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TESI DI DOTTORATO

SINGLE SETTING 3D MRI-US GUIDED FROZEN SECTION AND FOCAL CRYOABLATION OF THE INDEX LESION IN LOW/INTERMEDIATE RISK PROSTATE CANCER

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Ai miei più convinti sostenitori,

mamma, papà e Angela

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1. Epidemiology

Prostate cancer is the second most common cancer in men worldwide and the fifth most common cause of cancer death in men [1,2]. However, incidence varies notably, being highest in North America, Australia, northern and central Europe. Conversely, the lowest rates have been registered in southeastern and south-central Asia and northern Africa [1,3].

In the United States, approximately one in six White men and one in five Black men will be diagnosed with prostate cancer in their lifetime, with the likelihood increasing with age. In fact, prostate cancer is rare in men younger than 40 years, and it is uncommon in men younger than 50 years [2]. The peak of incidence is in the range 65-74 years, with a median age at diagnosis of 66 years [4].

According to data from the Surveillance, Epidemiology, and End Results (SEER) program and the Institute for Health Metrics and Evaluation (IHME) Global Burden of Disease (GBD) database, the incidence of prostate cancer has increased in men ages 15 to 40 years at a stable rate averaging 2% per year since 1990. These younger patients frequently present with more advanced cancer, are more likely to have distant disease at diagnosis [5] and have worse survival than middle-aged and older men.

In the early 1990s, the routinely use of serum PSA testing and standard digital rectal examination determined an increase in incidence rates of organ-confined prostate cancer, basically in asymptomatic men.

Subsequently, since 1992 incidence rates have declined progressively, from over 230 to less than 110 per 100,000 population in 2013-2017 [2,4].

In a recent review of around 800,000 cases of prostate cancer diagnosed in the decade 2004–2013, Authors found that although the incidence of low-risk prostate cancer decreased, the annual incidence of metastatic prostate cancer during those years increased, especially in men aged 55–69 years [6].

2. Aetiology and risk factors

Well-established risk factors for prostate include ethnicity, age and country of residence. There is important racial disparity in the incidence and mortality rates, Blacks and Caribbean men of African descent have highest incidence rates followed by Whites, Hispanics, and finally Asian men living in their native countries. Regarding prostate cancer–specific mortality, it is 2-fold higher in Blacks than in Whites [7]. Finally, countries with the highest socioeconomic index have highest incidence rates than poorer countries [8].

Additional risk factors include genetic predisposition and family history. Several mutations have been identified as associated with hereditary prostate cancer, including BRCA1, BRCA2, ATM, HOXB13, DNA mismatch repair genes, CHEK2, PALB2, NBN, and RAD51D [9]. Among these, BRCA1/2 and ATM mutations have been found in significantly higher rates for men with lethal prostate cancer [10].

Men with a family history (1 or more first-degree relatives) of breast cancer, have an increased risk of prostate cancer diagnosis and lethality by 21% and 34% respectively, compared with men without such history. Similarly, a family history of prostate cancer

increases risk by 68% and lethality by 72%, especially in case of earlier cancer onset in families [11].

Moreover, a family history of Lynch syndrome, including probands and their first- through fourth- degree relatives, give a 2-fold higher risk of prostate cancer compared with general population [12].

Overall, genetic testing remains a novel and developing research area. Probably, it could guide screening, risk stratification and treatment decision in the next future [13].

Increased body mass index (BMI) and metabolic syndrome have been inconsistently associated with increased prostate cancer incidence and possible recurrence after therapy [14-18]. Also smoking, activity and dietary supplementation and/or deficiency have demonstrated variable association with prostate cancer incidence and/or mortality [19]. Unfortunately, there is a low level of evidence, or conflicting evidence which suggests that further evaluation is required before they can be classified as risk factors.

3. Pathophysiology

70% of PCa occurs in the peripheral zone, 15-20% in the central zone and 10-15% in the transitional zone. Sometimes it could be unifocal, with evidence of a single lesion at MRI, but most PCa are multifocal, with synchronous involvement of multiple zones because of clonal and/or nonclonal tumours.

After the initial transformation events that determine a cell division rate which exceed the cell death rate with consequent uncontrolled cellular growth, further mutations

could occur, including the genes for p53 and retinoblastoma. These alterations can be responsible of tumour progression and metastasis.

Around 90% of PCa are acinar adenocarcinomas [20]. Ductal carcinoma and neuroendocrine carcinoma account for the majority of the remaining cases. After radiation or hormone treatment, it is possible to diagnose prostate carcinomas with squamous differentiation which constitutes less than 1% of all prostate carcinomas.

Rarely, cancers may arise from the urothelium of the prostatic urethra. These are not prostatic adenocarcinomas and are treated as urothelial cancers.

4. Natural history

A tumour initially confined to the gland can locally invade the surrounding organs and tissues over time. So, a tumour of the transitional-zone can spread to the bladder neck, while one of the peripheral-zone can reach the ejaculatory ducts and seminal vesicles. Extra-prostatic capsule extension and along the perineural or vascular spaces occurs relatively late.

The mechanism for distant metastasis is poorly understood. However, cancer spreads to bone early, often without significant lymph nodes involvement.

Several studies suggested a consistent number of undiagnosed prostate cancers, as noted in 25-40% of prostate specimens after radical cystectomy and as described in numerous autopsy studies. In particular it seems that the prevalence of incidental PCa at autopsy doubles every 14 years of life [21].

It is already clear that there is a spectrum of PCa, ranging from cancer that men die with to that which men die from. Indeed, low-grade PCa (Gleason 5-6) requires 10-15 years to develop into aggressive disease while higher-grade PCa can lead to death during a period of 10 years if left untreated [22].

On the other hand, with the advent of PSA testing in the 1990's, a considerable number of clinically insignificant PCa have been diagnosed and thus treated with a consequent overtreatment [23-25].

5. Diagnosis

Diagnosis of PCa is essentially based on elevation of the prostate-specific antigen (PSA) level and/or abnormal findings on digital rectal examination (DRE). Transrectal ultrasonography (TRUS) has been associated with a high false-positive rate, making it unsuitable as a screening tool. As recently remarked by the PRECISION Study, a valid alternative to TRUS is represented by multiparametric magnetic resonance imaging (MRI), even in men with a raised prostate-specific antigen level who have not undergone biopsy.

In the pre-PSA era, patients could have signs and symptoms related to lymphatic, hematogenous or contiguous local spread of tumour. In case of advanced disease, manifestations of metastases may include anaemia, weight loss, bone pain, pathologic fractures, neurologic deficits from spinal cord compression, lower extremity pain and oedema due to obstruction of venous and lymphatic vessels by nodal metastasis,

uremic symptoms due to urethral or ureteral obstruction caused by local tumour growth or retroperitoneal adenopathy, respectively.

Nowadays, PCa is basically diagnosed in patients who are asymptomatic or with obstructive symptoms (urinary frequency, urinary urgency, decreased urine stream) due to a concomitant benign prostatic hyperplasia of the prostate (BPH).

More rarely, PCa can be an incidental pathologic finding after a transurethral resection (TURP) for BPH.

5.1 Physical Examination

In case of advanced disease findings in patients may include cachexia, bony tenderness, lymphedema or deep venous thrombosis, adenopathy, overdistended bladder due to outlet obstruction. Neurologic examination, including assessment of external anal sphincter tone, should be performed to early detect possible spinal cord compression.

5.2 Digital rectal examination (DRE)

In current practice, most patients diagnosed with PCa have a normal DRE but abnormal PSA value.

DRE can individuate a suspicious solid nodule, an asymmetry of the gland, a difference in texture and bogginess of the prostate. However, these important findings should be considered in conjunction with the PSA level and can suggests the need for a biopsy.

DRE alone cannot reliably differentiate benign prostatic disease from cancer and biopsy is the only tool to confirm the diagnosis. Unfortunately, false-negative results often occur, so multiple biopsies may be needed before prostate cancer is definitely detected.

The DRE is examiner-dependent, and serial examinations over time are the best practise. If cancer is detected, the DRE represents the basis of clinical staging. For example, obliteration of the lateral sulcus or involvement of the seminal vesical often indicates locally advanced disease.

A systematic review and meta-analysis that included 7 studies with 9,241 patients has demonstrated that DRE performed by primary care physicians has poor diagnostic accuracy in screening for PCa. Sensitivity was estimated to be 0.51 and specificity was 0.59 with a positive predictive value of 0.41 [26].

5.3 Prostate-Specific Antigen (PSA)

Prostate-specific antigen (PSA), also known as gamma-seminoprotein or kallikrein-3 (KLK3), is a glycoprotein enzyme encoded by the KLK3 gene. It is a kallikrein-related peptidase and is secreted by the epithelial cells of the prostate gland.

PSA is produced for the ejaculate, where it liquefies semen in the seminal coagulum and allows sperm to swim freely [27]. It is also believed to dissolve cervical mucus, allowing the entry of sperm into the uterus [28].

PSA is present in small quantities in the serum of men with healthy prostates, but is often elevated in the presence of prostate cancer or other prostate disorders [29]. In

fact, it is not uniquely an indicator of prostate cancer, but may also detect prostatitis or benign prostatic hyperplasia [30].

When PSA testing was first introduced, the upper limit of normal range was set to 4 ng/mL, but subsequent studies have shown that no PSA level is associated to a zero-risk of prostate cancer. In fact, as the PSA level increases, the risk of PCa does the same. The probability of detecting cancer with biopsy is about 8% when the PSA is 1 ng/mL, about 25% with a PSA level of 4-10 ng/mL, and the likelihood is even higher when the PSA is above 10 ng/mL [31].

Basically, unless there is a suspicious DRE or a suspicious lesion at the MRI, experts do not suggest a biopsy under the cutoff level of 2.5-3 ng/mL.

In the European Randomized Study of Screening for Prostate Cancer (ERSPC) a PSA cutoff value of 3 ng/mL or higher was applied as an indication for lateralized sextant biopsy [32].

For men with an initial PSA < 3 ng/mL, the risk of developing aggressive prostate cancer and death has been found to significantly increase with PSA values in the 2-2.9 ng/mL range, although the overall risk of aggressive prostate cancer–related death remains limited [32].

According to Preston et al, PSA levels in midlife correlate with future risk of lethal prostate cancer. In men 40-59 years old with baseline PSA levels in the >90th percentile, the odds ratios for developing lethal prostate cancer is higher than in man with PSA levels at or below the median. In particular this odd ratio varies with the age at baseline measurement, being 8.7 in the range 40-49 years, 12.6 in the range 50-54 years and 6.9 in the range 55-59 years.

Shao et al, by means of a review of SEER database of men with newly diagnosed prostate cancer from 2004 to 2006, found that most patients with a PSA threshold below 4.0 ng/mL had low-risk disease but underwent aggressive local therapy. Shao et al suggested that in the absence of the ability to distinguish indolent from aggressive cancers, lowering the biopsy threshold might increase the risk of overdiagnosis and consequent overtreatment [33].

To overcome this problem, different approaches have been proposed for improving the accuracy of PSA, among these the velocity of PSA level increase and the percentage of free PSA.

5.3.1 PSA velocity

PSA velocity is calculated considering at least 3 consecutive measurements over at least 18-24 months. NCCN guidelines suggest to assess PSA velocity together to the PSA level, considering as suspicious the following: PSA velocity of 0.35 ng/mL/y, when the PSA is ≤2.5 ng/mL; PSA velocity of 0.75 ng/mL/y, when the PSA is 4–10 ng/mL.

However, the real value of PSA velocity is still controversial. Several studies have questioned its role concluding that it adds little to the predictive accuracy of high PSA levels or positive DRE and would substantially increase the number of men recommended for a biopsy [34].

5.3.2 Free PSA

Free PSA is generally used in men with very large glands when PSA level is of 4-10 ng/mL. It is considered normal when is above 25% and it is most useful in patients with a previous negative prostate biopsy. It helps to differentiate mildly elevated PSA levels due to cancer from benign prostatic hyperplasia.

The lower the percentage of free PSA, the higher the likelihood of cancer. In fact, cancer is found at prostate biopsy in only 8% of men with free PSA > 25%, but in more than half of those with free PSA < 10%.

Some experts recommend a biopsy when the free PSA is less than 18%; others when it is less than 12%. However, in healthy men with a PSA level of 4-10 ng/mL, many experts recommend biopsy independently of free-PSA test.

5.4 Prostate Biopsy

Prostate biopsy is the only way to confirm diagnosis of prostate cancer. For decades it has been performed via a transrectal approach, utilizing ultrasound guidance (TRUS) and a sextant protocol (6 cores) with consequent suboptimal sensitivity. Subsequently, new optimized protocols were progressively used, obtaining 12 cores from predetermined regions of the peripheral zone, where most prostate cancers arise [35].

In spite of these improvements, ultrasound-guided prostate biopsy fails to identify 20-30% of cancers and undergrades others [36]. Moreover, it is associated with several potential complications: haematuria, rectal bleeding, hematospermia, abdominal or perineal pain, urinary tract infection (UTI), lower urinary tract symptoms, acute

urinary retention, transient erectile dysfunction and sepsis requiring hospitalization in 0.5-4% of cases [37].

More recently, the trans perineal approach has gained popularity thanks to a comparable detection rates for clinically significant prostate cancer than TRUS biopsy [38], associated with lower complication rate (<1% UTI, 6.7% of acute urinary retention) [39].

5.5 Multiparametric Magnetic Resonance Imaging

In the last decades, magnetic resonance imaging (MRI) has progressively expand its indications in prostate cancer diagnosis process. In particular, it has been used in different clinical scenarios: active surveillance, surgical or local therapy planning, prior to prostate biopsy both in naïve patient and in patients with a prior negative biopsy and persistently elevated PSA.

Lesions detected on MRI are assessed using the Prostate Imaging–Reporting and Data System (PI-RADS) score which represents an estimation of the probability of detecting clinically significant cancer. Even if standard MRI techniques can be considered for initial evaluation of high-risk patients, multiparametric magnetic resonance imaging (mpMRI) can be used in the staging and characterization of prostate cancer. mpMRI images are defined as those acquired with at least one more sequence in addition to the anatomic T2-weighted images, such as diffusion-weighted and dynamic contrast images.

Images obtained with MRI can be overlapped with images obtained by ultrasound probe (fusion biopsy) in order to target the lesions of interest for biopsy.

MRI-guided and TRUS-guided biopsy have comparable detection rates of clinically significant prostate cancer in men with no prior biopsy and a suspicious elevated PSA. On the other hand, a negative MRI might lead to avoid unnecessary biopsy in patient with clinically insignificant or no prostate cancer, maintaining low rates of missing clinically significant prostate cancer [40]. These findings have been further confirmed in the PRECISION trial, in which MRI at the time of initial prostate biopsy demonstrated noninferiority to standard TRUS biopsy, with findings suggestive of superiority [41].

The role of MRI in the active surveillance for low-risk disease is currently being evaluated, with mixed results and a consistent suggestion that a targeted biopsy should not be omitted [42-44].

The 2016 EAU/ESTR/SIOG guidelines recommend mpMRI prior to a repeat biopsy (persistent suspicion of prostate cancer in spite of previous negative biopsies) which has to target any lesions seen.

Finally, the guidelines also recommend mpMRI for local staging and metastatic screening in predominantly Gleason pattern 4 intermediate risk patients and for local staging in high-risk localised prostate cancer [45].

6. Screening programs, overdiagnosis and overtreatment

Since the introduction and widespread use of PSA testing, the rate of metastatic disease at diagnosis has dropped by 50% and the rate of death from prostate cancer has been reduced by 70% [46]. At the same time, PSA screening determined an increase of

diagnosis and treatment of prostate cancer at early stages with the risk for potential overtreatment.

In a cohort study evaluating 10 years cancer specific survival, prostate cancer–specific mortality occurred in only 3%, 7% and 18% of patients with low, intermediate or high-risk disease, respectively. Nevertheless, 10-year mortality in men with more than three comorbid conditions was 26%, 40% and 71% for age groups < 60, 61-74, and >75 years, respectively [47]. Similar conclusions were stated in the PIVOT trial, remarking the importance to balance treatment, patient preference and life expectancy [48].

Several studies have investigated benefit and disadvantages of screening in prostate cancer; among these the Prostate, Colorectal, Lung, and Ovarian (PLCO) screening trial [49], the European Randomized Study of Screening for Prostate Cancer (ERSPC) [50] and the Göteborg trial [51].

The PLCO was conducted in the United States, including 38,340 men randomized between 1993-2001 to either annual PSA screening with a DRE for 6 years or usual care. At a median follow up of 15 years, only 2.7% of men died from prostate cancer and no reduction in cancer specific mortality was noted in the two arms [49]. This study demonstrated no difference between screening and opportunistic screening but has been criticized for his questionable clinical significance. In fact, the overall rate of screening for at least one PSA test during the study was 99% for the intervention arm and 86% for the control arm.

The ERSPC trial involved centers of 8 European countries, including 72,891 men in the PSA screening arm and 89,352 men in the control arm. After 13 years of follow-up, there was a significant reduction in prostate cancer-related mortality for the PSA screening arm. In particular, to prevent 1 death for prostate cancer, 781 men have to

be screened. Analogously, to prevent 1 death for prostate cancer, 27 patients with PCa have to be treated [50].

The Göteborg screening study involved 20,000 men aged 50-64 who had not had prior PSA testing. They were randomized to PSA screening or standard care. At 18 years of follow-up, to prevent one prostate cancer-related mortality, 231 men have to be screened and 10 men with PCa have to be treated [51].

On the basis of these data, the United States Preventive Services Task Force (USPSTF) initially recommended against all prostate cancer screening in 2012 [52-54], resulting in a significant decrease in the annual incidence of prostate cancer and an increase in the incidence of metastatic disease at the time of diagnosis in men older than 50 years of age. Moreover, emerging evidence suggests that these trends are also associated with worse survival [48]. For these reasons, USPSTF guidance was amended in 2018, providing a grade C recommendation for individualized screening for men aged 55-69 and a grade D for 70 years men or older [55].

Both surgery and radiotherapy are associated with morbidity, including possible erectile function and continence impairment. This is especially important in patients with comorbidities and limited life expectancy. For these reasons, evaluation of the competing risks and shared decision regarding screening and treatment are of paramount importance.

Collaboration between physicians and patients is then required to ensure that patient preference is accounted for in the decision-making process.

6.1 Guidelines recommendations on prostate cancer screening

The European Association of Urology (EAU) [56], the American Urological Association (AUA), the American Cancer Society (ACS) [57] and the National Comprehensive Cancer Network (NCCN) [58] provide guidelines regarding prostate cancer screening programs (Table 1). All of these take life expectancy into consideration both for screening and treatment. Early screening is generally recommended in men with a family history of prostate cancer, those of African-American origin, and those with a personal or family history of high-risk germline mutations such as those associated with DNA damage repair, including BRCA1/2 and ATM. DRE is not as sensitive as PSA and is not required by many of the guidelines panels. Repeated PSA testing occurs at differing intervals, there is no PSA value threshold that can be used to rule out prostate cancer, and PSA should be repeated at least once after initial elevation.

Table 1. Guidelines recommendations on prostate cancer screening

	Screening Tools	Ages to Screen	Repeat Testing Interval	Indication for Prostate Biopsy
NCCN (2018)	PSA and DRE	• 45-75 • >75 (only in very healthy men)	• PSA* < 1 ng/mL, 2-4 years • PSA* 1-3 ng/mL, 1-2 years	PSA > 3 ng/mL or Suspicious DRE
AUA (2018)	PSA	• Not indicated < 40 • Not recommended in men 40-54** • After shared decision making for men 55-69 • Not recommended for men >70 or men with life expectancy < 10-15 yrs	2 or more years	Consider PSA >3, however with additional consideration of factors affecting PSA***

EAU (2016)	PSA	Risk adapted model after shared decision making for men with a life-expectancy >10-15 years • Men >50 • Men >45 with a family history • Afro-American men >45 • Men with PSA >1 ng/mL at age 40 • Men with PSA >2 ng/mL at age 60	Risk adapted: • Every 2 years for men with PSA >1 ng/mL at age 40 or PSA >2 ng/mL at age 60 • Every 8 years for those not at risk	• PSA should be addressed continuously • Consider the addition of novel risk assays

*assuming normal DRE
**Screening should be individualized for men aged 40-54 and those at higher risk, such as African-American men, men with a family history of metastatic or lethal adenocarcinoma (including prostate, breast, ovarian, and pancreatic cancers), multiple affected first-degree relatives, family members with cancer detected at younger ages.
***A second confirmatory PSA should be obtained prior to biopsy

7. Histology

The majority of prostatic cancers are acinar adenocarcinomas. All histological variants of prostatic carcinoma can be allocated to two main groups [59]. The first group comprises histological variants of acinar adenocarcinoma which were defined in 2004 by the World Health Organization and include atrophic, pseudohyperplastic, foamy, colloid, signet ring, oncocytic and lymphoepithelioma-like carcinomas. The second group comprises histological variants of non-acinar carcinoma which account for about 5–10% of all prostate carcinomas. These include sarcomatoid carcinoma, ductal adenocarcinoma, urothelial carcinoma, squamous and adenosquamous carcinoma, basal cell carcinoma, and neuroendocrine tumours, specifically small-cell carcinoma. Recently characterized variants not present in the 2004 WHO classification, including microcystic adenocarcinoma, prostatic intraepithelial neoplasia-like adenocarcinoma, large-cell neuroendocrine carcinoma, and pleomorphic giant cell carcinoma, are also described.

From normal prostatic epithelium to invasive carcinoma there is a continuum of alterations. The architecture of the gland remains normal but the epithelial layers become multi-layered and crowded. Inside the cells, the nucleus becomes large and nucleoli visible.

Prostatic intraepithelial neoplasia (PIN) and atypical small acinar proliferation (ASAP) are considered precursor lesions to carcinoma. In the past, PIN was thought to antedate a carcinoma by 10 or more years. More recently, this concept is been abandoned and the term PIN is becoming less used in favour of atypical small acinar proliferation (ASAP), which is a proliferation of usually small acini with features suggestive of but not diagnostic of cancer.

Nowadays, when ASAP is identified in prostate biopsy specimens, further searching for invasive carcinoma are needed because it could be concurrently present up to 30% of cases. Repeat biopsies within 3-6 months are then recommended.

8. Grading

Histopathologic grading is an important factor influencing prognosis. Specimens obtained from a needle biopsy or a prostatectomy are graded using the Gleason Grading System, a two-number system in which the first number is assigned to the most common and the second number to the second most common cellular pattern. Each is graded on a scale of 1-5 and the sum of the two constitutes the overall grade [60]. Generally, the cut-off for prostate cancer starts with Gleason grade 3+3.

In 2010, the International Society of Urological Pathology (ISUP) modified the Gleason Grading system to a 5-grade system in which grade group 1 encompasses Gleason 3+3 disease, grade group 2 encompasses Gleason 3+4, grade group 3 encompasses Gleason 4+3, grade group 4 encompasses Gleason 8 (4+4, 5+3 and 3+5) and grade group 5 encompasses Gleason 9 and 10 (4+5, 5+4 and 5+5) [61].

9. Stage and risk stratification

For staging is widely used the tumour-node-metastasis (TNM) staging system of the American Joint Committee on Cancer (AJCC). The last revision of the AJCC system, introduced in January 2018, takes into account both the Gleason score and the grade group for staging [62].

Clinical staging is combined with grading and other clinical parameters, such as PSA, PSA density, volume of cancer in biopsy cores, to formulate clinical risk groups. Several risk stratification tools have been elaborate and one of the most used is those of NCCN.

However, all risk stratification systems are based on groups initially defined by D'Amico et al in 1998 [63] and are widely used in the decision-making process regarding both treatment and follow up scheme.

Table 2. Staging and prognostic groups

Stage	T	N	M	PSA	Grade Group
I	cT1a-c, cT2a	N0	M0	< 10 ng/ml	1
	pT2	N0	M0	< 10 ng/ml	1
IIA	cT1a-c, cT2a	N0	M0	≥10, < 20 ng/ml	1
	pT2	N0	M0	≥10, < 20 ng/ml	1
	cT2b-c	N0	M0	< 20 ng/ml	1
IIB	T1-2	N0	M0	< 20 ng/ml	2
II C	T1-2	N0	M0	< 20 ng/ml	3
	T1-2	N0	M0	< 20 ng/ml	4
IIIA	T1-2	N0	M0	≥20 ng/ml	1-4
IIIB	T3-4	N0	M0	Any	1-4
IIIC	Any	N0	M0	Any	5
IVA	Any	N1	M0	Any	Any
IVB	Any	Any	M1	Any	Any

9.1 Whitmore-Jewett classification

This classification divides prostate cancer into 4 stages, from A to D. To improve understanding of a subset of patients with hormone-insensitive prostate cancer, Crawford and Blumenstein [64], proposed more recently a further stratification of stage D.

Table 3. Whitmore-Jewett classification

Stage A	Tumour present, but not detectable clinically
Stage B	Tumour felt on physical examination but not spread outside the prostatic capsule

Stage C	Tumour extended through the capsule
Stage D	Tumour spread to other organs
D1	Involvement of pelvic lymph nodes
D1.5	Rising PSA level after failure of local therapy (i.e., biochemical failure)
D2	Metastatic disease to bone and other organs
D2.5	Rising PSA after nadir level
D3	Castrate-resistant prostate cancer
D3.5	Prostate cancer sensitive to hormones
D4	Prostate cancer insensitive to hormones

9.2 Nomograms and prediction models

In the last years, several prediction models have been developed with the intent to predict the local extension of prostate cancer, the probability of lymph nodes involvement, the time to biochemical failure, the time to the development of clinical metastatic disease, etc.

These models are essentially statistical tools which consider different parameters such as clinical stage, Gleason score, PSA level, MRI imaging, etc.

Nowadays, these are widely used in clinical practice by urologists. Among these, the Prostate Nomogram of the Memorial Sloan-Kettering Cancer Center [65], the Partin tables elaborated by experts at Johns Hopkins University, the Briganti Nomogram updated in 2018 [66], the CAPRA score and the PCPT risk calculator.

The Cancer of the Prostate Risk Assessment (CAPRA) score is calculated from the PSA level, the Gleason score, the percentage of biopsy cores positive for cancer, the clinical tumour stage and the patient age at diagnosis. In a large cohort of patients with clinically localized prostate cancer, the CAPRA score proved accurate for predicting metastases, cancer-specific mortality and all-cause mortality [67].

From the results of the Prostate Cancer Prevention Trial (PCPT), a risk calculator was created [68] including age, PSA score, ethnicity, family history, DRE and prior biopsy findings. The calculator predicts the chances for no cancer, low-grade and high-grade prostate cancer with the intent to guide treatment decision-making.

10. Treatment of localised PCa

Standard treatments for clinically localized prostate cancer include different options: watchful waiting, active surveillance, radical prostatectomy and radiation therapy.

10.1 Watchful waiting

Watchful waiting is defined as observation without any definitive local therapy. It has been evaluated in several key studies, such as PIVOT, VACURG and SPCG-4 trial. In the Prostate cancer Intervention Versus Observation Trial (PIVOT), conducted through the US Department of Veterans Affairs from 1994-2020, 731 men with localized prostate cancer were randomized to radical prostatectomy or observation [69]. Initially, there was no difference in survival, but at 22.1 years of follow-up, 68% of men assigned to surgery versus 73% of men assigned to observation had died; mean survival was 1 year longer in the surgical arm, especially in intermediate-risk disease [48].

In the Veterans Administration Cooperative Urological Research Group (VACURG) trial comparing observation with surgery for non-PSA-screening–detected prostate

cancers in 111 patients, minimal to no difference was found in survival at 23 years [70].

The Scandinavian Prostate Cancer Group Study Number 4 (SPCG-4) trial provided the most robust data comparing watchful waiting with surgery for non-PSA–detected prostate cancers. After 23.6 years of follow-up in 695 men there was a 12% absolute reduction in the risk of prostate cancer–related death for men undergoing surgery and a gain of 2.9 years in life expectancy [71].

These studies demonstrated a modest improvement in favor to local therapy, but at the same time they showed that many men might suffer from overtreatment of prostate cancer. This led to the development of active surveillance as a management modality.

10.2 Active surveillance

Active surveillance (AS) is indicated for very low, low and some favorable intermediate-risk prostate cancers. The objective is to delay potential curative intervention if needed, without missing the window for a cure. No single AS strategy is currently recommended, and the time to discontinue AS has not been established. However, it includes PSA monitoring at set intervals, confirmatory prostate biopsy and repeat prostate biopsies at predetermined time points (generally at 12-24 months).

According to several long-term cohort studies, 27-53% of patients undergo definitive therapy, while the rates of metastasis (0.12-6%) and prostate cancer–related mortality (0-1.5%) are low [72], even if not negligible. The randomized controlled ProtecT trial, which compared AS with both surgery and radiotherapy, demonstrated that, for patients with low-risk prostate cancer, AS is a safe option. Less than 1% of patients

died from prostate cancer over the study time period, but about half of the patients in the AS arm underwent surgery or radiotherapy due to disease progression [73].

10.3 Radical local therapies

Radical local therapy comprises surgical removal and irradiation of the prostate, with or without involvement of the draining pelvic lymph nodes. No randomized, prospective trials comparing radiation with surgery have been conducted, so decision generally takes into account patient preferences, short- and long-term adverse effects. In general, radiotherapy and surgery have similar effects on quality of life. Both can impair erectile function and continence temporary or definitively. In addition, surgery can involve immediate operative risks (bleeding, pain, infection) [74-76], while radiotherapy can be associated with a persistent fecal urgency and incontinence of gas, secondary malignancy in the radiation field and haemorrhagic cystitis [77-79]. Moreover, a systematic review and meta-analysis found that external beam radiation therapy (EBRT), but not brachytherapy, was consistently associated with increased odds for a second malignancy of the bladder, colon, and rectum. Absolute rates were low, 0.1-3.8% for bladder, 0.3-4.2% for colorectal and 0.3-1.2% for rectal cancers [80].

10.4 Surgical therapy

Surgical treatment for prostate cancer consists of removal of the prostate, seminal vesicles and the draining pelvic lymph nodes (when risk indicates removal) with re-

anastomosis of the bladder to the urethra. Prostatectomy has been performed via open surgical approaches, including perineal, suprapubic, retropubic, infrapubic, transrectal and sacral [81,82]. Radical retropubic prostatectomy (RRP) became the gold standard while the other approaches were progressively abandoned [83].

Since the introduction of the robotic surgical platform, robot-assisted radical prostatectomy (RARP) has rapidly spread worldwide (from 67% of all prostatectomies in 2010 to 85% in 2013 [84,85]), demonstrating oncological equivalence to RRP, with decreased blood loss and reduced hospital stay. Moreover, the development of techniques for sparing of the peri-prostatic nerves, bladder neck and space of Retzius, have led to improved functional outcomes in term of potency, continence and surgical margins positivity [86-89].

Independently of surgical approach, despite of favourable outcomes for low- and intermediate-risk disease, 25-50% of patients with high-risk disease experiences disease recurrence over 10 years.

10.5 Radiotherapy

Radiotherapy may be delivered in the form of EBRT or brachytherapy (insertion of radioactive seeds into the prostate). EBRT techniques include 3-dimensional conformal radiation therapy (3D-CRT) and intensity-modulated radiation therapy (IMRT) with hypofractionation.

Androgen ablation has been shown to improve survival in men with localized disease who are treated with external radiation. D'Amico et al reported higher overall survival

with the combination of radiation therapy and 6 months of ADT in men with intermediate-risk prostate cancer [90].

Jones et al found that short-term ADT increased overall survival in intermediate-risk—but not low-risk—men with stage T1b, T1c, T2a, or T2b prostate cancer and a PSA level ≤ 20 ng/mL. 10-y overall survival was 62% with combination therapy, versus 57% with radiotherapy alone; 10-y disease-specific mortality was 4% and 8%, respectively [91].

Valid comparisons of surgery and radiation therapy are impossible without data from randomized studies that track long-term survival rather than PSA recurrence. Variation in radiation techniques and dosage administered; the variable use of androgen ablation, which improves survival in intermediate- and high-risk disease; and the variable impact on the quality of life complicate comparison using uncontrolled studies.

Several randomized trials have evaluated the use of adjuvant radiation therapy to the prostatic bed following surgery for patients at high risk of recurrence (generally those with positive surgical margins or with seminal vesical invasion). Those include EORTC 22911 [92], SWOG 8794 [93], ARO 96-02/AUO AP 09/95 [94] and FinnProstataX [95] as well as the ongoing RAVES, GETUG-AFU 17 and RADICALS-RT studies. Recent research has further highlighted the role of early salvage radiation therapy (PSA < 0.5) with concomitant ADT for those with biochemical recurrence after prostatectomy. This is reflected in the current AUA/ASTRO guidelines [96].

10.6 Focal therapies

Several emerging therapies for the management of localized prostate cancer are gaining traction, though they are not yet routinely recommended. These include whole-, hemi-, and partial-gland ablative therapies such as cryoablation, high-intensity focused ultrasound (HIFU) and photodynamic therapy.

They are generally employed in patients with low-risk prostate cancer. Long-term safety and efficacy data remain unclear [97-101].

11. Abstract of the study

SINGLE SETTING 3D MRI-US GUIDED FROZEN SECTION AND FOCAL CRYOABLATION OF THE INDEX LESION IN LOW/INTERMEDIATE RISK PROSTATE CANCER

Objectives: To explore the reliability of frozen sections to diagnose prostate cancer (PCa) and to describe surgical steps of a 3D magnetic resonance imaging (MRI)–ultrasound (US)-guided prostate biopsy (PB) and focal cryoablation of the index lesion in a single setting procedure.

Patients and Methods: Patients with suspicious PCa, based on prostatic specific antigen (PSA) value and on a PIRADS 4 or 5 single lesion, as well as the steadfastness of avoiding any kind of radical treatment, were considered for enrolment. IRB and written informed consent were obtained from the patients.

The entire procedure was performed transperineally, in two consecutive surgical phases: 3D MRI–US-guided plus systematic template PB and real-time TRUS-guided focal cryoablation.

Three cores were taken from the index lesion (one for frozen section and two for final pathology), three cores from the surrounding area and systematic sampling was performed for the rest of the gland. Focal cryoablation of the index lesion was performed once confirmation of PCa was obtained by means of frozen sections.

Follow-up schedule included PSA test at 3-mo interval, MRI 3-mo and 1-yr postoperatively and prostate biopsy of the treated area at 1-yr.

Results: This report includes 14 patients with a minimum follow up time of 12 months. All patients were potent before treatment, complained no severe low urinary tract symptoms and denied consent to any radical treatment.

PCa diagnosis was histologically confirmed in all patients by frozen sections. All other cores were negative. At final histology, there was a Gleason score upgrade in three patients, from 3+3 to 3+4.

The postoperative course was uneventful and all patients were discharged on the first postoperative day.

Mean PSA value decreased from 6.37 (baseline) to 0.83 ng/mL at 3-mo evaluation.

Three-mo postoperative MRI images showed complete ablation of the index lesion in all patients.

Urinary continence and erectile function were preserved in all patients, without clinically meaningful changes at EPIC questionnaire.

At one-yr follow-up, eleven patients showed no signs of persistent or recurrent disease at MRI imaging and treated area biopsies; three patients had a suspicious area at MRI and they needed treatment for confirmed disease at biopsy.

Conclusion: Single setting 3D MRI–US-guided frozen section and focal cryoablation of the index lesion could represent a step forward towards a "patient-tailored" minimally invasive approach to diagnosis and cure of low and intermediate risk PCa.

12. Introduction and background

There is a growing interest in focal treatment for prostate cancer (PCa). Improvements in magnetic resonance imaging (MRI)–ultrasound (US) fusion imaging have led to significantly higher accuracy in the diagnosis of PCa and more interestingly in the identification of the index lesion. However, the necessity of histopathological confirmation of diagnosis has obliged to defer the treatment from prostatic biopsy.

In recent years, the progressive spread of focal therapy has sought to bridge the existing therapeutic empty space between two diametrically opposite solutions: active surveillance on the one hand, and radical prostatectomy and radiotherapy on the other hand.

13. Materials and methods

13.1 Patient selection

For this study, we considered patients with suspected low/intermediate risk PCa, based on PSA value and MRI proven PIRADS 4 or 5 single lesion. A metastatic evaluation was performed for patients with PSA >10 ng/mL to exclude those with possible non-organ confined disease. Finally, we excluded patients with previous prostate biopsy, PSA ≥ 20 ng/mL, cT3b/cN1/cM1 at MRI and Prostate Grade Group (PGG) 4 or 5 at frozen section. This allowed us to treat only patients with low/intermediate risk PCa.

After a detailed discussion about the limitations and benefits of focal cryoablation and the reliability of the frozen section for PCa, only patients who refused any radical

treatment were enrolled, and medical clearance and consent were obtained.

Patients were evaluated by history, physical examination, regular preoperative urine and blood tests, PSA and the use and number of pads for incontinence, and by validated questionnaires for sexual function (the International Index of Erectile Function, IIEF-5 [102]) and for low urinary tract symptoms (LUTS) (the International Prostate Symptom Score, I-PSS) [103].

13.2 Preoperative preparation

On the day before surgery, patients were given a laxative. Moreover, patients fasted for a minimum of 8 h and, 2 h before procedure, they received an enema and prophylactic antibiotic therapy intravenously.

13.3 Surgical technique

Fusion biopsy and cryoablation were performed in the same setting, as a one-day surgery. The entire procedure was performed transperineally, with the patient under spinal or general anaesthesia, in lithotomic position. The perineum was previously shaved and prepared, and the scrotum was suspended while an 18-French Foley catheter was inserted. In the meantime, the antibiotic was administered intravenously. We used the NavigoTM (UC-CARE Medical Systems, Yokneam, Israel.) and an argon/helium-gas-based third generation cryoablation system (Endocare; HeathTonics Inc., Austin, TX, USA). The NavigoTM System provides a 3D real-time orientation of both probe and targeted prostate areas. This real-time adjustment is generated thanks

to an electromagnetic transmitter, above the body sensor and the probe sensor. This tracking system allows both transrectal and trans perineal biopsies, and it can be combined with the US based on Endocare® Cryocare® Systems.

13.3.1 MRI–US fusion biopsies of the index lesion

After a US scan of the entire gland, the prostatic diameters were calculated using the NavigoTM System, while prostatic contours were scored and marked on the screen.
MRI images previously uploaded on NavigoTM System were fused with US images and a 3D model was created by NavigoTM software. The index lesion, previously marked on MRI images, was clearly integrated and visible in the 3D model.
Every prostatic biopsy was performed under MRI–US guidance, using a grid to track the exact location of prostate biopsies and as a guide for subsequent cryoprobes insertion.
Overall, three cores were taken from the index lesion (Figure 1), one of which was sent to the frozen section. Other two cores were sent for final histopathological examination to confirm Gleason score and to provide an accurate tumour staging.

13.3.2 Biopsies of the area surrounding the index lesion

Three cores were taken from the perilesional area (Figure 2) and they were sent to the frozen section to ensure a safety margin of index lesion ablation. The frozen section of these three cores must be negative to proceed with performing cryoablation. In case of positive core at one of these sites, a further biopsy should be performed at a distance

of 5 mm to ensure proper safety margins.

13.3.3 Systematic prostate biopsy

A systematic 6 core sampling of the contralateral lobe was performed (Figure 3) and cores taken were sent to the frozen section to exclude other PCa foci.

13.3.4 Frozen section

Frozen section as practiced today was first described by Dr Louis B. Wilson in 1905, who is hence considered the pioneer of the procedure.

The specimen is embedded in a gel-like medium called OCT (optimal cutting temperature compound) and rapidly freeze using a cryostat, basically at an optimal temperature between -16 º C and -20º C for prostatic tissue [104]. At this temperature, most tissues become rock-hard and can be cut with a microtome, obtaining slices of tissue (cryosections) which are picked up on a glass slide and stained with haematoxylin and eosin for microscopic examination.

Frozen section has multiple uses in urological field, being the most commonly used method for intra-operative assessment of surgical margins during radical prostatectomy [105].

Considering the limitations, it is certainly a time and resource consuming phase. Moreover, the difficulty of frozen section interpretation due to confounding artefacts, could lead to an upstaging at final histology. Even if widely used for intra-operative pathological evaluation of surgical margins, its complete accuracy and reliability in

prostate cancer diagnosis is still debatable.

In the present study, frozen sections were used for different purposes: first of all, to confirm the presence of PCa at the level of MRI index lesion (Figure 4); then, to shape the treatment area of focal cryoablation, obtaining negative cores at all three perilesional sites, at a minimum distance of 5 mm from the index lesion margins; finally, to exclude the presence of clinically significant PCa [106] in the rest of the gland.

Pathological evaluation was performed by a dedicated uropathologist assisted by technicians simultaneously working on 2 cryostats. In this way, preparation and transportation lasted approximately 15 minutes, while frozen section processing and evaluation took around 15 minutes for a total of 30-40 minutes. Permanent sections were always done for every specimen for final histologic evaluation.

13.3.5 Procedure planning

The 3D model created by NavigoTM allowed us to define accurately the area to be treated. Cryoablation planning is a crucial step to determine which probe to use and where to position them. The aim is creating an iceball shaped to inscribe completely the treatment area.

13.3.6 Cryoprobe and thermocouple placement

Three-four cryoprobes were placed around the index lesion, exactly at the level of the perilesional biopsies.

We specifically used V-ProbeTM by EndoCare which can create variable iceball sizes, from 1.5 to 5 cm, by sliding a blue tab to the desired isotherm length. They are perfectly suited to shape an iceball tailored to the planned treatment area (Figure 5). This permitted us to minimize any unintended injury to surrounding tissues while precisely targeting the index lesion. A thermometer probe was inserted into the index lesion, where the frozen section had confirmed the presence of PCa to allow active monitoring of the target temperature during the entire freezing process.

A second thermometer probe was placed at the level of the external sphincter to guarantee the maintenance of a temperature greater than 0º C during the entire treatment.

13.3.7 Urethral warming catheter placement

Urethral warming catheter was placed before starting cryoablation. It is a dual lumen urethral catheter which can be passed transurethrally to circulate warm saline and prevent the destruction of the urethral epithelium. This prevents transmural necrosis, maintaining the epithelial barrier for the containment of necrotic prostate tissue after treatment [107]. At the end of the procedure, it was replaced with a Foley catheter which was removed on the seventh postoperative day.

13.3.8 Focal cryoablation

Focal cryoablation was performed with two freezing cycles as standard. A homogeneous temperature reduction was observed on the screen while the US imaging

showed the progressive formation of the iceball. The target temperature of -40º C was maintained for ≥2 minutes per freezing cycle, for a total of ≥4 minutes. A thawing phase of 15 minutes was ensured between the two consecutive freezing cycles.

13.4 Postoperative course and follow-up

Patients were discharged on the first postoperative day. A urethral catheter was removed on the 7th postoperative day.

Continence was defined as the absence of any urine leakage. Erectile function was assessed by means of IIEF-5 score. LUTS were assessed using the I-PSS scoring system [103]. PSA was evaluated 1-mo after surgery and every 3-mo subsequently for the first one year. Three-month and 1-yr MRIs were performed [108] to confirm complete ablation of the treated area.

On the base of 1-yr MRI a targeted biopsy of the treated area and of all suspicious metachronous PCa foci was performed (Figure 6) according to the International Multidisciplinary Consensus on Trial Design for focal therapy in PCa [109]. Quality of life was assessed using the Expanded Prostate Cancer Index Composite (EPIC) questionnaire (version 2.2002, 32 items) [110] 1-mo, 3-mo and 1-yr after surgery.

14. Results

Overall, 14 patients underwent 3D MRI–US-guided frozen section and focal cryoablation of the index lesion with the described technique. All patients had a

clinical suspicion of PCa based on PSA test and a single MRI lesion with a PIRADS score of 4 or 5 and denied consent to any radical treatment. They were all potent and continent and complained of no severe LUTS before treatment. Baseline clinical data, 3-mo and 1-yr functional and oncologic outcomes were collected and reported (Table 1, 2, 3). Median follow up time was 15 months and all patients had a minimum follow up of 12 months.

Postoperative course was uneventful and all patients were discharged on the first postoperative day. Erectile function and urinary continence were preserved in all patients. Median IIEF-5 decreased slightly from 19 to 18 after treatment. All patients continued to use any pad, and median I-PSS score changed faintly from 12.5 to 11 after cryoablation.

Quality of life, assessed using the EPIC questionnaire, showed no clinically meaningful changes after treatment.

In all patients, all systematic and non-targeted biopsies were negative both at frozen section then at final histology. PCa was histologically confirmed in all 14 patients by frozen sections from the index lesion (7 PGG1, 4 PGG2, 3 PGG3), but at final histology there was a Gleason score upgrade in three patients, from 3+3 to 3+4.

Mean PSA values decreased from 6.37 (baseline) to 0.83 ng/mL at 3-mo evaluation and to 1.12 ng/mL at 1-yr follow-up. Mean PSA reduction was 86.9% and 82.3% at 3-mo and 1-yr evaluations, respectively. Three-mo postoperative MRI images showed complete ablation of the index lesion in all patients. At one-yr follow-up neither MRI imaging nor treated area biopsies showed signs of persistent or recurrent disease in eleven out of fourteen patients.

In three remaining patients, a suspicious area was observed at one-yr MRI. In one

patient retreatment was performed and histology confirmed a 4+3 ipsilateral PCa recurrence. The other two patients decided to underwent fusion biopsy which confirmed 3+4 PCa and radiotherapy was scheduled.

15. Discussion

According to a systematic review of the Literature, the negative predictive value of mpMRI ranges from 63 to 98% [111]. This indicates that in the presence of an index lesion without further foci, the risk of missing PCa is poor and consequently the risk of missing clinically significant cancer is reduced up to 2-7% [111]. Despite the significant advancements in imaging technologies, diagnosis of PCa is still based on prostate biopsy [112], which has itself potential complications with conceivable impact on quality of life [113]. In the attempt to reduce them, less numerous and more precise biopsies have been advocated. In this context, a recent study demonstrated that fusion biopsies outperform systematic biopsies, increasing the detection rate of clinically significant PCa [114].

Nevertheless, the treatment of PCa must be postponed at the availability of pathologic report of prostate biopsy, exposing the patient to the possible consequences of two separate and distinct surgical procedures.

Our study is a proof of concept to confirm reliability and diagnostic accuracy of frozen sections for PCa, which demonstrates feasibility and safety of performing a prostate biopsy and focal treatment in the same surgical session.

Increasing evidence supports the reliability of the frozen section in several oncological

contexts, like ovarian cancer [115] and endometrium [116]. This lay the foundations of a possible fusion of the diagnostic and therapeutic moment in those patients who are candidates for focal therapy with an expected reduction of costs, patients' stress and waiting list.

Although PCa is a multifocal pathology in 60–90% of patients, it is often possible to identify a dominant lesion, called "index lesion", characterized by a higher Gleason score than satellite lesions. This area is likely to be the tumour area that will drive patient's prognosis [117]. This assumption represents the rationale behind all organ-sparing therapies that aim for adequate oncological control through the sole treatment of the dominant lesion, minimizing undesired effects related to radical therapeutic options. Recent scientific evidence suggests that the treatment of index lesion is feasible, oncologically safe and characterized by excellent functional results [118,119].

Despite the small sample size, the diagnostic accuracy of frozen sections appeared comparable to that obtained by means of conventional prostate biopsy. Frozen section findings were confirmed through final histopathological evaluation in twelve out of fifteen cases (during eleven out of fourteen primary cryoablations and at the unique retreatment).

Our study introduced several original messages from a clinical standpoint: first of all, we reported the feasibility of combining diagnosis and focal treatment of PCa in a single surgical session; therefore, we confirmed the oncologic effectiveness of PCa focal ablation by means of 1-yr prostate biopsy, the functional benefits of such treatment in terms of erectile function and urinary continence preservations and finally the negligible impact on patients' QoL assessed by EPIC questionnaire [110]. We also

reported the feasibility of retreatment in a patient who had an ipsilateral recurrence at 1-yr follow-up and the reliability of mpMRI to assess the risk of PCa recurrence after treatment.

Our study is not exempt from criticism and limitations: first of all the small sample size, essentially due to the low enrolment that basically requires patient's motivation to refuse any kind of radical treatment; the need for trust in the diagnostic accuracy of mpMRI, with the consequent risk of omitting diagnosis of other PCa foci performing a 12-core "only" systematic prostate biopsy; the need for an expert uropathologist to optimize the diagnostic accuracy of frozen sections and therefore the risk of poor reproducibility in other centers.

Finally, defining the proper indication to an MRI–US-guided biopsy and focal ablation of PCa is beyond the scope of this study and will certainly require larger populations and reports from other centers. Similarly, a precise follow-up schedule after a focal treatment is still awaited and based on an International Multidisciplinary Consensus on Trial Design [109] with the potential risk of missing the "lethal clone" by treating the index lesion exclusively [120].

A continuously increasing role of mpMRI in different contexts, including diagnosis, treatment, surveillance and follow-up, is likely to be anticipated [121].

16. Conclusions

Diagnosis and focal treatment of a PCa index lesion in a single session is safe and feasible and represent a further step towards a minimally invasive and patient-tailored approach.

Further trials are needed to confirm the reliability of frozen section for PCa diagnosis and therapeutic effect of 3D MRI–US-guided focal cryoablation of the index lesion.

17. Appendix

Figure 1 – Bioptic sampling of the index lesion

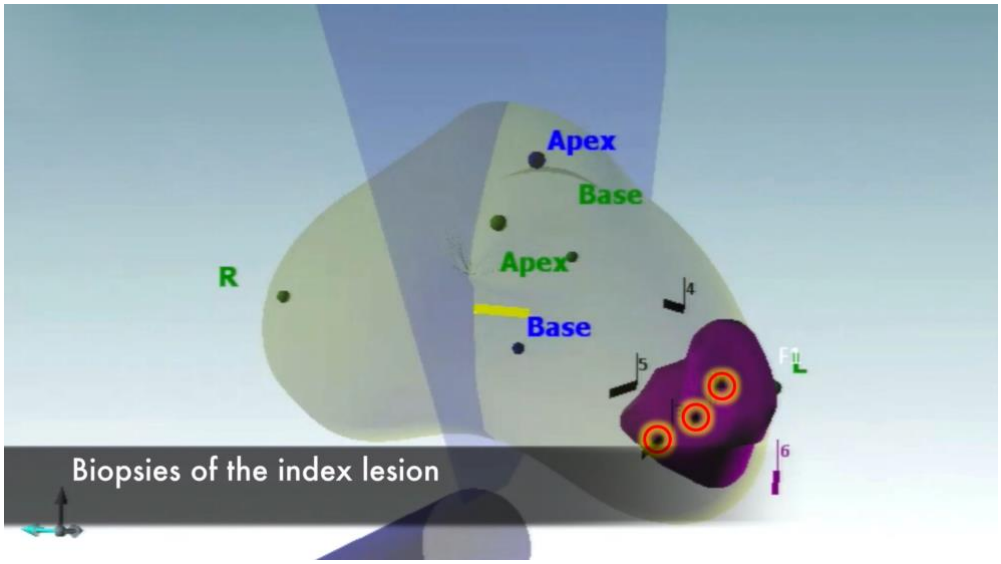


Figure 2 – Bioptic sampling of the perilesional area

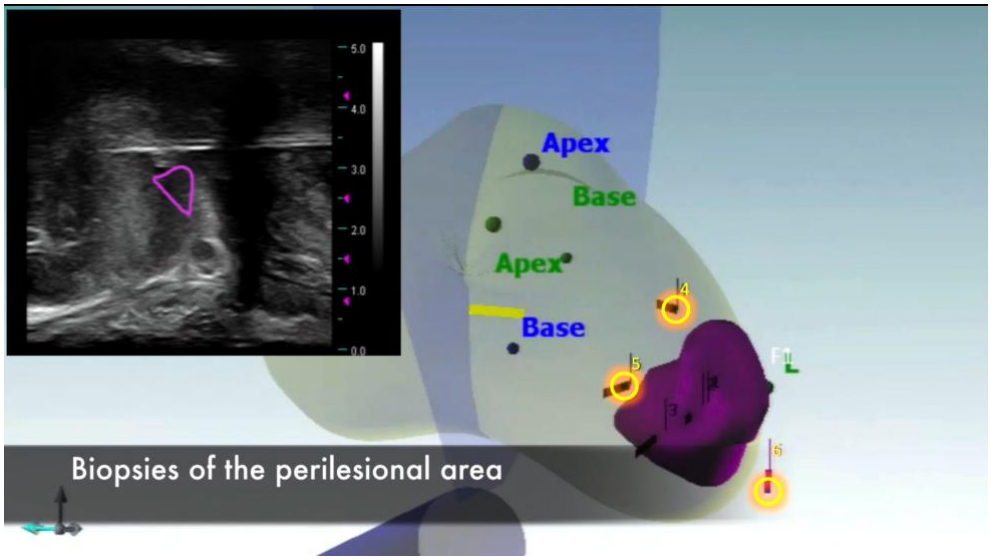


Figure 3 – Systematic 6 core sampling of the contralateral lobe

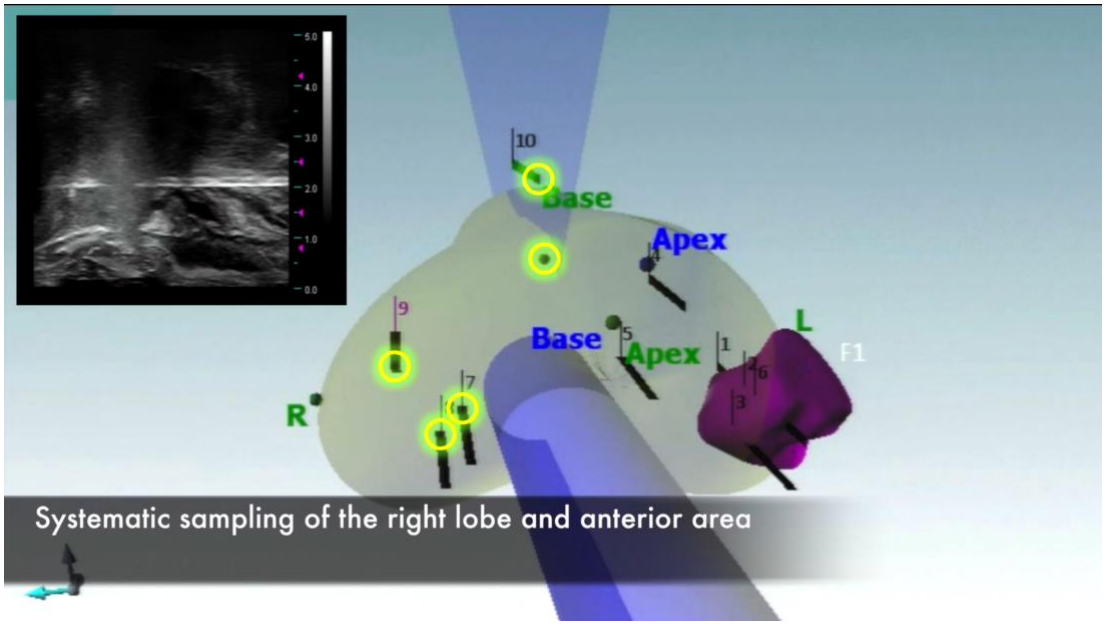


Figure 4 – Frozen section showing prostate cancer Gleason Score 3+3 (Hematoxylin / Eosin)

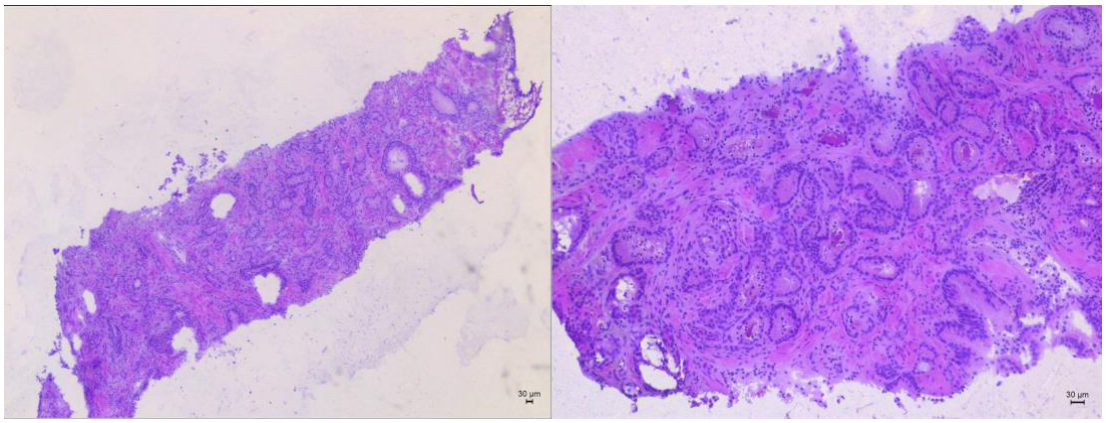


Figure 5 – Cryoprobes placement and shaping of the iceball tailored to the planned treatment area

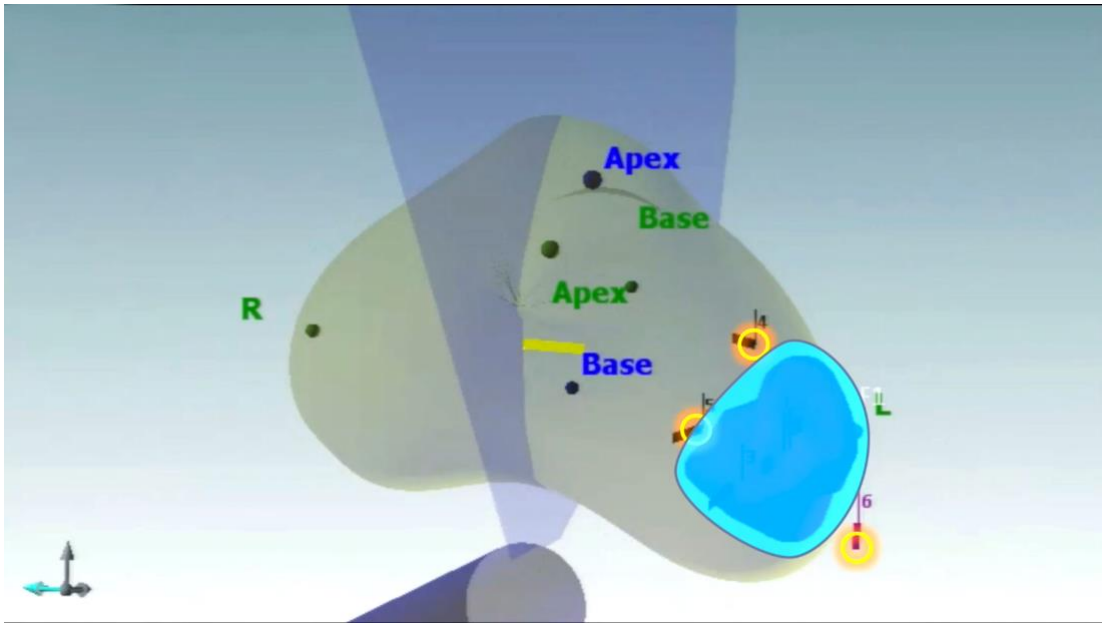
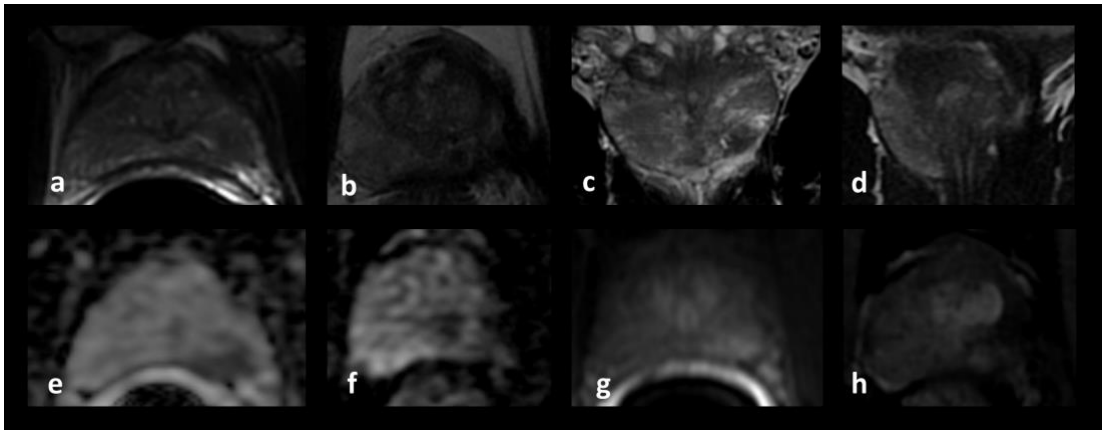


Figure 6 – Pre-operative and post-operative multiparametric-MR images.



a: pre-operative axial T2-weighted image.

b: post-operative axial T2-weighted image.

c: pre-operative coronal T2-weighted image.

d: post-operative coronal T2-weighted image.

e: pre-operative axial ADC (apparent diffusion coefficient) map image.

f: post-operative axial ADC map image.

g: pre-operative axial perfusion image.

h: post-operative axial perfusion image.

Table 1. Demographic and baseline clinical data

Characteristics	
Median Age, yr	64,5
Mean PSA, ng/mL	6,37
Median Body Mass Index, kg/m²	27,3
Digital rectal examination	Negative in all pts
MRI findings	PIRADS-4 in 9 pts PIRADS-5 in 5 pts
cT Stage	cT3a in 1 pt cT2b in 9 pts cT2a in 4 pts
Median ASA score	2
Median Index lesion volume, cc	15
Median IIEF-5 score	19
Median I-PSS score	12,5

Table 2. Three-month postoperative data

Characteristics	
Mean PSA, ng/mL	0.83
Digital rectal examination	Negative in all pts
MRI findings	Negative in all pts
Continence, yes/no	All pts continent
Median IIEF-5 score	18
Median IPSS score	11

Table 3. One-year postoperative data

Characteristics	
Mean PSA, ng/mL	1.12
Digital rectal examination	Positive in 2/14 pts
MRI findings	PIRADS-4 in 3 pts
Repeated biopsy	Negative in 11 pts 4+3 in 1 pt 3+4 in 2 pts
Continence, yes/no	All pts continent
Median IIEF-5 score	18
Median IPSS score	11

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