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Algorithmus zur Anwendung von Mikro-Ultraschall und Vergleich von Mikro-Ultraschall mit multiparametrischer MRT zur Früherkennung von Prostatakrebs

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Table of Contents

Abbreviations

AUC	Area under the curve
BPH	Benign prostatic hyperplasia
bpMRI	Biparametric magnetic resonance imaging
CEUS	Contrast-enhanced ultrasound
csPCa	Clinically significant prostate cancer
CT	Computed tomography
DCE	Dynamic contrast enhancement
DRE	Digital rectal examination
DWI	Diffusion-weighted imaging
EAU	European Association of Urology
ERSPC	European Randomized Study of Screening for Prostate Cancer

GG	Grade group
GS	Gleason score
HiTRUS	High-resolution transrectal ultrasound
ISUP	International society of urological pathology
IQR	Interquartile range
LoTRUS	Conventional transrectal ultrasound
MicroUS	Micro-ultrasound
mp-US	Multiparametric ultrasound
mpMRI	Multi-parametric magnetic resonance Imaging
MRI	Magnetic resonance imaging
NPV	Negative predictive value
PCa	Prostate cancer

PI-RADS	The standardized prostate imaging reporting system
PLCO	The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial
PPV	Positive predictive value
PRI-MUS	Prostate Risk Identification Using Micro-Ultrasound
PSA	Prostate-Specific Antigen
PSA-D	PSA density
RCs	Risk calculators
ROC	Receiver Operating Characteristic
SE	Strain Elastography
SWE	Shear-wave elastography
T1W1	T1-weighted images
T2W1	T2-weighted images

TGC	Time gain compensation
TPM-biopsy	Template prostate mapping biopsies
TRUS	Transrectal ultrasound
USPSTF	United states Preventive Services Task Force
WHO	World Health Organization

1. Introduction

1.1. Prostate cancer statistics

Prostate cancer (PCa) stands as the second most frequently diagnosed malignancy in men globally after lung cancer, with 1,414,259 new cases reported worldwide (2020)(World Cancer Research Fund International, *2020*). The mortality rate varies globally, and according to statistics from 2022 across 21 world regions the highest mortality rate was documented in the southern Africa, 29.7 per 100,000 men(Schafer et al., 2024). Given that the incidence of PCa increases with age, the mortality rate is notably elevated in men aged 55 to 74 and even more in individuals over 75 years. Furthermore, prostate cancer-specific mortality has exhibited an upward trend over the last decade, even in developed countries such as the USA and Germany. These statistics define the global significance of PCa. Despite technological advancements and the overall progress in the medical field, the increasing number of new cases and prostate cancer-related deaths persists(World health organization, 2021).

1.2. The role of different examinations in prostate cancer screening

The Prostate-Specific Antigen (PSA) has served as a basic, straightforward, and cost-effective tool for prostate cancer screening since the 1980s. However, the sensitivity of screening test varies depending on age and the PSA cut-off values('Prostate Specific Antigen - StatPearls - NCBI Bookshelf', no date). In 2002, the

US Preventive Services Task Force (USPSTF) initiated a shift in recommendations, advising for the restriction of PSA as a mass screening tool. Although its positive side lies in the early detection of prostate cancer, the consequences manifested on health outcome were inconclusive and mixed. The USPSTF initially issued recommendations in 2002, which were updated in 2008 to advise against prostate cancer screening for men over the age of 75. In 2012, the USPSTF further recommended against PSA screening for prostate cancer detection in all men, regardless of age, except for those undergoing PSA monitoring for survival-related purposes. This led to a decrease in the diagnosis of localized prostate cancer, an increase in late-stage and metastatic disease diagnoses, and a rise in prostate cancer-specific mortality. Similar trends were observed in Europe(Van Poppel *et al.*, 2021).

A large-scale study, the European Randomized Study of Screening for Prostate Cancer (ERSPC), was initiated in 1993 and involved 182,160 men aged 55 to 69 years. The primary objective was to identify prostate cancer mortality among individuals subjected to regular PSA screening. Prostate biopsy was performed in men with elevated PSA (cut-off 3.0-4.0 ng/ml). Randomized patients were stratified into screening and control groups. Over a 13-year period, prostate cancer-related deaths numbered 371 in the intervention group and 570 in the control group, 520 and 793 deaths at 16 years, respectively. The ERSPC study revealed that the reduction in prostate cancer-related mortality was linked to the extension of the follow-up period. Throughout the years, the incidence of prostate cancer in both the control and screening arms remained relatively constant.

However, at the 16-year follow-up, there was a difference favoring the screening group with cumulative PCa-specific incidence 13.3% and 10.3% in the control arm. In the control group, the incidence of prostate cancer gradually approached that of the screening arm; however, after 16 years, the screening arm still exhibited a 41% higher incidence. Notably, preventing one death from prostate cancer necessitated the involvement of 570 men at 16 years, compared to 1,947 men at 9 years. To prevent one prostate cancer-related death, the number of cases needed to diagnose decreased from 48 to 18 after 16 years of follow-up. The study concluded that prolonged follow-up reduced prostate cancer mortality over time. Additionally, the small beneficial effect of one-time screening, compared to serial PSA testing was obvious. Prolonged screening duration is anticipated to yield a significant reduction in prostate cancer mortality(Hugosson *et al.*, 2019).

The current guideline of the European Association of Urology (EAU) strongly advocates for the early performance of PSA testing solely in men exhibiting an elevated risk of PCa who are well-informed. Testing without adequate counseling on the advantages and disadvantages is discouraged. Additionally, a weak recommendation exists to propose an individual risk-adapted strategy for men with a life expectancy of 10-15 years(EAU Guidelines Office, 2024).

For the early detection of PCa, digital rectal examination (DRE) has been an integral component of screening for many years. Throughout this period, opinions on the utility of performing the examination have varied and, for the most part, remained understudied. The sensitivity and specificity of using this examination alone for PCa detection are both less than 60%(Halpern *et al.*, 2018). In the early phase of ERSPC study, DRE was initially excluded as a basic screening tool. However, following the establishment of an association between pathological DRE and PCa, the ERSPC study began screening with DRE in patients with elevated PSA. At present, the National Comprehensive Cancer Network (NCCN) guidelines recommend performing PSA and DRE simultaneously in all patients

for the early detection of PCa, emphasizing that it should not be considered a standalone diagnostic tool. Performing DRE in patients with an increased PSA is deemed beneficial(National comprehensive cancer Network, 2023). The Prostate, Lung, Colorectal, and Ovarian Cancer screening Trial (PLCO) enrolled men aged 55-74 years from 1993 to 2001. Men in the trial underwent PCa screening compared to usual care as the control, and the screening cohort included PSA and DRE examinations. During yearly examinations in the screening arm, PSA levels and DRE status were adjusted based on the current examination results. The primary focus of the statistical analysis was to identify the association between DRE, PSA, and the presence of clinically significant prostate cancer (CSPCa). The screening cohort was divided into three groups based on PSA levels (normal, indeterminate, and elevated) for statistical analysis. Over the 10 years, in men with a positive DRE, the presence of csPCa was higher compared to DRE non-suspect patients in all PSA groups. However, a stronger correlation was observed with the elevated PSA group. Based on the trial, it can be concluded that a pathological DRE was associated with a greater risk of csPCa. Additionally, individuals with an elevated PSA (>3ng/ml) and a positive DRE examination had a 9% higher incidence of csPCa compared to those with a negative DRE. Lastly, DRE was of lesser value in men with a normal PSA. However, a potential bias in the study was that participants with a suspect DRE and normal PSA values avoided biopsy, as recommended by clinicians, which may have led to the underdiagnosis of PCa cases(Halpern *et al.*, 2018).

Nowadays, various risk assessment calculations are available to predict individual risks for PCa and the necessity for biopsy. This plays an important role to minimize the number of unnecessary biopsies. A systematic review was conducted to identify current available risk calculators (RCs). Different calculators incorporate various characteristics, but most include serum biomarker levels and DRE; some also consider sociocultural properties, family history, and imaging methods. For instance, the ERSPC study includes MRI results in the RC. Some newly developed RCs use lifestyle factors and personal characteristics, such as

height and weight, for the calculations. The two most widely used RCs, based on the ERSPC trial and the Prostate Cancer Prevention Trial (PCPT), have been broadly accepted and are available. Additionally, they have been updated and adapted to the broader society beyond the original trial participants. A total of 36 articles were selected, pinpointing 18 risk calculators. The ERSPC and PCPT RCs demonstrated a decent area under the curve (AUC) ranging from 0.68 to 0.86 and 0.56 to 0.811, respectively. It should be noted that these two large trials were conducted in specific populations, namely North American and European, with an insignificant number of other non-American and non-European populations. However, through the systematic review, several calibrations were identified that recreated and validated those results in other cohorts, obtaining the same results as in those basic trials. An example is a Chinese cohort by Chen et al, which validated the ERSPC RC and led to a decrease in unnecessary biopsies. Even though advanced and novel imaging tools and different biomarkers exist today to identify PCa risk, RCs remain a very useful and easyto-use method. In the end, the systematic review indicated that what matters is not which risk calculation is better, but which will be adapted to a specific target population. The calibration of risk calculators for different populations developed in North America and Europe will have an equally positive outcome(Bandala-Jacques *et al.*, 2021).

PSA density (PSA-D) is a crucial factor that provides additional value during the screening for prostate cancer. PSA-D is calculated by dividing the serum PSA by prostate volume. Utilizing PSA-D enhances the credibility of PSA values, given that patients with benign prostatic hyperplasia (BPH) may present with elevated PSA, while PCa patients may sometimes exhibit lower PSA values. The range of PSA values between 4-10 ng/ml remains a gray zone in diagnosis. A trial conducted from 1986 to 1990 involved 61 participants, of whom 41 were scheduled for radical prostatectomy based on the clinical local stage of cancer. Among them, 20 presented with BPH and signs of subvesical obstruction. Prostate volume was determined by MRI for BPH patients with the multiplication of three dimensions of the prostate gland, and through the obtained specimen after surgery for PCa participants. No specific unit was assigned for the PSA-D

value. In the final analysis, all participants with a PSA-D >0.12 were diagnosed with PCa, with only two exceptions having a PSA-D <0.05. Among the subjects with PCa and lower PSA values, 12 were identified. Out of these 12 participants, 5 exhibited abnormal PSAD, 5 had an indefinite PSA-D, and 2 would not have been detected based on PSA-D. While the trial did not provide a direct recommendation based on the selected population, it is important to consider that PSA-D in males with no changes in digital rectal examination and an elevated PSA level could serve as a differentiating tool between benign hyperplasia and PCa(Benson *et al.*, 1992).

There are currently a number of blood-based biomarkers (such as PHI, 4K score, IsoPSA) as well as urine-based biomarkers (such as PCA3, Select MDX, etc.) that provide additional value in prostate cancer diagnosis. However, none of them is considered a standard diagnostic method.

1.3. Imaging modalities for diagnosing prostate cancer

When evaluating imaging modalities for the detection of PCa, various techniques are currently employed. For many years, the standard transrectal ultrasound (TRUS) has served as a basic imaging tool. However, TRUS exhibits limited efficacy in identifying prostate lesions associated with PCa, primarily due to the multifocal nature of prostate cancer. Analysis of removed prostate glands postradical prostatectomy revealed that the typical visual appearance of prostate cancer on TRUS is hypoechoic in 54.5% of cases, slightly hypoechoic in 22%, and isoechoic in 24%. Since the 1970s, TRUS has been available in Japanese and European centers for detection of PCa. Given the limitations of conventional TRUS, novel techniques have emerged to enhance its imaging potential. One

such advancement is the concept of multiparametric ultrasound (mp-US), which combines various innovations(Correas *et al.*, 2021). Modern ultrasound systems have integrated nonlinear imaging and noise reduction technologies, resulting in higher quality images compared to standard B-mode imaging. The frequency of ultrasound probes has generally increased to 7-9 MHz. Additionally, there is a growing trend toward computerizing transrectal ultrasound using artificial intelligence with deep learning algorithms to enhance interpretation accuracy. Prostate TRUS elastography encompasses strain elastography (SE) and shearwave elastography (SWE). The fundamental principle of elastography is to identify stiffer prostate tissue, which is indicative of cancerous lesions. SE requires compression of the prostate gland for a color-coded elastogram. Its sensitivity and specificity are 0.72 and 0.76, respectively, surpassing B-mode ultrasound. However, SE may struggle with benign lesions with inflammation, displaying similar tissue stiffness. SWE provides real-time color mapping of tissue stiffness with a sensitivity and negative predictive value range from 70 to 96%(Barr, Memo and Schaub, 2012; Woo *et al.*, 2015), but limitations include delayed image stabilization, large prostate glands, and excessive compressions. Another valuable ultrasound diagnostic method for PCa is contrast-enhanced ultrasound (CEUS). Intravenously administered contrast agents create colored ultrasound images, emphasizing irregular vessels supplying tumorous tissue. CEUS enhances the identification of hypervascularity in PCa nodules, increasing sensitivity from 54% to 93% compared to baseline Doppler examination. However, a limitation arises from the resonant frequency of most ultrasound contrasts (around 2MHz), whereas most ultrasound transducers operate within the range of 3 to 12MHz. Furthermore, the enhancement of prostate cancer tissue after contrast administration is momentary, lasting less than 20 seconds. Advanced ultrasound modalities include transrectal prostate elastography, contrast-enhanced US, improved B-mode, and micro-Doppler techniques. These techniques can be combined to define a novel US approach known as multiparametric US. The concept of multiparametric ultrasound is inspired from multiparametric magnetic resonance imaging, integrating enhanced B-mode

ultrasound, vascular imaging techniques, elastography, and perfusion imaging in a comprehensive approach(Correas *et al.*, 2021).

Magnetic Resonance Imaging has emerged as an exemplary modality, extensively investigated and currently serving as the standard diagnostic tool for PCa. MRI is included in the EAU guidelines as a standard diagnostic method before performing a prostate biopsy(EAU Guidelines Office, 2024). In addition, it is multifunctional and serves not only for the diagnosis of PCa but also for followup purposes, staging, whole-body scans to identify metastases, evaluating biochemical recurrence, performing MRI-fusion biopsies, sampling of target lesions, and composing a treatment plan. Different sequences can be used while performing imaging with MRI, for example, dynamic contrast enhancement (DCE) and diffusion-weighted imaging (DWI). As not all patients with increased PSA levels have PCa, MRI is a good filter before a prostate biopsy is indicated. That is why MRI is a powerful option with 93% sensitivity for detecting csPCa, as demonstrated by the PROMIS trial. Especially, multiparametric MRI (mpMRI) is the advanced imaging method that combines different sequences, such as the above-mentioned functional sequences DWI and DCE, with anatomical T1weighted images (T1W1) and multiplanar T2-weighted images (T2W1), thereby increasing the sensitivity and specificity of the MRI overall. There is also biparametric MRI (bpMRI), which uses only two sequences, T2W1 and DWI. In both cases, the standardized prostate imaging reporting system (PI-RADS), with a sensitivity of 0.89 and specificity of 0.72, is used to classify different suspicious lesions. For PI-RADS 1 and PI-RADS 2 lesions, the likelihood of encountering clinically significant cancer is highly unlikely and low, respectively. In contrast, with PI-RADS scores of 3, 4, and 5, the risk of presence of clinically significant cancer escalates with increasing PI-RADS score. A PI-RADS score of ≥3 has a positive predictive value (PPV) ranging between 27-48%(Fernandes *et al.*, 2022). Opinions regarding performing mpMRI or bpMRI are controversial in the medical field. A systematic review, a head-to-head comparison of biparametric and multiparametric MRI in the diagnosis of prostate cancer was performed. A total

of 20 studies were included in the qualitative and quantitative synthesis, selected from 2,464 articles retrieved from database searches. The primary rationale for researchers using multiparametric MRI is its high accuracy in detecting prostate cancer. In contrast, proponents of biparametric MRI argue that it offers advantages such as shorter examination time, avoidance of adverse events associated with gadolinium-based contrast agents, and a minimal risk of missing clinically significant prostate cancer. Overall, the findings indicate that the diagnostic performance of bpMRI and mpMRI is comparable in detecting prostate cancer(Woo *et al.*, 2018).

The PROMIS trial was the first prospective, multi-center trial that assessed the diagnostic accuracy of both mpMRI and TRUS biopsy. Each examination was conducted blinded to the results of the other, and the findings were reported according to the Standards for Reporting Diagnostic Accuracy. The primary objective of the study was to determine the proportion of patients identified by mpMRI as csPCa and to identify the percentage of males who could avoid prostate biopsy. Additionally, the study compared the sensitivity, specificity, positive predictive value, and negative predictive value of TRUS and mpMRI. The trial included biopsy-naive men with a clinical suspicion of prostate cancer. An important aspect of the trial was the use of transperineal template prostate mapping biopsies (TPM-biopsy), which samples the entire prostate gland at 5 mm intervals, providing high accuracy. All examinations were performed with multiparametric magnetic resonance imaging using a 1.5 Tesla magnetic field strength. The imaging protocols included T1-weighted and T2-weighted sequences, diffusion-weighted imaging, and dynamic contrast-enhanced imaging sequences with gadolinium(Ahmed *et al.*, 2017).

A Likert radiology reporting system, a 5-point scale ranging from "highly unlikely" (1) to "very likely" (5) for csPCa, was used to score the prostates. This scoring system was based on the outputs of a consensus group convened before the publication of the Prostate Imaging and Data Reporting System.

A total of 576 patients underwent both examinations in the trial. Initially, mpMRI was performed with results blinded to patients and physicians. Subsequently, patients underwent TPM biopsies, followed by TRUS biopsies, both conducted in one session. Out of 576 MRI tests, 418 were deemed significant, and 158 yielded no cancer or non-significant cancer results. After performing TPM biopsy, 213 cases were found to be significant (including MRI scores 3, 4, and 5), while 205 cases were false positives, indicating positive MRI results with no cancer or nonsignificant cases. Among MRI-rated non-significant results, 141 were true negative cases, and only 17 were significant cancer cases.

The final results demonstrated that mpMRI exhibited superior sensitivity (93% vs. 48%) and negative predictive value (NPV) (89% vs. 74%) compared to transrectal ultrasound biopsy. Conversely, TRUS biopsy showed higher specificity (96% vs. 41%) and positive predictive value (PPV) (90% vs. 51%) relative to mpMRI. The PROMIS trial provided level 1b evidence that using mpMRI as a triaging test could allow 25% of patients to safely avoid biopsy. The high NPV of the MRI offers reassurance that a negative result would likely exclude the diagnosis of csPCa.

Some limitations of the study include the exclusion of very large prostates (>100ml) since TPM biopsy could not cover the entire gland, potentially decreasing the number of true negatives. Additionally, performing TRUS biopsy after TPM biopsy, a relatively invasive test, may have led to poorer accuracy of the standard TRUS test due to swelling and distortion of the prostate tissue(Ahmed *et al.*, 2017).

The Precision trial was a multicenter, randomized, noninferiority study that involved a total of 500 men across 25 different centers, with patients randomized in a 1:1 ratio to either the MRI-targeted biopsy or standard biopsy group. One of the major inclusion criteria was the enrollment of biopsy-naïve patients referred

by clinicians based on elevated PSA, suspect DRE, or both, for undergoing prostate biopsy. Additionally, the inclusion criteria required a PSA value of 20 ng/ml or lower. In the MRI arm, male participants underwent mpMRI with T2weighted, diffusion-weighted, and dynamic contrast-enhanced sequences. In cases where the PI-RADS score was ≥3, participants underwent mpMRI-targeted biopsy. The primary outcome aimed to evaluate men with clinically significant cancer (Gleason score 3+4), while the secondary outcome focused on identifying the proportion of clinically insignificant (Gleason score 3+3) cancers. The trial results indicated that 38% of the MRI-targeted group were diagnosed with clinically significant prostate cancer, compared to 26% in the standard biopsy arm. Regarding the secondary outcome, fewer males were diagnosed with clinically insignificant PCa in the mpMRI arm (9%) than in the standard biopsy arm (22%). Furthermore, the superiority of the mpMRI arm was evident in the higher percentage of positive biopsy cores (44%) compared to the standard group (18%). In conclusion, the outcomes suggest the superiority of mpMRI-based biopsies over standard prostate biopsies in diagnosing more csPCa while minimizing the number of clinically insignificant PCa diagnoses. However, the trial has limitations, including variations in interpreting mpMRI results and only moderate agreement between site and central radiological readings. Additionally, participants with increased PSA levels and negative MRI results who did not undergo biopsy could not be excluded from having PCa. The use of only targeted mpMRI biopsies may pose a risk of missing clinically significant cancers(Kasivisvanathan *et al.*, 2018a).

When mpMRI identifies a PI-RADS ≥3 lesion, current recommendations from the EAU guidelines support the performance of targeted biopsies with perilesional sampling(EAU Guidelines Office, 2024).

A prospective, randomized, non-inferiority trial, STHLM3-MRI, was conducted between 2018 and 2020 in Sweden. A total of 12,750 men aged 50-74 years were invited and included in the trial. Participants were randomized if their PSA was 3 ng/ml or higher, or if the Stockholm3 test was 0.11 or higher. Both PSA and the Stockholm3 test, with a cutoff of 3 ng/ml and 0.11, respectively, were initially calibrated to achieve the same sensitivity for identifying clinically significant PCa. However, during the trial, the Stockholm3 test cutoff was upgraded to 0.15. In the standard arm, a systemic biopsy with 10 to 12 cores was performed, while in the experimental arm, a biparametric MRI was conducted. In the experimental arm, if pathological lesions were detected on MRI, targeted plus systematic biopsies were performed. About 3-4 cores per target lesion were collected using the BKMRI fusion system. The Stockholm3 test takes into account various clinical factors, including previous prostate biopsies, patient age, and levels of PSA, free

PSA, human kallikrein 2, β-microseminoprotein, and growth-differentiation factor15 in plasma. MRI is valuable on two levels in diagnosing PCa – first, by detecting suspicious prostate lesions through imaging, and second, by possibility to perform a target biopsy of those suspect lesions. The trial defined a clinically significant PI-RADS score of 3-5. Consequently, the combination of MRI-targeted biopsies with systematic biopsies, in conjunction with the Stockholm3 test, identified a higher proportion of men with clinically significant prostate cancer, totalling 3%, compared to 2.1% in the standard arm that employed PSA testing in conjunction with standard systematic biopsy. Simultaneously, there was a lower number of low-grade cancers identified in the experimental arm compared to the standard arm. Finally, the experimental arm resulted in a 69% reduction in overdiagnosis of PCa while maintaining the good ability to diagnose csPCa effectively. The Stockholm3 cutoff of 0.15 is comparable to a PSA cutoff of 3 ng/ml for maintaining sensitivity in csPCa diagnosis while reducing the number of MRIs and subsequent biopsies(Nordström *et al.*, 2021).

The IP1-PROSTAGRAM study demonstrated that screening men using MR imaging with a score of 4 or 5, followed by a biopsy, detected more csPCa defined as grade group (GG) ≥2, with a similar proportion of biopsied men as PSA ≥3. The harm-benefit relationship of the screening test is based on the sensitivity and specificity of the method. A high sensitivity test is superior to avoid missing a significant disease, provided that there is an acceptable second test to exclude false positive results. Although the PSA test with a threshold of 3 ng/ml has low sensitivity, PSA remains an affordable test, and previous studies have shown that lowering the PSA threshold could improve its sensitivity.

In the IP1-PROSTAGRAM study, 13 different pathways, combining variations of PSA alone, MRI alone, and a range of PSA-MRI combinations, were classified to detect prostate cancer with GG ≥2. The pathways were classified into three groups: single-test screening pathways (1 to 5) using PSA only or MRI only, multiple-test pathways with PSA ≥1 ng/ml as an initial triage test (6 to 9), and pathways with PSA ≥3 (9 to 13) as a first-line test. The reference pathway for all other pathways was pathway 1 with PSA ≥3, reflecting previous randomized controlled studies such as ERSPC. This means that patients with PSA ≥3 underwent systemic biopsy without any imaging, while every other pathway used MRI followed by a target biopsy. No further tests were performed with PSA <3.

ROC curve analysis determined the thresholds for PSA in combined PSA-MRI pathways. A total of 408 participants were included in the study. The results showed that the proportion of participants who underwent biopsies decreased continuously from pathway 3 to 13, but there was no linear relationship for detecting GG≥2 cancer across the pathways. Additionally, there was no difference in GG≥2 cancer detection between black ethnicity or a family history of PCa. The results also demonstrated that pathways with PSA as a first-line test, followed by MRI ≥4 as a follow-up test (in pathways 8, 9, 12, 13), had a significantly reduced biopsy rate compared to the reference pathway(EldredEvans *et al.*, 2023).

The PPV was low for pathway 1, primarily attributed to a low detection rate for GG ≥2. The trial results indicate that the pathways using PSA as a triage test and MRI score of ≥4 would recommend fewer males for the biopsy. The pathways with a

PSA value of ≥3 were associated with lower sensitivity for GG≥2 detection. Finally, the optimal screening pathway out of the above mentioned was suggested to be pathway 8, which combined an initial low PSA threshold of ≥1 and a positive PROSTAGRAM MRI score of ≥4 for identifying GG ≥2.

There are some limitations to the study, including those multiple comparisons between 13 pathways could have led to a type 1 error. Additionally, the study used only target biopsies. Furthermore, there is no consensus on defining csPCa, as GG2 and GG3 may not have an impact on the patient's quality and quantity of life.

The Göteborg screening study conducted the tenth and last screening round in which 20,000 men were randomized to a screening and control group. The screening pathways in this study included PSA ≥3, PSA ≥3 + PIRADS ≥3, and PSA ≥1.8 and PIRADS ≥3. The trial demonstrated a reduction in the prostate biopsy rate with the addition of MRI to PSA ≥3. (Eldred-Evans *et al.*, 2023).

1.4. The development of micro-ultrasound

Performing a prostate biopsy usually involves dedicated prostate imaging, primarily serving as a guide for needle placement and sample collection.

The first pilot clinical trial utilizing a novel 21-MHz high-resolution transrectal ultrasound (HiTRUS) center-frequency sagittal probe on a human prostate took place in 2010. To compare LoTRUS (low resolution transrectal ultrasound) with HiTRUS, a small trial involving 25 patients was conducted from 2010 to 2011. Patients with biopsy-proven PCa, scheduled for robot-assisted radical prostatectomy (RARP) with a prostate gland volume below 60cc, were prospectively recruited. Both ultrasound methods were performed in a single setting before RARP, and after the operation, actual areas of prostate cancer ≥5mm were identified and localized within a prostate sextant. A correlation between ultrasound and pathological findings was analyzed and compared sextant by sextant. Prostate size, Gleason score, and local tumor extent were recorded for each participant. In total, 69 cancerous prostate gland sextants were identified. LoTRUS (38% correct) identified 26 and missed 43 sextants, while HiTRUS (65% correct) identified 45 and missed 24 sextants. In every parameter (sensitivity, specificity, NPV, and PPV), HiTRUS performance exceeded that of LoTRUS(Pavlovich *et al.*, 2014).

1.5. Creation of microUS and PRI-MUS protocol

The development of preclinical ultrasound technologies has led to the creation of a new micro-ultrasound device (ExactVu™, Exact Imaging, Toronto, Canada) operating at 29MHz, in contrast to the 8-12MHz range of conventional TRUS for prostate examination. The heightened frequency of this technology provides special resolution down to 70 microns, resulting in a 300% improvement in

resolution over existing standard ultrasound, while maintaining sufficient depth for visualizing the anterior wall of the gland. This enhanced visualization is anticipated to facilitate the identification of suspicious lesions and, consequently, improve targeting during biopsies.

A novel protocol for this system, named PRI-MUS (Prostate Risk Identification Using Micro-Ultrasound), has been developed to standardize methodologies and establish a consistent imaging-based scoring system for differentiating the risk of malignant transformation in each segment of the prostate gland.

Micro-ultrasound (microUS) represents a further advance of B-Mode Prostate TRUS(Dias *et al.*, 2022).

The above-mentioned pilot study, encompassing 25 patients and slides from whole-mount radical prostatectomy, serves as the foundation for suggesting valuable imaging characteristics. Cine sequences were generated while obtaining each biopsy core using high-resolution ultrasound. Subsequently, 200 randomly selected micro-ultrasound cine sequences from 121 patients undergoing a single biopsy were analyzed by three investigators. The aim was to classify the prostate tissue as pathological (csPCa, Gleason sum >7) or normal. In the biopsied results, 100 samples were benign, while 50 were low-grade and 50 were high-grade PCa samples. Throughout the cine evaluation process, investigators were blinded to the pathological results.

The investigators established a set of defined points to evaluate the prostate based on predefined features, including identifying the prostate border, assessing border irregularities, analyzing the peripheral zone, and assigning a PRI-MUS score, among others. To reduce multiple risk variants, similar risk features were grouped together.

To validate the risk scale, an independent set of 100 biopsy cine sequences was reviewed by five investigators, particularly focusing on the surrounding tissue of the biopsy needle path while remaining blinded to pathology results. A table was

constructed to represent biopsy pathology results based on 10 different features of the microUS cine sequences.

It is important to note that an increasing PRI-MUS score was associated with the severity of cancer, including both the quantity and severity of the disease. The proposed protocol is specifically designed for assessing the peripheral zone of the prostate and is not applicable to other zones.

Table 1 presents the distribution of microUS imaging features and their association with prostate cancer detection. Findings indicate that features such as irregular prostate in the peripheral zone (PZ), irregular PZ border, mixed-echo lesions, and irregular shadowing exhibit the highest relative risk (RR = 2.01–2.02), suggesting a strong correlation with malignancy. Additionally, a heterogeneous "cauliflower/smudgy/mottled" appearance (RR = 1.48, 90% CI [1.11–1.97]) and bright echoes (RR = 1.24, 90% CI [0.89–1.73]) are also associated with an increased likelihood of cancer detection. Conversely, small regular ducts "Swiss cheese" (RR = 0.28, 90% CI [0.05–1.72]) and hyperechoic areas with or without ductal patches (RR = 0.49, 90% CI [0.31–0.78]) demonstrate a lower relative risk of malignancy.

Features such as mild heterogeneity (RR = 1.19, 90% CI [0.87–1.62]) and bright echoes in hyperechoic tissue (RR = 0.79, 90% CI [0.37–1.71]) show a moderate or neutral association with prostate cancer. These findings suggest that heterogeneous and irregular echotexture patterns are more frequently associated with prostate cancer, whereas more uniform or hyperechoic features may be indicative of benign conditions.

The PRI-MUS protocol was then formulated based on the results of this analysis (Table 2)(Ghai *et al.*, 2016a).

Table 1. PRI-MUS risk of carcinoma.

PZ peripheral zone. PRI-MUS Prostate Risk Identification Using Micro-Ultrasound. Adapted from (Ghai *et al.*, 2016a)

Feature	N	N (Cancer)	RR [90% CI]
Small regular ducts "Swiss cheese"	7	1	0.28 [0.05 - 1.72]
Hyperechoic, with or without ductal patches	50	14	0.49 [0.31 - 0.78]
Mild heterogeneity	42	24	1.19 [0.87 - 1.62]
Bright Echoes in hyperechoic tissue	10	4	0.79 [0.37 - 1.71]
Heterogeneous "Cauliflower/Smudgy/Mottled" appearance	32	22	1.48 [1.11 - 1.97]
Bright Echoes	30	18	1.24 [0.89 - 1.73]
Irregular Prostate (PZ)	1	1	2.01 [1.75 - 2.31]
Irregular PZ border	1	1	2.01 [1.75 - 2.31]
Mixed-echo lesions	2	2	2.02 [1.76 - 2.33]
Irregular Shadowing	12	11	1.94 [1.54 - 2.43]

Table 2. PRI-MUS risk of carcinoma
PRI-MUS Prostate Risk Identification Using Micro-Ultrasound
Adapted from (Ghai *et al.*, 2016a)

PRI-MUS Risk Score	Risk of Carcinoma	Findings
1	Very low	Small regular ducts 'Swiss cheese' with no other heterogeneity or bright echoes
2	Some	Hyperechoic, with or without ductal patches (possible ectatic glands or cysts)
3	Intermediate	Mild heterogeneity or bright echoes in hyperechoic tissue
4	Significant	Heterogenous 'Cauliflower/Smudgy/Mottled' appearance or Bright Echoes (possible comedonecrosis)
5	Very high	Irregular Shadowing (originating within the Prostate, not the prostate border) or Mixed-echo lesions or Irregular Prostate and/or PZ border

The protocol incorporates sonographic features characteristic of specific PRIMUS levels. For example, PRI-MUS 1, a benign tissue pattern, characterized by a ductal appearance, presents a "swiss cheese" pattern, as shown in Image 1. PRI-MUS 2 also represents a benign condition but without typical swiss cheese appearance, instead displaying hyperechogenicity with or without ductal patches. PRI-MUS 3 is considered a transitional score, exhibiting mild heterogeneity or bright echoes within hyperechoic tissue. While PRI-MUS 4 and 5 indicate highly suspicious lesions for prostate cancer. PRI-MUS 4 is characterized by a cauliflower-like, smudgy/mottled appearance or bright echoes (referred to as a "starry sky" pattern). PRI-MUS 5 presents as irregular shadowing, mixed echo lesions, or an irregular prostate/peripheral zone border.

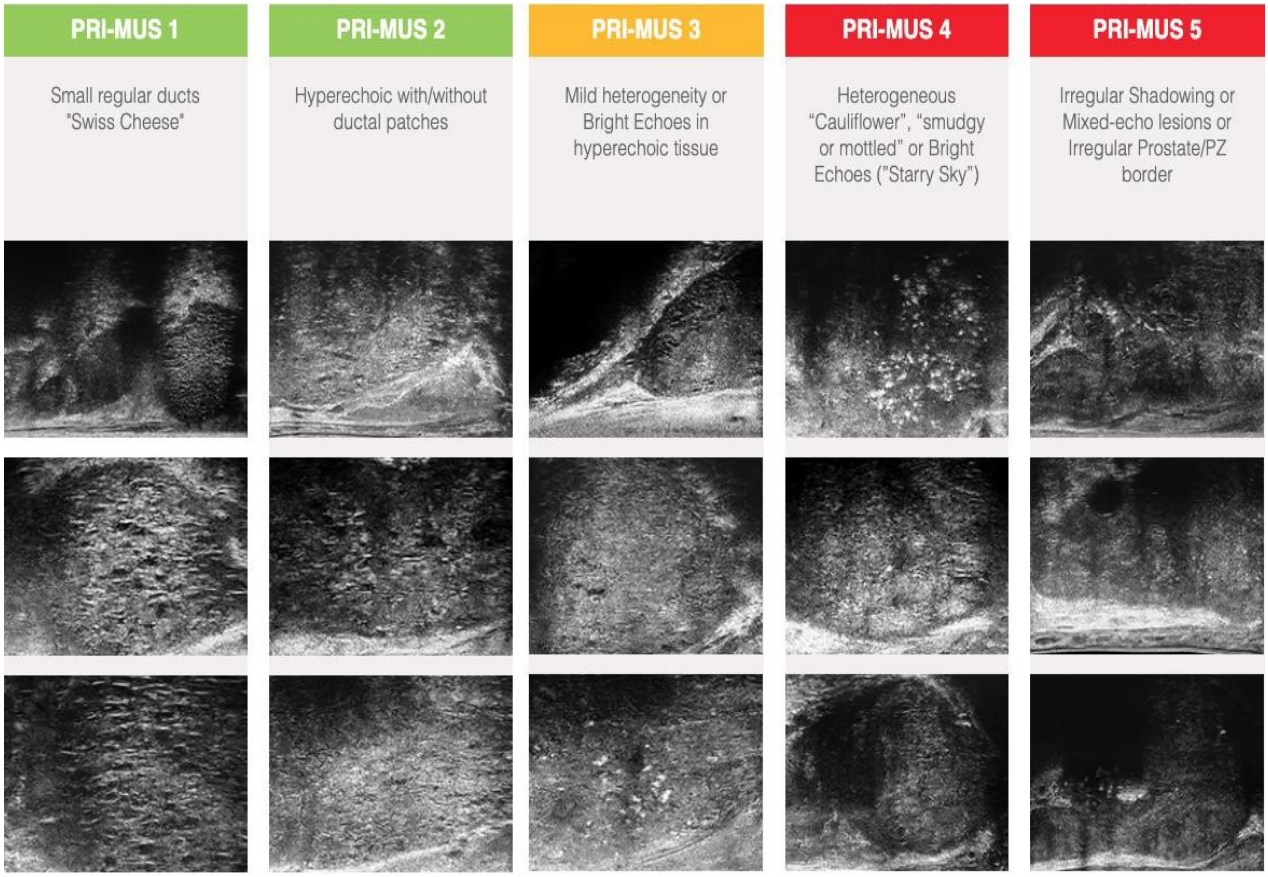


Image 1. Micro-ultrasound images of different PRI-MUS risk levels
PRI-MUS Prostate Risk Identification Using Micro-Ultrasound
PRI-MUS Prostate Risk Identification Using Micro-Ultrasound(Exact Imaging, no date)

1.6. Feasibility of microUS

While mpMRI has been extensively researched, it still misses up to 23% of csPCa cases(Eure *et al.*, 2018).

During 2017 and 2018, 104 subjects suspected of having PCa based on elevated PSA values, digital rectal examination, or a PI-RADS lesion score of at least 3, were enrolled in a prospective clinical trial. All patients underwent microUSguided targeted prostate biopsy, with operator blinded to multiparametric magnetic resonance imaging results. Targeted biopsies with microUS were followed by mpMRI/US fusion-targeted biopsies and a 12-core standard biopsy. Clinically significant prostate cancer was defined as a Gleason score of ≥ 7.

The study design inherently favored mpMRI identification of more lesions, as it was part of the inclusion criteria. The majority of PI-RADS lesions were classified as PI-RADS 4, comprising 58%. MicroUS identified 83 out of 104 lesions (80%), primarily PRI-MUS score 4 lesions (58%). In total, 56 out of 104 patients were diagnosed with PCa and 35 with csPCa. MicroUS detected 33 csPCa cases and 15 insignificant cancers, while missing 2 non-csPCa cases. MRI detected 35 csPCa cases, and 48 subjects had benign histology despite initially being rated as suspicious lesions on mpMRI. MicroUS achieved a high sensitivity of 94% (95% CI 81–98%), specificity of 28% (95% CI 18–39%), and high negative predictive value of 90% (95% CI 70–97%) for diagnosing csPCa. Sensitivity, specificity, and NPV values for mpMRI could not be determined, as only mpMRIpositive subjects were included. The inclusion of only mpMRI-positive subjects might have also affected the sensitivity of microUS(Lughezzani *et al.*, 2019).

Another trial prospectively validating the PRI-MUS protocol at a single center in Linz, Austria included 372 patients who underwent biopsy using ExactVu™ microUS from 2018 to 2019. For biopsy, an extended version of the sextant protocol was employed for prostate sampling, comprising 2 cores in the anterior prostate zone to exclude the presence of large anterior tumors. Pathology results confirmed clinically significant prostate cancer (GG>1) in 42% of patients, with an AUC of 0.76 for PRI-MUS in predicting GG>1. The accuracy varied across different prostate zones, with the highest accuracy observed in the prostate apex and the lowest in the prostate base. Since the PRI-MUS protocol was not validated for scoring in the anterior zone but only for the peripheral zone, targets from the anterior zone were sampled but often not assigned lesion scores. Nonetheless, in 33 out of 733 anterior samples with assigned PRI-MUS scores, the AUC was 0.80. It was found that not only did the prostate base exhibit the lowest PRI-MUS accuracy, but also the lowest number of csPCa cases. PPV for PRI-MUS ranged from 4.4% (PRI-MUS 1) to 71.4% (PRI-MUS 5). The study uncovered the potential of microUS in detecting cancer in the anterior prostate zone(Luger *et al.*, no date).

A single-institution study conducted in France by Cornud et al. evaluated the role of high-frequency microUS as a second-look examination due to limitations observed with other techniques, such as MRI-TRUS fusion and in-bore MRI guidance, in performing real-time biopsies with higher resolution than conventional TRUS. The investigators evaluated as a primary objective the value of microUS as a second-look examination following prostate bpMRI identifying suspicious lesions with a score >2. The secondary objective of the study was to identify lesions via microUS that were not detected by MRI. MRI lesions in the sagittal plane were correlated with images obtained using microUS in the parasagittal plane to perform fusion biopsies. For lesions visible only with ultrasound, real-time microUS-guided targeted biopsies were performed. It is

important to note that a PRI-MUS scoring system was not utilized for microUS lesions; instead, lesions were suspected based on the visualization of either homogeneous or heterogeneous hypoechoic lesions. Additionally, systematic biopsies were not performed, and a median core number collected was 4.

Out of 144 lesions, 114 were detected by both imaging methods, while 13 were visible only on MRI and 17 only on microUS. Histopathologically, 70 out of 144 lesions were deemed clinically significant, defined as Gleason score ≥ 7 components or any sample with a maximum cancer core >3mm for Gleason 3+3. The PCa detection rate was 21%, 63.9%, and 79.4% for bp-MRI scores 3, 4, and 5, respectively. Only MRI detected 13 lesions that turned out to be clinically nonsignificant, while out of the 17 lesions identified only by microUS, 4 were significant. Overall, in response to the primary objective, the cancer detection rate by a second-look microUS after bpMRI was 57.6% for all cancers and 51.4% for csPCa. All 74 csPCa were observed on microUS, achieving a sensitivity of 100%, not achieved by any other ultrasound method. In addressing the secondary objective of the study, microUS identified 4 csPCa lesions not seen by MRI, highlighting the potential of microUS(Cornud *et al.*, 2020).

A prospective registry involving 1,040 subjects across 11 different urological centers in North America and Europe was created. Various criteria, such as elevated PSA levels, suspicious DRE, or MRI findings, were utilized to register subjects, ensuring a real-world analysis. Targeted prostate biopsies, ranging from 1-3 cores, were obtained based on suspect lesions identified through either mpMRI with PI-RADS scores ≥3 or micro-ultrasound with PRI-MUS scores ≥3. This was followed by 12-14 core systematic samples. Clinically significant prostate cancer was defined as GG ≥2. In general, a 15% increase in csPCa diagnosis is observed with MRI compared to standard systematic biopsy. The primary objective of the trial was to determine the sensitivity and specificity of the selected imaging modalities. There were variations in biopsy methods across

different sites, including the use of cognitive fusion or fusion targeting systems and 9 out of 11 sites were unblinded to MRI results.

Of the 864 (83%) positive cancer cases identified, 364 (43%) were clinically significant. Micro-ultrasound identified suspicious lesions in 877 (84%) subjects, with only 25 cases (2%) where micro-ultrasound failed to detect csPCa. Detection of csPCa with mpMRI PI-RADS lesions increased with higher PI-RADS scores, with detection rates of 14%, 38%, and 62% for PI-RADS scores of 3, 4, and 5, respectively. The overall results for micro-ultrasound and mpMRI are presented in Table 4 below(Klotz *et al.*, 2020).

Multiparametric MRI failed to detect approximately 15% of csPCa cases. Microultrasound demonstrated superior sensitivity in diagnosing csPCa compared to MRI. According to this multicenter, prospective, large-scale analysis microUS appears to be a viable alternative concerning sensitivity, specificity, PPV, and NPV when compared with mpMRI(Klotz *et al.*, 2020).

Table 4. Overoll Outcomes: Comparison of mpMRI and micro-ultrasound microUS micro-ultrasound. mpMRI multiparametric MRI Adapted from (Klotz *et al.*, 2020).

Modality	**Sensitivity**	**Specificity**	**PPV**	**NPV**
mpMRI	90%	22%	43%	77%
microUS	94%	22%	44%	85%
P (non-inferiority)	<0.001	<0.001	<0.001	<0.001
P (superior)	0.03	0.45	0.32	0.04

Hofbauer et al. included a total of 203 patients in the first multicenter, prospective, non-inferiority comparative analysis of microUS and mpMRI. The trial was conducted in urology centers in Germany (Tübingen, Berlin) and Austria (Linz).

Patients were enrolled based on clinical suspicion of prostate cancer and mpMRI findings. MicroUS was assessed by a physician blinded to the mpMRI results and the patients' medical history. After documenting PRI-MUS suspicious lesions on microUS, physicians were unblinded to the mpMRI results, and targeted biopsies were performed alongside systematic biopsies.

A total of 79 out of 203 (39%) patients were diagnosed with clinically significant prostate cancer. MicroUS-targeted biopsies detected 58 out of 79 (73%) cases, while mpMRI-targeted biopsies identified 60 out of 79 (76%) (Image 2).

MicroUS detected 97% of the csPCa cases identified by mpMRI-targeted biopsy. However, performing a combined approach using microUS along with systematic biopsies provided a greater benefit, detecting 72 out of 79 (91%) cases of csPCa.

The study concluded the non-inferiority of microUS compared to mpMRI in a realworld biopsy population(Hofbauer *et al.*, 2022).

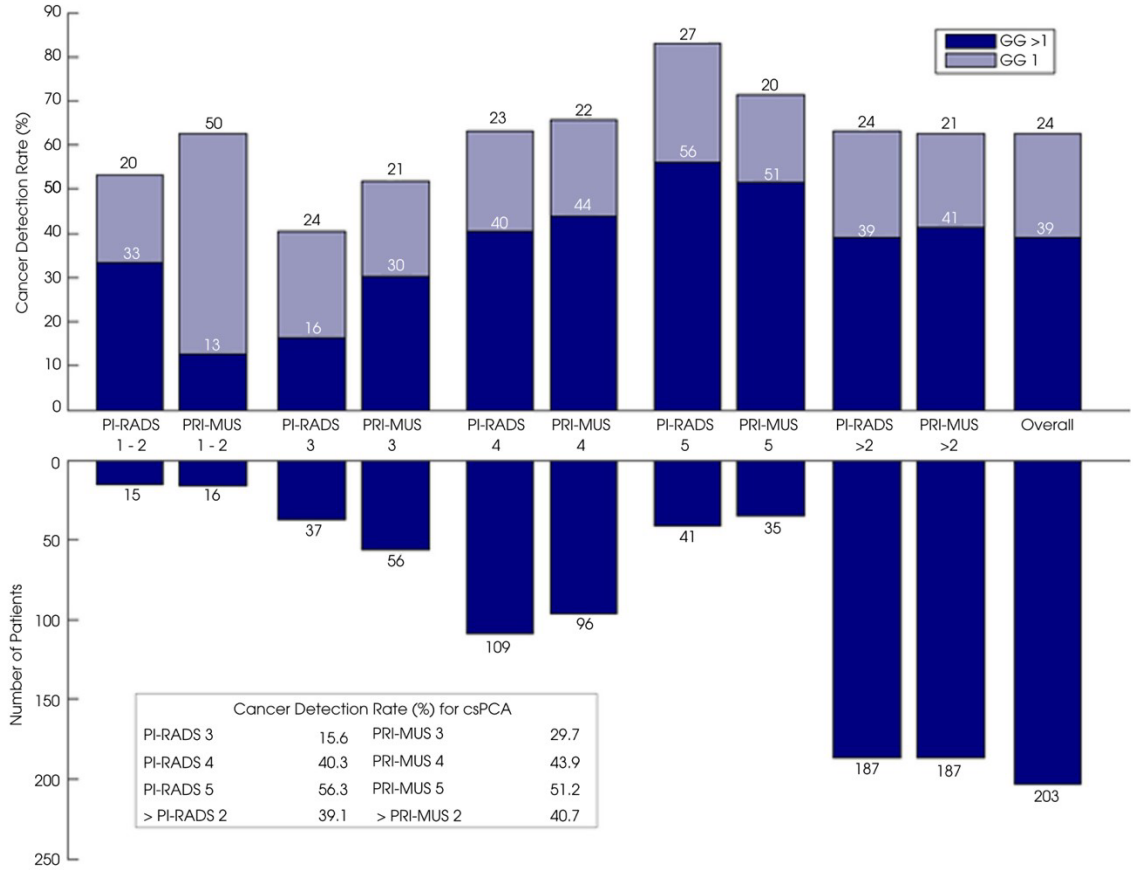


Image 2. Detection of clinically significant prostate cancer with microUS and mpMRI
PRI-MUS Prostate Risk Identification Using Micro-Ultrasound. PI-RADS Prostate Imaging Reporting and Data System (Hofbauer *et al.*, 2022)

Image 3 represents additional studies comparing microUS with mpMRI. Although the studies are still limited and mostly provide retrospective data, the published data is promising. For patients with contraindications for mpMRI, microUS already presents the best available option. However, whether it is possible to rely on microUS for the local staging of prostate cancer depends on further evaluation(Harland and Stenzl, 2021).

Year	First author	n	Sensitivity csPC		Specificity MUS	PPV csPC		NPV MUS	Detection rate csPC	
			MUS	MRI		MUS	MRI		MUS	MRI
2020	Cornud, F	118	100%	88%	23%				51.40%	
2019	Abouassaly, R	19							21%	11%
2019	Lughezzani, G	104	94%		28%	40%	34%	90%	40%%	23%
2020	Roja Claros, O	269							38%	23%
2020	Rodriguez Socarras, ME	194	98.9%	85.5%	29.3%	62.3%	56.3%	95.6%	24%	28%
2020	Wiemer, L.	159	95%		15%	52%		75%		
2020	Klotz, L	1040	94%	90%	22%	44%	43%	85%		

Image 3. Trials comparing microUS and MRI-guided prostate biopsies
MUS micro-ultrasound. csPC clinically significant prostate cancer. (Harland and Stenzl, 2021)

1.7. MicroUS features

MicroUS is an innovative technology operating at 29MHz, capable of achieving a resolution of 70 microns. This resolution enables interpreters to visualize alterations in the ductal structure of prostate gland tissue and identify prostate cancer. MicroUS offers high spatial resolution, while it does not incorporate functional imaging techniques such as diffusion-weighted imaging and dynamic contrast-enhanced sequences. For the detection of prostate lesions, ultrasound employs the PRI-MUS scoring system. Notably, microUS facilitates real-time visualization of suspect lesions(Basso Dias and Ghai, 2023).

The ExactVu system integrates a suite of technological advancements in transducer fabrication and image processing. This innovation enables the 29MHz system to achieve a satisfactory signal-to-noise ratio at a depth of 5 cm, surpassing the typical 3 cm depth associated with such frequencies. These advancements involve the development of ultrasonic crystals characterized by an exceptionally wide bandwidth and heightened sensitivity, which accounts for the inevitable downshift introduced by biological tissues. Moreover, optimizations in transmit pulse configurations and software-based image filtering enhance penetration capabilities and maximize imaging depth. While imaging performance remains optimal between 0 and 3 cm, particularly in the peripheral zone of all prostates, there is a gradual decline in lateral resolution and signal-to-noise ratio beyond the 3 to 5 cm depth range(Cornud *et al.*, 2020).

MicroUS utilizes high-frequency ultrasound, offering exceptional resolution that enhances anatomical detail and tissue differentiation. The 70 μm resolution enables the distinction of structures as close as 70 μm apart.

It is noteworthy that microUS utilizes an EV29L side-fire transducer, which provides several advantages compared to standard end-fire transducers. The side-fire transducer facilitates comfortable probe insertion and includes two needle ports with separate angles for the anesthesia needle and biopsy needle. The anesthesia port, with a shallow trajectory, is specifically designed to access the seminal vesicle/prostate junction without excessive rectal insertion. Additionally, its longer imaging surface enables comprehensive visualization of the entire prostate gland. In terms of prostate gland visualization, microUS offers special features, allowing imaging depths of up to 60mm.

2. Materials and Methods

2.1. On-site procedures

We share our experience with micro-ultrasound technology at the University Clinic of Tübingen (Eberhard-Karls University), specifically within the Department of Urology. Ethical approval for this study was obtained from the University of Tübingen (Approval No. 495/2023B02).

After assembling the device in the department in 2020 and completing intensive and official training from the Exact Imaging team members at our site, prostate examinations using microUS were implemented and gradually offered to a selected group of patients suspected of having prostate cancer.

Inclusion/Exclusion:

Men aged 18 years and older undergoing prostate biopsy due to suspected prostate cancer were included in the study retrospectively, between 2020 and 2022.

Clinical risk factors for prostate cancer included elevated PSA levels, suspicious digital rectal examination, family history of prostate cancer, or suspicious microultrasound/mpMRI lesions. Patients who had not undergone prior multiparametric MRI were also included.

Biopsy

Prostate biopsies were performed via transperineal approach. In patients with suspicious microUS lesions who had not undergone prior mpMRI, lesions with a

PRI-MUS score ≥3 were used as the target for biopsy. In cases where mpMRI had been performed previously, a microUS-mpMRI fusion biopsy was conducted, and targeted samples were collected based on both microUS and mpMRI findings. If target lesions overlapped between microUS and mpMRI, no additional targeted samples were collected.

The biopsy protocol was completed with systematic biopsies.

All microUS examinations at our center were primarily conducted by one experienced urologist who possessed specialized certification as an expert in microUS examinations and had extensive prior experience in diagnosing and biopsying prostate, both with microUS and mpMRI-fusion biopsies.

microUS Evaluation:

Each microUS examination adhered to a standard protocol and utilized the PRIMUS scoring system. Systematic biopsies, as well as targeted biopsies, were performed in all cases. Before conducting a biopsy, the prostate gland underwent ultrasound examination and scanning. Initially, the ultrasound scan began with the default setting of 50mm, allowing for visualization of the entire gland and its surrounding anatomy. However, for a comprehensive examination, including PRIMUS scoring or biopsy procedures, the 30mm mode was utilized. The TGC and gain knob settings were adjusted to obtain high-quality images of the gland.

Following evaluation of the gland, including determination of volume and assessment of all zones (peripheral, central, transitional, and anterior), as well as the seminal vesicles and prostate capsule, prostate lesions were identified and scored according to the PRI-MUS protocol. Suspicious lesions were characterized by a PRI-MUS score of ≥3 and exhibited features such as mild heterogeneity (mottled, smudgy, or cauliflower texture), presence of bright echoes, irregular shadowing, irregular peripheral zones or prostate margins, and mixed-echo lesions. High-risk anterior prostate gland features were identified separately and included focal lenticular lesions, storm-cloud lesions, lesions with irregular finger-like projections, and lesions of the anterior apical horn and anterolateral peripheral zone. Assessment for extraprostatic growth of cancer, capsular bulging, contact of cancer with the prostate capsule, and capsule deformation or invasion was also conducted.

During biopsy preparation, a standardized approach was employed for each procedure. The initial important step involved calming the patient and optimizing image quality, which included identifying artifacts or inadequate transducer preparation, such as gel bubbles or insufficient gel application. Subsequently, image size was set to 'small' (30mm) while employing the aforementioned image optimization techniques. Benign features such as ductal patterns, cysts, and calcifications were initially identified to prevent confusion with suspicious lesions. Suspicious lesions were then identified and recorded as cine loops. A local anesthetic (20 mL of 1% Xylocaine) was administered subcutaneously perineally, and 5 mL of 1% Xylocaine was injected at the apex on each side. Once adequate anesthesia was achieved and the patient and transducer were properly positioned, perineal prostate biopsy was conducted using either a microUS system alone or MRI/microUS fusion. Both microUS and MRI-targeted lesions were sampled, with the number of target cores varying based on lesion count and size, typically ranging from 2 to 3 cores, but with a maximum of 7 cores collected. This was followed by systematic biopsy, where samples were evenly spaced, considering evidence of previous needle tracks detected by microUS. Approximately 10-12 systematic cores were obtained per biopsy using an onscreen needle guide. Each core was visually inspected to ensure a 2cm

sample was collected before being sent to the local pathology department for examination.

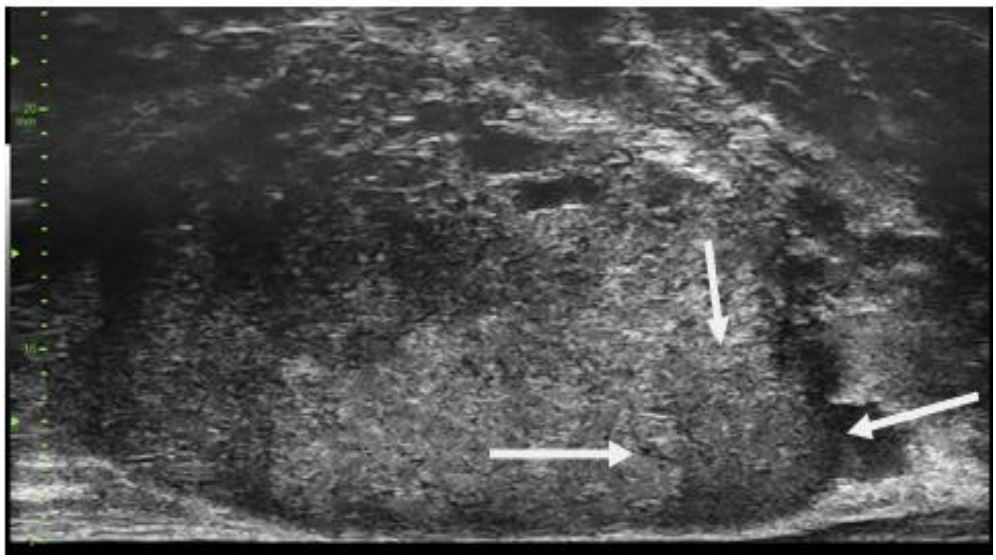


Image 4. PRI-MUS 4. Pathology: 3+4, ISUP 2.
PRI-MUS Prostate Risk Identification Using Micro-Ultrasound. ISUP international society of urological pathology

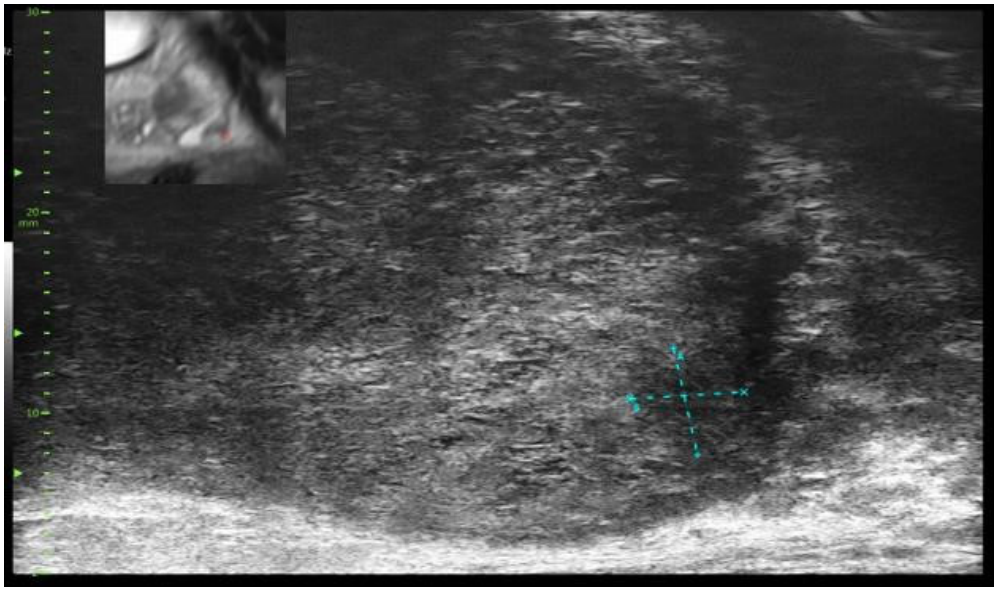


Image 5. PRI-MUS 4, target lesion Apex. Pathology: 3+4, ISUP 2
PRI-MUS Prostate Risk Identification Using Micro-Ultrasound. ISUP international society of urological pathology

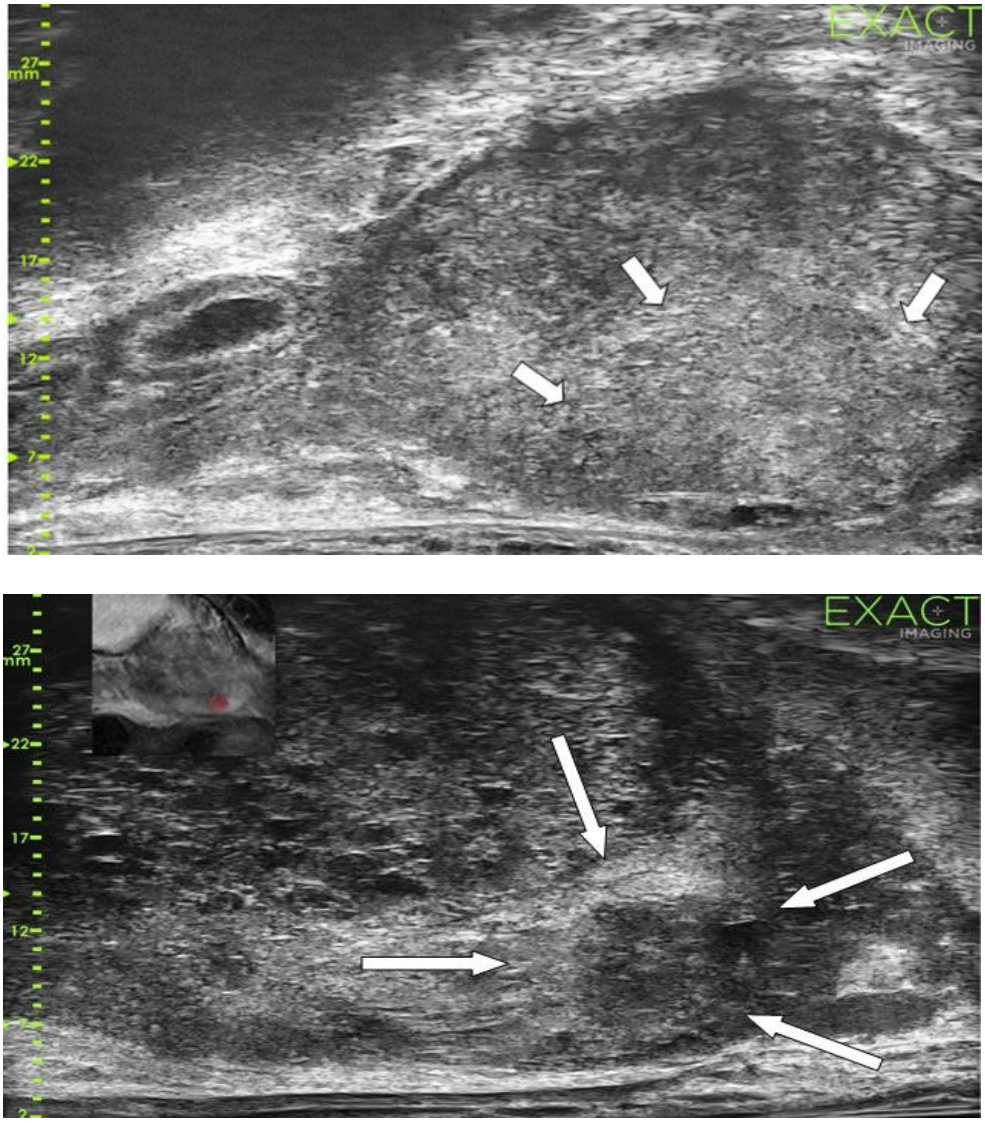


Image 6. upper image: right Apex target (PRI-MUS 5), ISUP 4, lower image: right middle lateral target (PRI-MUS 4) ISUP 3

ISUP international society of urological pathology. PRI-MUS Prostate Risk Identification Using Micro-Ultrasound

2.2. Data collection

The data from patients who underwent a prostate biopsy guided by microUS were retrospectively collected from the general clinic database. Special authorization was obtained to ensure compliance with data protection regulations for the collection and editing of personal data.

A data bank was generated to compile retrospective data from subjects who underwent microUS-guided biopsies at our department from 2020 to 2022. The database encompassed various parameters, including patient identification number, case number, subject age, biopsy date, iPSA level, prostate volume, PRI-MUS score, PI-RADS score, presence of benign/malignant histology, ISUP grade, digital rectal examination findings, PSA density, target biopsy results (positive/negative), systematic biopsy results (positive/negative), ISUP grades of target and systematic cores, number of positive target cores, number of positive systematic cores, total number of target cores, total number of systematic cores, TNM status, post-biopsy treatment modality (active surveillance, radical prostatectomy, radiation therapy with/without androgen deprivation therapy, clinical trial participation), and surgical margin positivity (R-status) in case of surgery.

The data for a total of 122 patients were obtained. Among these patients, 66 underwent prostate MRI prior to biopsy. Some of the patients who received MRI underwent microUS/MRI-fusion biopsy, with biopsy decisions guided by the combined results of both examinations.

The suspicious lesion identified by MicroUS and MRI was defined as ≥3 PRIMUS and PI-RADS, respectively. Significant prostate cancer was defined as ISUP ≥ 2 (Gleason score 3+4).

2.3. Statistical analysis of the data

The statistical analysis for the research was primarily conducted using IBM SPSS Statistics, version 28.0.0.0 (190), with supplementary analysis performed using Excel. Tables generated in Excel were imported and aligned to produce adapted tables within SPSS. Variables were appropriately adjusted and categorized into nominal, ordinal and metric types. Some variables required further subclassification based on their characteristics, such as histopathology results (benign and malignant), ISUP grades, or DRE findings (positive for suspicion and negative).

The Institute of Clinical Epidemiology and Applied Biometry was engaged to provide support for the statistical analysis. This collaboration aimed to develop a robust analytical framework and offer guidance on data creation and management. This service is part of a support system available to doctoral candidates at the University of Tübingen and is provided free of charge.

First, a table was created to summarize the general demographics of 122 patients (Table 6). Subsequently, specific tables were generated to illustrate the percentage and distribution of subjects with all PRI-MUS scores, as well as with all PI-RADS scores. True positive, true negative, false positive, and false negative rates, alongside sensitivity and specificity, were calculated for both microUS and MRI modalities. Multiple associations between PRI-MUS and ISUP scores, as well as between PI-RADS and ISUP scores for all risk groups (1 to 5), were examined. Descriptive statistics, frequencies and logistic regression analysis were extracted using SPSS (IBM SPSS Statistics, version 28.0.0.0 (190), with supplementary analysis performed using Excel.

3. Results

3.1. General statistical data

A total of 122 patients were included in the study. The median PSA level was 7.0 ng/ml with an interquartile range (IQR) of 5.4–10.3 ng/ml. The median age of the patients was 64 years (IQR: 64–68 years). The median prostate volume measured 45 ml (IQR: 28–58 ml), while the median PSA density was 0.17 ng/ml² (IQR: 0.11–0.27 ng/ml²).

A suspicious digital rectal examination was reported in seven patients.

PRI-MUS score was recorded for all 122 patients. Among them, 17 patients (13.9%) had a PRI-MUS score of 1 or 2, 33 patients (27.0%) had a score of 3, 57 patients (46.7%) had a score of 4, and 15 patients (12.3%) had a score of 5.

In the subgroup of 66 patients who underwent mpMRI, PI-RADS score was distributed as follows: 6 patients (9.1%) had a score of 1 or 2, 19 patients (28.8%) had a score of 3, 32 patients (48.5%) had a score of 4, and 9 patients (13.6%) had a score of 5.

These data provide an overview of the baseline patient characteristics and imaging-based risk stratification using both the PRI-MUS and PI-RADS scoring systems. The results illustrate the distribution of lesion suspicion levels, highlighting variations in risk assessment.

Table 6. Patient demographics
PRI-MUS Prostate Risk Identification Using Micro-Ultrasound. PI-RADS Prostate Imaging–Reporting and Data System. IQR interquartile range. DRE digital rectal examination

N	122
PSA median (IQR), ng/ml	7,0 (5,4-10,3)
Age median (IQR)	64 (64-68)
Prostate volume median (IQR), ml	45 (28-58)
PSA density median (IQR), ng/ml^2	0,17 (0,11-0,27)
Suspicious DRE	7
Highest PRI-MUS	N=122
1-2	17 (13.9%)
3	33 (27%)
4	57 (46,7%)
5	15 (12,3%)
Highest PI-RADS	N=66
1-2	6 (9,1%)
3	19 (28,8%)
4	32 (48,5%)
5	9 (13,6 %)

The distribution of PRI-MUS scores (Table 7) in the given group of patients, expressed as percentages, was as follows: PRI-MUS 2: 13.9%, PRI-MUS 3: 27%, PRI-MUS 4: 46.7%, and PRI-MUS 5: 12.3%. Among the 17 patients with a PRIMUS score of 2, five were diagnosed with prostate cancer in the pathology results. Among the 105 subjects with microUS-suspected prostate glands and a PRI-MUS score equal to or greater than 3, 70 were diagnosed with prostate cancer.

Table 7. The distribution of PRI-MUS score
PRI-MUS Prostate Risk Identification Using Micro-Ultrasound

PRI-MUS	Frequency	Percent %
2	17	13.9
3	33	27.0
4	57	46.7
5	15	12.3
Total	122	100.0

The distribution of PI-RADS scores (Table 8) expressed as percentages, was as follows: PI-RADS 2: 9.1%, PI-RADS 3: 28.8%, PI-RADS 4: 48.5%, and PI-RADS 5: 13.6%. Among the 6 patients with a PI-RADS score of 2, four were diagnosed with prostate cancer in the pathology results and among the 60 subjects with PIRADS-suspected prostate glands and a PI-RADS score equal to or greater than 3, 34 were diagnosed with prostate cancer.

Table 8. The distribution of PI-RADS score
PI-RADS Prostate Imaging–Reporting and Data System

PI-RADS	Frequency	Percent %
2	6	9.1
3	19	28.8
4	32	48.5
5	9	13.6
Total	66	100.0

Table 9 presents the diagnostic performance metrics (sensitivity, specificity, positive predictive value, and negative predictive value) for microUS and mpMRI. The values are expressed as percentages along with their 95% confidence intervals.

MicroUS correctly identified PCa in 70 cases (true positives), while accurately excluding the presence of cancer in 12 cases (true negatives). There were 35 false positive cases and 5 false negatives. Overall, the sensitivity of microUS was 93%, and the specificity was 25%.

For mpMRI with PI-RADS score ≥3, 34 cases were correctly identified as PCa (true positives), and 2 cases were accurately negative for cancer (true negatives). There were 26 false positive cases and 4 false negatives. The sensitivity of mpMRI was calculated as 89%, with a specificity of 7%.

The PPV for microUS (66%) is higher than that for mpMRI (56%). Also, the NPV for microUS (70%) is higher than that for mpMRI (33%).

Table 9. Diagnostic Performance of microUS and mpMRI microUS micro-ultrasound. mpMRI multiparametric MRI

%	microUS	mpMRI
Sensitivity	93% CI(0.84-0.97)	89% CI(0.74-0.96)
Specificity	25% CI(0.14-0.40)	7% CI(0.012-0.24)
PPV	66% CI(0.56-0.75)	56% CI(0.43-0.69)
NPV	70% CI(0.44-0.88)	33% CI(0.05-0.75)

3.2. Association between PRI-MUS/PI-RADS and ISUP grade

In the cohort studied, a single patient with PRI-MUS 2 was identified with ISUP ≥2 cancer. Among the 17 PRI-MUS 2 glands assessed, 12 were negative for cancer, 4 exhibited clinically insignificant cancers with ISUP 1, and 1 demonstrated clinically significant prostate cancer with ISUP 2 (Table 10).

Similarly, in patients undergoing mpMRI assessment of the prostate gland with a PI-RADS score of 2, three cases of csPCa were identified. Specifically, among the 6 patients with a PI-RADS score of 2, 1 exhibited no cancer, 2 were diagnosed with non-csPCa, 2 with ISUP 3, and 1 with ISUP 4 prostate cancers (Table 11).

Table 10. Distribution of Prostate Cancer absence or presence (ISUP 1-5) for PRI-MUS 2 scores
PRI-MUS Prostate Risk Identification Using Micro-Ultrasound. ISUP international society of urological pathology

Histology	Frequency	Percent %
No cancer	12	70.6
GL 3+3 (ISUP1)	4	23.5
GL 3+4 (ISUP 2)	1	5.9
Total	17	100

Table 11. Distribution of Prostate Cancer absence or presence (ISUP 1-5) for PI-RADS 2 scores
PI-RADS Prostate Imaging–Reporting and Data System. ISUP international society of urological pathology

Histology	Frequency	Percent %
No cancer	1	16.7
GL 3+3 (ISUP1)	2	33.3
GL 3+4 (ISUP 2)	2	33.3
GL 4+4 (ISUP 4)	1	16.7
Total	6	100

After re-filtering the dataset, cases with a PRI-MUS score of 3 or higher, and PSA levels of 4 or higher have been selected.

Pathology results confirmed clinically significant prostate cancer (ISUP score ≥

2) in 58 cases. Within the subgroup of 101 patients meeting the criteria of PRIMUS score equal to or greater than 3, 32 individuals showed no evidence of cancer, 11 were diagnosed with non-clinically significant prostate cancer, while 58 cases were clinically significant cancers (Table 12).

Similarly, applying the same filter to cases assessed via mpMRI, with a PI-RADS score equal to or greater than 3, revealed that 25 patients had csPCa, 21 were diagnosed as benign, and 6 were classified as non-clinically significant prostate cancer cases (Table 13).

Table 12. Distribution of Prostate Cancer absence or presence (ISUP 1-5) for PRI-MUS ≥ 3 and PSA ≥ 4 cohort GL Gleason score. PRI-MUS Prostate Risk Identification Using Micro-Ultrasound. ISUP international society of urological pathology

PRI-MUS ≥ 3 **PSA ≥ 4**	**Frequency**	**Percent %**
No cancer	32	31.7
GL 3+3 (ISUP1)	11	10.9
GL 3+4 (ISUP 2)	24	23.8
GL 4+3 (ISUP 3)	19	18.8
GL 4+4 (ISUP 4)	11	10.9
GL 4+5, 5+4 (ISUP 5)	4	4.0
Total	101	100

Table 13. Distribution of Prostate Cancer absence or presence (ISUP 1-5) for PI-RADS ≥ 3 and PSA ≥ 4 cohort PI-RADS Prostate Imaging–Reporting and Data System. ISUP international society of urological pathology

PI-RADS ≥ 3 **PSA ≥ 4**	**Frequency**	**Percent**
No cancer	21	40.4
GL 3+3 (ISUP1)	6	11.5
GL 3+4 (ISUP 2)	9	17.3
GL 4+3 (ISUP 3)	12	23.1
GL 4+4 (ISUP 4)	3	5.8
GL 4+5, 5+4 (ISUP 5)	1	1.9
Total	52	100

The total number of patients with PRI-MUS 4 and PRI-MUS 5 lesions was 72. Among this cohort, 16 were found to have no identified cancer, 6 exhibited ISUP 1 non-significant cancer pathology, and 50 presented with significant cancer.

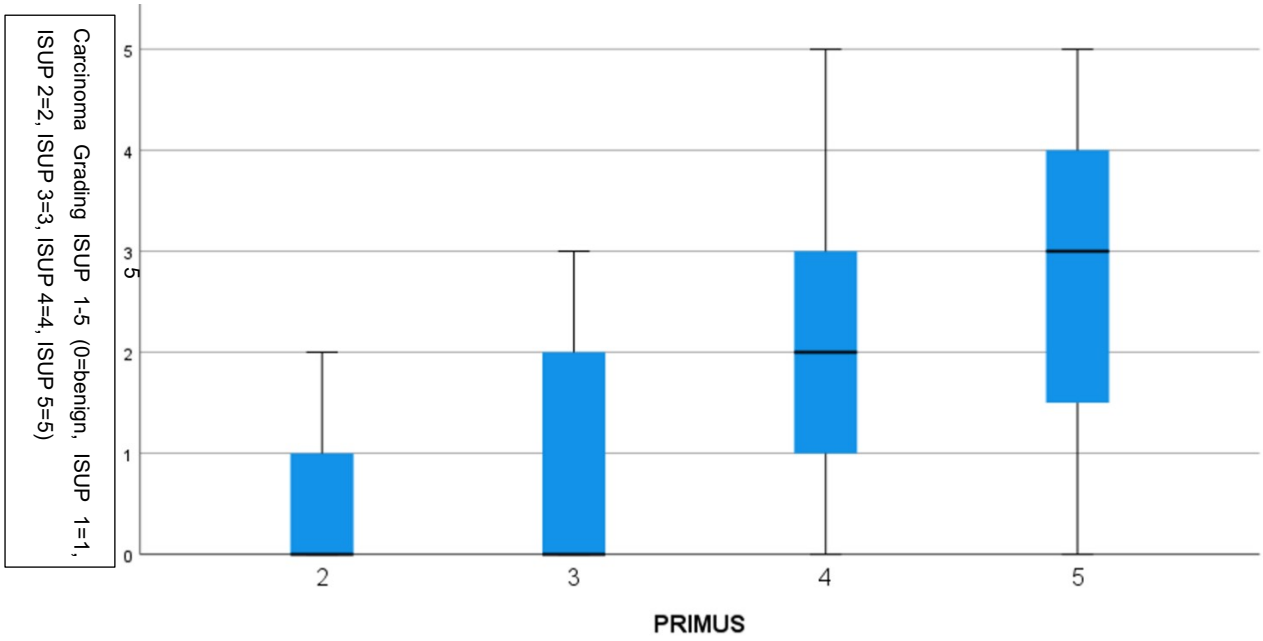
The total number of patients with PI-RADS 4 and PI-RADS 5 lesions on MRI was 41, among whom 14 were diagnosed with no cancer, 4 had non-clinically significant prostate cancer with ISUP 1, and 23 had significant prostate cancers.

Table 14. The summary comparison of microUS and mpMRI suspect lesions for identifying csPCa
PRI-MUS Prostate Risk Identification Using Micro-Ultrasound. PI-RADS Prostate Imaging–Reporting and Data System. ISUP international society of urological pathology. mpMRI multiparametric MRI

	MicroUS, N=122	mpMRI, N=66
PRI-MUS/PI-RADS 2, ISUP ≥2	n= 1 (6%)	n= 3 (50%)
PRI-MUS/PI-RADS ≥3 and PSA≥4, ISUP ≥2	n= 58 (57%)	n= 25 (48%)
PRI-MUS/PI-RADS 4 and 5, ISUP ≥2	n= 50 (70%)	n= 23 (56%)

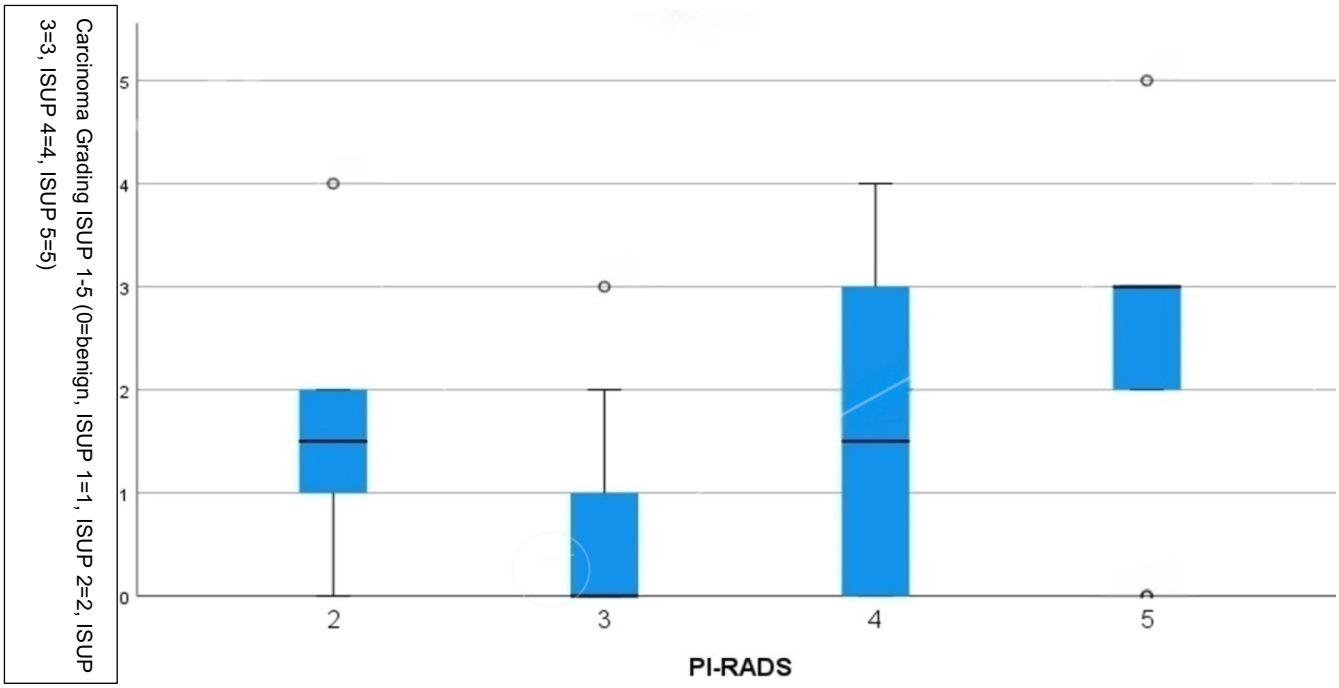
3.3. Distribution of ISUP Grade 1 to 5 in relation to microUS and mpMRI-derived Risk of Prostate Cancer Scoring Systems

The boxplot in Graph 1 illustrates the relationship between PRI-MUS 2 to 5 and ISUP 1 to 5 scores, with 0 indicating benign histology. It is evident that PRI-MUS 2 predominantly corresponds to benign conditions, non-csPCa, and a small number of ISUP 2 pathologies, whereas PRI-MUS 5 predominantly correlates with csPCa and ISUP scores of ≥ 2. The boxplot illustrates a discernible correlation, suggesting that higher PRI-MUS scores correspond to increased ISUP scores.



Graph 1. Boxplot ISUP-PRIMUS association
PRI-MUS Prostate Risk Identification Using Micro-Ultrasound. ISUP international society of urological pathology

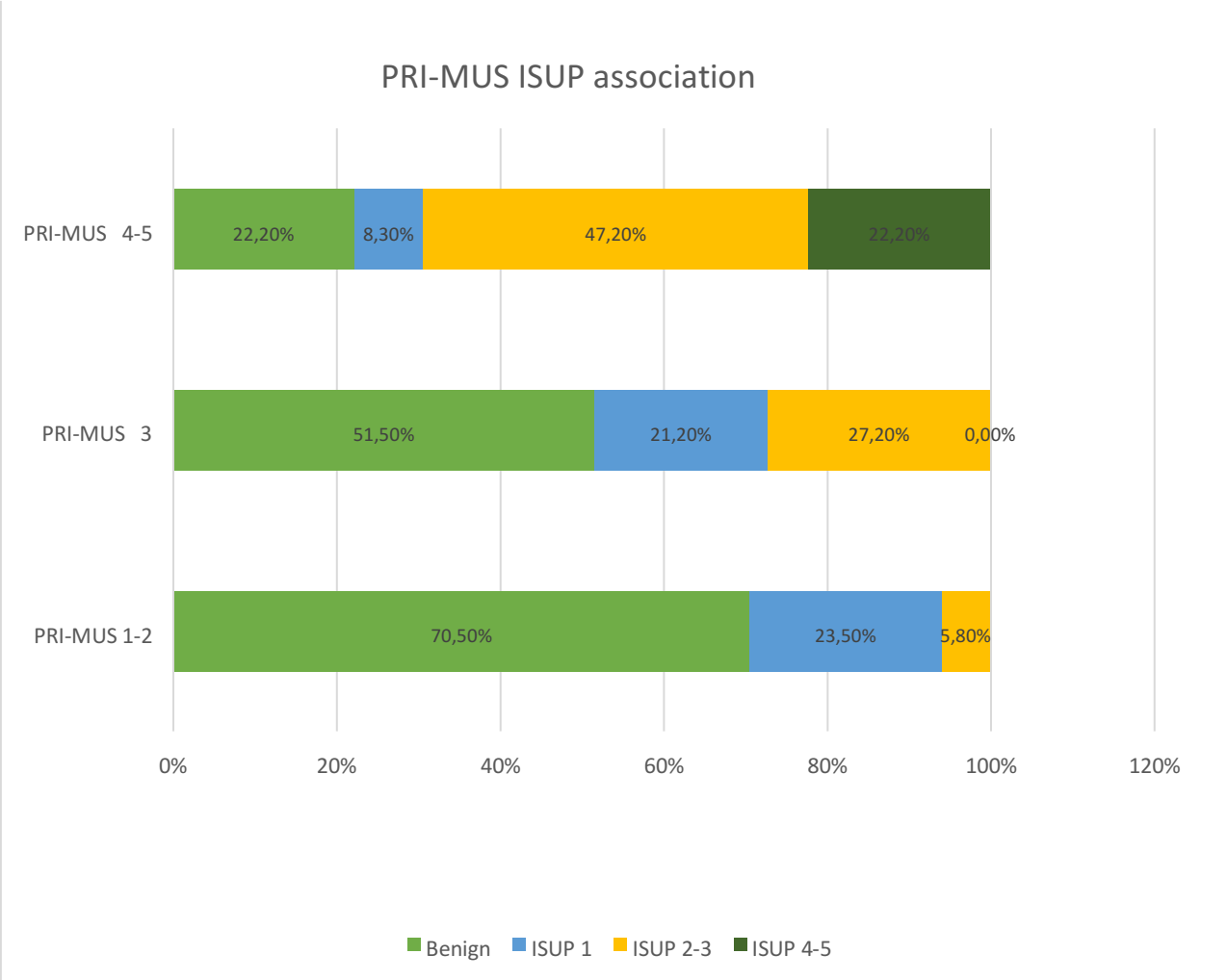
The boxplot in Graph 2 illustrates the relationship between PI-RADS scores (2 to 5) and ISUP grades (1 to 5), with 0 indicating benign histology. It is evident that PI-RADS 2 and 3 are predominantly associated with benign findings, nonclinically significant prostate cancer, and a small number of ISUP grade 2 cases. PI-RADS 4 includes approximately equal proportions of non-clinically significant and clinically significant prostate cancer, whereas PI-RADS 5 is predominantly associated with csPCa, ISUP grades ≥ 2.



Graph 2. Boxplot ISUP-PI-RADS association
PI-RADS Prostate Imaging–Reporting and Data System. ISUP international society of urological pathology

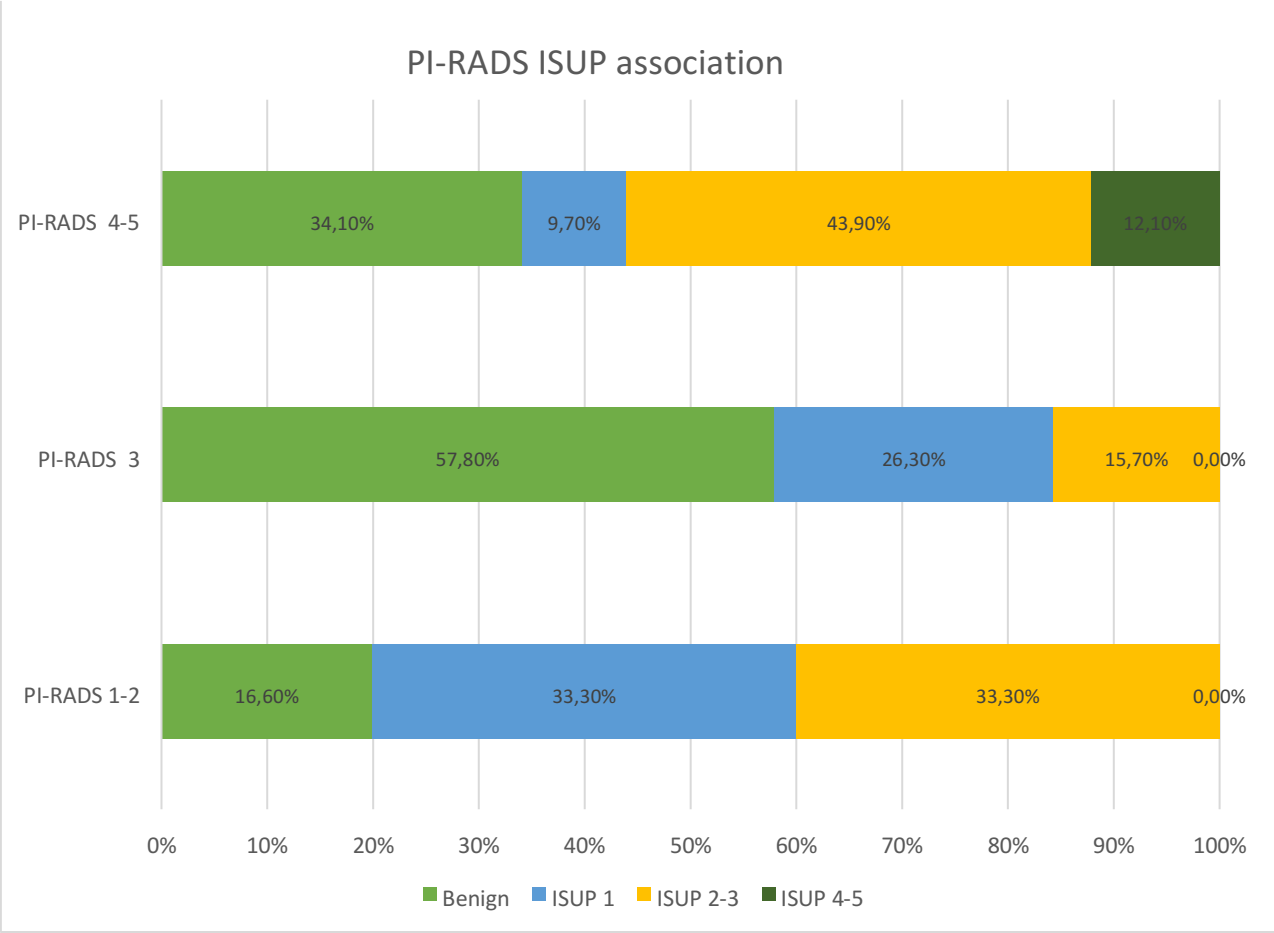
Bar Chart 1 illustrates the association between PRI-MUS and ISUP score. It reveals that lesions identified as PRI-MUS ≥3 are predominantly linked with higher ISUP scores (≥2) compared to lesions with PRI-MUS scores of 1-2. Specifically, PRI-MUS 3 lesions were associated with 27% of ISUP 2-3 cases, 21.2% of ISUP 1 cases, and 51% of benign cases. While PRI-MUS 4-5 lesions were associated with a higher proportion of ISUP 2-3 cases (47.2%), ISUP 4-5 cases (22.2%), with only 22.2% being benign and 8.3% being ISUP 1 cases.

In summary, PRI-MUS 1-2 is predominantly benign, with a small percentage of higher ISUP grades. PRI-MUS 3 shows a more balanced distribution, with significant portions in benign, ISUP 1, and ISUP 2-3 grades. PRI-MUS 4-5 indicates higher severity, with almost half of the cases falling into ISUP 2-3 and a notable portion in ISUP 4-5, while the benign cases are reduced significantly compared to lower PRI-MUS categories.



Bar Chart 1. Association of ISUP with PRI-MUS score
PRI-MUS Prostate Risk Identification Using Micro-Ultrasound. ISUP international society of urological pathology

Bar chart 2 represents the association of ISUP results with mpMRI-rated PIRADS scores. The percentage distribution of ISUP in PI-RADS 1-2 is as follows: 33.3% for ISUP 1 and 33.3% for ISUP 2-3, with 16.6% for benign cases. PI-RADS 3 exhibits 57% benign cases, 26.3% ISUP 1, and 15.7% ISUP 2-3 cases. PIRADS 4-5 lesions exhibit 12.1% ISUP 4-5 scores, while the remainder comprises 43.9% ISUP 2-3, 9.7% ISUP 1, and 34.1% benign cases.



Bar Chart 2. Association of ISUP with PI-RADS score
PI-RADS Prostate Imaging–Reporting and Data System. ISUP international society of urological pathology

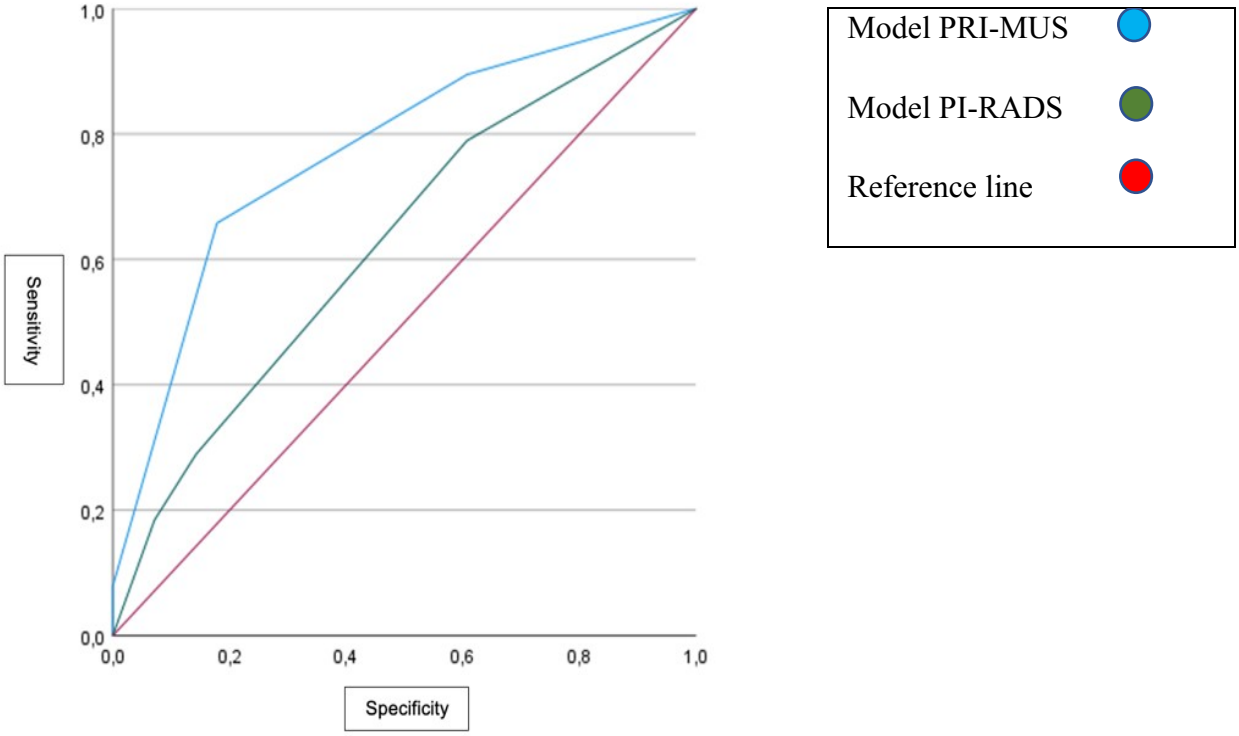
Table 15. Logistic Regression Analysis of PRI-MUS Scores and their Association with Prostate Cancer Diagnosis PRI-MUS Prostate Risk Identification Using Micro-Ultrasound

PRI-MUS	P value	Odds ratio	95% Confidence Interval for odds ratio	
PRI-MUS 3	0,276	2,000	0,574	6,968
PRI-MUS 4	0,001	7,371	2,209	24,598
PRI-MUS 5	0,007	9,600	1,863	49,481

As depicted in Table 15, the PRI-MUS 3 score exhibits an odds ratio of 2.000, indicating that patients with this score are twice as likely to have a PCa compared to those with a lower score. However, the P-value of 0.276 suggests that this finding lacks statistical significance. The broad confidence interval (0.574 to 6.968) underscores the considerable uncertainty surrounding the estimate. Conversely, the PRI-MUS 4 score demonstrates an odds ratio of 7.371, signifying a markedly higher likelihood of the PCa. The P-value of 0.001 indicates statistical significance, suggesting that the result is highly unlikely due to chance. The confidence interval (2.209 to 24.598) indicates a relatively precise estimate, though spanning a wide range. Moreover, the PRI-MUS 5 score exhibits the highest odds ratio at 9.600, implying that patients with this score are 9.6 times more likely to experience the outcome compared to those with a lower score. The P-value of 0.007 supports the statistical significance of this finding. The confidence interval (1.863 to 49.481), while wide, still supports the strength of the association.

The table demonstrates a consistent trend in which higher PRI-MUS scores are associated with increased odds of prostate cancer. Specifically, PRI-MUS 4 and 5 scores show significant associations with prostate cancer, indicated by their low

P-values and high odds ratios. Conversely, PRI-MUS 3 does not exhibit a statistically significant association. The confidence intervals further elucidate the reliability of these estimates, with PRI-MUS 4 and 5 displaying more robust and precise associations compared to PRI-MUS 3.



Graph 3. ROC-curve
PRI-MUS Prostate Risk Identification Using Micro-Ultrasound. PI-RADS Prostate Imaging Reporting and Data System

The ROC (Receiver Operating Characteristic) curve shown in Graph 3 compares the performance of two diagnostic models, the PRI-MUS model and the PI-RADS model, in distinguishing between the presence and absence of prostate cancer. PRI-MUS Model: This curve demonstrates the performance of the PRI-MUS model. It shows a higher sensitivity compared to the PI-RADS model, indicating better overall performance in distinguishing between the two classes. PI-RADS Model: This curve represents the performance of the PI-RADS model. It lies below the PRI-MUS curve for most of the range, suggesting it is less effective in differentiating between cancerous and non-cancerous cases compared to the PRI-MUS model. Reference Line: This diagonal line represents random chance (an AUC of 0.5). Any model performing along this line would be no better than random guessing.

The ROC curve analysis indicates that the PRI-MUS model outperforms the PIRADS model in terms of diagnostic accuracy, providing higher sensitivity at most false positive rates.

3.4. Outcomes of microUS-guided biopsy without prior mpMRI

We also calculated the outcomes of those men who received only microUSguided biopsy and had no prior mpMRI examinations performed. This analysis reflects the results that would have occurred with only microUS and no additional imaging. A total of 56 patients underwent microUS-guided biopsies based on a

PRI-MUS score of ≥3, without prior mpMRI. The number of patients who had a positive histology for cancer was 35, while 19 were benign cases. Clinically significant prostate cancer was diagnosed in 30 cases (53%), and 6 cases (10.7%) were non-clinically significant.

The detection rate for csPCa was in the cohort with previous mpMRI (43.9%) compared to microUS only (53%) (p=0.029). In the group of patients without mpMRI and PRI-MUS ≥4 (n=42, 77%), the detection rate for csPCa was 66.7% (n=28). (Table 16).

Table 16. Outcomes of microUS-Guided Biopsy without prior mpMRI
PRI-MUS Prostate Risk Identification Using Micro-Ultrasound. csPCa clinically significant prostate cancer. microUS micro-ultrasound. mpMRI multiparametric MRI

	microUS biopsies without prior mpMRI	microUS biopsies with prior mpMRI
Total subjects	56 (100%)	66 (100%)
Positive histology for cancer	35 (62.5%)	38 (57.7%)
Benign cases	19 (33.9%)	28 (42%)
Clinically significant PCa	30 (53%)	29 (43.9%)
Non-clinically significant cases	6 (10.7%)	9 (13.6%)
csPCas with PRI-MUS 4-5 (Total 42 (77%) PRI-MUS 4-5)	28 (66%)	
Non-csPCa with PRI-MUS 4-5 (Total 42 PRI-MUS 4-5)	14 (33%)	

In the group of 66 patients who underwent both imaging modalities prior to biopsy, a total of 51 PRI-MUS-suspicious lesions were identified, of which 29 were clinically significant. Simultaneously, 60 PI-RADS-suspicious lesions were identified, with 26 confirmed as csPCa. Additionaly, microUS detected three csPCa cases that were missed by mpMRI. Notably, mpMRI did not identify any csPCa lesions that were missed by microUS.

4. Discussion

A growing interest in the early diagnosis of prostate cancer aims primarily to reduce the number of unnecessary biopsies and the overdiagnosis of clinically insignificant cancers. The goal is to accurately identify patients who truly require a biopsy while employing a minimally invasive, accurate, user-friendly and costeffective technique.

In the modern world, a range of advanced technologies and techniques are employed for the diagnosis and biopsy of the prostate in patients with suspected prostate cancer. Magnetic Resonance Imaging has become an essential component in the early detection of prostate cancer, particularly when clinical suspicion arises from physical examination findings or elevated PCa biomarkers. The PSA screening presents a dual perspective: it contributes to the early diagnosis of prostate cancer, and it reduces metastatic disease, while on the other hand, PSA screening leads to the diagnosis of clinically insignificant prostate cancer, often followed by overtreatment. The positive and negative aspects of screening appear to balance each other out. The small beneficial effect of one-time screening, compared to serial PSA testing was observed (Hugosson *et al.*, 2019).

Current diagnostic protocols increasingly incorporate mpMRI, with many of the latest biopsy methods utilizing mpMRI guidance. These approaches include mostly fusion biopsies, which integrate mpMRI imaging to perform targeted biopsy procedures based on PI-RADS score.

The PRECISION trial demonstrated, with a 95% confidence interval, the superiority of multiparametric MRI with or without targeted biopsy over standard systematic biopsy. Currently, mpMRI is considered the gold standard for the detection and targeting of suspicious prostate lesions. Importantly, the trial showed that approximately 25% men were able to avoid unnecessary biopsy as a result of this approach(Kasivisvanathan et al., 2018b).

On the other hand, multiparametric MRI is an expensive imaging modality that is not widely available at high quality and remains accessible to a relatively limited patient number. Furthermore, its utilization is complex, particularly with regard to image interpretation.

And even though, mpMRI has revolutionized the diagnosis of PCa, with targeted biopsies that detect more significant cancer compared to systematic biopsies, it fails to detect approximately 15% of csPCa cases(Klotz et al., 2020).

Here, we can highlight the advantages of micro-ultrasound, most notably its comparable detection rate of clinically significant prostate cancer to multiparametric MRI.

MicroUS was introduced in 2014 by Pavlovich et al., this innovative system utilizes high-frequency ultrasound to achieve exceptional spatial resolution(Harland and Stenzl, 2021). The concept of this diagnostic approach is not related to MRI and does not require its evaluation for detecting suspicious lesions. It employs its own scoring system, the PRI-MUS classification, which is conceptually similar to the PI-RADS score and was originally developed by Ghai et al(Ghai *et al.*, 2016b). This system eliminates the need for external referral to the radiology department for MRI examinations. Consequently, patients can avoid additional examination time and the associated emotional stress related to cancer diagnoses. Basic urological assessments and microUS, can be conducted by a urologist in the office setting. Suspicious lesions can be identified using microUS, and a prostate biopsy can be performed immediately without additional preparation. This approach allows for both systematic and targeted lesionoriented biopsies. As a result, the biopsy procedure becomes less complex.

Our study confirms the high sensitivity of microUS for csPCa. A very high detection rate was found in patients with PRI-MUS 4/5. It should be further investigated whether microUS might serve as a pretest and mpMRI could be reserved for patients with PRI-MUS ≤3: an approach not directly suggested in previous studies.

The study highlights the strong correlation between PRI-MUS 4/5 lesions and high-risk, clinically significant prostate cancer. This finding could play an important role in clinical decision-making by enabling urologists to reduce the number of biopsies, optimize patient-specific risk assessment, and potentially limit the overuse of mpMRI. If prospective studies confirm this strong correlation, it could be suggested that men with PRI-MUS 4/5 lesions undergo direct targeted, perilesional plus systematic biopsies as current standard suggests, whereas those with PRI-MUS ≤3 lesions may still benefit from additional mpMRI to support clinical decision-making.

Our findings indicate that clinically significant prostate cancer was present in 69.7% of PRI-MUS 4-5 cases, compared to 56% of cases with PI-RADS 4-5 scores. In this study, microUS outperformed mpMRI in terms of sensitivity and specificity for detecting csPCa.

In the group of 66 patients who underwent both imaging modalities prior to biopsy, microUS detected three csPCa cases that were missed by mpMRI. Notably, mpMRI did not identify any csPCa lesions that were missed by microUS.

We performed various analyses to obtain the final results and to establish associations between the micro ultrasound-based PRI-MUS scoring system and prostate cancer detection rate. In our biopsy series, there were instances when biopsies were performed based on clinical suspicion or elevated PSA, even though imaging with either microUS or mpMRI showed no suspicion of cancer. This means that the prostate gland was rated as either PRI-MUS 2 or PI-RADS

2. The outcome was that clinically significant prostate cancer was missed in only

1 case (5.9%) with microUS, where PRI-MUS was 2. For mpMRI, there were 3 cases (50%) where the same was true, and the PI-RADS scoring missed the presence of clinically significant PCa.

Clinically significant prostate cancer, defined as ISUP >2, was detected in 57% of PRI-MUS ≥ 3 cases and in 48% of PI-RADS ≥3 cases.

Also, clinically non-significant cancers with ISUP <2 were less frequently diagnosed by microUS compared to MRI results. This means that in those cases where microUS and MRI identified suspicious lesions falsely, and histologically there was no presence of prostate cancer, fewer mistaken cases were identified by microUS (42.5%) then by mpMRI (51.9%).

These findings suggest that microUS demonstrates superior performance relative to mpMRI across all evaluated parameters.

Overall, our study demonstrated that micro-ultrasound had a sensitivity of 93% and a specificity of 25%, exceeding the sensitivity of multiparametric MRI, which was 89%, with a specificity of 7%. The positive predictive value of microUS was 66%, compared to 56% for mpMRI. Notably, the negative predictive value of microUS was 70%, considerably higher than the 33% observed for mpMRI. Although the negative predictive value of microUS is generally high across various studies, it remains quite variable.

A systematic review and meta-analysis compared the effectiveness of microUSguided and mpMRI-guided prostate biopsies in detecting both significant and insignificant prostate cancer. After an extensive search of databases including PubMed, Cochrane Library, and Scopus from 2020 onwards, 15 studies met the inclusion criteria. All subjects underwent consecutive microUS and mpMRItargeted prostate biopsies, in addition to 10-12 core systematic biopsies. The total number of subjects who received both methods was 1,391. Notably, 9 of these studies employed a cognitive approach, while 6 utilized standard MRI-

fusion biopsy technique. In 4 studies, the operators were blinded to the MRI results, whereas 11 studies were conducted with unblinded operators(Sountoulides *et al.*, 2021).

Consistent with the findings of our study, the meta-analysis indicates that microUS-guided prostate biopsy represents a viable alternative to mpMRI-guided biopsy, demonstrating comparable detection rates.

MicroUS identified 437 patients with clinically significant prostate cancer, whereas MRI identified 327 cases. Clinically nonsignificant cases numbered 95 and 102, respectively. It has also been demonstrated that MRI still misses 1635% of clinically significant prostate cancer cases, indicating that systematic biopsies should be performed alongside targeted biopsies(Sountoulides *et al.*, 2021).

Multicenter prospective registry comparing microUS and mpMRI demonstrated results similar to those of our study, with an overall sensitivity of 94% for microUS and 90% for mpMRI in diagnosing clinically significant prostate cancers. The specificity was relatively low for both modalities, at 22% and 23%, respectively. The trial ivolved 1040 subjects across 11 different urological centers(Klotz et al., 2020).

However, it should be noted that the nonrandomized design of the included studies and mostly retrospective study designs downgraded the strength of evidence.

Hofbauer et al. conducted a prospective non-inferiority comparative analysis involving 203 patients to evaluate the effectiveness of micro-ultrasound versus multiparametric MRI in detecting csPCa. The results demonstrated that microUS detected 97% of the csPCa identified by mpMRI-targeted biopsy. The study concluded that microUS is non-inferior to mpMRI in detecting suspicious prostate lesions and suggested that microUS may be as effective as mpMRI in identifying csPCa(Hofbauer *et al.*, 2022).

The prospective randomized PROMIS trial demonstrated superior results for mpMRI, reporting a sensitivity of 93% and a NPV of 89% for clinically significant prostate cancer. The specificity of mpMRI was 41%, with a PPV of 51%(Ahmed *et al.*, 2017). Several factors may account for the discrepancies between these findings and those of our study. Firstly, differences in study design can significantly impact outcomes. Prospective trials generally employ stricter inclusion criteria and are more standardized, whereas retrospective studies are more susceptible to selection bias. Secondly, sample size may influence the stability of estimates, with smaller studies potentially producing less reliable results. Additionally, the radiologist's level of experience plays a critical role in the interpretation and assessment of mpMRI results. Given the steep learning curve associated with mpMRI that may contribute to misinterpretation and diagnostic variability.

Modern prostate biopsy techniques frequently employed in clinical practice also often require general anesthesia. A notable example is the iSR'obot MonaLisa system (Biobot Surgical Ltd, Singapore), which is known for its high detection rate of clinically significant prostate cancer. The use of robotic technology, while beneficial for detection rates, significantly extends the procedural workflow compared to non-robotic biopsies. This extended duration makes the use of local anesthesia impractical, thereby necessitating general anesthesia. Additionally, current robotic systems lack the capability to provide a sagittal view of the needle trajectory, which is a limitation in these procedures(Miah *et al.*, 2020).

MicroUS offers significantly improved anatomical visualization of the prostate gland in a sagittal view, which facilitates precise tracking of prior biopsy needle trajectories. MicroUS eliminates the need for general anesthesia, requires no additional preparation time, and can be performed on an outpatient basis. This efficiency is important not only for patient comfort but also for physicians, who must perform preparatory tasks for mpMRI-fusion biopsies, including the generation of image drawings and synchronization with the ultrasound system. Furthermore, there is the challenge of interpreting mpMRI images, that also becomes the responsibility of urologists as operators and not only for radiologists,

who are primarily trained in interpreting radiological images. This task becomes more complex in daily practice, especially when considering that prostate biopsy is used for diagnosing the disease and is performed in multiple patients with suspected prostate cancer.

Magnetic resonance imaging is also associated with some other limitations, including its relative expense, a significant learning curve due to its complexity, accessibility limited to a wide range of people, and complexity and variation in interpretation. The use of contrast material during the examination should also be mentioned, as well as the potential toxicity associated with gadolinium.

Other contraindications to perform mpMRI should also be noted, such as claustrophobia, prosthetic implants, and pacemakers(Klotz *et al.*, 2020).

It is important to acknowledge that in certain countries or regions, magnetic resonance imaging (mp or bp) may not be readily accessible due to various factors, including resource limitations and healthcare infrastructure constraints.

MRI inter-reader variability, even among experienced radiologists, is a limiting factor of the modality. It is a well-known fact that mpMRI requires a high level of expertise. On the other hand, in favor of microUS, it should be mentioned that it has a short learning curve, which is also our personal experience. José Gregorio Pereira-Arias et al. also describe microUS as an easy-to-learn and cost-effective modality(Gregorio Pereira-Arias *et al.*, 2019). Although microUS appears to be an easy-to-learn, the fact that the operators in the aforementioned systematic analysis were new users may have contributed to a reduced detection rate of microUS in prostate cancer diagnosis.

MicroUS can demonstrate all its benefits, showcasing its affordability, simplicity, comparability, and non-inferiority in identifying csPCa. The high resolution of microUS, offers numerous benefits, including, the ability to perform live biopsies, and no need for extra materials such as contrast. With the high resolution provided by microUS, we can clearly visualize the previous biopsy tracks (image 7), enabling the precise distancing and collection of subsequent cores. This

results in an overall higher quality of the biopsy. Moreover, the learning curve appears to be short. Even when wanting to perform MRI fusion biopsy, microUS allows for its execution. The exceptions for those who cannot undergo microUSguided biopsy are those with anal stenosis or absence post-abdominal perineal resection; however, these limitations apply to any prostate biopsy, as any ultrasound probe should be inserted transrectally, even when conducting a perineal biopsy.

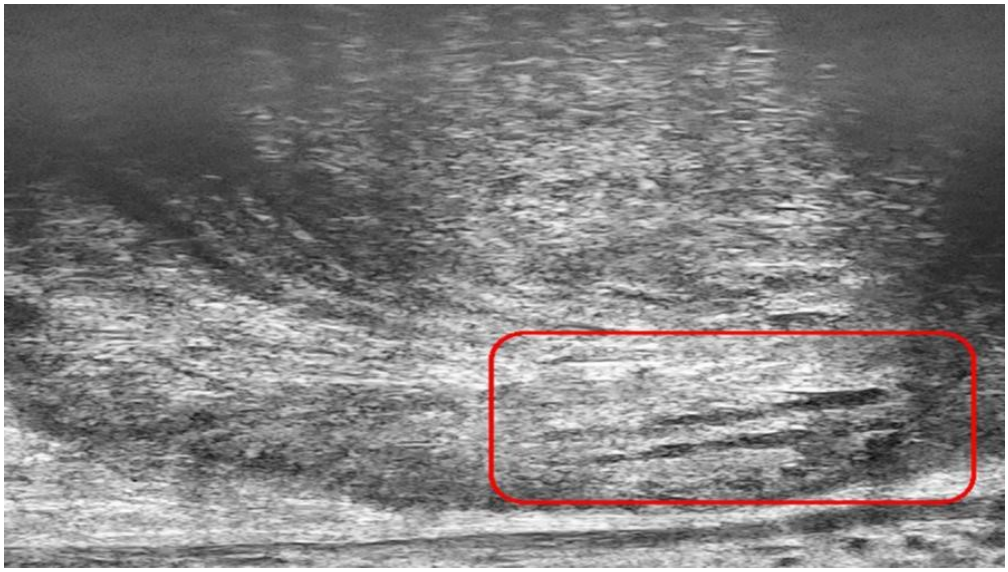


Image 7. Micro-ultrasound: tracks of preceding biopsy of the prostate (two years prior)
Harland N, Stenzl A. Micro-Ultrasound: a way to bring imaging for prostate cancer back to urology. *Prostate Int*. 2021;9(2):61-65. doi:10.1016/j.prnil.2020.12.002(Harland and Stenzl, 2021)

It is important to acknowledge several limitations of our study. The cohort consisted of 122 patients, of whom only 66 underwent both microUS and mpMRI. The relatively small sample size and the uneven distribution of imaging modalities may have influenced the study outcomes. Future research with a larger and more balanced cohort, including an equal number of microUS and mpMRI examinations, could potentially yield different results. Additionally, the use of retrospective data introduces some limitations. To enhance the reliability of the findings, prospective studies incorporating randomization are recommended.

While considering the effectiveness of the aforementioned imaging modalities in correctly identifying suspicious areas and providing better early diagnosis of clinically significant prostate cancer, it should still be mentioned that the need for performing systematic prostate biopsies is not eliminated. Current recommendations support performing targeted biopsies with perilesional sampling to minimize the chance of missing significant PCa.

An analysis of 108 microUS-guided prostate biopsies with a mean PSA of 12 ng/ml, where all subjects received both targeted and systematic prostate biopsies, revealed the following: PCa would have been diagnosed via only targeted biopsy in 22% of cases and via only systematic biopsy in 40% of cases. However, combining targeted and systematic biopsies detected prostate cancer in 44% of cases(Karazanashvili *et al.*, 2021). This suggests that microUS is an effective emerging technology for PCa diagnosis due to its superior visualization of the prostate anatomy and its ability to perform live biopsies. Nevertheless, the importance of performing both targeted and systematic biopsies remains critical.

From all the above-mentioned studies, it is apparent that there was a need for a prospective, randomized large trial to compare the outcomes of microUS and mpMRI in detecting clinically significant prostate cancer and to provide more evidence to support the significant number of studies already concluding the noninferiority of microUS compared to mpMRI for detecting csPCa.

To address this need, the most recent prospective, multicenter, three-arm randomized non-inferiority trial, known as the OPTIMUM trial, was conducted across 20 centers, enrolling a total of 678 participants.

The primary objective of the trial was to test the non-inferiority of the microUSalone arm compared to the MRI/ultrasound fusion arm in detecting csPCa. As a secondary objective, the study also compared detection rates between the MRI/US fusion and MRI/microUS fusion arms. The detection rate of Gleason Grade Group ≥2 prostate cancer using targeted biopsy alone was 38.0%

in the microUS group, 34.1% in the MRI/conventional US group, and 40.3% in the MRI/microUS group.

The study concluded that microUS-guided biopsy is a non-inferior alternative to MRI/conventional US fusion-guided biopsy for diagnosing clinically significant prostate cancer(Klotz *et al.*, 2022; Kinnaird *et al.*, 2025).

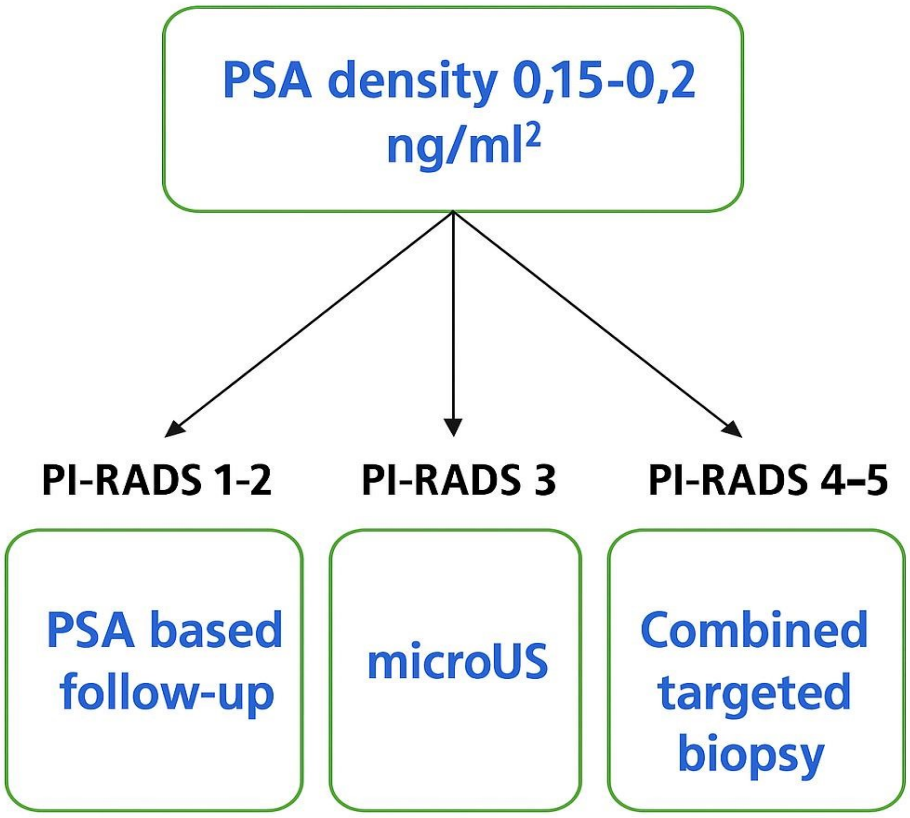
These findings may encourage the broader adoption of microUS in clinical practice, offering a patient-adapted approach to prostate biopsy.

An algorithm for diagnosing prostate cancer has been previously suggested, for example, by the University Hospital in Pilsen. This algorithm was primarily based on PSA levels and the prostate health index for initial patient stratification. It then continued with imaging techniques, biopsy, or active surveillance(Sedláčková *et al.*, 2021). However, this algorithm did not include examination and biopsy with microUS.

We have introduced a diagnostic pathway for prostate cancer in clinical practice, incorporating microUS examination as part of the early diagnosis strategy. This approach is based on recent advancements in knowledge and focuses on differentiating patients based on their PSA density. Specifically, we categorize patients with PSA densities of 0.15-0.2 ng/ml² (the so-called grey zone) and those with PSA densities greater than 0.2 ng/ml².

Friesbie et al. demonstrated that PSA density serves as a complementary measure to PI-RADS in identifying clinically significant prostate cancer, particularly in cases where mpMRI detects a PI-RADS 3 lesion. Given the high inter-reader variability associated with PI-RADS 3, which often results in unnecessary biopsies, a PSA-D cutoff of ≥0.15 ng/ml/cc is critical. The integration of PSA density enhances diagnostic accuracy, reducing the likelihood of unnecessary procedures and improving patient outcomes(Cash and Schostak, 2023).

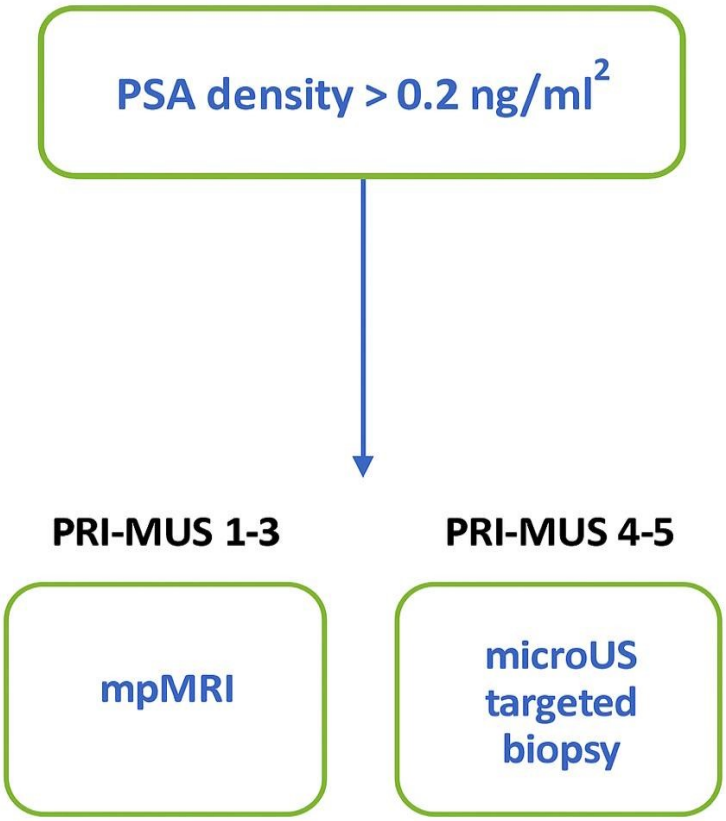
As per the current standard of care, patients at risk for prostate cancer should undergo prostate mpMRI prior to biopsy. Consequently, our algorithm suggests that patients with PSA density in the grey zone undergo mpMRI. Based on the outcome of the mpMRI, as indicated by the PI-RADS score, the subsequent steps are recommended as follows: in the case of PI-RADS 1-2, PSA-based follow-up; in the case of PI-RADS 3, additional examination with microUS to strengthen the results of mpMRI; and when getting PI-RADS 4-5, performing a combined targeted biopsy (Graph 4).



Graph 4. Suggested pathway for prostate cancer diagnosis
PI-RADS Prostate Imaging Reporting and Data System. microUS micro-ultrasound

For patients with PSA density >0.2ng/ml^2, the suggested pathway does not automatically require performing a prostate mpMRI. Instead, it recommends directly conducting an examination with microUS. If the result is PRI-MUS 1-3, an additional mpMRI should be performed to strengthen the outcomes before deciding whether to proceed with a biopsy. If the PI-RADS score is 4 or 5, an MRI-targeted biopsy should be performed. In the case of PRI-MUS 4-5, a microUS-guided prostate biopsy can be performed directly (Graph 5).

Integrating the algorithm into the diagnostic pathway may result in cost savings, a reduction in the number of biopsies performed, and a high cancer detection rate. The integration of the pathway should be further examined and validated in prospective, randomized large-scale studies.



Graph 5. Suggested pathway for prostate cancer diagnosis PRI-MUS Prostate Risk Identification Using Micro-Ultrasound

5. Conclusion

Introduction:

With the increasing incidence of prostate cancer, it is crucial to develop new effective, affordable, and cost-effective diagnostic tool for proper management of this global challenge. Performing a prostate biopsy typically involves dedicated prostate imaging. With mpMRI emerging as a standard of care for prostate cancer diagnosis, there is a room for an alternative urologist-performed, office-based prostate imaging. The development of preclinical ultrasound technologies has led to the creation of a micro-ultrasound device (ExactVu™, Exact Imaging, Toronto, Canada) that operates at 29MHz, in contrast to the 8-12MHz range of conventional TRUS for prostate examination. The PRI-MUS protocol for microUS, a scoring system for prostate cancer risk based on imaging, was developed to differentiate between low-risk and high-risk cancer patients.

Materials and Methods:

Data from patients who underwent prostate biopsy guided by micro-ultrasound at the Department of Urology, University Hospital of Tübingen, from 2020 to 2022, were retrospectively analyzed. The study included a total of 122 patients. Suspicious lesions were identified using microUS and multiparametric magnetic resonance imaging, with definitions set at PRI-MUS ≥3 and PI-RADS ≥3, respectively. Clinically significant prostate cancer was defined as an ISUP grade

≥2. The primary objective of this study was to evaluate the efficacy of microUS in diagnosing csPCa and to produce a diagnostic algorithm for early diagnosis of prostate cancer.

Results:

MicroUS detected csPCa in 69.7% of cases. Among 105 subjects with microUSsuspicious lesions, 70 (66.7%) were diagnosed with PCa, 58 (55,2%) were clinically significant. A strong correlation was observed between PRI-MUS scores and ISUP grades, where PRI-MUS 2 predominantly corresponded to benign conditions or non-csPCa, whereas PRI-MUS 4 (P=0.001), and 5 (p=0.007) predominantly correlated with csPCa.

Overall, microUS demonstrated a sensitivity of 93% and a specificity of 25%. The positive predictive value was 66%, and the negative predictive value was 70%. The detection rate for csPCa was in the cohort with previous mpMRI (43.9%) compared to microUS only (53%) (p=0.029). In the group of patients without mpMRI and PRI-MUS ≥4 (n=42, 77%), the detection rate for csPCa was 66.7% (n=28).

Conclusion:

Our findings indicate that clinically significant prostate cancer was present in 69.7% of PRI-MUS 4-5 cases, compared to 56% of cases with PI-RADS 4-5 scores. In this study, microUS outperformed mpMRI in terms of sensitivity and specificity for detecting csPCa.

This study confirms the high sensitivity of microUS for csPCa. A very high detection rate was found in patients with PRI-MUS 4/5. It should be further investigated whether microUS might serve as a pretest and mpMRI could be reserved for patients with PRI-MUS ≤3.

However, it is important to acknowledge the limitations of our study. The cohort was relatively small, consisting of 122 patients, with only 66 receiving both microUS and mpMRI imaging. The limited sample size and the imbalance in the number of microUS and mpMRI examinations may have influenced the study outcomes.

While limited by a small sample size and retrospective design, this study provides preliminary evidence supporting the use of microUS as a possible initial screening tool. Further prospective studies with larger cohorts are needed to validate these findings and to investigate the proposed diagnostic algorithm where mpMRI is reserved for patients with PRI-MUS scores ≤3. This algorithm, if proven effective, could potentially improve the efficiency of csPCa diagnosis by reducing the reliance on mpMRI and minimizing unnecessary biopsies. Also, it is important to acknowledge that in certain countries or regions, magnetic resonance imaging may not be readily accessible due to various factors, including resource limitations and healthcare infrastructure constraints. Consequently, future recommendations in the form of diagnostic algorithms should consider integrating micro-ultrasound into the prostate cancer diagnostic pathway. This could additionally reduce the reliance on mpMRI, thereby optimizing diagnostic strategies in settings where access to more expensive imaging techniques is limited.

Deutsche Zusammenfassung

Einleitung:

Mit der zunehmenden Inzidenz von Prostatakrebs ist es entscheidend neue, effektive, erschwingliche und kosteneffiziente diagnostische Werkzeuge zu entwickeln, um diese globale Herausforderung angemessen zu bewältigen. Die Durchführung einer Prostatabiopsie erfordert in der Regel spezielle ProstataBildgebung. Da die mpMRT als Standard in der Prostatakrebsdiagnostik etabliert ist, besteht noch Raum für eine alternative, von Urologen durchgeführte Bildgebung für Prostata Biopsie. Die Entwicklung präklinischer Ultraschalltechnologien hat zur Schaffung eines Mikro-Ultraschallgeräts (ExactVu™, Exact Imaging, Toronto, Kanada) geführt, das mit 29 MHz arbeitet, im Gegensatz zu den 8-12 MHz herkömmlicher TRUS zur Prostatauntersuchung. Das PRI-MUS-Protokoll für microUS, ein auf Bildgebung basierendes Bewertungssystem für das Prostatakrebsrisiko, wurde entwickelt, um zwischen Patienten mit niedrigem und hohem Krebsrisiko zu unterscheiden.

Material und Methoden:

Daten von Patienten, die sich von 2020 bis 2022 einer durch Mikro-Ultraschall gestützten Prostatabiopsie in der Urologischen Klinik des Universitätsklinikums Tübingen unterzogen, wurden retrospektiv analysiert. Die Studie umfasste insgesamt 122 Patienten. Verdächtige Läsionen wurden mittels microUS und mpMRI identifiziert, wobei die Definitionen auf PRI-MUS ≥3 und PI-RADS ≥3 festgelegt wurden. Klinisch signifikanter Prostatakrebs wurde als ISUP-Grad ≥2 definiert. Das primäre Ziel dieser Studie war es, die Wirksamkeit von microUS bei der Diagnose von klinisch signifikantem Prostatakrebs zu bewerten, sowie die Entwicklung eines diagnostischen Algorithmus zur Früherkennung von Prostatakrebs.

Ergebnisse:

MicroUS identifizierte csPCa in 69,7% der Fälle. Unter den 105 Patienten mit microUS-verdächtigen Läsionen wurden 70 (66,7 %) mit Prostatakrebs diagnostiziert, davon 58 (55,2 %) als klinisch signifikant. Es wurde eine starke Korrelation zwischen den PRI-MUS-Scores und den ISUP-Graden festgestellt, wobei PRI-MUS 2 überwiegend mit benignen Zuständen oder nicht-signifikantem Krebs korrelierte, während PRI-MUS 4 (p=0,001) und 5 (p=0,007) überwiegend mit csPCa assoziiert waren.

Insgesamt zeigte microUS eine Sensitivität von 93 % und eine Spezifität von 25 %. Der positive prädiktive Wert betrug 66 %, der negative prädiktive Wert 70 %. Die Detektionsrate von csPCa betrug in der Kohorte mit vorherigem mpMRT 43,9 %, im Vergleich zu 53 % bei alleiniger Anwendung von microUS (p=0,029). In der

Gruppe der Patienten ohne mpMRT und PRI-MUS ≥4 (n=42, 77 %) betrug die Detektionsrate für csPCa 66,7 % (n=28).

Schlussfolgerung:

Unsere Ergebnisse zeigen, dass klinisch signifikanter Prostatakrebs in 69,7 % der Fälle mit PRI-MUS 4-5 auftrat, verglichen mit 56 % der Fälle mit PI-RADS 45. In dieser Studie übertraf microUS mpMRT in Bezug auf Sensitivität und Spezifität zur Detektion von csPCa. Die Studie bestätigt die hohe Sensitivität von microUS für csPCa. Eine sehr hohe Detektionsrate wurde bei Patienten mit PRIMUS 4/5 festgestellt.

Es sollte weiter untersucht werden, ob microUS als Vorabtest dienen könnte und mpMRT für Patienten mit PRI-MUS ≤3 reserviert werden sollte.

Es ist jedoch wichtig, die Einschränkungen unserer Studie zu berücksichtigen. Die Kohorte war mit 122 Patienten relativ klein, wobei nur 66 Patienten sowohl microUS als auch mpMRT erhielten. Die begrenzte Stichprobengröße und das

Ungleichgewicht in der Anzahl der durchgeführten microUS- und mpMRTUntersuchungen könnten die Studienergebnisse beeinflusst haben.

Trotz der Limitationen durch die geringe Fallzahl und das retrospektive Studiendesign liefert diese Untersuchung erste Hinweise darauf, dass microUS als potenzielles Instrument für das initiale Prostatakarzinom-Screening geeignet sein könnte. Zukünftige prospektive Studien mit größeren Patientenkollektiven sind erforderlich, um diese Ergebnisse zu validieren und das vorgeschlagene diagnostische Algorithmusmodell zu überprüfen, bei dem mpMRT Patienten mit einem PRI-MUS-Score ≤3 vorbehalten ist. Falls sich dieser Algorithmus als effektiv erweist, könnte er zur Effizienzsteigerung bei der Diagnose des klinisch signifikanten Prostatakarzinoms beitragen, indem der Einsatz von mpMRT reduziert und unnötige Biopsien vermieden werden.

Zudem ist zu berücksichtigen, dass in bestimmten Ländern oder Regionen die Magnetresonanztomographie aufgrund unterschiedlicher Faktoren, wie begrenzter Ressourcen oder infrastruktureller Einschränkungen im Gesundheitssystem, nicht leicht verfügbar ist. Künftige Empfehlungen in Form diagnostischer Algorithmen sollten daher die Integration von Mikro-Ultraschall in den diagnostischen Pfad des Prostatakarzinoms in Betracht ziehen. Dies könnte zusätzlich die Abhängigkeit von der mpMRT verringern und die diagnostischen Strategien insbesondere in ressourcenlimitierten Settings optimieren.

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7. Explanations regarding the individual's own contribution

The study design was developed collaboratively by Karazanashvili N, Harland N, and Stenzl A, who conceptualized and designed the research study together. Karazanashvili N was solely responsible for data collection, gathering the necessary information, and creating a comprehensive database.

Karazanashvili N conducted the data analysis using advanced statistical methods and analytical tools.

The comprehensive textual body of the dissertation was also generated solely by Karazanashvili N.

Stenzl A provided supervision and guidance throughout the study.

The dissertation was solely created and written by N. Karazanashvili. N. Harland reviewed the text and provided suggestions for its improvement, and A. Stenzl conducted the final revision.

For the paper publication, Karazanashvili N took the lead in writing, while Harland N reviewed and edited the manuscript.

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