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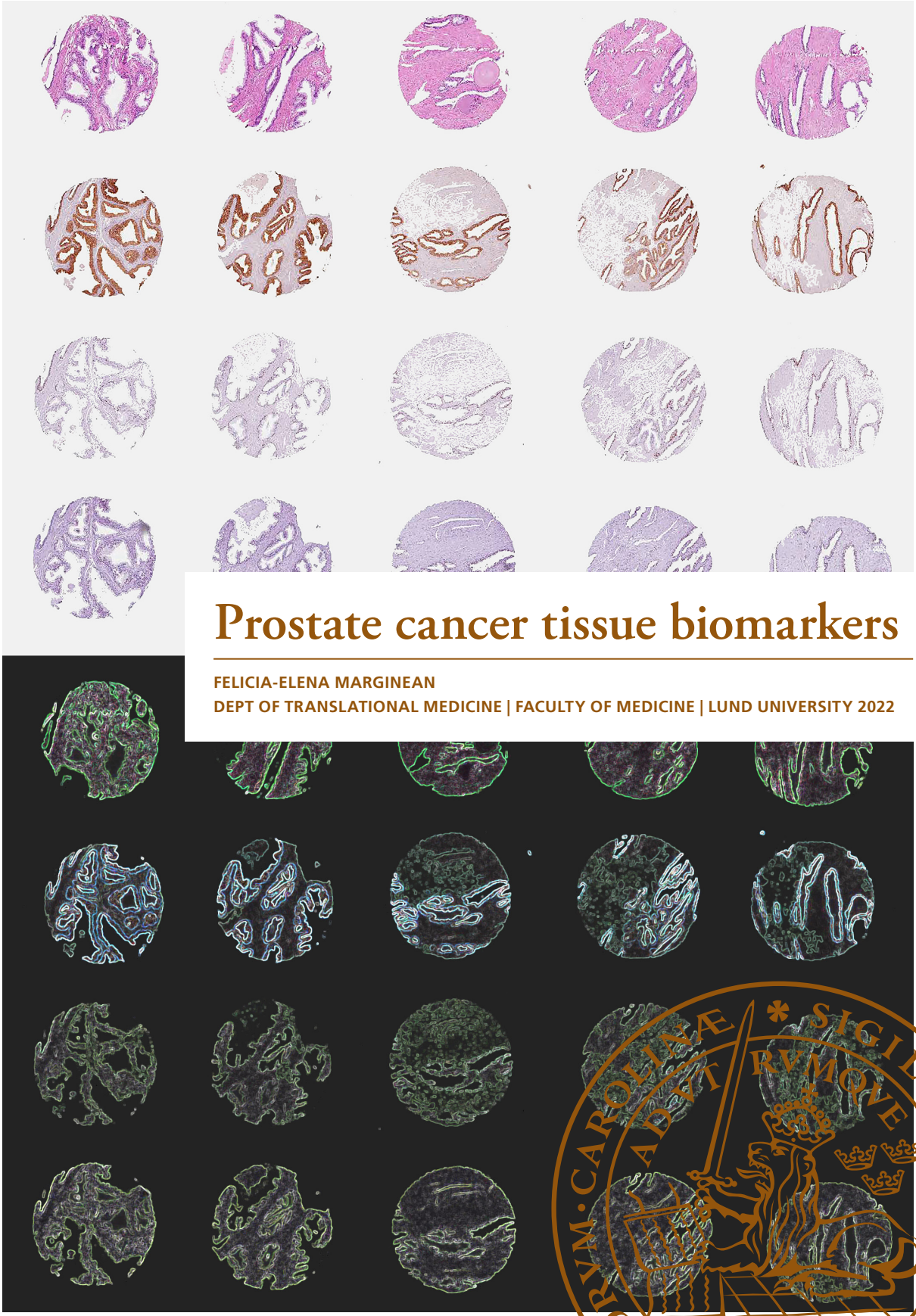

Prostate cancer tissue biomarkers

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Prostate cancer tissue biomarkers

Prostate cancer tissue biomarkers

Felicia-Elena Marginean

LUND
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DOCTORAL DISSERTATION

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Abstract Prostate cancer (PCa) is the second most common cancer in men worldwide. To optimize the best course of treatment for PCa, new biomarkers are required to determine prognosis, predict response to new treatments, and identify molecular targets.

The objective of this thesis was to evaluate different forms of Signal Transducer and Activator of Transcription 3 (STAT3) as PCa tissue biomarkers by immunohistochemistry and evaluate automated Gleason grading of cancer areas in prostate biopsies using an artificial intelligence (AI) based algorithm.

STAT3 and its activated forms, pSTAT3^{Tyr705} and pSTAT3^{Ser727}, are expressed in various cancers and implicated in the progression of PCa. The prognostic value of pSTAT3 at early disease stages has not yet been clearly identified.

We studied the expression levels of STAT3 in epithelial and stromal components of tissue obtained from hormone-naïve patients undergoing radical prostatectomy for localised PCa using tissue microarrays (TMAs). In the malignant epithelial glands, low expression of pSTAT3^{Tyr705} and pSTAT3^{Ser727} was associated with faster disease progression but did not significantly improve the prognostic value when added to Gleason score or pathological T stage (Paper 1).

Expression of pSTAT3^{Tyr705} and pSTAT3^{Ser727} in the stromal compartment, gave similar results as in the epithelium where low expression levels were correlated with reduced time until biochemical recurrence. In multivariable analysis, expression of pSTAT3^{Tyr705} and pSTAT3^{Ser727} did not add prognostic information when added to established prognostic factors like Gleason score, pathological T stage, and surgical margin status (Paper 2).

Expression of pSTAT3^{Tyr705} and interleukin-6 receptor (IL6R) were investigated in a unique TMA collection of metastatic tissues obtained from the rapid autopsy of castration-resistant prostate cancer patients (CRPC). Higher expression of pSTAT3^{Tyr705} and IL6R were found in bone metastases compared with lymph node and visceral metastases. STAT3 mRNA levels were also significantly higher in bone than in lymph node and visceral metastases, whereas no significant difference was observed in IL6R mRNA expression. These results suggest that targeting STAT3 may be a therapeutic option in patients with metastatic CRPC (Paper 3).

Visual inspection of histopathological slides is extremely time-consuming and hampered by inter- and intra-observer variability. We evaluated an AI-based algorithm for automated recognition and Gleason grading of cancer cells in PCa biopsies. The algorithm had a high sensitivity (100%) in detecting cancer areas, with a specificity of 68%. It was also good at detecting the different Gleason grades. The algorithm results were similar to the pathologist's analysis suggesting the algorithm to be a promising tool to improve the detection and diagnosis of PCa (Paper 4).

In conclusion, the expression of activated forms of STAT3 in hormone-naïve PCa is lower in cancer than in benign epithelium but has limited prognostic value. pSTAT3 and IL6R are highly expressed in PCa metastases, suggesting that inhibition of STAT3 may be a promising targeted therapy in metastatic CRPC. Applying an AI algorithm in a pathologist's daily workflow may be a good auxiliary tool to minimize the inter- and intra-observer variability and speed up the Gleason grading diagnostic process.

Keywords: Prostate cancer, biomarkers, STAT3, stroma, metastases, Artificial Intelligence, Gleason score, Biochemical recurrence

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Prostate cancer tissue biomarkers

Felicia-Elena Marginean

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MADE IN SWEDEN

Dedication: To my dear family and all my friends all around the world.

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List of papers

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I. Expression of tSTAT3, pSTAT3^{727} and pSTAT3^{705} in the epithelial cells of hormone-naïve prostate cancer:
Krzyzanowska A., Don-Doncow N., **Marginean F.E**., Gaber A., Watson R.W., Hellsten R., Bjartell A. 2019, *Prostate*, Volume 79(7), Pages: 784-797.

II. Nuclear expression of pSTAT3^{Tyr705} and pSTAT3^{Ser727} in the stromal compartment of localised hormone-naïve prostate cancer:
Marginean F.E., Krzyzanowska A., Hellsten R., Bjartell A. 2022, *Pathology - Research and Practice,* Volume 232, Page:153811.

III. Expression of STAT3 in prostate cancer metastases:
Don-Doncow N., **Marginean F**., Coleman, I., Nelson P. S., Ehrnström R., Krzyzanowska A., Morrissey C., Hellsten R., Bjartell, A. 2017, *European Urology*, Volume 71, Pages: 313-316.

IV. An artificial intelligence-based support tool for automation and standardisation of Gleason grading in prostate biopsies:
Marginean F., Arvidsson I., Simoulis A., Overgaard N.C., Åström K., Heyden A., Bjartell A., Krzyzanowska A. 2020, *Eur Urol Focus*, Volume 7, Pages: 995-1001.

Abbreviations

ADT	androgen deprivation therapy
AI	artificial intelligence
AMACR	alpha-methylacyl-CoA racemase
AS	active surveillance
BCR	biochemical recurrence
bpMRI	bi-parametric magnetic resonance imaging
BS	bone scintigraphy
CK34β12	cytokeratin 34β12
CNN	convolutional neural network
CRPC	castration-resistant prostate cancer
cT	clinical tumour stage
CT	computer tomography
CZ	central zone
DRE	digital rectal examination
EBRT	external beam radiotherapy
ELISA	enzyme-linked immunosorbent assay
GG	Gleason Group
GS	Gleason score
H score	intensity x percentage score
H&E	haematoxylin eosin staining
HDR	high-dose-rate
IHC	immunohistochemistry
IL-10	interleukin 10
IL-6	interleukin 6
IL-6R	interleukin 6-receptor
ISUP	International Society of Urologic Pathology
JAK	janus associated kinase
LDR	low-dose rate
MMPs	matrix metalloproteinases
mpMRI	multi-parametric magnetic resonance imaging
MRI	magnetic resonance imaging
NKX3.1	NK3 Homeobox 1

p63	tumor protein 63
PCa	prostate cancer
PCR	polymerase chain reaction
PET/CT	positron-emission tomography/computer tomography
pGS	pathological Gleason score
PSA	prostate-specific antigen
$pSTAT3^{Ser727}$	STAT3 phosphorylated at serine 727
$pSTAT3^{Tyr705}$	STAT3 phosphorylated at tyrosine 705
pT	pathological tumour stage
PZ	peripheral zone
RARP	robot-assisted radical prostatectomy
RP	radical prostatectomy
RRP	retropubic radical prostatectomy
RT	radiotherapy
SPECT	single-photon emission computed tomography
STAT3	signal transducer and activator of transcription 3
TMA	tissue microarray
TNM	tumor node metastasis
tSTAT3	total STAT3
TZ	transitional zone

Popular Science Summary (in English)

Prostate cancer (PCa) is a prevalent disease in men, the second most common cancer after lung cancer and one of the causes of death in men. PCa affects the general male population over 50 years.

The highest incidence tends to be in the more developed parts of the world. No etiologic agent is known for PCa. Age, family history, diet and smoking are some of the risk factors associated with prostate cancer.

However, the mortality rate in patients with PCa has registered an important decrease in the last decades due to the new methods of early detection prostate antigen specific (PSA) blood test, digital rectal examination, multiparametric magnetic resonance imaging (MP-MRI), as well as multiple treatment options from the initial stages till the metastatic disease.

The gold standard method of prostatic cancer diagnosis is the inspection of prostate tissue. Prostate tissue is provided by the urologist under image techniques, such as ultrasound or MP-MRI who takes multiple, systematic biopsy samples. These samples are then analysed under light microscope by the pathologist. The prostate tissue, under the microscope is divided into glands and stroma. The diagnosis of prostate cancer is based on the detection of abnormal architecture and disposition of the glands, which contain atypical cells and lack basal cells.

In 1966, Donald Gleason MD, classified PCa in grades determined by different glandular patterns, which change with the advancement of the cancer. The more advanced the cancer, the higher the grade. The Gleason grade is not only indicative of the prognosis of prostate cancer but also provides information about disease progression.

The treatment options of the prostate cancer depend on the disease stage at the moment of diagnosis, and include a variety of choices from less aggressive methods such as active surveillance, to curative local treatment by surgery, radiotherapy and androgen deprivation therapy

Hence, there are a variety of treatment options for PCa. Some cancers are not aggressive and can be monitored without treatment. Treatment can be offered when

the cancer turns aggressive. Curative treatments include surgery, radiotherapy or medication that blocks the production of male hormones (testosterone).

Many prostate cancers can be treated, however a small percentage of these are aggressive and can become resistant to treatment.

Therefore, it is important to be able to diagnose the cancer and determine how aggressive it could behave. This can be done with help of the tissue biomarkers – features that are present in the tissue and can predict the behavior of the cancer if it will become aggressive or not. These features could be different proteins or patterns in the tissue. Certain proteins can be detected in the tissue by means of a method called immunohistochemistry, whose presence can be noticed as a stained part of the tissue under the light of the microscope. Similarly, we can observe different patterns in the tissue under the microscope.

This thesis focuses on two biomarkers: STAT3 and Gleason grade.

STAT3 is a protein which participates in cell growth, proliferation, and differentiation. STAT3 is found in normal tissue where it is tightly controlled.

In cancer this control is lost, and STAT3 can change normal cells to become cancer cells. Analysing the levels of STAT3 in PCa tissue can give us indications on how the patient's disease can progress.

We studied the STAT3 levels in different tissue materials. In paper one and two we evaluated the levels of STAT3 on prostate tissue provided from the patients who underwent curative surgery.

The first paper showed that in patients with low levels of STAT3, the risk of cancer recurrence was elevated.

In the second paper we wanted to find the levels of STAT3 in the tissue surrounding the cancer glands. As expected, we found that low levels of STAT3 in the tissue surrounding the cancer also correlated with faster progression to metastatic disease.

In the third paper we evaluated the STAT3 levels in tissues from patients who died from PCa. The analysed tissue samples were from metastases such as bone, liver, lung, and lymph nodes. The highest levels were found in the bone, which is a common site for PCa metastases.

In the same study we evaluated the level of the most common cell receptor which activates STAT3, interleukin 6 receptor, and we found similar results as STAT3. These results point to STAT 3 as a promising target for anticancer drugs.

The second biomarker that occupied our research attention was the Gleason grade.

Prostate biopsy samples from patients are taken very commonly as long as this is the best method to detect the presence of the cancer. This generates a large volume of material that has to be examined by a pathologist under the light microscope. This

method is extremely time consuming and often there are disagreements between pathologists regarding the assessment of cancer grade in particular samples.

To make this process faster, more reliable and more uniform, there is a lot of interest in developing computerised artificial intelligence algorithms that can detect patterns on tissue and assign a grade.

In paper four, in collaboration with Dept of Mathematics in Lund and the software company Sectra we developed an algorithm which can detect and grade cancer on prostate tissue.

We found that the algorithm that we developed was very good in detecting cancer as well as estimating the severity of the cancer. We compared the results of the algorithm to two pathologists and found that the algorithm achieved optimal results.

These results point to this algorithm as a promising tool to improve the detection and diagnosis of prostate cancer.

In conclusion, this thesis found that STAT3 is a biomarker which low levels in tumour gland and the surrounding tissues are correlated with shorter time to disease progression. In the metastatic disease, high levels of STAT3 and IL-6 receptors were pointing out this biomarker as a promising drug target for therapy.

Applying an artificial intelligence algorithm to detect cancer on prostate tissue showed similar performance to that of the pathologist, demonstrating that it could be a valuable and user-friendly tool for prostate cancer diagnosis.

Resumen de la tesis.
(Summary in Spanish)

El cáncer de próstata es una enfermedad habitual en el hombre. Es el segundo cáncer más común después del cáncer de pulmón, y una de las principales causas de fallecimiento. Suele afectar a hombres mayores de 50 años.

Según datos epidemiológicos, la mayor incidencia de esta enfermedad se halla en las zonas mas desarrolladas del mundo, como son el Noroeste de Europa, Australia, y Norteamérica, con la tasa más alta reportada en los afroamericanos de Norteamérica. La menor incidencia se reporta en el continente asiático.

La edad, los antecedentes genéticos familiares, la dieta, y el tabaquismo son algunos factores de riesgo asociados a esta enfermedad.

La tasa de mortalidad en pacientes con cáncer de próstata (CaP) está en descenso, debido a la detección precoz con analíticas, tacto rectal, resonancias multiparamétricas, así como múltiples opciones de tratamiento.

La prueba más efectiva y segura que confirma la presencia del CaP es mediante el estudio detallado del tejido prostático. Las muestras de tejido obtenidas por el urólogo bajo control ecográfico, son examinadas mediante el microscopio óptico por el patólogo. El estudio microscópico del tejido prostático lo divide en dos componentes principales, glándulas y estroma. El diagnostico de CaP se basa en la observación de una disposición anormal de las glándulas, las cuales contienen células atípicas y carecen de cellulas basales.

En 1966, el doctor Donald Gleason, clasificó los diferentes patrones de disposion glándular según su arquitectura del 1 al 5, los determinaran los grados de Gleason (del 2 al 10). El grado de Gleason no solo indica el pronóstico de CaP, sino que también informa sobre la evolución de la enfermedad.

Hay multitud de opciones terapéuticas para el CaP, desde tratamientos poco agresivos como la supervisión activa, hasta tratamientos más agresivos como la cirugía, radioterapia, terapia a base de hormonas, o una combinación de ellos, dependiendo del estadio de la enfermedad en el momento del diagnóstico.

Aunque gran parte de los casos de CaP tienen tratamiento, sigue habiendo un pequeño porcentaje que tienen un comportamiento muy agresivo y la habilidad de desarrollar resistencia al tratamiento.

Por lo tanto, es importante diagnosticar el CaP y determinar lo agresivo que puede llegar a ser. Esto se puede hacer con la ayuda de biomarcadores que están presentes en el tejido y pueden predecir si el CaP se volverá agresivo o no con el tiempo. Los biomarcadores pueden ser diferentes proteínas y/o patrones del tejido prostático. La presencia de las proteínas se detecta mediante técnicas de inmmunohistoquimica que colorean las proteines estudiadas, las cuáles pueden ser evaluadas mediante el microscopio óptico,–al igual que los patrones arquitecturales de las glándulas malignas.

El estudio de esta tesis está centrado en dos biomarcadores: STAT3 y los grados de Gleason.

La STAT3 es una proteína que está presente en el tejido normal y participa activamente en la homeostasis celular (crecimiento, diferenciación, proliferación) de forma estrictamente controlada.

En el cáncer, este control se pierde, y gracias al STAT3 las células benignas se convierten en malignas, ocurriendo un crecimiento y proliferación incontrolados, y desarrollando la capacidad de evasión de la muerte celular programada y la habilidad de invasión y metástasis.

El estudio de los niveles del STAT3 en el tejido prostático maligno pueden aportar información sobre la evolución de la enfermedad.

Hemos estudiado los niveles del STAT3 en diferentes tejidos. En el primer y segundo articulo hemos analizado los niveles del STAT3 en muestras del tejido prostático maligno proveniente de pacientes que han sido intervenidos quirúrgicamente sin previo tratamiento hormonal o radioterapéutico. En este artículo hemos demostrado que niveles bajos de STAT3 en las glándulas malignas se correlaciona con la recidiva del CaP en un periodo de tiempo menor de lo habitual.

En el segundo artículo, hemos estudiado los niveles de STAT3 en el tejido que rodea las glándulas cancerosas. Como habíamos sospechado, aquí también los niveles bajos de STAT3 en estos tejidos se relacionan con la metástasis en un corto espacio de tiempo.

En el tercer artículo, hemos evaluado los niveles de STAT3 en pacientes que han muerto por CaP. El material analizado proviene del tejido metastásico en hueso, hígado, pulmón y ganglios linfáticos. Los niveles más altos de STAT3 se encontraron en los depósitos metastáticos óseos. En el mismo estudio hemos evaluado los niveles del receptor celular que más comúnmente activa el STAT3, el receptor de la interleukina 6, y hemos hallado resultados similares a los de STAT3.

Estos resultados señalan al STAT3 como una diana terapéutica anticancerígena prometedora.

El segundo biomarcador en el cual hemos centrado nuestra investigación son los grados de Gleason.

La biopsia prostática es el método habitual de diagnóstico del CaP. Esto genera un gran volumen de muestras para el patólogo, las cuales se analizan de forma tradicional mediante el microscopio óptico. Un método que además de requerir mucho tiempo, genera diferencias de interobservacion entre patólogos dedicados al estudio de los grados de Gleason. Para acelerar este proceso y estandarizarlo, hemos desarrollado un algoritmo basado en inteligencia artificial para detectar diversos patrones y asignar el grado de Gleason correspondiente.

Este proyecto de inteligencia artificial es presentado en el artículo número cuatro. Gracias a la colaboración del departamento de Matemáticas de Lund y la compañía de software Sectra se hizo posible el desarrollo de un algoritmo con el cual podemos detectar el grado de cáncer en las muestras de tejido prostático.

El algoritmo tiene la capacidad de detectar las áreas de cáncer al igual que predecir la severidad. La comparación de los resultados proporcionados por el algoritmo con los resultados de dos patólogos independientes determinó una correlación óptima.

Estos resultados hacen del algoritmo una herramienta a tener en cuenta para mejorar la detección y el diagnóstico del CaP.

En conclusión, en esta tesis hemos demostrado que STAT3 es un biomarcador que correlaciona niveles bajos en las glándulas malignas y en tejidos circundantes al tumor con metástasis tempranas. En la enfermedad metastásica, los altos niveles de receptores para STAT3 y IL-6 apuntan a una prometedora diana terapéutica.

La aplicación de los algoritmos de inteligencia artificial en la detección de cáncer y la determinación de los grados de Gleason han demostrado resultados similares a los diagnósticos y gradación determinados por los patólogos.

En comparación con el método tradicional, este nuevo método basado en inteligencia artificial aporta como ventajas una disminución significativa en el tiempo dedicado al análisis y una mayor precisión y uniformidad en el diagnóstico del CaP. Por lo tanto, puede ser una herramienta muy valiosa para el trabajo del patólogo.

Rezumatul tezei doctorale (Summary in Romanian)

Cancerul de prostată este una dintre bolile cele mai comune la bărbați.

Este al doilea cancer ca frecvență la bărbați după cancerul de plămâni, făcând parte dintre bolile cauzatoare de moarte.

In general, afectează bărbații începând cu vârsta de 50 de ani.

Incidența cea mai ridicată a acestei boli se întâlnește in populația masculină din zonele lumii dezvoltate.

Nu se cunoaște un factor etiologic, dar vârsta avansată, bagajul genetic familial, alimentația și fumatul sunt unii dintre factori de risc asociați cancerului de prostată.

Cu toate acestea, rata mortalității la pacienți afectați de aceasta boala, a înregistrat un declin important datorită noilor metode de depistare precoce ca testul de sânge pentru determinarea antigenului specific prostatic, examenului rectal, rezonanța magnetică precum și datorită amplului evantai de tratamente disponibile începând cu stadiile precoce ale bolii pâna la cel metastatic .

Testul cel mai sigur pentru confirmarea diagnosticul cancerului de prostată este examenul microscopic al țesutului prostatic.

Țesutul prostatic este obținut de către urolog, prin biopsii repetate ale glandei prostatice sub control ecografic sau rezonanță magnetică sistematică.

Aceste biopsii, dupa o prelucrare specială, sunt examinate de către anatomopatolog cu ajutorul microscopului optic.

Microscopic țesutul prostatic se divide in glande și stromă. Detectarea unei dispoziții anormale a glandelor alcătuite din celule atipice confirmă diagnosticul de cancer de prostată.

Doctorul Donald Gleason, in 1966 a clasificat dispoziția glandelor prostatice tumorale in diverse modele pe care le a numit gradele Gleason. Sunt in număr de 5, de la 1 la 5, si sunt corelate cu agresivitatea cancerului, de la cel indolent, gradul 1, la cel cu potențial metastatic, gradul 5.

Acest grad Gleason nu doar confirma diagnosticul de cancer de prostată, ci oferă și informații despre evoluția bolii.

Opțiunile de tratament sunt multiple, de la cele mai puțin agresive ca stricta supraveghere pana la cele cu scop curativ precum chirurgia, radioterapia, tratament hormonal cu antiandrogeni, depinzând de stadiul bolii in momentul diagnosticului.

Majoritatea cancerelui de prostată este tratat cu success, dar exista o mică proporție care are un comportament agresiv si care devine rezistent la tratament pe parcursul lui.

De aceea este importantă diagnosticarea corectă a cancerului pentru a caracteriza agresivitatea si comportamentul lui.

Acest lucru se poate face cu ajutorul biomarkerilor tisulari - caracteristici speciale ale țesutului tumoral care pot da informații despre agresivitatea evoulției cancerului. Aceste caracteristici sunt reprezentate de diverse proteine sau de modele de organizare ale glandelor tumorale in țesut.

Anumite proteine se pot detecta in țesuturi cu ajutorul metodei numite imunohistochimie, cu ajutorul căreia se observă prezența acestora sub forma de marcaje colorate sub lumina microscopului optic. La fel se pot recunoaște diferite modele de distribuție in țesutul canceros.

Aceasta teza de doctorat se centrează pe doi biomarkeri: STAT3 si gradul Gleason.

STAT3 este o proteina care participa in ciclul celular in procesele de creștere, proliferare, diferențiere. Se găsește in țesutul normal si este sub un strict control.

În cancer acest control se pierde si astfel STAT3 are capacitatea de a determina celulele normale sa devină celule canceroase. Prin analiza nivelelor de STAT3 in țesuturile canceroase putem obține informații despre evoluția bolii.

Am studiat nivelurile de STAT3 in materialele prelevate din țesuturi diferite.

În primul și în al doilea articol am evaluat nivelurile de STAT3 in țesuturile prostatice provenite de la pacienții tratati chirurgical prin extirparea glandei prostatice in scop curativ.

În primul articol am observat că pacienții cu niveluri scăzute de STAT 3 in glandele canceroase au avut tendința de recidivă a cancerului in scurt timp.

În al doilea articol am studiat nivelurile de STAT 3 in țesutul din jurul glandelor tumorale. Cum am prevăzut, si in acest țesut nivelurile scăzute de STAT3 au fost relaționate cu progresul rapid spre metastazarea cancerului.

În al treilea articol am evaluat nivelurile de STAT3 în țesutul metastatic provenit de la pacienți decedați de cancer de prostată.

Biopsiile leziunilor metastatice au fost recoltate din metastaze din os, ficat, plămân si ganglioni limfatici. Nivelurile cele mai înalte de STAT3 le-am găsit in metastazele de os, care este unul dintre locurile cele mai frecvente de metastază.

În același timp am studiat nivelurile receptorului cel mai comun interleukine -6 (IL-6) care activează STAT3 si rezultatele au fost similare cu cele ale STAT3.

In baza acestor rezultate STAT3 este un candidat favorit in terapiile anticancer pentru cancerul de prostata.

Al doilea biomarker pe care ne am concentrat atenția în această teză este gradul Gleason.

Biopsiile de prostată sunt frecvent prelevate de la pacienți, fiind proba cea mai eficientă pentru detectarea cancerului.

Aceata practică generează un volum de muncă mare pentru patolog. Examinarea țesutului cu ajutorului microscopului optic este un procedeu care necesita timp lung si adesea cu incongruențe in privința desemnării gradului Gleason.

Pentru a scurta acest timp de examinare cât si pentru uniformizarea detectării gradului Gleason un important interes s-a conferit dezvoltării algoritmilor bazați pe inteligența artificială.

In articolul 4, in colaborare cu departamentul de Matematică al Universității din Lund și compania de software Sectra am conceput și dezvoltat un algoritm care poate detecta zonele de cancer si gradul Gleason în același timp.

Rezultatele obținute de către algoritm au fost foarte bune in detectarea cancerului și a severității lui.

Am comparat rezultatele obținute de algoritm cu cele obținute de doi patologi și am găsit că performanța algoritmului este optima.

In baza rezultatelor obținute acest algoritm este văzut ca o unealtă utila in munca patologului, cu minim de timp consumat si o mai buna detectare si caracterizare a cancerului.

În concluzie această teză a evidențiat că nivelurile scăzute ale biomarkerului STAT3 atât în glandele canceroase cat și în țesutul din jur sunt corelate cu progresul rapid al boli.

Nivelurile ridicate ale STAT3 si IL-6 in boala metastatică reprezintă o țintă terapeutica promițătoare.

Utilizarea algoritmului bazat pe inteligența artificială pentru depistarea cancerului si corecta clasificare a gradului Gleason a demonstrat rezultate similare cu cele obținute de patolog, indicând acestă nouă tehnică ca o viitoare unealtă simplă în rutina patologului pentru obținerea unei ridicate acurateți în diagnosticul cancerului de prostată.

Résumé de thèse de doctorat (Summary in French)

Le cancer de la prostate est une maladie prévalente chez l´homme, le deuxième cancer le plus fréquent après le cancer du poumon et l'une des causes de décès chez l´homme. Le cancer de la prostate touche en général la population masculine de plus de 50 ans.

L'incidence la plus élevée pour ce type de cancer se trouve plus fréquemment dans les régions les plus développées du monde.

Aucun agent étiologique est connu pour le cancer de la prostate. L'âge, les antécédents familiaux, l'alimentation et le tabagisme sont quelques-uns des facteurs de risque associés au cancer de la prostate.

Cependant, le taux de mortalité chez les patients atteints d'un cancer de la prostate a enregistré une baisse importante au cours des dernières décennies grâce aux nouvelles méthodes de détection précoce de l'antigène prostatique spécifique (PSA), au toucher rectal et à l'imagerie par résonance magnétique multiparamétrique (MP-MRI), ainsi que de multiples options de traitement depuis les stades initiaux jusqu'à la maladie métastatique.

La méthode de référence pour le diagnostic du cancer de la prostate est l'examen du tissu prostatique. Le tissu prostatique est fourni par l'urologue qui s´assiste de techniques d'imagerie, telles que l'échographie ou la MP-IRM (résonance magnétique paramétrique multiparamétrique) pour réaliser des biopsies multiples et systématiques. Ces échantillons de tissue sont ensuite analysés au microscope optique par le pathologiste. Le tissu prostatique, vu au microscope, est divisé en glandes et en stroma. Le diagnostic du cancer de la prostate est basé sur la détection d´anomalies de l´architecture des glandes, qui contiennent en outre des cellules atypiques et ont perdu leurs cellules basales.

En 1966, Donald Gleason MD a classé les cancers de la prostate en fonction de grades déterminés par les différentes architectures glandulaires; plus le cancer est avancé, moins les glandes sont bien définies et plus le grade est elevé. Le grade de Gleason est non seulement indicatif du pronostic du cancer de la prostate, mais fournit également des informations sur la progression de la maladie.

Les options de traitement du cancer de la prostate dépendent du stade de la maladie au moment du diagnostic et comprennent une variété de choix allant des méthodes les moins agressives comme la surveillance active, jusqu'au traitement local curatif par chirurgie, radiothérapie et thérapie de privation des récepteurs aux androgènes.

Par conséquent, il existe une variété d'options de traitement pour le cancer de la prostate.

Certains cancers ne sont pas agressifs et peuvent être surveillés sans traitement. Un traitement peut être proposé lorsque le cancer devient agressif. Les traitements curatifs comprennent la chirurgie, la radiothérapie ou des médicaments qui bloquent la production d'hormones mâles (testostérone).

De nombreux cancers de la prostate peuvent être traités curativement, mais un petit pourcentage d'entre eux sont agressifs et peuvent devenir résistants au traitement.

Par conséquent, il est important de pouvoir diagnostiquer le cancer et de déterminer son comportement agressif. Cela est rendu possible par le biais des biomarqueurs tissulaires - des caractéristiques présentes dans les tissus et qui peuvent prédire le comportement du cancer, s'il deviendra agressif ou non. Ces caractéristiques peuvent être différentes protéines ou différents arrangements des glandes dans le tissu. Certaines protéines peuvent être détectées dans le tissu au moyen d'une méthode appelée immunohistochimie: leur présence est notée comme une partie colorée du tissu en microscopie optique. Les différents arrangements des glandes cancéreuses sont aussi visibles en microscopie optique.

Cette thèse se concentre sur deux biomarqueurs: STAT3 et le grade de Gleason.

STAT3 est une protéine qui participe au cycle cellulaire pour les étapes de croissance, proliferation et différenciation. STAT3 se trouve dans les tissus normaux où il est étroitement régulé.

Dans le cancer, cette régulation disparaît: STAT3 peut ainsi transformer des cellules normales en cellules cancéreuses. L'analyse des niveaux de STAT3 dans les tissus cancéreux de la prostate pourrait nous donner des indications sur la façon dont la maladie peut progresser pour chaque patient.

Nous avons étudié les niveaux de STAT3 dans différents tissus. Dans les articles un et deux, nous avons évalué les niveaux de STAT3 sur le tissu prostatique fourni par les patients ayant subi une chirurgie curative.

Le premier article a montré que chez les patients présentant de faibles niveaux de STAT3, le risque de récidive du cancer était plus élevé.

Dans le deuxième article, nous voulions étudier les niveaux de STAT3 dans les tissus entourant les glandes cancéreuses. En accord avec nos prévisions, nous avons constaté que de faibles niveaux de STAT3 dans les tissus entourant le cancer étaient également corrélés à une progression plus rapide vers la maladie métastatique.

Dans le troisième article, nous avons évalué les niveaux de STAT3 dans les tissus de patients décédés de cancer de la prostate. Les échantillons de tissus analysés provenaient de métastases provenant des os, du foie, des poumons et des ganglions lymphatiques. Les niveaux les plus élevés de STAT3 ont été trouvés dans l'os, qui est un site commun pour les métastases PCa.

Dans la même étude, nous avons évalué le niveau du récepteur cellulaire qui active le plus souvent STAT3, le récepteur de l'interleukine 6, et nous avons trouvé des résultats similaires à ceux de STAT3.

Ces résultats désignent STAT 3 comme une cible prometteuse pour les médicaments anticancéreux.

Le deuxième biomarqueur qui a fait l'objet de nos recherches était le grade de Gleason.

Des biopsies de la prostate de patients sont prélevés très couramment puisqu´il s´agit de la meilleure méthode pour détecter la présence de cancer. Cela génère un grand volume de matériel qui doit être examiné par un pathologiste au microscope. Cette méthode est chronophage et il existe des désaccords entre les pathologistes concernant l'évaluation du grade de Gleason pour certaines biopsies.

Pour rendre ce processus plus rapide, plus fiable et plus uniforme, nous nous intéressons beaucoup au développement d'algorithmes informatisés d'intelligence artificielle capables de détecter des arrangements spécifiques des glandes sur les tissus et de leur attribuer un grade de Gleason.

Dans le quatrième article, en collaboration avec le département de mathématiques de Lund et la société de logiciels Sectra, nous avons développé un algorithme capable de détecter et de classer le cancer sur le tissu prostatique.

Nous avons constaté que l'algorithme que nous avons développé était très bon pour détecter le cancer ainsi que pour estimer la gravité du cancer. Nous avons comparé les résultats de l'algorithme à deux pathologistes et avons constaté que l'algorithme obtenait des résultats optimaux.

Ces résultats indiquent que cet algorithme est un outil prometteur pour améliorer la détection et le diagnostic du cancer de la prostate.

En conclusion, cette thèse a révélé que STAT3 est un biomarqueur dont les faibles niveaux dans la glande tumorale et les tissus environnants sont corrélés avec un délai de progression de la maladie plus court. Dans la maladie métastatique, des niveaux élevés de récepteurs STAT3 et IL-6 indiquent que ce biomarqueur pourrait constituer une cible thérapeutique prometteuse.

L'application d'un algorithme d'intelligence artificielle pour détecter le cancer dans le tissu prostatique a montré des performances similaires à celles du pathologiste,

démontrant qu'il pourrait s'agir d'un outil précieux et facile d´utilisation pour le diagnostic du cancer de la prostate.

Populärvetenskaplig sammanfattning (Summary in Swedish)

Prostatacancer är en vanlig sjukdom hos män, den näst vanligaste cancersjukdomen efter lungcancer och en av dödsorsakerna hos män. Prostatacancer drabbar den allmänna manliga befolkningen över 50 år. Den högsta förekomsten tenderar att befinna sig i de mer utvecklade delarna av världen.

Ingen känd etiologi finns för prostatacancer. Ålder, ärftlighet, kost och rökning är några av de riskfaktorer som är förknippade med prostatacancer.

Dödligheten hos patienter med prostatacancer har dock registrerat en betydande minskning under de senaste decennierna i samband med de nya metoderna för tidig upptäckt av prostata specifikt antigen (PSA) blodprov, rektalpalpation och multiparametrisk magnetisk resonanstomografi (mpMRI), samt med de flera behandlingsalternativ, från de första stadierna av sjukdomen till fjärrmetastaser.

Diagnos av prostatacancer bygger på undersökningen av prostatavävnad. Prostatavävnad tillhandahålls av urologer efter ultraljudsledda systematiska biopsier eller idag allt vanligare riktade biopsier efter magnetkameraundersökning. Dessa prover analyseras sedan under ljusmikroskop av patologen. Prostatavävnaden är histologiskt uppdelad i körtlar och stroma. Diagnosen av prostatacancer är baserad på upptäckten av en avvikande fördelning av körtlarna, som dessutom innehåller atypiska celler och saknar basalceller.

Redan år 1966 introducerade patologen Donald Gleason en klassifikation baserad på tumörcellerna växtmönster. Ju mer avancerad cancer, desto mindre välorganiserat mönster av tumörceller. Gleason graderingen ger värdefull information om sjukdomens prognos och kan vägleda behandling.

Behandlingsalternativen för prostatacancer beror på sjukdomsstadiet vid diagnostillfället. Behandlingar varierar från exspektans med aktiv övervakning, till kurativ behandlingen med kirurgi eller strålbehandling till bromsande, palliativ behandling som blockerar produktionen av manliga hormoner (testosteron).

Många prostatacancrar kan behandlas, men en liten andel av dessa är aggressiva och kan bli resistenta mot behandling.

Därför är det viktigt att kunna diagnostisera cancern och avgöra hur aggressiv den kan bete sig. Detta kan göras med hjälp av vävnadsbiomarkörerna – egenskaper som finns i vävnaden och kan förutsäga cancerns beteende. Dessa egenskaper kan vara olika proteiner eller mönster i vävnaden. Vissa proteiner kan detekteras i vävnaden med hjälp av en metod som kallas immunhistokemi, och ses då som en färgad del av vävnaden vid mikroskopundersökning. På samma sätt kan vi observera olika mönster i vävnaden under mikroskopet.

Detta avhandlingsarbete fokuserar på två biomarkörer: STAT3 och Gleasons grad.

STAT3 är ett protein som är involverad i tumörcellers tillväxt och överlevnad. STAT3 finns i normal vävnad där dess aktivitet är hårt reglerat. I cancer går denna kontroll förlorad. Att analysera nivåerna av STAT3 i prostatacancervävnad kan ge oss indikationer på hur patientens sjukdom kan fortskrida.

Vi studerade STAT3-nivåerna i olika vävnadsmaterial.

I de två första artiklarna utvärderade vi nivåerna av STAT3 i prostatavävnad från patienter som genomgick kirurgi i botande syfte.

Den första uppsatsen visade att hos patienter med låga nivåer av STAT3 var risken för recidiv av cancern förhöjd. I den andra artikeln ville vi hitta nivåerna av STAT3 i vävnaden som omger cancerkörtlarna. Som förväntat fann vi att låga nivåer av STAT3 i vävnaden som omger cancern också korrelerade med snabbare progression till metastaserande sjukdom.

I den tredje artikeln utvärderade vi STAT3-nivåerna i olika vävnader från patienter som dog av spridd prostatacancer. De analyserade vävnadsproverna var från metastaser från ben, lever, lungor och lymfkörtlar. De högsta nivåerna hittades i skelettmetastaser, som är en vanlig lokalisation för prostatacancer med spridning.

I samma studie utvärderade vi nivån av den vanligaste cellreceptorn som aktiverar STAT3, interleukin 6-receptorn, och vi hittade liknande resultat som för STAT3. Våra resultat pekar på att blockering av STAT3 kan vara en ny behandling vid prostatacancer med spridning till skelettet där tidigare behandling inte längre har effekt.

Den andra biomarkören som var i fokus i vår forskning var Gleason-graden.

Prostatabiopsier är standardmetoden för att diagnostisera prostatacancer. Det är en stor volym av prover som måste mikroskop undersökas av en erfaren patolog. Det är extremt tidskrävande och ofta finns det meningsskiljaktigheter mellan patologer om bedömningen av cancergraden för vissa prover.

För att göra processen snabbare, mer tillförlitlig och mer enhetlig finns det ett stort intresse för att utveckla datoriserade algoritmer för artificiell intelligens som kan upptäcka mönster i vävnaden och ange en Gleason grad.

I artikeln fyra har vi i samarbete med Matematikcentrum i Lund och mjukvaruföretaget Sectra utvecklat en algoritm som kan upptäcka och gradera cancer på prostatavävnad.

Vi fann att algoritmen som vi utvecklade hade god precision för att upptäcka cancerceller samt att bestämma Gleason grad. Vi jämförde resultaten av algoritmen med två patologer och fann att algoritmen gav bra resultat.

Dessa resultat pekar på denna algoritm som ett lovande verktyg för att förbättra upptäckten och diagnosen av prostatacancer.

Sammanfattningsvis fann denna avhandling att STAT3 är en biomarkör där låga nivåer i tumörcellerna och i omgivande vävnad är korrelerade med kortare tid till sjukdomsprogression vid tidigare obehandlad prostatacancer. Vid spridd sjukdom där hormonbehandling inte längre har god effekt, kan blockering av aktiverat STAT3 utvecklas till nya behandlingar vid terapiresistent prostatacancer.

Att tillämpa en artificiell intelligens algoritm för att upptäcka cancer på prostatavävnad visade liknande prestanda som erfarna patologer, vilket visar att det kan vara ett värdefullt och användarvänligt verktyg för diagnos av prostatacancer.

The prostate gland

Anatomy and physiology

The prostate gland is the largest accessory sex gland in men and is found in all mammals. It is approximately the size of a chestnut and weighs 20–30 g. It is placed in the pelvic cavity, below the urinary bladder, in front of the rectum and above the fascia of the urogenital diaphragm. The prostate gland arrives at maturity at puberty. The prostate gland secretes a slightly acidic fluid rich in simple sugars, enzymes (one of them prostate-specific antigen -PSA), immunoglobins, and zinc which represents 20% of seminal fluid. The prostate fluid, together with the other semen components, has an alkaline pH which neutralizes the acidity of the vagina. In this way, the sperm is protected from damage, and the motility and fertility of the spermatozoa are increased (1). The whole development, maturation, and maintenance process is under androgen control, with contributions from somatotrophic hormones, retinoic acid, and oestrogen (2).

Macroscopy

The prostate gland includes four regions: three glandular zones (the peripheral, transition, and central zones) and a fourth, non-glandular region, the anterior fibromuscular stroma (3) (Figures 1 and 2).

The largest zone is the peripheral zone. It is the site of most prostate cancers (PCa). The second zone is the transitional zone which includes part of the urethra and usually harbours the benign lesions, for example benign prostatic hyperplasia. The third glandular zone is the central zone which is crossed by the ejaculatory ducts which open into the urethra. The anterior part of the prostate is the non-glandular part, formed by smooth and skeletal muscle bundles and fat. Prostate lymphatic drainage to the retroperitoneal lymph node chain is done through the pelvic lymphatics system.

Nerves from the prostatic plexus serve the prostate gland. Branches of the internal iliac artery supply the arterial blood (4).

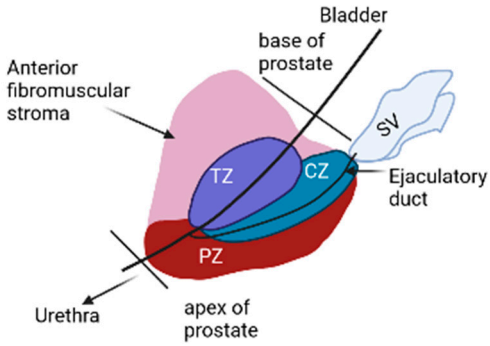

Figure 1. Normal prostate anatomy. SV seminal vesicle; PZ, peripheral zone; TZ, transitional zone, CZ-central zone.

Microscopy

All three glandular zones include ducts and acini, with glands composed of two-cell layers (Figure 2).

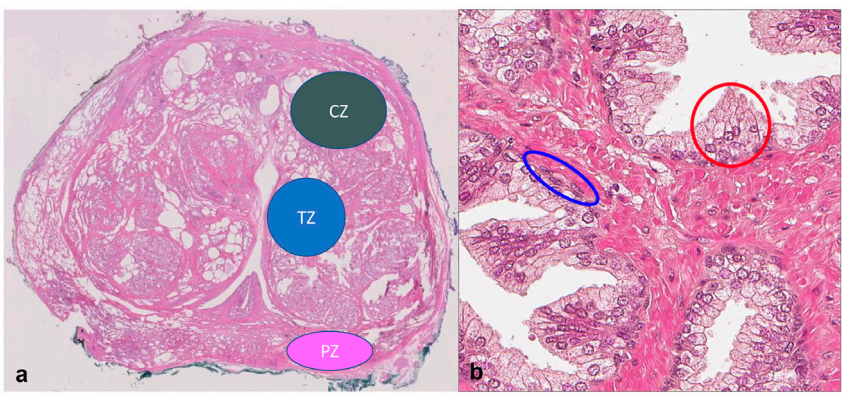

Figure 2: Histology of the prostate gland. (a) Transversal section of the prostate gland stained with haematoxylin-eosin (H&E) with the three zones highlighted. CZ = central zone, TZ = transitional zone, PZ = peripheral zone. (b) High magnification of a prostate gland, demonstrating acini with the luminal layer (red circle) and the basal layer (blue circle).

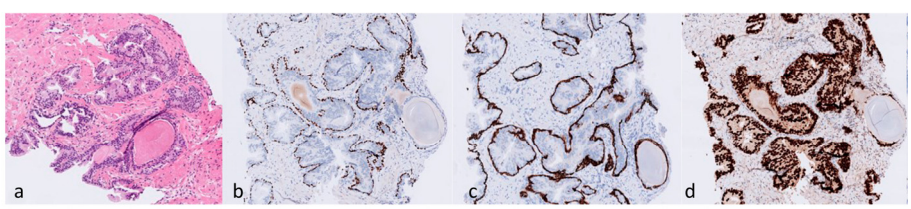

Figure 3. Normal prostate acini. H&E staining and different immunohistochemistry staining. (a) H&E, (b) p63 nuclear staining in basal cells, (c) CK34β12 cytoplasmic staining in basal cells, (d) NKX3.1 nuclear staining is mainly restricted to the luminal epithelial cells.

The luminal layer (the most superficial, adjacent to the gland`s lumen) contains secretory cells. These cells are epithelial cells, columnar or pseudostratified, and secrete enzymes, hormones, and other substances. One of these enzymes, the prostate-specific antigen (PSA), is clinically relevant. Once this glycoprotein enzyme, is secreted, it is released into the urethra. To detect the cells from the luminal layers, immunohistochemical (IHC) staining for PSA or NKX3.1 can be used (5) (Figure 3).

The luminal cells are surrounded the basal cell layer. Basal cells can be detected with IHC staining for high-molecular-weight cytokeratin (34ßE12) or p63 (Figures 3 and 4). These cells are missing in PCa.

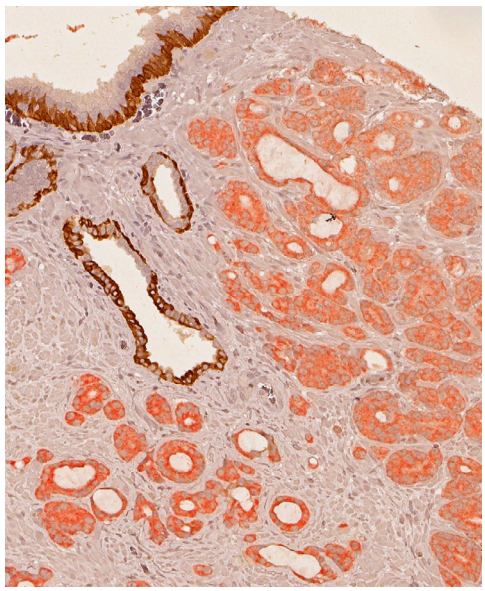

Figure 4. Triple immunohistochemical staining: AMACR (pink), CK34β12 (brown), and p63 (brown). Basal cell layer, stained with p63 in the nucleus and CK34β12 in the cytoplasm, is present in the normal prostate acini. Cancerous luminal epithelial cells are positive for AMACR.

Among these basal cells, it is possible to identify scattered neuroendocrine cells, which are detected using neuroendocrine markers, such as synaptophysin, chromogranin, or CD56, as well as rare stem cells.

The two cell layers, luminal and basal, are surrounded by the basement membrane, which contains two types of collagens (IV and V) (Figure 5), glycosaminoglycans, polysaccharides, and glucolipids, and represents the interface to the stromal compartment.

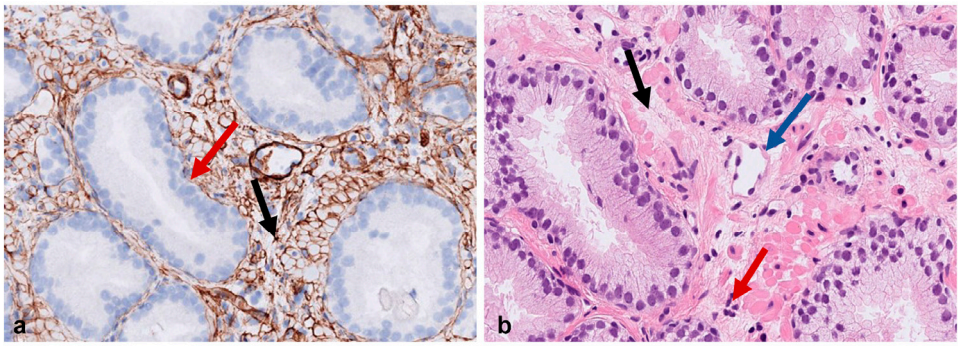

Figure 5. (a) Collagen IV staining basement membrane (red arrow) and the non-epithelial compartment (black arrow). (b) H&E staining of non-epithelial compartment: stroma and tissue matrix with fibroblasts (red arrow), endothelial cells from the capillary and lymphatics (blue arrow), and extra cellular matrix (black arrow).

The non-epithelial compartment includes an extracellular matrix with a variety of cells, such as smooth muscle cells, fibroblasts, endothelial cells (capillary and lymphatic structures), neuroendocrine cells, axons (from nerve structures), and immune cells, which may be sparse or in larger infiltrates (Figure 6) (6) (7, 8).

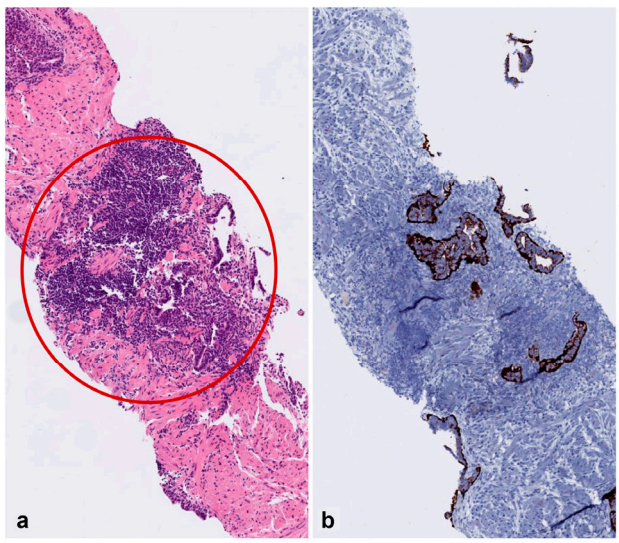

Figure 6. (a) H&E staining of normal prostate acini infiltrated by immune cells (red circle). (b) High molecular weight cytokeratin CK34β12 staining in normal prostate acini.

Prostate cancer

Prostate cancer general considerations

PCa is a heterogeneous disease with a range of survival from long (in case of localised disease and low risk of progression) to short (the aggressive type with rapid spread and poor prognosis).

The choice of treatment for PCa depends on a series of parameters that include the risk group, the patient's general health, life expectancy, and patient choice. In the case of localised PCa, where the patient has a long life expectancy (>10 years), the treatment can vary and might be active surveillance, radiotherapy, or surgery (9).

Epidemiology

In terms of incidence, PCa is the third most common cancer globally, surpassed only by breast and lung cancer (10). In men, PCa is the second most common cancer, and the fifth-highest cancer-related cause of death, after lung, liver, colorectum and stomach cancer (11). In Sweden, PCa is the most frequent malignancy in men and ranks first as a cause of cancer-related death (12).

PCa incidence is highest in elderly men (≥65 years old) and is rarely detected before 40 years. Among patients ≥ 75 years, 75% die of PCa. In patients older than 82 years, only 50% of them have a cause of death of PCa. The PCa incidence is highest in developed countries (e.g., North America and Scandinavia) and lowest in China. The highest worldwide incidence was detected among African-American men (13).

The mortality rate from PCa has declined during the last 20 years due to increased detection in the early stages (largely the result of the implementation of PSA screening programs) and limiting disease progression to advanced stages due to aggressive treatment for PCa with novel therapeutic options (14) (15).

Risk factors

Risk factors are defined as anything that can trigger a disease. In PCa, the well-known risk factors are age, ethnic origin, and genetic mutations.

Age has an important role in PCa, both as a risk factor and when determining treatment options. In white men without a previous family history, the predisposition to PCa is increased above 50 years of age. In men whose family history includes one member with PCa, the risk for disease is doubled (or quadrupled if there are two or more cases in the family). If the father has PCa, the risk is higher in his brother relative to his son (16, 17).

In black men, those ≥ 40 years old are considered at increased risk, with a doubled rate in incidence and mortality compared to other groups.

In patients with the above risk factors, PCa diagnoses are typically before 60 years old (18) and, on average, 6–7 years earlier than those who are the first member in the family with a diagnosis of PCa. Despite the earlier manifestation, the course of the disease is not more aggressive than the normal population with PCa (19).

The true hereditary represents only a small percentage of patients with PCa. Among black North American men, African American men have the highest risk for PCa, driven by genetic linkage in Chromosome 1q (20, 21).

Other reported risk factors include germline mutations, obesity, and alcohol and tobacco use (22).

Preventive methods for PCa, such as no smoking, sustaining daily physical activity, and maintaining a healthy weight, are not supported by medical evidence and should only be considered general lifestyle recommendations. Nevertheless, such preventive methods help protect against damage to epithelial cells caused by persistent oxidative stress (22-25).

Clinical diagnosis

PCa at an early stage generally gives no symptoms. The symptoms appear in locally advanced or metastatic disease. Among the symptoms of locally advanced disease, the most common are the urinary symptoms (such as haematuria, renal failure, hematospermia, and impotence), and in metastatic disease, pathological fracture, bone pain, back pain, anaemia, oedema, and disseminated intravascular coagulation (26).

The first steps in PCa diagnosis include serum prostate-specific antigen (PSA) quantification, digital rectal examination (DRE), and systemic transrectal ultrasound (TRUS)-guided biopsies (27).

Digital rectal examination

Digital rectal examination is recommended in the clinical diagnosis of PCa in cases with elevated levels of PSA. An abnormal DRE combined with an elevated PSA test was associated with a high rate in the detection of PCa. DRE increases the sensitivity and specificity of the PSA test (28, 29). Compared with the gold standard, the prostate core biopsy, DRE alone has a low sensitivity and specificity of around 60% (30). The low sensitivity and specificity are due to difficulties detecting cancer based on its anatomical location, the palpable tumour size, and examinator experience (31).

Imaging MRI and ultrasonography

Transrectal ultrasound is the first choice for primary PCa diagnosis but with limitations regarding poor image resolution and to interpret tumour extension (32).

Magnetic resonance imaging (MRI) is a sensitive and specific tool for detecting localised and metastatic PCa but is less performant in detecting the involvement of lymph nodes. Today, multiparametric or bi-parametric magnetic resonance imaging (mpMRI, bpMRI) is a valuable tool in detecting localised and locally advanced PCa and for the guidance of targeted biopsies.

Bone scintigraphy (BS) is the standard method for assessing bone metastases. The method uses Technetium 99 which reacts with bisphosphonate from osteoblastic PCa metastases. The radioactive material is visible in the “active” osteoblastic areas. A higher sensitivity of BS can be obtained by combining BS with single-photon emission computed tomography (SPECT) (22, 33).

A new method which is more and more used for detecting recurrent disease and in primary staging of intermediate and high-risk PCa is the PET/CT - Positron emission tomography/computed tomography (34). Compared with conventional imaging methods, this technique evaluates the disease based on a combination of metabolic and morphological processes.

Prostate biopsy

The number of systematic prostate biopsies recommended is between 8 and 12, depending on the prostate size. They can be obtained by an ultrasonography-guided transrectal or transperineal approach, and today, centres are increasingly adopting targeted MRI fusion biopsies (35-38).

PCa diagnoses are based on a series of features, including architecture (atypical glands, glomerular shape of glands, isolated cells), nuclear features (prominent nucleoli), intraluminal contents (crystalloids), desmoplastic stroma, and absence of basal cells with the help of immunohistochemistry (IHC).

The acinar prostatic adenocarcinoma has many variants that can mimic benign lesions. Among these are the atrophic, pseudohyperplastic, microcystic, foamy gland, mucinous (colloid), signet ring-like, pleomorphic giant cell, and sarcomatoid variants (39).

Gleason score/ISUP

The gold standard in the diagnosis of PCa, the Gleason score, was developed by Dr Donald Gleason between 1966 and 1974. The Gleason score is based on the patterns of malignant epithelial proliferation. Dr Gleason defined five patterns from the most differentiated to the least differentiated (Figure 7).

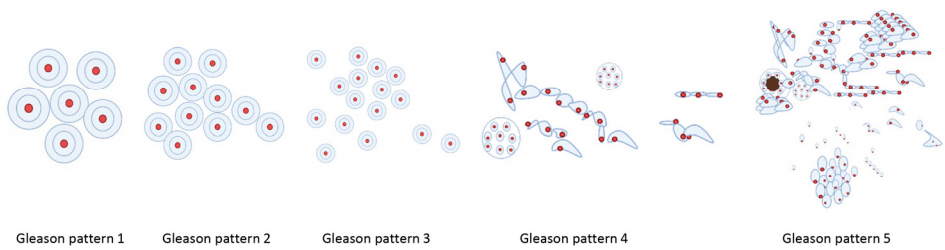

Figure 7. Gleason pattern 1 is a circumscribed nodule that includes uniform, separated acini. Gleason pattern 2 is similar to pattern 1 but with a looser architecture. Gleason pattern 3 is characterized by discrete, well-defined glands. Gleason pattern 4 is characterized by poorly formed or fused poorly formed glands, cribriform, and glomeruloid patterns. Gleason pattern 5 is characterized by solid sheets of cancer, small cords, nests, and individual single cells. Solid nests with comedo-necrosis or cribriform glands with central necrosis are also seen.

During the years, the Gleason scoring system has been modified on three occasions: a major modification in 2005 and two minor modifications in 2014 and 2019. These modifications were based on patients' clinical follow-ups and implemented by the International Society of Urological Pathology (ISUP).

In 2005, Gleason patterns 1 and 2 were eliminated from the Gleason scale by the ISUP due to their poor reproducibility and poor correspondence with the grading in

the prostatectomy samples. They are extremely rare, occasionally found in transurethral resection specimens (TURPs), in the transitional zone or possibly at the apex (40).

Until 2005, the Gleason score (GS) was the sum of the predominant patterns with the most prevalence. In 2005, the ISUP decided that the score should be the sum of the most predominant pattern and the highest grade pattern. In 2014, there was a further slight modification due to a highly clinically significant distinction between GS 7 (3+4) and GS 7 (4+3). The ISUP has introduced the group grading system (five groups) (Table 1) (41-43).

Table 1 ISUP classification of prostate cancers

Gleason score	ISUP Group
Gleason score ≤ (3+3)	Group 1
Gleason score (3+4),	Group 2
Gleason score (4+3),	Group 3
Gleason score (4+4=8, 5+3=8, 3+5=8)	Group 4
Gleason score (5+4 or 5+5).	Group 5

The presence of Gleason pattern 4 in ISUP Group 2 (Gleason score 3+4=7) and Group 3 (Gleason score 4+3=7) has an important role in patient's outcome and treatment decisions, which is why it was decided to separate them into two distinct ISUP groups.

Gleason pattern 4 includes four different architectural growth patterns of the glands: ill-formed glands, fused glands, glomeruloid glands, and cribriform glands. Among these four architectural patterns, the cribriform pattern is of particular interest due to its clinical implications (44, 45) (46).

Among pathologists, the ill-formed gland pattern of Gleason 4 pattern is the poorest reproducible pattern by the core needle biopsy (47). This is because of the similarity of Gleason pattern 3 on tangentially sectioned glands. It is recommended not to grade ill-formed fused glands as Gleason pattern 4 when they represent less than 5% of an area with well-defined Gleason pattern 3 glands (48).

On the contrary, the glomeruloid and cribriform patterns have high reproducibility (47, 49). The cribriform pattern can be found in intraductal cancer and invasive PCa. Intraductal cribriform PCa can be found mixed with the invasive PCa pattern Gleason 4 and 5 (Figure 8).

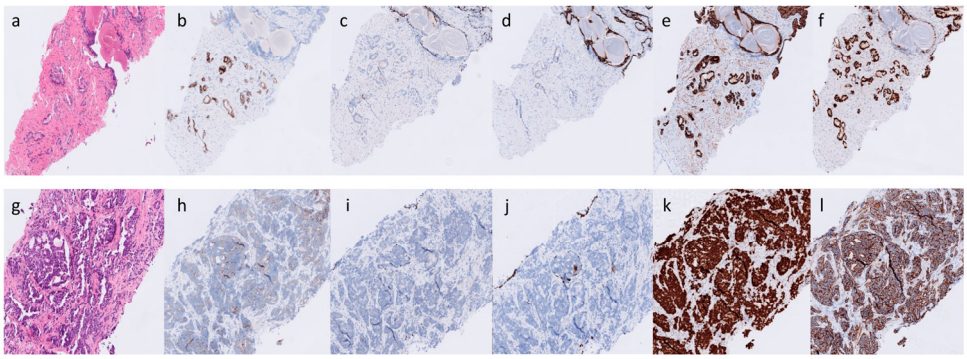

Figure 8. Biopsy staining examples with Gleason patterns 3 (top row, (a-f) and 4 and 5 (bottom row, (g-l). (a), (g) H&E. (b), (h) -AMACR cytoplasmic immunoreactivity staining in the luminal cells. (c), (i) p63 nuclear staining in the basal cells of benign glands and intraductal prostate cancer. (d), (j) CK34β12 cytoplasmic staining in basal cells of benign glands and intraductal prostate. (e), (k) NKX3.nuclear staining in benign and malignant luminal epithelial cells. (f), (l) CK AE1/AE3 cytokeratin staining with immunoreactivity staining in the epithelial cells.

Although morphologically, intraductal and invasive cribriform PCas are identical, genetically, they are two different entities with more loss of heterogeneity in intraductal PCa compared with invasive PCa (45). Intraductal and invasive cribriform PCas are associated with worse outcomes and germline mutations in DNA repair genes, BRCA2, and others (50) (42, 51).

The cribriform invasive Gleason 4 pattern was a strong prognostic marker for metastasis and disease-specific death post radical prostatectomy (51).

In September 2019, the ISUP included the cribriform Gleason pattern 4 in the guidelines for reporting biopsy and prostatectomy diagnosis (52). The presentence of Gleason pattern 4 in ISUP groups 2 and 3 has to be reported as a percentage in the biopsies and the presence and significance of the invasive cribriform pattern in percentages. The presence of intraductal cancer (IDC) will be included in the percentage of invasive. In case there is no evidence of invasive cancer in the biopsy, the IDC must be mentioned but not graded and not considered cancer. In radical prostatectomy diagnosis, tertiary Gleason patterns 4 and 5 must be reported if they represent ≥5% of the tumour volume. The presence and percentage of invasive and intraductal cribriform patterns have to be reflected in the pathology report in the biopsy and the prostatectomy.

Staging of Prostate Cancer

PCa staging is based on the Tumor Node Metastasis (TNM) classification system, which characterizes cancer. Tumour (T) provides information on the size of cancer. Node (N) refers to the number of lymph nodes infiltrated by the cancer cells. Metastasis (M) is characterised by cancer spreading to other organs. The treatment and the prognosis of the disease are based on this classification (39).

Clinical staging (cT-stage) (Figure 9) describes the tumour extension at digital rectal examination (DRE) and pathological stage (pT) describes the tumour extension upon histopathological examination of the surgical specimen. It follows the clinical staging except for clinical stage T1c and the T2 substage. After radical prostatectomy, the localised organ-confined PCa is considered T2, and the substages are no longer recognized. N-stage describes: the presence or absence of disease in local regional lymph nodes (Figure 10).

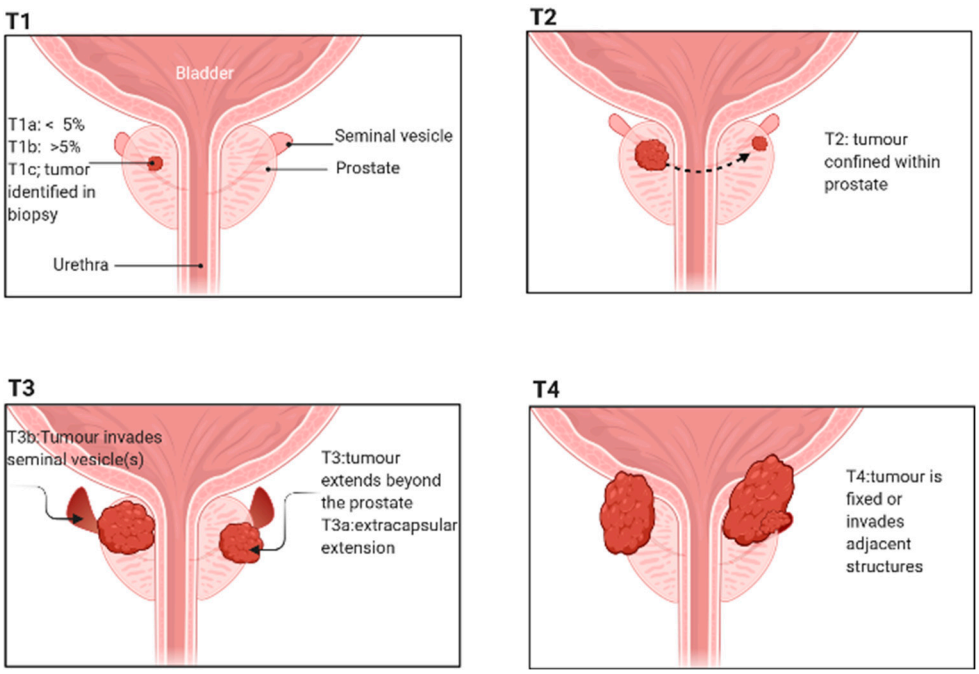

Figure 9. Staging of carcinomas of the prostate.

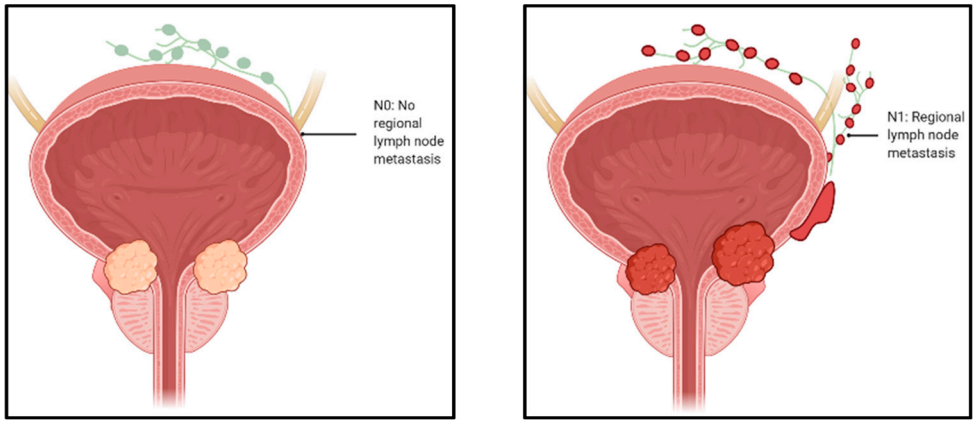

Figure 10: N-stage regional lymph node status .

Metastasis-stage (M) is the presence or absence of the disease in other organs, such as bone, liver, and lungs. Different imaging methods can detect metastases, including bone scintigraphy, computer tomography (CT), and positron emission tomography (PET).

Risk Classification

Risk classification was first implemented by D'Amico et al. and included PSA levels, cT with DRE, and the biopsy Gleason score. At the moment of diagnosis, the patients can be stratified into low, intermediate, or high risk (53). Several modifications have been proposed, and the different risk groups have been divided into subgroups, as exemplified by the Swedish National Guidelines for Prostate Cancer guidelines (54) (Table 2).

Table 2. Risk classification groups for localised prostate cancer. Table adapted from the Swedish National Guidelines for Prostate Cancer (54).

Localised Pca				
Risk group	**PSA (ng/mL)**	**Gleason score**	**ISUP group**	**cT-stage**
Very low risk	<10	≤6	Group 1	cT1
Very low risk	In total, ≤ 8 mm of cancer in ≤ 4 out of 8-12 biopsy cores. PSA density < 0.15 µg/cm			
Low risk	<10	≤6	Group 1	cT1-2a
Intermediate risk	10 to 19	7	Group 2/3	cT2b
High-risk	≥20	8 to 10	Group 4/5	cT2c
High-risk		≥8 or > half of biopsy cores with Gleason 4+3=7		
Locally advanced Pca	Any PSA	Any Gleason score	Any ISUP group	cT3-4 or cN+

Treatment of Prostate Cancer

Once the suspicion of PCa, based on DRE and/or PSA, is confirmed by the histopathological examination, different treatment options are available. The optimal treatment is made based on disease status and with life expectancy and quality of life in mind (55) (22).

PCa is not only an organ disease but also a complex, multifaceted issue with mental and physical implications. Therefore, the best course of treatment should take into account the patient's wishes to provide the best quality of life (56).

Treatment for localised PCa

Active surveillance

Active surveillance is one of the conservative treatment forms in men with clinically localised PCa that do not need urgent curative treatment. These patients are monitored under a strict periodic control in order to detect the cases which switch from an indolent disease to an aggressive one (57, 58). This control includes a series of tests such as PSA, mpMRI, clinical examination and prostate biopsies.

Another conservative treatment is watchful waiting. This is a treatment recommended in elderly patients with a short life expectancy of less than 5 years and who have other pathologies. The patients are monitored for local or systemic disease progression. In the case of progression, symptomatic treatments are applied. (22).

Radiotherapy

Radiotherapy is a curative treatment option, but it is also used for palliative care and the treatment of metastatic disease. There are two ways to apply this form of therapy. One is by external beam radiotherapy (EBRT), using gamma radiations focussed on the prostate tissue but with effects on the surrounding tissues. The second is brachytherapy. Brachytherapy is localised radiotherapy that uses radioactive sources (seeds) inserted into the prostate gland. If a low dose rate is needed (LDR), these seeds are iodine or palladium, with a half-life of ~60 days. At the high-dose-rate (HDR), iridium seeds are used.

Combined radiotherapy with hormonal treatment is the standard method for intermediate and high-risk PCa, particularly for locally advanced diseases.

A disadvantage of radiotherapy involves the effects on surrounding tissue that can be shorter or longer, including proctitis and cystitis, and fistulas in severe cases.

Surgery

Surgical removal of the prostatic gland and surrounding tissue could be done in the classic open way retropubic radical prostatectomy (RRP) or, more commonly, robot-assisted radical prostatectomy (RARP).

Today the RARP is preferred upon RRP. Advantages of RARP include less bleeding, optical three-dimensional visualization with better identification of crucial parts (which leads to easier vesicourethral anastomosis), and preservation of the neurovascular bundles, thereby improving postoperative functional outcomes. Regarding oncologic and functional outcomes, in the long term, in patients with localised PCa, RARP is a safer technique than RRP (59).

Treatment for Advanced Prostate Cancer

Treatment in patients with metastatic disease as the first manifestation

According to the European Association of Urology (EAU) guideline recommendations, multiple treatments are available to patients with metastatic disease at first manifestation (22). These include systemic androgen receptor deprivation therapy (ADT), local palliative radiotherapy (RT) or targeted RT in those with spinal cord compression or pathological fracture, combined systemic ADT with chemotherapy or with the second generation of antiandrogens, androgen synthesis inhibitors, or more recently developed therapies.

Treatment of prostate cancer in patients with biochemical recurrence

Salvage RT is the recommended treatment for patients with biochemical recurrence (BCR) after RP with curative intent. In those with BCR after salvage high intensity focused ultrasound or salvage cryosurgical ablation and salvage brachytherapy are recommended. In patients with recurrence of disease after ADT, when the disease becomes “castration-resistant”, the therapy options are multiple. It is recommended that a multidisciplinary team proposes the optimal treatment for prolonging life based on the first-line treatment, symptoms, disease extension, and the patient's wishes.

Biomarkers

Definition of Biomarkers

A biomarker is an objective parameter that can measure normal or pathogenic biological processes or responses to medical treatment (60). A biomarker can be detected in tissue or body fluids (e.g., blood, urine, semen). Biomarkers encompass a variety of molecules, including DNA, mRNA, enzymes, metabolites, transcription factors, and cell surface receptors. Their detection includes various methods depending on whether they are intra- and/or extracellular. Among them, the most used are: the classic immunohistochemistry in tissue (IHC), polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), electrophoresis, Raman spectroscopy, and colorimetric and fluorescence assays (61, 62), Patterns in histological preparations can also provide prognostic information. The Gleason score is an important prognostic factor in PCa and could be considered a histologic biomarker.

A good biomarker can identify tumours from a very early stage, confirm the diagnosis, follow up on the treatment efficiency, and detect clinical recurrence.

Depending on the information provided, biomarkers can be classified into diagnostic, prognostic, or predictive.

Diagnostic biomarkers are biological parameters that detect the presence of a disease or a particular medical condition. Based on this particular condition, the patient receives adequate treatment or is enrolled in a clinical trial studying a particular disease (63).

Prognostic biomarkers supply information about the natural course of the disease. A biomarker to be a prognostic factor must be significant, independent, and suitable for clinical use (64).

Predictive biomarkers are pathophysiologic markers that can predict response to specific treatments (63).

All these biomarkers gather and classify information that can be used to make the best clinical decisions and provide optimal treatment.

Prostate Cancer Biomarkers

Screening is a medical tool that aims to identify disease in healthy asymptomatic populations. Early diagnosis detects the disease at a very early stage among people with symptoms. Several biomarkers are used or have been proposed for screening or early detection of PCa.

Blood Biomarkers

The most common prostate-related screening biomarker is Prostate-specific antigen (PSA) in blood.

PSA was discovered by Flocks in 1960, purified in tissue in 1979 by Wang and quantified in blood by Papsidero in 1980. In 1987, Stamey stated its value as a predictive PCa marker based on clinical trials (65). It is produced by secretory cells and has a role in semen for liquefaction and protection. It is detected in serum with a normal value between 2 and 4 ng/ml (66).

An elevated level of total PSA in blood indicates the presence of PCa, but at moderately increased values (3–10 ng/ml). The specificity is low due to poor discrimination between benign and malignant processes, meaning that it is a prostate-specific biomarker but not cancer-specific. The blood test for total PSA has been evaluated in screening programs for early detection of PCa, but the findings of small insignificant tumours caused overdiagnosis and overtreatment (67). The conclusion was that the risk does not outweigh the benefits, and therefore, general screening programs have not been implemented (68). Despite its controversial results, there is evidence that the rate of mortality and advanced-stage disease decreased due to the screening programs (69).

Different molecular forms of PSA have been identified and measured, such as total PSA (tPSA), which is the common PSA test, free PSA (fPSA), intact PSA, pro-PSA, and PSA in complex with other proteins (e.g., α-1 antichymotrypsin, α-1-proteinase inhibitor, and α-2 macroglobulin) with varying prognostic values. Based on the above studies, researchers have developed multiplex tests to improve the accuracy of PSA to detect PCa and avoid unnecessary biopsies. A few tests are commercially available (70).

The four kallikrein (4K) score test measures free, intact, and total PSA and kallikrein-like peptidase 2, and the test also considers age, DRE, and prior biopsy status (71).

The Prostate Health Index (PHI) test includes three individual PSA in a mathematical equation: (–p2PSA/fPSA)×√PSAtPSA, tPSA and pro-PSA. It has better results than the %fPSA and is recommended by the European Association of Urology (EAU) in risk calculators (72-74).

Both of the above tests are intended to avoid overdiagnosis by reducing the number of unnecessary prostate biopsies. There are prospective multicenter studies showing that both 4K and PHI are superior to the ratio of free-to-total PSA in predicting clinically significant PCa on biopsy in men with a PSA value of 2–10 ng/ml (73, 75)

The STHLM3 model has been developed to avoid unnecessary prostate biopsies and decrease the high false-positive rates of the PSA test in the screening population for the detection of high-risk PCa (Gleason score ≥7). This model is a combination of a blood test of different proteins (PSA, free PSA, intact PSA, hK2, MSMB, MIC1), a gene panel and clinical information, including age, family history, previous prostate biopsy, digital rectal examination. It was found to have better specificity and the same sensitivity as the PSA test alone, with a cut-off of 3 ng/ml. It had a high success rate in detecting high-risk PCa (Gleason score ≥7) and reducing prostate biopsies with benign diagnosis (76, 77).

More recently, a new variant of the STHLM3 model involved adding MRI to better stratify the patients in the screening population. The first results show that the combination of MRI and the STHLM3 test increases precision, decreases over detection, and decreases the number of men that need a prostate biopsy (78). In another study using the same STHLM3 model and comparing MRI with targeted and standard prostate biopsy, MRI and standard biopsy had the same powers to detect clinically significant PCa in a screening population, but MRI with targeted biopsy was inferior in the detection of clinically insignificant cancer (79)

A microsimulation study was performed to evaluate the screening cost/effectiveness of the STHLM3 test combined with MRI compared to only PSA screening. The STHLM3 model combined with MRI and PSA ≥ 2 resulted in a 60% cost reduction compared with the traditional screening using just PSA (80).

AR-V7 is a biomarker representing the splice variant of the androgen receptor, variant 7. It can be detected in circulating tumour cells and is associated with castration resistance to enzalutamide and abiraterone (81). It has been explored as a prognostic biomarker as well as a therapy target (82) (83).

Genetic Biomarkers

Germline mutations in DNA repair genes have been associated with an increased risk of PCa. Implementation of germline testing and genetic counselling has been proposed for the early detection of clinically significant PCa. Mutations in the *BRCA2* and *HOXB13* genes have been correlated to an increased risk of clinically significant PCa. Other genes with known mutations of importance are ATM, CHEK2, BRCA1, and MMR genes (MLH1, MSH2, MSH6 and PMS2) (84-86) (87); (88-93). However, none of these genes is tested routinely in the clinic.

Urine Biomarkers

The first urine test for detection of PCa was Progensa based on the PCa gene 3 (PCA3), and it measures a non-coding mRNA obtained after prostatic massage (94, 95).

The most common promoter genes in PCa are TMPRSS2-ETS fusion genes which is an androgen-regulated promoter and coding sequence of the ETS family. It was found in the early stage of the PCa with implications for diagnostic and therapy. In urine, the combination between TMPRSS2-ERG and PCA3 improves the sensitivity in PCa diagnosis (96). A commercial assay called the Michigan Prostate Score (MiPS), which combines all these 3 biomarkers, TMPRSS2-ERG and PCA3, has improved cancer prediction (97). A more recently developed test is SelectMDX, which is a multimodal model measuring the mRNA levels of the *HOXC6* and *DLX1* genes. The test has been shown to predict high-grade PCa (98).

Even though the above tests are available, screening of urine biomarkers are not generally recommended for detection and stratification of PCa.

Tissue Biomarkers

Commercial tissue biomarker tests are available to improve patients' stratification and decide on the optimal treatment of primary cancer.

The Oncotype Dx®, Oncotype DX Genomic Prostate Score (GPS) assay is a genomic assay that includes a 17-gene panel designed for risk assessment and as a predictive marker for disease aggressiveness. It is used at the time of diagnosis to guide the treatment options. In combination with clinical risk factors, this test can predict biochemical recurrence within 3 years and death because of metastatic PCa within 10 years (99).

Prolaris® is a genomic test that measures cell cycle progression. This test provides information for patients with low or intermediate risk of PCa prostate for choosing active surveillance or extensive treatment. In patients with intermediate or high-risk PCa, the test can help in avoiding additional and intensive therapy (100).

Decipher® is a genomic test that uses the expression of 22 selected RNA markers. It can be used as a prognostic marker in patients with prostate diagnosis in biopsy but also in those with radical prostatectomy. In both categories, this test helps develop the treatment strategy and can predict metastases and cancer-specific mortality. Patients with previous biochemical recurrence can help in the decision of salvage therapy versus salvage radiotherapy (101).

The ProMark® is a protein-based test used as a prognostic test in early-stage PCa, providing information about cancer’s behaviour and the possibility of choosing the active surveillance or currant treatment (102).

An expert panel from an initiative of ASCO has made a statement that none of these assays is recommended for routine clinical use as they have not been prospectively tested or shown to improve long-term outcomes, including survival and quality of life (102) However, they may provide additional information on top of clinical parameters.

Apart from the above mentioned commercially used biomarkers, many tissue types and proteins can be studied with the aid of immunohistochemistry to identify new prognostic markers and attempt to elucidate possible disease mechanisms. Tissue microarrays (TMAs) are among the most used methods for such biomarker studies (103).

A TMA is a collection of small, multiple tissue samples on a single slide. The tissue samples can come from different patients or from different tissues from one patient. The precise and orderly arrangement makes it possible to trace each patient and tissue source and connect the sample with corresponding clinical information. It is a quick and cost-efficient method for the evaluation of multiple biomarkers, generating quantitative data for many patients, using small amounts of reagents in a short time; therefore, this method is commonly used in oncology to study new biomarkers. The selection of the material and the classification of the samples during the construction of a TMA are important. The sampling is usually done with the help of a pathologist in order to correctly identify the sampled areas (for example, the cancer tissue). The small sampling areas in a TMA can be a limitation, not representing the whole tissue and limiting the study of tumour heterogeneity. A larger cohort size tends to dilute some of the sampling errors (104). Nevertheless, TMAs have been a valuable tool in biomarker discovery in the last 20 years.

Signal transducer and activator of transcription 3

Out of many proteins expressed in the prostate, the transcription factor Signal Transducer and Activator of Transcription 3 (STAT3) has been proposed as a tissue biomarker of interest in PCa. STAT3 is one of the seven proteins of the STAT family. It is found in many cells, especially in epithelial and hematopoietic cells. STAT3 plays an important role in normal cells' homeostasis, cellular growth, differentiation, apoptosis, and inflammation (105). STAT3 is activated by phosphorylation via Janus associated kinases (JAK), SRC, or non-receptor tyrosine kinases. The activation of STAT3 may be induced by inflammatory cytokines (e.g., IL-6, IL-10) and growth factors (e.g., VEGF, EGF), among other factors (106-109).

STAT3 may be activated by phosphorylation at tyrosine 705 ($pSTAT3^{Tyr705}$) and serine 727 ($pSTAT3^{Ser727}$). Upon phosphorylation, STAT3 is dimerized and transferred to the nucleus, where it binds to STAT3 DNA binding sites and initiates the transcription of multiple genes (Figure 11) (110) (111). In normal cells, this process is under tight control. $pSTAT3^{Ser727}$ might also have a non-transcriptional role in mitochondria, which are involved in cell respiration (112).

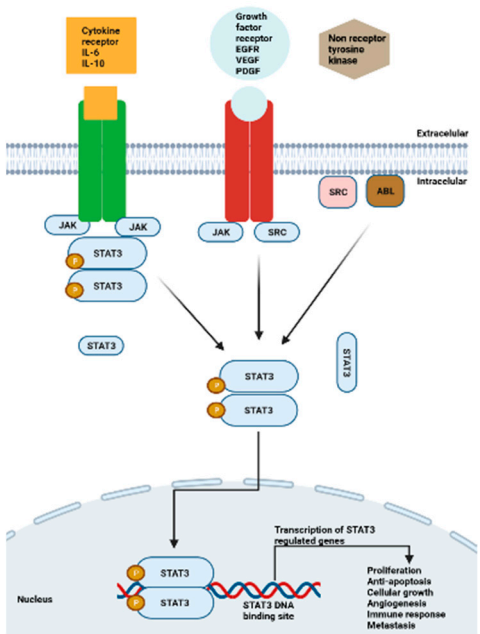

Figure 11. Overview of the STAT3 signalling pathway

STAT3 modulates various genes involved in various functions such as cell growth (cyclin D1, c-myc), apoptosis (survivin, BCL-XL, MCL-1), angiogenesis (vascular endothelial growth factor VEGF), metastases (matrix metalloproteinases MMPs) and immune escape (IL-6, IL-10) (113).

In cancer, STAT3 may be constitutively activated by loss of the negative regulation of STAT3, excessive stimulation of STAT3, positive feedback loops keeping persistent STAT3 activation and hyperactivation by somatic mutations (110).

Aberrant activation of STAT3 may lead to uncontrolled cell proliferation, improved cell avoidance of programmed cell death, increased cell migration, invasion, survival, greater therapy resistance and increased metastatic potential (108, 109, 111).

The activated forms of STAT3 are found in numerous cancers, such as breast, colon, prostate, lung and thyroid (114).

STAT3 in Prostate Cancer

In PCa, the activated forms of STAT3 have been studied at different stages, from early localised disease to metastatic CRPC. Many studies aimed to evaluate the prognostic and predictive biomarker potential of STAT3 expression.

Many studies have found that elevated levels of $pSTAT3^{Tyr705}$ were higher in the tumour cells compared with the normal epithelial cells (115-117), yet no difference in expression was found in another study (118).

High levels of $pSTAT3^{Tyr705}$ were associated with a worse prognosis (115, 117, 119, 120). In some studies, high expression levels of $pSTAT3^{Tyr705}$ were associated with a high Gleason score (115, 117, 121). In others, low expression levels of pSTAT3 were associated with an increased Gleason score (122). And in yet others, no association was observed (118, 119). $pSTAT3^{Ser727}$ expression was not associated with survival, Gleason score, or time to relapse (119).

The above studies include a series of limitations in their methodology, such as doubtful staining (119, 121), low number of patients (116, 118, 119), unclear tissue origin (if it comes from treated patients or hormone-naïve) (116), thus making results unreliable. Due to these methodology issues, it is difficult to extrapolate the expression status of pSTAT3 in PCa tissue. Not many studies have been performed on the expression of STAT3 in metastatic disease. Abdulghani et al. observed high levels of pSTAT3 in lymph node metastases and lower levels in metastases located in bone and other organs (123)

STAT3 is involved in the crosstalk between the tumour cell and tumour microenvironment. Activated STAT3 in the tumour microenvironment modulates the normal cell response, helping the tumour cells to develop the capacity for

invasion, angiogenesis, metastasis, and drug resistance (124) (125-127). Studies in different tissue types have reported opposite results regarding $pSTAT3^{Tyr705}$ expression in the microenvironment. In breast cancer, high expression levels were correlated with a good prognosis (128). On the contrary, in colon cancer, the increased level was related to reduced overall survival (129). To date, not much is known about the pSTAT3 in the PCa tissue microenvironment.

IL-6 is one of the common inflammatory cytokines that activate STAT3. High levels of IL-6 in serum were reported to be involved in developing hormone-refractory PCa and lower survival rates (119) (130).

STAT3 inhibitors have been proposed as targeted therapy for PCa (109, 131). However, as yet, no STAT3 inhibitors have been clinically approved. The small molecule STAT3 inhibitor galiellalactone decreased tumour growth, increased apoptosis, and reduced metastatic spread in an *in vivo* model of metastatic PCa (132). However, it is difficult to compare animal models that simulate advanced disease to tissues studies.

Digital pathology

The study of tiny objects through a lens began 2000 years ago. At the end of the 16th century, the Dutch eyeglass dealer Janssen and his son designed and assembled the first prototype of the modern optical microscope and telescope. Years later, a Dutch scientist, von Anthony van Leeuwenhoek, achieved a 300-times magnification using a single lens. In medicine, in the 1700s, the Italian Marcello Malpighi was the first scientist who described blood capillaries using an optical microscope (133). The desire for greater and deeper details resulted in continuous advances in microscopy.

In 1999, microscope slides were scanned for the first time into a digital image. However, due to issues of image quality, computing power, and storage space, the use of digitized images in histology was not common before the 2010s (134). During the last decade, digital microscope imaging has become more common due to improved image quality and computing power. Nowadays, digitised images have a high resolution, allow for an easy workflow and data stocking, and can be combined with different algorithms to aid diagnosis (135).

Digital pathology (assessing digital tissue images on a computer) is becoming routine in pathology labs. The advantages over the traditional microscope are numerous: the possibility to share the same image among labs without frontiers for expertise in research, the possibility to draw very precise annotations on the images, and the possibility to use algorithms to perform certain tasks (136) (137). All these advantages help improve the diagnosis and the clinical workflow (138). The weak point of this technique, at the moment, is that a 2D scanned image does not permit analyses at different depth levels, such as can be achieved with the classic optical microscope.

Artificial Intelligence (AI)

Artificial intelligence (AI) is a “smart” machine, a computer with a complex programme that simulates a limited human intelligence due to a limited database (139). Compared with the classical statistical methods working with databases, AI works by a non-transparent mechanism, and for this reason, it was named a black box (140) (Figure 12).

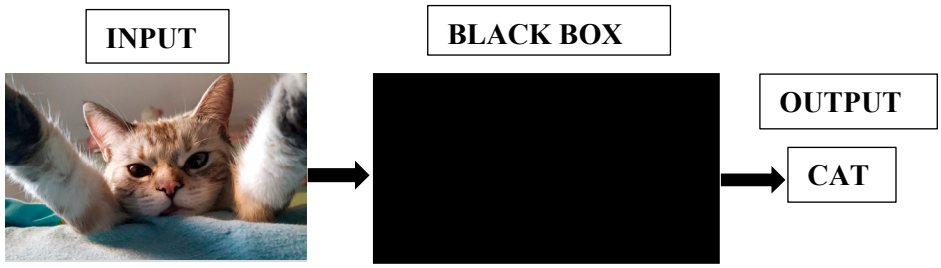

Figure 12: Example of AI algorithm illustrated as a black box processing the input image and the output prediction result.

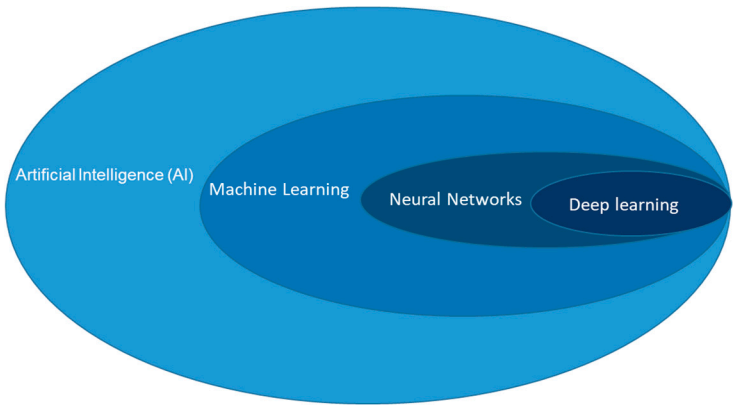

Figure 13: Illustration of AI and its subunits: machine learning, artificial neural networks, and deep learning.

The machine learning algorithm is a subunit of AI. It is designed to learn by receiving information from a database or image (ground truth) or another type of feedback. This is called the training process for machine learning algorithms and represents a crucial part of a good performance. The more data is used in training, the better the result will be (Figure 13).

A neural network is a way to "design" the machine learning algorithm. Deep learning is a deep neural network (141). Convolutional Neural Network (CNN) is a deep neural network that is specifically designed to handle images as input in an efficient way (142)

The machine learning algorithm can be optimized for a specific task based on the dataset or the feedback. The algorithm is tested on new data and compared with the ground truth. One can apply machine learning to many aspects of pathology, for example, assessing Gleason grading.

To build this kind of algorithm, one needs many examples for training. These examples could be in the form of specific images. For example, in Figure 12, cat

images are used to train the algorithm to identify images of cats. To train an algorithm to detect cancer and classify it into different Gleason patterns, one must provide many examples of different Gleason patterns. Such examples are obtained by annotating (by a pathologist or trained person) digitally scanned slides. Once the training annotations are loaded to the computer, they are integrated into the computer database. The more data that is loaded, the better performance is expected. The algorithm learns by taking one such image as input, applying the pixel weights and producing an output. The actual learning is then when it compares its output with the ground truth: the annotations that were given to the image (benign, G3, G4, G5). If its output is incorrect, it will modify the weights to make a better prediction next time. This process is done by iterating all the training data many times until the output prediction is as similar to the ground truth as possible for each of the examples in the training data.

The material used can be challenging for the algorithm depending on the colour intensity and image quality. The final version of the algorithm is adjusted for these details before being evaluated. The performance of the final algorithm version is done on a validation set of slides. The validation set includes slides previously annotated but not used in training. The first step that the algorithm has to pass is to recognize cancer from the other structures. The next step is that the algorithm needs to classify cancer into the different Gleason patterns and quantify them. If algorithm performance on the validation set of slides is satisfactory, the next step is testing. For testing, the algorithm is applied to an external set of slides without previous annotations.

Convolutional Neural Networks (CNNs) in histopathology

Digitised slides open the possibility to develop and use mathematical algorithms for histopathological tasks, such as the detection and characterization of cancer (143). In the last decade there has been an exponential rise in the numbers of published papers covering the topic of AI in cancer detection.

In 2017 a large study was published where CNNs were used to detect skin cancer on dermatology images (144). In this study Google's CNN algorithm was tested against 21 dermatologists to classify different subtypes of skin cancer. The new AI method was recognized to be on par with dermatologist level performance. Another interesting study with good results for an AI algorithm reported detecting the metastases in breast lymph nodes. The algorithm performance was compared with 11 pathologists and was shown to improve the pathologists' accuracy (145). In PCa Litjens et al were one of the first to report an AUC of 0.9 using a deep learning algorithm, to distinguish between cancer and noncancer (146).

Since then, there has been a huge increase in publications applying machine learning to prostate pathology images (147-154).

In 2021, the US Food and Drugs Administration approved the first and unique software (The Paige System) to enhance the accuracy and confidence in prostate biopsy diagnosis. According to the Royal College of Pathology UK, the diagnosis of prostate biopsies can take up to 28 days. This AI-based software system showed a time reduction of 65.5%, a sensitivity of 1.0 (CI 0.93 to 1.0), specificity of 0.78 (CI 0.64 to 0.89) and a negative prognostic value of 1.0 (CI 0.91 to 1). This AI system is recommended for automated screening of diagnostic prostate biopsies. Only those flagged by the system need to be reviewed by a diagnostic pathologist, thus being a useful tool for speeding up the process (155, 156).

The present investigation

Aims

The thesis aimed to evaluate:

1. The prognostic value of expression of active forms of STAT3 in the epithelium of localised hormone-naïve PCa.
2. The prognostic value of the expression of active forms of STAT3 in the stromal compartment of localised hormone-naïve PCa.
3. The expression of STAT3 and IL-6R in the metastatic tissue at different locations obtained from the rapid autopsy of patients with castration resistant PCa.
4. The accuracy of an AI-based algorithm for automated detection and Gleason grading in prostate biopsies.

Paper 1: Expression of tSTAT3, pSTAT3^{Ser727}, and pSTAT3^{Tyr705} in the epithelial cells of hormone-naïve prostate cancer

Patients with localised PCa and high lifetime expectancy (>10-years) are candidates for different options of treatment, such as active surveillance, RT, or surgery with RP. Those undergoing RP are commonly hormone-naïve, and the surgery has curative intent. The prognosis in most cases is excellent, but there is a small percentage whose prognosis is worse and have a high risk for disease recurrence due to the cancers aggressiveness.

In different malignancies, including PCa, overexpression of active (phosphorylated) STAT3 has been identified to promote cancer cell survival, proliferation, angiogenesis, metastasis and drug resistance (114).

In this present study, we evaluated the expression levels of total STAT3 (tSTAT3) and two phosphorylated forms of STAT3 (pSTAT3^{Tyr705} and pSTAT3^{Ser727}) in localised PCa. We focused on the expression levels in the PCa epithelium in both the nucleus and cytoplasm and the value as a prognostic biomarker. The study was conducted on tissue microarrays (TMA) from two cohorts of patients with localised hormone-naïve PCa, one from Malmö, Sweden, which included 300 patients and one from Dublin, Ireland, with 99 patients.

Results and Discussion

In the Malmö cohort, nuclear expression levels of tSTAT3, pSTAT3^{Ser727}, and pSTAT3^{Tyr705} in the epithelial cells of the cancerous glands were found to be significantly lower compared with the benign glands. Decreased nuclear expression levels of pSTAT3^{Ser727} and pSTAT3^{Tyr705} were correlated with increased Gleason score. Nuclear expression levels of tSTAT3 in individual cores of epithelial cells in cancerous glands, whose Gleason score was provided, were lower than the nuclear expression levels of tSTAT3 in cores of the benign epithelial cells.

Cytoplasmic expression levels of tSTAT3 and pSTAT3^{Ser727} but not pSTAT3^{Tyr705} were detected for evaluation. The average H score (intensity x percentage) of cytoplasmic tSTAT3 expression was lower in cancer cores compared to benign cores. No significant differences were found in the cytoplasmic H score of pSTAT3^{Ser727} in cancer cores compared with benign cores. However, higher cytoplasmic expression levels of pSTAT3^{Ser727} in cancer cores were correlated with Gleason score < 7, ISUP- grade 1.

In the Dublin cohort, comparable results were found with few differences. Significantly higher nuclear expression of tSTAT3 levels was found in the benign

cores compared with cancer cores from the same patient. There was a tendency for lower nuclear expression levels of pSTAT3^{Ser727} in the cancer cores compared with benign cores. Lower nuclear expression in all these three markers was associated with a higher Gleason score. This is different to the Malmö cohort, where only pSTAT3^{Tyr705} and pSTAT3 ^{Ser727} showed a progressive decrease. In evaluating cytoplasmic levels, tSTAT3 was significantly higher in the benign cores compared with those from cancer cores in the same patient, but when stratifying by Gleason score, no correlations were found.

We have used BCR obtained from the medical records as endpoint for disease progression. BCR was observed as a rise in PSA level to at least 0.2 ng/mL.

Shorter time to BCR was correlated with low expression levels of pSTAT3^{Tyr705} and pSTAT3^{Ser727} in the cancerous glands. For tSTAT3, the expression levels in nuclei and cytoplasm did not significantly correlate with the time to BCR in the Malmö cohort. Higher cytoplasmic tSTAT3 was associated with reduced time to BCR in the Dublin cohort.

We have used multivariable Cox regression to evaluate the prognostic values of these three markers. Adding the data of tSTAT3, pSTAT3^{Try705}, and pSTAT3^{Ser727} to Gleason score or pathological T stage, did not improve the prognostic values in the multivariable model.

In conclusion, this study reports that low pSTAT3^{Tyr705} and pSTAT3^{Ser727} expression in epithelial cells of cancerous prostatic glands in hormone-naïve PCa was associated with faster disease progression. None of the three investigated markers improved the prognostic value of the traditional markers. The results are contrary to previously published studies that reported that higher levels of pSTAT3 indicate disease progression (115, 116, 118, 119).

Paper 2: Nuclear expression of pSTAT3^{Tyr705} and pSTAT3^{Ser727} in the stromal compartment of localised hormone-naïve prostate cancer

In Paper 1, we showed that low expression of pSTAT3^{Tyr705} and pSTAT3^{Ser727} in epithelial cells of cancerous prostatic glands in hormone-naïve PCa was associated with a shorter time to disease progression.

Next, we became interested in the expression levels of STAT3 in the stromal compartment comprising of non-epithelial structures (matrix, fibroblasts, inflammatory cells, fibro myofibroblasts) concerning disease progression.

Using parts of the previous TMA material (Malmö cohort), we designed a study to evaluate pSTAT3^{Tyr705} and pSTAT3^{Ser727} nuclear expression levels in cancer and non-cancer stromal compartments and their prognostic value.

We selected tissue material from 225 patients who had at least one non-cancer stromal compartment core and one cancer stromal compartment core, as well as medical records of follow up.

We defined the stromal compartment as all the structures found in the prostatic tissue, excluding benign and malignant epithelial cells, blood vessels, and lymphatic vessels.

Results and Discussion

We found nuclear expression of pSTAT3^{Tyr705} and pSTAT3^{Ser727} in fibroblast-like and immune cell-like stromal cells in both cancer and non-cancer stromal areas in hormone-naïve prostatic tissue obtained from radical prostatectomies.

Higher nuclear expression levels of pSTAT3^{Tyr705} and pSTAT3^{Ser727} were seen in the non-cancer stromal cores compared with the stroma of cancer cores. Stratifying H score nuclear expression levels of pSTAT3^{Tyr705} and pSTAT3^{Ser727} by ISUP grade, significantly lower nuclear expression levels were detected in the cancer stromal compartment cores compared with the non-cancer stromal cores in ISUP grades 2 and 3.

A lower H score of pSTAT3^{Ser727} levels expression was found in ISUP grade 2 and 3 patients compared to ISUP grade 1 patients. Similar results were found for pSTAT3^{Tyr705} but were not statistically significant. No significant differences in pSTAT3^{Tyr705} and pSTAT3^{Ser727} expression levels were observed in non-cancer cores from patients with different ISUP grades. Correlation of nuclear expression

levels was found only between cancer cores or non-cancer cores but not between cancer and non-cancer cores.

Low expression levels of pSTAT3^{Tyr705} and pSTAT3^{Ser727} in the stroma in the cancer cores and in non-cancer cores were associated with shorter times to BCR. Nuclear expression levels of cancer-core pSTAT3^{Tyr705} and pSTAT3^{Ser727} individually had a prognostic value in univariate Cox regression analysis. The prognostic value of pSTAT3^{Tyr705} and pSTAT3^{Ser727} resulted to be similar to the classically used prognostic biomarkers of pT, pGS and surgical margin status.

The results obtained in the present study are similar to those we observed for the expression of pSTAT3 in the epithelium, where a higher level of nuclear expression of pSTAT3^{Tyr705} and pSTAT3^{Ser727} in non-cancer cores compared to cancer cores and lower expression of both forms of pSTAT3 correlated with shorter time to BCR.

Different results have been reported in other cancer types. For example, in the stromal compartment of breast cancer, high expression in the stromal compartment was associated with a good prognosis (128). In colorectal cancer, increased expression of pSTAT3 in the stromal compartment was correlated with reduced overall survival (129).

Paper 3: Expression of STAT3 in prostate cancer metastases.

PCa metastases are typically located in regional and distant lymph nodes, the pelvis, and axial skeleton, and at later stages also in visceral organs like lung, liver, and brain. The osteoblastic process detected in bone marrow is judged as a hallmark for metastatic disease in patients with PCa.

Our study aimed to measure the levels of the activated form of STAT3, $pSTAT3^{Tyr705}$, and IL-6R using IHC and mRNA expression on the TMA metastatic material from a rapid autopsy program. TMA material was provided by Washington University and included metastasis from bone, lymph node, and viscera.

Results and Discussion

By IHC, we evaluated the expression of $pSTAT3^{Tyr705}$ and IL-6R in 223 metastatic samples from 71 CRPC patients: 119 from bone, 52 from lymph nodes, and 52 from visceral metastases. Twenty-four patients had matched tissue samples with at least one sample of bone, lymph node, and visceral metastases.

On the non-matched tissue samples, the protein level of $pSTAT3^{Tyr705}$ was significantly higher in bone metastases than in lymph node metastases and visceral metastases.

In bone metastasis, IL-6R showed higher protein levels than lymph node metastases but no differences from visceral metastases. In the matched metastatic samples, protein levels of $pSTAT3^{Tyr705}$ and IL-6R were significantly higher in the bone metastases compared with lymph node or visceral metastases.

mRNA analyses were available from 149 metastases samples from 63 patients: 20 bone, 68 lymph nodes, and 61 visceral metastases. mRNA levels of STAT3 were significantly higher in bone than in lymph node and visceral metastases. Regarding mRNA IL-6R levels, no significant differences were seen among the different metastases' sites

Based on these results, we conclude that protein levels of $pSTAT3^{Tyr705}$ detected by IHC in bone metastases are correlated with STAT3 mRNA levels and are significantly higher compared to the lymph node and visceral metastasis.

The presence of the activated form of STAT3 in the metastases opens the necessity of further investigation to compare the primary tumour with the metastasis for a better understanding of disease progression. Targeting STAT3 in metastatic disease can offer new options for treatment.

Paper 4: An artificial intelligence-based support tool for automation and standardisation of Gleason grading in prostate biopsies.

PCa prognosis is based on the Gleason scoring system. Gleason scoring system limitations are due to a shortage of pathologists, histopathology caseload and the inter- and intra-observer variability.

This study proposes using new technology, an algorithm based on AI as a practical tool to solve the limitations of the traditional method.

Results and Discussion

To build the algorithm, for training, we have used 698 digitally scanned prostate biopsies from 174 patients. Each slide was manually annotated by two specialist pathologists and assigned the correspondent Gleason pattern. Numerous adjustments to improve the algorithm performance were included in the training process. To achieve the goal of this project, we first focused on the algorithm's ability to differentiate cancer areas from non-cancer areas. The second objective was to develop the capacity to quantitatively assign the different Gleason patterns to the detected cancer areas.

Once we were satisfied with the algorithm's performance on the validation slide set, we tested it on 37 digitised annotated biopsies from 21 patients scanned using two different scanners.

We found that the algorithm achieved a good capacity to detect cancer areas and to correctly classify the different Gleason patterns. The results were similarly reproducible on scans from two different scanners. To detect the cancer areas in the prostate tissue biopsies, the algorithm sensitivity was 100%, with a sensitivity of 68%. To assess the different Gleason patterns in detected cancer areas, the algorithm showed a sensitivity of 89% for Gleason pattern 3 and 77% specificity. When assigning Gleason pattern 4, sensitivity was 91%, and specificity was 79%. For Gleason pattern 5, sensitivity was 80%, and specificity was 98%.

We compared the algorithm's results with the annotations performed by two pathologists and found that the algorithm achieved optimal results. Interobserver variability between the two pathologists was similar to that between the algorithm and pathologist. These results point to this algorithm as a promising tool to improve the detection and diagnosis of PCa.

Conclusion and future perspectives

There are now multiple treatment methods for PCa when detected early, but there remains a small percentage of PCas with aggressive behaviour and poor prognosis. Among the treatment options, ADT is often used. Despite an initial good response, most patients become resistant to this therapy within a few years. Based on these observations, new biomarkers are needed to prognosticate the evolution of the disease. This thesis explored whether STAT3 has potential as a biomarker.

Low pSTAT3 levels in both epithelial and stromal tissue compartments from hormone-naïve patients were correlated to a shorter time to BCR. However, this prognostic value did not add to the prognostic power of the classical biomarkers (pT stage and GS). In CRPC patients, higher levels of pSTAT3 were found in bone metastasis compared to the metastasis in the lymph nodes and other visceral metastases. Similar results were found for IL6R levels. This data suggests that STAT3 is an important therapeutic target in advanced PCa.

Considering that increased expression of pSTAT3 was found in the metastatic tissue, one would expect high levels of pSTAT3 to be correlated with a shorter time to BCR at earlier stages of the disease. However, we found opposite results indicating that the expression of STAT3 is differently regulated in hormone-naïve PCa than in CRPC. We can conclude that activation of STAT3 occurs in later stages of the disease where inhibition of STAT3 may be a therapeutic option. Preclinical studies from our group have shown that STAT3 inhibitory drugs reduce tumour volume and metastatic spread (132) (157) .

Further studies on the expression of pSTAT3 in patient tissue should include a comparison of tissues from the same patient before and during ADT. For the evaluation of STAT3, it would be interesting to identify the mechanism and moment when the tumour became resistant to treatment.

An important aspect is that we have used tissue cores from cancer and from benign areas in a TMA setting with limited information regarding tumour heterogeneity. From the same patients, we collected cancer and non-cancer tissue cores from different parts of the prostate but we cannot rule out the possibility of a field effect. It would also be of interest to study the expression levels of active forms of STAT3 in immune cells (127).

The second aim of this thesis focuses on the Gleason score. Gleason grading, the gold standard in PCa, is an extremely time-consuming method with wide intra- and interobserver variability. We proposed a new method to examine prostatic tissue using an AI algorithm. Such an algorithm would standardize and speed up the Gleason grading process.

The performance of the AI algorithm described in the fourth paper was promising. Considering the vast number of similar studies published in recent years and

numerous IT companies working on similar products, we have not a plan for our algorithm to be developed commercially at this moment. However, it proves a useful research tool to study the various biopsy and prostatectomy cohorts that our lab has access to. It would also be interesting to re-train our algorithm to recognise the cribriform pattern, as this pattern has a high prognostic value.

In the future, it would be of interest to combine biomarker studies with the possibilities of digital image analysis. In our prior studies, biomarker scoring was evaluated by eye, requiring many hours of work and introducing subjectivity, even with the scoring criteria previously established. Once the high performance of digital algorithms is obtained, this method will allow fast and objective scoring. A study of the expression of STAT3 in the normal prostatic tissue and specifically in different immune tumour cell subpopulations using complex technical IHC and digital image analysis methods will provide valuable information for a better understanding of the interaction between the tumour cells and adjacent tumour microenvironment.

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