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Assessment of prognostic factors of prostate cancer – Limitations and possibilities of morphology

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Assessment of prognostic factors of prostate cancer – Limitations and possibilities of morphology

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

Prostate cancer is a leading cause of cancer morbidity and mortality. It is a morphologically, genetically, and clinically heterogeneous disease. Stage and grade are important predictors of patient outcome. Extraprostatic extension (EPE) of prostate cancer is a key component of staging but it is not fully understood how its histopathological characteristics correlate with outcome. Gleason grading takes the morphological heterogeneity into account and is considered one of the best prognostic factors of prostate cancer. The grading system has evolved considerably over time and it is essential to understand how this affects its utility.

The aim of this thesis was to classify patients with EPE into prognostic groups and to evaluate Gleason grading trends over time and how grading reproducibility can be improved.

We reviewed 1051 radical prostatectomy (RP) specimens and found 470 cases with EPE. Men with EPE had a higher risk of biochemical recurrence. When stratified by the extent and other pathological features of EPE, radial extent predicted recurrence, while perineural invasion at the site of EPE and circumferential extent did not.

We analyzed trends in Gleason grading practices in Sweden and assessed the impact of the 2005 International Society of Urological Pathology (ISUP) revision. Data on 97,168 men with a primary diagnosis of prostate cancer in needle biopsy from 1998 to 2011 were obtained from the National Prostate Cancer Register (NPCR). There was a shift towards higher Gleason scores (GS) at diagnosis over the period but more evident after the ISUP revision. The trend remained when stage migration was factored in. This grade inflation has consequences for therapy decisions, such as the eligibility for curative treatment or active surveillance.

The concordance between GS in biopsies and subsequent RP specimens was analyzed in 15,598 men registered by the NPCR between 2000 and 2012. The agreement improved from 55% to 68% during the period, but most of the improvement occurred before 2005. When adjusted for GS and year of diagnosis, the GS prediction became less accurate over time.

A limitation of Gleason grading is that it is subjective and suffers from interobserver variability. We analyzed causes of disagreement in 87 prostate cancer biopsies, included in a reference image database for standardization of pathology. A group of 23 international experts failed to reach consensus in 41% of cases. The most frequent cause of disagreement was between GS 3+3 with tangential cutting artifacts and GS 3+4 with poorly formed or fused glands. An artificial intelligence (AI) system trained in grading assessed the grades of non-consensus cases and obtained a weighted kappa value of 0.53 compared to 0.50 for the pathologists, placing AI as the sixth most reproducible observer.

In conclusion, prostate cancer is a heterogeneous disease calling for individualized diagnosis and treatment. These studies have highlighted some limitations of histopathological prognostic factors and suggested more standardized assessments. In a near future, AI may serve as a decision support for more consistent diagnoses.

POPULÄRVETENSKAPLIG SAMMANFATTNING

En av fem män i Sverige får prostatacancer under sin livstid. Det betyder att nästan alla känner någon som har prostatacancer. Det är den vanligaste cancersjukdomen bland män i Sverige och varje år diagnostiseras över 10 000 män med prostatacancer och över 2000 män dör av sjukdomen. Samtidigt klarar sig flertalet patienter utan att få spridd sjukdom oavsett om de behandlas. Botande behandling är bortoperation av hela prostatan eller strålbehandling. Båda metoderna är behäftade med biverkningar som sänker livskvaliteten, såsom inkontinens och impotens. En av våra största utmaningar i vården av dessa patienter är att lära oss förutsäga vilka patienter som har en aggressiv sjukdom och som behöver tidigt insatt behandling för bot. Många riskfaktorer är kända såsom hög tumörgrad och stor tumörbörda med spridning utanför prostata (extraprostatisk växt och inväxt i sädesblåsorna). Ytterligare klassificering behövs dock för att avgöra vilka patienter som behöver ytterligare behandling efter kirurgi för att undvika återfall.

Syftet med vår första studie var att analysera patienter som hade cancer som växte utanför prostatakörteln. Ungefär hälften av dessa patienter har i tidigare studier visats återfalla i sjukdom efter kirurgi. Vi gick igenom 1051 prostator som opererats bort på grund av cancer. Patienter med cancer utanför prostatakörteln hade högre risk för att få stigande PSA efter kirurgi vilket i en del fall betyder sjukdomsåterfall. När vi klassificerade extraprostatisk växt genom att mäta djupväxt, horisontell utbredning, växt vid nerver och anatomisk lokalisation fann vi att djupväxten av cancer utanför prostatakörteln i många fall kunde förutsäga återfall. De andra karakteristika korrelerade inte till prognos. Att mäta djupväxt av extraprostatisk växt kan bidra till att avgöra vilka patienter som kan ha nytta av ytterligare behandling efter kirurgi.

Tumörgrad undersöks genom mikroskopi på prostatabiopsier och på hela körteln efter kirurgi. Ju mindre lik normala prostatakörtlar desto aggressivare cancer. Tumörgraderingen är en av de starkaste faktorerna för prognos och är därför av yttersta vikt i behandlingsbeslutet. Gradering av prostatacancer har förändrats över tid. Större förändringar gjordes 2005. I en studie baserat på data från nationella prostatacancerregistret (NPCR) mellan 1998 och 2011, analyserade vi nästan 100 000 fall och undersökte hur tumörgradering av prostatacancer har förändrats över tid och hur förändringarna från 2005 har påverkat graderingen. Under denna tidsperiod skedde en gradvis högre gradering trots att den genomsnittliga tumörbördan minskade. Detta är viktigt att rapportera eftersom det har betydelse för behandlingsbeslut och kan medföra att patienter överbehandlas.

I en uppföljande studie analyserade vi hur väl graderingen stämde överens mellan prostatabiopsi och efterföljande operationspreparat (hela prostatan) från över 15 000 patienter registrerade av NPCR mellan 2000 och 2012. Under denna period tycktes överensstämmelsen av tumörgrad öka. En förklaring kan dock vara att man numera använder färre grader vilket gör att det blir lättare att förutsäga rätt grad.

En svaghet med tumörgradering är att den är subjektiv och tolkningen kan skilja mellan läkare. I den fjärde studien i denna avhandling analyserade vi olika anledningar till att patologer inte var överens om gradering i 87 prostatacancer biopsier. I 41% kunde internationella experter inte komma överens om tumörgrad. Den vanligaste anledningen till detta var oenigheten om det rörde sig om en låggradig cancer med artefakter eller en höggradig cancer som inte kunde forma prostatakörtlar. Vår grupp på Karolinska Institutet har deltagit i utvecklingen av ett artificiellt intelligent (AI) system som kan identifiera prostatacancer och bedöma tumörgrad. Vi fann att detta AI-system kunde gradera med samma reproducerbarhet som internationella experter.

Sammanfattningsvis är prostatacancer en sjukdom där prognosen varierar stort och individanpassad handläggning och behandling är viktigt. Dessa studier har belyst svagheter hos några prognostiska faktorer som tumörgrad och cancerväxt utanför prostata och föreslagit sätt att standardisera bedömning. Vidare tror vi att AI kommer att stödja läkare i diagnostik av prostatacancer i en nära framtid.

LIST OF SCIENTIFIC PAPERS

I. **Danneman D**, Wiklund F, Wiklund P, Egevad L.
Prognostic significance of histopathological features of extraprostatic extension of prostate cancer
Histopathology. 2013 Oct;63(4):580-9.

II. **Danneman D**, Drevin L, Robinson D, Stattin P, Egevad L.
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BJU Int. 2015 Feb;115(2):248-55.

III. **Danneman D**, Drevin L, Delahunt B, Samaratunga H, Robinson D, Bratt O, Loeb S, Stattin P, Egevad L.
Accuracy of prostate biopsies for predicting Gleason score in radical prostatectomy specimens: nationwide trends 2000-2012
BJU Int. 2017 Jan;119(1):50-56

IV. Egevad L, **Swanberg D**, Delahunt B, Ström P, Kartasalo K, Olsson H, Berney D M, Bostwick D G, Evans A J, Humphrey P A, Iczkowski K A, Kench J G, Kristiansen G, Leite K R M, McKenney J K, Oxley J, Pan Chin-Chen, Samaratunga H, Srigley J R, Takahashi H, Tsuzuki, T.
Identification of areas of grading difficulties in prostate cancer and comparison with artificial intelligence assisted grading
Virchows Arch. 2020 Dec;477(6):777-786

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LIST OF ABBREVIATIONS

ADT	Androgen deprivation therapy
AI	Artificial intelligence
AR	Androgen receptor
BCR	Biochemical recurrence
BPH	Benign prostatic hyperplasia
CNV	Copy number variation
cT	Clinical stage
DRE	Digital rectal examination
DNN	Deep neural network
EPE	Extraprostatic extension
FFPE	Formalin-fixed paraffin-embedded
GS	Gleason score
HGPIN	High-grade prostatic intraepithelial neoplasia
H&E	Hematoxylin and eosin
ISUP	International Society of Urological Pathology
MRI	Magnetic resonance imaging
NPCR	National Prostate Cancer Register
pT	Pathological stage
PIA	Proliferative inflammatory atrophy
PNI	Perineural invasion
PSA	Prostate-specific antigen
RP	Radical prostatectomy
RT	Radiotherapy
SNP	Single nucleotide polymorphism
s-PSA	Serum prostate-specific antigen
SVI	Seminal vesicle invasion
TNM	Tumor, node, metastasis
TRUS	Transrectal ultrasound
TURP	Transurethral resection of the prostate

1 INTRODUCTION

1.1 PROSTATE CANCER WORLDWIDE AND IN SWEDEN

Being the second most common cancer in men and the fifth leading cause of death makes prostate cancer a leading cause of morbidity and mortality worldwide. The highest prevalence and incidence of prostate cancer is found in developed countries, which include Northern and Western Europe, Australia/New Zealand, and North America. The incidence is also high in less developed regions including the Caribbean, Southern Africa, while it is less common in Asia and Northern Africa. The difference in incidence between countries mainly reflects the use of serum prostate-specific antigen (s-PSA) and the efforts to detect the disease but risk factors such as age, race, family history of prostate cancer, and certain genetic traits have also been identified (1, 2).

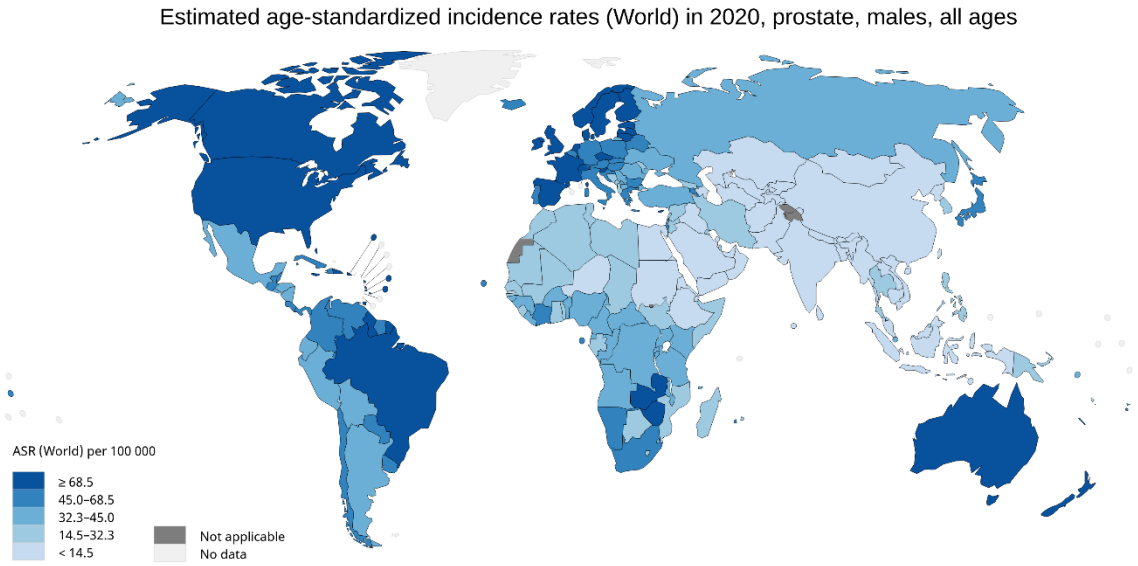


Figure 1. Prostate cancer incidence rate worldwide in 2020. The highest incidence is found in most Western countries. Reprinted with permission from http://gco.iarc.fr/today.

In Sweden, one in five men will get a prostate cancer diagnosis during their lifetime. In 2019, 10 984 men were diagnosed with prostate cancer and 2220 men died of their disease (3). The incidence doubled between 1990 and 2004, mainly due to increased s-PSA testing and increased life expectancy. In recent years, there have been stabilizing or decreasing trends in incidence and mortality rates in many developed countries. The decrease in incidence likely reflects a reduction in the use of s-PSA testing and depletion of indolent cancers in the

general population. In contrast, prostate cancer incidence continues to rise in some countries in Asia and Eastern Europe. Moreover, regional patterns of mortality rates do not follow those of incidence, with the highest mortality rates in the Caribbean, Southern Africa, and Micronesia/Polynesia (1, 4).

Prostate cancer is the leading cause of cancer death among men in Sweden. Since the beginning of the millennium, mortality rates have decreased by approximately 20% (5). Similar trends are seen in several high-income countries, most likely because of earlier detection and improved treatment (4). According to Statistics Sweden's prognosis, the number of men over 80-years old will increase by 50% in Sweden from 2018 until 2028 (6). Thus, even if more men are cured and others live longer with their disease, prostate cancer mortality will most likely not decrease further.

Prostate cancer is an age-dependent disease. The median age for getting prostate cancer in Sweden is 70 years old. However, it is important to remember that a significant number of patients are diagnosed before 65. In 2016, 141 Swedish patients got a prostate cancer diagnosis before the age of 50 (7).

Several autopsy studies have demonstrated that there is a substantial prevalence of clinically undetected tumors. A meta-analysis from 2015 (8), which included 29 studies between 1948 and 2013, showed that prostate cancer was seen in 5% of men under the age of 30 and 50% of men over 80 years old. The prevalence doubled about every 14 years. Thus, one of the unique features of prostate cancer is that many men live with latent, clinically undetected tumors, and many patients who are not treated never show evidence of metastasis (9), while others develop aggressive, metastatic disease and die of their disease. Therefore, one of our greatest challenges is to predict which patients have aggressive prostate cancer that is still manageable with early curative treatment and who have clinically insignificant cancer and would benefit from active surveillance instead of risking side effects from radiotherapy (RT) and surgery.

1.2 ETIOLOGY

For a disease as common as prostate cancer relatively little is known about its etiology. It appears the development of prostate cancer is caused by a combination of environmental and genetic factors (10-12). Advanced age, ethnicity, genetics, and familiar history are the only well-established risk factors (2).

Genetic susceptibility

Prostate cancer is among the most heritable of human cancers (13) but it is complex (14). Approximately 57% of the risk has been estimated to be attributed to genetic factors (13, 15). Men with first-degree relatives diagnosed with prostate cancer have about 1.5-2.5 times higher risk of prostate cancer development compared to the general population (16). They also have an earlier onset of their disease (17).

Mutations in the BRCA2, HOXB13, and ATM genes are rare but strongly penetrant (16). Men with mutations in these genes have a 2-4 fold higher risk of prostate cancer in their lifetime than the general population and often a more aggressive disease. Mutations in BRCA2 and ATM can also differentiate the risk between indolent and lethal disease (16, 18-23). The evidence for BRCA1 is weaker (16).

Only 40% of men with identifiable mutations in BRCA1, BRCA2, and ATM reported a family history of prostate cancer (22). Therefore, family history is not a reliable risk indicator. Families with mutations in the BCRA2 gene are commonly identified after investigations of young to middle-aged women with breast cancer and ovarian cancer. In Sweden, genetic testing is performed in families with BRCA2 mutation, and in these patients regular s-PSA testing is recommended from 40 years of age.

Moreover, mutation G84E in the gene HOXB13 gives approximately three times the risk for prostate cancer (24). Sweden has the highest prevalence of this mutation: 1.3% of the population and 4.5% of men with prostate cancer (19).

Genome-wide association studies have identified single nucleotide polymorphisms (SNPs) that are associated with prostate cancer risk. These are more common than germline mutations, but each SNP is associated with a rather small risk. In combination, however, they seem to have a cumulative effect resulting in a significantly increased risk for prostate cancer. Over 200 prostate cancer risk-associated SNPs have been identified (25-28).

It is important to remember that the difference by race is multifactorial, including biological differences, but also unequal access to prostate cancer screening, diagnosis, and treatment. Prostate cancer has a higher prevalence in Western countries (29). Migrant population data confirm lifestyle and environment as risk factors, as low-risk Asian men who move to high-risk geographical areas (such as the USA) develop an increased risk of prostate cancer (30, 31). Though, for the migrant population, the annual risk is half compared to whites born in the USA indicating a genetic component as well (31). Moreover, men with African ancestry

have been shown to have a higher incidence of prostate cancer as well as a higher mortality than other ethnic groups (32-34), which also reflects differences in genetic susceptibility and tumor biology. These men have a higher incidence of SPOP mutations, and fewer TMPRSS2-ERG fusions and PTEN deletions (35-38) compared to white men of European descent.

Potential dietary and other environmental risk factors

Environmental factors play an important role in prostate cancer initiation and progression. Increased obesity and unhealthy diets may be part of the explanation behind the incidence rise of prostate cancer in some parts of the world (39, 40). Obesity has been linked to prostate cancer (2) and a recent study showed that obese men had a higher risk to die from prostate cancer, regardless of tumor stage (41).

Red and processed meat, high intake of saturated and animal fat have been implicated in prostate cancer risk and progression. Intake of tomatoes and cooked vegetables containing lycopene are associated with a lower risk of aggressive prostate cancer. Studies have also indicated that moderate physical activity is associated with a lower risk of dying from prostate cancer. Tobacco smokers seem to have an increased risk of dying from prostate cancer, although smoking is not generally considered to increase the risk of prostate cancer (2, 42). However, none of these factors have a sufficiently strong association to justify recommendations for prevention of prostate cancer.

1.3 BIOLOGY, ANATOMY, AND PHYSIOLOGY OF THE PROSTATE

The prostate is an accessory sex gland of the male reproductive system and located below the urine bladder, where it surrounds the prostatic part of the urethra. It is composed of 30 to 50 tubuoalveolar glands and a fibromuscular stroma. The epithelial cells of the glands produce several components of the semen including PSA which serves to liquefy semen (43). Other components make the semen slightly alkaline, which neutralizes the acidity in the vagina and prolongs the lifespan of the sperms. The growth of the prostate is dependent on many local and systemic hormones. Testosterone is the main hormone acting on the prostate.

Interestingly, thousands of other species of mammals have prostates but except for the dog, no other species that age in zoos or captivity have a significant incidence of prostate cancer that results in prostate cancer diagnosis and death (10).

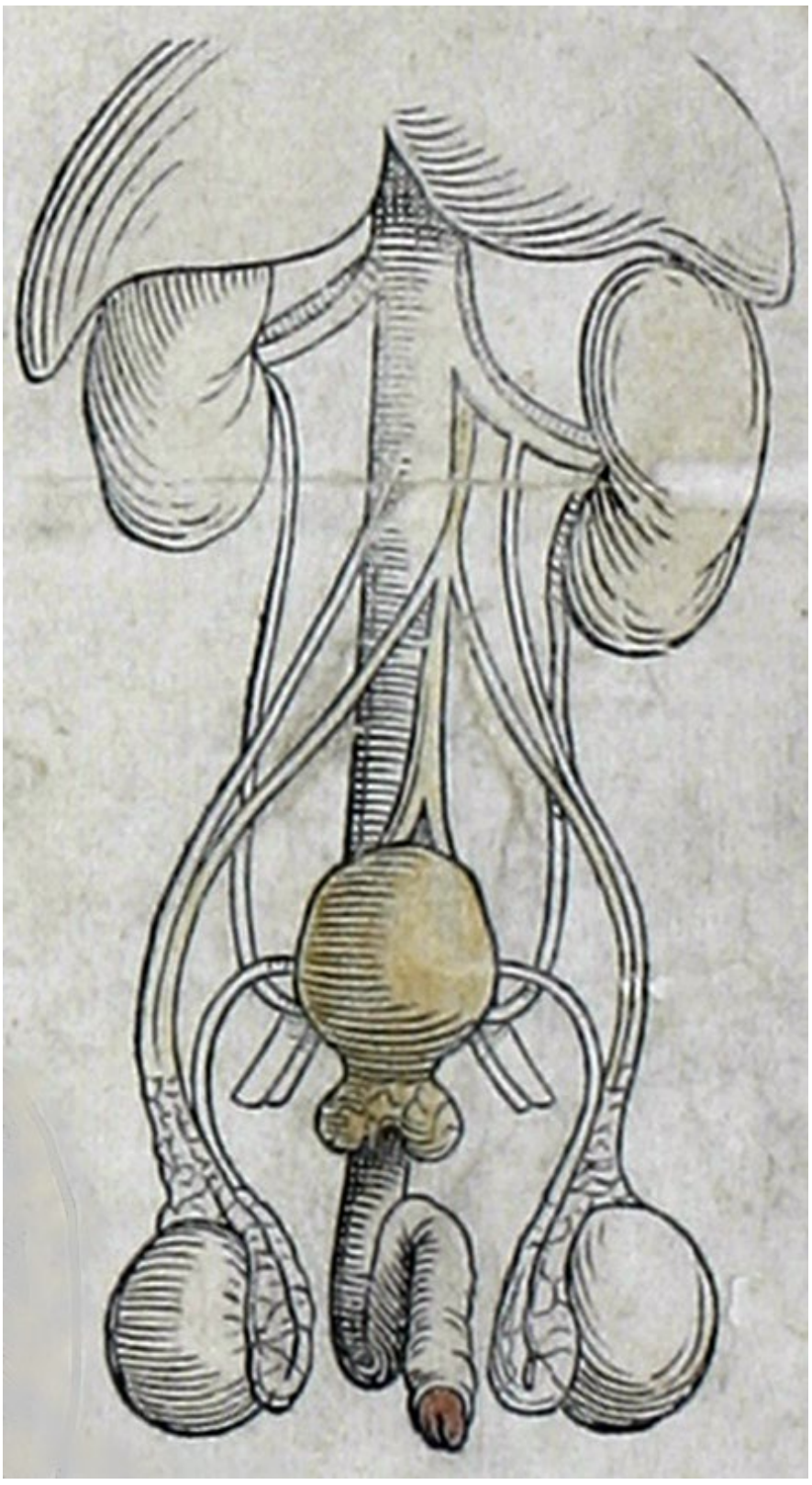


Figure 2. The first illustration of the prostate. From Andreus Versalius' Tabulae anatomicae sex, 1538, Hunterian Museum Glasgow (with permission from the University of Glasgow Library, Special Collections).

1.4 GENOMIC PROFILE OF PROSTATE CANCER

The first draft of the human genome sequence was published in 2001. Since then, advancements in DNA sequencing and computational biology have evolved rapidly. Like all cancers, prostate cancer develops as a result of the activation of oncogenes and inactivation of tumor suppressors by point mutations, copy number variations (CNVs), and structural rearrangements. The first description of the whole-genome landscape of localized prostate cancer was published in 2011 by Berger et al. (44). They showed that structural rearrangements, often as multiple complex events, are frequently occurring in prostate cancer, leading to gain or loss of function, and often initiating carcinogenesis. Moreover, studies show that localized prostate cancer has a relatively low mutation burden, while advanced disease shows a higher frequency of mutations (44-48).

It is important to distinguish between localized early stages of prostate cancer and advanced, metastatic disease. Alterations occurring early in disease initiation defines core subtypes of prostate cancers. In time, significant selective pressure often through many courses of treatment causes the accumulation of alterations, and the severity of those adds to the molecular and tumor complexity.

The most common structural rearrangement detected in about half of prostate cancers is the gene fusion between the androgen-regulated gene TMPRSS2 and a member of the ETS transcription factor family (49-51). The TMPRSS2-ERG gene fusion leads to overexpression of the oncogenic ETS transcription factor.

Frequently mutated genes in localized prostate cancer are SPOP, FOXA1, IDH1, TP53, and PTEN, although no single gene was mutated >10%-12% according to meta-analyses of prostate cancer exome sequencing studies (45, 52, 53). Tumors with mutations in SPOP seem to lack ETS rearrangements, suggesting different subclasses of prostate cancer (45). Moreover, approximately 15%-20% of prostate cancer have mutations in genes encoding epigenetic machinery components. Most frequent is the DNA methylation alterations including CpG island promoter methylation of the tumor suppressor gene GSTP1 which may occur in early stages of disease (54).

In advanced prostate cancer, the most frequent genomic alterations are point mutation or copy number gain of Androgen Receptor (AR) gene, TMPRSS2-ERG fusion, PTEN loss, TP53 inactivation, RB loss, a gain of 8q24 (MYC), and BRCA2 loss (46, 47).

Genomic alterations in the AR gene are critical for survival and proliferation of cancer cells. Alterations of the AR gene are restricted to late-stage disease of prostate cancer (45, 47, 55-57). In metastatic prostate cancer, almost every case is initially sensitive to androgen deprivation or AR blockade and thus the main therapeutic target, but most cases will ultimately progress to castration-resistant prostate cancer. This transition is associated with AR gene amplification, mutations, rearrangements, and/or activation of AR splice variants (58, 59). In a few cases, an AR negative phenotype causes castration resistance and a small subset show neuroendocrine differentiation (60).

PTEN is a tumor suppressor gene that is frequently mutated or deleted in prostate cancer. It is associated with a worse prognosis and mostly found in advanced, castration-resistant prostate cancer, although it can be altered in localized tumors as well (47, 52).

Moreover, up to 20% of metastatic prostate cancer appear to have germline or somatic alterations in DNA repair genes (e.g. BRCA1, BRCA2, ATM, etc.). These defects can make cancer cells vulnerable to PARP (Poly(ADP-ribose)polymerase) inhibition (23, 52, 61-63).

More about genetic susceptibility for developing prostate cancer is presented in *Etiology and Risk factors*.

Heterogeneity and origin of prostate cancer

Prostate cancer is in most cases multifocal and the different foci often show histological heterogeneity with differences in cell morphology, growth patterns, and architecture (64, 65). Until recently, it was not clear if the different tumor foci evolved from the same precursor clone or independent cancer clones. Even though studies have shown monoclonal origin (66), the majority of studies that have investigated the different molecular characteristics and expression profiles indicate that the different tumor foci arise independently (67-73). Lindberg et al. presented evidence of somatically independent tumors after performing whole-exome sequencing on multiple tumor foci from four patients after RP (67). Recently it was shown that intratumor heterogeneity also occurs with many alterations in subclonal populations within the same tumor focus (70).

1.5 PATHOGENESIS

Precursors, chronic inflammation, and infection

High-grade prostatic intraepithelial neoplasia (HGPIN) is an established precursor lesion of prostate cancer but it is unclear which tumors originate from HGPIN and if there are other precursors of prostate cancer (74). Similar to cancer, HGPIN is usually found to be of

multifocal distribution with significant genetic heterogeneity (65, 75, 76). It is defined as acini and ducts where the epithelial cells have nuclear and cytoplasmic features characteristic of invasive adenocarcinoma cells, such as enlarged nucleoli.

Three molecular alterations are frequently present in HGPIN: GSTP1 methylation (and loss of protein expression), telomere shortening, and MYC overexpression (77). Many studies have found the TMPRSS2-ERG gene fusion in HGPIN (49, 78), although one study examining HGPIN lesion from surgical specimens without cancer only found approximately 5% to have ERG overexpression (79).

Chronic inflammation is often seen in benign tissue of the prostate. Whether infection and/or inflammation is causative for prostate cancer is unclear. One theory is that the western diet causes inflammation and oxidative stress that induce mutagenesis. In the presence of inflammation, atrophic epithelial cells are often seen. Interestingly, these cells are more proliferative compared with nearby benign luminal epithelial cells (80). Proliferative inflammatory atrophy (PIA) is considered a risk factor for prostate cancer since it is often seen to merge with and potentially transition into HGPIN and more rarely into small adenocarcinoma lesions (81-83).

Several genotype and phenotype similarities have been described in atrophy, HGPIN, and cancer. A subset of PIA has somatic GSTP1 hypermethylation, also seen in HGPIN and cancer (82). A gain in 8q24.12–24.13 (including MYC) has been shown in atrophy (84). However, the TMPRSS2-ERG fusion that is a frequent finding in prostate cancer has not been found in atrophy (78) and some studies report a lack of association between atrophy and prostate cancer (85). Thus, inflammation and atrophy are involved in cancerogenesis but whether PIA is a precursor of prostate cancer and how the gradual transition to cancer would happen is still unclear.

1.6 CLINICAL FEATURES AND DIAGNOSIS OF PROSTATE CANCER

Patients with early stages of prostate cancer usually have no symptoms. In cases of advanced disease, tumors can obstruct the urethra with symptoms similar to the ones presented in benign prostate hyperplasia (BPH) including urinary obstruction, weakened urinary stream, and urinary retention. Haematuria and haematospermia are rare. Patients may present with weight loss, fatigue, and bone pain due to pathological fractures in cases with metastatic disease. Metastases to bone and lymph nodes are most common in advanced prostate cancer whereas metastases to liver, lung, pleura, and adrenal glands occur, but are rare (86).

Prostate cancer may be clinically suspected of elevated s-PSA and/or abnormal digital rectal examination (DRE). The patient is then usually investigated with ultrasound, core biopsy, and recently magnetic resonance imaging (MRI). The diagnosis is usually established by histopathological examination of prostate needle biopsy samples, but it can also be diagnosed in transurethral resection of the prostate (TURP)-material, transvesical prostate resection specimens, and other surgical specimens from the pelvic region.

1.6.1 PSA

Like most findings in medicine, many scientists contributed to the discovery of PSA. The history is interesting and surrounded by controversy. It was Wang et al. who purified PSA (87). In the 1980s it was clinically introduced and used for monitoring treatment (88). The finding of this biomarker made it possible to diagnose prostate cancer at an earlier stage (89).

PSA is a glycoprotein that is a serine protease present in the prostatic epithelium (90, 91). It is secreted in the seminal fluid and responsible for semen liquefaction (43). Most prostate acinar adenocarcinomas produce PSA (92). Cancer disrupts the basement membrane which leads to leakage of more PSA into the bloodstream. However, poorly differentiated cancer cells can lose their ability to produce PSA and approximately 13% of high-grade cancers are negative for PSA by immunohistochemistry (93). Another limitation of PSA as a diagnostic marker is that it is commonly elevated in benign diagnoses such as benign prostatic hyperplasia (BPH). Despite suboptimal sensitivity and specificity, PSA-testing is widely used for screening for prostate cancer. It is also used as a test for recurrence and disease progression after RP.

1.6.2 Other diagnostic tests

The STHLM3 study (94), evaluated a new model for prostate cancer detection. The test is a combination of clinical variables, plasma protein markers, and genetic polymorphisms (232 SNPs). The model was tested in the Stockholm region in men aged 50-69 and identified clinically significant high-risk cancers better than s-PSA alone. In addition, this model has shown to substantially reduce unnecessary biopsies, while maintaining the same sensitivity to diagnose clinically significant prostate cancer (95). To further improve the specificity in detecting prostate cancer and to reduce the risk of overdiagnoses a study evaluating the combination of STHLM3 test with MRI and targeted biopsies is ongoing (96).

1.6.3 DRE

In the diagnostic process of prostate cancer, DRE is a routine examination. It is fast, cost-effective, and safe. However, it has relatively low sensitivity and misses 25% to 50% of prostate cancers (97). Therefore, if prostate cancer is suspected, patients should be further investigated, regardless of the DRE result.

1.6.4 Core biopsies

A patient with elevated s-PSA and/or suspected DRE finding is usually further investigated with 10-12 systematic 18-gauge core biopsies for diagnosis, guided by transrectal ultrasound (TRUS). The biopsies are then evaluated histopathologically. A well-known limitation of biopsies is that the samples only include a small fraction of the prostate and may not represent the morphology of the targeted lesion, which may lead to undergrading of tumors.

1.6.5 Magnetic resonance imaging (MRI)

Since the first international consensus was published in 2011 with recommendations on how to use MRI in clinical practice (98), it has become more and more important in the diagnostic process of prostate cancer. An option to systematic core biopsies guided by TRUS is MRI-targeted biopsies. MRI is performed and the region of interest (ROI) is registered. Then MRI-targeted biopsies can be performed via visual (cognitive) registration, MRI-US fusion (software-assisted fusion), or direct in-bore (in-gantry). The level of cancer suspicion is reported according to the Prostate Imaging Reporting and Data System (PI-RADS) (99). With or without targeted biopsy, MRI has been shown to reduce the number of unnecessary biopsies by a quarter, thus leading to less overdetection of clinically insignificant cancer, and more detection of clinically significant cancers (100, 101).

1.6.6 The diagnosis

The pathologist evaluates the biopsies searching for morphological signs of prostate cancer. As cancer is identified it is assessed and reported according to systematic pathological reports.

More about the pathological report and prognostic factors are presented in *Histopathology.*

1.6.7 The dilemma of prostate cancer screening

For screening to be valuable, it must reduce disease-specific morbidity and/or mortality. Early detection of prostate cancer does not necessarily correlate with improved clinical outcome. There is a risk of overdiagnosis, meaning that screening finds indolent cancers that would not have become clinically significant during the patient's lifetime and instead put patients at risk of prostate biopsy-associated side effects such as infection, anxiety, and potential treatment side effects.

Prostate cancer mortality decreased by nearly 30% in the United States during the 1990s which is similar to Sweden and other Western countries. Etzioni et al. (102) estimated that 45% to 70% of the decrease might have been due to earlier stages at diagnosis because of PSA testing. Other factors, such as improved treatments, did also contribute to improved prostate cancer outcomes.

A meta-analysis published in 2018, included five randomized controlled trials and reported a small reduction in disease-specific mortality over 10 years but no effect on overall mortality (103). Included in this meta-analysis was the ERSPC-study (104). It is believed to be the one with the lowest risk of bias. According to the ERSPC study, lives can be saved with prostate cancer screening. The survival benefit increased from 9 to 13 years of follow-up and stabilized in the 16-year follow-up (RR for prostate cancer mortality 0.85 at 9 years, 0.79 at 13 years, 0.80 at 16-years follow-up) (105). Also included in the meta-analysis was the PLCO-trial (106) that did not show a difference in prostate cancer-specific mortality between the intervention and controlled arm. A major limitation of this study was the contamination of the controlled arm with a high rate of s-PSA testing and thus it can rather be viewed as a comparison of organized versus opportunistic screening.

Another study of annual s-PSA screening in men 55-69 years showed nine fewer prostate cancer deaths per 1000 men followed for their lifetime and a total of 73 life-years gained. When using simulation modeling taking the value of each potential benefit and harm, screening did not improve quality-adjusted life years (QALYs) even if mortality was reduced (gain of 56 QALYs with 95% CI ranging from loss of 21 QALYs to a gain of 97 QALYs) (107). Hence, large randomized controlled trials report somewhat contradicting results regarding reduction of mortality. In summary, prostate cancer screening has, at most, a limited benefit on prostate cancer-specific mortality.

Until recently in Sweden, men have only had s-PSA tests on their own initiative, so-called opportunistic screening. This is unorganized, takes a lot of resources, and leads to inequity as

less-educated men are less prone to take the test. A Swedish study published in 2015 (108) reported that organized screening reduced prostate cancer mortality but was associated with overdiagnosis. Opportunistic s-PSA testing, however, had little if any effect on prostate cancer mortality and was related to more overdiagnosis with almost twice the number of men needed to be diagnosed to save one man from dying from prostate cancer compared to organized screening programs.

The National Board of Health and Welfare in Sweden does not up until today recommend a national population-based screening. Instead, men > 50 years without symptoms, with a life expectancy > 15 years who wish to take a PSA-test should be engaged in shared decision making after receiving written information. In the case of high hereditary risk, the screening can begin as early as 40 years of age.

1.7 TUMOR CLASSIFICATION AND PROGNOSTIC FACTORS

1.7.1 TNM

The clinical stage (cT) is the clinician's best estimate of the extent of a patient's disease. The most widely used staging system for prostate cancer is the American Joint Committee on Cancer (AJCC) tumor, node, metastasis (TNM) system. It describes the extent and size of the primary tumor (T), the involvement of regional lymph nodes (N), and the presence of distant metastases (M). The pathological stage (pT) is likely to be more accurate since it is established after RP and thus after the entire prostate has been examined histopathologically. The TNM system has changed through the years. In 2017 the 8th edition was published and is the one used today (109). It is described in detail in Table 1. In 1997, the 5th edition was published, in 2002 the 6th edition, and in 2009 the 7th edition. Revisions since the previously used editions include that ISUP grade should be reported in addition to GS based on the ISUP 2014 revision. Pathologically organ confined tumors, pT2 are no longer subcategorized and pT3a includes microscopic bladder neck invasion.

Table 1. TNM classification of prostate cancer according to the AJCC TNM system.

Category	Criteria
cT (primary tumor)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Clinically unapparent tumor that is not palpable
T1a	Tumor incidental histologic finding ≤ 5% of tissue resected
T1b	Tumor incidental histologic finding > 5% of tissue resected
T1c	Tumor identified by needle biopsy in one or both sides, but not palpable
T2	Tumor is palpable and confined within the prostate
T2a	Tumor involves one half of one side or less
T2b	Tumor involves more than one half of one side but not both sides
T2c	Tumor involves both sides
T3	Extraprostatic tumor that is not fixed or does not invade adjacent structures
T3a	Extraprostatic extension (unilateral or bilateral)
T3b	Tumor invades seminal vesicle(s)
T4	Tumor is fixed or invades adjacent structures other than seminal vesicles, such as external sphincter, rectum, bladder, levator muscles, and/or pelvic wall
N (regional lymph nodes)	
NX	Lymph nodes were not assessed
N0	No positive regional lymph nodes
N1	Metastases in regional lymph nodes
M (distant metastasis)	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis
M1a	Non-regional lymph node(s)
M1b	Bone(s)
M1c	Other site(s) with or without bone disease

1.7.2 PROGNOSIS AND PROGNOSTIC GROUPS

Untreated prostate cancer without signs of metastases is classified into different risk groups based on TNM-staging, GS, s-PSA, number of cores positive for cancer, linear extent, and/or proportion of prostate tissue involved by cancer. This classification is based on the original study from 1998 by D'Amico et al. (110). These risk groups were recently revised in Sweden and today patients are classified into very-low, low, intermediate, high, and very-high risk groups (111). The very-low risk group includes men with the lowest risk of having significant cancer and favorable tumor features such as limited extent on biopsy cores, ISUP grade 1, and low PSA density (112, 113), and should be managed with active surveillance or watchful waiting.

The prognosis for men with prostate cancer varies significantly. Patients with low-risk cancers have less than 10% risk to die of prostate cancer within 10 to 15 years from diagnosis without curative treatment, while the risk is 20% for patients in the moderate-risk group. For patients with high-risk cancer, the risk is 20% to 30% to die within 5 years if not treated (114). The median survival for patients with distant metastases at diagnosis has previously been 2.5 years (115) but has improved as new treatments have been introduced for these patients.

After RP and RT, approximately one-third of men progress with elevated s-PSA (7, 116). Several postoperative prognostic factors, apart from GS, may be recognized by the pathologist on the RP specimen such as extraprostatic extension (EPE), seminal vesicle invasion (SVI), perineural invasion (PNI), and positive surgical margin (117). A positive surgical margin at RP is associated with an increased risk of biochemical recurrence (BCR) but whether it is a predictor of survival is uncertain (118).

However, the majority of men with elevated s-PSA after RP do not seem to develop distant metastases or die of prostate cancer. The risk of progression with metastatic disease after RP is dependent on GS, time to PSA-relapse, and the time it takes for PSA to double (119, 120). It is important to remember that levels of s-PSA $\leq$ 0.09 ng/ml and even values between 0.1–0.2 ng/ml are often due to remaining benign prostate gland tissue (121).

1.7.3 **EPE**

Cancer extending beyond the prostate gland, EPE, has long been associated with worse prognosis and predicts both cancer progression and survival (9, 122-129). Consequently, it is included in the TNM staging system where it is classified as pT3a (130). The definition of EPE is the extension of the tumor beyond the boundaries of the prostate into periprostatic adipose or connective tissue. As opposed to some other organs, e.g. the spleen and kidney, the prostate does not have a true histological capsule (131). In some areas of the prostate, a fibromuscular band can be identified at the edge of the gland while in other regions it is very difficult to identify the boundary of the gland (130). Therefore, it can be difficult to accurately identify EPE. The pathologist identifies EPE according to specific guidelines (132). At the posterior, posterior-lateral, and lateral regions of the gland, a tumor admixed with periprostatic fat is most easily recognized as EPE. Here the capsular plane may appear as a fibromuscular condensation of the stroma. Tumor in adipose tissue almost always indicates EPE (130). Intraprostatic fat has been described but is very rare (133). At the posterior-lateral site, EPE can also be diagnosed if cancer is identified within loose

connective tissue or perineural spaces of the neurovascular bundles, even in the absence of adipose tissue (130). One difficulty can be to determine where the edge is and whether cancer bulges out of the gland as cancer that extends out of the prostate gland may induce a dense desmoplastic response in the periprostatic tissue (132). To confirm EPE in this situation the pathologist extrapolates the outline of the gland from an adjacent area with no tumor in extraprostatic tissue. At the anterior, apex, and bladder neck region of the prostate, the boundary is poorly defined. Anteriorly smooth muscle and prostatic stroma merge with extra-prostatic smooth muscle (130). Moreover, benign prostate glands are admixed with extraprostatic skeletal muscle of the urogenital diaphragm at the apical region and pathologists disagree if EPE can be assessed here (132). There is a high concordance among pathologists for the diagnosis of SVI but much lower for EPE (94% and 58%, respectively) (134).

The most common location of EPE is in the posterior-lateral region, which is where most of the tumors are located. Although pathologists tend to report the location of EPE it was unclear if the location had a significant prognostic value (130).

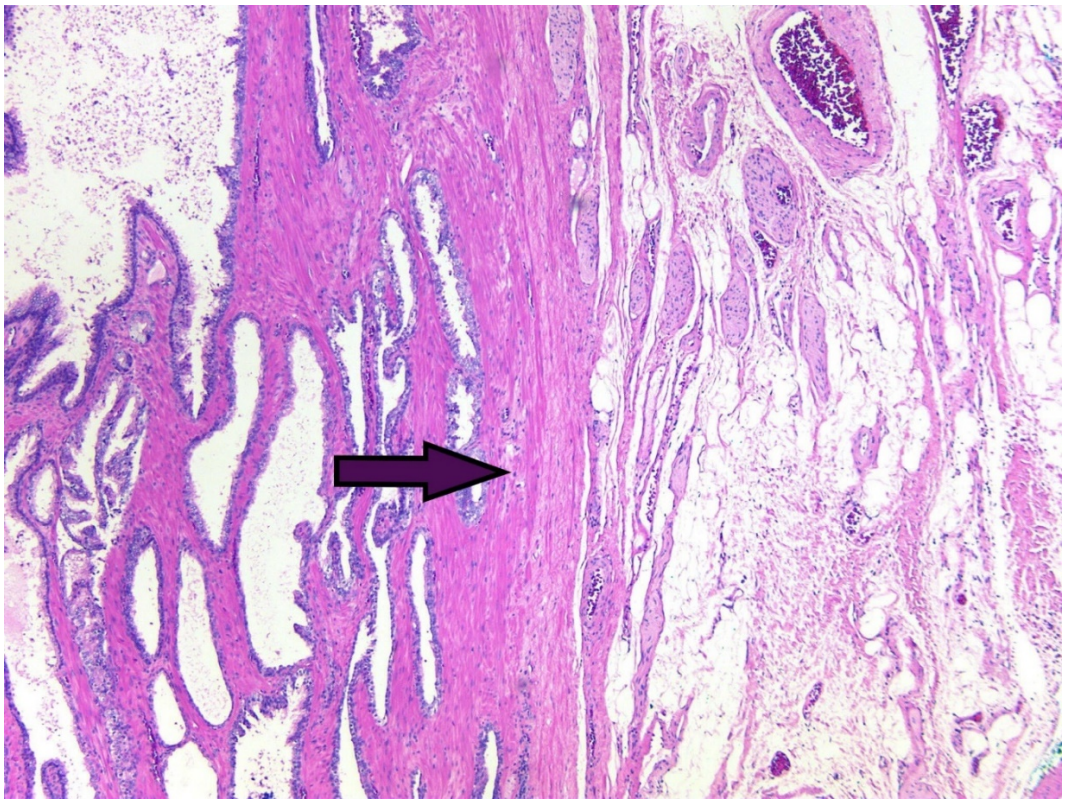


Figure 3. The arrow indicates the border of the prostate. Benign prostatic glands are shown to the left of the arrow and to the right, adipose tissue outside the confines of the prostate. Nerves and blood vessels are also illustrated. Hematoxylin and eosin (HE), 5x lens magnification. Reprinted from Danneman et al. Prognostic significance of histopathological features of extraprostatic extension of prostate cancer. Histopathology. 2013;63(4)580-9, with permission from Wiley.

There has been a downward trend in EPE, largely due to the introduction of PSA resulting in earlier detection (135). Still, a significant number of prostate adenocarcinoma patients who undergo RP are diagnosed with EPE which was not apparent before surgery. The rate of EPE decreased from 66% to 25% over several years from when s-PSA became commonly used in the United States (136). Other studies report EPE in 15% to 60% of RP specimens (123-126, 137-142). Even though EPE is a well-established risk factor, the clinical outcome varies within this patient group. Data on outcomes after RP in pT3 cancers are limited as data on this subset are often reported with other pathological features such as SVI and positive surgical margin. A positive surgical margin occurs in the same location as EPE in less than 50% of cases according to one study (143) and is seen more commonly at the apex, base, and posterior aspect of the prostate, whereas EPE occurs more often in the posterolateral mid prostate.

Studies have reported long-term freedom of BCR from 46% to 90% for patients with pT3a disease (144-147). When EPE cases have concurrent positive surgical margins and high GS the risk increases with BCR-free rates as low as 27% (146). Therefore, a major issue for many years has been to accurately identify and classify EPE into prognostic categories to decide who could benefit from adjuvant therapy after RP.

There is a general agreement among pathologists that EPE should be quantified (86, 130). Methods such as linear dimension, radial extension, and volumetric measurements of EPE have been studied (122, 128, 140, 148-151). Epstein et al. (125) quantified EPE as focal when there were a few glands outside the prostate and established (or non-focal) if anything more than that was seen. They found a significantly higher risk of progression in the established EPE group with 8-years progression-free survival for established and focal EPE at 65% and 82%, respectively. Wheeler et al. (124) used a more objective assessment of the extent by defining focal EPE as cancer extending outside the prostate in < 1 high-power field on ≤ 2 separate sections and established EPE as anything more than that. They showed 5-year progression-free rates of 42% and 73% in the established and focal group, respectively. They also introduced an intermediate level of prostate capsular invasion, a concept that has not gained general acceptance, as the prostate does not have a true capsule.

Another histopathological factor that has been reported as a predictor of progression is PNI. Ravery et al. (152) found that PNI correlated with progression but did not perform a multivariate analysis. By contrast, Maru et al. (153) found in a multivariate analysis, that the diameter of the largest focus of PNI was associated with other established prognostic factors and the probability of progression after radical prostatectomy (RP). In the same study, the

presence of PNI did not predict progression. However, until our first study, there were to our knowledge no studies examining the prognostic value of PNI at the site of EPE.

As previously described local stage (T) is evaluated by DRE and TRUS. However, the prediction of pT3 prostate cancer by these tools is known to have low accuracy (154). The prediction of EPE before curative treatment is of high value since the knowledge of the presence and location of EPE is likely to reduce positive surgical margins since the surgeon can select patients eligible for nerve-sparing technique (155). MRI is by far the best predictor of EPE at RP (156) but the sensitivity is low. According to a meta-analysis (157) sensitivities and specificities of MRI in the detection of EPE range from 41% to 92% and from 65% to 100%, respectively. As of today, the recommendation is to consider MRI before curative treatment for local staging in the high and intermediate risk groups with predominant Gleason pattern 4 (158).

1.7.4 Histopathology

Acinar adenocarcinoma is by far the most common tumor type of prostate cancer. Less than 5% of prostate cancers are other subtypes including ductal, mucinous, foamy gland, and rare forms of cancers such as neuroendocrine tumors, sarcomatoid carcinoma, and basal cell carcinoma.

The gland is divided into three anatomical zones: the peripheral, central, and transition zones. Most prostate cancers are acinar adenocarcinomas and arise in the peripheral zone (PZ) (159, 160). The transition zone (TZ) is where BPH arises although cancer is seen here as well (161). Tumors in the central zone are rare (159, 160). There are often multiple tumors, on average 2-3 tumor nodules in 68% to 98% of cases (64, 162).

From the 1960s until the Gleason grading system was widespread, prostate cancer was diagnosed by cytology on fine-needle aspiration biopsy, as described by Franzen et al (163). The cytological malignancy grading was assessed by the degree of differentiation and nuclear atypia similar to the former WHO grading 1-3 (164).

The microscopic diagnostic criteria rely on a combination of architectural and cytological features that can be divided into major and minor criteria. Major criteria include infiltrative growth patterns, absence of basal cells, and prominent nucleoli. Infiltrative growth pattern is typically small atypical glands spread between benign glands. In difficult cases, the absence of basal cells may need to be confirmed by immunohistochemical stains. Minor criteria include amphophilic cytoplasm, nuclear hyperchromasia, intraluminal amorphous

eosinophilic material, intraluminal crystalloids, blue-tinged mucinous secretions, adjacent HGPIN, and periacinar retraction clefting. Only a few features are pathognomonic for prostate cancer: circumferential PNI, glomerulations, and mucinous fibroplasia. These features are, however, uncommon in small prostate cancer foci and therefore have limited diagnostic value on needle biopsies (86, 165, 166).

1.7.5 The Gleason grading system

The Gleason grading system was introduced by Donald F. Gleason in 1966 (167) and is used worldwide. It is one of the most powerful prognostic factors for patients with prostate cancer as it is an independent predictor of BCR, metastasis, and prostate cancer-specific mortality (168-173). It is based on the glandular architecture assessed at low magnification. Nuclear and cytoplasmic features are not factored into the grading system. One of the strengths of the Gleason grading system is that it takes into account the morphological heterogeneity of prostate cancer with different growth patterns and glandular architecture within the same prostate. Nevertheless, the heterogeneity remains a challenge when predicting the overall cancer grade from biopsy specimens, because of the limited amount of tissue sampled, often more than one tumor focus, and that the foci often show different growth patterns and architecture.

The Gleason grading system divides prostate cancer into five patterns, where 1 is the most differentiated and 5 the least differentiated, and the sum of the two most common patterns generates the GS. Tumors with higher GS are more aggressive and associated with worse outcome (174).

The current system differs significantly from the original. Minor revisions of this grading system were done during the 1960s and 1970s (175) and major changes following the International Society of Urological Pathology (ISUP) consensus conferences in 2005, 2014, and 2019, and a Genitourinary Pathology Society (GUPS) “2019 White Paper” (176-179).

Among the most important changes in the 2005 revision was a modification of the reporting of GS on needle biopsies to always include the highest grade in the GS, regardless of its amount (176). This was done with the purpose to improve the agreement between biopsy and RP grade. In some RP specimens, the prostate cancer may consist of more than two patterns, with a higher pattern representing the smallest volume. In such cases, it was decided that it should be referred to as a minor high-grade pattern. If the higher grade makes up more than 5% of the total tumor volume it should become the secondary pattern in the GS since it is associated with a worse prognosis (180, 181).

The introduction of immunohistochemistry for basal cells has almost eliminated the diagnosis of Gleason patterns 1 and 2, and it was clarified that GS 2-5 should no longer be assigned on needle biopsies and rarely in RP specimens. Moreover, since cribriform cancer glands had been shown to be associated with worse prognosis (182), it was the consensus that most of these patterns be diagnosed as Gleason pattern 4 with only rare cribriform lesions meeting the diagnostic criteria for pattern 3.

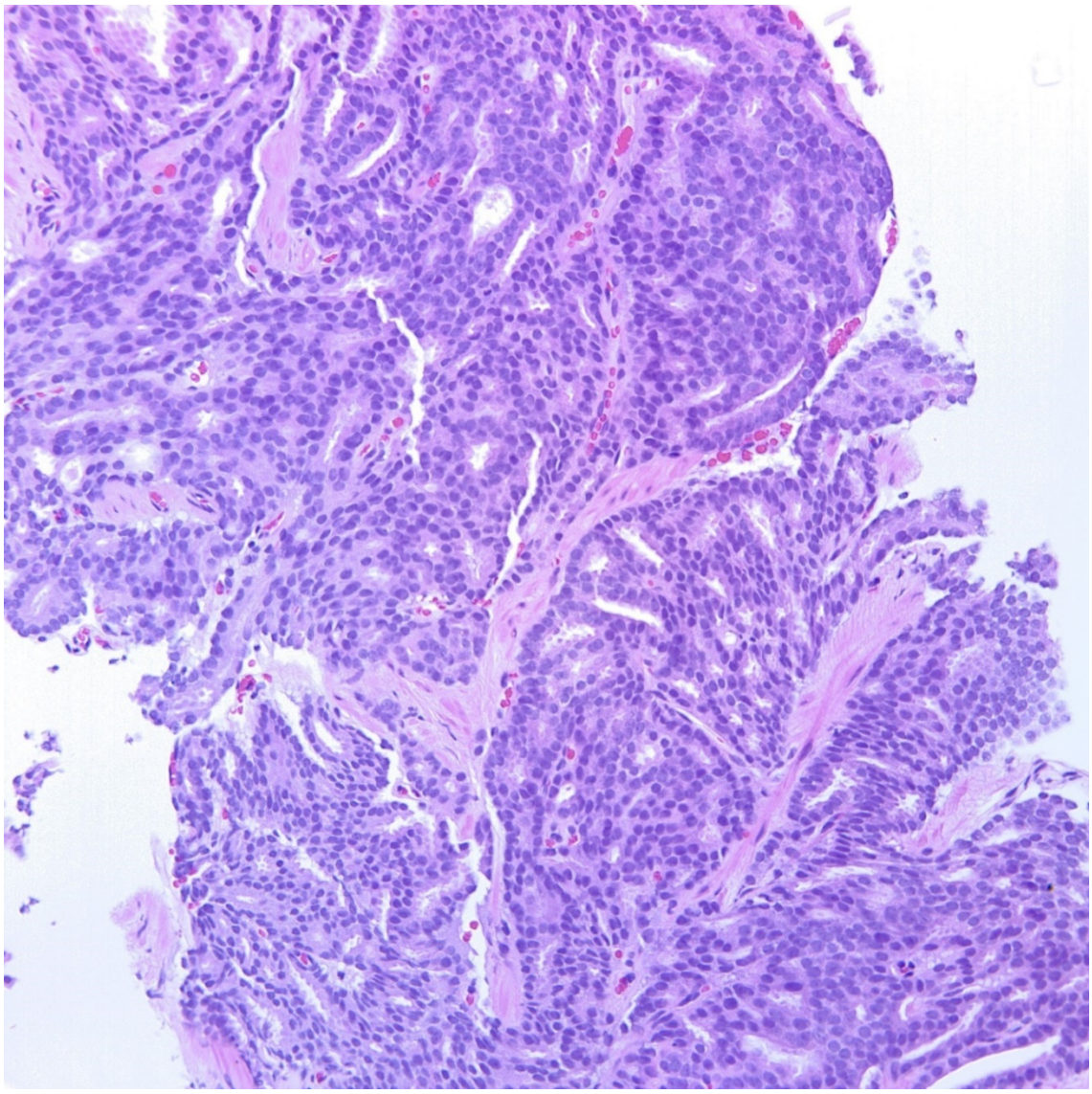


Figure 4. A core needle biopsy with an example of cribriform growth pattern of prostate cancer included in Gleason pattern 4. H&E, 20x lens magnification.

Since then, numerous studies have confirmed the adverse prognosis of cribriform glands (183-187) and at the 2014 ISUP conference, all cribriform cancers and glomeruloid glands were included in Gleason pattern 4. Moreover, it was suggested that GS should be grouped in

ISUP grades 1 (GS 2-6), 2 (GS 3+4=7), 3 (GS 4+3=7), 4 (GS 8), and 5 (GS 9-10). They are also referred to as ISUP grade groups or Gleason grade groups.

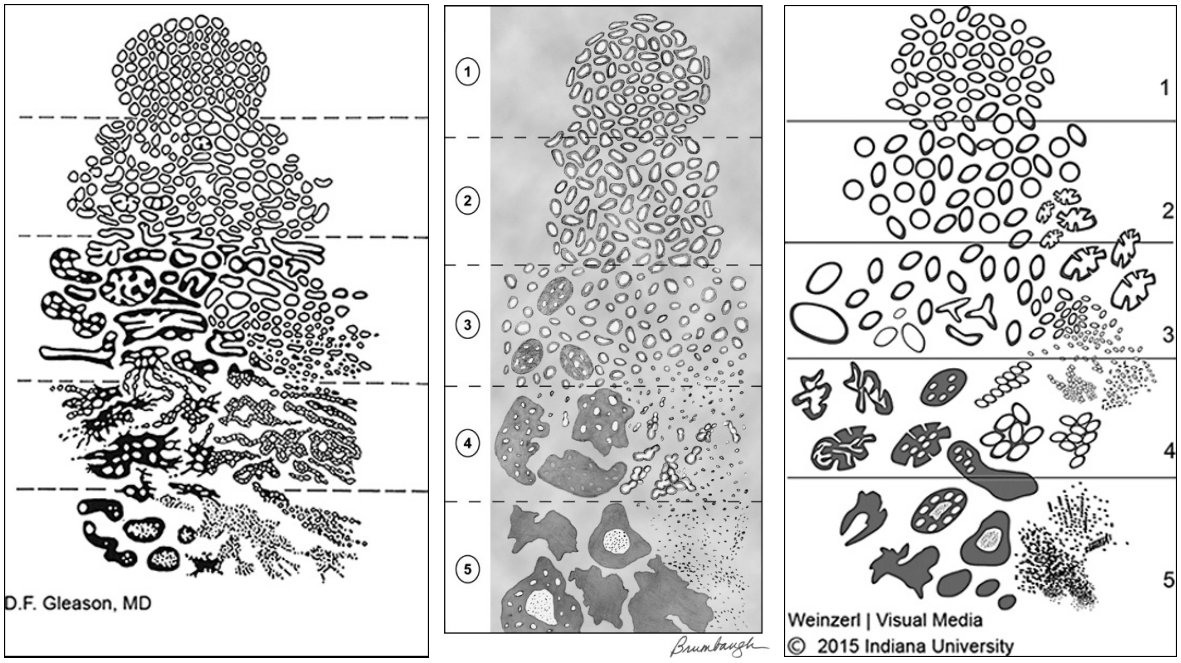


Figure 5. Evolution of the Gleason grading system. To the left, the original Gleason grading system. Reprinted from Gleason DF. Histologic grading of prostate cancer: a perspective. Hum Pathol. 1992;23:273-279, (with permission from Elsevier). In the middle, schematic Gleason grading system after the revision by ISUP in 2005. Reprinted from Epstein et al. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. Am J Surg Pathol. 2005;29(9):1228-42, (with permission from Wolters Kluwer). To the right, ISUP 2014 Gleason. Reprinted from Epstein et al. The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: Definition of Grading Patterns and Proposal for a New Grading System. Am J Surg Pathol. 2016;40(2):244-52, (with permission from Wolters Kluwer).

The prognostic value of the five ISUP grades has been confirmed in several studies (168-173, 188) and they were also accepted in the 4th edition of the WHO classification of prostate cancer (86). Cancers with GS 4+3=7 have a worse outcome than those with GS 3+4=7 (189, 190). Another strength of ISUP grades may be that a scale from 1-5 is more intuitive for patients. A patient would probably be less angst-ridden with an ISUP grade 1 cancer than with a GS 6 cancer as the latter may be misinterpreted as a tumor with intermediate aggressiveness on a scale from 2 to 10. At the ISUP consensus meeting in 2014, it was decided that both GS and ISUP grades should be reported (177) and at the last meeting in 2019, 93% of the participants reported both (178). However, whether the ISUP grades add any valuable information has been questioned recently (191).

The 2019 grading recommendations proposed by ISUP and GUPS need validation and there are also differences between the recommendations from the two bodies (192). Percentage of

Gleason pattern 4 in GS 7 (ISUP grade 2 and 3) cancers on biopsies, as well as the presence of cribriform pattern in GS 7 and 8 tumors (ISUP grade 2-4), should be reported since studies have shown that these features have prognostic and clinical significance (187, 193-196). These recommendations were the same from both societies. To allow comparisons and analyses of cohorts, pathologists have been recommended to specify which variant of recommendations is being used while awaiting more evidence.

Still, the Gleason grading system relies on the subjective interpretation of these grading rules and reproducibility is a well-known problem (197-199). A reference image database, The Pathology Imagebase, was therefore created for the purpose to standardize grading by online publication of images. The image library was generated by a large panel of expert uropathologists from around the world and serves to calibrate grading (200, 201).

1.7.6 The pathological report

The pathological anatomic diagnosis reported should include grade, by using the Gleason grading system, the number of biopsy cores with cancer, and the linear extent in mm or percent of the cancer length of the biopsy (202). Lymphovascular invasion, EPE, and SVI should also be reported but are uncommon on needle biopsies.

The RP specimen should be assessed according to zonal location and laterality of the main tumors, measurements of the biggest tumor foci, grades given separately for the main tumors, and an overview of the smaller tumor foci. In addition, EPE, SVI, and microscopic bladder neck invasion should be reported (202). If there is a positive surgical margin this should be stated and specified according to its location and extent.

1.7.7 Grading and Artificial intelligence

The Deep-Blue defeated the reigned world chess champion in 1997. It was one of the earliest examples of an artificial intelligent (AI) system and it could generate and evaluate about 200 million chess positions per second (203). Nowadays, AI is widely used in science, business, and healthcare. The use of AI to diagnose or predict disease risk is developing rapidly.

AI can be defined as computer systems able to do things that normally would require human intelligence, such as visual perception, speech recognition, and decision making. Machine learning is a subset of AI that gives the system the ability to automatically learn and improve from experience without being programmed in every step. In machine learning different algorithms are used. One example is deep neural networks (DNN) that are inspired by the structure of the human brain. In the medical field, DNN is often used (204).

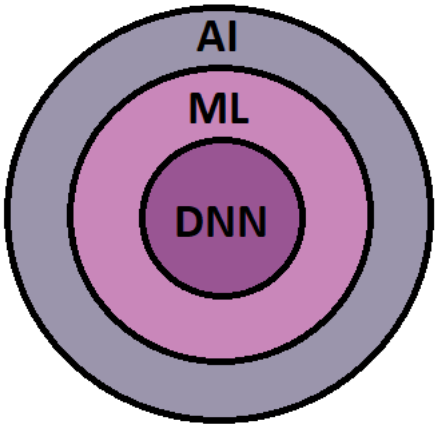


Figure 6. DNN is an example of machine learning (ML) and ML is a subset of AI.

With the introduction of digitalization and whole-slide images of prostate biopsies, computer-assisted prostate cancer grading using AI became possible. Litjens et al. (205) was the first group to use DNNs for the detection of prostate cancer on prostate biopsies. They showed that the AI system could safely exclude 32% percent of benign biopsies. A series of studies has shown that AI-based algorithms can perform prostate cancer grading and in the majority at the level of expert pathologists (206-212). Ström and colleagues (210) developed an AI system based on DNNs with the ability to safely separate benign and malignant prostate biopsies, with a high precision quantify cancer, and grade with the same accuracy as leading expert pathologists. A strength of their study was that they used prospectively controlled, population-based data from the STHLM3 trial (94).

The hopes and expectations for AI are high. For example, it may reduce the workload for pathologists and improve reproducibility (213). Interestingly, in the 2019 ISUP conference meeting, more than 70% of participating pathologists were positive to use AI in grading in the future (178).

1.8 TREATMENT

“Is cure possible for those for whom it is necessary, and is cure necessary for those in whom it is possible?”

This was said by Willet F. Whitmore, a New York specialist in Urology (214) who referred to prostate cancer. It illustrates that many patients who are cured of their cancer are overtreated, while therapy is inadequate for many men with aggressive tumors. Today, we still struggle to identify patients that most likely will benefit from therapy and to improve treatment options for aggressive or advanced tumors.

1.8.1 Active surveillance

For men with an early stage diagnosed prostate cancer that grows slowly, active surveillance can be an option. This means that the patient is followed by regular controls with DRE and s-PSA. In some cases, biopsies are also included. If the disease progresses, gives symptoms, or if the patient wishes, treatment can be initiated. In 2019, 92% of very-low-risk patients in Sweden were on active surveillance (5).

1.8.2 Curative treatment

Non-metastatic prostate cancer can be cured either by RP or RT. In Sweden, approximately half of the patients are treated with RT or RP with curative intention (7). Only one randomized controlled trial has studied the difference between RP and RT and showed no significant difference in prostate cancer-specific mortality at a median of 10 years (215). However, there were very few deaths in both groups, four in the RT group and five in the RP group which makes it difficult to show a potential difference between treatments. A meta-analysis of previous observational studies analyzing treatment for clinically localized prostate cancer showed a twice as high risk to die from prostate cancer in the RT group compared to the RP group (216). However, the difference in effect is likely explained by a selection of patients, and that those treated with RT had a worse prognosis already before treatment even if some of the confounding factors were adjusted for (217). Therefore, potential side effects and the patient's preference must be considered in the treatment decision.

1.8.2.1 Surgery

The first surgical removal of the prostate for prostate cancer (radical perineal prostatectomy) was first performed in 1904 by Hugh H. Young at Johns Hopkins Hospital. Today, RP is done by retropubic, laparoscopic, or robot-assisted approach. Robot-assisted laparoscopic RP

was first reported in 2000 and advantages of this modality include 3D stereoscopic visualization and intuitive finger-controlled movements and it is becoming the dominant surgical approach for prostate cancer (218). Whether nerve-sparing technique should be used depends on the tumor's characteristics, patient age, and preoperative erectile function. Most patients can be offered, at least, a unilateral nerve-sparing surgical technique which results in a lower risk for permanent urinary incontinence (219). When there is a high risk for EPE nerve-sparing technique is not recommended.

1.8.2.2 Radiotherapy

Today, RT can be given with higher precision and therefore patients can be treated with higher doses and lower risk for side effects. Curative external RT is given to intermediate and high-risk prostate cancers. RT may be given either as external radiation or as brachytherapy, sometimes both methods are combined. Neoadjuvant and adjuvant hormonal treatment is given to most patients with high-risk tumors. For patients with cT3 tumors, including the EPE group, RT in combination with hormonal therapy is usually recommended over surgery (220). No randomized trial has so far compared primary surgery to primary RT for men with high-risk locally advanced prostate cancer, but a Scandinavian multicenter study is ongoing.

1.8.2.3 Side effects

Both RP and RT have side effects such as urinary incontinence and impotence. Patients treated with RT can also have bowel symptoms. After RP most men have urinary leakage when the catheter is removed after one to two weeks. Higher age and less degree of nerve-sparing technique increase the risk for permanent urinary incontinence (219). After surgery, most men have erectile dysfunction, but the function can improve if a nerve-sparing technique is used. The risk for permanent erectile dysfunction varies between 10% and 100% depending on age, surgical technique, and the patient's erectile capacity before surgery (221).

Observational studies have shown a higher risk of incontinence after surgery and greater urinary bother after RT, but there seem to be no long-term differences. Also, worse erectile function appears more frequently early after surgery but the long-term risk, according to observational studies, seems to be similar (222). The risk for impotence is higher with age, comorbidity, and if the patient has received neoadjuvant or adjuvant hormonal therapy.

1.8.2.4 Hormone therapy

Androgen deprivation therapy (ADT) can be curatively intended when a combination of antiandrogen and medical castration is given as neoadjuvant therapy together with RT.

1.8.2.5 Salvage and adjuvant RT

For patients with slowly rising s-PSA after surgery (two consecutive values over 0.2 ng/ml) and suspected local recurrence, postoperative RT is commonly recommended, and it is then called salvage RT.

If the patient has a high risk of recurrence after surgery, adjuvant RT is considered even if s-PSA is below 0.1 ng/ml. In Sweden, high risk includes extensive positive surgical margins and especially if there is a Gleason pattern 5 involved (223).

In breast cancer, adjuvant RT following surgery has been shown to improve overall survival (224). The aim of adjuvant RT is sterilization of residual tumor cells following surgery to diminish the risk of local recurrence and subsequent spread of the disease. By this logic, delaying RT until the time of BCR may decrease the chance of secondary cure.

Recently, a French randomized controlled trial, although it lacked statistical power, demonstrated no benefit for event-free survival in patients assigned to adjuvant RT compared to salvage RT. Moreover, adjuvant RT increased the risk for permanent impotence and permanent urinary leakage (225).

A recent systematic review (226) concluded that adjuvant therapy based on EPE or positive surgical margins is beneficial in terms of disease control, but it is unclear whether it affects overall survival and the risk for distant metastasis. Adjuvant RT has been shown to reduce BCR by 20% for patients with positive surgical margins or pT3, without metastases to lymph nodes (227-229). Thompson et al. (227) showed a decreased risk for distant metastasis and prostate cancer-specific mortality. On the contrary, Bolla et al. (228) reported no significant difference in overall survival with a follow-up of more than 10 years. Approximately half of the patients that got adjuvant RT had no signs of recurrence of their disease after 5 years. Hence, if all patients with positive surgical margins or pT3 would receive adjuvant treatment it would result in significant overtreatment which is the argument why adjuvant RT is not recommended for these patients. Patients with EPE and negative surgical margins did not seem to benefit as much from adjuvant radiotherapy as patients with EPE and positive surgical margins (228).

There are several limitations of these studies. Firstly, the adverse pathological features such as EPE, SVI, and positive surgical margins are mixed. For example, in one study 33% had SVI alone or together with positive surgical margins and EPE (227). Further, patients with a detectable PSA were not an exclusion criterion or trigger for treatment in the control arm, allowing patients with high postoperative s-PSA values to be randomized. Approximately 30% had elevated s-PSA after surgery in these two studies (227, 228). Today this population would routinely be given postoperative RT and as described above. Adjuvant RT is applied to patients in whom the postoperative s-PSA is undetectable (< 0.1 ng/ml).

Today BCR is defined as a s-PSA level of 0.2 ng/ml and is the most common value at which salvage RT is initiated. However, RT given at PSA levels lower than 0.20 ng/ml may yield better outcomes, and thus the s-PSA value at which RT should start is not clear (226).

1.8.3 Palliative treatment

In, 1941, Charles B. Huggins won the Nobel Prize for showing that metastatic prostate cancer responded to androgen deprivation by either castration or estrogen medication. Castration is achieved surgically by orchidectomy or medically through luteinizing hormone releasing hormone agonist/antagonist. Antiandrogen (bicalutamide) is usually given to men with locally advanced prostate cancer, without metastasis, and where curative treatment is not an option due to high age and/or co-morbidity. From the 1940s ADT has been the standard of care for men with an initial diagnosis of metastatic prostate cancer. Today, ADT combined with chemotherapy (docetaxel), or second-generation antiandrogen therapy (abiraterone), have both been shown to improved survival and are thus an option in palliative treatment (230). In the case of restricted metastatic disease and a life expectancy of more than five years, RT towards the primary tumor can be an option. For asymptomatic patients with limited metastasis, bicalutamide alone is an option.

Most of these patients initially respond to castration therapy. However, sooner or later the disease becomes resistant to such therapy by mechanisms of resistance such as AR overexpression, AR mutations, and increased production of steroids by the tumor itself (231). Chemotherapy such as docetaxel, second-generation antiandrogen treatment, and radioisotope are treatment options for patients with metastatic castration-resistant disease.

1.8.4 Personalized prostate cancer medicine

Since the millennium advancements in whole-genome and exome sequencing have evolved rapidly. In prostate cancer, localized disease has a relatively low mutation burden, while mutations accumulate in advanced disease (44). Subclasses of prostate cancer have been

proposed (45). This is essential for the development of biomarkers and new treatments. We are in an era that moves towards personalized cancer medicine. The heterogeneity of prostate cancer is a challenge, but as more molecular subtypes of prostate cancer are identified treatment may be customized based on the molecular signature.

Blood samples containing tumor biomarkers, so-called liquid biopsies, could be part of detection, prognostication, and treatment decisions. In cases with multiple tumors of different aggressiveness, liquid biopsies may also aid in multifocal genomic profiling since the most aggressive cancer is represented by the highest fraction of circulating DNA in the blood (232). Another possibility could be to obtain new liquid biopsies after a specific treatment and if another tumor clone is isolated then change the treatment accordingly.

1.9 SUMMARY

Prostate cancer is a genetically, morphologically, and clinically heterogeneous disease. This heterogeneity poses a challenge when distinguishing a patient with potentially fatal prostate cancer from one where active surveillance is sufficient.

It is difficult to diagnose EPE before surgery and thus many men have been shown to have cancer outside the confines of the prostate when they undergo RP. Moreover, it is a heterogeneous group of patients with variable outcomes, and most of these patients do not have progression of the disease. That is why it is important to classify patients with EPE after RP into prognostic groups to identify those that could benefit from adjuvant treatment.

The Gleason grading system has been used worldwide for many decades and is still one of the strongest prognostic and predictive factors of prostate cancer. One of its strengths is that it takes the morphological heterogeneity of prostate cancer into account. It has undergone some minor and major changes, and these must be carefully evaluated since the grade is important for the treatment decision.

A well-known challenge of the Gleason grading system is reproducibility as it relies on the subjective interpretation of grading rules. The rapidly evolved AI systems are now used in healthcare. In prostate cancer grading the expectations are high with the belief that AI will improve the grading process. A method based on AI for diagnosis and grading of prostate cancer was recently developed by researchers from our group and if validated the AI system may play an important role in clinical practice.

2 AIMS

The overall aim of this thesis was to investigate prognostic factors and pathological features of prostate cancer. The specific aims were:

1. To evaluate histopathological variables of EPE to identify patient groups with increased risk of BCR after RP.

2. To study long-term trends in Gleason grading in a nationwide population and to assess the impact of the ISUP revision of the Gleason grading system in 2005.

3. To investigate, in a nationwide population-based cohort, whether the agreement between GS and ISUP grade in needle biopsies and RP specimens had changed between 2000 and 2012.

4. To analyze prostate cancer biopsies that are difficult to grade and where expert pathologists have not reached a consensus. In particular, we aimed to compare expert grading with AI-assisted grading and analyze the nature and reason for grading disagreement.

3 MATERIAL AND METHODS

3.1 STUDY 1

3.1.1 Patients, tissue collection, and preparation

Between 1998 and 2005, 1156 men underwent RP at the Karolinska University Hospital, in Solna, Stockholm. After excluding 105 patients, due to neoadjuvant treatment, TURP before surgery, or unavailable histological slides or clinical follow-up data, 1051 patients remained for analysis.

After overnight fixation in 4% buffered formalin, the prostate was inked, sliced horizontally at 4 mm, and totally embedded. Slices were whole-mounted or cut into two or six segments, usually quadrants. The apex and base were cut in sagittal sections or examined by the shave technique.

The seminal vesicles were cut longitudinally and totally embedded. The surgical margin was considered positive if cancer reached the ink. The specimens were dehydrated and paraffin-embedded and sections were cut at 4 µm and stained with H&E (hematoxylin and eosin).

All slides were reviewed by the author, using a microscope (Leica DM 1000 LED). Tumors were outlined with Indian ink on the glass slides and evaluated for the presence of EPE which was later confirmed together with the senior pathologist (prof. Lars Egevad, LE).

3.1.2 Examination protocol and measurements of extraprostatic tumor

Before the microscopic review of the radical prostatectomy specimens, the author and LE elaborated a protocol for a detailed assessment of EPE (Figure 8). The horizontal and axial location of EPE and its extent were noted. We classified the EPE group according to Epstein et al. (125), where focal EPE was defined as only a few neoplastic glands outside of the prostate in no more than two sections. More extensive EPE than that was called established EPE. Further, we classified EPE to be focal if the tumor outside of the prostate was less than a depth of 1 high-power field on no more than two sections. Any amount of EPE greater than this was designated as established EPE, according to the definition elaborated by Wheeler et al. (124). If the tumor extended beyond the confines of the gland on only one side of the prostate, EPE was recorded as unilateral. If EPE were present on both sides of the prostate, EPE was designated as bilateral. Tumors extending outside of the prostate in only one focus were analyzed against tumors outside of the gland in more than one focus. Likewise, tumors with EPE in only one section were analyzed against tumors with EPE in more than one section. We recorded PNI at the site of EPE but PNI within the confines of the prostate was not considered.

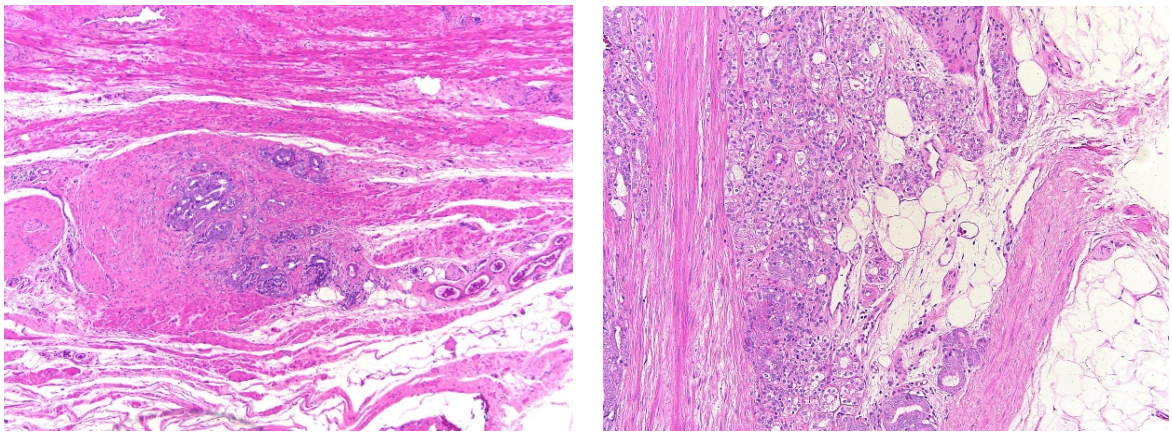
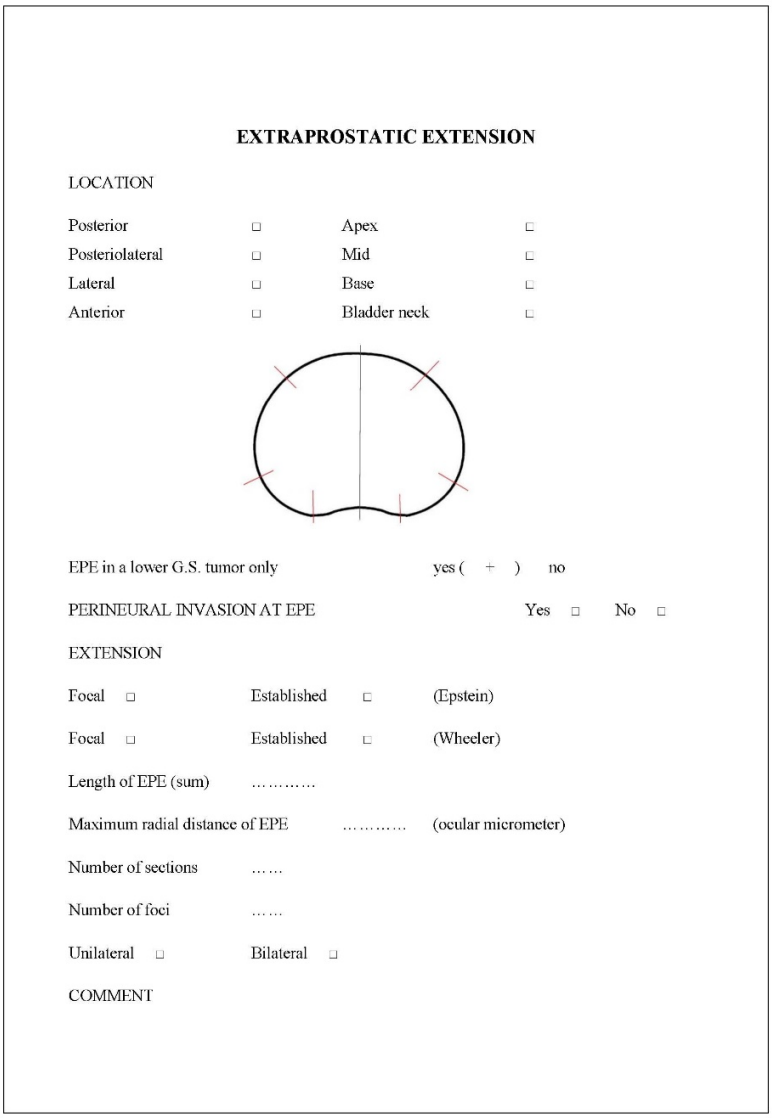


Figure 7. The picture to the left is an example of focal EPE. HE, 5x lens magnification. Established EPE is illustrated to the right. HE, 10x lens magnification. Reprinted from Danneman et al. Prognostic significance of histopathological features of extraprostatic extension of prostate cancer. Histopathology. 2013;63(4)580-9, with permission from Wiley.



EXTRAPROSTATIC EXTENSION

LOCATION

Posterior	□	Apex	□
Posteriolateral	□	Mid	□
Lateral	□	Base	□
Anterior	□	Bladder neck	□

EPE in a lower G.S. tumor only yes (+) no

PERINEURAL INVASION AT EPE Yes □ No □

EXTENSION

Focal □ Established □ (Epstein)

Focal □ Established □ (Wheeler)

Length of EPE (sum) ……

Maximum radial distance of EPE …… (ocular micrometer)

Number of sections ……

Number of foci ……

Unilateral □ Bilateral □

COMMENT

Figure 8. Protocol for detailed assessment of EPE.

The circumferential length of EPE was defined as the length of extraprostatic tumor parallel to the outer margin of the prostatic stroma. The measurement was made with a ruler directly on the slides. In specimens containing more than one focus of EPE, the circumferential length was calculated as the sum of lengths of all extraprostatic lesions. The radial distance of EPE was defined according to Sung et al. (151) as the distance that the tumor protrudes perpendicularly beyond the outer margin of the prostate stroma. The measurement was made with an ocular micrometer up to 2 mm. If the radial distance exceeded 2 mm it was measured with a ruler. The focus of maximum radial distance was recorded in specimens with multiple foci of EPE.

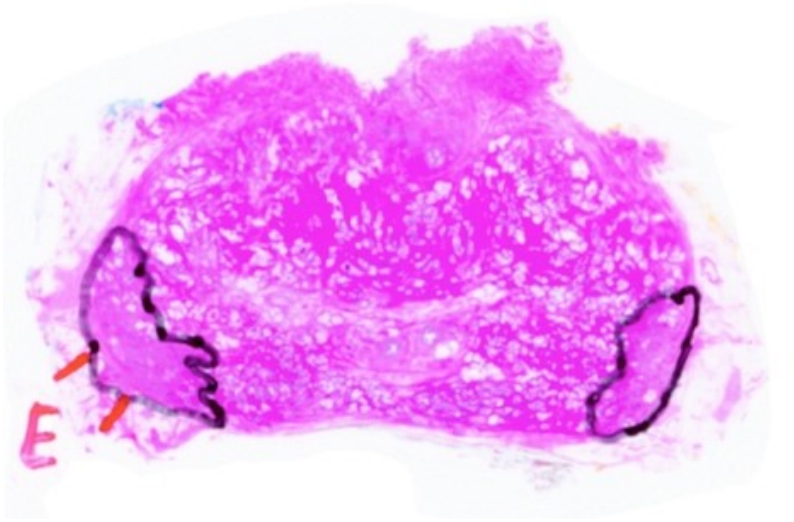


Figure 9. Whole mount section of radical prostatectomy specimen. EPE is seen in the posterior-lateral corner at the neurovascular bundle. E = extraprostatic extension. The circumferential length is measured between the two red lines.

The GSs of the original reports were used and the GS of the case was based on the individual tumor focus with the highest GS. Although the assessment of the GS was undertaken before the ISUP 2005 revision of the Gleason grading system, our laboratory applied most of the new rules long before 2005. The exception was that GS 5 was used relatively frequently before 2005. With contemporary grading, this would in most cases correspond to a GS 6. In six cases slides were not available, but an assessment of some measures could be made from scanned tumor maps with annotations of EPE.

3.1.3 Clinical follow-up

Clinical follow-up was done at the urology clinic at Karolinska University Hospital, Stockholm. The patient records were reviewed, and preoperative stage and pre- and postoperative s-PSA values were recorded. Clinical signs of metastatic disease and vital status were collected. Six to eight months after surgery s-PSA was measured and one or two times annually thereafter. The definition of BCR was s-PSA above 0.2 ng/ml.

3.1.4 Statistical analysis

Cox regression models were used to perform univariate time-to-event analysis of histopathological variables, with BCR as an outcome variable. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated to assess the effects of EPE, and various characteristics of EPE, on BCR. The Kaplan-Meier procedure was applied to estimate recurrence-free survival among patients. To adjust for established progression factors (GS, preoperative s-PSA, age, and cT), multivariate Cox regression analysis was performed. In the

multivariate model, tumor grade was entered by GS (sum). We considered p-values of < 0.05 to be statistically significant. All analyses were performed using the statistics program R (R Foundation for Statistical Computing, Auckland, New Zealand).

3.2 STUDY 2 AND 3

3.2.1 Study population and data collection

The second and third studies of this thesis were register studies based on data from the National Prostate Cancer Register (NPCR). All prostate cancers diagnosed in Sweden are reported to the NPCR, a nationwide population-based register that covers more than 98% of all prostate cancers compared with the National Cancer Registry to which reporting is mandatory by law.

Several separate forms are used in the registration process and include histopathological and clinical data such as s-PSA, number of biopsies, tumor grade, and tumor stage. In a second step, records about further investigation and treatment are registered.

In Study 2 we included 97 168 men who were diagnosed with prostate cancer based on assessment of TRUS-guided needle biopsies between 1998 and 2011. We included all these men and analyzed GS, Gleason patterns, cT, and s-PSA levels at diagnosis. The coverage of GS, cT, and s-PSA in NPCR was high: 97%, 99%, and 99% of all cases.

In the third study, we also used data from NPCR. Our inclusion criteria were men diagnosed between 2000 and 2012 with cT T1-2 M0/X (*Union Internationale Contre le Cancer* TNM 2009) prostate cancer on needle biopsies, aged ≤ 70 years at diagnosis, s-PSA < 20 ng/ml, who had undergone RP within 6 months from diagnosis and had complete data for primary, secondary, and tertiary Gleason patterns. This resulted in the inclusion of 15 598 men. We chose to study this subset of patients to allow comparison over the entire period since data on RP specimens were only gathered for this subset between 1997 and 2006. Of all cancers reported during this period, only 0.09% (92/97 260) were GS $3 + 5 = 8$ or $5 + 3 = 8$ and we decided to exclude these cases.

3.2.2 Statistical analysis Study 2

To adjust for the changing distributions of cT and s-PSA during the study period we standardized the GS by using the distribution in 1998 as standard with the following categorization: four levels of cT (T1-T4) were combined with three levels of s-PSA (above or below median s-PSA or missing value).

To answer the question of whether there had been a trend shift in Gleason grading in 2005 when the revision was done, logistic regression was used to demonstrate an association between GS 2-6 and the year of diagnosis. We adjusted for cT and s-PSA using the same categorization as above. We then compared two such models, one with broken-line regression and one without, using the chi-square test to see if the broken-line regression better explained the data.

Logistic regression was also used for trend test with the year of diagnosis as the continuous variable and the proportion of interest as the outcome. To evaluate the proportion of GS 3+4 among GS 7 tumors, the same test was used. After standardization, the chi-square test was used to compare the period before and after 2005. We also compared median s-PSA in 1998 and 2011 and for this, we used the Mann-Whitney test.

3.2.3 Statistical analysis Study 3

For each year we analyzed the proportion of men with agreement, undergrading, and overgrading between needle biopsy and subsequent RP specimen. Both GS and ISUP grades 1-5 were analyzed. Undergrading was defined as lower grade in needle biopsy than in RP specimen and overgrading the opposite. The chi-squared test was used to compare results before and after 2005 when the ISUP revision of the Gleason grading system was published.

Using logistic regression, odds ratios (ORs) and 95% CIs were calculated to evaluate if the prediction of RP GS and ISUP grade had changed over time. An OR > 1 indicates an improved correlation. To analyze if there was a trend shift in 2005, hence if the slope of the regression changed after ISUP 2005, the logistic regression models were compared using Analysis of Variance (ANOVA) with broken-line regression.

Like the second study, all tests were two-sided and a $p < 0.05$ was considered statistically significant. The statistical analyses were performed using R statistical program package (version 2.15.1).

3.3 STUDY 4

3.3.1 Study population and data collection

A reference image database was hosted by ISUP to establish an international standard in prostate cancer grading. Prostate cancer cases have been uploaded here and independently graded by 24 acknowledged experts in urological pathology representing geographic regions from around the world (200, 201).

For these cases, the grading options were GS 3 + 3 = 6, 3 + 4 = 7, 4 + 3 =7, 4 + 4 = 8, and 9-10 (also known as ISUP grades 1-5) and other (specified). Grading was done by review of microphotographs from each case. Cases reaching agreement among two-thirds of the experts were considered consensus cases and automatically moved to a public compartment of the database.

Each case was obtained from a single core from the STHLM3 study, a population-based screening study undertaken among men aged 50-69 years (94). The cases were uploaded between May and September 2015. These cases were included to show different morphology and challenging cases, thus there was an overrepresentation of high-grade cancers. We included 87 cases of the 90 biopsies since glass slides were available for scanning and AI-analysis for these cases. All non-consensus cases in the database were reviewed, first by the author and then together with LE. We identified reasons for disagreement and categorized them.

The scanned images were processed by AI as previously described (210). The AI system has two convolutional DNN ensembles, each consisting of 30 Inception V3 models pre-trained on ImageNet, with classification layers adapted to our outcome. Training of the system was done using 6682 digitized needle biopsies from 976 randomly selected participants in the STHLM3 study done between May 2012 and December 2014. The system was then evaluated by the ability to identify prostate cancer and to predict the extent and Gleason grade for independent and external test sets comprising 1961 biopsies from 218 men (94, 210). None of the 87 cases had been part of the dataset used for training or validation of the AI system.

3.3.2 Statistical analysis Study 4

To compare expert grading with AI-assisted grading we calculated weighted kappa values. For all observers weighted kappa values were calculated using O´Connell and Dobson estimators (233). To evaluate the average agreement for each case linear weights were used. The mean weighted kappas by a pathologist were calculate using Schouten`s methodology (234). To assess the agreement for a specific grade, the results were dichotomized and unweighted kappas used. All kappas were calculated using the Magree package in R. Bootstrap was used for computing 95% confidence intervals.

3.4 ETHICAL CONSIDERATIONS

Studies I-IV were approved by the Regional Ethics Review Board in Stockholm (2006/1014-31, 2013/153-31, and 2012/572-31/1). Study II and III have the same record number.

4 RESULTS AND DISCUSSION

4.1 STUDY I: THE RADIAL EXTENT OF EPE IS ASSOCIATED WITH A HIGHER RISK OF BCR

Main findings and general discussion

The clinical outcome in patients with EPE varies (144-147). Therefore, we aimed to find better ways to classify these patients for better treatment and management decisions. For this reason, we retrospectively reviewed RP specimens from the Karolinska University Hospital in Solna and identified cases with EPE and correlated various histopathological features to BCR.

Of the cohort including 1051 RP specimens between 1998 and 2005, EPE was observed in 470 (44.7%) cases. This number is rather high but within the range of 25% - 60% reported by others (124, 126, 138-141, 235). Moreover, 33% of this cohort had a positive surgical margin. A recent report from NPCR (5) showed that the positive surgical margin rate in Sweden has been quite stable, approximately one-third, since 2009, which is on the same levels as in this study period.

In the decade before and during this study period, a trend towards earlier detection of prostate cancers was seen, mainly due to the use of s-PSA, which resulted in more patients being diagnosed with a lower tumor stage. In the USA, the incidence of EPE on RP specimens decreased from 66% to 25% between 1987 and 2001 (136). Still, a significant number of patients that undergo RP are diagnosed with EPE that was not apparent before surgery. Moreover, there is a current trend towards surgery in patients with more advanced stages that might also increase the incidence of EPE.

Patients with EPE had a higher progression rate compared to patients with organ-confined disease (HR 1.4, 95% CI 1.1–1.8, p = 0.007). Among the 470 patients with EPE, 133 (28.3%) experienced BCR compared to 109 (18.8%) with organ-confined prostate cancer. However, the effect of EPE on disease progression was attenuated and no longer significant in a multivariate analysis where we adjusted for GS, cT, preoperative s-PSA level, and age at surgery. Some previous studies have reported EPE as an independent prognostic factor, whereas others have not (124, 126, 141). Although the prostate boundary cannot be considered a true capsule and not an efficient barrier to metastasis, cancer that invades outside the prostate can be an indicator that the cancer cells have acquired the ability to move

into other tissue compartments and metastasize.

Several studies have tried to classify cancers with EPE into prognostic groups. We evaluated several of these methods in one of the largest cohorts of EPE patients in the literature (Figure 10). We assessed both Epstein et al. (125) and Wheeler et al. (124) methods and found a significant difference in outcome between focal and established EPE, in line with those studies. These two methods identify very similar groups and thus the HRs were of the same magnitude. Only 7.9% of our patients had focal EPE, whereas, in the cohort studied by Epstein et al. (141), 20% had focal EPE. A possible explanation is that the tumors included in our cohort were larger and of higher stage. Tumors showing only focal EPE seem to be less aggressive and even if cut across have little potential for regrowth and progression (125). There is also a better chance of complete surgical removal when EPE is only focal and a reduction of the rate of positive surgical margins reduces the likelihood of BCR.

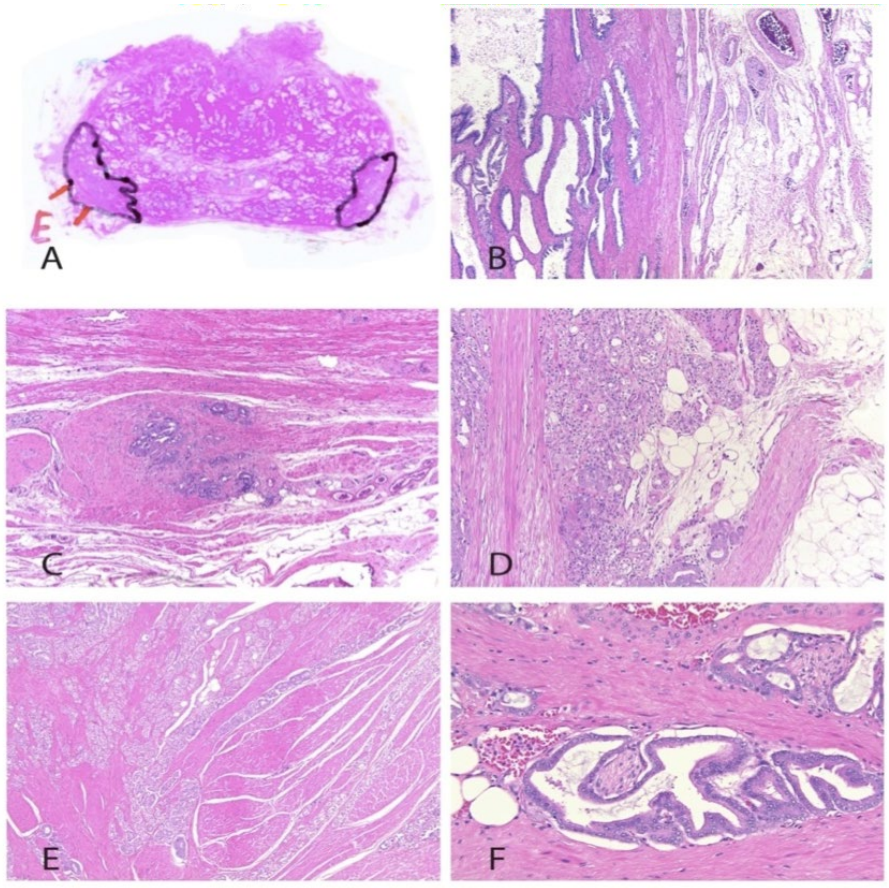


Figure 10. A. Circumferential distance of EPE: whole mount section of RP specimen. B. The edge of the prostate. C. Focal EPE D. Established EPE. E EPE into the bladder neck. F. EPE with PNI. All sections: H&E. Images B, C, D, and F are reused from Danneman et al. Prognostic significance of histopathological features of extraprostatic extension of prostate cancer. Histopathology. 2013;63(4)580-9, with permission from Wiley.

We showed that the radial distance was a useful classifier of EPE. When EPE was dichotomized by the median radial distance (1.1 mm) this cut-off predicted BCR (HR_{crude} 1.5, 95% CI 1.1–2.2, p = 0.015; Figure 11). Many groups have examined the radial distance. Sohayda et al. (140) also found the median radial distance to be 1.1 mm, whereas Chao et al. (149) reported a median of 2.4 mm. Davis et al. were first to quantify the radial distance by using an ocular micrometer but they did not study its relation with BCR (139). Sung et al. (151) proposed that pathologists should measure the maximum radial distance with an ocular micrometer, which they showed was an independent prognostic indicator for BCR. They proposed 0.7 mm as a cut-off. However, the validity of their findings has been debated as GS did not correlate with BCR after RP. Normally, GS is a strong predictor for recurrence. Van Veggel et al. (150) also found 0.7 mm to be the optimal cut-off.

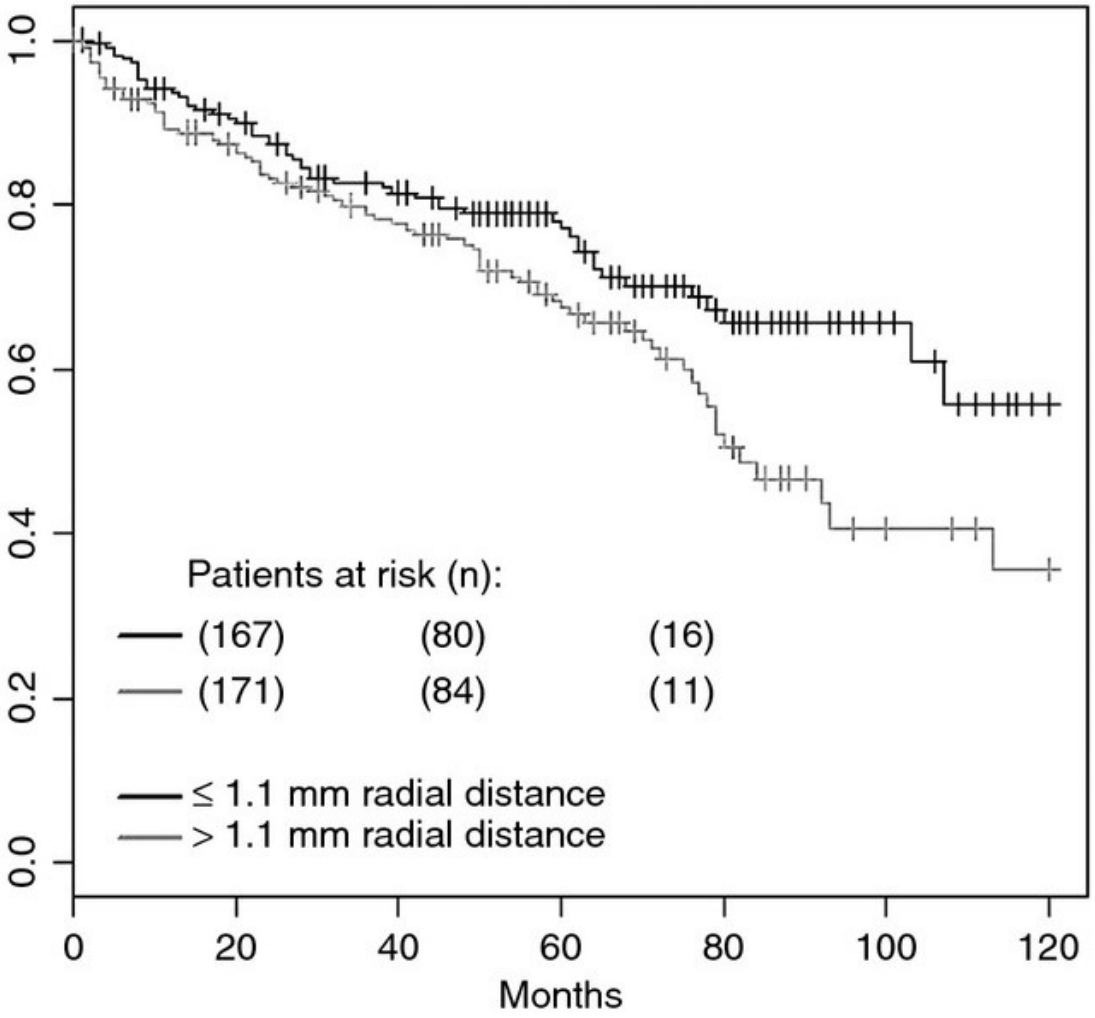


Figure 11. Correlation of radial distance with BCR. Number of patients with radial distance ≤1.1mm: 233. Number of patients with radial distance >1.1mm: 232. Reprinted from Danneman et al. Prognostic significance of histopathological features of extraprostatic extension of prostate cancer. Histopathology. 2013;63(4)580-9, with permission from Wiley.

To enable comparison with previous studies we evaluated other cut-offs as well (0.6 mm and 0.75 mm) and they also were associated with worse prognosis and differed only marginally in their ability to predict recurrence. However, radial distance as a continuous variable was not significantly correlated with outcome in our cohort. We concluded that a practical approach is needed to classify EPE and proposed using the diameter of a 20x field (usually corresponding to 1.1 mm). Given the difficulty in identifying the edge of the prostate, the micrometer precision of decimals of millimeters is not reasonable. The potential for radial distance to predict recurrence is similar to that of Epstein's or Wheeler's criteria and thus the method with the strongest association with prognosis may differ from cohort to cohort. Therefore, the choice of the preferred method is more a matter of what is practical and reproducible.

Our study also included evaluation of circumferential extent, number of sections or foci of EPE, and laterality. Neither of those features was found to classify EPE as a predictor of BCR after RP, which all is in line with previous studies (151, 236). To our knowledge, we were the first to evaluate the prognostic value of PNI at the site of EPE. The presence of PNI did not correlate with outcome. Negative findings are also important as the pathologist report should be as focused as possible on histopathological features that have a prognostic value.

Strengths and Limitations

This study was based on a very large series of RP specimens and the number of cases with EPE was at the time the largest in the world literature. All cases have undergone a centralized review. In addition, all prostates have been uniformly totally embedded which is not the case in some other institutions. We have a high rate of EPE and a high rate of BCR, ie. many events, which is a strength in the statistical analysis.

The optimal outcome measure for research purpose would be disease-specific survival, but the high survival rates in prostate cancer patients would result in too few events to allow any conclusions. It is important to emphasize that BCR does not translate to disease-specific mortality which is a limitation (119).

This cohort study spans over almost 8 years but since prostate cancer grows relatively slow, the optimal follow-up time would be at least 10-15 years. In the current study, the mean follow-up time was 44.7 months and 242 patients had BCR. Too few patients with recurrence may be an explanation of why EPE was not found to be an independent predictor of outcome in an adjusted Cox analysis. Besides, cT and EPE depend on each other, and thus questionable if cT should be included in a multivariate analysis.

Earlier studies have shown that the majority of BCR occur within the first few years after RP (119, 237). Consistent with this, Loeb et al. (238) investigated over 10 000 men treated with prostatectomy of which 16% had BCR and found that 77% of the BCR occurred within five years after RP.

The study is retrospective which is necessary to obtain a sufficiently long follow-up, but this also means that we have a different spectrum of tumors compared to modern practice. There has been a considerable stage shift over time, and it can be argued that the relatively advanced cases of our series do not reflect current practices with very small PSA-detected cancers.

The interpretation of EPE is to some extent subjective and it cannot be excluded that the classification would have been done differently by other experts. Thus, the results need to be interpreted with some caution, and validation in an independent cohort would be of value. Moreover, there is a methodological problem with measuring the radial distance as it is often difficult to identify the edge of the prostate. The cut-off of 1.1 mm corresponds to the diameter of a 20x field which could be a more practical measurement of EPE and probably a more reproducible approach.

In summary, we found that, in a large cohort, EPE was associated with BCR after RP but not as an independent predictor. The radial distance was found to predict BCR and may be used to classify EPE into more reliable prognostic groups.

4.2 STUDY II: THERE HAS BEEN AN INFLATION IN GLEASON GRADING BETWEEN 1998 AND 2011

Main findings and general discussion

The revision of the Gleason grading system by ISUP in 2005 (176) had the potential to cause a shift towards higher grading. Despite lower stages and lower PSA levels at diagnosis, several studies had shown a trend towards higher prostate cancer grading in recent decades (239). Some studies have reported an upgrading in the years after the ISUP revision (240). However, it was unclear how much the ISUP revision contributed to this. Therefore, in our second study, based on data from NPCR between 1998 and 2011, spanning several years before and after the new grading recommendations in 2005, we analyzed long-term trends in Gleason grading and the impact of the ISUP revision in a national cohort.

A GS of 2-5 was reported in 27% in 1998 and 1% in 2001 (OR 0.80, $P_{trend} < 0.001$; Figure 12(A)). Thus, GS 2-5 was almost entirely abandoned during the end of the studied period. This has little clinical relevance, as the diagnosis of GS 2-5 and GS 6 would not affect treatment decisions.

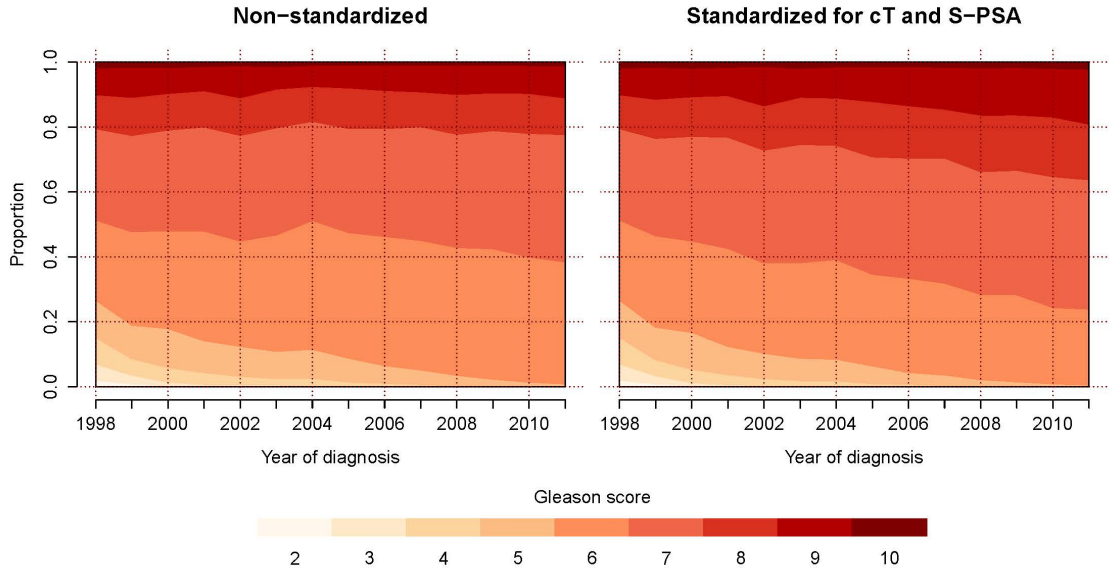


Figure 12. (A) GS from 1998 to 2011. (B) GS standardized for cT and s-PSA. Reprinted from Danneman et al. Gleason inflation 1998-2011: a registry study of 97,168 men. BJU Int. 2015;15(2):248-55, with permission from Wiley.

We also analyzed how stage and s-PSA had changed over the study period. Newly diagnosed prostate cancers were stage T1c in 20% in 1998 and 51% in 2011, while T2 and T3 tumors decreased from 40% and 31% respectively, in 1998 to 29% and 16% in 2011 ($p < 0.001$). A concurrent decrease in median s-PSA at diagnosis was seen from 20.5 ng/ml in 1998, to 8.9 ng/ml in 2011 ($p < 0.001$). Similar findings have been reported by others (241) and reflect a stage migration with cancer being detected at earlier stages.

A clinically important observation was the decrease of GS 6 tumors and concurrent increase of GS 7-10 cancers. GS 6 increased until 2004-2005 when there was a trend shift and a decline began (Figure 12(A)). A broken-line regression analysis of change of distribution of GS over time showed a slightly steeper decrease in the use of GS 2-6 after 2005 ($p < 0.001$). Tumors assigned GS 7-10 were diagnosed in 52% before 2005 and 57% afterward ($p < 0.001$). We standardized GS distributions for cT and s-PSA with 1998 as a baseline (Figure 12(B)). After standardization, GS 7-10 tumors were diagnosed in 59% and 72%, before and after 2005 respectively ($p < 0.001$)

Another clinically important finding is the increase of GS 7-10 among low-risk tumors (stage T1c and s-PSA 4-10 ng/ml) from 16% in 1998 to 40% in 2011 (OR 1.12, $p_{trend} < 0.001$; Figure 13(A)). Moreover, in high-risk tumors (stage T3 and s-PSA 20-50 ng/ml) GS 7-10 increased from 65% in 1998 to 94% in 2011 (OR 1.16, $p_{trend} < 0.001$; Figure 13(B)). We observed a concurrent stage shift towards lower stages at diagnosis. As stage and grade correlate this should have resulted in lower grades at diagnosis. Instead, we saw an upgrading among all prostate cancers, including low-risk tumors.

The percentage of GS 7 tumors increased over the years studied (Figure 12(A)). Previous studies have shown different prognosis between GS 3 + 4 = 7 and GS 4 + 3 = 7 tumors (189, 190). Primary and secondary Gleason patterns of GS 7 were reported in 75% from 2000 and always reported from 2007. Therefore, we evaluated the proportion of these tumors between 2000 and 2011 and observed a weak trend towards increasing proportion of GS 3 + 4 = 7 over the period (OR 1.01, $p_{trend} < 0.02$)

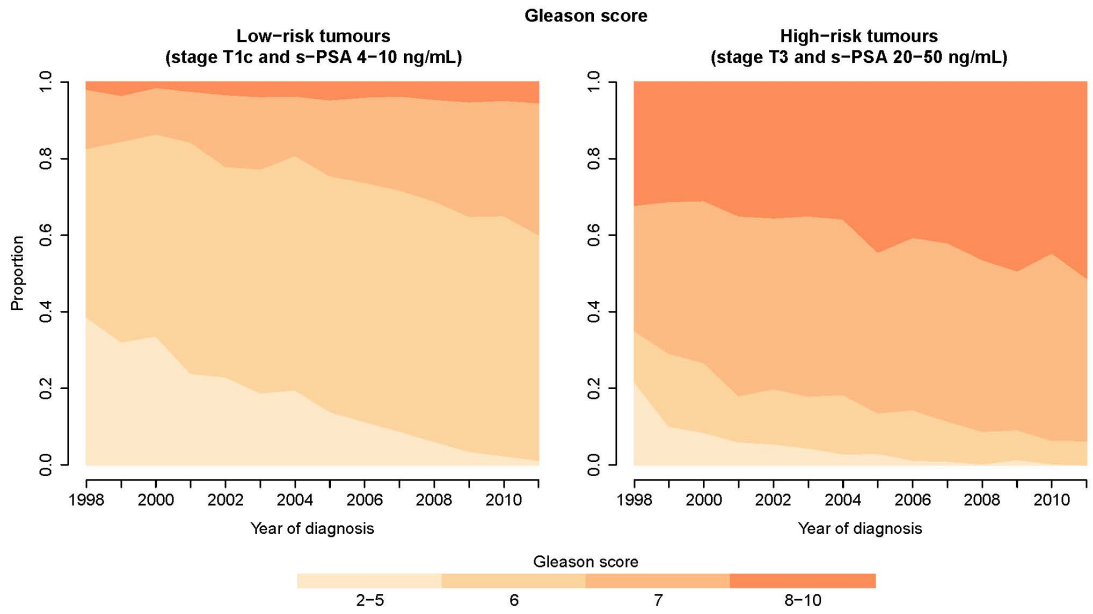


Figure 13. (A) GS, low-risk tumors (stage T1c and s-PSA 4-10 ng/ml), 1998-2011. (B) GS, high-risk tumors (stage T3 and s-PSA 20-50 ng/ml), 1998-2011. Reprinted from Danneman et al. Gleason inflation 1998-2011: a registry study of 97,168 men. BJU Int. 2015;15(2):248-55, with permission from Wiley.

There are two main reasons why grade inflation is problematic. First, comparison with historical datasets will be difficult. If higher GS are assigned over time, treatment effects will seem to improve in each grade category (239). Secondly, and arguably even more important, is the risk of overtreatment. By upgrading, many tumors previously classified as low-risk were considered high-risk tumors at the end of this period. A GS ≥ 7 excludes the patient from active surveillance in most cases (242). Thus, the distinction between GS 6 and higher grades is important for the decision of whether patients should be offered primary treatment with curative intention or active surveillance. It is problematic that six years after the ISUP revision, the upgrading trend had still not slowed down.

We must, however, consider that contemporary GS may more accurately reflect tumor prognosis than earlier grade assignments. Some changes in grading routines result from a better understanding of tumor biology. One example is that some of the Gleason pattern 1 cases may have been benign proliferations, e.g. adenoses. Today the pathologist can use immunohistochemistry to stain for basal cells to distinguish between benign and malignant glands, thus revealing that some of these lesions are in fact benign. Moreover, there is evidence that cribriform cancer, previously part of Gleason pattern 3 (and in early publications even Gleason pattern 2), is an aggressive disease and part of Gleason pattern 4 (183-187, 196). Prostate biopsies protocols have become more extensive, which increases the likelihood of finding a high-grade component and this may also have contributed.

As GS 2-5 has almost been abandoned, the lowest GS is 6 and can therefore only change in one direction. Tumors assigned a GS 8-9 also increased, while the proportion of rare GS 10 cases remained stable. Hence, we must also consider a spectrum bias in this study since the lowest GS cannot go lower and that a GS of 10 cannot increase.

Strengths and Limitations

In this study, we evaluated the incidence of certain histopathological features based on information from a cancer registry. A strength of the study is that it is based on data from a national cohort, thus minimizing selection bias. By design, retrospective registry studies also enable the inclusion of large datasets. The national cohort data were retrieved from NPCR, which has high coverage of prostate cancer patients and high data quality. The quality of the register data is assured by chronologic reporting and validation by linking with other registries. The considerable amount of research based on these data is an indirect quality assessment.

A limitation of the study design is that no central review was done. Previous studies have compared conventional and modified Gleason grading by re-grading of biopsies (243-248) but several of these studies were limited by small sample sizes. Our objective was not to regrade biopsies but to evaluate the impact of the ISUP 2005 revision in real-life practices by comparing the grade distribution over time in routinely graded biopsies. We also analyzed grading practices among the six healthcare regions of Sweden and found similar trends in all regions and a high level of grading consistency.

In summary, we confirmed a considerable shift upwards of the Gleason grading from 1998 to 2011. This had been a gradual process that started several years before the ISUP revision but became clearer after 2005. When adjusted for stage migration the upgrading became even more evident. Gleason grading has a central role for treatment decisions and quality assurance of this measure is therefore of great clinical importance.

4.3 STUDY III: IMPROVED GRADE CORRELATION BETWEEN BIOPSY AND RP SPECIMENS FROM 2000 TO 2012 WAS EXPLAINED BY A NARROWER DISTRIBUTION OF GRADE CATEGORIES

Main findings and general discussion

Needle biopsy samples only a small fraction of the prostate gland and may therefore not be representative of the tumor morphology. In our second study, we showed a shift towards higher Gleason grading. Whether the higher grading on biopsy was associated with improved agreement between needle biopsy and RP specimens was unclear (240, 245-247, 249-251). Therefore, in a large nationwide population-based cohort, including data from academic and non-academic hospitals, we investigated if the correlation between GS and ISUP grade in biopsy and RP specimen had changed over time.

The distribution of GS and ISUP grades in biopsies and RP specimens between 2000 and 2012 is illustrated in Figure 14. As described in our previous study, GS 2-5 was almost entirely abandoned with a reduction from 21% in 2000 to 0.2% in 2012. ISUP 1 tumors decreased from 52% to 23%, ISUP 2 increased from 33% to 46% and ISUP 3 increased from 8% to 22% over the studied years. The highest grades, ISUP 4 and 5 were relatively stable over time.

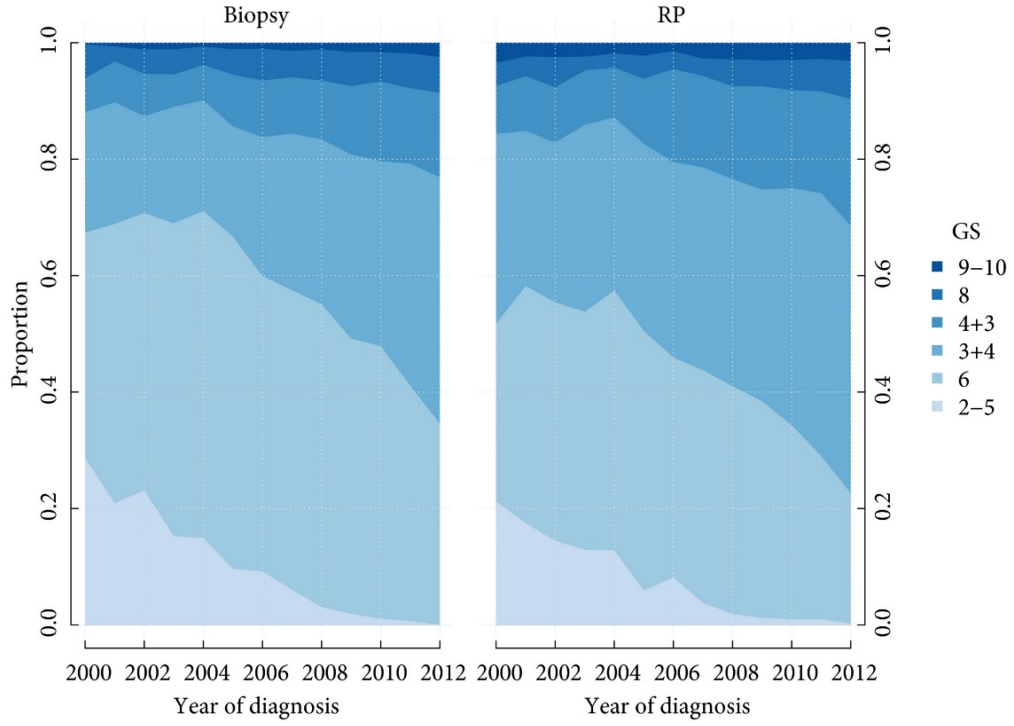


Figure 14. Distributions of GS in biopsy (left) and RP specimen (right), 2000-2012. Reprinted from Danneman et al. Accuracy of prostate biopsies for predicting Gleason score in radical prostatectomy specimens: nationwide trends 2000-2012. BJU Int. 2017;119(1):50-56, with permission from Wiley.

Prediction of RP GS improved from 55% to 68% between 2000 and 2012. Most of the increase occurred before 2005 (nine percentage points; $p < 0.001$). Our previous study indicated that some of the changes in grading practices were implemented before the ISUP consensus meeting in 2005, which explains why most of the improvements occurred before 2005.

The improved agreement appears to be the result of a decrease undergrading of biopsies (32% in 2000 to 22% in 2012; Figure 15). This may partly be attributed to the 2005 ISUP revision which changed both pattern interpretation and reporting routines. For example, poorly formed glands became accepted as a feature of Gleason pattern 4, and most cribriform cancers were now also included in this pattern, but the main explanation is probably the inclusion of minimal foci of Gleason pattern 4 in the GS (176). As a result, the percentage of biopsies with GS 7 doubled (Figure 14).

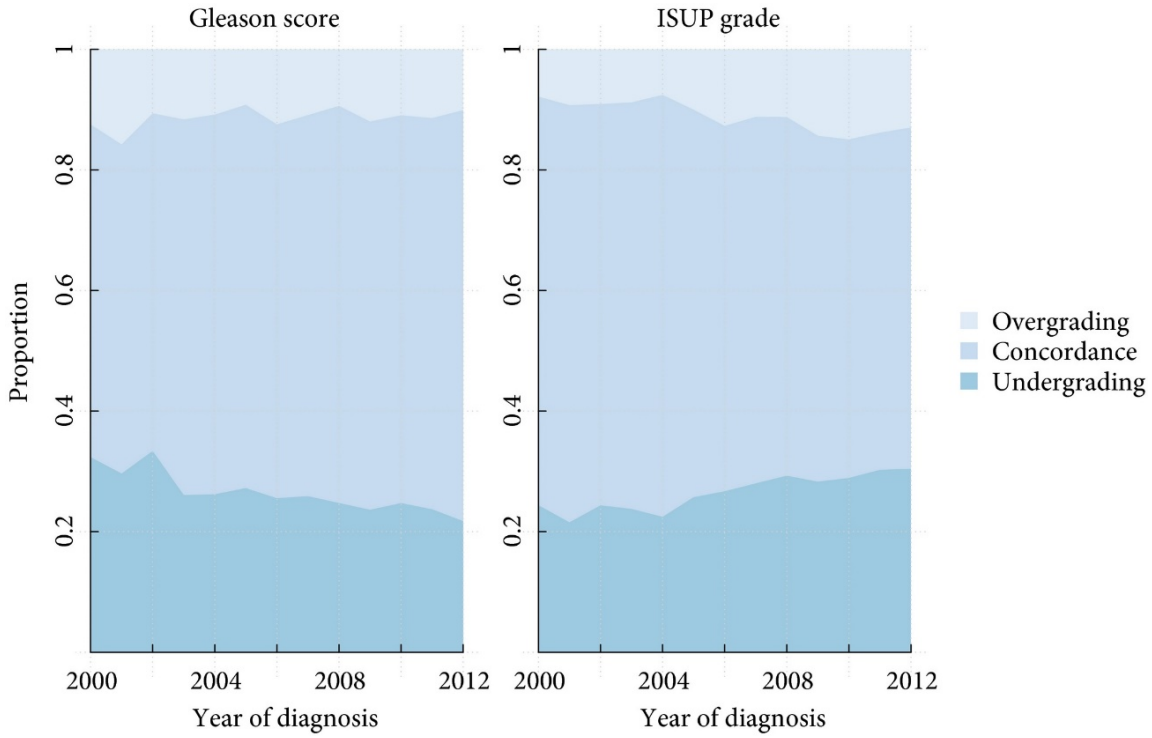


Figure 15. Agreement between biopsy and subsequent RP specimen grade using GS (left) and ISUP grade (right), 2000-2012. Undergrading = lower grade assigned to the biopsy than to the RP specimen; overgrading = vice versa. Reprinted from Danneman et al. Accuracy of prostate biopsies for predicting Gleason score in radical prostatectomy specimens: nationwide trends 2000-2012. BJU Int. 2017;119(1):50-56, with permission from Wiley.

Another explanation for these observations may be the extensive biopsy protocol established during this period. Obtaining more biopsies increases the chance that biopsy GS will agree with RP GS (252-254). In addition, a stage shift to lower stages may have induced a grade shift and improved grade prediction.

Studies addressing the correlation of GS on needle biopsy and RP specimen after the 2005 revision showed varying results (240, 245-247, 249-251), although the majority demonstrated improvement correlation (245-247, 250).

We also evaluated the agreement between biopsy and RP specimen according to grouped GS, i.e. ISUP grades, and found a decreased correlation, from 68% in 2000 to 57% in 2012 (Figure 15). Undergrading explained most of the discrepancy and increased from 24% to 30%. Overgrading went from 8% to 13%. Agreement decreased for ISUP grade 1 to 3 but increased for ISUP grade 4. No consistent time trend was seen in ISUP grade 5 cancers, probably since there were too few cases.

Thus, it may appear that the modified Gleason grading better predict GS on RP specimen. However, we must also consider a bias as the number of grade categories decreased. For example, GS 2-5 were almost entirely abandoned. Achieving a correct prediction of RP grade will automatically become easier if the number of grade categories decreases. When this was adjusted for, by including biopsy GS and years of diagnosis in a multivariate analysis we found a decreased correlation over time (OR 0.98; $p < 0.002$). Hence, the seemingly improved correlation is explained by a narrower distribution of grades in the ISUP 2005 modified grading.

This may also be the reason why we saw a decreased grade correlation between biopsy and RP specimen when ISUP grades were analyzed. The grade agreement was weakest for ISUP grades 2 and 3. Undergrading stood for most of the discrepancy among ISUP grade 2 cases and increased from 12% to 25%. In ISUP grade 3, overgrading predominated and increased from 24% to 31%. Thus, the decreased ability to predict ISUP grade on RP specimen is likely explained by the separation of GS 7 tumors to ISUP grade 2 (3 + 4) and 3 (4 + 3). When proportions of Gleason patterns are factored in, grade assessment becomes even more difficult. However, this separation is necessary since they better predict outcome (168, 169, 188-190).

Strengths and Limitations

The strengths of this study are that it includes many patients and its nationwide population-based design, which minimizes selection bias. However, we only included men aged < 70 years with cT T1-2 M0/X and s-PSA < 20 ng/ml. This is a limitation but was necessary due to incomplete data collection for the NPCR in the first decade of the studied period.

Grading routines have changed gradually. This is also a limitation since it is impossible to know retrospectively the rationale of the composition of the grade and why it is difficult to retrospectively translate GS to ISUP grades. The most accurate way to study ISUP grades would be to re-grade all the biopsies and RP specimens, but this would neither be feasible, nor relevant as the aim of studies 2 and 3 was to evaluate changes in grading practices over time. Moreover, interobserver variability is a well-known problem in pathology. In this study period, many pathologists were involved in grading, and biopsy and RP specimen were often graded by different pathologists. In centers with a small number of uropathologists, the agreement may be better, whereas our results are representative of real-world practice in Sweden.

An accurate GS and ISUP grade between biopsy and RP specimen is important for prostate cancer patients and treatment decisions. Incorrect grade reporting on biopsy can lead to under- or overtreatment. Therefore, revision of the Gleason grading system needs to be evaluated to ensure that the correlation of grade is not weakened. The true validity of the revised Gleason grading system is its correlation with patient outcome.

In summary, we analyzed grade agreement between biopsy and subsequent RP specimen from a national cancer registry between 2000 and 2012 and an uncorrected comparison seemed to indicate an improvement of GS concordance. However, multivariate analysis indicated that the observed improvement was explained by data compression into fewer categories.

4.4 STUDY IV: AN ARTIFICIAL INTELLIGENCE ALGORITHM GRADES PROSTATE CANCER WITH THE SAME ACCURACY AS EXPERT PATHOLOGISTS

Main findings and general discussion

A strength of the Gleason grading system is that it takes the morphological heterogeneity of prostate cancer into consideration and it includes several architectural descriptors such as cribriform glands, glandular fusions, and single cell invasion. Grading systems used in other cancer types are often based on the separation of a continuous range of features into an ordinal scale such as mild, moderate, or severe nuclear atypia. The Gleason grading system, however, does not use cellular details such as nuclear atypia or mitoses and therefore has the advantage of not factoring in conflicting features. Still, it relies on the subjective interpretation of these grading rules and intra- and interobserver variability is a challenge (197-199, 201).

An AI system for the detection and grading of prostate cancer in needle biopsies was recently developed by our group (210). The system was shown to perform within the range of expert uropathologists. The focus of the current study was to analyze the cases in the Pathology Imagebase reference library that were difficult to grade and failed to reach consensus among expert uropathologists. We aimed to investigate reasons for disagreement and study the performance of an AI system in problematic cases. Our outcome measurement for performance was weighted kappa values.

A substantial agreement was reached for the experts with an overall weighted kappa of 0.67. A 2/3 consensus was reached in 58.6% for the 87 cases, while 41.4% failed to reach consensus. This is a low level of agreement, but it is important to remember that these cases were selected to represent problematic scenarios and there was an enrichment of more complex tumors of higher grade. The distributions of grades are illustrated in Figure 16 by the size of the blue circles. Grades assigned by AI are marked with red dots.

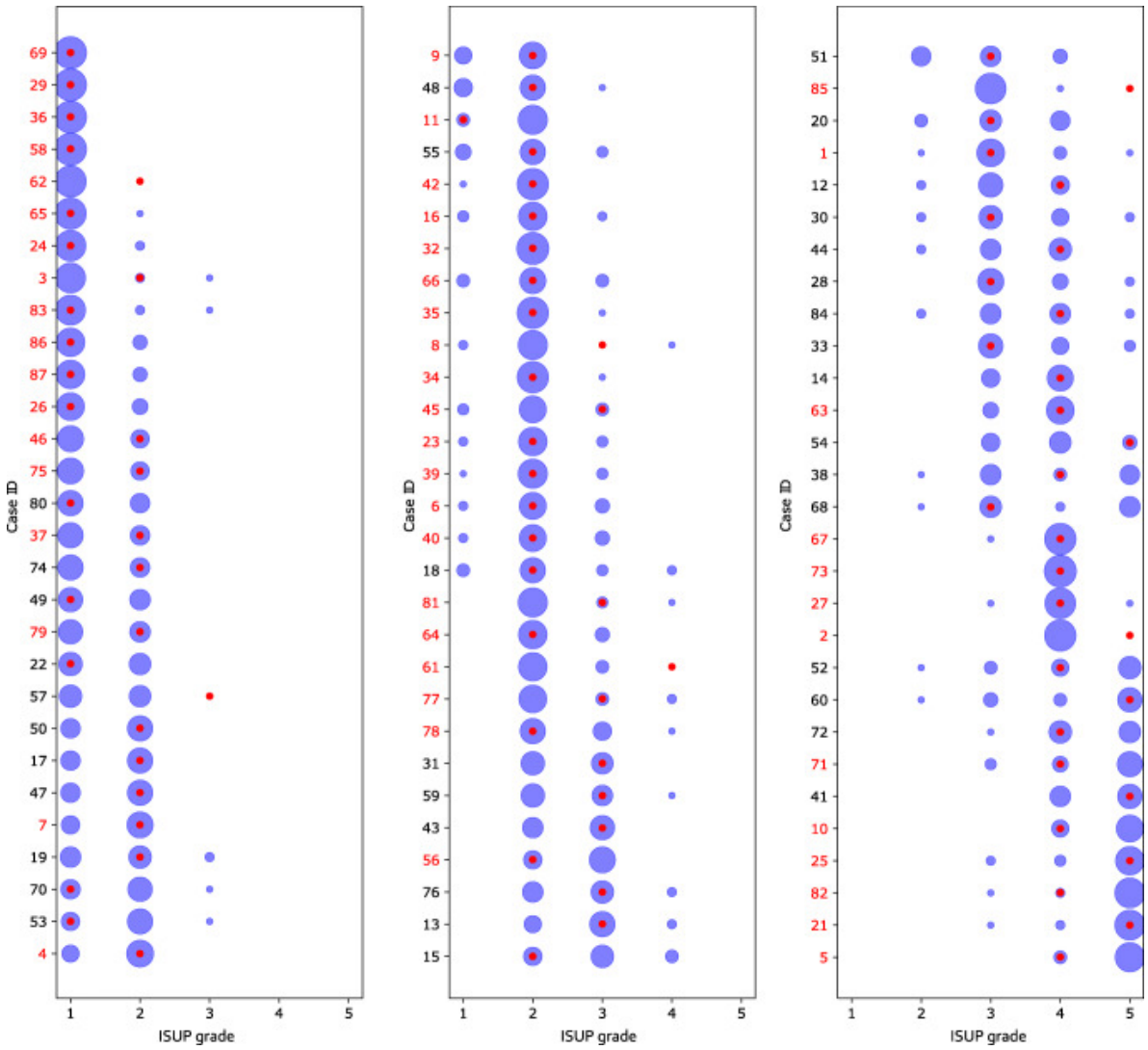


Figure 16. Grading performance relative to ISUP expert panel on Imagebase. The distribution of ISUP grades given by the 23 pathologists from the ISUP expert panel and the AI for each of the 87 case IDs in Imagebase. Each row corresponds to one case, and the cases are organized into three plots according to average ISUP grade increasing from left to right, and from top to bottom. The areas of the blue circles represent the proportion of pathologists who voted for a specific ISUP grade (x-axis). The red dot indicates the ISUP score given by the AI. Reprinted from Egevad et al. Identification of areas of grading difficulties in prostate cancer and comparison with artificial intelligence assisted grading. Virchows Arch. 2020; 477(6):777-786, with permission from Springer Nature.

We identified four frequently observed reasons for disagreement among the non-consensus cases (Table 2). The distinction between GS 3 + 3 and tangential cutting and GS 3 +4 with poorly formed or fused glands was the most common reason for disagreement. Others have also shown that the reproducibility of grading of poorly formed and fused glands is poor (196, 255). At an ISUP consensus meeting in 2014, it was decided that more than occasional elements of these structures need to be present to qualify for Gleason pattern 4 (177). The AI system chose the higher grade in 8 of the 13 cases. Fused and poorly formed glands are illustrated in Figure 17.

Table 2. Reasons for disagreement between pathologists among the non-consensus cases of ISUP Imagebase and results of AI.

Causes of disagreement	Number of cases	AI results
GS 3 + 3 with tangential cutting vs. GS 3 + 4 with poorly formed or fused glands	13	3 + 4 in 8/13
GS 3 + 4 vs 4 + 3	7	4 + 3 in 6/7
GS 4 + 3 vs. 4 + 4	8	4 + 4 in 4/8
Identification of small component of Gleason pattern 5	6	4 + 5/5 + 4 in 2/6
Other (a possible glomeruloid body, mucinous cancer)	2	3 + 3 and 4 + 3
Total non-consensus	36	

The majority of the other grading problems identified could be summarized as difficulties in the estimation of the proportion of grades. Cancers with different proportion of Gleason pattern 3 are reported as 3 + 4, 4 + 3 and 4 + 4 (with < 5% pattern 3). The grading of these GS has previously been shown to be problematic (201). The separation between GS 3 + 4 and 4 + 3 tumors is important since they have different prognostic impacts (166, 167, 184-186). The outcome of GS 4 + 3 = 7 and 4 + 4 = 8 differ less in recent studies (169, 171, 173) and thus the distinction between them may not be of clinical importance.

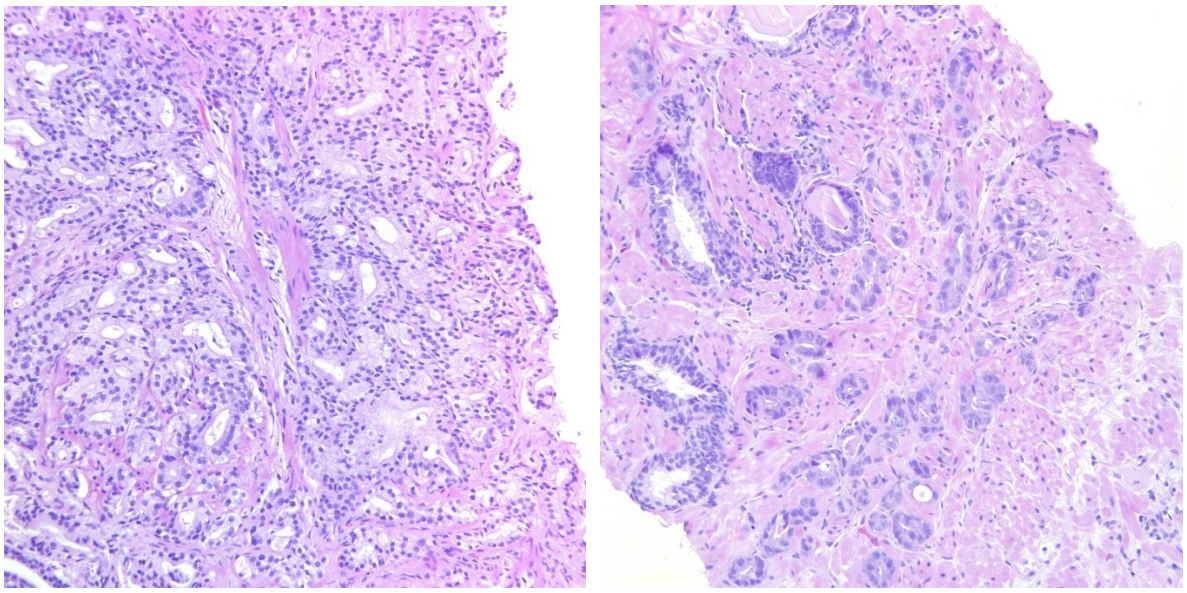


Figure 17. Fused prostate cancer glands (left picture). An example of poorly formed prostate cancer glands is illustrated to the right. H&E, 20x lens magnification.

The AI grades were the same as the majority vote in 61.1% of non-consensus cases. In six cases AI chose a lower grade than most of the experts and in eight cases the AI grade was higher. The AI system and the pathologist agreed in 72% of the GS 3 + 3 = 6 tumors, which

were the cases with the best overall agreement. The lowest concordance was seen in GS 4 + 3 = 7 tumors with an agreement of only 38.6%.

The weighted kappas of the AI system against the observers for all cases, the consensus cases, and non-consensus cases were 0.63, 0.66, and 0.53, respectively. Previous studies have reported moderate to substantial levels of reproducibility among experts in uropathology (199) and low to moderate reproducibility among general pathologists (198). Among all cases, the weighted kappa of the AI system was the fourth lowest but still within the range of leading international experts. Among the non-consensus cases, AI had the sixth best-weighted kappa.

Table 3. Classification of interobserver variability with kappa.

Kappa (*K*)	**Agreement**
<0	No agreement other than based on coincidence
0.1─0.20	Slight agreement
0.21─0.40	Fair agreement
0.41─0.60	Moderate agreement
0.61─0.80	Substantial agreement
0.81─0.99	Almost perfect agreement
1	Perfect agreement

Thus, the AI graded with similar accuracy as that of leading uropathologists, suggesting that AI may help to set a standard for pathologists by reducing subjectivity. It may also be used for external control and second opinions. Moreover, it can be used to provide support in parts of the world where pathology expertise is not available.

However, it is important to remember that an AI system is dependent on the environment in which it has been trained and has no deeper insight into optimal grading than provided by that specific training dataset. Therefore, it can be argued that the AI system is not better at grading than an expert pathologist. However, AI might be more consistent in grading and thus improve reproducibility. The reproducibility of AI was greater in the non-consensus cases than in the consensus cases. An explanation can be that the experts graded with better reproducibility in the consensus cases compared to the non-consensus cases.

In six cases, the AI system assigned grades that were not supported by any of the experts. In two of those, an ISUP grade 5 was seemingly overgraded due to occasional single cells that

were disregarded by the experts. This emphasizes that there is a need for fine-tuning the AI algorithms in more challenging grading scenarios. Moreover, there is a need for the AI system to be trained in unusual morphological variants of prostate cancer. In this study, AI ignored one case of glomeruloid pattern resulting in GS 3 + 3 = 6.

It seems that a major challenge for pathologists, as well as for the AI system, is to determine proportions of grades when there is a combination of patterns in high-grade cancers. The training dataset was enriched with high-grade cancers (210) but it may be necessary to use more complex cases in the training dataset for further improvement. Continuous interaction between humans and machine learning may also improve the skills of the pathologist and increase their reproducibility.

Despite an increasing understanding of the biology of prostate cancer and the significance of morphological features, grading of cancer is still based on a somewhat arbitrarily translation of a continuous scale into ordinal data. By training the AI system against large datasets with known outcome the system can be refined. Moreover, results from genetic and clinical studies will add further information that can be integrated into AI.

Limitations

A limitation of this study was that the experts graded microphotographs while the AI system used scanned slides. This limits comparability but was necessary since the current AI system only is able to grade scanned slides, while microphotographs were necessary for the utility of Imagebase as an accessible tool for pathologists around the world.

An extensive, prospective, validation of these algorithms is necessary before being rolled out broadly into clinical practice. Moreover, it is of importance that the ability of AI-based grading to predict clinical outcome is further analyzed.

In summary, we have illustrated reasons for disagreement in the grading of prostate cancer and shown that AI can perform at the level of expert uropathologists. This novel technique has the potential to improve prostate cancer grading in several ways, including improvement of reproducibility, standardization, quantification, automation, external quality control, and education.

5 CONCLUSIONS

We confirmed that patients with EPE have a poorer prognosis than those with organ-confined prostate cancer. The radial extent of EPE predicts recurrence after RP, but the circumferential extent, number of sections and foci of EPE, PNI at the site of EPE, and laterality do not. If validated, the proposed radial extent method may allow for more reproducible quantitation of EPE. These results may contribute to better classify patients into prognostic groups. Importantly, this may facilitate optimal treatment for long-term disease control and minimal treatment morbidity.

In a nationwide-based registry study including 97 168 men, we demonstrated that prostate cancers, despite being diagnosed at earlier stages, were gradually graded higher between 1998 and 2011 with a more evident trend shift after the ISUP 2005 revision. Among low-risk tumors (stage cT1 and s-PSA 4-10 ng/ml), GS 7-10 increased from 16% in 1998 to 40% in 2011. Grades including GS 2-5 were almost entirely abandoned towards the end of the study period. This inflation of the Gleason grading system is important to consider since the GS is one of the most important prognostic factors taken into account in the treatment decisions.

We further analyzed the prediction of grade on RP specimen from biopsy by using national cancer registry data from 15 598 men which is the largest series reported. We showed that the GS concordance seemed to improve between 2000 and 2012, but when a reduction in grade categories was factored in, the correlation decreased somewhat. We also found a worsened grade agreement if ISUP grade would have been used, partly because of the separation of GS 7 tumors into ISUP 2 and 3.

Lastly, we analyzed 87 prostate cancers that expert pathologists found difficult to grade. Experts failed to reach a consensus in 41% of the cases. Among all the cases and non-consensus cases, the weighted kappa was 0.67 (range 0.60-0.73) and 0.50 (range 0.40-0.57), respectively. We identified the distinction between GS 3 + 3 with tangential cutting artifacts versus GS 3 + 4 with poorly formed or fused glands as the most frequent reason for disagreement among the non-consensus cases. Further, we evaluated the performance of AI by comparing its grading against expert pathologists. Weighted kappas for all cases and non-consensus cases were 0.63 and 0.53, respectively, placing AI as the observer with the fourth-lowest reproducibility of all cases and the sixth-best of the non-consensus cases out of 23 observers in total. AI can thus make consistent decisions and it may serve as decision support for improved reproducibility in prostate cancer grading.

6 SUMMARY AND FUTURE PERSPECTIVES

Since the first study of this thesis was published in 2013 a lot has happened in the research field of prostate cancer. The introduction of MRI-targeted biopsies has improved prostate cancer diagnosis as more clinically significant cancers are detected and fewer clinically insignificant cancers compared to systematic biopsies (101). Robot-assisted RP has become a standard procedure for localized prostate cancer in most developed countries. Screening for prostate cancer is still a hot topic worldwide. In Sweden, as in most countries, population-based screening is not recommended as of today. Instead, shared decision-making is used, meaning that patients are encouraged to make informed decisions as to whether the benefits of screening outweigh the harms.

As our understanding of the molecular basis of prostate cancer deepens the possibility for personalized genomic profiling will increase and thus help to provide the most effective treatment for each patient. An example of a new therapy is abiraterone that targets AR and has been shown to prolong survival in men with castration-resistant prostate cancer (256). Moreover, patients with germline BRCA mutations have been shown to have a high response rate to the PARP inhibitor olaparib (257).

Nevertheless, histopathology is and will remain an important tool in the risk classification and management of prostate cancer. For such prognostic and predictive factors to improve, it is crucial to understand the possibilities and limitations of morphology. In the first study, we evaluated the prognostic significance of EPE and recommended the use of radial distance as a method to classify patients with EPE. At the ISUP consensus meeting in 2009, a majority of delegates stratified EPE as focal or established (130). The Epstein criteria were most often used but there was no consensus as to the method of classification. The International Collaboration on Cancer Reporting recommends using focal and established EPE by either Epstein's definition or Wheeler’s (258). This has until this day not been widely adopted in Sweden where national guidelines only state that pathologists should report EPE and not the extent (259).

Prostate cancer is a heterogeneous disease and the subset of patients with EPE needs to be further subclassified as the prognosis varies for these patients as well (144-147). Therefore, it is important that Swedish guidelines also include a method to stratify these patients.

Moreover, we reported considerable inflation of the Gleason grading over the last decades. In economics, inflation reflects a reduction in the purchasing power per unit of money, meaning a loss of real value. The shift towards higher grading is important to report as comparison

with historical datasets will be difficult and there is a risk for overtreatment. Especially with a concurrent stage shift towards lower stage at diagnosis. To date, there is no study reporting stabilization of prostate cancer grading, which is worrying. We also demonstrated that although the grade concordance between biopsy and RP specimens seemed to improve over time, this was rather an effect of changed grade distribution.

As people are getting older the cancer incidence rises. Adding the fact that we are heading towards more individualized treatment options, this results in pathologists going through many slides. Therefore, a clinical application of an AI system that can detect, quantify and grade prostate cancer could reduce the workload for pathologists and letting them focus on difficult cases. Known limitations in prostate cancer grading are intra- and interobserver variability as well as over- and undergrading. An AI system with expert-level performance might provide a second opinion and aid in standardizing grading. It may also provide pathology expertise in parts of the world where there is a shortage of trained pathologists. AI will most likely play an important role in the management of prostate cancer patients in a near future. Though, understanding its limitations and possibilities is essential for making it a helpful tool without jeopardizing patient safety.

We are in the middle of a pandemic and it seems it should at least be mentioned in this thesis. In NPCR, preliminary data shows a 40% reduction of prostate cancer diagnosis in the spring of 2020 compared to the same period in the last five years (5). The full extent of the impact of the COVID-19 pandemic is not known but delayed diagnoses and treatment will most likely affect prostate cancer patients, in particular those with aggressive cancer. Sadly, this decline in cancer incidence will most likely be followed by patients presenting with tumors in more advanced stages and a subsequent increase in cancer mortality.

Lastly, as a researcher and physician, it can be a challenge to apply statistics and research findings to each specific patient. For a disease like prostate cancer, it may be even trickier as it is a heterogeneous disease, and our prognostic models are far from perfect.

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