymph node dissection (PLND). A limited PLND has been abandoned, since only a small number of the LNs draining the prostate are removed during this procedure. The exact template for an extended pelvic lymph node dissection (ePLND) is still being discussed, and there is agreement that it should at least include the tissue medially from the external iliac artery, the obturator fossa, the area of the internal iliac vessels and the tissue around the common iliac artery, at least up to the level of the ureteric crossing [114]. Some authors also include the tissue laterally of the external iliac artery and all the tissue in the area of the common iliac artery and the presacral nodes [115].

It seems that ePLND can also have a therapeutic role in patients with LN metastases [116, 117]. It might therefore be curative and at least beneficial for patients with limited LN metastases. It is also very useful in the selection of patients for post-operative EBRT.

As per EAU guidelines, an ePLND should be performed if the estimated risk of lymph node metastases is >5%, though which nomogram should be used has not yet been determined [51].
At our institution, the indication for ePLND is based on a modified risk stratification; all patients with unfavourable intermediate-risk or high-risk PC are treated with ePLND, while in low-risk or favourable intermediate-risk cases an ePLND is not performed during RP.

Radiation therapy

Radiation therapy (RT) is a treatment in which ionizing radiation is used with the intent of killing cancer cells. It is a widely used treatment modality in PC treatment, both as a primary treatment and an adjuvant/salvage treatment. RT can be given as External Beam Radiation Therapy (EBRT) or as brachytherapy. Today, mostly photon-radiation is being used, while proton-radiation is also being tested to some level, but have not been proven to be superior to EBRT [118].

External beam radiation therapy

In EBRT, the radiation is given as external photon therapy, which comes after image modulation/guiding. The position of the prostate depends on many factors such as rectal- and bladder- filling, and gold markers can be used for a more accurate targeting of the prostate. In these cases, three small gold markers are placed within the prostate using TRUS. The exact location of the prostate can then be visualized before EBRT is given, and in this way a more targeted radiation can be applied.

In most cases, EBRT is a multimodal treatment since EBRT, in combination with androgen derivation therapy (ADT), has been shown to lead to lower rates of BCR, a local recurrence and to a better survival. In low-risk cases, it can be applied without ADT. In intermediate-risk patients, a short-term ADT is recommended, which consists of approximately six months of ADT [119]. In some of the unfavourable intermediate-risk patients, a longer duration can be discussed. In high-risk patients, a long-term ADT consisting of two-three years of ADT is recommended, particularly in locally advanced PC (stage T3/T4) [120]. For the duration of ADT, several other factors such as

comorbidity and the performance status must be taken into consideration when planning the length of ADT.

Dose escalation is recommended, and a total dose of at least 74-78 Gy should be given. Dose escalation has been shown to reduce BCR rates, but has not shown significant overall survival benefit [121].

EBRT can also be given to patients with a locally advanced disease by including a larger field of radiation, i.e. parts of the bladder, seminal vesicles or rectum.

Brachytherapy

Brachytherapy is a radiation treatment modality used in PC. Instead of using an external radiation source as in EBRT, radioactive seeds are being implanted in the prostate, and the radiation dose is administered as internal radiation. Brachytherapy can be used alone or in combination with EBRT. Brachytherapy can further be divided into two groups: high-dose rate (HDR) and low-dose rate (LDR) brachytherapy. LDR brachytherapy is a treatment option in patients with low-risk PC and prostates with a volume <50cc. HDR brachytherapy is mostly used together with EBRT to boost the radiation dose given to the prostate. The results are comparable to high-dose EBRT, with brachytherapy serving as an option to boost the radiation dose applied [122-125].

Active surveillance

As a result of an increasing awareness in overtreatment, active surveillance (AS), has been introduced. AS is a concept in which patients with a newly diagnosed PC are offered close surveillance instead of an immediate curative approach. The inclusion criteria differ from different cohorts, as in most series only low-risk patients are included [126, 127]. Some series have also included intermediate-risk patients in an AS protocol [128]. AS consists of a follow-up after a pre-planned scheme, which includes regular PSA measurements, DRE and repeat biopsies. If the patient experiences progression, a radical treatment is offered to him. The outcome of radical treatment does not seem to be affected by delayed compared to immediate treatment. The EAU guidelines are still restrictive since they only recommend AS as an option for a subgroup

of low-risk patients. In addition to the low-risk criteria, there is a limitation of a maximum of two cores involved and <50% PC per core. The National Comprehensive Cancer Network (NCCN) recommends active surveillance for low-risk patients, but they also state that AS can be offered to patients with a favourable intermediate-risk disease [106, 129]. Considering the high probability of overtreatment in the favourable intermediate-risk group, many patients in this risk group can most likely be safely managed with AS.

Watchful waiting

This is an approach for patients in whom a radical intent is not indicated, as the life expectancy should be <10 years. This is a palliative approach with the goal of minimizing the side effects from treatment. It is a symptom-guided approach in which only complications to PC are treated, i.e. symptoms due to a local progression or to metastatic disease. Examples of treatment are androgen deprivation therapy, treatment of the bladder outlet obstruction or EBRT of symptomatic metastases. The follow-up is patient adjusted, and no predefined follow-up scheme is used. Watchful waiting should only be applied to patients in whom radical treatment is not supposed to be of any benefit.

High-intensity focused ultrasound

High-intensity focused ultrasound (Hifu) is a treatment option in which US waves are being used in treatment. The waves are being focused, and produce cell death through mechanical and thermal effects. It can be used for focal therapy of PC, as well as for treatment of radio-recurrent PC [130]. In focal therapy, the index tumour or several tumours are treated, thereby leaving the rest of the prostate untreated. It has been shown to have a therapeutic effect in PC, but comparable studies are missing [131]. It is still considered an investigational method, and only recommended to be used in clinical studies.

Cryotherapy

Cryotherapy induces cell death through freezing techniques, e.g. cryo needles are being placed within the prostate under TRUS guidance, and thermo censors are used to

monitor the procedure. It can be used for focal therapy or for whole-gland treatment. Studies have shown a biochemical free survival rate of 36-92% [132]. A study with a longer follow-up has shown an approximate 60-70% biochemical disease-free survival [133]. Cryosurgery is also being tested as part of cryo-immunotherapy in a clinical trial for palliative castration resistant PC patients. Furthermore, cryotherapy is still considered investigational, and should only be offered in clinical studies.

Metastatic disease

The cornerstone in the management of M1 disease is androgen deprivation therapy (ADT). The goal of ADT is to lower testosterone to castration levels. This can be achieved by surgical castration consisting of a bilateral orchiectomy, or through the use of drugs. The most common drugs used are luteinizing-hormone-releasing hormone (LHRH) agonists and antagonists.

LHRH agonists

The pituitary LHRH receptors are being stimulated, leading to a temporary increase in serum follicular stimulating hormone (FSH) and LH, thus following an increase in testosterone level (testosterone surge). Since the drugs overstimulate the LHRH receptors, the receptors are being down-regulated, leading to a fall in FSH-, LH- and testosterone levels in serum. Because of the testosterone surge, it is recommended to use a testosterone receptor antagonist until castration levels of testosterone are reached. The substances are given as depot injections, and exist as formulas for a duration of 1, 2, 3, 6 or12 months.

LHRH antagonists

LHRH antagonists bind to the LHRH receptors in the pituitary glands, and lead to a rapid decrease in serum levels of LH and FSH and consecutively to a rapid decrease in serum testosterone levels [134]. Castration levels are mostly reached within three days. The antagonists do not have a testosterone surge, instead having a rapid onset of the therapeutic effect. One study has shown a possible better progression-free survival over LHRH agonists; however, the results need to be verified [135]. In patients with

symptomatic metastatic disease, it can be used to a quicker relief of symptoms due to its rapid reach of castration levels of testosterone. Nevertheless, it only exists as monthly depot injections, and this limits its use.

Chemotherapy

Until recently, chemotherapy has been used solely in castration-resistant PC. The Systemic Therapy in Advancing or Metastatic Prostate cancer: Evaluation of Drug Efficacy (STAMPEDE) trial has shown the survival benefit of Docetaxel given at the time of initiation of ADT, i.e. in hormone-naive patients. It was followed by an increase in adverse effects, and is now recommended to use in adequately fit hormone-naive metastatic patients at the initiation of ADT [136, 137].

Aims of the Thesis

General aims

At the start of these studies, the management of patients with a suspicion of PC was unsatisfying, as a PSA was the only biomarker in normal clinical use. The use of imaging has mostly been limited to TRUS solely as a guiding tool for biopsies, rather than as a useful imaging modality. Because of having a benign disease, many patients with PSA elevation had several biopsy sessions performed, and it was still difficult to be certain that the patients did not have PC. It was necessary to investigate emerging methods to see if they could contribute to a faster and more precise diagnosis, and to investigate the possibility of better identifying patients at risk for PC before biopsy, with an aim of saving some patients the risk and discomfort of prostate biopsy.

Specific aims for the different papers

Paper I

The aim was to test RTE and PCA3 in a clinical setting, and to validate each method alone and combined in patients planned for a radical prostatectomy. The results are tested against the gold standard of the whole-mount section pathology of RP specimens. This is necessary for validation of the methods, and to decide whether it is worthwhile to continue performing studies on prostate biopsies.

Paper II

Here, the aim was to test RTE and targeted biopsies in an initial biopsy setting, testing the ability of RTE to identify patients with PC and testing whether RTE with targeted biopsies can replace systematic biopsies or be of additional value.

Paper III

The aim in this paper was to test the ability of PCA3 and Hansen´s PCA3-based nomogram to predict PC probability in a Norwegian cohort, with the goal of reducing unnecessary biopsies.

Paper IV

The aim of the last paper was to evaluate prospectively the capability of RTE and PCA 3 alone and combined to predict clinically significant PC in patients admitted for initial prostate biopsy.

Material and Methods

For all papers

Ethical considerations

All patients were given oral and written information about the study, and they gave their written informed consent to participate in the studies. The studies were approved from the Regional Committee for Medical and Health Research Ethics in Western Norway with registration number 223.08.

PCA 3

DRE was performed in all patients for determination of clinical stage (cT-stage), and was also used for the necessary three strokes per lobe prostatic massage needed for the PCA3 test. A first stray urine sampling was performed directly after DRE, as post-DRE urine has a higher yield of the prostatic tissue cells needed for the analyses. We used the Progensa™ (Gen-Probe Inc. San Diego, CA, USA), and the PCA3 tests were analysed by the Fürst Medical Laboratory in Oslo, Norway. The urine samples were prepared, stored and shipped according to the instructions given by the manufacturer.

Paper I

Patients

Forty consecutive patients with known PC planned for RP were examined in the period from November 2009 to September 2010 with PCA3 and RTE before RP.

B-mode TRUS, RTE and report form

A Hitachi 8500 with a V53W transrectal end-fire probe was used for the TRUS examinations. A B-mode ultrasound was performed at first, and the prostate volume (Pvol) was calculated using the Hitachi software after measuring the length, height and width of the prostate. The machine was equipped with RTE software. RTE was performed as previously described under RTE in the Introduction part of this thesis. Hard and reproducible lesions were measured, and any lesion larger than 5 mm was

considered to be malignant. The identified lesions were marked on a clinical report form (CRF), and the location of the lesion was determined by a six-zone regions of interests (ROI) described in the anatomy section of this thesis.

Before the onset of the study, the examiner visited Professor Ferdinand Frauscher and Professor Friedrich Aigner at the University Hospital of Innsbruck, Austria. These doctors are highly skilled in the use of RTE, and are international experts on the method, and advices on US probes and US hardware and software were given. This resulted in the use of an end-fire probe for RTE. The examiner had performed at least 20 RTEs before the inclusion of patients in the study.

The presence and location of the lesions were compared to whole-mount section pathology of the RP specimen. The uropathologists made detailed maps of the whole-mount sections, in which the foci are presented in a 1:1 relationship; these drawings and the pathology reports were compared to the report forms from RTE. An investigator-independent urologist, together with a uropathologist, evaluated the correlation between RTE and the pathology in a consensus.

We decided to leave out the insignificant cancers from the analysis by using the definition by Epstein of a tumour volume < 0.2 cc, and no Gleason grade 4 or 5 present [22].

Papers II – IV

The same consecutive patient cohort is investigated in all three papers, and the same procedures have been performed on all patients. The methods and patient characteristics are presented together, and for each paper the methods used are more closely described in subchapters.

Patients

A total of 127 consecutive patients were included in the study in the period from February 2011 to June 2012. The inclusion criteria were:

- A PSA between 3.0–25.0 ng/ml and/or pathological DRE

- Age < 75 years
- No prior biopsies within the last five years
- Amenable for radical treatment

All patients were admitted from general physicians because of an elevated PSA and/or a pathological DRE as a result of early detection. All patients meeting the inclusion criteria in this period were asked to be part of the study, and only very few (<5) patients declined to participate.

Transrectal ultrasound

All patients were examined in the left decubital position. A Hitachi Preirus ultrasound machine equipped with an RTE module (Hi-RTE) was used for all examinations. A six cubic centimetre (cc) Lidocaine 10mg/ml were given as periprostatic local infiltration anaesthesia. All patients received an antibiotic prophylaxis consisting of 1,000 mg of Ciprofloxacin (Ciproxin ®) administered before biopsy.

RTE and targeted biopsies

A V53W transrectal end-fire probe was used for RTE and targeted biopsies, with the method for RTE further described in Paper I. The same six ROI described in Paper I were used. The prostate was initially examined in total with B-mode and RTE. The Pvol was calculated using the software after measuring the height, width and length of the prostate. For RTE, only reproducible hard lesions were considered to be suspicious of malignancy, and targeted biopsies were obtained from such lesions. A maximum of five targeted biopsies were obtained, and in patients with suspected lesions, the location and number of targeted biopsies were marked on the CRF. Total core length, length of cancer tissue and Gleason grade and score were recorded separately for each biopsy core.

Systematic biopsies

After RTE, another urologist unaware of the RTE results performed a TRUS with a 10-core systematic biopsies in all patients. This urologist is an experienced urologist highly skilled in the TRUS-guided systematic biopsy technique. For this examination, a CC531 transrectal simultaneous biplane probe was used. All

biopsies were assigned to predefined regions in accordance with the biopsy scheme described in the anatomy section of this thesis. Total core length, length of cancer tissue and Gleason grade and score were recorded separately for each biopsy core.

Paper II

The paper focuses on RTE with targeted biopsies and a systematic biopsy in the initial biopsy setting. All biopsy data from the initial biopsies, the rebiopsies performed within six months after the end of inclusion, as well as the histo-pathological gross-section pathology for patients treated with RP, are included in the analyses of the performance of RTE by ROI analyses.

Paper III

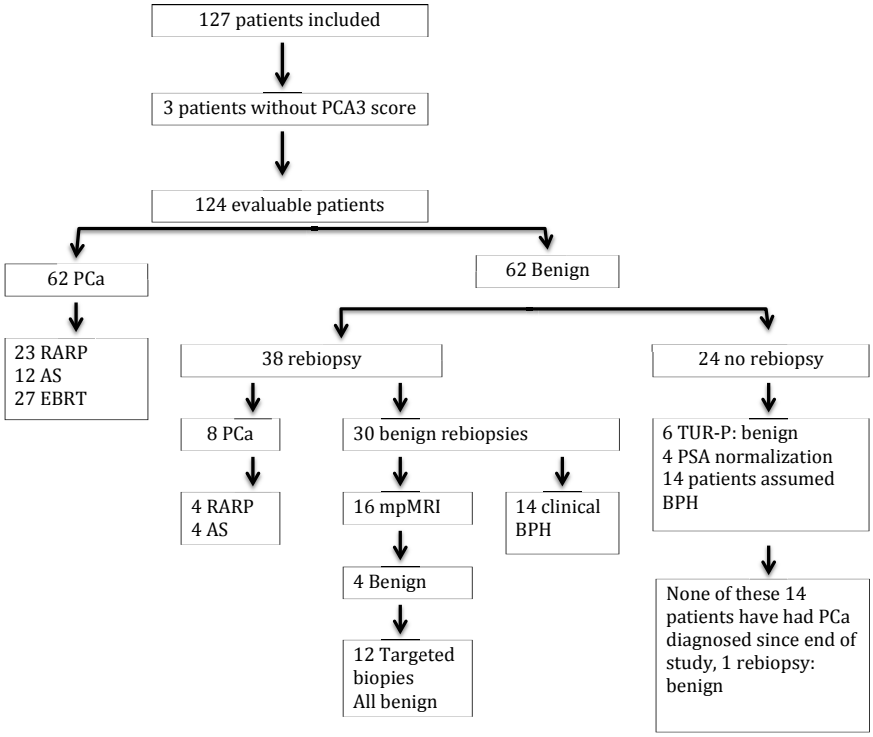
This paper focuses on the performance of PCA3 in an initial biopsy setting, and the results are used to externally validate Hansen´s nomogram [34]. It includes only the systematic biopsies from the initial biopsies, as this is the same as those used in the internal validation of the nomogram. The predicted probability of PC according to Hansen´s nomogram was calculated after receiving the coefficients for the logistic regression analysis from Hansen et al. We then performed a logistic regression on our own data by entering the same parameters, and compared these data with the estimated probability calculated by Hansen´s nomogram. From the authors of Hansen´s nomogram, a threshold value of 30% is suggested as a biopsy indication, which represents a 115 point score when applied to the individual patient.
The performance of PCA3 alone is estimated with cut-off values of 21 and 35.

Paper IV

In this paper, all available histo-pathological data from initial biopsies and rebiopsies are included, as well as follow-up data from the patients with benign biopsies, with a mean observation time for these patients of 46.7 ± 1.5 months (median 44.4, range 41–55). For details, see Figure 7.

Figure 7:

Flowchart for patients included in the papers III and IV.



Legend to figure:
Flowchart of the 127 included patients in this study. The numbers are indicating the number of patients in each group.
Abbreviations: PCA3: Prostate cancer gene 3. PCa: Prostate cancer. RARP: Robotic assisted radical prostatectomy. AS: Active surveillance. EBRT: External beam radiation therapy. TUR-P: Transurethral resection of prostate. PSA: Prostate specific antigen.

The focus of this paper is to investigate the combination of RTE and PCA3 as selection tools before biopsy. In this paper, we have defined a positive RTE in line with Paper II as a reproducible visible hard lesion, and we have used RTE by patient and not by ROI. We have focused on the ability of these methods combined for the detection of clinically significant PC, and whether these tools can contribute to a reduction in unnecessary biopsies, as well as a reduction of overdiagnosis and overtreatment. A PCA3 score of 35 is chosen for the analyses, and we defined four different groups for the analyses:

Group 1: Both RTE and PCA3 positive

Group 2: RTE positive and PCA3 negative

Group 3: RTE negative and PCA3 positive

Group 4: Both RTE and PCA3 negative

A logistic regression analysis was performed, entering age, PSA and Pvol with the addition of a dichotomized PCA3 score of 35 and RTE together. These results are presented in ROC curves, and the results are presented as AUC for both models. The results from the univariate and multiple logistic regression models are also presented in a table as OR with p-value by the use of the Likelihood Ratio test.

Statistics

To be able to draw conclusions based on a limited study population, different statistical methods have been used.

For continuous data, such as PSA, Pvol and PCA3 score, the t-test was used. The numbers are presented as means and median, and the uncertainty of the means are presented as either a standard error of mean (SEM) or standard deviation (SD), or by presenting the 95% confidence interval (95%CI). For nominal data, such as a comparison of groups with different Gleason scores, a Mann-Whitney U-test was used. Cross-tables were used to find differences between groups of categorical data, and a chi-square test was used to determine the significance level of these differences. A p-value <0.05 was considered as the limit for statistical significance.

For the calculation of sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and accuracy, cross-tables were used, and the values were calculated in the standard manner [138]. In Papers I and II, these parameters are presented per ROI, while in Paper IV they are calculated per patient.

Logistic regression analyses were performed in univariate and multiple models in Papers II and IV, whereas the likelihood ratio test was used to find the p-value. In Paper III, a multiple logistic regression model was developed to estimate our own calculated scores based on the variables of PSA, DRE and Pvol, with a dichotomized PCA3 score of 21 and age. A Hansen´s score was calculated by using the same parameter based on the coefficients received from the authors of the Hansen nomogram. The intraclass correlation (ICC) between Hansen´s score and our own score was calculated and expressed as a Cronbach´s alpha. Both the performance of Hansen´s score in our material and our own score were expressed using receiver operating curves (ROC), and presented as an area under the curve (AUC).
In Paper III, a statistician developed the multiple logistic regression models. In Papers II and IV, the same statistician was consulted regarding the correct use of the regression analyses.
The statistical calculations were performed using IBM® SPSS® Statistics software in different versions.

Results

Paper I

Out of a total of 40 patients, three patients had insignificant cancers, hence leaving 37 patients for the analyses.

Table 3:

The table below shows the patient characteristics

PSA	10.7 (10; 3.3–33)
Pathological T-stage	
pT2	29 (78)
pT3	8 (22)
PCA3 score	64 (51; 11–258)
<35	11 (30)
>35	26 (70)
RP specimen Gleason grade and score	
Grade 3+3, score 6	16 (43)
Grade 3+4, score 7a	20 (54)
Grade 4+3, score 7b	0
Grade 4+4, score 8	1 (3)

Data are shown as n (%) or mean (median; range).

PCA3

The mean PCA3 score was 64 (median: 51, range: 11 – 258). A PCA3 score of ≥ 35 (positive test) was observed in 26 patients, leaving 11 patients with a false negative PCA3 test. No significant differences were found regarding the PCA3 score and the Gleason score. The percentage of positive PCA3 tests in pT2 patients was 63%, in pT3 88%, although this difference was not statistically significant (p=0.23).

RTE

One or more tumours with a correct location were found in 33 of 37 patients (89%), while the largest tumour was correctly identified in 27 patients (73%). There was a close to statistically significant ($p = 0.079$, exact Chi-square test) difference between pT3 and pT2 tumours, as all eight pT3 tumours were identified by RTE (100%), but only 19 of 29 pT2 tumours (66%) were correctly identified. The RTE was false negative in four patients, and had false positive lesions in four patients.

RTE and PCA3 combined

Only one patient had both a negative PCA3 score and a negative RTE, leading to a 97% detection rate. The patient with both tests negative had a small tumour of 1.1 cc, with the tumour only constituting 2.6% of the total Pvol.

Paper II

PC was diagnosed in 64 patients in the initial biopsy setting, whereas another eight patients were found to have PC on rebiopsies within the next six months, thus yielding a detection rate of 50% for the initial biopsies and 56% in total.

For the initial biopsies, the distribution of patients according to the D´Amico criteria were 13 low-, 24 intermediate- and 27 high-risk.

In the initial biopsy setting, all patients had a 10-core systematic biopsy, yielding a total of 1,270 systematic biopsies, of which 236 biopsies with PC were found. Eighty-six patients had one or more suspect lesions on RTE, and a total of 287 targeted biopsies were obtained, of which 80 exhibited PC. This gave a significantly higher frequency of positive cores in the targeted biopsies compared to the systematic biopsies ($p<0.001$). There was also a trend towards a higher fraction of PC in the targeted than in the systematic biopsies (42% vs. 33%, $p=0.087$). The group of patients with PC in the targeted biopsies also had a significantly higher Gleason score than those found in systematic biopsies.

In the initial biopsies, three patients were diagnosed solely on the RTE-targeted biopsies; 31 were only found on the systematic biopsies and 30 had PC in both RTE-targeted and systematic biopsies.

A multiple logistic regression model was used to identify the markers for high-risk PC. Moreover, the Pvol and positive RTE came out as independent markers for the detection of high-risk PC.

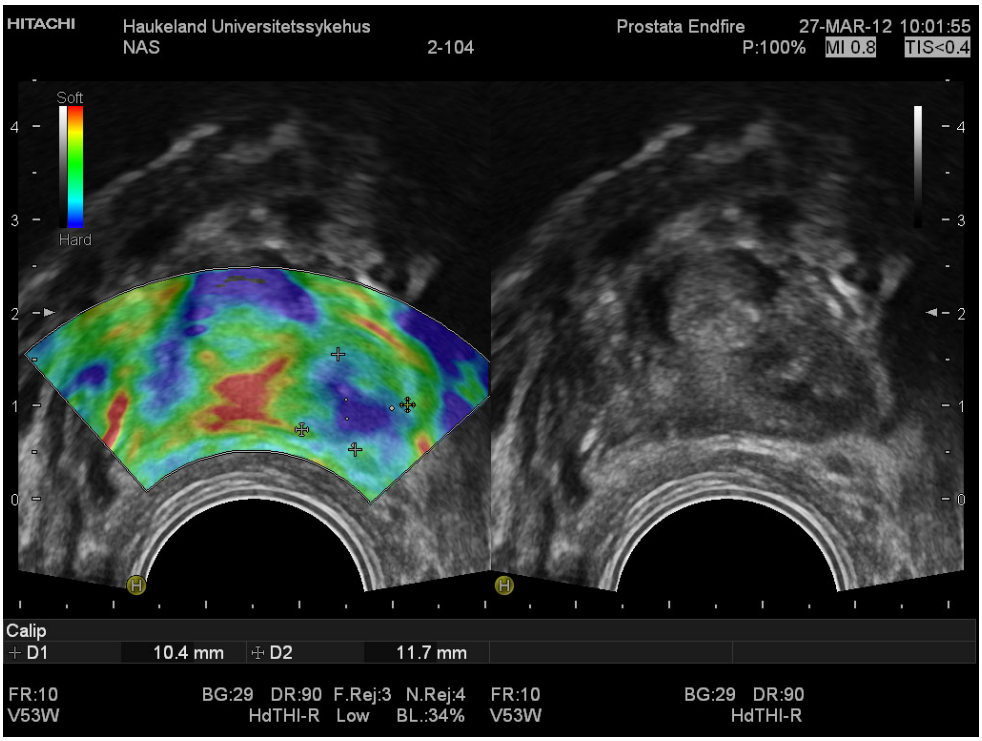
For the calculation on the performance of RTE by ROI, all the available histo-pathological data were used, including data from the initial biopsies, rebiopsies, targeted biopsies and from the gross-section pathology of those patients treated with RARP. The calculations were done for any PC, and for high-grade PC defined here as Gleason grade 4+3, score 7 and higher, see Table 4.

Table 4:

	Sensitivity (%)	**Specificity (%)**	**NPV (%)**	**PPV (%)**	**Accuracy (%)**
Any PC (All Gleason scores)	42	83	79	49	72
High grade PC (Gleason score 7b-10)	60	80	97	20	78

Figure 8:

RTE shows a tumour located in the peripheral zone left apex, two targeted biopsies revealed a Gleason grade 4+5, score 9 tumour



Paper III

In three patients, PCA3 score could not be calculated because the urine did not contain enough cells, leaving 124 evaluable patients. The systematic initial biopsies detected PC in 59 patients (47.6%), and in 65 patients (52.4%) no PC was found. For the distribution of cT-stage and Gleason grade and score, see Table 5. Table 6 presents the PSA and PCA3 score.

Table 5:
Distribution of clinical stage (cT), Gleason grade and score of the patients with prostate cancer on initial systematic biopsies (n = 59)

	Number of patients (%)
Clinical stage (cT)	
T1c	27 (45.8)
T2a	10 (16.9)
T2b	6 (10.2)
T2c	10 (16.9)
T3a	6 (10.2)
Gleason grade and score	
3+2 = 5	1 (1.7)
3+3 = 6	19 (32.2)
3+4 = 7a	22 (37.2)
4+3 = 7b	6 (10.2)
4+4= 8	4 (6.8)
4+5 = 9	5 (8.5)
5+4 = 9	2 (3.4)

Table 6:
The table below shows the patient characteristics for 124 evaluable patients, both with and without prostate cancer (PC) regarding PSA and PCA3 score. P-values are calculated for the difference of means between groups, both with and without PCa at initial biopsies using the Student's t-test.

Clinical parameter	PC (n=59)	No PC(n=65)	p-value	Total (n=124)
PSA (ng/ml) *mean (SD; median)*	10.2 (5.3; 8.1)	8.11 (3.9; 6.6)	0.014	9.1 (4.7; 7.2)
PCA3-score *mean (SD; median)*	79.6 (70.1; 55.0)	29.1 (25.9; 19.0)	<0.001	53.1 (57.5; 33.5)

In this paper, the definition of low-grade PC (LGPC) and high-grade PC from Vickers was used, with LGPC defined as a Gleason score of ≤ 6 and HGPC as a Gleason score ≥ 7 [139]. There were no statistically significant differences for PCA3 score or PSA between these two groups.

When comparing the Hansen´s nomogram to our own estimated score, there was a high ICC expressed as a Cronbach´s alpha of 0.959.
The AUC of the ROC using a Hansen´s nomogram was 0.806. By adding the PCA3 to our own estimated score, the AUC was increased from 0.819 to 0.842.
When applying the suggested 30% calculated probability of PC as a biopsy indication, 47 patients (38%) would have been advised against biopsy. Nine of these patients had PC

on the initial systematic biopsies, three had LGP and six had HGPC. After EAU risk stratification, there were two low-, five intermediate- and two high-risk PCs in this group. When applying a 20% threshold value, 22 patients would have been advised against biopsy, and among these only one was low-risk PC.

For PCA3 alone, the performance was tested for two different cut-off values, see Table 7.

Table 7:
Diagnostic performance of prostate cancer gene 3 (PCA3) score for cut-off values of 21 and 35, expressed as sensitivity, specificity, negative predictive value (NPV), positive predicted value (PPV) and accuracy for 124 evaluable patients

PCA3 cut-off	Sensitivity	Specificity	NPV	PPV	Accuracy
>35	71%	72%	73%	70%	72%
>21	81%	55%	77%	62%	67%

Paper IV

In three patients the urine did not contain enough cells for the PCA3 analysis, thereby resulting in 124 evaluable patients. A total of 70 patients were diagnosed with PC.

For distribution of clinical stage, a Gleason grade and score and risk stratification according to EAU risk groups was used, see Table 8.

Table 8:

	Number	%
Gleason grade and score		
3+2=5	1	1
3+3=6	26	21
3+4=7a	19	15
4+3=7b	11	9
4+4=8	6	5
4+5=9	5	4
5+4=9	2	2
Clinical stage		
T1c	35	50
T2a	12	17
T2b	6	9
T2c	11	16
T3a	6	9
EAU-risk		
Low-risk	21	30
Intermediate-risk	32	46
High-risk	17	24

RTE was positive in 85 patients and negative in 39. The mean PCA3 score was 73.6 in patients with PC and 26.6 in patients without PC, and this difference is statistically significant ($p<0.001$). For the performance of RTE, PCA3 and both parameters combined, see Table 9. In this table, we have analysed for all PC, for the group of intermediate-risk and high-risk together, and for high-risk alone.

Table 9:

Risk group	Parameter	Sensitivity	Specificity	NPV	PPV
Any PC	RTE	74%	39%	54%	61%
	PCA3	64%	78%	66%	80%
	Combination	91%	26%	70%	62%
IR and HR PC	RTE	86%	43%	82%	51%
	PCA3	71%	66%	78%	58%
	Combination	96%	24%	90%	55%
HR PC	RTE	88%	35%	95%	18%
	PCA3	82%	57%	95%	23%
	Combination	100%	19%	100%	16%

We split the patients into four groups as described earlier.

Group 1:

In total, 44 patients, 38 with PC and six without. Thirty patients had intermediate- or high-risk PC in this group.

Group2:

In total 41 patients, 14 with PC and 27 without PC.

Group 3:

In total 16 patients, 10 with PC and six without PC.

Group 4:

In total 23 patients, 15 without PC and 8 with PC, of whom two had an intermediate-risk and six had low-risk PC.

Univariate and multiple logistic regression models for predicting intermediate- and high-risk PC were developed, and the results are given in the table presented below. In these analyses, PCA3 came out as a significant marker for high- and intermediate-risk PC. RTE came out as a significant marker in the univariate analysis ($p=0.001$), while in the multiple logistic regression model it was close to significant ($p=0.068$).

Table 10:

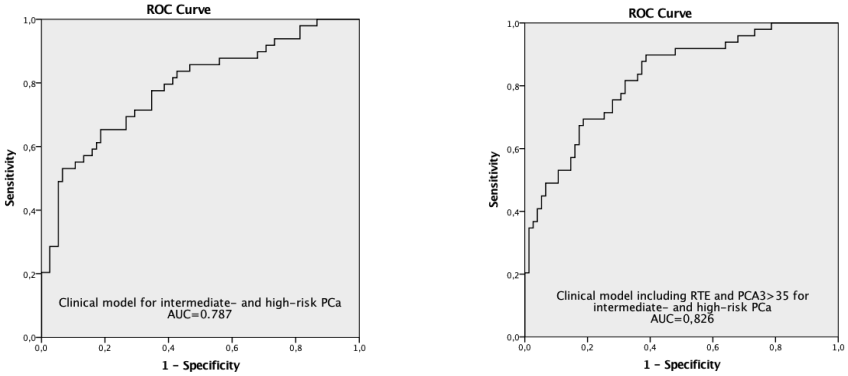
Logistic regression model entering age, PSA, Pvol, Positive RTE and a dichotomized PCA3 score of 35

	Simple			Multiple					
	Unadjusted			Fully adjusted			Final model		
Variables	**OR**	**95% CI**	**p-value****	**OR**	**95% CI**	**p-value****	**OR**	**95% CI**	**p-value****
Age (cont. in years)	1.04	(0.98,1.10)	0.188	1.04	(0.96, 1.13)	0.287			
PSA (cont. in ng/ml)	1.18	(1.08, 1.29)	<0.001	1.19	(1.07, 1.34)	0.001	0.18	(0.03, 1.14)	0.001
Pvol (cont. in ml)	0.98	(0.96, 0.99)	0.003	0.97	(0.95, 0.99)	0.005	1.04	(1.04, 1.07)	0.009
Positive RTE (Y/N)	4.46	(1.78, 11.22)	0.001	2.73	(0.96, 7.79)	0.052	2.56	(0.91, 7.23)	0.068
PCA3 (>35 vs. <35)	5.00	(2.28, 10.95)	<0.001	3.31	(1.27, 8.63)	0.013	4.12	(1.71, 9.91)	0.001

*** P-value by the use of the Likelihood Ratio test.*

The results from the logistic regression were also expressed in the ROC curves presented below in Figure 9. The addition of PCA3 and RTE led to an increase in the AUC of 0.039.

Figure 9:



Discussion

RTE

In Paper I the ability to identify PC lesions was clearly demonstrated. The total tumour detection rate was 89% for any lesion and 73% for the largest tumour with the correct location. Pallwein et al. investigated 15 patients planned for RP. In their analyses per cancer foci the RP-whole mount section pathology revealed a total of 35 foci, in 28 of these the RTE was positive leading to a sensitivity of 80% per foci[67]. They found at least one cancer area in all 15 patients leading to a sensitivity of 100% per patient compared to our rate of 89% per patient. Salomon et al. included 109 patients planned for RP in their evaluation on RTE[140]. They used the same six-zone distribution as in our studies and analysed the results per ROI and found a sensitivity of 75.4% per ROI. This is in line with our results of 73% sensitivity for the largest tumour with correct location. Some later studies have also included mp-MRI in addition to RTE and evaluated these methods head to head in the same patients planned for RP[141, 142]. In the study of Pelzer et al. they found an overall sensitivity of PC detection per patient of 92% for RTE and 84% for mp-MRI[141]. On the ROI analyses RTE performed better in the apical and middle parts of the prostate; mp-MRI was better at the base and in the TZ. In the paper from Junker et al. they found comparable results between mp-MRI and RTE, thus enlightening some of the issues of RTE regarding prostate size[142]. This is in line with our findings in paper 2 where RTE performed better in smaller prostates. Goddi et al. described this challenge; the normal RTE pattern in prostates enlarged due to BPH is unevenly inelastic, thus producing hard benign lesions[143].

There were four false positive lesions on RTE in our first study; this was of course a highly selected material since all patients had already been diagnosed with PC and were planned for RP. To help limit the bias as much as possible, the examiner was blinded to the biopsy results and to other investigations such as mp-MRI. Another challenge evaluating RTE in this material was the comparison of RTE to the whole-mount section pathology. The RP specimen were sliced at 90 degrees to the rectum, which is a different angle than the projections from the end-fire probe; these projections will also differ for the individual patients because of difference in anatomy. The prostate was further divided into the previously described six regions, so it was sometimes difficult to decide

whether the RTE suspected lesion was at the correct location according to the RP whole mount section pathology. In addition, the RTE examiner must decide in real-time about the location, and there are no anatomical structures to decide between the areas. To help overcome this challenge, an RTE investigator independent urologist, together with an uropathologist, performed a consensus reading, and the results from this reading were used in the further analyses. Another limitation to this paper is that one urologist performed all the RTE examinations, hence leaving it impossible to evaluate the inter-observer variability. Even with these considerations, the method seemed to be promising and worth examining further in a biopsy setting.

Because RTE is a real-time examination, it has some limitations regarding learning curve and inter-observer variability. Since one urologist performed all the examinations, the latter has not been addressed. When it comes to the learning curve, there is evidence supporting that there is a learning curve, but that the novice can achieve good results after approximately 30-60 examinations [144]. In our studies, the examiner performing RTE had training by Professor Ferdinand Frauscher and Professor Friedrich Aigner at the University Hospital of Innsbruck, Austria. This is considered an expert centre in the field of TRUS and RTE. RTE was also tested in at least 20 patients before inclusion started, which means that at least for Papers II-IV, the learning curve should be a minor issue.

RTE can also be used in a biopsy setting for targeted biopsies. This topic is addressed in Paper II of this thesis. Three patients (4.7%) were diagnosed by RTE targeted biopsies alone, 30 (46.9%) were diagnosed on both the targeted and systematic biopsies, whereas in 31 patients (48.4%) PC was found only on the systematic biopsies. The detection rate of the targeted biopsies compared to the systematic biopsies is lower than the one described by Aigner et al.; they found a higher detection rate by RTE and targeted biopsies than for the systematic biopsies[70]. Their material is different from ours as they only included patients in whom the PSA levels were between 1.25 and 4 ng/ml and the two materials can therefore not be directly compared. In the paper from Salomon et al. the total detection rate in the primary biopsy setting was 51.7%, this is in line with our own results[72]. Of the 297 patients with PC on the initial biopsies they found that 259 (87%) were detected by systematic biopsies and 140 (47%) were

detected on the RTE targeted biopsies. In 38 patients (12.8%) the diagnosis was based solely on the RTE targeted biopsies. By adding the RTE targeted biopsies to the systematic biopsies they found an incremental detection rate of 14.7%. This increase is higher than in our paper where the addition of RTE targeted biopsies led to an incremental detection rate of 5%. They concluded, that the RTE targeted biopsies have too low sensitivity used alone, but that the RTE targeted biopsies lead to an incremental detection rate in the initial biopsies. These findings are more in line with our own results in paper II than the results from Aigner et al.

There were some differences between the targeted and the systematic biopsies. The frequency of positive cores was 28% for the targeted biopsies and 19% for the systematic biopsies ($p<0.001$). In the study of Aigner et al. they found a frequency of positive cores in the targeted biopsies of 24%, which is similar to our results [70]. We also found a fraction of cancer tissue for the positive biopsies of 42% in the targeted compared to 33% in the systematic biopsies, which is a difference close to statistical significance in favour of the targeted biopsies ($p=0.087$).

We found that the targeted biopsies influenced the distribution of Gleason score, and for some patients it had a clinical important influence. In one patient the systematic biopsies revealed only one positive biopsy with a Gleason score of 6, the RTE targeted biopsies revealed that the patient had an aggressive tumour with a Gleason score of 8 (grade 4+4). Both Salomon et al. and Brock et al. noted that RTE targeted biopsies lead to an incremental detection rate in a biopsy setting[71, 72]. In the paper from Salomon et al. they also addressed the matter of Gleason score in the biopsies. In line with our results they found that the addition of RTE targeted biopsies led to an upgrading of Gleason grade; Some patients with a Gleason grade of 3 on the systematic biopsies had a Gleason grade of 4 or 5 on the RTE targeted biopsies.

In our study (Paper II) we found that a positive RTE was an independent marker for high-risk PC, and also that RTE-positive patients had a significantly higher Gleason score than RTE-negative patients. This is in line with the publications from both Aigner et al. and Nelson et al. [70, 145]. In a recent publication Boehm et al. showed that RTE-targeted biopsies improved the agreement of the biopsy Gleason score and the final

Gleason score found at radical prostatectomy specimen, suggesting that the biopsies are more representative [73]. Brock et al. did not find an association between RTE and Gleason score, which might be because of the difference in the distribution of patients. In our study, 80% of the patients were either intermediate- or high-risk patients, whereas in the study from Brock et al. most patients were low-risk patients[71]. The difference in distribution may have several explanations. One possibility is the inter-observer variability in Gleason grading and scoring. The uropathologists at our institution are scoring according to the ISUP criteria, leaving this as a less probable explanation. There is some evidence supporting a high prevalence of high-risk PC in the Norwegian population; Hernes et al. showed that 42% of the patients amenable for curative treatment had a high-risk PC [146].

When analysing the performance per ROI, we included all available histo-pathological data to get the performance as exact as possible. We did analyses for both any PC and for high-grade PC. The sensitivity increased from 42% to 60%, while the specificity was 80% in both categories. More important was the high NPV for a high-grade PC of 97%. This demonstrates that RTE may be used as a selection tool before biopsy since a negative RTE argues against a large aggressive tumour, and that a positive RTE can strengthen the indication to perform a prostate biopsy.

In our study we used periprostatic local infiltration anaesthesia. This may lead to a difference in strain if injected intraprostatically. To help reduce this risk, caution was taken to inject it only periprostatically, and the volume was reduced to 3 cc per injection. We do not think that this has compromised our results.

Another modality for targeted biopsies is mp-MRI. In the publication from Siddiqui et al. they concluded that mp-MRI-targeted biopsies led to an increased detection of high-risk PC and a decreased detection of low-risk PC, and they also questioned the need for systematic biopsies[59]. In this paper they excluded the patients with a normal mp-MRI and only approximately 20% of the patients were in the initial biopsy setting. Filson et al. also addressed the use of mp-MRI and targeted biopsies in a large study, in which many of the patients were biopsy-naïve[57]. In this study all patients, including those with a normal mp-MRI, had a systematic biopsy. Clinically significant PC was found in

17% of the patients with a normal mp-MRI. This demonstrates one of the challenges of targeted biopsies both for mp-MRI and for RTE; some significant cancers will be missed when targeted biopsies are used alone.

In the fourth paper, we have looked into the matter of using RTE as a selection tool before biopsy, in this case we have analysed RTE per patient, and not per ROI. A strength of this study is that we have a long follow-up of all the patients without PC in the initial biopsy setting. In the group of patients without rebiopsies, 14 patients had clinical BPH as the reason for not performing a rebiopsy. In this group, the Pvol was a mean of 98 ml (median 83.5, 95%CI 70.5-126.0), and the PSA was only slightly elevated to a mean of 7.7 (median 6.7, 95%CI: 6.1-9.4). This strengthens the probability of BPH as the reason for the slight elevation in PSA level. In the group of patients with benign rebiopsies, we also performed an mp-MRI in 16 patients. In 12 patients, the mp-MRI was considered abnormal and targeted biopsies were performed; none of these patients were diagnosed with PC. This might have several explanations. Firstly, there was only a maximum of PI-RADS grade 3 abnormalities. Secondly, all patients in this group had been biopsied with initial and repeat biopsies, which can make the MRI pictures more difficult to interpret. Thirdly, we did not have any anterior tumours in this group, as these are the tumours most likely to be missed by systematic biopsies [81, 82]. With the reasons mentioned above we believe that in this paper we are as close to the true prevalence as possible at the time of the examinations.

PCA3

When we started the inclusion of patients, PCA3 was a novel and promising marker. In our first paper, we investigated PCA3 in patients with known PC. In this series, we are only able to evaluate the sensitivity of the test; since all patients had a diagnosed PC, we cannot make any statements about specificity or predictive values. PCA3 must be recognized as a continuous marker, with an increasing risk of PC with increasing values. Even though it is a continuous marker, threshold values are suggested for the clinical use. The cut-off value is still to be defined.

In Paper I, we used a cut-off value of 35 for our calculations. This was at that time the most investigated value, thus resulting in a sensitivity for the detection of PC of 70%.

This was in line with other comparative studies[147, 148]. Durand et al. showed that the PCA3 score was correlated to Gleason score, as the PCA3 score was higher in patients with a Gleason score ≥ 7 than 6 or lower. Even though it was significant, the clinical impact was considered modest [148]. Durand et al. also showed significant associations between tumour volume and clinical stage. In Paper I, we were not able to find any association between Gleason score or tumour volume and PCA3 score. We found a difference between the percentage of positive PCA3 tests (with cut-off of 35) and pathological stage, although this difference was not statistically significant. There are several possible explanations for these differences. The most important contributing factor is probably the study size; we only had 37 eligible patients compared to the study of Durand et al., which had 160 patients included. The larger study population makes it possible to identify smaller differences between the groups.

In Paper III, we have validated Hansen´s nomogram. In this paper, a PCA3 score of 21 was used as cut-off value, as this was the value used by Hansen et al [34]. We also tested a cut-off value of 35. The sensitivity (71% vs. 81%) increases with a decreasing PCA3 score but at the cost of the specificity (72% vs. 55%), thereby leading to a large number of false positive tests.

We found a high ICC (Cronbach´s alpha=0.959) between our estimated scores and the scores developed using Hansen´s nomogram, thus suggesting a high reliability of the nomogram. There was a statistically significant difference between the calculated probability using Hansen´s nomogram and our own score, since Hansen´s nomogram predicted a slightly higher probability. The difference was only 0.02 and is negligible for clinical use, even if statistically significant (p=0.033).

In Hansen´s nomogram, PCA3 only affected the biopsy decision for a small subset of patients; in our cohort, this was the case for only 17 patients. Albern and Freedland discussed this matter in an editorial comment, [149] and because the biopsy decision is only influenced for a small subset of patients, it cannot be justified to use in all patients before the initial biopsy.

In the paper by Hansen et al., they suggested a threshold probability of 30% as a biopsy indication. In our less screened cohort, this would lead to a substantial reduction of biopsies of 37.9%, but at the cost of missing many significant cases of PC. In our material, we would miss a total of nine patients with PC, of whom six had high-grade PC. This is higher than in other publications. Both Hansen et al. and Ruffion et al. showed a lower probability of missing high-grade PC than our results indicate [34, 150]. This is probably because our cohort was less screened, and that there seems to be a difference in the distribution of patients regarding risk stratification. For this lesser screened cohort, a 20% suggested probability was safer to implement, in this case 22 patients (17.8%) would have been advised against prostate biopsy. Within this group, only one low-risk PC was found at the initial biopsy. This underscores the need for an external validation of nomograms before taking them into clinical use; this matter is also thoroughly described by Turo et al. [151].

In the fourth paper, PCA3 has been tested as a marker to identify clinically significant PC. We have defined this as PC classified as intermediate- or high-risk PC according to EAU guidelines. This is in line with current evidence suggesting a major risk of overtreatment when treating low-risk PC. Vickers et al. described the results from SPCG4 trials, concluding that there is a huge risk of overtreatment in patients with a Gleason score of 6 and a T1 tumour. Wilt et al. concluded the same in the summary after the Prostate Cancer Intervention Versus Observation Trial (PIVOT) [110, 111]. We decided to use a PCA3 score of 35 as a cut-off value for the analysis. When used alone, we achieved a sensitivity of 71% and a specificity of 66% for the group of clinically significant PC. If applied to a cohort of patients as a tool for biopsy decision, we would miss a substantial number of patients in need of diagnosis and treatment.

In a logistic regression analysis PCA3 came out as an independent marker for predicting significant PC. When applied to a clinical model, together with RTE, it also produced an increase in the AUC. This is in line with earlier publications, in which PCA3 has been shown to increase the diagnostic performance of nomograms; this is discussed earlier. PCA3 has also been shown to be of value when combined with mp-MRI [35].

PCA3 can be seen as a promising marker, but it also has several issues that need to be addressed. No clear cut-off value exists for the clinical use. Different values have been described and tested in different cohorts, hence making it more difficult to implement in daily clinical use. Since it only affects the biopsy decision in some cases, it is hard to justify being used in all patients as a pre-biopsy decision tool.

Most studies had previously investigated PCA3 for the repeat biopsy setting; this is also the indication for the test according to the FDA, which in this setting suggests a score of 25 as cut-off value[38]. Auprich et al. investigated the role of PCA3 in the first, second and third or more rebiopsy. The PCA3 performed best in the first repeat biopsy, and showed in this setting a capability to reduce the number of rebiopsies by 72% when using a threshold sensitivity of 75%; this was achieved with a PCA3 score of 44 [28]. When applying a sensitivity threshold of 85%, the cut-off value was 34 and 50% of the biopsies could be avoided. The performance of PCA3 was worse in the second and third rebiopsy. Considering this, PCA3 can be considered a helpful tool in decision-making before performing a rebiopsy, especially before the first rebiopsy [28]. Another issue to be addressed in the paper of Auprich et al. would be whether or not rebiopsies are beneficial at all, since the majority (72.7%) of the patients diagnosed with PC had a biopsy Gleason score of 6. For most of these patients, a rebiopsy would only imply an increased risk of overdiagnosis and overtreatment. This is in line with our own results. We detected an additional eight patients with PC on the rebiopsies. Of these patients six were low-risk patients and two had favourable intermediate-risk PC. Considering the evidence regarding overtreatment these patients probably have no benefit from further diagnostic procedures and treatment[110, 111].

Combination of PCA3 and RTE

To the best of our knowledge, the papers on the combinative use of RTE and PCA3 are the first publications combining these two markers.

Paper I must be considered a methodological study to investigate the ability of PCA3 and RTE, whether alone or in combination, of detecting PC. The ability of RTE and PCA3 alone has been previously discussed. In Paper I, the two markers seem to be of additional value when used in combination. Only one patient had a negative RTE and a

negative PCA3 score, therefore leading to a detection rate of 97% of significant PC. In this publication, we decided to use the very strict definition of Epstein et al. for significant PC, which differs from Paper IV [22]. Later publications have suggested a tumour volume of 0.5 cc as the limit; in our study, only three patients had a tumour volume of 0.2-0.5 cc, while all of them had a secondary Gleason pattern grade 4 and were not considered insignificant [152]. In past years, the matter of overdiagnosis and overtreatment has been elucidated and given a lot more consideration. Two randomized clinical trials investigating the effect of RP have presented results clearly demonstrating a substantial risk of overtreatment of all low-risk PCs, but also showing a PC-specific survival benefit for intermediate- and high-risk PC patients [109-111]. This is also the reason that we have defined significant PC as intermediate- and high-risk PC in the fourth paper. The combinative use of PCA3 and RTE has a capability of detecting 96% of these patients. If both examinations come out negative, there is a very small risk of missing clinically significant PC, this is mirrored in the high NPV of 90% for the group of intermediate and high-risk PC together. If only applied to the high-risk group, the NPV is 100%. Since the study sample is relatively small and includes only 17 high-risk PC patients, these results must be interpreted with caution. On the other hand, these results support the findings from the first paper. In this setting, we can reduce the number of unnecessary biopsies by approximately 19%, with a low risk of missing clinically significant PC.

The considerations of implementing these markers in the initial biopsy setting are the increase in costs. RTE is a software module that can be installed on several Hitachi ultrasound machines. After installation, there are no costs related to the use of RTE when performing a TRUS, and it is also quick to perform. Since both parameters have to be negative if used as a biopsy decision tool, one could make an argument to measure PCA3 in all patients with a negative RTE; in our cohort, 41 patients had a negative RTE. In these patients, urine could be sampled after TRUS and sent for analysis. The patients with a negative PCA3 score could then safely be advised against having a prostate biopsy. In this way, it is possible to reduce the costs and only do the PCA3 analysis in those patients where it affects the biopsy decision. Approximately 20% of the patients could then be spared the discomfort and risk of a prostate biopsy, with only a small risk of missing clinically significant PC.

Conclusions

- RTE has the ability of visualizing and detect PC especially in smaller prostates.
- RTE can be used for targeted biopsies. Although the sensitivity is too low to replace systematic biopsies, RTE contributes to a higher detection rate and to more representative biopsies.
- RTE is an independent marker for detection of high-risk PC. It also has a high NPV for detection of these cancers, and RTE can be used as a selection tool in a pre-biopsy setting for better identification of patients in need of prostate biopsy.
- Hansen´s nomogram is valid in our cohort and PCA3 contributes significantly to the AUC when added to the nomogram.
- The combined use of PCA3 and RTE is better than each method alone and can be used to reduce the number of prostate biopsies.

Future Aspects

This thesis leaves some questions to be addressed in future research:

Targeted prostate biopsy is a field of growing interest. In our study we have used RTE for the targeted biopsies, while other studies have investigated mp-MRI with targeted biopsies. There is evidence that both methods are contributing to a more accurate diagnosis. In an initial biopsy setting it is unclear which imaging procedure performs the best. The approach of using real-rime examinations, like the different ultrasound modalities, is appealing because of the effectiveness both regarding costs and time. It would be of interest to perform a randomized clinical trial testing one group using a multiparametric ultrasound examination including B-mode, RTE and Doppler examination as the method for imaging and targeted biopsies, and one group using mp-MRI for imaging and image fusion with ultrasound for targeted biopsies.

PCA3 and TMPRSS2 have shown to be of additive value. It would be of interest to examine further the role of different biomarkers used combined in an initial biopsy setting. By testing a panel of different biomarkers it could be possible to identify markers with additive value and so to perform a better selection of patients before prostate biopsy. Such a study might contribute in reducing the overdiagnosis and overtreatment in prostate cancer.

Errata

Table 10 contains errors regarding OR for PSA and PVol. The correct table follows below:

	Simple			Multiple					
	Unadjusted			Fully adjusted			Final model		
Variables	**OR**	**95% CI**	**p-value****	**OR**	**95% CI**	**p-value****	**OR**	**95% CI**	**p-value****
Age (cont. in years)	1.04	(0.98,1.10)	0.188	1.04	(0.96, 1.13)	0.287			
PSA (cont. in ng/ml)	1.18	(1.08, 1.29)	<0.001	1.19	(1.07, 1.34)	0.001	1.19	(1.06, 1.33)	0.001
Pvol (cont. in ml)	0.98	(0.96, 0.99)	0.003	0.97	(0.95, 0.99)	0.005	0.98	(0.96, 0.99)	0.009
Positive RTE (Y/N)	4.46	(1.78, 11.22)	0.001	2.73	(0.96, 7.79)	0.052	2.56	(0.91, 7.23)	0.068
PCA3 (>35 vs. <35)	5.00	(2.28, 10.95)	<0.001	3.31	(1.27, 8.63)	0.013	4.12	(1.71, 9.91)	0.001

*** P-value by the use of the Likelihood Ratio test.*

This is the same table as used in Paper 4, there it appears as table 3. The editorial office of BMC Urology has been informed of the errata.

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A positive real-time elastography is an independent marker for detection of high-risk prostate cancers in the primary biopsy setting

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Objective

- To evaluate the performance of real-time elastography (RTE) in an initial biopsy setting.

Patients and Methods

- In the period from February 2011 to June 2012, 127 consecutive patients were included in the study.
- We used a Hitachi Preirus with Hi-RTE module, a prostate end-fire transrectal probe was used for RTE and for targeted biopsies, and a simultaneous biplane probe was used for the standard systematic biopsies.
- The peripheral zone of the prostate was divided into six regions, and each biopsy obtained was referred to a specific region.
- All patients were first examined with RTE and, if cancer was suspected, targeted biopsies were taken. A standard systematic 10-core biopsy was then taken in all patients.

Results

- In all, 64 (50%) patients were diagnosed with prostate cancer in the initial biopsy setting. Three patients were diagnosed solely on RTE-targeted biopsies, 31 were found only in systematic biopsies, and 30 were correctly diagnosed with both methods.
- In the RTE-positive group there was a significantly higher frequency of positive cores, a lower prostate volume, a higher Gleason score, and a higher fraction of cancer tissue in each core.
- In a multiple regression model RTE was an independent marker for high-risk cancer.
- The sensitivity of 42% for all prostate cancers increased to 60% for high-grade prostate cancers.
- Similarly, the negative predictive value increased from 79% to 97%. An additional eight patients were diagnosed with prostate cancer during the study period.

Conclusions

- A positive RTE is an independent marker for detection of high-risk prostate cancer, and a negative RTE argues against such.
- RTE with targeted biopsies cannot replace systematic biopsies, but provides valuable additional information about the tumours.

Keywords

real-time elastography, prostate cancer, prostate biopsy, high-risk cancers, diagnosis, treatment

Introduction

Prostate cancer is the most common cancer in men, accounting for 4299 new cases in Norway in 2009. The incidence was 110 per 100 000 (world standard), and is increasing. The principal tools for detection of prostate cancer are serum PSA level and DRE. PSA and DRE have low specificity, and they do not differentiate between aggressive and indolent disease. Currently the diagnostic standard of care is to perform B-mode TRUS-guided systematic biopsy of the prostate [1,2]. According to European Association of Urology guidelines there is a need for at least two series of biopsies with at least 10 cores in the first series and 12 cores in the repeat biopsy series to exclude prostate cancer the cause of an elevated PSA level [3]. Even after two series of biopsies there will still be men with undetected but significant prostate cancer in the group. On the other hand, due to the low specificity of PSA testing, many men will have to undergo unnecessary prostate biopsies. There is a definite need for improvement in the diagnostic tools in prostate cancer and several new methods are emerging. Real-time elastography (RTE) is an ultrasound (US) method that can be helpful in detecting prostate cancer [4,5]. The advantage of RTE is the possibility for the operator to place the biopsy needle into a

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suspicious area at the same session without the need for additional assessments or the use of contrast enhancement.

In the present study, our primary goal was to compare the ability of RTE-targeted biopsy with the standard 10-core systematic biopsy for detection of clinically significant prostate cancer in the initial biopsy setting, and to evaluate whether RTE increases the detection rate of high-risk cancers.

Patients and Methods

The study was performed in the period from February 2011 to June 2012. The 127 patients gave their oral and written consent to participate in the study after receiving written and oral information about the study. The study protocol was approved by the Regional Committee for Medical and Health Research Ethics (REC) in Western Norway. The patients' characteristics are given in Table 1. The patients were included using active inclusion and the inclusion criteria were:

1. PSA level of 3.0–25.0 ng/mL and/or pathological DRE
2. Age <75 years
3. No prior biopsies within the last 5 years
4. Amenable for radical treatment

All patients were referred by GPs, either because of an elevated PSA level or because of a suspicious DRE. Based on the referral from the GPs, consecutive patients meeting the inclusion criteria were offered inclusion in the study. In all, 127 patients were subsequently included. Very few patients (less than five) refused to participate in the study.

US, RTE, Targeted Biopsies, Standard Biopsies and Report Forms

All patients were examined in the left lateral decubitus using a Hitachi Preirus US machine. The Hitachi Preirus was equipped with an RTE module (Hi-RTE). A V53W transrectal end-fire probe was used for RTE and targeted biopsies. A CC531 transrectal simultaneous biplane probe was used for standard systematic biopsies. RTE displays a colour-coded strain map called an elastogram, which is superimposed on the B-mode images in real-time. RTE visualises strain in the tissue using the extended combined autocorrelation method (ECAM) [6,7].

Table 1 The patients' characteristics.

Variable	Value
Mean (SEM; median, range):	
Age, years	64.2 (0.6;65, 38–76)
PSA level, ng/mL	9.2 (0.4;7.2, 2.2–24.4)
N (%):	
Reason for referral:	
Elevated PSA only	68 (54)
Positive DRE by GP (only)	3 (2)
Family history of prostate cancer	5 (4)
Symptoms	51 (40)
DRE evaluation by Urologist:	
Normal/ BPH	87 (69)
Suspicion of cancer	32 (25)
Inconclusive	8 (6)
Mean (SEM; median, range)prostate volume, mL	61.4 (2.8;53, 23–186)
Positive (reproducible) RTE (low strain–hard lesions), *n* (%)	86 (68)

The examination was performed using the default settings of the elastography software (E-dyn 4, frame rejection 6, noise rejection 4, smoothing 2, and persistence 3). Minimal compression and decompression of the prostate performed by the transrectal probe produced the RTE images. The machine gives feedback on the screen about the quality of the compression/decompression cycles. With training it is not hard to get consistent elastograms. The elastograms were presented simultaneously with the B-mode US images on a split-screen monitor. Hi-RTE software displays elastograms using a scale from red (highest strain; soft), through green (average strain; intermediate), to blue (low strain; hard). Figure 1 shows an elastogram suspicious of cancer.

The prostate was divided into six peripheral zone (PZ) regions excluding the transition zone (TZ) from the investigation [8]. Each region was examined for cancer suspicious lesions with both methods starting with B-mode. Prostate volume was calculated after measuring height, width, and length of prostate using the Hitachi software. The whole gland was then examined using RTE. If there were any suspicious (blue) areas, the examiner (Y.N.) would check their reproducibility. All reproducible suspicious areas would then undergo targeted biopsies. A maximum of five targeted biopsies were obtained. Irreproducible areas or fluctuant areas were considered inconclusive and would not undergo targeted biopsies. In the statistical analysis they were considered benign.

In patients with pathological RTE the location of the suspicious areas and the number of the targeted biopsies were registered. A standard 10-core biopsy was then taken in all patients. The 10-core systematic biopsy consisted of TRUS-guided standard sextant biopsy, supplemented with four lateral cores from the mid-prostate and the apex. For the biopsy scheme see Fig. 2. The urologist (S.A.H.) performing the standard biopsies did not have any knowledge of the results from the RTE examination. Hence, the patients served as their own control group for the performance of RTE and targeted biopsies vs systematic 10-core biopsies.

A standardised clinical report form was used during the procedure and kept for later analysis. Prostate volume, DRE findings, and RTE findings were marked on the clinical report form. Individual biopsy cores were numbered and assigned to a prostate region before being examined by a uro-pathologist (O.J.H., K.G.).

All patients received antibiotic prophylaxis with ciprofloxacin (1 g orally) before the procedure. In all patients, 6 mL

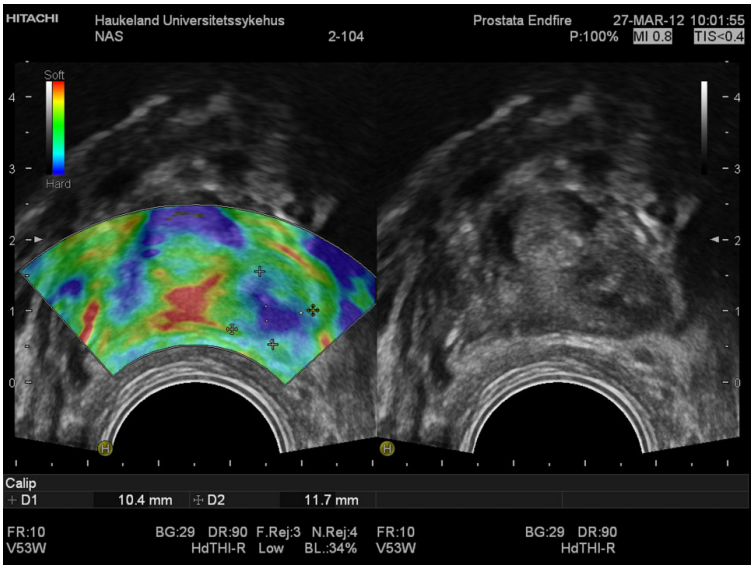

Fig. 1 A typical elastogram (left) and the B-mode image (right). It shows a hard lesion (blue) in the left PZ at the apex. The lesion is marked for measurement (+ signs), and it is ≈10 × 11 mm. The red area is the urethra; the anterior aspect of the prostate cannot be evaluated by RTE (the blue area anteriorly). Three targeted biopsies were obtained from the lesion in the left PZ and all three revealed a Gleason grade 5 + 4, score 9 tumour.

lidocaine (10 mg/mL) was administered as periprostatic infiltration anaesthesia.

Patients with a negative primary biopsy were followed up either because of urinary symptoms or because of a persistent, high suspicion of prostate cancer. A repeat biopsy was performed in patients with persisting indication for biopsy.

The biopsy and radical prostatectomy (RP) specimen results were retrieved from the pathology reports prepared by two study uro-pathologists (K.G., O.J.H.). The total core length, length of cancer tissue in each biopsy, and biopsy Gleason grade and score were recorded. In patients with several positive cores with different Gleason scores the highest Gleason score was used. In patients treated with robot-assisted RP (RARP) the information from the final pathology reports, based on whole-mount section histopathology and detailed in separate schemes, were used for the final analysis of the RTE performance.

Statistics

Standard descriptive statistics were used. Mean values are presented as the mean (SEM). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated on a per-region basis. As gold standard we employed RTE-targeted biopsies, systematic 10-core biopsies, and repeat biopsies of 12 systematic cores including two cores from the TZ. In the 27 cases where patients underwent RARP, we also used final pathology if it provided additional information.

Comparisons between different groups were performed by cross-tables and exact chi-square test, Mann–Whitney *U*-test, and *t*-test for categorical, ordinal and continuous data, respectively.

The multiple logistic regression models were performed, without pre-selection of the variables, in a backward Likelihood Ratio test manner. A $P < 0.05$ was considered to indicate statistical significance.

Results

Prostate cancer was diagnosed in 64 (50%) of the 127 patients during the initial biopsy session. As all patients had a systematic 10-core biopsy series, the total number of cores was 1270. In all, 86 patients had suspicious lesions on RTE and were biopsied with targeted biopsies; a median of three cores was obtained. A total of 287 targeted biopsy cores were obtained. In only three patients was prostate cancer detected solely by RTE-targeted biopsies. In all, 30 of the 64 prostate cancers detected at initial biopsy were diagnosed on both RTE-targeted biopsies and systematic biopsies, and 31 of 64 on systematic biopsies alone. However, the frequency of positive cores was significantly higher in the RTE-targeted biopsies than in the standard systematic biopsies (80/287 vs 236/1270, $P < 0.001$). There was a trend towards a higher fraction of cancer in the targeted biopsies, with an average of 42% of the total core length compared with 33% in the standard biopsies

Fig. 2 The different prostatic zones used in the study (posterior view) and the standard biopsy pattern used in the study (posterior view).

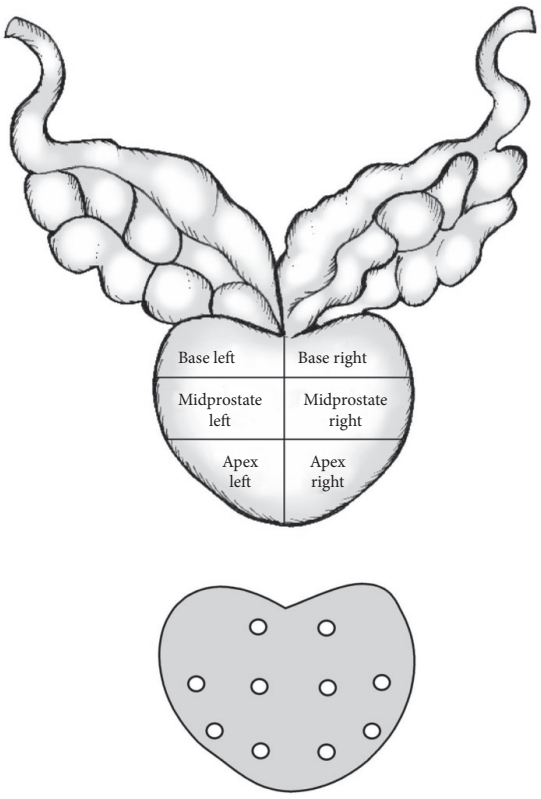

Table 2 Initial biopsy results.

Variable	Value
Total number of RTE-targeted biopsies*	287
Total number of systematic biopsies†	1270
Median (range) number of RTE-targeted biopsies	3 (0–5)
Median number of systematic biopsies	10
N (%):	
Patients with prostate cancer	64 (50)
Patients with prostate cancer in RTE-targeted biopsies only	3 (5)
Patients with prostate cancer in systematic biopsies only	31 (48)
Patients with prostate cancer in both RTE-targeted AND systematic biopsies	30 (47)

**The number of patients that underwent RTE-targeted biopsies was 86, †All 127 patients underwent systematic biopsies.*

($P = 0.087$). The primary biopsy results are shown in Table 2 and the zone distribution of positive cores of the different biopsy methods is given in Table 3. Table 4 shows the tumour characteristics found on biopsy.

According to the D'Amico criteria [9] 42% of the patients were in the high-risk group, 38% were in the intermediate-risk group, and 20% had low-risk prostate cancer.

A subgroup analysis was performed between the cancers detected on both RTE-targeted and systematic biopsies (group I, $n = 30$). The prostate cancers only found on systematic 10-core biopsies constituted group II ($n = 31$). In the systematic biopsies, the fraction of cancer of the total core length was 41% in group I and 26% in group II ($P = 0.01$). In group I, the mean (SEM) number of systematic positive cores was 5.1 (0.5) vs 2.7 (0.3) in group II ($P < 0.001$). Furthermore, the biopsy Gleason score was significantly higher within group I when comparing with the systematic biopsies only. If the biopsy results from the targeted biopsies were added to group I and the highest Gleason score was used, the differences became even more pronounced (Table 5). Furthermore, Group I had a significantly lower mean prostate volume than group II, at 44.0 (2.6) vs 68.0 (3.5) mL ($P < 0.001$).

A multiple regression model was used to identify markers for high-risk cancers. Five different parameters were entered for analysis; prostate volume, positive RTE (low strain), symptoms, study period, and age. The PSA level and DRE findings could not be included in such a model, as these are key criteria in D'Amico's risk stratification [9].

A positive RTE and low prostate volume were independent markers for high-risk cancers. Details are shown in Table 6.

During the 6-month period after primary biopsy, four patients underwent TURP for symptomatic BPH with a benign histology, and 36 patients had a repeated systematic TRUS-guided 12-core biopsy, of which two cores were sampled from the TZ. At histology, another eight patients were diagnosed with prostate cancer. Six of these patients were low-risk cancers and two were intermediate risk. One of the intermediate-risk prostate cancers was localised at the base of the prostate; this was detected after multiparametric MRI-guided TRUS biopsy. Four of these patients were treated with RARP, and four were followed according to an active surveillance protocol. In all, 72 (57%) of 127 patients were diagnosed with prostate cancer.

For calculations of sensitivity, specificity, PPV, NPV and accuracy of RTE, we divided the prostate into six regions. In all, 762 regions in 127 patients were analysed. All these regions were analysed for all cancers and for high-grade cancers, using the information from the systematic biopsies, the RTE-guided biopsies, repeat biopsies, and pathology reports from patients treated by RARP. The sensitivity increased from 42% for all prostate cancers to 60% for high-grade prostate cancers. Further results are shown in Table 7.

Discussion

One of the main findings of the present study was the capability of RTE to identify high-risk prostate cancer.

Table 3 The zone distribution of positive biopsies.

	Apex	Mid-prostate	Base	>1 region	Total
N **(%):**					
RTE-targeted biopsy series:	2 (6)	4 (12)	4 (12)	4 (12)	14 (42)
Right	8 (24)	4 (12)	2 (4)	2 (6)	16 (48)
Left	2 (6)	0	0	1 (3)	3 (9)
Bilateral	12 (36)	8 (24)	6 (9)	7 (21)	33 (100)
Total					
Systematic 10-core biopsy series:					
Right	3 (5)	5 (8)	4 (7)	8 (13)	20 (33)
Left	3 (5)	1 (2)	2 (3)	5 (8)	11 (18)
Bilateral	2 (3)	1 (2)	0	27 (44)	30 (49)
Total	8 (13)	7 (11)	6 (10)	40 (66)	61 (100)

Table 4 Tumour characteristics and treatment.

Variable	Total	RTE-targeted biopsies results	Systematic 10-core biopsies results
	n **= 64**	***n*** **= 33**	***n*** **= 61**
N (%)			
Clinical tumour stage (cT)			
T1c	28 (46)		
T2a-b	16 (25)		
T2c	10 (16)		
T3a	7 (11)		
Gleason Score			
5 (3+2)	1 (2)	0	1 (2)
6 (3+3)	20 (31)	13 (39)	19 (31)
7a (3+4)	20 (31)	6 (18)	24 (39)
7b (4+3)	10 (16)	7 (21)	6 (10)
8 (4+4)	6 (9)	3 (9)	4 (7)
9 (4+5)	5 (8)	3 (9)	5 (8)
9 (5+4)	2 (3)	1 (3)	2 (3)
D'Amico risk stratification:			
High risk	27 (42)		
Intermediate	24 (38)		
Low risk	13 (20)		
Treatment			
RARP	23 (36)		
External beam radiation therapy	29 (45)		
Active surveillance	12 (19)		

Table 5 Impact of RTE-targeted biopsies on Gleason score in 61 patients detected by systematic 10-core biopsy.

Gleason score	RTE-positive patients (Group I) Only systematic biopsy results (*n* = 30)	RTE-positive patients (Group I) Systematic and targeted biopsy results (*n* = 30)	RTE-negative patients (Group II) Systematic biopsy results (*n* = 31)
N (%):			
5 (3+2)	0	0	1 (3)
6 (3+3)	5 (17)	4 (13)	14 (45)
7a (3+4)	14 (47)*	10 (33)	10 (32)**
7b (4+3)	2 (7)	6 (20)**	4 (13)
8 (4+4)	3 (10)	4 (13)	1 (3)
9 (4+5 or 5+4)	6 (20)	6 (20)	1 (3)

**Median Gleason Score was 7a (3+4) in both groups; however, the rank distribution within the groups are significantly different (P = 0.007, Mann–Whitney U-test). **Median Gleason Score was 7b (4+3) in group I and 7a (3+4) in group II. The rank distribution within the groups are significantly different (P = 0.001, Mann–Whitney U-test).*

Table 6 Logistic regression analyses for detection of high-risk prostate cancer[†] in the primary biopsy setting.

Variables	Simple		Multiple			
	Unadjusted		Fully adjusted		Final model	
	OR 95% CI*	P**	OR 95% CI*	P**	OR 95% CI*	P**
Age (continuous)	1.01 (0.94,1.08)	0.860	1.03 (0.96,1.11)	0.376		
Study period (second vs first half)	0.77 (0.33, 1.81)	0.545	1.07 (0.41,2.77)	0.891		
Symptoms (yes vs no)	1.03 (0.43, 2.45)	0.944	1.23 (0.47,3.23)	0.673		
Positive RTE (yes vs no)	5.89 (1.31, 26.4)	0.005	4.56 (0.95,21.8)	0.030	4.20 (0.89,19.6)	0.038
Prostate volume (≥53.1 vs <53.1 mL)[‡]	7.69 (2.44, 25)	<0.001	6.67 (2.04,20)	<0.001	6.25 (2.04,20)	<0.001

*[†]High-risk cancer of the prostate according to the risk stratification proposed by D'Amico [9]; [‡]threshold for dichotomy placed at median volume 53.1 mL; **P values are calculated by the Likelihood Ratio (LR) test, while the CI* are calculated by use of the Wald coefficient under an assumption of a normal distribution. The contradictory results, in regard to statistical significance, given by the CI and the P value for the RTE variable, are explained by these different methods of calculation. However, the LR-test should be regarded as the most accurate.*

Table 7 Sensitivity, specificity, NPV, PPV and accuracy of RTE for the detection of prostate cancer.

	Sensitivity, %	Specificity, %	NPV, %	PPV, %	Accuracy (%)
All prostate cancers (all Gleason scores)	42	83	79	49	72
High-grade prostate cancer (Gleason 7b–10)	60	80	97	20	78

Cancers identified with RTE showed a significantly higher Gleason score than those with a negative RTE. This is consistent with the findings of Nelson et al. [10] and Aigner et al. [11], while Brock et al. [12] were not able to find an association between RTE and Gleason score. This discrepancy may be explained by different distributions of Gleason scores in the patient series. In the series of Brock et al. [12], most of the patients were Gleason score 4–6. In the present study most patients were, on the contrary, intermediate- or high-risk cancers. Nelson et al. [10] found a positive association between RTE and intermediate- and high-grade cancers.

Another important result in the present study was the relation of both positive RTE and prostate volume with prostate cancer. The patients with RTE-positive cancers had significantly smaller prostates (mean 44 vs 68 mL). In patients for whom there was a suspicion of prostate cancer, the combination of a small prostate and a positive RTE did lead to an increased risk of being diagnosed with high-risk prostate cancer. In RTE-positive cancers we found significantly more positive cores (5.1 vs 2.7), and the fraction of cancer tissue in the cores was significantly higher in this group (41% vs 26%). The reference standard for the diagnostic performance of RTE consisted of all the biopsy results including repeat biopsy within 6 months. This reference standard is close to the true prevalence of prostate cancer in the study cohort. The sensitivity of a 42% detection of prostate cancer on a per-region basis is higher than the 24% reported by Taverna et al. [13] and 31% in the study of Cochlin et al. [14], but lower than the reported sensitivity of 61% in the study by Brock et al. [12]. This discrepancy in sensitivity may be explained by differences in technology, study design, and study population, as well as well-known inter-observer variability. Although the skilled examiner usually achieves higher sensitivity, a novice achieves valid results after some 50 examinations when he is properly trained [15]. As RTE is a real-time examination there will be differences in the interpretation of the images produced. As the present study is a single examiner study, the inter-observer variability could not be addressed.

In our experience RTE does not seem to be a good method to identify cancer lesions in large glands, which demonstrates one of the challenges for RTE. In glands with a large TZ, RTE shows a lower sensitivity. In patients with BPH the inner gland compresses the PZ. The PZ then appears thinned out and difficult to examine. The normal RTE pattern of BPH in large prostates is unevenly inelastic in most cases, producing hard lesions indistinguishable from cancer [16].

The low sensitivity precludes the use of RTE-targeted biopsy alone, and at present it has to be combined with systematic biopsies. However, in the present study RTE showed a high specificity of 83%, and a high NPV of 79%, for detection of prostate cancer. Although RTE showed too low a sensitivity to be used alone, RTE is of clinical value because it showed high specificity. The specificity remained high (80%) in the present study also for high-grade tumours. Furthermore, in a high-grade setting, the sensitivity increased to 60%. However, most important was the NPV of 97%. In our opinion, if verified in later studies, this could make a place for RTE routinely used as a component of multiparametric US assessment for excluding high-risk prostate cancer in a screening setting.

In all, 50% of the patients were diagnosed with prostate cancer at initial biopsy using systematic biopsies and RTE-guided biopsies. This is comparable to the 51% detection rate published by Brock et al. [12] in their study of combining RTE-targeted and systemic biopsies. They found a significantly higher cancer detection rate using RTE-guided systematic biopsies vs B-mode TRUS-guided systematic biopsies (51% vs 39%).

In the present series, the number of patients with high-risk cancer (42%) was also higher than in most cohorts. In a population-based study of initial management of prostate cancer in Norway in 2004, Hernes et al. [17] found 42% high-risk cancers among 1650 patients amenable for curative treatment. In Norway a population-based screening programme has not been implemented, but an increased use of erratic screening has led to an increased number of PSA-detected cancers. As many patients have only measured their PSA level once, there might be more patients with larger tumours than in heavily screened populations.

We have evaluated RTE with targeted biopsies and compared it with standard systematic biopsies. We did both examinations in all patients. Some studies have investigated RTE with targeted biopsies and compared it with standard biopsies. Aigner et al. [11] reported similar detection rates using RTE and targeted biopsies compared with 10-core standard systematic biopsies, despite the use of fewer cores.

We found that the cores sampled by RTE-targeted biopsies frequently had more cancer, and also a higher fraction of cancer tissue in each biopsy. This will for some patients make a difference in treatment options. For instance, in one patient we found, by standard systematic biopsies, a Gleason score 6 (3+3) tumour in one of 10 biopsies; the RTE-guided biopsies revealed a Gleason score 8 (grade 4+4) tumour. This patient was treated with RARP. Without the RTE-guided biopsies he would most likely have be recommended for active surveillance.

The study is limited by being a single-centre, single examiner study and by the number of patients. The RTE method is observer dependent. Although there is a learning curve for RTE, valid results may be achieved after ≈50 examinations [15].

In conclusion, a positive RTE is an independent marker for detection of high-risk prostate cancer and a negative RTE argues clearly against the presence of such. The relationship of RTE and prostate size in the patients with prostate cancer may imply a better diagnostic performance of RTE in more normal-sized prostates. RTE with targeted biopsies cannot replace systematic biopsies, but RTE provides valuable additional information in the initial biopsy setting.

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Conflict of Interest

The authors have nothing to disclose, and the study was carried out with funding from the institutions mentioned on the title page and by a grant from the URO-BERGEN research foundation. The authors alone are responsible for the content and writing of the paper.

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Abbreviations: (N)(P)PV, (negative) (positive) predictive value; (RA)RP, robot-assisted radical prostatectomy; PZ, peripheral zone; RTE, real-time elastography; TZ, transitional zone; US, ultrasound/ultrasonography.

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RESEARCH ARTICLE Open Access

A positive Real-Time Elastography (RTE) combined with a Prostate Cancer Gene 3 (PCA3) score above 35 convey a high probability of intermediate- or high-risk prostate cancer in patient admitted for primary prostate biopsy

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Abstract

Background: The standard of care in patients with suspected prostate cancer (PCa) is systematic prostate biopsies. This approach leads to unnecessary biopsies in patients without PCa and also to the detection of clinical insignificant PCa. Better tools are wanted. We have evaluated the performance of real-time elastography (RTE) combined with prostate cancer gene 3 (PCA3) in an initial biopsy setting with the goal of better identifying patients in need of prostate biopsies.

Methods: 127 patients were included in this study; three were excluded because of not measureable PCA3 score leading to 124 evaluable patients. A cut-off value of 35 was used for PCA3. All patients were examined with a Hitachi Preirus with an endfire probe for RTE, a maximum of five targeted biopsies were obtained from suspicious lesions detected by RTE. All patients then had a 10-core systematic biopsy performed by another urologist unaware of the RTE results. The study includes follow-up data for a minimum of three years; all available histopathological data are included in the analysis.

Results: There was a significant difference in PCA3 score: 26.6 for benign disease, 73.6 for cancer patients ($p < 0.001$). 70 patients (56 %) were diagnosed with prostate cancer in the study period, 21 (30 %) low-risk, 32 (46 %) intermediate-risk and 17 (24 %) high-risk. RTE and PCA3 were significant markers for predicting intermediate- and high-risk PCa ($p = 0.001$). The combination of RTE and PCA3 had a sensitivity of 96 % and a negative predictive value (NPV) of 90 % for the group of intermediate- and high-risk PCa together and a NPV for high-risk PCa of 100 %. If both parameters are positive there is a high probability of detecting intermediate- or high-risk PCa, if both parameters are negative there is only a small chance of missing prostate cancer with documented treatment benefit.

Conclusions: RTE and PCA3 may be used as pre-biopsy examinations to reduce the number of prostate biopsies.

Keywords: Prostate cancer, Ultrasound, Diagnosis, PCA3, Real-time elastography, RTE

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Background

The mainstay in the diagnosis of prostate cancer (PCa) is biopsy-driven by serum prostate-specific antigen (PSA) and digital rectal examination (DRE). There is really no level of PSA that excludes PCa, and many benign prostatic diseases may cause PSA elevation. The threshold value of PSA for prostate biopsy is arbitrarily chosen, which is dependent on the age of the patient, life expectancy and the size of the prostate. It is well recognized that PSA screening results in both the over-diagnosis and overtreatment of prostate cancer [1–3]. Furthermore, a lot of men with benign disease are going through prostate biopsy without any beneficial effects. There is also an increase in biopsy-related infections because of antibiotic resistant bacteria, and some of these infections can be lethal [4, 5]. There is a need to better identify those men not harboring PCa to avoid unnecessary biopsies and related complications.

Currently, there is little enthusiasm for population-based PSA screening, and in May 2012 the U.S. Preventive Services Task Force recommended against routine PSA screening [6]. Moreover, European Association of Urology (EAU) Guidelines (2013) do not support programmed mass PSA screening, while recommending early detection in well-informed men [7].

To assist in the decision to perform prostate biopsy, nomograms have been created. The US Food and Drug Administration has approved prostate cancer gene 3 (PCA3) as a predictive test prior to performing a repeat biopsy. PCA3 has shown to enhance the performance of nomograms based on initial biopsy results [8, 9].

Standard systematic prostate biopsy is performed by placing a biopsy needle in 10 to 12 prostate sectors of the peripheral zone under transrectal ultrasound (US) guidance. Cancer in the central or anterior part of the prostate may be overlooked, and insignificant cancer detected with such biopsy regimens [10].

Imaging techniques, specifically advanced US and multiparametric MRI (mpMRI), are evolving, and thereby making it possible to identify areas suspected of harboring PCa [11, 12]. Targeted biopsy guided by RTE detects high-grade cancer, although it misses some significant cancers compared with a systematic 10-core biopsy [13, 14]. mpMRI, together with fusion into real-time US, is practical for targeted biopsy but this approach also misses significant PCa [15].

In a prospective series of patients undergoing radical prostatectomy, the combination of RTE and PCA3 detected 97 % of significant PCa [16]. The present study was undertaken to evaluate prospectively the capability of RTE and PCA 3 to predict clinically significant PCa in patients admitted for initial prostate biopsy.

Methods

The study was carried out in the outpatient clinic of the Department of Urology at Haukeland University Hospital from February 2011 to June 2012. The Regional Committee for Medical and Health Research Ethics in Western Norway approved the study.

A total of 127 consecutive patients were included using active inclusion, with only a very small amount of patients declining to participate. The inclusion criteria were a PSA level 3 – 25 ng/ml, age ≤75 years and no prior biopsies within the last five years, in addition to the patients being amenable for radical treatment.

At first, DRE was performed in all patients to determine clinical stage (cT) and to perform the prostatic massage needed before urine sampling. Before further evaluation, the first stray urine was captured and transferred to the transportation tubes needed for the PCA3 analysis. We used Progensa™ PCA3 analysis, and the tests were analyzed at the Fürst Medical Laboratory in Oslo, Norway. After the urine test, all patients were given a single dose of Ciprofloxacin 1 g as an antibiotic prophylaxis. All patients were examined in the left decubital position, with the ultrasound procedures being thoroughly previously described [16]. In brief, all patients were examined using a Hitachi Preirus Ultrasound machine with software for RTE. They were first examined using a V53W transrectal end-fire probe for B-mode evaluation, determination of prostate volume (Pvol), RTE and targeted biopsies. The peripheral zone (PZ) of the prostate was divided in six region of interest (ROI), one at the base, one at the mid prostate and one at the apex on each side. All RTE-reproducible hard lesions of more than 5 mm were allocated to the corresponding ROI. Furthermore, two to four targeted biopsies were taken from suspicious ROIs. A CC531 transrectal simultaneous biplane probe was used for standard systematic biopsies. In the same setting a different urologist blinded for the RTE results performed a 10-core systematic biopsy from the six ROIs. The biopsies were fixated in formaldehyde and analyzed by two uro-pathologists. Total core length, as well as the length of cancer tissue and Gleason grade and score, was separately recorded for each biopsy core.

In the statistical analyses, we included not only the results and outcomes of the initial biopsy, but also at least three years of follow-up data for the patients. If there was a clinically persisting suspicion of PCa after the initial biopsies, patients were monitored closely (see Fig. 1). Repeat biopsies were performed in 38 patients within the next six months, while in 24 patients no repeat biopsy was performed. Sixteen patients with benign repeat biopsies went through a mpMRI of the prostate, and in 12 of these we performed targeted biopsies of suspicious areas by TRUS guided biopsies with a “cognitive fusion” of

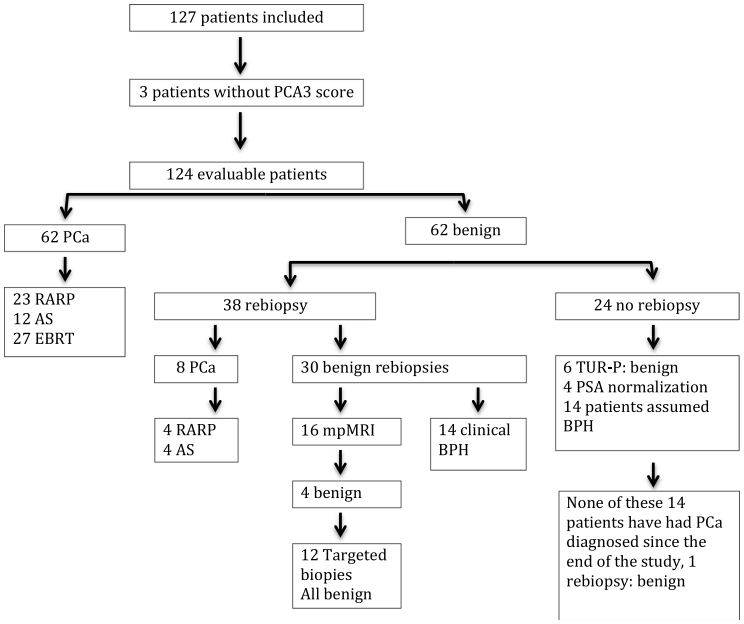

Fig. 1 Flowchart of the 127 included patients in this study. The numbers indicate the number of patients in each group. Abbreviations: PCA3: Prostate cancer gene 3; PCa: Prostate cancer; RARP: Robotic assisted radical prostatectomy; AS: Active surveillance; EBRT: External beam radiation therapy; TUR-P: Transurethral resection of prostate; PSA: Prostate specific antigen

mpMRI. Together with an uro-radiologist, a trained urologist performed such biopsies. All biopsy data were included in the analyses. Among those 24 patients with no repeat biopsy, four patients experienced a normalization of PSA levels at follow-up, six were admitted for TUR-P with a benign pathology, and in 14 patients benign prostatic hyperplasia was assumed as the reason for a slight elevation in PSA level. The medical records for these patients and the registry at our department of pathology were examined in October 2015 to identify whether PCa had been diagnosed since the end of inclusion. The mean observation time for these patients is 46.7 ± 1.5 months (median 44.4, range 41–55). The medical records for the 14 patients with benign repeat biopsies were also examined at the same time, though none have had PCa diagnosed in this period.

Statistical analyses

Standard descriptive statistics were used and presented as mean and median. A 95 % confidence interval (CI) was calculated. Negative predictive value (NPV), positive predictive value (PPV), sensitivity and specificity were calculated for RTE by ROI and by patient, for PCA3 using a cut-off value of 35 and for a combination of both. Different groups were compared using the exact Chi-square test, a Mann–Whitney U-test and the t-test for categorical, ordinal and continuous data, respectively. A multiple logistic regression model was estimated entering the clinical parameters age, Pvol and PSA alone, or combined with a dichotomized PCA3 score of 35 and positive RTE by patient. DRE is commonly used in such clinical models but we excluded DRE from the model because DRE and RTE both are parameters expressing tissue stiffness. The performance of the calculations was expressed as the area under the curve (AUC) of the receiver operating curves (ROC). A 95 %CI was calculated for the AUC and displayed in parenthesis after AUC.

Results

In three patients the urine did not contain enough cells for the PCA3 analysis resulting in 124 evaluable patients.

A total of 70 (56 %) patients were diagnosed with PCa, of whom 62 were identified in the initial biopsy setting and eight patients at the repeat biopsy. The inclusion of these eight patients did not alter the diagnostic performance of RTE by ROI as the sensitivity, specificity, PPV and NPV were 43, 84, 49 and 80 %, respectively; the false positive rate was 16 % and the false negative rate 12 %.

According to the European Association of Urology (EAU) risk stratification, there were 21 (30 %) low-, 32

(46 %) intermediate- and 17 (24 %) high-risk cancers [7]. In the eight patients detected with PCa on the repeat biopsies six were low-risk and two were intermediate-risk cancers, there were no high-risk PCa in this group.

The distribution of PSA, PCA3 score, Pvol, age and proportion of positive DRE for all patients and for patients with and without PCa is found in Table 1. The p-values are calculated for the difference between the groups with PCa and without PCa. The clinical stage, biopsy Gleason grade and score and risk stratification according to EAU guidelines are also detailed in Table 1.

RTE was positive in 85 cases and negative in 39 . The average PCA3 score in patients with PCa was significantly higher compared with normal or benign disease (73.6 vs. 26.6, $p < 0.001$). For PSA, there were no statistical significant differences between those patients with- and those without PCa (9.7 vs. 8.3, $p = 0.09$).

The sensitivity, specificity, PPV and NPV of RTE by patient, PCA3 at score 35 and the combination of both for any PCa, for intermediate- and high-risk PCa together, and for high-risk PCa alone, are shown in Table 2.

In univariate logistic regression analysis a positive RTE was a highly significant predictor of intermediate- risk and high-risk PCa (Table 3).

Entering PCA3 and RTE in a clinical model encompassing age, PSA and Pvol; PSA, Pvol and PCA3 were independent predictors of intermediate-risk and high-risk PCa while RTE showed a tendency toward significance (Table 3).

The results of the logistic regression analyses were also expressed in a ROC curve that yielded an AUC of 0.826 (0.752-0.899) for the complete model and 0.787 (0.703-0.872) for the clinical model alone (Fig. 2).

To evaluate the clinical impact of the combination of PCA3 and RTE, we utilized the most commonly used cut-off value of 35 for PCA3, and allocated the patients into four groups.

Group 1 included patients for whom both RTE and PCA3 were positive. Patients with a positive RTE and negative PCA3 were put into Group 2, and RTE negative and PCA3 positive patients were allocated to Group 3. Finally, Group 4 encompassed patients negative for RTE,

Table 1 Patient characteristics of 124 patients of whom 70 were diagnosed with PCa

Variable		Total ($n = 124$)	PCa ($n = 70$)	No PCa ($n = 54$)	p-value*
PSA	Mean (Median; 95 %CI)	9.1 (7.2; 8.3-9.9)	9.7 (7.7; 8.5-11.0)	8.3 (6.7; 7.2-9.4)	0.090*
PCA3-score	Mean (Median; 95 %CI)	53.1 (33.5; 42.9-63.4)	73.6 (53.5; 57.7-89.6)	26.6 (19.0; 19.9-33-2)	<0.001*
Prostate volume	Mean (Median; 95 %CI)	60.0 (53.0; 54.7-65.4)	49.9 (43.5; 44.7-55.1)	73.2 (66.5; 63.8-82.5)	<0.001*
Age	Mean (Median; 95 %CI)	64.0 (65.1; 62.9-65.2)	64.9 (65.7; 63.5-66.2)	62.9 (63.0; 61.0-64.9)	0.094*
Positive DRE	Number (%)	31 (25 %)	22 (31 %)	9 (17 %)	0,060**
Clinical stage	Number (%)				
T1c			35 (50 %)		
T2a			12 (17 %)		
T2b			6 (9 %)		
T2c			11 (16 %)		
T3a			6 (9 %)		
Gleason score	Number (%)				
3 + 2 = 5			1 (1 %)		
3 + 3 = 6			21 (26 %)		
3 + 4 = 7a			15 (19 %)		
4 + 3 = 7b			9 (11 %)		
4 + 4 = 8			5 (6 %)		
4 + 5 = 9			4 (5 %)		
5 + 4 = 9			2 (2 %)		
EAU-risk	Number (%)				
Low-risk			21 (30 %)		
Intermediate-risk			32 (46 %)		
High-risk			17 (24 %)		

PCa prostate cancer, *PSA* prostate specific antigen, *PCA3* prostate cancer gene 3, *DRE* digital rectal examination is considered positive if there was suspicion of PCa
**p*-value is estimated for the difference of means between the group with PCa and the group without PCa using the t-test
***p*-value is estimated for the difference of proportions between the group with PCa and the group without PCa using Chi-square test

Table 2 This table shows the diagnostic performance of RTE and PCA3 score with cut-off 35 for the group of any PCa, for the combined group of intermediate-and high-risk PCa, and for high-risk PCa

	Parameter	Sensitivity	Specificity	NPV	PPV
Any PCa	RTE	74 %	39 %	54 %	61 %
	PCA3	64 %	78 %	66 %	80 %
	Combination	91 %	26 %	70 %	62 %
IR and HR PCa	RTE	86 %	43 %	82 %	51 %
	PCA3	71 %	66 %	78 %	58 %
	Combination	96 %	24 %	90 %	55 %
HR PCa	RTE	88 %	35 %	95 %	18 %
	PCA3	82 %	57 %	95 %	23 %
	Combination	100 %	19 %	100 %	16 %

Abbreviations: *PCa* prostate cancer, *IR* intermediate-risk, *HR* high-risk, *RTE* real-time elastography, *PCA3* prostate cancer gene 3, *NPV* negative predictive value, *PPV* positive predictive value

as well as PCA3. Group 1 encompassed 44 patients; 30 had a high- or intermediate-risk PCa, eight a low-risk PCa and six a benign prostate. If both tests were positive, we found a high (86 %) probability of PCa at biopsy. On the other hand, of 23 patients with a PCA3 below 35 and a negative RTE (Group 4), eight patients were diagnosed with PCa, including six with low-risk cancer and two with intermediate-risk cancer, while 15 patients did not have any cancer. There was no high-risk PCa in this group. Omitting a biopsy in this group would imply a 9 % likelihood of missing PCa of clinical importance. In Group 2, 14 patients were diagnosed with cancer and 27 without cancer. There were 16 patients with a PCA3 score equal to or higher than 35 and a negative RTE (Group 3); ten patients had cancer and no cancer was found in the other six. The results achieved from pre-biopsy PCA3 urinary tests and RTE assessments in both Group 1 and Group 4 are informative and may be of benefit in the decision-making process as to whether to perform a biopsy or not.

Out of 70 patients for whom PCa was diagnosed, 27 underwent radical prostatectomy, 27 received external radiotherapy and 16 opted for active surveillance.

Discussion

There is a changing wind in the way we detect and treat PCa as a consequence of the well-known over-diagnosis and overtreatment of PCa, in addition to the documented increasing rate of post-biopsy infections [4, 5]. There is an ongoing search for new biomarkers and the development of improved methods for identifying clinically significant PCa. Evolving evidences show the benefit of PCA3 in the decision-making process of performing repeat biopsies in men where the initial biopsy is negative.

Both RTE and mpMRI are capable of identifying PCa that is not visualized on B-mode ultrasound [17, 18].

To the best of our knowledge, the present paper is the first to present prospective data on the combination of pre-biopsy PCA3 and RTE by patient in predicting PCa in an unselected series of men admitted for an initial biopsy.

The most important findings are the high sensitivity as well as NPV in predicting intermediate-risk and high-risk PCa (Table 2). PCA3 and RTE appeared to be of benefit mostly in patients if both parameters were positive or negative. If both parameters are positive, there is good reason to perform a biopsy and there is a high probability of detecting aggressive disease. Additionally, avoiding a biopsy in which PCA3 and RTE are negative carries a small risk of missing patients harboring a clinically significant PCa. In this series we found 32 intermediate-risk PCa and 17 high-risk PCa. By using RTE and PCA3 as selection criteria for performing a biopsy, 23 patients would have been advised against having a biopsy; only two of these patients had intermediate-risk PCa and no patients had high-risk PCa. One could argue that the reduction of unnecessary biopsies is relatively small since only 23 patients (19 %) would have been advised against biopsy. On the other hand, these patients could safely be advised

Table 3 Logistic regression analyses for predicting high and intermediate risk prostate cancer ($n = 124$)

	Simple			Multiple					
	Unadjusted			Fully adjusted			Final model		
Variables	OR	95 % CI	*p*-value**	OR	95 % CI	*p*-value**	OR	95 % CI	*p*-value**
Age (cont. in years)	1.04	(0.98,1.10)	0.188	1.04	(0.96, 1.13)	0.287			
PSA (cont. in ng/ml)	1.18	(1.08, 1.29)	<0.001	1.19	(1.07, 1.34)	0.001	1.18	(1.03, 1.14)	0.001
Pvol. (cont. in ml)	0.98	(0.96, 0.99)	0.003	0.97	(0.95, 0.99)	0.005	1.04	(1.04, 1.07)	0.009
Positive RTE (Y/N)	4.46	(1.78, 11.22)	0.001	2.73	(0.96, 7.79)	0.052	2.56	(0.91, 7.23)	0.068
PCA3 (>35 vs. <35)	5.00	(2.28, 10.95)	<0.001	3.31	(1.27, 8.63)	0.013	4.12	(1.71, 9.91)	0.001

Abbreviations: *RTE*: real-time elastography, *Cont* continuous, *Y/N* yes/no, *OR* odds ratio, *CI* confidence interval, *Pvol* prostate volume
***p*-value by the use of the Likelihood Ratio test

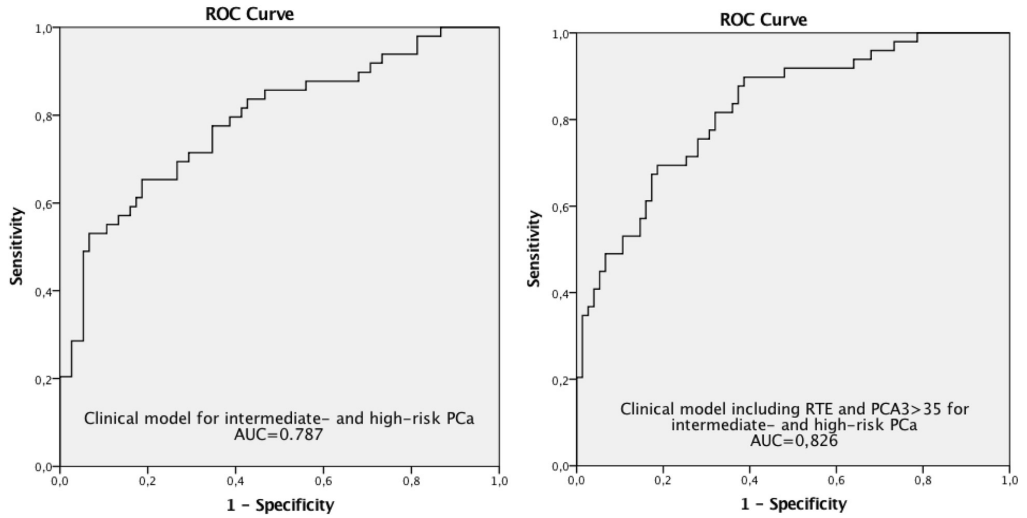

Fig. 2 ROC curves for the regression analyses for the group of intermediate- and high-risk PCa. The addition of PCA3 score >35 and RTE lead to an increase in AUC of 0.039. Abbreviations: PCa = Prostate cancer, ROC = receiver operating curves, PCA3 = prostate cancer gene 3, AUC = area under curve

against biopsy, as every reduction of unnecessary biopsies is a step in the right direction in reducing over-diagnosis and overtreatment of low-risk PCa. These findings are in line with our previous study of the combination of PCA3 and RTE in a smaller series of radically operated PCa patients [16].

In the logistic regression analysis PCA3 as well as a positive RTE contributed to the clinical model although RTE achieved a p-value close to significance (0. 068). In a ROC analysis, the full model with PCA3 and RTE achieved an AUC of 0.826. In univariate analysis a positive RTE is a highly significant predictor of PCa.

No definite threshold of PCA3 score has been agreed upon as yet, although a score of 35 is most frequently used as a cut-off value. In our study, we tested two different PCA3 score thresholds of 21 and 35, respectively. A threshold score of 35 provided the most optimal PPV of 80 %, which is the same figure found in a prospective randomized study by Wei et al., using a PCA3 score threshold of 60 in the initial biopsy setting [19]. In our analyses, we utilized a PCA3 score of 35 as the threshold value.

A strength of this study is that it includes histopathological data on initial biopsies, repeat biopsies as well as data of further follow-up, including mpMRI targeted biopsies of suspected lesions. No patients in the group diagnosed with a benign disease have been diagnosed with PCa in the period since the study inclusion was closed in June 2012. For all 14 patients with a presumably benign reason for an elevated PSA, both medical records and records for the regional pathology laboratory were checked. We believe that we are as close as possible to the true prevalence of PCa in the study population at the time of the examinations. This makes this study different from other studies investigating PCA3 [8, 20] and RTE [21, 22], in which the performance of these markers has been solely evaluated at the initial biopsy.

In this series of patients, a total of 70 patients were diagnosed with PCa, including 21 who were classified as low-risk and 49 as either high- or intermediate-risk.

Analyzing the group of PCa patients harboring either high- or intermediate-risk PCa, the combination of RTE and PCA3 correctly identified 47 of these patients. That means we correctly identified 96 % of the patients harboring PCa in need of treatment in a pre-biopsy setting. This result may be used to reduce the number of unnecessary biopsies at a small risk of missing PCa in need of treatment.

The present study has some limitations. Firstly, it is a single center, single investigator study. RTE like all US investigations are real-time examinations and are operator dependent and an inter-observer investigation would have been of value. As to the learning curve, it has been shown that after about 30 RTE the novice is achieving comparable results to experienced US operators [23]. Secondly, a relatively small number of patients are included. Thirdly, there is a limited number of patients with high-risk PCa, although the findings are in

line with our previously published paper on patients planned for radical prostatectomy [16].

Conclusions

In patients with a positive RTE combined with a PCA3 score above 35 there is a high probability of detecting intermediate- or high-risk PCa. The combination of these markers correctly identified 47 of 49 (96 %) patients in need of a further diagnostic work-up. The high NPV of the combination of PCA3 and RTE makes it possible to avoid some 20 % of the prostate biopsies without missing high-risk PCa. If applied to the upper age group, in which a missing low-risk PCa may be seen as an advantage, the use of RTE and PCA3 may be implemented as pre-biopsy examinations to reduce the number of prostate biopsies.

Abbreviations

AUC, area under the curve; CI, confidence interval; DRE, digital rectal examination; EAU, European Association of Urology; mpMRI, multiparametric magnetic resonance imaging; NPV, negative predictive value; PCa, prostate cancer; PCA3, Prostate cancer gene 3; PPV, positive predictive value; PSA, prostate-specific antigen; Pvol, prostate volume; ROC, receiver operating curve; RTE, real-time elastography; TRUS, transrectal ultrasound; US, ultrasound

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Availability of data and materials

The approval from the ethical committee and the informed consent do not cover full open publication of the dataset. The raw data will be made available in unidentified form on request, if needed contact corresponding author.

Authors' contributions

YN: Project development, Data collection and analysis, Manuscript writing. SAH: Project development, Data Collection and analysis, Manuscript editing. OJH: Data collection and analysis, Manuscript editing. KG: Data collection and analysis, Manuscript editing. JF: Data collection and analysis, Manuscript editing. LAA: Data analysis, Manuscript editing. CB: Project development, Data analysis, Manuscript editing. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests. The authors alone are responsible for the content and writing of the paper.

Consent for publication

Not applicable.

Ethics approval and consent to participate

All patients were given oral and written information about the study, and they gave their written informed consent to participate in the studies. The studies were approved from the Regional Committee for Medical and Health Research Ethics in Western Norway with registration number 223.08.

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