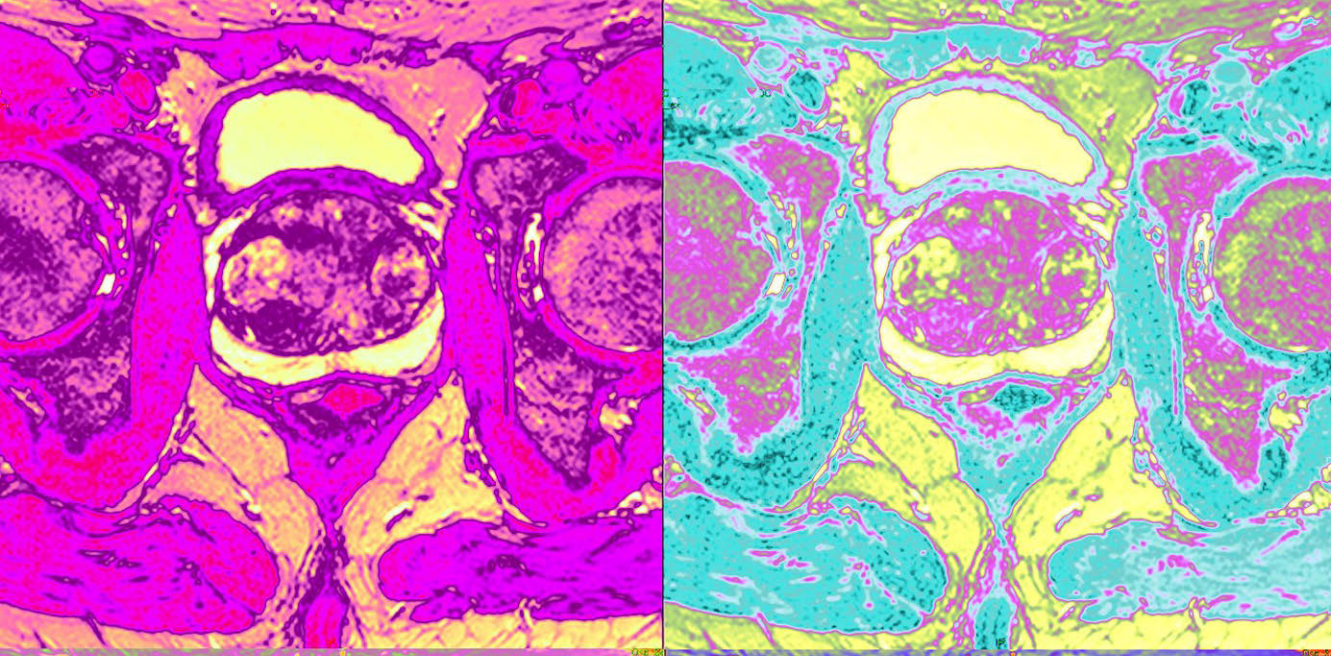

CHALLENGES IN DIAGNOSTICS OF PROSTATE CANCER

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The originality of this thesis has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

Cover art: Juha Knaapila

ISBN 978-951-29-8037-6 (print)
ISBN 978-951-29-8038-3 (pdf)
Painosalama Oy, Turku, Finland 2020

Emilialle

UNIVERSITY OF TURKU
Faculty of Medicine, Department of Clinical Medicine, Surgery
JUHA KNAAPILA: Challenges in Diagnostics of Prostate Cancer
Doctoral Dissertation, 157 pp.
Doctoral Programme in Clinical Research
May 2020

ABSTRACT

Prostate cancer (PCa) is the most frequently diagnosed cancer in men and it is one of the leading causes of men's cancer deaths in the developed countries. It can be considered as a continuum of neoplasms with aggressiveness ranging from cancers with no effect on the patients' life expectancy to highly aggressive cancers. Contrary to all other solid-organ cancers, PCa diagnosis is not traditionally based on imaging or visual examination (e.g. endoscopy), but systematic biopsies. Convincing evidence of prostate MRIs' high sensitivity in detecting clinically significant PCa (CSPCa) and tendency to discriminate insignificant PCas have recently been published. However, the role of imaging is still under debate. Additionally, infectious prostate biopsy complications have increased lately, which has been suspected to have risen from a globally increasing antibiotic resistance e.g. an increased resistance to an antibiotic prophylaxis.

In the first substudy, we prospectively investigated the prevalence of transrectal prostate biopsy complications. The rate of complications was low, even though intestinal bacterial antibiotic resistance for prophylaxis was significant. In the second substudy, we prospectively determined the prevalence and risk factors for antibiotic resistance of intestinal *Escherichia coli* in men undergoing prostate biopsies. A rate of fluoroquinolone resistant *Escherichia coli* was 13% while international traveling was a significant risk factor. In the third substudy, we externally validated an optimal combination strategy of PSA density and MRI score for selecting men to prostate biopsies. Like in the study to be validated, PSA density has only a minor additional value to the MRI score. In the fourth substudy, we investigated an impact of prostate MRI in an initial PCa diagnostics. In a prospective study cohort using prebiopsy prostate MRI, the rate of CSPCa was significantly higher in initial biopsies and significantly few CSPCa were diagnosed during the follow-up comparing to a cohort with traditional PCa diagnostics. In the fifth substudy, we analyzed performance measures of MRI in CSPCa diagnostics. In the substudy, MRI demonstrated an excellent negative predictive value in ruling out CSPCa.

As a conclusion, prebiopsy prostate MRI should be performed to all men in a suspicion of localized prostate cancer. A biopsy decision should be based on men's individual risk for having significant PCa in the patient level. From a point of view of an individual man, the most significant risk for an unnecessary biopsy could be a diagnosis of insignificant PCa, not a lethal complication.

KEYWORDS: prostate, cancer, diagnostics, biparametric, mri, negative, predictive value, fluoroquinolone, resistance, e.coli, escherichia coli,

TURUN YLIOPISTO
Lääketieteellinen tiedekunta, Kliininen laitos, Kirurgia
JUHA KNAAPILA: Haasteet eturauhassyövän diagnostiikassa
Väitöskirja, 157 s.
Turun kliininen tohtoriohjelma
Toukokuu 2020

TIIVISTELMÄ

Eturauhassyöpä (PCa) on miesten yleisin syöpä ja toiseksi yleisin syöpäkuolemien aiheuttaja kehittyneissä maissa. Sitä voidaan pitää jatkumona merkityksettömistä ja elinajanennusteeseen vaikuttamattomista syövistä erittäin aggressiivisiin syöpiin. Toisin kuin muiden kiinteiden elinten syöpien suhteen, PCa:ää ei perinteisesti ole diagnosoitu kuvantamisen tai tähystystutkimusten/inspektion perusteella vaan systemaattisilla biopsioilla. MRI:sta on viime aikoina julkaistu vakuuttavaa näyttöä sen korkeasta herkkyydestä kliinisesti merkittävien eturauhassyöpien (CSPCa) suhteen ja taipumuksesta jättää merkityksettömät syövät löytämättä. Kuvantamisella ei kuitenkaan ole vielä vakiintunutta roolia diagnostiikassa. Lisäksi eturauhasbiopsioista seuraavien infektioiden määrä on lisääntynyt, minkä epäillään johtuvan maailmanlaajuisesti lisääntyneestä antibioottiresistenssistä eli toisin sanoen lisääntyneestä resistenssistä profylaktiselle antibiootille.

Ensimmäisessä osatyössä tutkimme eturauhasbiopsioiden komplikaatioita prospektiivisessa aineistossa, joita todettiin vähän huolimatta merkittävästä antibioottiresistenssistä annetulle profylaksialle. Toisessa osatyössä tutkimme prospektiivisesti suoliston *Escherichia colin* antibioottiresistenssin vallitsevuutta ja sen riskitekijöitä eturauhasbiopsioitavilla miehillä. Tutkimuksessa fluorokinoloniresistentti *Escherichia coli* todettiin 13 %:lla. Ulkomaanmatkailu oli tälle merkittävä riskitekijä. Kolmannessa osatyössä validoimme optimaalista strategiaa PSA tiheyden ja MRI tuloksen yhdistelmälle biopsoitavien miesten valinnassa. Kuten validoitavassa tutkimuksessa, PSA tiheyden tuoma lisäarvo oli vähäinen. Neljännessä osatyössä tutkimme MRI:n vaikutusta CSPCa:n primääridiagnostiikassa. Prospektiivisessa aineistossa MRI kuvattuja miehiä, CSPCa:n määrä ensimmäisissä biopsioissa oli merkittävästi korkeampi ja seurannassa todettujen CSPCa:n määrä merkittävästi alempi verrattuna perinteisellä diagnostiikalla tutkittuihin. Viidennessä osatyössä tutkimme MRI:n tehokkuusarvoja CSPCa:n diagnostiikassa. Totesimme MRI:n negatiivisen ennustearvon olevan erinomainen CSPCa:n poissulussa.

Yhteenvetona, MRI pitäisi tehdä ennen biopsioita kaikille miehille, joilla epäillään paikallista PCa:ää. Päätös biopsioista tulisi perustua yksilölliseen riskiin potilaskohtaisesti merkittävälle PCa:lle. Yksittäisen miehen näkökulmasta, mahdollisesti oleellisin riski tarpeettomilla biopsioilla on merkityksettömän PCa:n diagnoosi, ei hengenvaarallinen biopsiakomplikaatio.

AVAINSANAT: eturauhassyöpä, eturauhasbiopsiat, diagnostiikka, mri, biparametrinen, fluorokinoloniresistenssi, escherichia coli, e. coli

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Abbreviations

3CEF	3rd generations cephalosporins
5ARI	5-alphareductase
ADC	apparent diffusion coefficient
ADT	androgen deprivation therapy
AUC	area under the receiver operating characteristic curve
BCR	biochemical recurrence
BPH	benign prostate hyperplasia
bpMRI	biparametric MRI
CI	confidence interval
CSPCa	clinically significant prostate cancer
CT	computer tomography
DCA	decision curve analysis
DCE	dynamic contrast enhanced imaging
DR	detection rate
DRE	digital rectal examination
DWI	diffusion weighted imaging
E. coli	Escherichia coli
EAU	European association of urology
EBRT	external beam radiotherapy
ESBL	extended-spectrum beta-lactamase
EUCAST	The European Committee on Antimicrobial Susceptibility Testing
GGG	Gleason Grade Group
HIFU	high intensity focused ultrasound
IDC-P	intraductal carcinoma of prostate
INR	international normalized ratio
insPCa	clinically insignificant prostate cancer
IQR	interquartile range
mpMRI	multiparametric MRI
MRI	magnetic resonance imaging
NECP	neuroendocrine cancer of prostate
NICE	National Institute of Health and Care Excellence
NPV	negative predictive value

OR	odds ratio
PCa	prostate cancer
PET	positron emission tomography
PHI	prostate health index
PI-RADS	Prostate Imaging Reporting and Data System
PPV	positive predictive value
PSA	prostate specific antigen
PCA3	prostate cancer gene 3
RCT	randomized, controlled trial
RR	relative risk
T2W	T2-weighted imaging
TRUS	transrectal ultrasound
TRUS-Bx	transrectal, ultrasound guided prostate biopsies
TURP	transurethral resection of prostate
TYKS	Turku University Hospital

List of original publications

This doctoral thesis is based on the following five original publications including one submitted manuscript, which are referred in the by the Roman numerals I–V.

I Knaapila J, Gunell M, Syvänen K, Ettala O, Kähkönen E, Lamminen T, Seppänen M, Jambor I, Rannikko A, Riikonen J, Munukka E, Eerola E, Hakanen AJ, Boström PJ. Prevalence of Complications Leading to a Health Care Contact After Transrectal Prostate Biopsies: A Prospective, Controlled, Multicenter Study Based on a Selected Study Cohort. Eur Urol Focus. 2019 May;5(3):443–448

II Knaapila J, Kallio H, Hakanen AJ, Syvänen K, Ettala O, Kähkönen E, Lamminen T, Seppänen M, Jambor I, Rannikko A, Riikonen J, Munukka E, Eerola E, Gunell M, Boström PJ. Antibiotic susceptibility of intestinal Escherichia coli in men undergoing transrectal prostate biopsies: a prospective, registered, multicentre study. BJU Int. 2018 Aug;122(2):203–210

III Knaapila J, Jambor I, Perez IM, Ettala O, Taimen P, Verho J, Kiviniemi A, Pahikkala T, Merisaari H, Lamminen T, Saunavaara J, Aronen HJ, Syvänen KT, Boström PJ. Pre-biopsy IMPROD biparametric magnetic resonance imaging combined with prostate specific antigen density in the diagnosis of prostate cancer: An external validation study. Eur Urol Oncol. 2019 Sep 6. pii: S2588-9311(19)30134-8.

IV Knaapila J, Autio V, Jambor I, Ettala O, Verho J, Kiviniemi A, Taimen P, Perez IM, Aronen HJ, Syvänen KT, Boström PJ. Impact of Biparametric Prebiopsy Prostate Magnetic Resonance Imaging on the Diagnostics of clinically significant Prostate Cancer in Biopsy naïve Men. Scand J Urol. 2020 Jan 9:1–7

V Knaapila J, Jambor I, Ettala O, Taimen P, Verho J, Perez IM, Kiviniemi A, Pahikkala T, Merisaari H, Lamminen T, Saunavaara J, Aronen HJ, Syvänen KT, Boström PJ. Negative predictive value of biparametric prostate MRI in excluding significant prostate cancer: a pooled data analysis based on clinical data from four prospective, registered studies. (submitted manuscript)

1 Introduction

Prostate cancer (PCa) is the most frequently diagnosed cancer in men and it is one of the leading causes of men's cancer deaths in the developed countries (Center et al., 2012; Fitzmaurice et al., 2015; Torre et al., 2015). In addition, according to an estimation, PCa causes 11% of cancer-related health care costs in the European Union (Luengo-Fernandez et al., 2013). Prostate cancer can be considered as a continuum of neoplasms with aggressiveness ranging from cancers with no effect on the patients' life expectancy to highly aggressive cancers (Barocas et al., 2017; Bell et al., 2015; Bill-Axelson et al., 2018; Epstein, Zelefsky, et al., 2016; Jahn et al., 2015). Hence, not all PCas demand a definitive treatment. After an introduction of a prostate specific antigen (PSA) for clinical use in the 1980s, which was initially recommended for the surveillance of PCa after radical treatments, the number of PCa diagnoses has dramatically increased (Center et al., 2012; Stamey et al., 1987). The increase in PCa diagnoses is mainly a consequence of opportunistic PSA testing. However, a corresponding trend is not seen in PCa mortality (Center et al., 2012). Although, after the introduction of PSA, PCa is diagnosed more in an earlier stage (Seikkula et al., 2017).

Usually, a localized PCa gives no symptoms, or the symptoms cannot be distinguished from common and benign conditions, for example benign prostate hyperplasia (Hollingsworth & Wilt, 2014). Suspicion of a localized PCa in symptomatic and also in asymptomatic men derives usually from an increased PSA level or an abnormal finding in digital rectal examination (DRE) (Mottet et al., 2018; NICE, 2019). Contrary to all other solid-organ cancers, PCa diagnosis is not traditionally based on imaging or visual examination (e.g. endoscopy), but random biopsies (i.e. systematic biopsies) from the prostate (Mottet et al., 2018; NICE, 2019). Even if the systematic biopsies are usually taken under transrectal ultrasound (TRUS) guidance, the sensitivity of TRUS in detecting PCa is low. TRUS is mainly used for the guidance of biopsy cores, not primarily to visualize pathological lesions (Halpern & Strup, 2000; Onur et al., 2004; Smeenge et al., 2012). The role of imaging, which usually means prostate magnetic resonance imaging (MRI) in PCa diagnostics is still under debate (Mottet et al., 2018). In addition, the guidelines do not primarily recommend to take biopsies only from suspicious lesions visible in MRI, since all significant lesions may not be visible in MRI (Mottet et al., 2018; NICE, 2019). Thus,

systematic biopsies still remain to be a cornerstone of the PCa diagnostics (Mottet et al., 2018).

The traditional diagnostic protocol is suboptimal for modern diagnostic demands. Firstly, PSA is an organ-specific, not cancer-specific marker. The probability of PCa rises along with rising PSA level, thus there is no threshold level for PSA to rule out clinically significant PCa (CSPCa) (Thompson et al., 2006). PCa screening using PSA has also been widely studied, however, it seems not to decrease PCa-specific or overall mortality and thus it is not recommended by the guidelines (Ilic et al., 2013; Mottet et al., 2018; NICE, 2019). Additionally, DREs have very low sensitivity to PCa (Catalona et al., 1994; Naji et al., 2018).

After a suspicion of PCa has emerged, the traditional diagnostic protocol with systematic biopsies cannot rule out CSPCa even with moderate probability, because systematic biopsies give only limited information about the whole prostate gland pathology (Ahdoot et al., 2020; Drost et al., 2019; Hu et al., 2012). In addition, PCa is commonly a multifocal disease, thus systematic biopsies may not present the most malignant histology of the gland (Choi et al., 2019). Therefore, the biopsy procedure should be repeated, when a suspicion of a PCa or its more malignant histology, prevails.

Repeated prostate biopsies obviously increase the probability of biopsy-related complications. Urinary tract infection is the most significant complication following transrectal ultrasound guided prostate biopsies (TRUS-Bx). Infectious prostate biopsy complications have increased lately, which has been suspected to have risen from a globally increasing antibiotic resistance e.g. an increased resistance to an antibiotic prophylaxis (Borghesi et al., 2016; Carignan et al., 2012; CDDEP, 2016; Lahdensuo et al., 2016; Mottet et al., 2018; Nam et al., 2013). Prostate biopsies taken via a transperineal approach might be a safer procedure, however, it is demanding for outpatient use due to a demand for spinal anesthesia (Eldred-Evans et al., 2016; Xue et al., 2017).

Systematic (contrary to lesion-specific) prostate biopsies also include an additional issue: diagnoses of clinically insignificant PCas (insPCa). The deficiencies in contemporary diagnostics in ruling out CSPCa drifts to radical treatments of cancers with a low malignant potential, which contains a potential to major adverse effects.

Convincing evidence of prostate MRIs' high sensitivity in detecting CSPCa, and its tend to discriminate insPCas, has recently been presented (Ahmed et al., 2017; Kasivisvanathan et al., 2018; Moldovan et al., 2017; Rouvière et al., 2019; van der Leest et al., 2019). The future in PCa diagnostics seems to drift towards the use of prostate MRI as an essential part of initial PCa diagnostics. In addition, other imaging modalities e.g. various ultrasound applications, biomarkers and gene analytics are under intensive research (Kretschmer & Tilki, 2017; Sarkar & Das, 2016). There are still issues e.g. in the validation of the methods and breakthrough is not likely in the

near future. Hence, the histological diagnosis of PCa still remains to be the base for all the treatment planning and it is not evident that biopsies will be abandoned in PCa diagnostics. Thus, a safe biopsy procedure remains a key issue also in the future.

2 Review of the literature

2.1 Characteristics of prostate cancer

2.1.1 Prostate gland and its function

The prostate gland's function is the production of seminal plasma, which provides a suitable environment for the survival and function of spermatozoa (Wein et al., 2016). Anatomically, it is divided into four histologically distinct regions: the central zone, peripheral zone, transitional zone and fibromuscular stroma (McNeal, 1981). The central zone is located in the basis of the prostate, which is the most adjacent part to the urinary bladder, and includes an orifice where prostatic ducts drain into the urethra (verumontanum). The peripheral zone is the largest part of the glandular prostate, and it surrounds the prostatic urethra distally from the verumontanum and lateral and posterior parts of the prostate; thus it is the only region of the prostate which could be reached by DRE (Aaron et al., 2016; McNeal, 1981). Two thirds of PCas are located in the peripheral zone (McNeal et al., 1988; Terris et al., 1995). The transitional zone surrounds the urethra proximally from the verumontanum and it is a typical origin of benign prostate hyperplasia (BPH) (McNeal, 1981). The fibromuscular stroma is a non-glandular region covering the prostate anteriorly from the bladder neck to the apex of the prostate (McNeal, 1981).

2.1.2 Incidence, etiology and risk factors

There is not an unambiguous way to define the worldwide incidence and mortality rates for cancer. It is especially difficult for PCa, which constitutes a significant proportion of indolent cancers diagnosed by an extensive PSA screening. Variation is also derived from the definitions and registries used. However, several general assumptions can be made.

Prostate cancer is the most common cancer in men by total incidence, but just in the 8th place in total cancer mortality in men (Fitzmaurice et al., 2015). Comparing age standardized incidence rates, PCa is the second most common cancer in men worldwide after lung cancer in both developed and non-developed countries (Bray et al., 2018). Using the age standardized mortality rates, PCa is the

6th most common cancer to cause cancer-related deaths in developed countries, and the second most common in non-developed countries (Bray et al., 2018). Age standardized incidence has a major geographical variation: The highest age standardized incidence rates are in Western, high-income regions, Australia/New Zealand, Europe and North America, and the lowest in Asia (Bray et al., 2018). The highest and the lowest region had about a 17-fold difference in incidence (Bray et al., 2018). However, a similar difference is not seen in mortality rates, which vary only with about eight-fold magnitude (Bray et al., 2018). In Finland, 5446 PCa were diagnosed and 912 men died from PCa in 2017, which makes it the most common diagnosed cancer and the second most common cancer to cause cancer-related deaths in men (Suomen Syöpärekisteri, 2019).

Etiology of PCa is largely unknown. The well-established risk factors for PCa are advanced age, ethnicity, hormonal status and family history including genetic factors (Bostwick et al., 2004; Rawla, 2019).

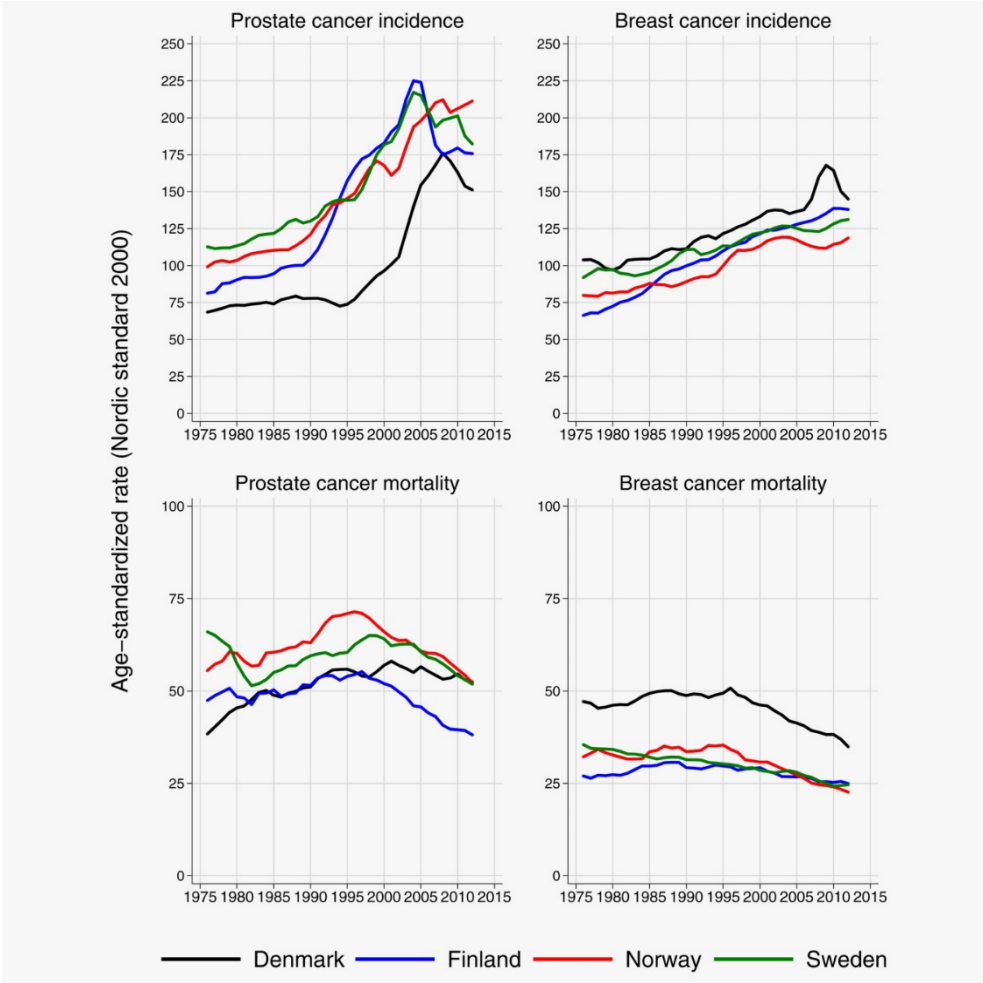
Advanced age is the most prominent risk factor for PCa, and it is suspect to arise mainly from oxidative processes (Bostwick et al., 2004). In Finland, PCa is commonly diagnosed in men aged 65–69 years, but it is extremely rare in men younger than 40 years of age (Suomen Syöpärekisteri, 2017). Ethnicity-related differences in incidence and mortality are significant. Incidence of PCa is highest in African-Americans; they also likely present more advanced disease and their stage-specific mortality is worse than in white men in the United States (Bostwick et al., 2004). Variation by ethnicity is suspected to derive from environmental and demographic factors in addition to genetics (Bostwick et al., 2004; Rawla, 2019). According to the traditional ”androgen hypothesis” which was introduced already in the 1940s, PCa is an androgen-dependent cancer (Rawla, 2019). In addition, androgen deprivation therapy (ADT) is known to be effective as a treatment especially in advanced PCa. However, the role of androgens in the carcinogenesis of PCa is controversial and high androgen levels have no clear connection to the development or severity of a PCa, although men with low free testosterone levels might be in lower risk for PCa (Bostwick et al., 2004; Michaud et al., 2015; Watts et al., 2018).

Evidence is quite consistent about the significance of family history to the PCa risk, and it is noted to include an even stronger association to family history than in colon and breast cancer (Bostwick et al., 2004). Prostate cancer diagnosed in first-degree relatives is highly associated with one’s risk for PCa diagnosis and PCa related death, and the risk cumulates with an increasing number of first-degree relatives having PCa and their younger age in PCa diagnosis (Brandt et al., 2009). Familial PCa is reported to be more fatal than incidental PCas (Hemminki, 2012). Also many genetic factors have been associated with PCa, and significant germline mutations as BRCA and HOXB13 should be noted as risk factors for PCa (Lynch et al., 2016). Especially BRCA2 mutations is associated with an adverse prognosis (Castro et al.,

2015). As a conclusion, familial PCa should be suspected when first-degree relatives have PCa diagnoses especially in younger age, or in case of known germline mutations.

After introduction of PSA testing in the middle of the 1980s, the incidence rate as well as the age standardized incidence rate of PCa have dramatically increased, especially in the developed countries; however, the trend has stabilized in recent years (Center et al., 2012; Fitzmaurice et al., 2015). A corresponding trend is not seen with mortality rates (Center et al., 2012). In the Nordic countries, the trend is even more prominently seen, as presented in comparison to trends of breast cancer (**Figure 1**). From the late 1980s, age standardized incidence of PCa doubled to the year 2005, and since, a slight decrease is seen (Kvåle et al., 2017). According to a recent data from Finnish Cancer Registry, the incidence of PCa in Finland is again increasing (Suomen Syöpärekisteri, 2017). The age-standardized mortality rate has slightly increased over the decades to the middle of the 1990s, subsequently only a moderate decreasing trend is seen in the mortality rates (Kvåle et al., 2017; Suomen Syöpärekisteri, 2017). In addition, a stage-migration is seen in diagnosed PCas which are nowadays diagnosed and treated in an earlier stage (Mouraviev et al., 2011; Seikkula et al., 2017). Although an introduction of PSA has indisputably the foremost effect on the increased PCa incidence, also the aging of the population from the 1980s should be noted.

Figure 1. Observed age-standardized rates of prostate and breast cancer incidence and mortality in Denmark, Finland, Norway and Sweden (three-year moving averages, all ages). Figure from study of Kvåle et al., reprinted with a permission (Kvåle et al., 2017).



2.1.3 Pathology and staging

Hanahan et al. have presented the six hallmarks of cancer originally in the year 2000 and the work was revised in 2011 (Hanahan & Weinberg, 2000, 2011). The hallmarks comprise biological capabilities acquired during the multistep development of human tumors (Hanahan & Weinberg, 2000, 2011). The hallmarks include: sustaining proliferative signaling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis and resisting cell death (Hanahan & Weinberg, 2011). Prostate cancer includes a wide range of neoplasms with different malignant potential, thus low-grade PCas' status as a cancer has been questioned in the light of the mentioned hallmarks (Ahmed et al., 2012).

The vast majority of prostate neoplasms are adenocarcinomas. Classification of adenocarcinomas is still based on the Gleason grading system, which was originally introduced by Gleason et al. in 1966 (Gleason, 1966). During the last 50 years, surprisingly slight modifications have been made to the original grading system (Epstein et al., 2005). However, in 2014, the International Society of Urological Pathology (ISUP) consensus conference presented a proposal for a new grading system, which is based on the original Gleason scoring system (Epstein, Egevad, et al., 2016). The original system is based on prostate tissue morphology graded to 1–5, and the Gleason score is calculated by using the sum of first and, if present, the second most common pattern seen in the prostate specimen. If there is only one type of pattern seen, the second most common value is the same as the first one. Thus, the Gleason score can theoretically be between 2–10. However, patterns 1–2 are rarely reported, because they are recognized as biologically similar as the Gleason pattern 3, and the Gleason score is practically always between 6–10 (Epstein et al., 2005). The new grading system, which ISUP introduced in 2014, divides PCas to Gleason grade groups (**Table 1**, [GGG]) 1–5, aiming to correspond more accurately to the prognosis of current cancer (Epstein, Egevad, et al., 2016).

Table 1. ISUP Gleason grade group system (Epstein, Egevad, et al., 2016).

Gleason score	ISUP Gleason grade group (GGG)
3+3	1
3+4	2
4+3	3
4+4, 3+5, 5+3	4
4+5, 5+4, 5+5	5

Intraductal carcinoma of the prostate (IDC-P) is a histological subtype of adenocarcinomas, and it is independently associated with a worse prognosis (Guo & Epstein, 2006; Tsuzuki, 2015). In prostatectomy specimen, IDC-P is also associated with other adverse prognostic markers, including higher lesion size and Gleason score (Wilcox et al., 1998). In the 2014 ISUP consensus conference, it was discovered that an IDC-P without invasive carcinoma should not be assigned a Gleason grade (Epstein, Egevad, et al., 2016).

Cribriform PCa is a histological pattern, primarily graded as a subtype of Gleason 3+3, however its significance to prognosis has not been realized until the last decades (Iczkowski et al., 2018). The cribriform pattern is associated with adverse prognostic features independently from an underlying Gleason pattern (Iczkowski et al., 2018). In the 2014 ISUP consensus conference, it was also discovered that cribriform glands should be assigned a Gleason pattern 4, regardless of the morphology (Epstein, Egevad, et al., 2016).

Neuroendocrine carcinoma (NECP) is an aggressive subtype of PCa. It consists of heterogeneous types of carcinomas differentiated from neuroendocrine cells of prostate (Parimi et al., 2014). The majority of NECPs are small cell carcinomas, which can be considered as identical with small cell carcinoma of the lung (Parimi et al., 2014; Wein et al., 2016). It occurs in about a half of the cases in pure form without concomitant adenocarcinoma, or it may also occur with adenocarcinoma or have been developed from it (Parimi et al., 2014; Wein et al., 2016). It has been presumed that differentiation to NECP could be linked to androgen resistance following long-term ADT, and the degree of neuroendocrine differentiation increases along with PCa adenocarcinoma progression and in response to ADT (Parimi et al., 2014). Typically, NECP is not producing PSA and it is resistant to ADT (Parimi et al., 2014). Other extremely rare types of PCas are mucinous carcinoma, lymphomas and mesenchymal tumors i.e. sarcomas. In addition, urothelial carcinoma could occur in prostate also without a concomitant bladder involvement.

Prostate cancer is commonly a multifocal cancer; thus, there could be histologically different-graded cancer focuses in the same gland (Wein et al., 2016). A tumor focus with the largest volume (index lesion) is presumed to be a main driving factor for tumor progression and cancer prognosis and most satellite lesions do not appear to be life-threatening (Mouraviev et al., 2011). The genetic background of the multiple tumors in the same prostate is under discussion, however it seems that there is significant genetic alteration among tumor foci (Wei et al., 2017). In the study of Choi et al., PCa occurred as multifocal in 60.5% and bilateral in 82% of cases in a large series of prostatectomy specimens (Choi et al., 2019). According to the aforementioned study, the index tumor has often, but not always, the most malignant histology of the gland: in 6.7% of specimens the secondary tumor on the contralateral lobe had higher Gleason score than the primary tumor (Choi et al., 2019).

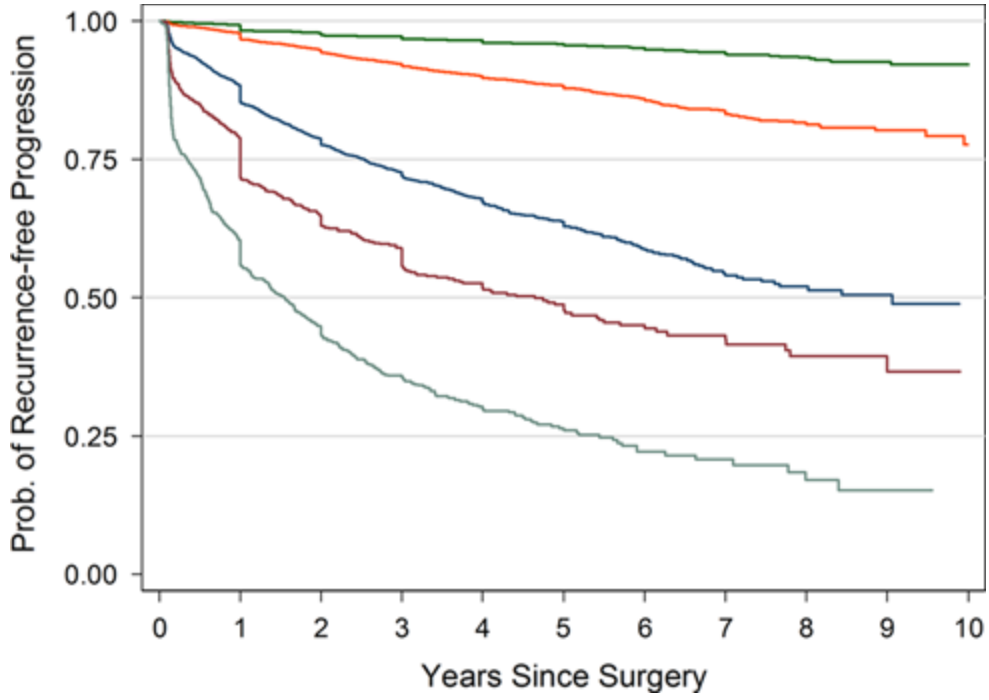
2.1.4 Prognosis, treatments and natural course

As a whole, PCa is a cancer with a good prognosis; after a PCa diagnosis, 92% of men are alive at five years and in general, an 80-year-old man has only a 1.6% risk of dying from PCa in Finland (Suomen Syöpärekisteri, 2017). Thus, PCa could be considered mostly as a chronic disease of aging men. However, the statistics relate only that a majority of PCas are cancers with good prognosis, but they discriminate the fact that PCa is a very heterogeneous group of malignancies from indolent to highly aggressive cancers.

In a landmark study of Epstein et al., the authors presented an independent and substantial influence of the cancer grade to a biochemical recurrence (BCR) rate when PCa is treated with radical prostatectomy or radiation therapy (Epstein, Zelefsky, et al., 2016). In the study, Gleason scores were grouped to the GGGs based on a prognosis and in this new grade grouping system differentiate PCas elegantly by a

prognosis (Epstein, Zelefsky, et al., 2016). Biochemical recurrence free rates after prostatectomy stratified by GGGs are demonstrated in **Figure 2** (Epstein, Zelefsky, et al., 2016). As an example of significance of PCa histological grade, men treated with radical prostatectomy and having Gleason ≤6 PCa in prostatectomy specimen, a five-year BCR rate was 96% (95% confidence interval [CI] 95–96%), whereas in men with Gleason score 8–10, the rate was 26% (95% CI 23–30%) (Epstein, Zelefsky, et al., 2016).

Figure 2. Recurrence-free progression following radical prostatectomy stratified by prostatectomy Gleason grade. Green line: Gleason score 6, grade group 1. Orange line: Gleason score 3 + 4, grade group 2. Dark blue line: Gleason score 4 + 3, grade group 3. Brown line: Gleason score 8, grade group 4. Gray line: Gleason score ≥9, grade group 5. Figure from the study of Epstein et al., reprinted with a permission (Epstein, Zelefsky, et al., 2016).



Although a BCR precedes almost exclusively the clinical progression of PCa, not all patients with BCR die of PCa (Brockman et al., 2015; Killian et al., 1985; Kuriyama et al., 1981; Partin, Pound, Clemens, Epstein, & Walsh, 1993). However, the power of the GGGs to also predict PCa-specific mortality has been recently retrospectively validated in a Finnish prostatectomy cohort (Erickson et al., 2018). Additionally, a similar significance of the cancer grade for the prognosis is seen in the mortality rates in a prospective SPCG-4 study where 3/88 (3.4%) men having Gleason score 2–6 PCa in prostatectomy specimen died from PCa in a mean follow-up of 23 years (Bill-

Axelson et al., 2018). The relative age-group adjusted risk for death from PCa increases in men having Gleason score 3+4, 4+3 and 8–9 to 1.91, 11.78 and 20.06 in prostatectomy specimen, respectively. Interestingly, when the surgical margin status and extraprostatic extension were taken into account in a multivariate analysis, the risk for death from PCa was practically equal (0.99) comparing Gleason 3+3 to Gleason 3+4 (Bill-Axelson et al., 2018).

Clinical and pathological staging of PCa with Tumor Node Metastasis (TNM) classification was reassessed in 2010 at American Joint Committee on Cancer to correspond more accurately with the prognosis of PCa (**Table 2**) (Cheng et al., 2012). The system classifies PCa to various prognostic groups by a tumor extent and spreading. However, the histological grade of PCa also remains an important and independent prognostic factor in addition to surgical margin status and extracapsular extension in prostatectomy specimen, especially in high grade PCas (Bill-Axelson et al., 2018; Catalona & Smith, 1998; Cheng et al., 1999; Epstein, Zelefsky, et al., 2016; Pound et al., 1997). As linked to the mentioned parameters, an initial PSA level predicts the pathological stage and spreading of a PCa (Gleave et al., 1996; Partin et al., 1997; Thompson et al., 2004). Today in the PSA era, the significance of preoperative clinical stage for prognosis after radical prostatectomy has been questioned (Catalona & Smith, 1998; Reese et al., 2010).

Table 2. American Joint Committee on Cancer clinical and pathological TNM classification of prostatic tumors (Cheng et al., 2012).

clinical TNM classification		pathological TNM classification	
TX	Primary tumour cannot be assessed	pTX	–
T0	No evidence of primary tumor	pT0	–
T1	Clinically inapparent tumor neither palpable nor visible by imaging	pT1	There is no pathological T1 classification
T1a	Tumor incidental histological finding in ≤5% of tissue resected	pT1a	–
T1b	Tumor incidental histological finding in >5% of tissue resected	pT1b	–
T1c	Tumor identified by needle biopsy (e.g. because of elevated PSA)	pT1c	–
T2	Tumor confined within prostate	pT2	Organ confined
T2a	Tumor involves ≤one-half of one lobe	pT2a	Unilateral, one-half of one side or less
T2b	Tumor involves >one-half of one lobe but not both lobes	pT2b	Unilateral, involving more than one-half of side but not both sides
T2c	Tumor involves both lobes	pT2c	Bilateral disease
T3	Tumor extends through the prostate capsule	pT3	Extraprostatic extension
T3a	Extracapsular extension (unilateral or bilateral)	pT3a	Extraprostatic extension or microscopic invasion of bladder neck
T3b	Tumor invades seminal vesicle(s)	pT3b	Seminal vesicle invasion
T4	Tumor is fixed or invades adjacent structures other than seminal vesicles such as external sphincter, rectum, bladder, levator muscles, and/or pelvic wall	pT4	Invasion of rectum, levator muscle, and/or pelvic wall
NX	Regional lymph nodes were not assessed	pNX	Regional nodes not sampled
N0	No regional lymph node metastasis	pN0	No positive regional nodes
N1	Metastases in regional lymph node(s)	pN1	Metastases in regional node(s)
M0	No distant metastasis	pM0	No distant metastasis
M1	Distant metastasis	pM1	Distant metastasis
M1a	Non-regional lymph node(s)	pM1a	Non-regional lymph node(s)
M1b	Bone(s)	pM1b	Bone(s)
M1c	Other site(s) with or without bone disease	pM1c	Other site(s) with or without bone disease. When more than one site of metastasis is present, the most advanced category is used. pM1c is most advanced

The tumor volume in prostatectomy specimen has a significant influence on the prognosis; larger tumor volumes have been associated with more aggressive behavior of a PCa (D'Amico, Whittington, Malkowicz, Schultz, Kaplan, et al., 1998; D'Amico et al., 1997; McNeal et al., 1986; McNeal et al., 1988). This became distinctly evident in a study of Bostwick et al., where men with PCa volume of 0.5 ml, 10 ml, and 20 ml had a probability to have metastases in 2.8%, 29.5% and 87.4%, respectively (Bostwick et al., 1993). Traditionally, PCa lesions smaller than 0.5 ml are considered unlikely to reach a clinically significant size in an aspect of long doubling time of PCa, however this threshold is nowadays assumed to be too restrictive (Stamey et al., 1993; Ting et al., 2015; Wolters et al., 2011). Nonetheless, independently from tumor volume, the PCa stage, histological grade and surgical margin status are still the most important prognostic parameters of PCa (Epstein, Zelefsky, et al., 2016; Han et al., 2001; Kupelian et al., 1996; Wein et al., 2016). Therefore, tumor volume is more like an additional parameter, linked with stage and grade when evaluating the aggressiveness of PCa. The classic "Epstein criteria" for insPCa in prostatectomy specimen (index tumor volume of <0.5 cm^3, no Gleason 4 or 5 pattern, and organ confined pT2 disease) takes these into account (Epstein et al., 1994; Van der Kwast & Roobol, 2013).

Gleason 3+3 (GGG 1) graded PCa is commonly considered as insPCa without almost any metastatic potential (Eggener et al., 2015; Ross et al., 2012). Therefore its status as a cancer has been questioned (Ahmed et al., 2012). However, a Gleason 3+3 PCa diagnosed in prostate biopsies cannot be assumed as insignificant without additional parameters since the biopsies give only limited information about the whole gland pathology. Hence, the true pathology is only available from a pathological analysis of prostatectomy or autopsy specimen. The issue is nicely demonstrated in the results of an SPCG-study, where radical prostatectomy and watchful waiting were prospectively compared in 15 years' follow-up period: When low risk PCa was defined by Gleason score < 7 and PSA level < 10 ng/ml, intention-to-treat analysis showed cumulative PCa specific mortality in 6.8% of men in the prostatectomy group and in 11% of men in the watchful waiting group without a statistically significant difference between the treatment modalities (Bill-Axelson et al., 2011). However, when the biopsy results were compared with a prostatectomy specimen, in 6 of 7 men who died from PCa in the prostatectomy group, the Gleason score was upgraded in a prostatectomy specimen (Bill-Axelson et al., 2011). Additionally, it has been hypothesized that Gleason 3+3 PCas, especially tumors with high volume, could evolve to a CSPCa in the course of time (Lepor & Donin, 2014; Wein et al., 2016; Whittemore et al., 1991). However, the evidence concerning this potential is not entirely coherent and it might be a result of sampling error i.e. initial biopsies did not hit the highest grade PCa foci in the gland.

D'Amico et al. introduced a division in to the four groups for pretreatment risk of PCa for BCR using the prognostic factors described above (D'Amico, Whittington,

Malkowicz, Schultz, Blank, et al., 1998). The system with only minor modifications is widely adopted as a risk stratification strategy in the management of PCa (Mottet et al., 2018; NICE, 2019; Sanda et al., 2018). **Table 3** presents the risk grouping according to the European Association of Urology (EAU) guidelines (Mottet et al., 2018).

Table 3. EAU risk groups for BCR of localized and locally advanced PCa (Mottet et al., 2018). The cT category only refers to the DRE finding (Mottet et al., 2018).

Low risk	Intermediate risk	High risk	
PSA < 10 ng/ml, Gleason score < 7 (ISUP grade group 1) and cT1-cT2a	PSA 10-20 ng/ml or Gleason score = 7 (ISUP grade group 2–3) or cT2b	PSA > 20 ng/ml or Gleason score > 7 (ISUP grade group 3–5) or cT2c	any PSA any Gleason score (any ISUP grade) cT3–4 or cN+
Localized			Locally advanced

The prognosis of a PCa additionally and obviously depends from treatments chosen and recently the topic has been under intensive and interesting research. The traditional radical treatments for clinically localized PCa are prostatectomy and various radiation therapy modalities (Mottet et al., 2018). Radiation therapy is also used as a part of multimodal therapy in locally advanced PCa (Mottet et al., 2018). Both treatment modalities include significant adverse effects, but they are currently the two available potentially curative treatments for PCa. After a prostatectomy, 17–29% of men have some level of urinary incontinence and a significant proportion (19–56% with varying definitions) of men have erectile dysfunction at the timepoint of 12 months (De Carlo et al., 2014). For some patients, these disorders remain permanent. Radiation therapy is better tolerated at least in a short term; however, it can cause urinary and bowel irritation symptoms during the therapy which usually subside. However, late complications after the most commonly used conventionally fractioned external beam radiation therapy (EBRT) also exist, more commonly gross hematuria (10–14%), rectal bleeding (19–28%), and urinary obstruction (6–9%) (Matta et al., 2019). The complication rates are mainly higher in other radiation therapy modalities (Matta et al., 2019).

Because of the significant adverse effects of the radical treatments, for low-risk patients who are suitable for radical treatments, active surveillance is also a viable option. An active surveillance patient is followed intensively with PSA measurements, clinical status, repeated prostate biopsies and nowadays, also with MRI (Mottet et al., 2018; van den Bergh et al., 2007). If there are signs of disease progression, active treatment still remains an option. In frail patients and patients with low life expectancy, all stages of PCas could be treated with passive surveillance ("watchful waiting") where ADT is initiated only after symptomatic progression

(Mottet et al., 2018). In a patient with a locally advanced or metastasized PCa, ADT is recommended, with or without additional chemotherapy (Mottet et al., 2018).

In a large, prospective ProtecT study, prostatectomy, radiation therapy and active surveillance as treatment alternatives for clinically localized PCa were compared in a randomized setting (Neal et al., 2019). In the study, active surveillance was performed with repeated PSA measurements, and within a 10-years surveillance period, roughly 40% were referred to active treatment (Neal et al., 2019). After 10 years, there were no statistically significant differences in PCa specific or overall mortality among the randomized treatment modality groups (Neal et al., 2019). However, there was significantly more metastatic progression in the active surveillance group, and when the radical treatment groups were combined and compared to the active surveillance group, a reduction in the PCa specific death rate was noted in the radically treated group (Neal et al., 2019). Understandably, there were substantially less adverse effects in the active surveillance group comparing to radically treated men (Neal et al., 2019). In the PIVOT trial, prostatectomy and active surveillance were compared in randomized setting in clinically localized PCa (Wilt et al., 2017). During almost 20 years of follow-up (median 12.7 years), no statistically significant difference in PCa specific mortality was seen between the groups, and definitive treatment occurred in about 20% of patients the in surveillance group (Wilt et al., 2017). In SPCG-4 study, men with localized PCa were randomly assigned to the prostatectomy group and watchful waiting group (n=695) and 29 years' follow-up data were analyzed. The study results favor prostatectomy: after 23 years of follow-up, the mean years of life gained in the prostatectomy group were 2.9 and the results are even more prominent in younger (<65 years) patients (Bill-Axelson et al., 2018). Significantly lower mortality was observed in the prostatectomy group, both death from any cause (relative risk [RR] prostatectomy vs. watchful waiting, 95% CI: 0.74, 0.62–0.87) and death from PCa (RR prostatectomy vs. watchful waiting, 95% CI: 0.55, 0.41–0.74) (Bill-Axelson et al., 2018).

Although PCa is commonly a multifocal disease, due to the invasiveness and significant adverse effects of the radical treatments, there is emerging research concerning mini-invasive, cancer-focused therapies for PCa, i.e. high intensity focused ultrasound (HIFU), cryotherapies and photodynamic therapies, but the role of these alternatives is so far investigational (Mottet et al., 2018). Therefore, a reliable and sensitive imaging modality for adequate identification of each significant cancer foci is essential for the further development of these methods (Mouraviev et al., 2011). Contemporary ultrasound based applications do not yet meet the accuracy demands of focal therapies (Smeenge et al., 2012). Thus, developing prostate MRI seems to be a considerable basis for pre/intraoperative imaging in these new focal therapies.

2.2 Contemporary diagnostic protocol of prostate cancer

2.2.1 Guidelines for primary diagnostics and risk stratification

Recommendations of PCa diagnostics vary only minimally between the leading guidelines. In Finland, most urologists adhere to the EAU guidelines, which is the one discussed below in this chapter (Mottet et al., 2018).

Suspicion of a PCa usually arises from an elevated PSA level or abnormal DRE. Sometimes, the histological diagnosis could be obtained incidentally by prostate surgery, like simple prostatectomy or TURP. However, according to the EAU guidelines, TURP should not be used as a diagnostic tool in PCa detection, even if it was formerly used for diagnostic purposes.

According to the EAU guidelines, a suspicious finding in DRE or an elevated PSA level is an indication for prostate biopsies with certain considerations. An elevated PSA level should be repeated with a new sample prior the biopsy decision due to interfering factors. The guidelines give no cut-off value for PSA level suspicious to CSPCa. However, if the PSA level is between 2–10 ng/ml, in asymptomatic men with a normal DRE finding, the guidelines recommend offering further risk assessment to avoid unnecessary biopsies. As tools for the risk assessment, the guidelines recommend using a risk calculator, imaging or an additional serum or urine-based test (prostate cancer gene 3 [PCA3] marker, SelectMDX, Mi Prostate score, ExoDX, and prostate health index [PHI] and 4Kscore). The guidelines outline that PSA dynamics and doubling time give no additional information in primary diagnostics compared to PSA alone, also the value of free PSA/PSA ratio is limited, and it should not be used if total PSA is over 10 ng/ml.

The EAU guidelines discuss widely and extensively the multiparametric MRI (mpMRI) in PCa diagnostics. It concludes that mpMRI should be performed to biopsy naïve patients, however, the strength of evidence is weak. Nevertheless, due to lack of evidence, mpMRI should not be used in screening for PCa. When mpMRI is done, the guidelines recommend with support of a strong level of evidence that both systematic and targeted biopsies should be performed if a cancer-suspicious lesion is present. With a weak strength of evidence, the guidelines give an option to omit (systematic) biopsies based on shared decision making with the patient, if there is no suspicious lesion in mpMRI and a clinical suspicion of a PCa is low. After a cancer-negative biopsy the guidelines recommend mpMRI before repeat biopsies and even if mpMRI is negative, systematic biopsies should be performed based on shared decision making, if clinical suspicion of PCa is high. The level of evidence is strong with the mentioned recommendations. In the re-biopsy setting, the guidelines give an option to take only mpMRI targeted biopsies with the support of weak strength of

evidence. Finally, the guidelines outline that only a systematic biopsy is an acceptable approach, if mpMRI is unavailable.

According to the EAU guidelines, after cancer-negative biopsies, repeat biopsies are indicated if the PSA level is rising or persistently elevated, or there is a suspicious finding in DRE. Also repeat biopsies should be performed, if there was atypical small acinar proliferation, extensive high grade intraepithelial neoplasia (HGPIN), a few atypical glands immediately adjacent to high-grade prostatic intraepithelial neoplasia or IDC-P as a solitary finding in prior biopsies. Also, a positive finding in mpMRI (performed with the prior biopsies) is a repeat biopsy indication. In men with an elevated risk for PCa and prior cancer negative biopsies, the guidelines recommend considering additional serum, urine and tissue-based tests for selecting men to repeat biopsies. Saturation biopsies (>20 biopsies taken via transperineal approach) may be performed as repeat biopsies.

According to the EAU guidelines, prostate biopsies should be taken with TRUS guidance and via transrectal or perineal approach. Local anesthesia should be used. When taking systematic (baseline) biopsies, at least eight biopsy cores from the peripheral gland should be taken if prostate size is about 30 cc and 10–12 biopsy cores should be taken in larger prostates. Seminal vesicle biopsies are recommended if it has a decisive impact on treatment. Transitional zone biopsies should be limited to repeat biopsies. Prior prostate biopsies and oral or intravenous antibiotic prophylaxis is recommended by the guidelines. In transrectal ultrasound guided prostate biopsies (TRUS-Bx), ciprofloxacin and in transperineal biopsies, single dose of cephazolin are recommended antibiotic regimens. To risk patients, TRUS biopsies with prior rectal swab culture or targeted antibiotic prophylaxis should be offered. Rectal disinfection with povidone-iodine may be considered.

2.2.2 Laboratory tests and clinical examination

The PSA is the most widely used biomarker in PCa diagnostics. In clinical urology, it was introduced in the 1980s to be an useful marker in surveillance of radically treated PCa (Kuriyama et al., 1981; Stamey et al., 1987). The PSA is a member of the kallikrein gene family (also known as human kallikrein peptidase 3 [hK3]). It is practically exclusively produced in the prostate luminal epithelial cells and it is in high concentrations secreted to the seminal fluid where its function is to balance coagulation (Wein et al., 2016). It is essential that PSA is an organ-, not a cancer-specific biomarker (Wein et al., 2016). On a per cell basis, PSA expression is similar between benign and malignant prostate cells; however, elevated serum PSA levels are probably due to disruption of cellular architecture within the prostate gland (Wein et al., 2016). Expression of PSA is strongly influenced by androgens, and the PSA level is also affected by benign conditions and medications, especially advanced age,

prostate volume, BPH, prostatitis and 5-alphareductase (5ARI) medication (Stamey et al., 2004; Wein et al., 2016).

Although PSA is an excellent tool for the surveillance of radically treated PCa, it is a poor marker in diagnostics of PCa regardless its wide usage for diagnostic purposes (Brockman et al., 2015; Thompson et al., 2005). Formerly, prostate biopsies were recommended for men with normal DRE if the PSA value was over 4 ng/ml, i.e. a PSA level below the value 4 ng/ml was considered as normal (Catalona et al., 1994). In the landmark study of Thompson et al., the rate of PCas below the level of 4 ng/ml were 15% in a surveillance period of seven years, and no threshold value ruling out CSPCa were obtained (Thompson et al., 2004). However, a moderate correlation with an increasing PSA level and more aggressive PCa histology among men with PSA levels less than 4 ng/ml were presented in the study (Thompson et al., 2004). In another study with a large cohort of PSA screened men, the area under the receiver operating characteristic curve (AUC) for PSA discriminating any PCa to benign pathology was found to be 0.678 and with significant vs. benign or insPCa (Gleason score < 7), 0.782 (Thompson et al., 2005). Also, PSA velocity in PCa diagnostics has been under intensive research. However, the results are controversial and there is no convincing evidence that PSA velocity has an additional value to PSA level alone in PCa diagnostics (Loughlin, 2014). As a conclusion, when using PSA as a diagnostics tool for PCa, it should be taken into account in addition to the mentioned benign conditions that elevates PSA level, that there is no threshold value for ruling out PCa, and the probability of PCa rises with elevating PSA levels (Stamey et al., 2004; Thompson et al., 2005; Thompson et al., 2004). Thus, in low PSA levels, additional parameters are needed to an adequate biopsy decision.

The prostate specific antigen in PCa screening has been widely used, however its use for the purpose remained a very controversial topic (Loeb, 2014). The major issue in PSA screening is a high false positive rate, which drives to patient anxiety, unnecessary biopsies, and thus unnecessary biopsy related complications in addition to unnecessary diagnoses of insPCas. The Cochrane systematic review from 2013 concludes that among studies with 7–20 years' follow-up, there were no statistically significant differences in PCa-specific or overall mortality in men randomized to the screening and control groups, however overall PCa incidence was significantly higher in PSA screened men (risk ratio: 1.3; 95% CI: 1.02–1.65) and in the screening cohort, diagnosed PCas more commonly localized (risk ratio: 0.80, 95% CI: 0.73–0.87) (Ilic et al., 2013). In 2014, results of a 13-year follow up in The European Randomized Study of Screening for Prostate Cancer were published: in the study, a lower risk for PCa specific mortality was noted in the screened men, but there was no significant difference in overall mortality (Schröder et al., 2014). In addition, the incidence of PCa was 1.57-fold higher in the screening arm (Schröder et al., 2014). Similar results of PSA screening with a decrease in PCa specific mortality and no significant difference in overall mortality was seen in the Swedish Gothenburg randomized

population-based prostate cancer screening trial, where results of PSA screening were investigated in a 17-year study period (Carlsson et al., 2017). In the trial, the number needed to invite PSA screening and the number of PCas needed to diagnose to prevent one PCa death were 176 and 16, respectively (Carlsson et al., 2017).

The European Association of Urology recommends an individualized risk-adapted strategy for early detection to well-informed men with a good performance status and a life-expectancy of at least ten to fifteen years, thus systematical PSA screening to all men is not recommended (Mottet et al., 2018). Although many prospective high-volume studies have been published, PSA screening is still under investigation.

There is no significant role for PSA density (PSA divided with prostate volume) as an individual marker in early detection of PCa according to the EAU and National Institute of Health and Care Excellence (NICE) guidelines (Mottet et al., 2018; NICE, 2019). However, in the recent studies, PSA density seems to be a better predictor for CSPCa than the PSA level alone, especially in higher PSA levels and in repeat biopsy setting (Jue et al., 2017; Nordström et al., 2017). According to the studies, PSA density should be taken as an additional component for prostate biopsy decision-making, when estimating an individual risk for a CSPCa. However, as a PSA derivative, there is no threshold value in ruling out CSPCa.

Within serum, PSA circulates bound to proteases and in unbound form (free PSA) and the lower ratio of free PSA and PSA is detectable in men with a PCa (Wein et al., 2016). The usage of free PSA/PSA ratio is useful in PSA levels lower than 10 ng/ml, due to the rising positive predictive value (PPV) of total PSA in levels more than 10 ng/ml (Wein et al., 2016). A recent meta-analysis presents poor outcomes of free PSA/PSA ratio in PCa diagnostics: pooled sensitivity and specificity were 0.70 and 0.58, respectively, when total PSA was between 4–10 ng/ml (Huang et al., 2018). According to the EAU guidelines, the free PSA ratio has limited clinical value in the light of novel serum tests (Mottet et al., 2018).

Feasibility of PSA kinetics (velocity and doubling time) have been studied aiming to increase PSA sensitivity in primary diagnostics of PCa, and its usage has been recommended in several guidelines (Carter et al., 2007; Loughlin, 2014). However, its feasibility has been questioned in recent studies (Loughlin, 2014). It seems that the additional value given by PSA velocity to increase sensitivity of PSA is minimal or none, and its usage is not recommended by contemporary EAU guidelines (D'Amico, Whittington, Malkowicz, Schultz, Kaplan, et al., 1998; Mottet et al., 2018).

The EAU guidelines mention several commercial diagnostic tests listed below that could be used as a part of individual risk assessment when DRE is normal and PSA is between 2–10ng/ml (Mottet et al., 2018). Prostate cancer gene 3 and SelectMDX are urine-based microRNA markers. Prostate cancer gene 3 is shown to outperform PSA testing predicting PCa in subsequent biopsy, however they performed similarly in predicting high-grade PCas (Wein et al., 2016). SelectMDX measuring HOXC6 and

DLX1 mRNA levels, which were shown to be good predictors for the detection of high-grade PCa (Van Neste et al., 2016). The Michigan Prostate score is based on a urine test and it combines information from transmembrane proteinase TMPRSS2 and ERG-gene fusion and PCA3. TMPRSS2:ERG fusion which is present in at least 50% of screened PCas. The ExoDX measures exomes secreted by PCa cells from urine in which ERG and PCA3 is analyzed resulting in a good NPV for high grade PCa (Donovan et al., 2015). The prostate health index and 4Kscore are serum tests using kallikreins expressed from the prostate as a part of a multifactorial score, and various combinations of these kallikreins outperformed PSA and free PSA/PSA ratio in the detection of PCa and are additionally closely correlated with an increasing Gleason score (Wein et al., 2016).

A clinical examination in the context of PCa diagnostics means DRE and TRUS. A local extent of PCa can also be estimated with MRI, which is discussed later (see chapter 2.3. MRI in primary diagnostics of prostate cancer). Distant spreading of PCa is routinely evaluated with bone scan and computer tomography (CT) in patients with increased risk of non-localized disease (Mottet et al., 2018). According to the guidelines, distant spreading should be evaluated from men in the intermediate risk group (see **Table 3**) with GGG ≥3 (Mottet et al., 2018). In addition, various applications of positron emission tomography (PET) could be used in staging of PCa, however the clinical significance of PET findings still remain unclear (Mottet et al., 2018). However, PET is not routinely used to evaluate the local extent of PCa in the primary diagnostics.

Digital rectal examination has relatively poor value as an individual test in PCa diagnostics: In the recent meta-analysis, DRE performed by a primary care physician had a sensitivity of 0.59 and specificity of 0.51 (Naji et al., 2018). However, DRE has limited complementary value in combination with PSA in an early detection of PCa, because PSA and DRE not necessarily detect the same cancers (Catalona et al., 1994; Wein et al., 2016).

Transrectal ultrasound has been used in PCa diagnostics from the 1980s. Ultrasound guided biopsies have been the gold standard in PCa diagnostics for the last decades. However, traditional grayscale ultrasound has relatively poor value in visualizing PCa lesions, and according to the EAU guidelines, biopsies targeted to lesions visible in traditional ultrasound cannot replace systematic biopsies (Mottet et al., 2018). According to the consensus meeting concerning the role of ultrasound in PCa focal therapies, conventional ultrasound without biopsy is not suitable for diagnosing and staging PCa and it should be used solely for identifying the location of the prostate, directing biopsies and assessing gland volume, as well as anatomical variations (Smeenge et al., 2012).

Onur et al. presented with the prospectively collected study cohort in the 1990, that in biopsies from hypoechogenic i.e. cancer suspicious areas or isoechogenic areas in TRUS from any location (base, middle, apex, transitional zone) of prostate, have

no significant difference in cancer detection rate (Onur et al., 2004). In addition, prostates with hypoechogenic lesions did not have significantly more PCas than prostates without them (Onur et al., 2004). However, prostate size or the Gleason score of cancers detected were not defined in the study. In a more recent study from the early 2000s, Toi et al., presented in a large prospective series of men quite opposite results: in prostates with a suspicious lesion visible in TRUS, a PCa was detected almost twice as likely than when there were no visible lesions and 83.8% had PCa detected in a biopsy core taken from the visible lesion (Toi et al., 2007). In addition, the TRUS finding is also correlated with the tumor grade; a Gleason score of ≥7 was found twice as frequently in the TRUS positive group as in the TRUS negative group (Toi et al., 2007). Aforementioned differences between the groups were all significant (Toi et al., 2007). An obvious explanation for the difference in results seems to be the evolution of ultrasound technique in a course of time.

The European Association of Urology guidelines do not recommend using TRUS for local staging of PCa (Mottet et al., 2018). According to the study of Hamper et al. from the 1980s, the outcomes in evaluating extraprostatic extension with ultrasound is moderate (Hamper et al., 1991). It is worth mentioning that the cancer detection rate was quite good in the study; a PCa was detected in 76% of hypoechogenic lesions, however hypoechogenic lesions detected without a cancer were not reported (Hamper et al., 1991). In the study, a positive predictive value (PPV) for capsular penetration was 80% and a negative predictive value (NPV) was 85% while the ultrasound became more accurate with increasing penetration depth (Hamper et al., 1991).

New interesting applications of ultrasound technology, e.g. ultrasound elastography and contrast-exchanged ultrasound, are emerging in PCa diagnostics with a promising results (Sarkar & Das, 2016). However, their role in PCa diagnostics or staging is not yet defined by the guidelines (Mottet et al., 2018).

2.2.3 Prostate biopsies

2.2.3.1 Procedures and outcomes

After a suspicion of PCa has emerged, systematic prostate biopsies using an 18 gauge needle gun and TRUS guidance with or without ultrasound/MRI/DRE lesion-targeted biopsies remain to be the cornerstones of PCa diagnostics, especially in biopsy naïve patients (Mottet et al., 2018). According to the guidelines, depending on prostate size, 8–12 biopsy cores are taken from the prostate systematically from the template distributed regions (Mottet et al., 2018; NICE, 2019). An increasing core number over 12 is not more conclusive in diagnostics; however, additional targeted biopsies from suspicious lesions are acceptable to take (Mottet et al., 2018; Shariat & Roehrborn, 2008). Saturation biopsies (i.e. > 12 systematic biopsy cores) increase the detection

rates of both CSPCa and insPCa, thus as a procedure demanding additional anesthetic requirements, it could be more useful in a repeat biopsy setting (Li et al., 2014; Walz et al., 2006; Wein et al., 2016).

Commonly, prostate biopsies can be taken via the transperineal or transrectal route. In the transrectal biopsy procedure, biopsy needles penetrate rectal mucosa whereas in the transperineal biopsy procedure, they penetrate the sterilized perineum. Theoretically, the transperineal biopsy procedure should then be more sterile. Anatomically, transperineal prostate biopsies could also be the better approach in biopsing anterior and apical lesions and, in addition, the longitudinal orientation could be more efficient in biopsing the peripheral zone (Eldred-Evans et al., 2016; Wein et al., 2016). Obviously, the transperineal approach is the only way to perform prostate biopsies in patients with no access to the rectum. However, in the recent meta-analysis, the approaches have no significant differences in cancer detection and complication rates in systematic biopsies (Xue et al., 2017). The TRUS-Bx procedure is performed using a local infiltration anesthesia to the periprosthetic region, while the transperineal procedure requires at least an additional infiltration anesthesia to perineal skin and subcutis (Wein et al., 2016). These requirements of anesthesia and sterilized conditions make transperineal biopsies less practical in outpatient use comparing to the transrectal approach.

Taking into account the central role of systematic prostate biopsies in the traditional PCa diagnostics, their sensitivity in diagnostics of CSPCa is low. In a study of Epstein et al., authors investigated a large series of prostatectomy specimens, in which the Gleason scores from 5–6 were upgraded in 36% of cases and if tertiary patterns were ignored, 25% of the whole cohort were upgraded (Epstein et al., 2012). In the autopsy study of Haas et al., an NPV of traditional 12-core biopsy protocol in excised prostates was 80% for CSPCa (Haas et al., 2007). In a recent study of Ahdoot et al., the Gleason score was upgraded to CSPCa from systematic biopsies in 30.2% of cases in a series of 404 prostatectomy specimens (Ahdoot et al., 2020).

An alternative to systematic biopsies is template mapping biopsies, in which the prostate gland is transperineally saturated with biopsy cores using a grid of e.g. 5 mm, which theoretically cannot miss (spherical) lesions larger than 5 mm. In a study of Crawford et al., in 25 men, transperineal mapping biopsies from prostates with a 5mm grid with mean 49 cores (27–110) per patient, were compared to the pathology of whole mount prostatectomy specimen (Crawford et al., 2013). In the study, 18 of 64 lesions diagnosed in the prostatectomy specimen were missed by transperineal mapping prostate biopsies, but a CSPCa was in only one missed lesion (Crawford et al., 2013). In the study, CSPCa was defined as lesions ≥ 0.5 cm^3 or Gleason score ≥ 7 (Crawford et al., 2013). However, the prostate with the missed CSPCa lesion has an additional CSPCa focus diagnosed by the study biopsies (Crawford et al., 2013). Marra et al. analyzed biopsy results and prostatectomy specimens from 204 retrospectively collected men, which underwent saturation template mapping biopsies

with a 5mm grid with a mean of 30 biopsies per patient and subsequent radical prostatectomy (Marra et al., 2017). In the study, the PCa histology in 12.7% of patients was upgraded, and 11.8% were downgraded from prostate biopsies in an analysis of prostatectomy specimen (Marra et al., 2017). As a conclusion, for now, transperineal template mapping biopsies are quite a demanding alternative for TRUS-Bx in primary diagnostics of a PCa. In addition, the limitations in sensitivity and specificity should be noted when used as a reference in studies concerning targeted prostate biopsies.

2.2.3.2 Antibiotic prophylaxis

The EAU guidelines recommend an use of antibiotic prophylaxis in TRUS-Bx and transperineal prostate biopsies (Mottet et al., 2018). In the TRUS-Bx, fluoroquinolones is recommended as an antibiotic regimen, while in transperineal biopsies only a single dose of intravenous cephazolin is recommended (Mottet et al., 2018). However, the guidelines do not give recommendations for a dose or timing for the prophylaxis (Mottet et al., 2018).

As an additional prophylactic intervention, the EAU guidelines now recommend to consider a rectal disinfection with povidone-iodine prior biopsies (Mottet et al., 2018). According to the studies, it is simple and affordable, not associated with the selection of resistant bacteria and seems to reduce infectious complications after transrectal prostate biopsies (Pilatz et al., 2019; Roberts et al., 2017).

In a Cochrane review from 2011, the effectiveness of antibiotic prophylaxis in transrectal prostate biopsies was evaluated (Zani et al., 2011). In the analysis, the authors presented that in placebo/no treatment controlled trials, antibiotic prophylaxis was significantly more effective against infectious complications classified as bacteriuria, urinary tract infection and hospitalization (Zani et al., 2011). Moreover, analyzing studies that directly compared different antibiotics, there was no difference between fluoroquinolones and other classes of antibiotics (Zani et al., 2011). In addition, using a long course of antibiotics or multiple antibiotic regimens in prophylaxis had significant reducing effect to bacteriuria, but not for other measured variables (Zani et al., 2011). Most of the studies in the aforementioned review were published 20 years ago, which should be taken into account in applying the results to today's practice in an evolving bacterial resistance environment (CDDEP, 2019).

Recently, infectious complications of transrectal prostate biopsies have increased, and it is suspected to arise from an increasing fluoroquinolone resistance rate of the most common pathogen causing biopsy related infections, *Escherichia coli* (E. coli) (See chapter: 2.2.3.3 Biopsy complications). Therefore, numerous alternative and additional antibiotics to fluoroquinolones in addition to targeting antibiotic prophylaxis have been investigated, aiming to reduce the complication rate (Loeb et al., 2013). In a meta-analysis of Roberts et al., fosfomycin significantly outperformed fluoroquinolones in reducing post-TRUS-Bx complications when comparing the rates

of overall infectious complications and infectious complications caused by fluoroquinolone resistant organisms (Roberts et al., 2018). However, in the study, there was no significant difference in the rate of infectious complications caused by fluoroquinolone sensitive organims (Roberts et al., 2018). As a conclusion, while fosfomycin is relatively safe, and have favourable pharmacological features for use as TRUS-Bx prophylaxis, it is promising in reducing infectious complications after TRUS-Bx (Noreikaite et al., 2018; Roberts et al., 2018). In addition, targeted antibiotic prophylaxis might have an effect on reducing complications, however the influence on the complication rate was minor and high-quality evidence is still missing (Pilatz et al., 2019; Scott et al., 2018).

Fluoroquinolone resistance and its adverse effects have recently been under intensive public discussion. In March 2019, the European Commission has banned fluoroquinolones as a prophylaxis in urological procedures including TRUS-Bx (Bonkat et al., 2019). At the moment (January 2020), EAU guidelines are not yet adapted to this regulation; however, possible alternatives might be fosfomycin or a rectal swab culture targeted prophylaxis, as well as the change to transperineal prostate biopsy (Bonkat et al., 2019).

2.2.3.3 Biopsy complications

In a systematic review by Loeb et al., complications of TRUS-Bx were primarily investigated (Loeb et al., 2013). In this systematic review, the frequency of infection varies among studies, with most studies reporting infectious complications requiring hospitalization in 0–6.3% of cases (Loeb et al., 2013). Also serious infectious complications occur: Incidence of sepsis and bacteremia varies among studies between 0.62% and 3.06% (de Jesus et al., 2006; Pinkhasov et al., 2012; Simsir et al., 2010; Steensels et al., 2012; Zaytoun et al., 2011). Lahdensuo et al. reported in their retrospective study cohort a mean bacteremia rate of 0.7% in patients after TRUS-Bx in southern Finland (Lahdensuo et al., 2016). Carmignati et al. presented, in a prospective, multicenter study, urosepsis in 10/447 (2.2%) patients after TRUS-Bx (Carmignani et al., 2012).

There is also a significant number of non-infectious biopsy complications. In the systematic review of Loeb et al., the rate of hematuria varies between 10–84%, however significant bleeding requiring hospitalization occurs in less than 1% of cases (Loeb et al., 2013). Rate of rectal bleeding were 1.3–45% and urinary retention occurs in 0.2–1.7% of cases (Loeb et al., 2013).

In the aforementioned systematic review of Loeb et al., an additional analysis was made concerning complications of transperineal biopsies: In the analysis, the rate of urinary tract infections varies between 0–1.6%, and the rate of prolonged or severe hematuria occurs in 0–5.2% (Loeb et al., 2013). Urinary retention was reported in 1.6–8.8% of cases (Loeb et al., 2013). There is no clear evidence supporting the hypothesis

that transperineal prostate biopsies cause less infectious complications compared to the transrectal approach (Loeb et al., 2013; Pilatz et al., 2019). However, urinary retention might be more prevalent after transperineal biopsies (Loeb et al., 2013; Moran et al., 2006).

An increased number of biopsy cores does not seem to cause more infectious biopsy complications, however the influence on bleeding complications is controversial (Loeb et al., 2013; Pilatz et al., 2019).

The current EAU guidelines do not recommend interrupting anticoagulation antiplatelet therapy prior to prostate biopsies (Mottet et al., 2018). According to a review of Culkin et al., an uninterrupted use of aspirin does not increase the risk of moderate/severe hematuria or rectal bleeding after TRUS guided biopsies (Culkin et al., 2014). In the study of Ihezue et al., there were no significant differences in the rate of rectal bleeding after TRUS guided prostate biopsies between a patient group using warfarin medication and a control group (Ihezue et al., 2005). In the study, hematuria was significantly more common in the warfarin group; however, there were no significant differences in the severity of bleeding complications between the groups (Ihezue et al., 2005). As a conclusion, severe bleeding complications after prostate biopsies are rare and their prevalence does not seem to depend on anticoagulation/antiplatelet therapy; however, risks following interruption of anticoagulation/antiplatelet therapy could be more harmful.

There is a worrying trend of increasing infectious complications after prostate biopsies in recent years, however a similar trend is not seen in other types of biopsy complications (Borghesi et al., 2016; Carignan et al., 2012; Nam et al., 2013). In a retrospective registry study of 75 000 biopsied men, hospital admission rates due to infection after prostate biopsies from 1996 to 2005 have increased from 0.6% to 3.6% (Nam et al., 2013). According to the mentioned study, the mortality rate during 30 days after prostate biopsies was 0.09%, however there was no similar increasing trend in the mortality rate as was in infectious complications (Nam et al., 2013). In a retrospective registry study conducted in Finland, bacteremic complications after prostate biopsies have increased from 0.5% in 2005 to 1.2% in 2012 and 53% were caused by a fluoroquinolone resistant organism (Lahdensuo et al., 2016). Feliciano et. al presented similar results: The incidence of infectious complications after TRUS-Bx were three times higher and specifically infectious complications caused by fluoroquinolone resistant organisms were also 3.3 to 4.3 times higher in 2006 compared to the incidence in 2004 and 2005 (Feliciano et al., 2008). In addition, post-biopsy urine and blood cultures of 79% of patients with infectious complications included a fluoroquinolone resistant organism (Feliciano et al., 2008). It has been a widely accepted hypothesis that the increasing rate of infectious complications after TRUS-Bx arises from a globally increasing fluoroquinolone resistance rate of E. coli strains (Borghesi et al., 2016; Loeb et al., 2013; Wagenlehner et al., 2013).

In determining an ability of fluoroquinolone resistant organisms to cause post-TRUS-Bx infections, it might not be a fruitful approach to dichotomize pathogens as resistant or susceptible to fluoroquinolones. In the study of Kalalahti et al., also a moderately decreased fluoroquinolone susceptibility of an organism seems to be associated with higher infectious TRUS-Bx complication rates (Kalalahti et al., 2018).

In an international prospective multicenter study, no significant risk factors for infectious complications emerged after prostate biopsies, which were mainly performed by the transrectal approach (Wagenlehner et al., 2013). In a prospective study of Steensels et al., rectal cultures from patients having TRUS-Bx were investigated, and fluoroquinolone resistant E. coli seems to be a significant risk factor for infectious biopsy complications (Steensels et al., 2012). In a systematic review investigating risk factors for infectious complications after TRUS-Bx, all significant risk factors were presumably associated with an increased risk of antibiotic resistant strains (urogenital infection, antibiotic use, international travel, hospital exposure, bacteriuria, previous TRUS-Bx, and resistance of fecal flora to antibiotic prophylaxis) (Roberts et al., 2017). However, also a higher comorbidity has been associated with a significantly increased risk of hospitalization after TRUS-Bx (Carignan et al., 2012; Roberts et al., 2017).

2.2.3.4 Emerging antibiotic resistance of E. coli

Escherichia coli as a concept consists of a wide variety of bacterial strains with varying virulence potential (Vaara et al., 2010). It is a gram-negative, aerobic coliform and a main aerobic pathogen of the human bowel (Vaara et al., 2010). It is the most common pathogen causing infections after prostate biopsies and also the most common fluoroquinolone resistant pathogen: In a series of 63 men admitted to hospital due to an infection after prostate biopsies, E. coli was a predominant organism in 84% of urine and 91% of blood cultures (Loeb et al., 2012). In a prospective study of Taylor et al., the rectal flora of 865 patients having TRUS-Bx during 2009–2011 were investigated (Taylor et al., 2013). In the study, fluoroquinolone resistant gram-negative coliforms were detected in 19% of men and the rate of E. coli in these gram-negative and fluoroquinolone resistant strains was 90% (Taylor et al., 2013). There is an worldwide increase in the fluoroquinolone resistance of bacterial strains, including E. coli, and it has a significant clinical relevance to PCa diagnostics, because fluoroquinolones are still recommended as an antibiotic prophylaxis prior TRUS-Bx (See chapter: 2.2.3.3 Biopsy complications) (CDDEP, 2019; Mottet et al., 2018).

Additionally, E. coli is the most common pathogen producing extended spectrum beta lactamases (ESBL), and its prevalence is also increasing worldwide (Ben-Ami et al., 2009). Extended spectrum beta lactamases are a heterogeneous family of commonly plasmid mediated enzymes that inactivate and confer resistance to most beta-lactam antibiotics, including penicillins and cephalosporins (Brolund &

Sandegren, 2016; Vaara et al., 2010). A clinical relevance of ESBL producing strains in PCa diagnostics is that cephalosporins are commonly used as a first line parenteral antimicrobial therapy in community-acquired infections in emergency medicine, including men's urinary tract infections. In the aforementioned prospective study of Taylor et al., 4.6% of men having prostate biopsies harbored an ESBL producing strain in their rectal bacterial flora (Taylor et al., 2013). In addition, ESBL producing pathogens show greater resistance to fluoroquinolones than the average; over 70% of ESBL strains were also fluoroquinolone resistant in an international multi-institutional study (Ben-Ami et al., 2009).

Colonization with resistant E. coli strains mainly occurs by indigestion and the resistant strains are suspected to arise especially from a wide and off-label usage of antibiotics in food animals (Collignon, 2009). However, the antibiotic usage of an individual could also influence the colonization of resistant organisms: In the registry study of Kim et al., an inpatient use of fluoroquinolones, beta-lactams/beta-lactamase inhibitors and carbapenems significantly increased E. coli strains' resistance to fluoroquinolones in various isolates (Kim et al., 2018). In addition, carbapenem and fluoroquinolone usage increased resistance of E. coli to 3rd-generation cephalosporins (3CEF) (Kim et al., 2018).

In the previously mentioned prospective study of Taylor et al., risk factors for carriage of a ciprofloxacin resistant strain in men undergoing prostate biopsies were also analyzed (Taylor et al., 2013). In the study, 19% of men were found to have ciprofloxacin resistant gram-negative organism in the rectal flora and in a multivariate analysis, a heart valve replacement and ciprofloxacin use within three months were the only significant risk factors for the carriage of the organism (Taylor et al., 2013). Additionally, infectious complications were observed in the study: an infectious complication occurred in 3.6% of the patient population and 48% of these patients grew ciprofloxacin-resistant organisms on their pre-biopsy rectal swab culture, while fluoroquinolone resistant gram-negative coliforms were detected in 19% of the whole study cohort (Taylor et al., 2013). It should be mentioned that diabetes and suppressed immunity were not significant risk factors for fluoroquinolone resistance in the study (Taylor et al., 2013). Mulder et al. studied risk factors for fluoroquinolone resistance in patients having community acquired urinary tract infections (Mulder et al., 2017). In the study, the rate of ciprofloxacin resistant E. coli were 10.2% and in a multivariate analysis, higher age and recent fluoroquinolone prescriptions were detected as risk factors for ciprofloxacin resistance, while no association between fluoroquinolone use more than one year before the culture and ciprofloxacin resistance could be demonstrated (Mulder et al., 2017). However, the study population consisted mainly of women (Mulder et al., 2017).

Ben-Ami et al. reviewed the risk factors for community acquired infection due to an ESBL producing organism (Ben-Ami et al., 2009). In the study, the most common ESBL producing pathogen was E. coli, which occurs in 87.6% of the analyzed patient

specific isolates and the rate of ESBL producing strains among the E. coli isolates was 33.6% (Ben-Ami et al., 2009). In a multivariate analysis, statistically significant predictors for infection due to ESBL-producing enterobacteriaceae were age ≥ 65 years, recent use of any antibiotic, recent hospitalization, residence in a long-term care facility, and male sex (Ben-Ami et al., 2009).

An interesting study about colonization of antibiotic resistant E. coli strains was carried out by Kennedy et al. with volunteers traveling internationally (Kennedy & Collignon, 2010). In the study, pre-travel and post-travel incidence of ciprofloxacin resistant E. coli were 3.9% and 33.3%, respectively and pre-travel and post-travel incidence of 3CEF resistant E. coli (mostly ESBL producing) strains were 2.0% and 25.5%, respectively (Kennedy & Collignon, 2010). The differences were all significant. Study patients with resistant E. coli were more likely to have developed gastroenteritis or have taken antibiotics whilst traveling (Kennedy & Collignon, 2010). Over a half of the patients had gentamicin, ciprofloxacin and/or 3CEF resistant E. coli after traveling in Asia (excluding Japan), South America, Middle East, Africa or India (Kennedy & Collignon, 2010). The majority of study patients cleared all ciprofloxacin and 3CEF resistant E. coli within two months and clearance of 3CEF resistant strains was the most rapid; however, at least 18% of those returning with ciprofloxacin/3CEF resistant strains remained persistently colonized at six months post-travel (Kennedy & Collignon, 2010).

Similar results were presented by Kantele et al. in a study investigating risk factors for ESBL colonization within Finnish volunteers traveling from outside the Nordic countries (Kantele et al., 2015). In the study, 21% of travelers were colonized by ESBL producing strain while in a multivariate analysis, significant risk factors for the colonization were travels to sub-Saharan Africa, tourist diarrhea, having an antibiotic use for tourist diarrhea and having meals with the locals (Kantele et al., 2015).

2.3 MRI in primary diagnostics of prostate cancer

2.3.1 Basics of prostate MRI sequences and reporting systems

Prostate MRI is multiparametric, i.e. includes several sequences, including anatomical (T1 and T2 weighted [T2W] imaging) and functional (diffusion weighted imaging [DWI], dynamic contrast exchanged [DCE] imaging and MRI spectroscopy) sequences (Sarkar & Das, 2016). The gold standard in prostate MRI imaging is mpMRI, including T2W imaging, DWI and DCE imaging sequences (Mottet et al., 2018; NICE, 2019).

T2 weighted imaging is an anatomical imaging sequence, with excellent soft tissue contrast and depiction of the zonal anatomy of the prostate, thus it is the most practical

sequence to identify defects in the zonal anatomy i.e. extracapsular extension by PCa cells (Sarkar & Das, 2016). In T2W imaging, the water-rich peripheral zone appears in bright, while PCa appears usually as a rounded or ill-defined low signal intensity lesion in the peripheral zone. Rarely BPH in the peripheral zone could give a false positive finding (Sarkar & Das, 2016; Wein et al., 2016). However, several other conditions mimic the PCa appearance as well (Sarkar & Das, 2016). The transitional zone appears relatively darker in T2W imaging where it is often harder to distinguish PCa from BPH (Sarkar & Das, 2016). T1 weighted images are obtained to determine, if hemorrhage is present in the prostate, which can give rise to a false positive interpretation in T2 weighted imaging (Wein et al., 2016). T2 weighted imaging has a high specificity, but low sensitivity to detect PCa, therefore multiparametric assessment is needed (Wein et al., 2016).

Diffusion weighted imaging measures the diffusion of water molecules in the magnetic field (Brownian motion) in extracellular space, therefore a high cellular density, which is typical in PCa, makes contrast to images decreasing extracellular space (Sarkar & Das, 2016; Wein et al., 2016). In DWI, a duration and strength of the magnetic field, measured by b-values, can be changed and apparent diffusion coefficient (ADC) maps can be made for cancer detection (Sarkar & Das, 2016). The b-values present a threshold for detecting tissue restriction (Wein et al., 2016). Apparent diffusion coefficient values have been found to predict cancer aggressiveness (Sarkar & Das, 2016; Wein et al., 2016).

Dynamic contrast enhanced imaging is a T1 weighted sequence using a contrast medium, typically gadolinium, which rapidly diffuses to the extracellular space (Sarkar & Das, 2016). The DCE made before, during and after intravenous injection of the contrast medium, and the increased tumor microvessel density and higher permeability make differences in the enhancement of tumor tissue (Sarkar & Das, 2016).

Magnetic resonance spectroscopic imaging is based on determining the cellular metabolite concentrations in the prostate tissue (Sarkar & Das, 2016). The cancerous tissue has lower levels of citrate and the levels of choline increase with higher cellular density, cell membrane turnover, and phospholipid metabolism during PCa and the choline-citrate ratio is measured by the sequence (Sarkar & Das, 2016).

The most widely accepted reporting system for prostate MRI is the Prostate Imaging Reporting and Data System (PI-RADS), and the EAU guidelines recommend to adhere to PI-RADS guidelines in mpMRI interpretation (Mottet et al., 2018). The last update to the PI-RADS system was in 2019 to version 2.1 (Turkbey et al., 2019). The system is a five-tiered estimation of the probability of CSPCa, based on a combination of T2W, DWI and DCE MRI sequences, while DCE plays a minor role in determining PI-RADS (American College of Radiology®, 2019). Significant prostate cancer is defined in PI-RADS v2.1 scoring system by Gleason score $\geq$ 3+4, and/or tumor volume $\geq$ 0.5 cc, and/or tumor extraprostatic extension (American

College of Radiology®, 2019). PI-RADS score is given to every cancer-suspicious lesion in the prostate gland, based on a combination of mpMRI findings only and not incorporating any clinical factors (American College of Radiology®, 2019). Although, PI-RADS v2.1 does not include detailed recommendations for the management of suspicious lesions (American College of Radiology®, 2019). However, according to the PI-RADS v2.1, biopsy should be considered for PI-RADS 4 or 5, but not for PI-RADS 1 or 2, while a decision to biopsy PI-RADS category 3 lesion should be based on other factors than the mpMRI score, including the laboratory/clinical history and local preferences, expertise and standards of care (American College of Radiology®, 2019).

PI-RADS version 2.1 assessment categories (American College of Radiology®, 2019):

- PI-RADS 1: very low (clinically significant cancer is highly unlikely to be present)
- PI-RADS 2: low (clinically significant cancer is unlikely to be present)
- PI-RADS 3: intermediate (the presence of clinically significant cancer is equivocal)
- PI-RADS 4: high (clinically significant cancer is likely to be present)
- PI-RADS 5: very high (clinically significant cancer is highly likely to be present)

PI-RADS version 2.1 scoring is based only on the radiographic appearance of the lesion (American College of Radiology®, 2019). T2W and DWI lesional scores are reported in a scale of 1–5 and the contribution of sequence score to the final score is depending on the location of the lesion (American College of Radiology®, 2019). The dynamic contrast enhancement score is dichotomized, and its significance is in upgrading score 3 to 4 in peripheral zone lesions (American College of Radiology®, 2019). Generally, a difference between PI-RADS version 2.1 scores 4 and 5 is depending only on a diameter (cutoff 1.5cm) or a definite extraprostatic extension/invasive behavior of the lesion (American College of Radiology®, 2019).

Due to a multifocal nature of PCa, in the PI-RADS version 2.1 scoring, up to four lesions should be identified, and the lesion with the highest PI-RADS version 2.1 score should be defined as an index lesion, or if there are several lesions with the same PI-RADS version 2.1 score, the lesion with extraprostatic extension, or secondarily, the largest lesion should be defined as the index lesion (American College of Radiology®, 2019).

Another, less used alternative for PI-RADS scoring in prostate MRI reporting is the five-step tiered Likert scoring, in which suspicion categories for CSPCa are analogous with PI-RADS scoring. The Likert system varies between studies, and it is

more a general impression of the performing radiologist of the level of CSPCa suspicion, and it is not bound to any structured reporting system. The main differences of the Likert system comparing to the PI-RADS system in PCa diagnostics is an opportunity to patient based (contrary to lesion-based) analysis and combination of non-prespecified imaging, biochemical data and reader's experience (Khoo et al., 2019). According to the Khoo et al. prospective, multicenter study, the Likert scoring system is a valid alternative to PI-RADS system in experienced centers, with no significant differences in cancer detection rates (DR) of CSPCas or insPCas (Khoo et al., 2019).

Rosenkranz et al. investigated in a retrospectively collected cohort an inter-reader reproducibility of mpMRI interpretation with PI-RADS (initial version) and Likert scoring systems among three radiologists with different grades of experience (Rosenkrantz et al., 2013). In the analysis, generally agreement between experienced readers in Likert and PI-RADS scoring system was strong (Rosenkrantz et al., 2013). However, in transition zone lesions, agreement was lower with both reporting systems (Rosenkrantz et al., 2013). Agreement between experienced and inexperienced readers was slightly better in Likert than in PI-RADS scoring system (Rosenkrantz et al., 2013).

2.3.2 MRI in prostate cancer diagnostics

Multiparametric MRI has now been adopted to EAU guidelines as a complementary part of initial PCa diagnostics, if available (Mottet et al., 2018). Formerly, mpMRI was recommended only after cancer negative biopsies in prevailing suspicion of PCa. The role of MRI in the guidelines is handled in more detail in chapter 2.2.1: Guidelines for primary diagnostics and risk stratification.

Recently, high quality prospective (**Table 4**) studies concerning the sensitivity of mpMRI in diagnostics of PCa have resulted quite unanimously in its good sensitivity to CSPCa in addition to its tendency to discriminate insPCas as described in more detail below.

Table 4. Details of prospective studies concerning mpMRI in PCa diagnostics.

study name	author	year	n	reference standard	CSPCa definition (in current comparison)	definition for a CSPCa suspicious lesion
PROMIS	Ahmed et al.	2017	576	transperineal template mapping biopsies	Gleason ≥ 7	Likert score 3–5
PRECISION	Kasivisvanathan et al.	2018	500	systematic transrectal biopsies	Gleason ≥ 7	PI-RADS version 2 score 3–5
4M	Van der Leest et al.	2019	626	systematic transrectal biopsies	Gleason ≥ 7	PI-RADS version 2 score 3–5
MRI-FIRST	Rouvière et al.	2019	275	systematic transrectal biopsies	Gleason ≥ 7	Likert score 3–5

In a multicenter PROMIS trial, mpMRI targeted and TRUS guided systematic biopsy results were compared against transperineal template mapping biopsies (Ahmed et al., 2017). In the study, NPV of mpMRI and TRUS guided systematic biopsies in ruling out CSPCa were 76% and 63% ($p<0.0001$), respectively, while a prevalence of CSPCa was 53% (Ahmed et al., 2017).

In an international, multicenter, randomized, controlled PRECISION trial, a cohort of men with prebiopsy mpMRI was compared to a cohort of men having a traditional diagnostics with only systematic prostate biopsies (Kasivisvanathan et al., 2018). In the mpMRI cohort, only lesion targeted biopsies from lesions suspicious to a CSPCa in mpMRI, if present, were taken without performing additional systematic biopsies (Kasivisvanathan et al., 2018). In the MRI cohort, CSPCa were diagnosed in 38% of men, and in 26% of men in the systematic biopsy cohort ($p=0.005$) (Kasivisvanathan et al., 2018). In addition, fewer insPCas were diagnosed in the MRI cohort (9% vs. 22%; $p<0.001$) and the percentage of PCa in biopsy cores was higher in the MRI group (Kasivisvanathan et al., 2018).

In a multicenter 4M trial, prebiopsy mpMRI were performed and systematic biopsies were taken from all patients (van der Leest et al., 2019). Additional in-bore mpMRI targeted biopsies were taken, if suspicious lesions were present in the mpMRI (van der Leest et al., 2019). In the study, there were no statistically significant differences in detection rates of CSPCa in systematic biopsies and MRI-targeted biopsies while the prevalence of CSPCa was 32% in combined biopsies (van der Leest et al., 2019). Additionally, there were no significant differences in Gleason ≥ 4+3 prevalence in MRI targeted biopsies comparing to the systematic biopsies, while the prevalence of Gleason ≥ 4+3 PCa in combined biopsies was 15% (van der Leest et al.,

2019). However, the rate of insPCa was significantly lower in MRI targeted biopsies than in systematic biopsies (14.1% vs. 24.8%; p<0.0001) (van der Leest et al., 2019).

Finally, in a multi-institutional MRI-FIRST study, systematic biopsies were performed to all patients and additional targeted biopsies were taken if cancer-suspicious lesions were present in mpMRI (Rouvière et al., 2019). In the analysis, there was no significant difference in the prevalence of CSPCa in MRI guided biopsies (29.9%) comparing to systematic biopsies (32.3%), while the prevalence of CSPCa in combined biopsies was 37% (Rouvière et al., 2019). However, MRI biopsies missed 5.2% of CSPCas detected in systematic biopsies, and conversely, systematic biopsies missed 7.6% of CSPCas detected in MRI-targeted biopsies (Rouvière et al., 2019). Interestingly, the prevalence of Gleason ≥ 4+3 PCas was significantly higher in the target biopsies comparing to systematic biopsies, and added value of targeted biopsies to the systematic biopsies in detection of Gleason ≥ 4+3 PCa was 6.0% (p=0.0095), while the prevalence of Gleason ≥ 4+3 PCas was 21.1% in the combined biopsies (Rouvière et al., 2019).

Two of the mentioned studies provided prevalence of CSPCas and insPCas in MRI targeted biopsies in various MRI suspicion scores, which are presented in **Table 5**.

Table 5. Prevalence of CSPCa (Gleason ≥ 7) and insPCa in MRI targeted biopsies in relation to mpMRI score in the prospective 4M and MRI-FIRST studies (Rouvière et al., 2019; van der Leest et al., 2019). Rate of insPCas in systematic biopsies in men with MRI suspicion level of 1-2 were 20% in 4M trial and 31% in MRI-FIRST trial, while definition of insPCa were Gleason score 3+3 in 4M trial, and Gleason score 3+3 with maximum cancer core length < 6 mm in MRI-FIRST trial (Rouvière et al., 2019; van der Leest et al., 2019).

mpMRI score	mpMRI targeted biopsies			
	4M		MRI-FIRST	
	CSPCa %	insPCa %	CSPCa %	insPCa %
3	18	18	12	5
4	40	32	31	15
5	70	26	77	0

Ahdoot et al. compared mpMRI targeted and systematic biopsy results in a large cohort of men (n=2103) having detectable lesions in mpMRI (Ahdoot et al., 2020). In the study, additional GGG ≥ 2 PCas were diagnosed with mpMRI targeted biopsies in 12.7% of men comparing to only systematic biopsies, while systematic biopsies diagnosed additional GGG ≥ 2 PCas in 5.8% of men, comparing to only mpMRI targeted biopsies (Ahdoot et al., 2020). Additionally, new insPCas (GGG 1) were diagnosed in 3.5% of men with mpMRI targeted biopsies and 7.8% of men with systematic biopsies (Ahdoot et al., 2020). All the differences were statistically significant. The overall prevalences of PCa and CSPCa in the study biopsies were 62.4% and 43.7%, respectively (Ahdoot et al., 2020).

In a meta-analysis of 48 included studies, Moldovan et al. investigated NPV of mpMRI in ruling out CSPCa (Moldovan et al., 2017). In the study, the median NPV for CSPCa was 88.1% while significant cancer prevalence median was 32.9% (Moldovan et al., 2017). The meta-analysis presented the commonly known fact, that when cancer prevalence decreases, NPV increases: in the analysis, when overall PCa prevalence was 50%, mpMRIs NPV and PPV was 0.76 and 0.64, while PCa prevalence was 30%, NPV and PPV was 0.88 and 0.43 in detection on CSPCa, respectively (Moldovan et al., 2017). However, there was great variation in the definitions of CSPCa in addition to the study protocol and reference standards (Moldovan et al., 2017).

In a meta-analysis by Kasivisvanathan et al., DRs of MRI-guided biopsies to TRUS-guided systematic biopsies were compared (Kasivisvanathan et al., 2019). In the paired data cohort of 56 studies, the DR of Gleason ≥ 3+4 PCa was 1.09 ($p=0.018$) which favors slightly, but statistically significantly MRI-guided biopsies in sensitivity; however, the DR increases with a stricter definition of CSPCa (Kasivisvanathan et al., 2019). More evident results were seen in DRs of insPCa defined as Gleason 3+3: in the analysis of 46 paired data cohorts, DR for insPCa was 0.74 ($p<0.0001$) favoring MRI-targeted biopsies (Kasivisvanathan et al., 2019). The proportion of men with CSPCa missed by MRI targeted biopsies, but detected by the addition of systematic biopsies in the analysis of 56 studies, was 13% ($p<0.0001$) (Kasivisvanathan et al., 2019). Similar results are seen in the mentioned study of Ahdoot et al., where in the analysis of 404 whole mount prostatectomy specimens, GGG was upgraded to ≥ 2 in 18.3% of MRI targeted biopsies and in 30.2% of systematic biopsies while only in 6.7% of combination biopsies a GGG was upgraded to ≥ 2 (Ahdoot et al., 2020). These results indicate that MRI targeted and systematic biopsies might diagnose different CSPCas, however, targeting errors should be also taken into account.

In a Cochrane meta-analysis of Drost et al., the authors concluded that prostate MRI with or without targeted biopsies has the most favorable accuracy in CSPCa detection while the DR of insPCa is reduced (Drost et al., 2019). In the meta-analysis, a pooled analysis was made concerning the studies comparing MRI targeted biopsies to systematic biopsies using template-guided mapping biopsies as a reference standard in CSPCa (Gleason ≥ 7) detection (Drost et al., 2019). In the analysis, using a baseline prevalence of 30%, pooled sensitivity and specificity of MRI targeted biopsies were 0.80 and 0.94, respectively, while sensitivity of systematic biopsies was 0.63 (Drost et al., 2019).

MRI-targeted biopsies are commonly taken using the cognitive approach or MRI-TRUS-fusion where the MRI-suspicious lesion in the prostate is marked by the radiologist and is visible real-time in TRUS. Also, biopsies can be taken in the MRI-scanner (in-bore target biopsies). However, there is no evidence of significant

difference in CSPCa detection between the methods (Monda et al., 2018; Rouvière et al., 2019; Wegelin et al., 2019).

As a conclusion, there is high-quality evidence about the mpMRI's great sensitivity for CSPCa and tendency to discriminate insPCas, still standard biopsies find CSPCas that are not visible in mpMRI. However, the specificity measures should also be taken into account. As **Table 5** clearly presents, even the highest suspicion level is not definitely a guarantee for CSPCa, and in the mentioned studies, PPV of mpMRI is low. Obviously, targeting errors should be considered.

2.3.3 Biparametric MRI protocol

Biparametric MRI (bpMRI) is performed without a contrast medium, thus it includes only T2W and DWI sequences. The benefits of the bpMRI protocol are a faster MRI scanning process, mainly due to the lack of need for intravenous access and also due to a shorter interpretation time. In addition, the use of gadolinium contrast medium in mpMRI and gadolinium deposits on the brain have been under discussion; however, there are no reliable data regarding its clinical or biological significance, if any (Gulani et al., 2017). In the study of Porter et al., time reserved for an mpMRI scan is 45 min, and the average interpretation time is about 21 min (Porter et al., 2019). Correspondingly, the time reserved for bpMRI is 15 min and the average interpretation time is about 16 min (Porter et al., 2019). While the significance of DCE is under debate, bpMRI appears to be an attractive alternative to mpMRI due to its rapid and thus more cost-effective imaging protocol (Porter et al., 2019).

According to the PI-RADS version 2.1, bpMRI and mpMRI have no difference in grading of transitional zone lesions, however peripheral zone lesions graded to PI-RADS version 2.1 score 3 cannot be upgraded to 4 in bpMRI, which will impact the ratio of PI-RADS 3/4 (American College of Radiology®, 2019). While mpMRI is still the gold standard of prostate imaging, further research is needed to validate bpMRI's position in diagnostics of PCa (American College of Radiology®, 2019). However, the PI-RADS steering committee recommends mpMRI over bpMRI in men with high and/or prevailing suspicion of CSPCa, after prostatic interventions or 5ARI therapy, or patients with hip implantation or other considerations that are expected to yield degraded DWI (American College of Radiology®, 2019).

Biparametric MRI and mpMRI have been compared in various studies. Di Campli et al. retrospectively compared bpMRI and mpMRI (Di Campli et al., 2018). In the study (n=85), CSPCa was defined as Gleason ≥ 7 and its prevalence was 48%, while systematic biopsies (42/85) or a prostatectomy specimen (43/85) was used as a reference standard (Di Campli et al., 2018). There were no significant differences in AUC values between the mpMRI and bpMRI in diagnostics of CSPCa (Di Campli et al., 2018). Additionally bpMRI and mpMRI were compared among three radiologists with a different grade of experience using PI-RADS version 2 reporting system: In

the analysis, there were no significant differences within the imaging method used between AUC values of the most experienced reader comparing to the less experienced in the diagnostics of CSPCa (Gleason score ≥ 7) (Di Campli et al., 2018).

In the study of Schimöller et al., 235 MRI scanned men were retrospectively included in the study, where in-bore biopsies of 200 lesions from 115 included men were analyzed (Schimmöller et al., 2014). In the analysis, AUC values for T2W+DWI and T2W+DWI+DCE in diagnostics of significant (Gleason ≥ 4+3) PCa in lesion level were 0.847 (95% CI 0.798–0.895) and 0.871 (95% CI 0.832–0.911), respectively, i.e. no statistically significant differences were detected between bpMRI and mpMRI methods (Schimmöller et al., 2014). Additionally, differences between the methods were also statistically insignificant when comparing the detection of transitional and peripheral zone lesions (Schimmöller et al., 2014).

Biparametric MRI have also been investigated in a high-quality setting. In a prospective, single-institutional BIDOC study (n=1020), all included men underwent bpMRI and systematic TRUS-guided biopsies were taken (Boesen et al., 2018). Additional targeted TRUS-MRI-fusion guided biopsies were taken from men with PCa suspicious (modified PI-RADS version 2 score 3–5) lesions present in bpMRI (Boesen et al., 2018). In the study, standard biopsy strategy (all men, only systematic biopsies taken) were compared to the combined biopsy strategy (men with a suspicious lesion in MRI present and systematic+targeted biopsies taken) (Boesen et al., 2018). In the study, 70% of men had suspicious lesions present in MRI and thus combination biopsies were taken (Boesen et al., 2018). There were no statistically significant differences in the amount of CSPCa (Gleason ≥ 3+4) diagnosed in the patient subgroup with combined biopsies, comparing to standard biopsy results of a whole study cohort, while the total CSPCa prevalence was 47% (Boesen et al., 2018). Additionally, significantly less insPCas were diagnosed with combined biopsy strategy (relative difference -42%, 95% CI -53% to -28%) (Boesen et al., 2018).

When a more strict definition for a CSPCa (Gleason ≥ 4+3) was used, significantly more CSPCas were diagnosed with the combined biopsy strategy cohort (relative difference 11%, 95% CI 0.6% to 21%) and significantly less insPCas were diagnosed (relative difference -40%, 95% CI -49% to -29%) (Boesen et al., 2018). The NPVs for CSPCa with the definitions of Gleason ≥ 3+4 and Gleason ≥ 4+3 of bpMRI targeted biopsies were 93% and 97%, respectively, when combined biopsies to all men were used as a reference standard (Boesen et al., 2018).

2.3.4 Diagnostic performance of MRI combined with additional parameters

Recommendations of the EAU guidelines for patient selection prior initial prostate biopsies are presented in more detail in Chapter 2.2.1: Guidelines for primary diagnostics and risk stratification.

Even the EAU guidelines recommend mpMRI prior initial biopsies when there is a suspicion of a CSPCa, considering the well-known issues in PCa diagnostics, including the lack of cut-off value for PSA in ruling out CSPCa, poor sensitivity of DRE in addition to the risk for biopsy complications and diagnosing insPCas, the question about relevant patient selection for prostate biopsies is still open. In summary, the question is: is there some objective way to omit systematic biopsies, when the level of suspicion in MRI is low or equivalent and conversely, is there some subgroup of patients, whose negative MRI is not excluding CSPCa in an acceptable probability? Additional clinical parameters provide an interesting approach to the mentioned issues.

PSA density might be the most investigated additional parameter to be used in combination with MRI. In a large, prospectively collected cohort, Distler et al. investigated PSA density in combination of mpMRI in ruling out CSPCa (Gleason ≥ 7) (Distler et al., 2017). In the study, there was statistically significant improvement in AUC values to PI-RADS scoring in a combination of PI-RADS + PSA density (AUC 0.752 vs. AUC 0.789) (Distler et al., 2017). Also NPV of negative mpMRI (PI-RADS < 3) in ruling out CSPCa was analyzed in PSA density groups of <0.07 ng/ml/ml, 0.07–0.15 ng/ml/ml and >0.15 ng/ml/ml: the NPV of whole cohort was 79.4%, while it increased to 88.9% in PSA density group 0.07–0.15. However, the prevalence of CSPCa in the whole cohort was 43.4% and in the mentioned subgroup 24% (Distler et al., 2017).

Boesen et al. investigated using the previously described BIDOC dataset an optimal biopsy strategy to biopsies using PSA density and bpMRI score (Boesen et al., 2019). In a decision curve analysis (DCA), the best strategy was restricting biopsies to men with (modified) PI-RADS version 2 score of 4–5 or PSA density ≥ 0.15 ng/ml/ml (Boesen et al., 2019). Using this strategy, NPV for CSPCa (Gleason ≥ 7) was 0.96 and the strategy would reduce prostate biopsies by 41% missing a CSPCa in 5% of men (Boesen et al., 2019).

In a study of Panebianco et al., large surveillance data was analyzed concerning patients with non-suspicious mpMRI (PI-RADS 1–2) (Panebianco et al., 2018). In biopsy naïve men, CSPCa diagnosis-free survival probability at 24 months was 95%, and the values remained unchanged at 48 months, while the median follow up time was 38 months (Panebianco et al., 2018). The statistically significant predictors for having CSPCa during the follow-up period were increasing PSA, PSA density and age, however, the previous biopsies did not increased the risk (Panebianco et al., 2018). In the study, PCa was defined as insignificant if active surveillance criteria were fulfilled, or PCa was defined as low risk stage in the prostatectomy specimen (Panebianco et al., 2018).

Thompson et al. prospectively investigated the NPV of mpMRI (PI-RADS < 3) in combination with PSA and DRE using transperineal mapping biopsies or radical prostatectomy specimen as a reference standard (Thompson et al., 2016). Significant

PCa was defined as Gleason score 7–10 with greater than 5% having Gleason grade 4, 20% or more cores positive, or 7 mm or more PCa in any core, while the prevalence of CSPCa were 41.6% (Thompson et al., 2016). In the study (n=344), NPV of mpMRI for ruling out CSPCa were 92% in a whole cohort, while among a subgroup of men with prebiopsy PSA > 10.0 ng/ml or abnormal DRE, NPV of mpMRI was even 100%, while 11% of men would have been avoided biopsies without missing any CSPCa (Thompson et al., 2016). However, patients with normal DRE and a PSA less than 10.0 ng/ml, the NPV of mpMRI were 90% in ruling out CSPCa (Thompson et al., 2016).

In the previously described MRI-FIRST study, the authors made an additional analysis to compare CSPCa detection rates of systematic and targeted biopsies in various clinical subgroups (Rouvière et al., 2019). In the analysis of clinical stage, PSA (cut-off 10 ng/ml), prostate volume (cut-off 50 ml), and cognitive/fusion guidance, no statistically significant difference in detection rates of CSPCa were found (Rouvière et al., 2019).

As a conclusion, the prior studies give no unanimous answer, which patients could safely avoid biopsies after a negative MRI or in case of an equivalent MRI suspicion. However, PSA with its derivates seems to be the most promising approach. Again, the studies cited in this review quite coherently present that NPV decreases when prevalence increases.

3 Aims of the study

Novel imaging methods are now changing diagnostics of PCa almost as radically as the invention of PSA did in the late 1980s. Also, analogously with the introduction of PSA, prostate MRI, especially in initial PCa diagnostics, is still seeking its position as a part of modern PCa diagnostics. Even though the recent studies have demonstrated very good NPV of prostate MRI in ruling out CSPCa and its trend to discriminate insPCas, initial PCa diagnostics based only on MRI targeted biopsies without systematic biopsies are not yet a viable practice and the question about patient selection for biopsies is still open. Considering the risk of biopsy complications, emerging antibiotic resistance and unnecessary and even harmful diagnoses of insignificant cancers, ideally the PCa diagnostics should shift towards the lesion-based approach, thus reducing the need for unnecessary biopsies.

This doctoral thesis consists of five studies (below: "Study I–V"). Specifically, the aims of the studies are to

- survey the prevalence of TRUS-Bx complications,
- survey the prevalence and risk factors for intestinal E. coli antibiotic resistance in men undergoing prostate biopsies,
- validate the bpMRI score and PSA density combination strategy in selecting men for prostate biopsies,
- investigate the impact of prebiopsy prostate bpMRI on the prevalence of CSPCa in an initial biopsy session, and
- investigate the NPV of IMPROD bpMRI as a whole and in clinical subgroups.

4 Patients and methods

4.1 Study population

The study population for the five substudies of this doctoral thesis is from four prospective, registered trials conducted in Turku University Central Hospital (TYKS) between 2013–2017, investigating an IMPROD bpMRI protocol and IMPROD Likert scoring system in PCa diagnostics, as described more detail in **Table 6** and below. In addition, a control cohort was retrospectively collected to Study IV.

Table 6. Details of prospective studies used in this doctoral thesis.

study	Clinicaltrials.gov identifier	n	essential inclusion criterias	essential exclusion criterias
IMPROD	NCT01864135	175	suspicion of a PCa based on PSA level of 2.5–20 ng/ml or abnormal DRE	previous prostate biopsies within 6 months, previous prostate surgery or previous PCa diagnosis
MULTI-IMPROD	NCT02241122	364	suspicion of a PCa based on PSA level of 2.5–20 ng/ml or abnormal DRE	previous prostate biopsies within 6 months, previous prostate surgery or previous PCa diagnosis
IMPROD2.0	NCT02844829	69	suspicion of a PCa based on PSA level of 2.5–20 ng/ml or abnormal DRE	previous prostate biopsies, previous prostate surgery or previous PCa diagnosis
PROMANEG	NCT02388126	257	suspicion of a PCa based on PSA level of 2.5–20 ng/ml or abnormal DRE and/or previous negative prostate biopsies. Also patients in active surveillance of PCa were included.	

In a prospective, single center IMPROD trial, prebiopsy IMPROD bpMRI and IMPROD Likert scoring system in diagnostics of CSPCa were investigated. Systematic biopsies were performed on all the included patients. In addition, on men

with an IMPROD bpMRI Likert scored 3–5 lesion in IMPROD bpMRI, additional lesion-targeted biopsies were performed.

A prospective, multicenter MULTI-IMPROD trial was conducted in four centers in Finland. The aim of the trial was to validate the results of the IMPROD trial in a multicenter setting. In the study, systematic biopsies were performed on all the included patients. In addition, on men with IMPROD bpMRI Likert score 3–5 lesions in IMPROD bpMRI, additional lesion-targeted biopsies were performed. The men included in the study filled in a detailed questionnaire form concerning their medical history, medication, smoking, recent traveling and recent antibiotic usage. In addition, in a prostate biopsy session, rectal swab samples were taken, and the samples were cultured in a microbiology laboratory to discover the antibiotic resistance profile of intestinal E. coli.

In a prospective, single-center, IMPROD2.0 trial, prebiopsy IMPROD bpMRI and IMPROD bpMRI Likert scoring system in diagnostics of CSPCa was investigated. Systematic biopsies were performed on the all included patients. In addition, on men with an IMPROD bpMRI Likert scored 3–5 lesion in IMPROD bpMRI, additional lesion-targeted biopsies were performed.

In a prospective, single-center, PROMANEG trial, prebiopsy IMPROD bpMRI and IMPROD bpMRI Likert scoring system were investigated in diagnostics and also in active surveillance of PCa. According to the study protocol, systematic biopsies were performed on all the included patients. In addition, from men with an IMPROD bpMRI Likert score 3–5 lesion in IMPROD bpMRI, MRI-targeted biopsies were taken.

The population of Study I and Study II is based on the MULTI-IMPROD trial. Study III is based on the population of both the IMPROD and MULTI-IMPROD trials. In Study I and in a risk factor analysis of Study II, only patients with a full data available (questionnaire form and cultured rectal swab sample) were included. Study IV is based on a population of all the previously described four studies. However, patients with prior PCa diagnosis or prior prostate biopsies were excluded. In addition, a control cohort was retrospectively collected for the study from a patient registry using a prostate biopsy laboratory code and PSA level under 20 ng/ml as a criteria in a search algorithm, aiming to collect comparable patients who had initial prostate biopsies taken in TYKS between 2010–2013, i.e. prior the clinical use of prostate MRI. Study V is also based on the population of all the described four studies. However, patients with a prior PCa diagnosis were excluded.

4.2 Study methods

4.2.1 MRI protocol and reporting system

In all four MRI studies, the IMPROD bpMRI protocol and IMPROD bpMRI Likert scoring system was used, which has been developed in TYKS since 2013. The IMPROD bpMRI protocol and IMPROD Likert bpMRI reporting system are public and freely available on the study server.

IMPROD bpMRI was performed using body array coils (no endorectal coil) and 3 Tesla MRI scanners in Turku (Verio, Siemens), Tampere (Skyra, Siemens), Helsinki (Skyra, Siemens), while a 1.5T (Aera, Siemens) MRI scanner was used in Pori. The imaging consisted of T2W acquisitions in axial and sagittal planes, three separate DWI and corresponding calculated ADC maps fitted using mono-exponential fit. Dynamic contrast enhanced imaging was not performed, thus an intravenous contrast agent was not used. Diffusion weighted imaging datasets were collected in three separate acquisitions: 1) b-values 0,100,200,300,500 s/mm^2; 2) b values 0, 1500 s/mm^2; 3) values 0, 2000 s/mm^2. The imaging protocol was carefully optimized to allow comparable image quality at 1.5T and at 3T. The overall imaging time using 3T scanners was 13–17 minutes including shimming and calibration while the corresponding time at 1.5T was about 3 minutes longer. Only routinely available magnetic resonance acquisition and post-processing methods were used. Patients have a rectal enema prior the MRI scan.

All imaging data sets were reported using a five-step tiered IMPROD bpMRI Likert scoring system by a local radiologist and confirmed centrally by one designated central reader with six years of experience in prostate MRI in the beginning of the first IMPROD trial, to guarantee reporting integrity before each biopsy procedure. IMPROD bpMRI Likert scoring system is very similar to PI-RADS version 2.1, however the final IMPROD bpMRI Likert score is not based on an arithmetical combination of ratings for each method (T2W, DWI), but the “overall impression score” of a lesion to be a significant cancer or not. IMPROD bpMRI Likert scoring system does not include additional clinical data.

The central reader was unaware of all clinical data. In all the studies, the prostate volume was primarily estimated with TRUS. The cognitive biopsy targeting method was mainly used in all the studies, with the exception of one center in the MULTI-IMPROD trial, where MRI-TRUS fusion was used (n=58). The prostate volume was estimated using TRUS with the exception of 17 cases, where MRI based volume estimation was used due TRUS based prostate volume estimation was not reported.

4.2.2 Antibiotic susceptibility testing

During the TRUS-Bx procedure in MULTI-IMPROD trial, fecal samples were collected with sterile rectal swab (Copan floq swab). Fecal swabs were sent to the Medical microbiology laboratory for culturing. The samples were directly cultured on a CHROMagar™ Orientation plate. A ciprofloxacin disc (Oxoid, 5 µg) was added on top of the plate to select the patient's most resistant bacteria. The plates were incubated over night at +35 °C. The following day, the most resistant E. coli strain was selected for pure culture, i.e. a pink or dark rose colony nearest to the ciprofloxacin disc. Antimicrobial susceptibility testing of E. coli isolates was performed with the disc diffusion method using Oxoid susceptibility discs (Thermo Scientific, Helsinki, Finland). Susceptibility was tested against ampicillin (10µg), amoxicillin-clavulanic acid (30µg), mecillinam (10µg), cefoxitin (30µg), cefotaxime (5µg), ceftazidime (10µg), meropenem (10µg), ciprofloxacin (5µg), gentamicin (10µg), nitrofurantoin (100µg), trimethoprim (5µg) and trimethoprim-sulphamethoxazole (25µg) on Mueller-Hinton agar (Oxoid, Thermo Scientific, Helsinki, Finland) plates according to EUCAST criteria (The European Committee on Antimicrobial Susceptibility Testing, 2017). Ciprofloxacin-resistant strains (inhibition zone <24 mm) were re-tested and resistance to fluoroquinolones was confirmed with nalidixic acid (30µg), levofloxacin (5µg) and pefloxacin (5µg) discs.

Isolates showing reduced susceptibility to cefotaxime and ceftazidime (inhibition zone <21 mm and <22 mm, respectively) were tested for ESBL production with the combination disc test (Rosco ESBL and AmpC confirmation Kit, Rosco, Denmark). Bacterial strains were incubated with cefotaxime and cefotaxime-clavulanic acid as well as ceftazidime and ceftazidime-clavulanic acid discs. If the inhibition zone around the antimicrobial disc with clavulanic acid was ≥5 mm greater than the inhibition zone around the antimicrobial disc without clavulanic acid, the strain was determined to be an ESBL producer. In this study, E. coli strains with 3CEF resistance were used instead of ESBL production in all analysis.

Before collecting the rectal swab samples and a TRUS-Bx procedure, antibiotic prophylaxis was administered according to the guidelines of each research center.

EUCAST guidelines for determining antimicrobial susceptibility are updated annually. In the risk factor analysis, the most recent EUCAST 2017 clinical breakpoint criteria was used (The European Committee on Antimicrobial Susceptibility Testing, 2017). As the criteria for defining fluoroquinolone resistant E. coli strains changed remarkably in the latest EUCAST update, we analyzed fluoroquinoloneresistance rates in E. coli strains also according to the prior EUCAST criteria (The European Committee on Antimicrobial Susceptibility Testing, 2016). The definition of 3CEF resistance of E. coli did not change during the study period.

4.2.3 Methods of the individual studies

Study I

In the study, we aimed at prospectively investigating the prevalence and risk factors for biopsy complications in men undergoing TRUS-Bx. After an MRI scan, the included men underwent TRUS guided systematic biopsies in addition to 2–4 lesion-targeted biopsies, if suspicious lesions were present in the MRI. During the biopsy session, patients had antibiotic prophylaxis according to the local guidelines, and it was prospectively registered in detail. After a follow-up period of 30 days, complications following the TRUS-Bx were collected from medical records and analyzed with the data from the questionnaire form and determined antibiotic resistance profile of E. coli in the rectal swab sample. Rectal bleeding, urinary infections, hematuria, and other urinary symptoms leading to an emergency department visit, were registered as biopsy related complications. The prevalence of complications was analyzed in clinical subgroups of prostate volume, age, diabetes and anticoagulation/antiplatelet therapy, which was selected on the basis of a clinical experience and prior studies.

Study II

In the study, we aimed to prospectively determine the prevalence and risk factors for antibiotic resistance of intestinal E. coli in men undergoing TRUS-Bx. During a biopsy session, rectal swab samples were taken and cultured in a microbiology laboratory to determine the antibiotic resistance profile of E. coli. In the study, we analyzed resistance for fluoroquinolones and 3CEF resistance, as well as the ESBL production of the E. coli stains. Fluoroquinolone susceptibility was reported in categories ”susceptible”, ”intermediate susceptible” and ”resistant” using the criteria of The European Committee on Antimicrobial Susceptibility Testing from 2017 and additional analyses were made using the mentioned criteria valid to 2016, due to remarkable changes of the fluoroquinolone susceptibility criteria in the time of the manuscript publication (The European Committee on Antimicrobial Susceptibility Testing, 2016, 2017). In the risk factor analysis, we used the data from the questionnaire form and medical records, and analyzed with the determined antibiotic resistance profile of E. coli.

The patients without E. coli growth in the rectal swab sample were analyzed as fluoroquinolone susceptible in the risk factor analysis. E. coli strains as well as fluoroquinolone resistant and intermediate resistant E. coli strains were analyzed together as one variant.

The risk factor categories were selected on the basis of earlier studies and clinical experience. The risk factor categories were international traveling during the last year,

being a current smoker or quitted smoking during the last two years, antibiotic therapy during the last year, diabetes, and age. The majority of the patients did not recall the specific name of the antibiotic used. As the study consent did not allow us to review study patients' antimicrobial prescriptions, the data on antimicrobial usage was based on the patients' recollections of their previous antimicrobial courses. Therefore, the previous use of antibiotics was analyzed as a whole, without dividing into different antibiotic classes.

Study III

In the study, we aimed at validating the optimal method combination strategy of PSA density and bpMRI score for selecting men to prostate biopsies, which was presented by Boensen et al. (Boesen et al., 2019). In this retrospective external validation study, we used our prospectively collected data and performed similar statistical analyses as in the validated study, which are described in more detail in chapter 4.3: Statistical analysis. In the study, CSPCa were defined as Gleason score ≥ 7 in any biopsy core (standard or targeted) taken. The prostate volume was estimated using TRUS, with the exception of 17 cases where MRI based volume estimation was used due TRUS based prostate volume estimation was not reported.

Study IV

In the study, we analyzed the rate of CSPCa from biopsies taken in an initial biopsy session among men undergoing prebiopsy prostate MRI and systematic prostate biopsies with additional lesion-targeted biopsies. Using follow-up data, we also investigated the delay and number of biopsy sessions performed for diagnosis of CSPCa. With a retrospectively collected control cohort of men, whose initial biopsies were taken prior to clinical use of prostate MRI, we determined the clinical significance of prostate MRI in the PCa diagnostics comparing it to the traditional diagnostic protocol. In the study, CSPCa was defined as Gleason score ≥ 7 in any individual biopsy core taken, combining targeted and systematic biopsies.

Study V

In the study, we aimed at determining in a prospectively collected cohort, an NPV of IMPROD bpMRI and IMPROD Likert scoring system in ruling out CSPCa. In an additional analysis, we investigated NPV of IMPROD bpMRI in various clinical subgroups. We also analyzed other performance measures of IMPROD bpMRI in CSPCa diagnostics in addition to AUC values for IMPROD Likert scoring system as presented in more detail in chapter 4.3 Statistical analysis. In the study, CSPCa was

defined as Gleason score ≥ 7 in any individual biopsy core taken, combining targeted and standard biopsies.

4.3 Statistical analysis

Study I

Patient characteristics were summarized using descriptive statistics. Prevalence of complications during the follow up period were analyzed as a whole and divided to subclasses of infectious complications, rectal bleeding, hematuria, and urinary retention. Due to the low amount of biopsy-related complications, a further risk factor analysis was not made. Antibiotic susceptibility of E. coli was reported as prevalence rates.

Study II

Patient characteristics were summarized using descriptive statistics. Prevalence of E. coli growth, its fluoroquinolone and 3CEF resistance in patient level, were described in total and dividing to individual results from the included study centers. Also, the prevalence of ESBL producing strains was reported.

Risk factors for having a fluoroquinolone or 3CEF resistant E. coli strain were analyzed using odds ratios (OR) with 95% CIs derived from univariate and multivariate logistic regression analysis. Statistical significance was defined as $p<0.05$. Binary variables in the risk factor analysis were international traveling during the last year, being a current smoker or quitted smoking during the last two years, antibiotic therapy during the last year and diabetes, and a continuous variable was age. The statistical analyses were made with IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp. Released 2013. Armonk, NY).

Study III

Patient characteristics were grouped according to the biopsy results and summarized using descriptive statistics. The Wilcoxon rank-sum test was used to compare no PCa/insPCa against CSPCa for continuous variables. The chi-squared test for homogeneity was used to assess the difference between no PCa/insPCa and CSPCa within the same PSA density groups as defined in the validated study. The combination of IMPROD bpMRI Likert score with PSA density thresholds was evaluated using a chi-squared test to determine the effect of PSA density as predictive value within each IMPROD bpMRI Likert score group.

The sensitivity, specificity, PPV, NPV and Youden's J index (sensitivity + specificity - 1) were calculated to evaluate various IMPROD bpMRI Likert score and PSA density thresholds for detecting and ruling out CSPCa. Furthermore, net benefit analysis for biopsy strategies that combined IMPROD bpMRI Likert score with PSA density thresholds was performed to compare the benefit of detecting CSPCa against the risk of unnecessary biopsies. A DCA for all proposed biopsy strategies was carried out using thresholds' probabilities ranging from 0% to 25% (Vickers et al., 2016).

Statistical analysis was conducted using R v. 3.4.3 software (R Foundation for Statistical Computing, Vienna, Austria). Results with a two-sided $p<0.05$ was considered statistically significant.

Study IV

Patient baseline characteristics and follow up times were summarized separately in the both study cohorts (MRI group and non-MRI group) using descriptive statistics and the significance of differences between the study groups was calculated using Pearson's chi-square test for nominal variables and with an independent samples T-test for continuous variables. Descriptive statistics and Pearson's chi-square test were also used to compare the study group's initial biopsy results and biopsy/prostatectomy results during the follow up. All the pathology results were divided using GGGs.

Delay to CSPCa diagnosis during the follow-up was presented using a Kaplan-Meier graph, restricting it to the data of the first two years due to a significant difference in length of follow-up periods between the study groups. Statistical significance was defined as $p<0.05$ in all analyses. All the statistical analysis was made with SPSS version 24 for Windows (IBM Corp., Armonk, N.Y., USA).

Study V

Patient characteristics were summarized using descriptive statistics. Prevalence of PCa and CSPCa were also summarized using descriptive statistics, and an AUC value for IMPROD bpMRI Likert scoring system was determined for the whole study cohort and additionally for subcohorts of the four included studies. Performance measures (NPV, PPV, specificity, sensitivity) of IMPROD bpMRI for CSPCa were determined in suspicion levels of IMPROD bpMRI Likert 3–5 and IMPROD bpMRI Likert 4–5 for the whole study cohort and additionally for subcohorts of the four included studies.

The study population was divided to subgroups of PSA level, PSA density, prostate volume, free PSA/PSA ratio, age, history of previous biopsies, and 5ARI medication. The dichotomizing cut-off values for continuous variables were determined by mean value of the study cohort in age and free PSA/PSA ratio. In PSA, prostate volume and PSA density, the most dichotomizing cut-off values were determined by dividing the patients in value groups and analyzing the NPV in a

suspicion level of IMPROD Likert 4–5 separately in the different groups. The performance measures of IMPROD bpMRI for CSPCa were determined in all the subgroups separately in suspicion levels of IMPROD Likert 3–5 and IMPROD Likert 4–5. Statistical significance was defined as $p<0.05$.

The data analyses were performed using JMP Pro version 13 for Windows (SAS Institute Inc., Cary, N.C., USA) in exception of the ROC analysis which was made using SPSS version 24 for Windows (IBM Corp., Armonk, N.Y., USA). The performance measures were determined with IVD performance add-in v12.0 for JMP.

4.4 Ethics

All the four included prospective studies were conducted in compliance with the current revision of the Declaration of Helsinki guiding physicians and medical research involving human subjects (64th World Medical Association General Assembly, Fortaleza, Brazil, 2013). Prior to commencement of each of the studies, the study protocol, the patient information sheet and the informed consent form were approved by the ethics committee of the Hospital District of Southwest Finland. Patient data from the patient registry for the control cohort of Study III were collected with a permission of the Hospital District of Southwest Finland.

5 Results

5.1 Study I

In Study I, we aimed at surveying the prevalence of TRUS-Bx complications. In total, 359 patients were recruited to the study. However, 294 biopsied patients with a cultured microbiological sample and full data available were included in the final analysis (**Figure 3**). **Table 7** presents the characteristics of the study cohort.

Figure 3. Flowchart of the patient inclusion to the final analysis.

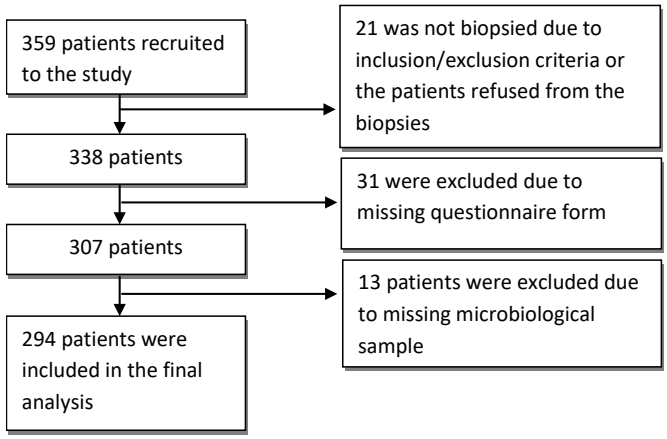


Table 7. Study patient characteristics.

	Included (294)
age, mean (range)	64 (29–82) years
PSA, mean (range)	7.6 (1.4–30.0) ng/ml
prostate weight, mean (range)	45 (14–131) g
5ARI medication (%)	30 (10%)
alpha blocker therapy (%)	38 (13%)
diabetes (%)	34 (12%)
anticoagulation/antiplatelet therapy (%)	52 (18%)

All biopsy-related complications leading to physical contact to a health care unit that occurred during the 30-day follow-up period are listed in **Table 8**. The total rate of complications was 4.1% (12/294).

No septic or other infectious complications requiring hospitalization followed the TRUS guided biopsies during the follow-up period. Only two minor urinary tract infections occurred, and they were treated with per oral antibiotics in an outpatient setting. Both patients received fluoroquinolone-based prophylaxis prior to biopsies. One of them had an E. coli strain with reduced fluoroquinolone susceptibility in the pre-biopsy rectal swab culture.

Significant hematuria occurred in seven study patients and rectal bleeding in one study patient. Two out of 52 patients with anticoagulation/antiplatelet medication (3.8%) had a bleeding complication. The rate of bleeding without anticoagulation/antiplatelet use was 2.5% (6/242).

Three patients experienced an urinary retention. One study patient visited an emergency department due to urinary discomfort symptoms but without a diagnosed infection or a urinary retention. No mortality during the follow-up period was observed. Only one patient was hospitalized during the follow-up period (hematuria complication).

Table 8. Complications related to prostate biopsies leading to a visit to a health care unit within 30 days follow-up period after a TRUS-Bx. There was no mortality and only one patient was hospitalized during the follow-up period (hematuria complication).

	total (% of study population)
any complication	12/294 (4.1%)[1]
infection	2/294 (0.7%)
rectal bleeding	1/294 (0.3%)
urinary retention	3/294 (1.0%)
hematuria	7/294 (2.4%)

[1] One patient had both hematuria and urinary retention.

The antibiotic prophylaxis given to the patients was based on guidelines of each institution and is summarized in **Table 9**. Fluoroquinolone based antibiotic (ciprofloxacin or levofloxacin) was the most common prophylactic antibiotic and it was administered to 97% (284/294) of the patients. Ten patients had fosfomycin and one patient had fosfomycin + ciprofloxacin as a prophylactic antibiotic. Antibiotic prophylaxis was compared with the results of the cultured E. coli fluoroquinolone and fosfomycin susceptibility profiles of the rectal swab samples. From this point of view, only 89% (262/294) of the study patients got an effective antibiotic prophylaxis against their intestinal E. coli pathogens. Interestingly, all patients who had fosfomycin as an antibiotic prophylaxis had either fosfomycin susceptible E. coli or

no E. coli growth in the rectal swab sample. The rate of fluoroquinolone resistant E. coli strains in patients with fosfomycin as an antibiotic prophylaxis was 36% (4/11).

Table 9. Antibiotic prophylaxis given to the study patients. Antibiotic prophylaxis was administered according to the guidelines of each center.

	n	mean time		No E. coli growth or cultured E. coli strain in rectal swab sample susceptible to given prophylactic antibiotic (%)
		before biopsies	after biopsies	
levofloxacin 500 mg 1x1	3	32 min		3 / 3 (100%)
levofloxacin 500 mg 1x2	121	34 min	11 h 22 min	103 / 121 (85%)
ciprofloxacin 500 mg 1x1	109	1 h 12 min		102 / 109 (94%)
ciprofloxacin 500 mg 1x2	2	1 h 45 min	7 h 45 min	1 / 2 (50%)
ciprofloxacin 750 mg 1x1	46	1 h 0 min		40 / 46 (87%)
ciprofloxacin 750 mg 1x2	1	5h 30 min	6 h 0 min	1 / 1 (100%)
fosfomycin 3000 mg 1x1	10	2 h 0 min		10 / 10 (100%)
other[1]	2			2 / 2 (100%)
average		57 min	11 h 4 min	262 / 294 (89%)

[1] Other prophylactic antibiotic prophylaxis was fosfomycin 3000 mg + ciprofloxacin 500 mg one hour before biopsies and in another patient levofloxacin 500 mg 2 h 10 min before and 9 h 50 min after biopsies + additional levofloxacin 250 mg daily five days after biopsies.

The fluoroquinolone resistance rates in the patients' pre-biopsy rectal swab samples are demonstrated in **Table 10**. According to The European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2017 criteria, 12% (36/294) of the study patients had a fluoroquinolone resistant E. coli strain and 6% (18/294) had a 3CEF resistant E. coli strain (The European Committee on Antimicrobial Susceptibility Testing, 2017).

Table 10. Antibiotic susceptibility profiles in study patients rectal swab culture.

	n	%
E. coli growth in rectal swab sample	262	89% (262 / 294)
no E. coli growth in rectal swab sample	32	9% (27 / 294)
fluoroquinolone-resistant E. coli	36	12% (36 / 294)[1]
fluoroquinolone-intermediate E. coli	9	3% (9 / 294)
fluoroquinolone-susceptible E. coli	217	74% (217 / 294)
E. coli resistant to 3rd generations cephalosporins	21	7% (18 / 294)[1]

[1] Patients with E. coli growth in rectal swab culture, 14% and 8% was resistant to fluoroquinolones and 3rd generations cephalosporins, respectively.

The risk factor analysis for biopsy complications is presented in **Table 11**. The patients having E. coli strain resistant to prophylactic antibiotics had no infectious or non-infectious complications.

Table 11. Risk factors for complications leading to a visit to a health care unit after a TRUS-Bx. Total 32/294 of patients (11%) had a E. coli strain resistant to given antibiotic prophylaxis in their intestinal bacterial flora. None of them had infectious or non-infectious complications leading to a visit to health care unit after biopsies.

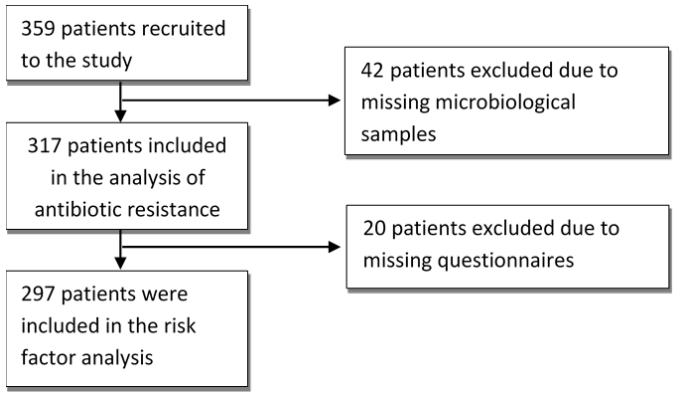
	n	complication rate
prostate weight over 40 g	128	7/128 (5.5%)[1]
prostate weight 40 g or less	144	5/144 (3.5%)[1]
age over 70 years	53	2/53 (3.8%)
age 70 years or less	241	10/241 (4.1%)
diabetes	34	2/34 (6.0%)
no diabetes	260	10/260 (3.8%)
anticoagulation/antiplatelet therapy	52	2/52 (3.8%)
no anticoagulation/antiplatelet therapy	242	10/242 (4.1%)

[1)] Prostate weight was measured in 272 of 294 patients.

5.2 Study II

In Study II, we aimed at surveying the prevalence and risk factors for intestinal E. coli antibiotic resistance in men undergoing prostate biopsies. The collection of patient data is demonstrated in **Figure 4**. A total of 359 patients from four study centers were recruited to the study. Microbiological samples were available from 317 patients. A total of 62 patients were excluded from the initial study population due to missing questionnaires or microbiological samples, i.e. 297 patients were included in the risk factor analysis to investigate underlying reasons for E. coli resistance.

Figure 4. Flowchart of the patient inclusion to the final analysis.



Study patient characteristics are presented in **Table 12**. The mean age was 64 and the mean prostate size was 45 g. A total of 55% of the patients had travelled internationally during the preceding year.

Table 12. Study patient characteristics.

number of patients with the full data available	297
age, mean (range)	64 (29–82) years
PSA, mean (range)	7.6 (1.4–30) ng/ml
prostate weight, mean (range)	45 (14–131) g
diabetes, n (%)	35 (12)
current smoker or quitted smoking during the last 2 years, n (%)	58 (20)
international traveling during the last year, n (%)	163 (55)
antibiotic therapy during the last year, n (%)	99 (33)

The results of the rectal swab cultures are presented in **Table 13**. According to EUCAST 2017 criteria, the rates of fluoroquinolone and 3CEF resistant E. coli strains in patient level were 13% and 8%, respectively, whereas the rates of fluoroquinolone and 3CEF resistant strains among patients having E. coli strain were 14% and 8%, respectively (The European Committee on Antimicrobial Susceptibility Testing, 2017). Patients having 3CEF resistant E. coli strain, 13/21 (62%) were ESBL-producers and 21/24 (88%) were fluoroquinolone resistant. Differences in the fluoroquinolone resistance rates between patients from different study centers were notable in our study: Highest rates were in the Helsinki (18%) and lowest in the Pori (5%) study center.

Table 13. Results of cultured rectal swab samples antimicrobial susceptibility testing for E. coli strains in the study population.

	n	E. coli growth (%)	no E. coli growth (%)	3CEF resistant E. coli (%)[3]
All	317	283 / 317 (89%)	34 / 317 (11%)	24 / 317 (8%)[4,5]
Turku	130	122 / 130 (94%)	8 / 130 (6%)	12 / 130 (9%)
Helsinki	56	48 / 56 (86%)	8 / 56 (14%)	6 / 56 (11%)
Tampere	56	48 / 56 (86%)	8 / 56 (14%)	4 / 56 (7%)
Pori	75	65 / 75 (87%)	10 / 75 (13%)	2 / 75 (3%)

EUCAST 2016

	fluoroquinolone resistant E. coli (%)	fluoroquinolone intermediate susceptible E. coli (%)	fluoroquinolone susceptible E. coli (%)
All	22 / 317 (7%)[2]	9 / 317 (3%)[2]	286 / 317 (90%)[2]
Turku	13 / 130 (10%)	2 / 130 (2%)	115 / 130 (88%)
Helsinki	1 / 56 (2%)	6 / 56 (11%)	49 / 56 (88%)
Tampere	5 / 56 (9%)	1 / 56 (2%)	50 / 56 (89%)
Pori	3 / 75 (4%)	0 / 76 (0%)	72 / 75 (96%)

EUCAST 2017

	fluoroquinolone resistant E. coli (%)	fluoroquinolone intermediate susceptible E. coli (%)	fluoroquinolone susceptible E. coli (%)
All	40 / 317 (13%)[1]	11 / 320 (3%)[1]	267 / 317 (84%)[1]
Turku	20 / 130 (15%)	1 / 130 (1%)	109 / 130 (84%)
Helsinki	10 / 56 (18%)	3 / 56 (5%)	43 / 56 (77%)
Tampere	6 / 56 (11%)	2 / 56 (4%)	48 / 56 (85%)
Pori	4 / 75 (5%)	4 / 75 (5%)	67 / 75 (89%)

[1)] According to EUCAST 2017 criteria, the amount of fluoroquinolone resistance, fluoroquinolone intermediate susceptible, fluoroquinolone susceptible E. coli strains in patients having E. coli strain in their rectal swab sample was 40/283 (14%), 10/283 (4%), and 233/283 (82%), respectively.
[2)] According to EUCAST 2016 criteria, the amount of fluoroquinolone resistance, fluoroquinolone intermediate susceptible, fluoroquinolone susceptible E. coli strains in patients having E. coli strain in their rectal swab sample was 22/283 (8%), 9/283 (3%), and 252/283 (89%), respectively.
[3)] There were no changes in the EUCAST criteria defining 3CEF resistant *E. coli* in 2016/2017.
[4)] According to EUCAST 2017 criteria, the amount of 3CEF resistant strain in patients having E. coli strain was 24/283 (8%).
[5)] Of the 3CEF resistant E. coli strains, 21/24 (88%) were also fluoroquinolone resistant, and of the 21 tested 3CEF resistant strains, 13 (62%) were ESBL-producers.

The risk factor analysis for antibiotic resistance is presented in **Table 14** and **Table 15**. In univariate analysis, smoking, diabetes, recent use of antibiotics and age did not have statistically significant influence on the risk of having fluoroquinolone resistant E. coli strains. In univariate and multivariate analysis, unspecified international traveling during the preceding year significantly increased the risk of colonization

with the fluoroquinolone resistant E. coli strains, OR 3.592, p=0.001. Surprisingly, in multivariate analysis, recent (unspecified) antibiotic therapy during the preceding year reduced the risk of having the fluoroquinolone resistant E. coli strain, OR 0.442, p=0.035. None of the risk factors had significant influence on the 3CEF resistance rates in E. coli strains in univariate or multivariate analysis. Although no significant risk factors were found, we identified a trend of an increasing risk for 3CEF resistance E. coli strain in patients with international traveling and smoking history.

The rate of patients having fluoroquinolone resistant E. coli strain in the rectal swab sample was compared to the patients' traveling history during the preceding year. The results are presented in **Figure 5**. Traveling, especially to Asia or Japan, considerably increased the risk of a fluoroquinolone resistant E. coli strain in the rectal swab sample.

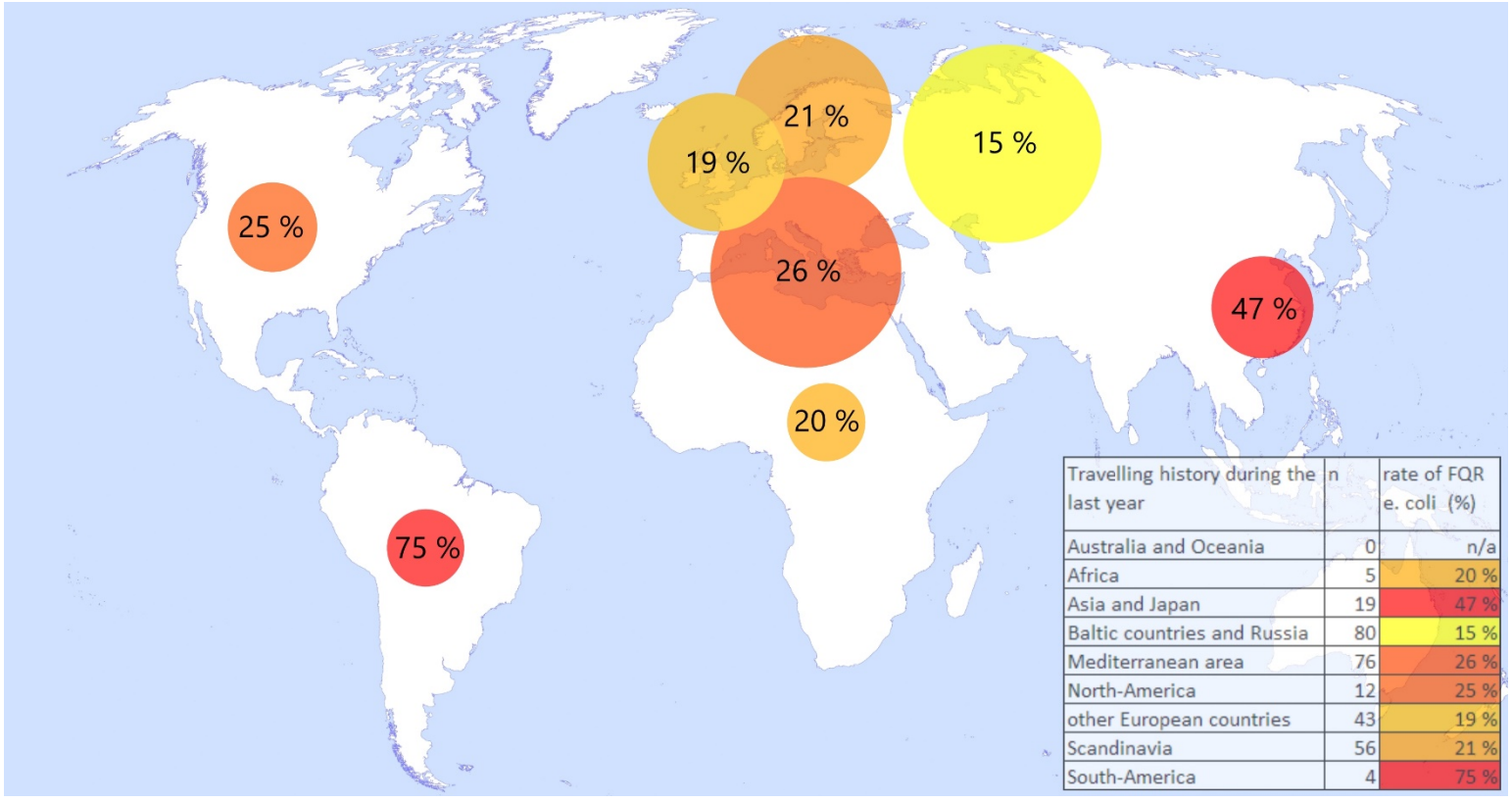
Table 14. Analysis of risk factors for study patients having a E. coli strain with decreased susceptibility (resistant or intermediate susceptible) to fluoroquinolones.

	fluoroquinolone resistant or intermediate susceptible E. coli	fluoroquinolone sensitive E. coli or no E. coli	univariate analysis				multivariate analysis			
				95% CI				95% CI		
			O.R.	Lower	Upper	p	O.R.	Lower	Upper	p
international traveling during the last year, n (%)	36 (22)	127 (78)	3.170	1.544	6.507	0.002	3.592	1.704	7.571	0.001
no international traveling during the last year, n (%)	11 (8%)	123 (92%)	reference				reference			
current smoker or quitted smoking during the last two years, n (%)	10 (17%)	48 (83%)	1.137	0.529	2.447	0.742	1.547	0.683	3.505	0.296
non-smoker at least last two years, n (%)	37 (15%)	202 (85%)	reference				reference			
antibiotic therapy during the last year, n (%)	11 (11%)	88 (89%)	0.563	0.273	1.160	0.119	0.442	0.206	0.946	0.035
no antibiotic therapy during the last year, n (%)	36 (18%)	162 (82%)	reference				reference			
diabetes, n (%)	6 (17%)	29 (83%)	1.115	0.436	2.855	0.820	1.549	0.570	4.209	0.391
no diabetes, n (%)	41 (16%)	221 (84%)	reference				reference			
age, mean (SD)	63 (8)	64 (8)	0.982	0.945	1.021	0.366	0.982	0.941	1.026	0.425

Table 15. Analysis of risk factors for study patients having a E. coli strain resistant to 3rd generations cephalosporins.

	3CEF resistant E. coli	3CEF sensitive E. coli or no E. coli	univariate analysis				multivariate analysis			
				95% CI				95% CI		
			O.R.	Lower	Upper	p	O.R.	Lower	Upper	p
international traveling during the last year, n (%)	14 (9)	149 (91)	2.228	0.778	6.377	0.135	2.330	0.789	6.879	0.126
no international traveling during the last year, n (%)	5 (4)	129 (96)	reference				reference			
current smoker or quitted during the last two years, n (%)	5 (11)	53 (89)	1.553	0.532	4.528	0.420	1.754	0.582	5.291	0.318
non-smoker at least last two years, n (%)	14 (6)	225 (94)	reference				reference			
antibiotic therapy during the last year, n (%)	6 (6)	93 (94)	1.013	0.371	2.765	0.980	0.911	0.324	2.560	0.860
no antibiotic therapy during the last year, n (%)	13 (7)	185 (93)	reference				reference			
diabetes, n (%)	1 (3%)	34 (97)	0.385	0.050	2.989	0.362	0.473	0.059	3.804	0.481
no diabetes, n (%)	18 (7)	244 (93)	reference				reference			
mean age (SD)	62,3 (7)	64,4 (8)	0.967	0.916	1.022	0.238	0.973	0.917	1.032	0.363

Figure 5. The rate of fluoroquinolone resistant (FQR) E. coli strains in our study patients rectal swab samples linked to international traveling during the preceding year. The size of the circle correlates with the number of patients with traveling history to the designated region. Reprinted with a permission.



Travelling history during the last year	n	rate of FQR e. coli (%)
Australia and Oceania	0	n/a
Africa	5	20 %
Asia and Japan	19	47 %
Baltic countries and Russia	80	15 %
Mediterranean area	76	26 %
North-America	12	25 %
other European countries	43	19 %
Scandinavia	56	21 %
South-America	4	75 %

5.3 Study III

In Study III, we aimed at validating the bpMRI score and PSA density combination strategy in selecting men for prostate biopsies. In total, 499 men were included, 161 patients from the IMPROD trial and 338 patients from the Multi-IMPROD trial. Baseline characteristics of the enrolled men are presented in **Table 16**. The median age was 65 years, median PSA was 7.1 ng/ml, median PSA density was 0.18 ng/ml/ml and median prostate volume was 39 ml. Based on the combination of standard and targeted biopsies, no PCa, insPCa, CSPCa was detected in 186 (37%), 84 (17%) and 229 (46%) men, respectively.

Table 16. Patient characteristics for men with benign prostate biopsy, insignificant (Gleason 3+3) prostate cancer, and significant prostate cancer (Gleason >3+3), respectively.

Clinical Characteristics		No PCa (n=186)	Insignificant PCa (n=84)	Significant PCa (n=229)	p-value	Total (n=499)
Age (yr), median (IQR)		63 (58–67)	64 (59–68)	67 (62–71)	<0.001	65 (59–69)
PSA (ng/ml), median (IQR)		6.3 (4.6–8.6)	6.4 (5.3–8.6)	7.7 (6.2–10)	<0.001	7.1 (5.4–9.3)
Prostate volume, median (IQR)		44 (34–62)	37 (28–49)	35 (27–46)	<0.001	39 (29–53)
PSA density (ng/ml/ml), median (IQR)		0.14 (0.10–0.19)	0.17 (0.12–0.24)	0.22 (0.16–0.31)	<0.001	0.18 (0.12–0.26)
cT_{DRE} category, n (%)						
cT_x		127 (68)	-	1 (1)		128
cT_{1c}		53 (28)	66 (79)	97 (42)		216
cT_2		-	5 (6)	18 (8)		23
cT_{2a}		5 (3)	4 (5)	35 (15)		44
cT_{2b}		1 (1)	-	7 (3)		8
cT_{2c}		-	8 (10)	44 (19)		52
cT_3		-	1 (1)	12 (5)		13
cT_{3a}		-	-	14 (6)		14
cT_4		-	-	1 (1)		1
PSA density group, n (%)						
1	< 0.10	45 (24)	13 (16)	16 (7)	<0.001	74 (15)
2	0.10 – 0.14	59 (32)	20 (24)	34 (15)		113 (23)
3	0.15 – 0.19	39 (21)	18 (21)	51 (22)		108 (22)
4	≥ 0.20	43 (23)	33 (39)	128 (56)		204 (41)

Prostatectomy was performed on 32% (161/499) of the included men, and Gleason score was upgraded in 24% (38/161) of the cases from the study biopsy results. In patients having IMPROD Likert scored 3 bpMRI finding, in a prostatectomy

specimen, Gleason score was upgraded in 21% (3/14) of men from the study biopsy results.

Figure 6 presents a distribution of PCas within the IMPROD bpMRI Likert and PSA density groups. In men with an equivocal or high suspicion of PCa (IMPROD bpMRI Likert score 3–5), the amount of CSPCas rises with higher PSA density.

Figure 6. Detection rates of prostate cancers divided to various risk groups. Distribution of PCas within PSA density groups and IMPROD bpMRI Likert score groups. Reprinted with a permission.

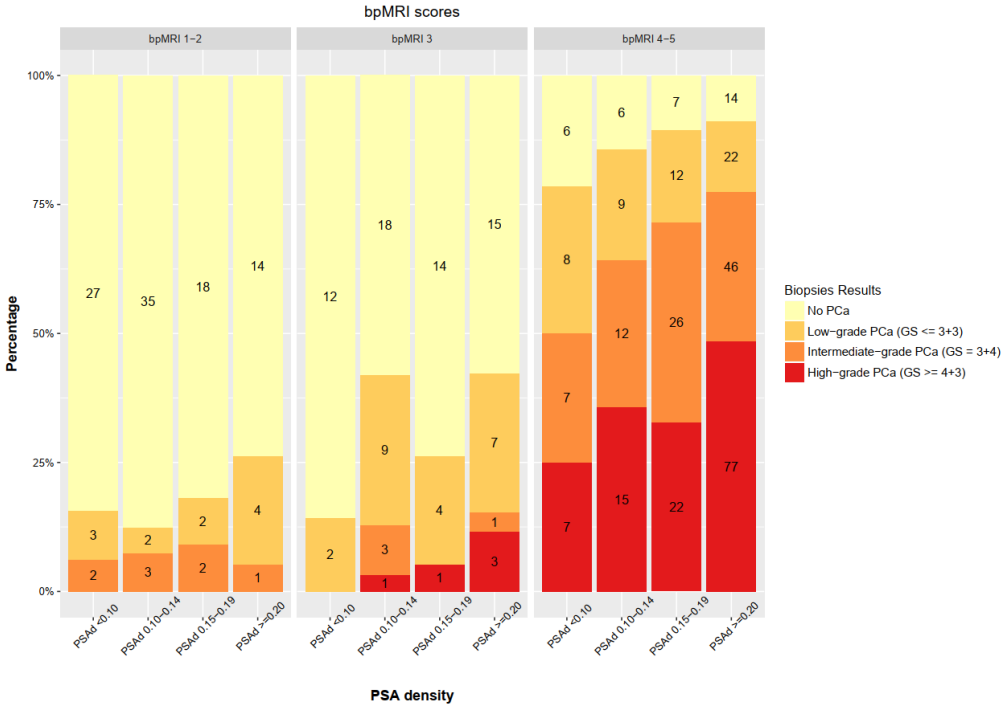


Table 17 presents the performance measures for CSPCa detection at various thresholds of IMPROD bpMRI Likert scores and PSA density values. The NPVs for ruling out CSPCa in suspicion levels of IMPROD bpMRI Likert 3–5 and IMPROD bpMRI Likert 4–5 were 93% and 92%, respectively, while the corresponding PPVs were 57% and 72% respectively. The NPVs for PSA density of ≥ 0.20 ng/ml/ml and ≥ 0.15 ng/ml/ml were 66% and 73% respectively, while the corresponding PPVs were 63% and 57%, respectively. Youden's J index values in patient groups with IMPROD bpMRI Likert scores 4–5 and 3–5 were 0.61 and 0.35, respectively. The highest Youden's J index value in the PSA density groups were 0.29 in patients with PSA density ≥ 0.15.

Table 17. Sensitivity, specificity and predictive values for CSPCa detection at various thresholds of bpMRI score and PSA density.

Restricted Biopsies to	Avoided biopsies, n (%)	Missed significant PCa, n (%)	Sensitivity (CI 95%)	Specificity (CI 95%)	PPV (CI 95%)	NPV (CI 95%)	Youden's J index	AUC (CI 95%)
bpMRI Likert score, n (%)								
Likert ≥ 5, n=216 (43)	283 (57)	51 (22)	0.78 (0.72–0.83)	0.86 (0.81–0.90)	0.82 (0.77–0.87)	0.82 (0.77–0.86)	0.64	0.82 (0.79–0.85)
Likert ≥ 4, n=296 (59)	203 (41)	17 (7)	0.93 (0.88–0.96)	0.69 (0.63–0.74)	0.72 (0.66–0.77)	0.92 (0.87–0.95)	0.61	0.81 (0.78–0.84)
Likert ≥ 3, n=386 (77)	113 (29)	8 (3)	0.97 (0.93–0.98)	0.39 (0.33–0.45)	0.57 (0.52–0.62)	0.93 (0.87–0.97)	0.35	0.68 (0.65–0.71)
PSA density, n (%)								
PSA density ≥ 0.20, n=204 (41)	295 (59)	101 (44)	0.56 (0.49–0.62)	0.72 (0.66–0.77)	0.63 (0.56–0.69)	0.66 (0.60–0.71)	0.28	0.64 (0.60–0.68)
PSA density ≥ 0.15, n=312 (63)	187 (37)	50 (22)	0.78 (0.72–0.83)	0.51 (0.45–0.57)	0.57 (0.52–0.63)	0.73 (0.66–0.79)	0.29	0.64 (0.60–0.68)
PSA density ≥ 0.10, n=425 (85)	74 (15)	16 (7)	0.93 (0.89–0.96)	0.21 (0.17–0.27)	0.50 (0.45–0.55)	0.78 (0.67–0.87)	0.14	0.57 (0.54–0.60)

Table 18 summarizes the effect of PSA density on predictive values for detecting and ruling out CSPCa in each IMPROD bpMRI Likert score group. In men with only IMPROD bpMRI Likert scored 3 lesion present (n=90), restricting biopsies within this group to men with PSA density ≥ 0.20 ng/ml/ml, 64 (71%, 64/90) men from the group would have avoided biopsies and five CSPCas would have been missed in the study biopsies. Restricting biopsies to men with IMPROD bpMRI Likert 4–5 lesion, 17 (17/229, 7% of all CSPCas diagnosed in the study) CSPCas would have been missed, and with IMPROD bpMRI Likert 3–5 lesion, eight (8/229, 3% of all CSPCas diagnosed in the study) CSPCas would have been missed in the study biopsies.

Table 18. Effect of PSA density on predictive values for detecting and ruling out significant prostate cancer (Gleason score ≥ 3+4) in each IMPROD bpMRI suspicion group.

	Total		Diagnostic evaluation		
bpMRI	**Total n (%)**	**Significant PCa n (%)**	**PPV (CI 95%)**	**NPV (CI 95%)**	**p value***
bpMRI Likert score 4–5 and	296 (59)	212 (93)			
PSA density ≥ 0.20	159 (54)	123 (58)	0.77 (0.70–0.84)	0.23 (0.16–0.30)	0.018
PSA density < 0.20	137 (46)	89 (42)	0.65 (0.56–0.73)	0.35 (0.27–0.44)	
PSA density ≥ 0.15	226 (76)	171 (81)	0.76 (0.70–0.81)	0.24 (0.19–0.30)	0.006
PSA density < 0.15	70 (24)	41 (19)	0.59 (0.46–0.70)	0.41 (0.30–0.54)	
PSA density ≥ 0.10	268 (91)	198 (93)	0.74 (0.68–0.79)	0.26 (0.21–0.32)	0.008
PSA density < 0.10	28 (9)	14 (7)	0.50 (0.31–0.69)	0.50 (0.31–0.69)	
bpMRI Likert score 3 and	90 (18)	9 (4)			
PSA density ≥ 0.20	26 (29)	4 (44)	0.15 (0.04–0.35)	0.85 (0.65–0.96)	
PSA density < 0.20	64 (71)	5 (56)	0.08 (0.03–0.17)	0.92 (0.83–0.97)	
PSA density ≥ 0.15	45 (50)	5 (56)	0.11 (0.04–0.24)	0.89 (0.79–0.98)	
PSA density < 0.15	45 (50)	4 (44)	0.09 (0.02–0.21)	0.91 (0.76–0.96)	
PSA density ≥ 0.10	76 (84)	9 (100)	0.12 (0.06–0.21)	0.88 (0.79–0.94)	
PSA density < 0.10	14 (16)	0 (0)	0.0 (0.00–0.23)	1.0 (0.77–1.00)	
bpMRI Likert score 1–2 and	113 (23)	8 (3)			
PSA density ≥ 0.20	19 (17)	1 (13)	0.05 (0.00–0.26)	0.95 (0.74–1.00)	
PSA density < 0.20	94 (83)	7 (87)	0.07 (0.03–0.15)	0.93 (0.85–0.97)	
PSA density ≥ 0.15	41 (36)	3 (38)	0.07 (0.02–0.20)	0.93 (0.80–0.98)	
PSA density < 0.15	72 (64)	5 (63)	0.07 (0.02–0.15)	0.93 (0.85–0.98)	
PSA density ≥ 0.10	81 (72)	6 (75)	0.07 (0.03–0.15)	0.93 (0.85–0.97)	
PSA density < 0.10	32 (28)	2 (25)	0.06 (0.01–0.21)	0.94 (0.79–0.99)	

* Chi-squared test p value.

According to the DCA of different biopsy strategies (**Figure 7**), the optimal biopsy strategy would be to restrict biopsies to men with IMPROD bpMRI Likert score 4–5 lesion or IMPROD bpMRI Likert score 3 lesion with PSA density ≥ 0.20 ng/ml/cc.

Figure 7. Decision curve analysis showing the net benefit of different biopsy strategies for detection of CSPCa across 0 to 25% threshold probabilities. Reprinted with a permission.

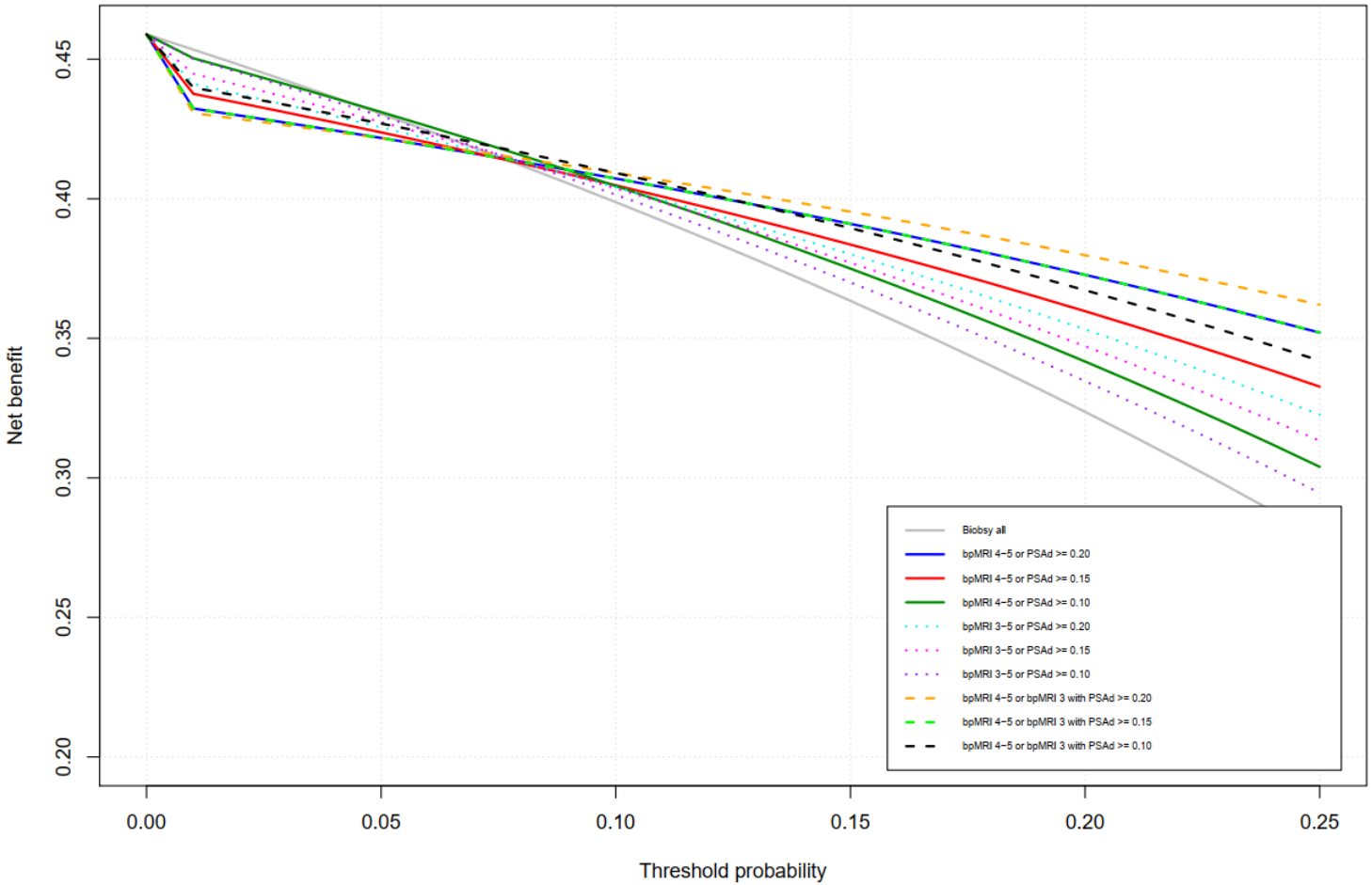


Table 20 presents combination strategies in various IMPROD bpMRI Likert score groups and PSA density thresholds. Using the optimal strategy based on the DCA, i.e. restricting biopsies to men with IMPROD bpMRI Likert scored 4–5 lesion or IMPROD bpMRI Likert scored 3 lesion with PSA density ≥ 0.20 ng/ml/ml, the strategy gives NPV of 93% and PPV of 67% in diagnostics of a CSPCa. Using this strategy, 35% (177/499) of men would have avoided biopsies and only 13 CSPCas would have been missed (13/229, 6% of all diagnosed CSPCas in the study). The majority (11/13) of the missed PCas were classified as Gleason 3+4, except two, which were graded as Gleason 4+4 and Gleason 4+3. **Table 19** presents the amount of PCa and CSPCa in biopsy cores in patients missed by the optimal strategy.

In contrast, the optimal strategy in the Boesen et al. study (bpMRI score 4–5 or PSA density ≥ 0.15 ng/ml/ml) gives NPV of 92% and PPV of 58% in ruling out CSPCa using our study material (Boesen et al., 2019). Moreover, nine CSPCas (9/229, 4% of all diagnosed CSPCas in the study) would have been missed, and 117 men (117/499, 23% of the study patients) would have avoided biopsies with the strategy. The missed cancers were classified as Gleason 3+4, with the exception of one which was classified as Gleason 4+4.

Table 19. Amount of prostate cancer and significant prostate cancer (Gleason score ≥ 3+4) in biopsies taken from patients who did not fulfill the biopsy criteria of the DCA based optimal biopsy strategy (IMPROD bpMRI Likert score 4–5 or IMPROD bpMRI Likert score 3 with PSA density ≥0.20 ng/ml/cc).

Patient	Highest Gleason score	Biopsy cores with prostate cancer graded as Gleason ≥ 3+4	Total prostate cancer positive biopsy cores	Biopsy cores taken
1	3+4	1	1	12
2	3+4	7	7	16
3	3+4	2	3	14
4	4+4	3	4	16
5	3+4	1	3	12
6	3+4	1	2	12
7	3+4	5	6	14
8	4+3	6	6	14
9	3+4	2	2	14
10	3+4	1	1	14
11	3+4	2	2	12
12	3+4	1	7	12
13	3+4	2	4	12

Table 20. Results of different biopsy strategies and the sensitivity, specificity, and predictive values for detecting and ruling out CSPCa when IMPROD bpMRI Likert scores are combined with various PSA density thresholds.

Restrict Biopsies to	Biopsies		Insignificant PCa		Significant PCa		Diagnostic Evaluation (Significant PCa)				
	Performed n (%)	Avoided n (%)	Detected n (%)	Avoided n (%)	Detected n (%)	Missed n (%)	Sensitivity (CI 95%)	Specificity (CI 95%)	PPV (CI 95%)	NPV (CI 95%)	AUC (CI 95%)
All men	**499**	**0**	**84**	**0**	**229**	**0**	**Ref**	**Ref**	**Ref**	**Ref**	**Ref**
IMPROD bpMRI Likert score = 3-5, or	386 (77)	113 (23)	73 (87)	11 (13)	221 (97)	8 (3)	0.97 (0.93, 0.98)	0.39 (0.33, 0.45)	0.57 (0.52, 0.62)	0.93 (0.87, 0.97)	0.68 (0.65, 0.71)
PSA density ≥ 0.20	405 (81)	94 (19)	77 (92)	7 (8)	222 (97)	7 (3)	0.97 (0.94, 0.99)	0.32 (0.27, 0.38)	0.55 (0.50, 0.60)	0.93 (0.85, 0.97)	0.65 (0.62, 0.68)
PSA density ≥ 0.15	427 (86)	72 (14)	79 (94)	5 (6)	224 (98)	5 (2)	0.98 (0.95, 0.99)	0.25 (0.20, 0.30)	0.52 (0.48, 0.57)	0.93 (0.85, 0.98)	0.61 (0.58, 0.64)
PSA density ≥ 0.10	467 (94)	32 (6)	81 (96)	3 (4)	227 (99)	2 (1)	0.99 (0.97, 1.00)	0.11 (0.08, 0.15)	0.49 (0.44, 0.53)	0.94 (0.79, 0.99)	0.55 (0.53, 0.57)
IMPROD bpMRI Likert score = 4-5, or	296 (59)	203 (41)	51 (61)	33 (39)	212 (93)	17 (7)	0.93 (0.88, 0.96)	0.69 (0.63, 0.74)	0.72 (0.66, 0.77)	0.92 (0.87, 0.95)	0.81 (0.78, 0.84)
PSA density ≥ 0.20	341 (68)	158 (32)	62 (74)	22 (26)	217 (95)	12 (5)	0.95 (0.91, 0.97)	0.54 (0.48, 0.60)	0.64 (0.58, 0.69)	0.92 (0.87, 0.96)	0.74 (0.71, 0.77)
PSA density ≥ 0.15	382 (77)	117 (23)	68 (81)	16 (19)	220 (96)	9 (4)	0.96 (0.93, 0.98)	0.40 (0.34, 0.46)	0.58 (0.52, 0.63)	0.92 (0.86, 0.96)	0.68 (0.65, 0.71
PSA density ≥ 0.10	453 (91)	46 (9)	79 (94)	5 (6)	227 (99)	2 (1)	0.99 (0.97, 1.00)	0.16 (0.12, 0.21)	0.5 (0.45, 0.55)	0.96 (0.85, 0.99)	0.58 (0.56, 0.6)
(IMPROD bpMRI Likert score = 4-5) or IMPROD bpMRI Likert score = 3, with											
PSA density ≥ 0.20	322 (65)	177 (35)	58 (69)	26 (31)	216 (94)	13 (6)	0.94 (0.90, 0.97)	0.61 (0.55, 0.67)	0.67 (0.62, 0.72)	0.93 (0.88, 0.96)	0.78 (0.75, 0.81)
PSA density ≥ 0.15	341 (68)	158 (32)	62 (74)	22 (26)	217 (95)	12 (5)	0.95 (0.91, 0.97)	0.54 (0.48, 0.60)	0.64 (0.58, 0.69)	0.92 (0.87, 0.96)	0.74 (0.71, 0.77)
PSA density ≥ 0.10	372 (75)	127 (25)	71 (85)	13 (15)	221 (97)	8 (3)	0.97 (0.93, 0.98)	0.44 (0.38, 0.50)	0.59 (0.54, 0.64)	0.94 (0.88, 0.97)	0.70 (0.67, 0.73)

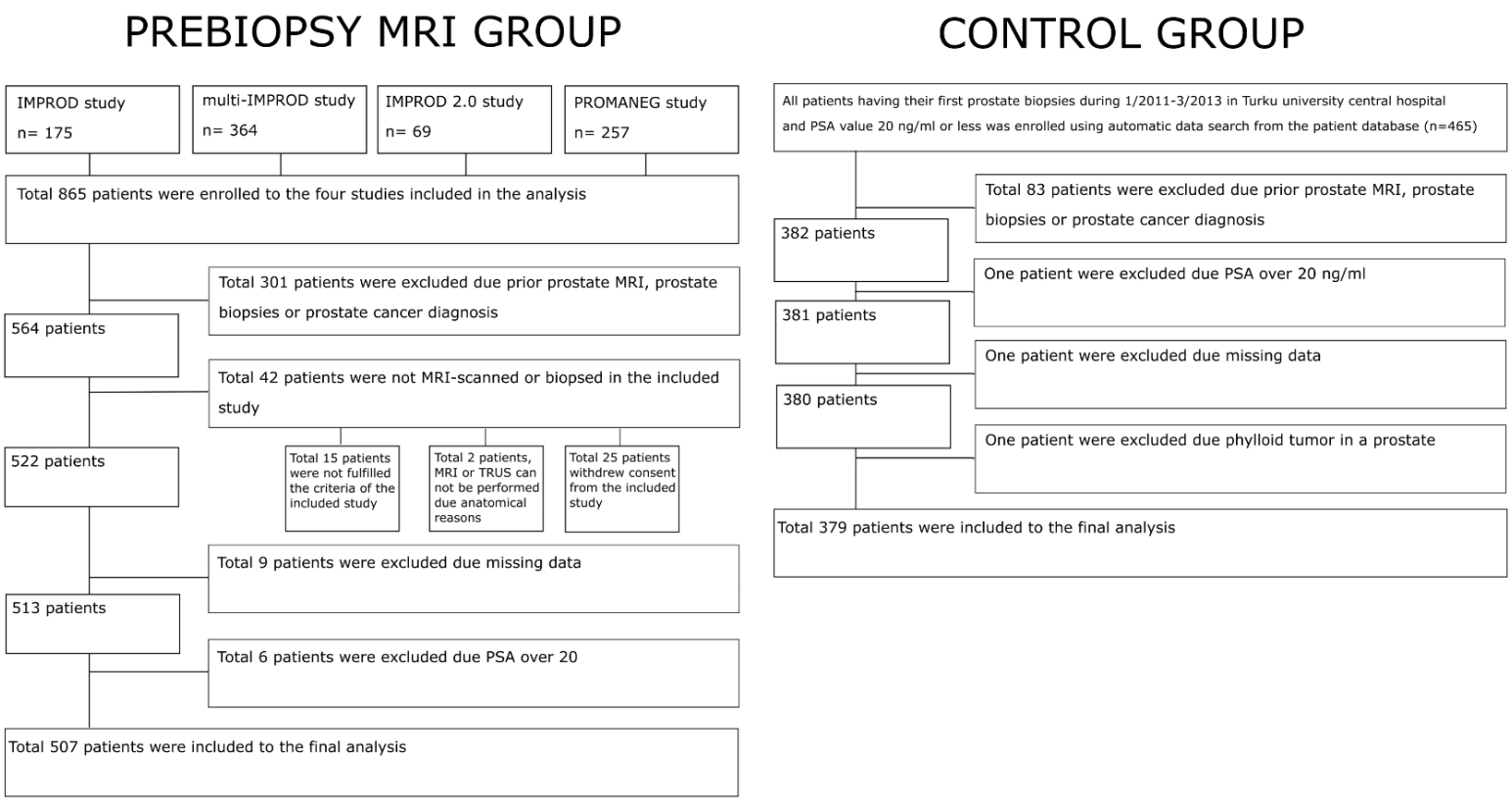
5.4 Study IV

In Study IV, we investigated the impact of prebiopsy prostate bpMRI on the prevalence of CSPCa in an initial biopsy session. Baseline characteristics of the study groups are presented in **Table 21** and a flowchart of the patient inclusion is presented in **Figure 8**. The MRI group consists of 507 men and the non-MRI group of 379 men. There was a significant ($p<0.05$) difference in the mean age and a mean PSA level between the study groups: The mean age and the mean PSA were in the MRI group 64 years and 7.6 ng/ml, respectively, and in the non-MRI group 68 years and 8.2 ng/ml, respectively. Mean prostate volumes were in the MRI group and in the non-MRI group 43 ml and 44 ml, respectively, and the difference was not significant. Due to the study setting, mean follow-up times differ substantially between the study groups, 736 days in the MRI group and 2381 days in the non-MRI group ($p<0.05$).

Table 21. Baseline characteristics of the study patients.

	non-MRI group	MRI group	p
n	379	507	
mean age years (range)	68 (39–91)	64 (29–82)	<0.001
mean PSA ng/ml (range)	8.2 (0.42–19)	7.6 (1.2–20)	0.020
mean prostate volume ml (range)	44 (16–120)	43 (14–150)	0.245
mean follow-up time, days (range)	2381 (1941–2758)	736 (36–1596)	<0.001

Figure 8. A flowchart of the study patient inclusion.



The results of initial biopsies are presented in **Table 22**. Significant PCa (GGG 2-5) were diagnosed with initial biopsies in 48% of men in the MRI group and in 34% of men in the non-MRI group (p<0.001). The rate of insPCa (GGG 1) was 16% in the both study groups. When all the biopsy results were divided to the GGGs, significantly (p<0.05) higher rates of GGG 3 and GGG 4 graded PCas were discovered in the MRI group.

Table 22. Histological diagnosis in initial biopsies.

	non-MRI group	MRI group	p
n	379	507	
benign (%)	190 (50)	179 (35)	<0.001
any PCa (%)	189 (50)	328 (65)	<0.001
Gleason grade group 2-5 PCa (%)	130 (34)	245 (48)	<0.001
Gleson grade group 1 (%)	59 (16)	83 (16)	0.747
Gleson grade group 2 (%)	50 (13)	89 (18)	0.077
Gleson grade group 3 (%)	17 (5)	57 (11)	<0.001
Gleson grade group 4 (%)	20 (5)	52 (10)	0.007
Gleson grade group 5 (%)	43 (11)	47 (9)	0.312

Upgraded biopsy results during the follow-up are presented in **Table 23**. In men with a benign or a GGG 1 histology in initial biopsies, upgrading histology in re-biopsies was in 5% of men in the MRI group and in 19% of men in the non-MRI group (p=0.001). In addition, histology of the initial biopsies upgraded to a highly aggressive (GGG ≥ 3) PCa in re-biopsies in 1% and 11% PCa of the men in the MRI group and the non-MRI group (p<0.001), respectively. We made an additional analysis on the non-MRI group, dividing it to men having MRI and men without MRI during the follow-up. Substantially more CSPCa, and also more aggressive PCas were found in men having MRI during the follow-up.

Table 23. Biopsy findings during the follow-up.

	MRI group	non-MRI group	p	non-MRI group		p
				MRI before re-biopsies subgroup	no MRI before re-biopsies subgroup	
n	379	507		23	226	
benign or GGG 1 (%) in primary biopsies	262 (69)	249 (49)	<0.001	23 (100)	226 (100)	
GGG ≥ 2 PCa in rebiopsies if primary biopsies benign or GGG 1 (%)	13 (5)	47 (19)	0.001	12 (52)	35 (25)	<0.001
GGG ≥ 3 PCa in rebiopsies if primary biopsies benign or GGG 1 (%)	3 (1)	27 (11)	<0.001	6 (26)	21 (9)	0.014

Histological findings of prostatectomy specimen compared to the initial biopsy results are presented in **Table 24**. Upgrading histology in prostatectomy specimen was substantially and significantly (p=0.002) more common in the non-MRI group (57%) than in the MRI group (36%), and capsule invasive PCa was significantly more common in the non-MRI group.

Table 24. Prostatectomy findings during the follow-up.

	MRI group	non-MRI group	p
n	379	507	
prostatectomy (% of patients in the cohort)	173 (46)	77 (20)	<0.001
upgrading Gleason grade in prostatectomy specimen (% of prostatectomies in the cohort)	62 (36)	44 (57)	0.002
pT3-pT4 in prostatectomy specimen (% of prostatectomies in the cohort)	71 (41)	43 (56)	0.030

A diagnostic delay and a number of biopsy sets taken for the diagnosis of CSPCa are presented in **Figure 9** and **Figure 10**. In **Figure 9**, a Kaplain-Meier graph of first two years of the follow-up indicates a trend that more CSPCas were diagnosed during the follow-up in the non-MRI group in comparison to the MRI group. The same trend is seen in **Figure 10**, which presents how many biopsy sets had to be taken for the diagnosis of CSPCa.

Figure 9. A Kaplan-Meier graph of a two years' follow-up period of the study patients. The graph presents the prevalence of CSPCa in the study groups during the first two years of follow-up. Reprinted with a permission.

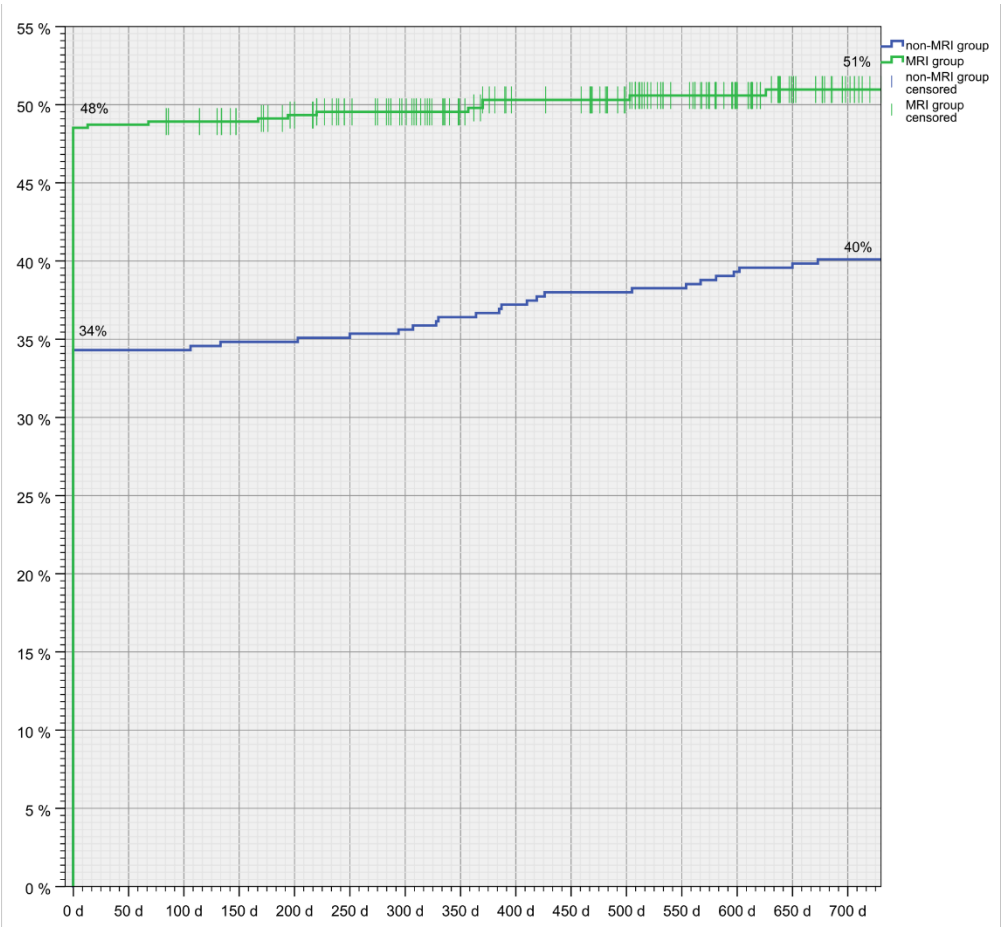
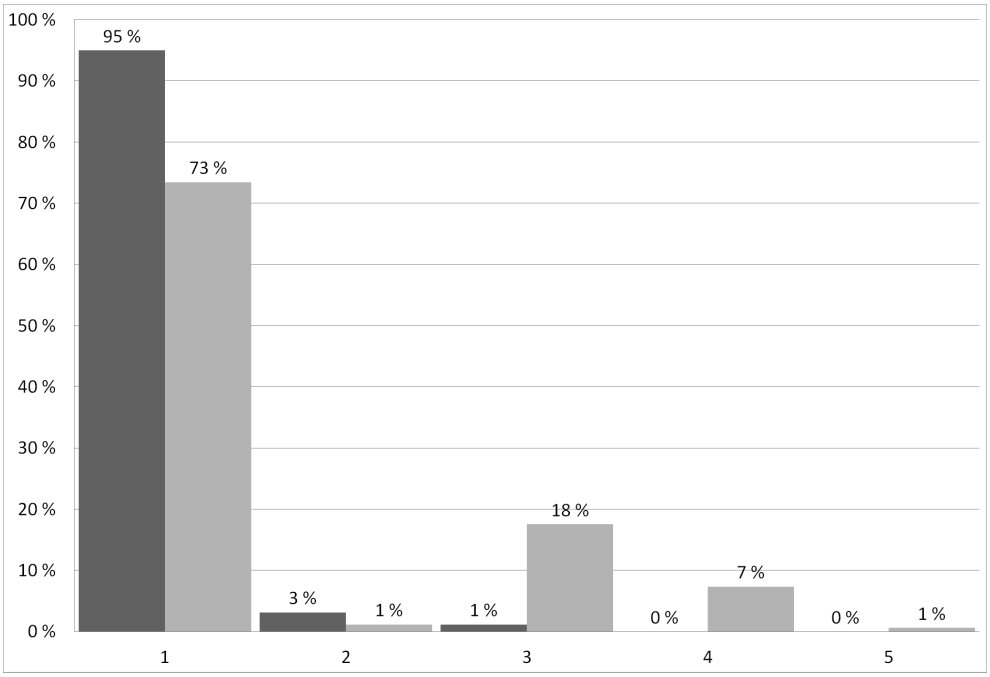


Figure 10. A graph presenting how many biopsy sessions had to be performed for a diagnosis of a CSPCa during the follow-up. The dark grey bar presents the MRI group and the light grey bar presents the non-MRI group (control group). Reprinted with a permission.



5.5 Study V

In Study V, we investigated the NPV of IMPROD bpMRI as a whole and in clinical subgroups. A total of 865 patients were recruited to the studies and 673 of these patients were included into this pooled data analysis. The median delay from MRI to study biopsies was 10 days. **Table 25** summarizes the study patient characteristics and **Figure 11** includes a flowchart of patient inclusion to the final analysis. The mean age of the included men was 65 years and mean PSA level was 9.7 ng/ml. Of the study population, 76% were prostate biopsy and prostatic surgery naïve. Significant PCa was found in 320 (48%) and any grade of PCa in 425 (63%) in the study biopsies. Characteristics of different MRI studies is depicted in **Table 26**. In total, the AUC of IMPROD bpMRI and IMPROD Likert scoring system in CSPCa diagnostics was 0.863.

Table 25. Study patient characteristics.

age, mean (range)	65 (29–83)	years
PSA, mean (range)	9.7 (1.2–380)	ng/ml
free PSA/PSA ratio, mean (range)	14.9 (0.50–47.9)	%
prostate volume, mean (range)	44 (10–200)	ml
PSA density, mean (range)	0.24 (0.025–3)	ng/l/ml
5-alpha reductase therapy n (%)	94 (14)	
previous prostatic procedures		
none, n (%)	513 (76)	
prostate biopsies, n (%)	153 (23)	
prostatic surgery, n (%)	14 (2)	
total number of biopsy cores, mean (range)	13 (2–20)	
prostate cancer status in study biopsies		
bening, n (%)	248 (37)	
Gleason score 3+3, n (%)	105 (16)	
Gleason score 3+4, n (%)	130 (19)	
Gleason score 4+3, n (%)	69 (10)	
Gleason score 4+4, n (%)	59 (9)	
Gleason score >4+4, n (%)	62 (9)	

Figure 11. Flowchart of patient inclusion to the final analysis.

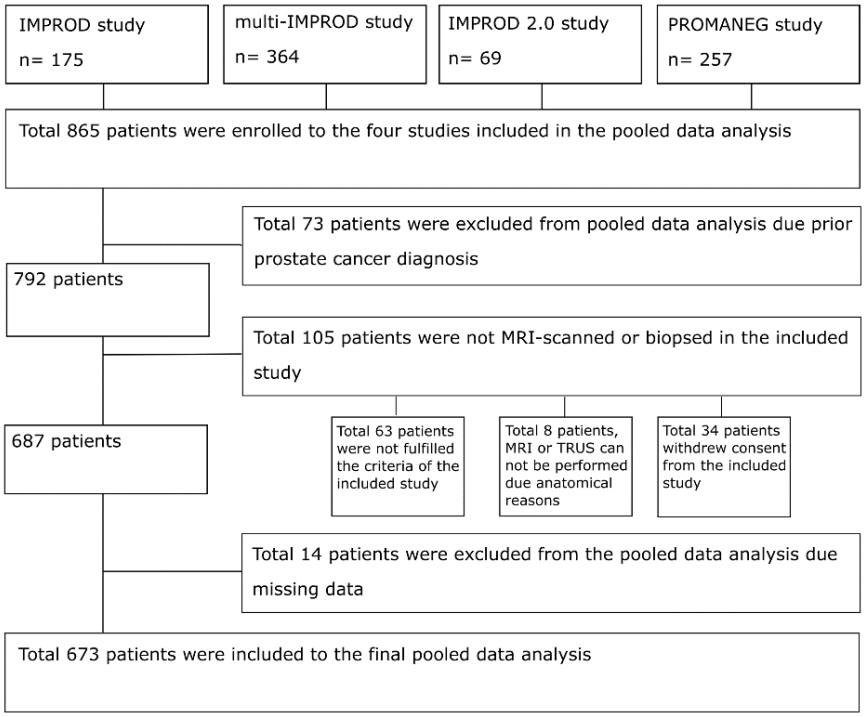


Table 26. Prospective studies included in the pooled analysis. Number of patients included in the studies, PCa rates, and the AUC values of prebiopsy IMPROD bpMRI for a CSPCa are presented.

study	n	PCa in the study biopsies n (%)	CSPCa in the study biopsies n (%)	AUC for a CSPCa
IMPROD	162	106 (65)	81 (50)	0.873
Multi-IMPROD	338	207 (61)	146 (43)	0.861
IMPROD 2.0	56	42 (75)	36 (64)	0.807
PROMANEG	117	70 (60)	57 (49)	0.865
total	673	425 (63)	320 (48)	0.863

Table 27 summarizes the parameters of IMPROD bpMRI in CSPCa diagnostics. A total of 80% and 62% of the study patients had IMPROD bpMRI Likert scores between 3–5 and 4–5, respectively. The NPVs of the IMPROD bpMRI Likert score groups 1–2 and 1–3 for ruling out CSPCa were 0.932 and 0.914, respectively. The corresponding PPVs for CSPCa in the IMPROD bpMRI Likert score groups 3–5 and 4–5 were 0.575 and 0.713, respectively.

Table 27. The diagnostic parameters of IMPROD bpMRI for CSPCa.

MRI suspicion score	study		diagnostic parameters to a CSPCa			
			NPV	PPV	sensitivity	specificity
IMPROD Likert 3-5	IMPROD	123 (76)	0.897	0.626	0.951	0.432
	Multi-IMPROD	263 (78)	0.947	0.540	0.973	0.370
	IMPROD 2.0	50 (89)	1.000	0.720	1.000	0.300
	PROMANEG	105 (90)	0.917	0.533	0.982	0.183
	total	541 (80)	0.932	0.575	0.972	0.348
IMPROD Likert 4-5	IMPROD	99 (61)	0.921	0.768	0.938	0.716
	Multi-IMPROD	197 (58)	0.915	0.680	0.918	0.672
	IMPROD 2.0	44 (79)	0.833	0.773	0.944	0.500
	PROMANEG	78 (67)	0.923	0.692	0.947	0.600
	total	418 (62)	0.914	0.713	0.931	0.660

Table 28 and **Table 29** present the subgroup characteristics and performance measures of IMPROD bpMRI in the study population in the score groups of IMPROD bpMRI Likert 3–5 and IMPROD bpMRI Likert 4–5. In the analysis, no clear outlier with a higher NPV in ruling out CSPCa were present. The highest NPV of the IMPROD Likert score group 3–5 was in a subgroup of men with a PSA level ≥10 ng/ml (0.966). Correspondingly, the highest NPVs of the IMPROD Likert score group 4–5 were in subgroups of men with PSA density <0.25 ng/ml/ml (0.941) or prostate volume ≥40 ml (0.936). However, the subgroup of men with PSA density ≥0.25

ng/ml/ml stood out with low NPV in both IMPROD Likert 3–5 (0.875) and IMPROD Likert 4–5 (0.757) score groups.

Table 28. Subgroup analysis of the study population.

		n/total (%)	significant PCas n/total (%)
prostate volume	≥ 40 ml	298/625 (48%)	102/298 (34%)
	< 40 ml	327/625 (52%)	193/327 (59%)
PSA	≥ 10 ng/ml	180/673 (27%)	101/180 (56%)
	< 10 ng/ml	493/673 (73%)	219/493 (44%)
PSA density	≥ 0.25 ng/ml/ml	191/625 (31%)	131/191 (69%)
	< 0.25 ng/ml/ml	434/625 (69%)	164/434 (38%)
free PSA/PSA ratio	≥ 10%	415/530 (78%)	173/415 (42%)
	< 10%	115/530 (22%)	65/115 (57%)
age	≥ 65 years	382/673 (57%)	218/382 (57%)
	< 65 years	291/673 (43%)	102/291 (35%)
previous biopsies	yes	153/673 (23%)	65/153 (42%)
	no	520/673 (77%)	255/520 (49%)
5-alpha reductase therapy	yes	94/673 (14%)	44/94 (47%)
	no	579/673 (86%)	276/579 (48%)

Table 29. The diagnostic parameters of IMPROD bpMRI for CSPCa in suspicion levels of IMPROD Likert 3–5 and IMPROD Likert 4–5 in different clinical subgroups.

suspicion level			NPV	PPV	sensitivity	specificity
IMPROD Likert 3-5	prostate volume	≥ 40 ml	0.945	0.469	0.951	0.439
		< 40 ml	0.895	0.654	0.979	0.254
	PSA	≥ 10 ng/ml	0.966	0.662	0.990	0.354
		< 10 ng/ml	0.922	0.541	0.963	0.347
	PSA density	≥ 0.25 ng/ml/ml	0.875	0.737	0.985	0.233
		< 0.25 ng/ml/ml	0.938	0.489	0.957	0.393
	free PSA/PSA ratio	≥ 10%	0.938	0.525	0.965	0.376
		< 10%	0.941	0.653	0.985	0.320
	age	≥ 65 years	0.949	0.666	0.986	0.341
		< 65 years	0.918	0,440	0.941	0.354
	previous biopsies	yes	0.900	0.504	0.954	0.307
		no	0.941	0.596	0.976	0.362
	5-alpha reductase therapy	yes	0.952	0.589	0.977	0.400
		no	0.928	0.573	0.971	0.340
IMPROD Likert 4-5	prostate volume	≥ 40 ml	0.936	0.648	0.902	0.745
		< 40 ml	0.869	0.749	0.943	0.545
	PSA	≥ 10 ng/ml	0.900	0.792	0.941	0.684
		< 10 ng/ml	0.918	0.681	0.927	0.653
	PSA density	≥ 0.25 ng/ml/ml	0.757	0.792	0.931	0.467
		< 0.25 ng/ml/ml	0.941	0.658	0.927	0.707
	free PSA/PSA ratio	≥ 10%	0.927	0.678	0.925	0.686
		< 10%	0.875	0.735	0.938	0.560
	age	≥ 65 years	0.909	0.793	0.950	0.671
		< 65 years	0.918	0.580	0.892	0.651
	previous biopsies	yes	0.903	0.648	0.908	0.636
		no	0.917	0.731	0.937	0.668
	5-alpha reductase therapy	yes	0.929	0.788	0.932	0.780
		no	0.911	0.702	0.931	0.640

Of the study patients classified as MRI negative by IMPROD Likert score of 1–3 (255/673 [38% of all the study patients]), 22 had CSPCa in the study biopsies. Of these 22 patients, four had Gleason 5 patterns in the study biopsies. Accordingly, of the study patients classified as MRI negative by IMPROD Likert score of 1–2 (132/673 [20% of all the study patients]), nine had CSPCa in the study biopsies. Of these nine patients, seven had Gleason 3+4 graded PCa and two Gleason 4+3 graded PCa. Thus, 20% of the study patients with suspected PCa could have been excluded

from prostate biopsies without missing any Gleason >7 PCa and missing only nine CSPCas (9/320 [3% of all CSPCas diagnosed in the study]) in the study biopsies.

Negative predictive values for different patient subcohorts defined by prostate volume, PSA and PSA density value groups are presented in **Figure 12**, **Figure 13** and **Figure 14**. The diagnostic accuracy of IMPROD bpMRI in ruling out CSPCa seems to increase in patients having larger prostates. We found no similar pattern in increasing PSA values. Also, higher NPV was seen in patients with PSA density <0.25 ng/ml/ml.

Figure 12. Negative predictive value of IMPROD bpMRI for CSPCa in prostate volume subgroups. IMPROD Likert 4–5 graded lesion in MRI report was used as a positive for CSPCa. (prev=prevalence).

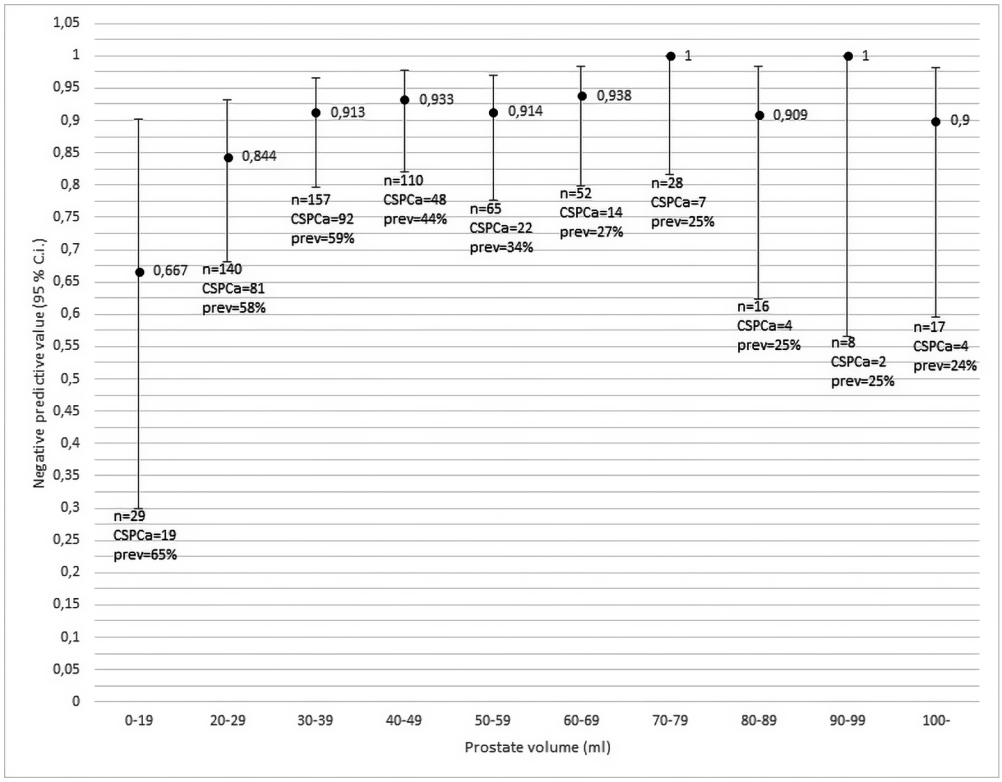


Figure 13. Negative predictive value of IMPROD bpMRI for CSPCa in different PSA level subgroups. IMPROD Likert 4–5 graded lesion in MRI report was used as a positive for CSPCa. (prev=prevalence).

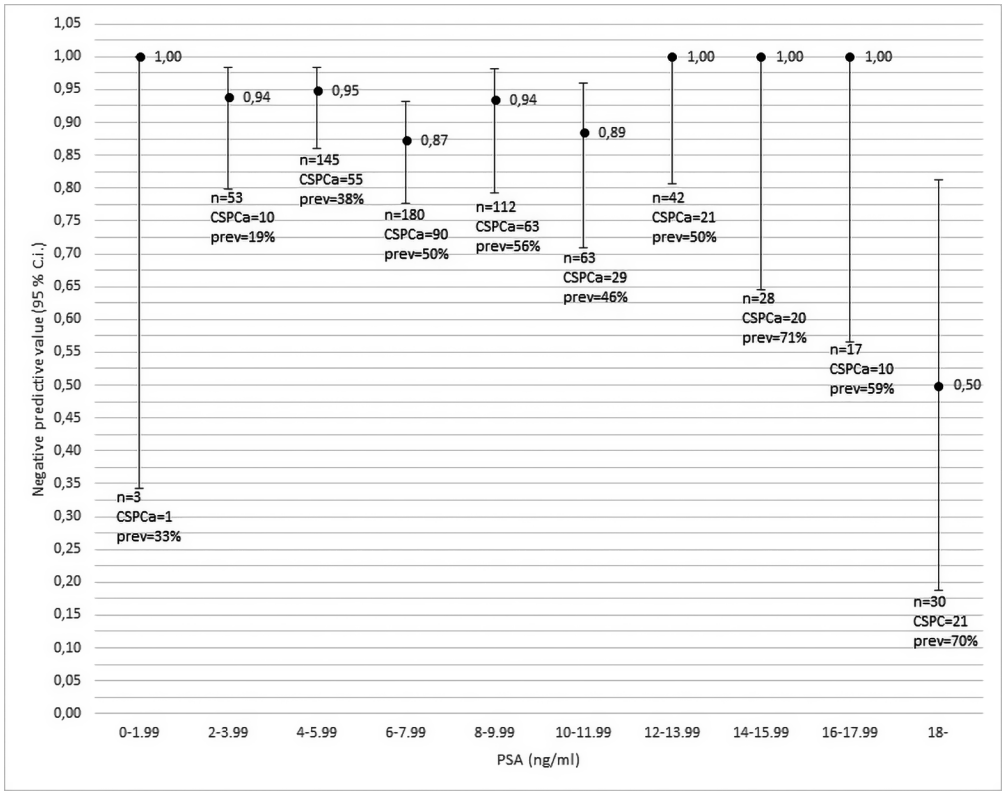
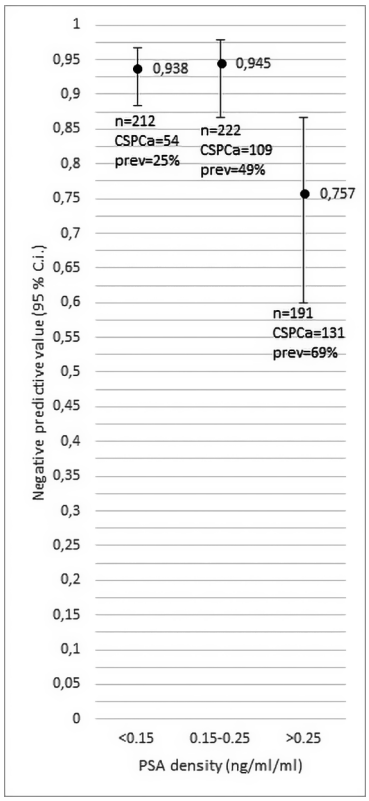


Figure 14. Negative predictive value of IMPROD bpMRI for CSPCa in different PSA level subgroups. IMPROD Likert 4–5 graded lesion in MRI report was used as a positive for CSPCa. Negative predictive value of IMPROD bpMRI for CSPCa in in different PSA density groups. IMPROD Likert 4–5 graded lesion in the MRI report was used as a positive for CSPCa. (prev=prevalence).



6 Discussion

6.1 Main findings and discussion of the substudies

6.1.1 Summary

Traditional PCa diagnostics with systematic prostate biopsies is inevitably shifting towards an MRI and lesion-based approach, however, the role of MRI in the modern diagnostic protocol is still under a debate. Additionally, the globally increasing E. coli fluoroquinolone resistance rate and rate of infectious biopsy complications requires improved prophylaxis and advanced patient selection for prostate biopsies.

In this doctoral thesis, we investigated means to improve PCa diagnostics with MRI and biopsy safety especially from microbiological aspects. The methods included were to survey a prevalence of TRUS-Bx complications, prevalence and risk factors for intestinal E. coli antibiotic resistance in men undergoing prostate biopsies, validate the bpMRI score and PSA density combination strategy in selecting men for prostate biopsies, investigate the impact of prebiopsy prostate bpMRI on the prevalence of CSPCa in an initial biopsy session, and investigate the NPV of IMPROD bpMRI as a whole and in clinical subgroups.

In the microbiological section of the doctoral thesis, we noted quite high prevalence of fluoroquinolone-resistant E. coli strains among men undergoing TRUS-Bx, which was consistent with the findings of prior studies. In addition, recent traveling was noted as a risk factor for fluoroquinolone resistance also in our study. Contrary to findings in prior studies, the rate of infectious biopsy complications was however low.

In the MRI section of the doctoral thesis, we demonstrated a high NPV of IMPROD bpMRI in ruling out CSPCa and its diagnostic superiority with almost all measured variables comparing to the traditional diagnostic protocol, which is also a consistent finding with the prior studies. In the subgroup analysis, no clinical subgroups with improved bpMRI performance appeared. Additionally, PSA density gives only minor additional value to diagnostics in case of equivocal CSPCa suspicion, which validates the results of Boesen et al. (Boesen et al., 2019).

6.1.2 Study I

In this prospective, multicenter study, the rate of biopsy-related complications was 4.1% during the follow-up period of 30 days. None of the patients got a septic complication and only two (0.7%) had a minor infectious complication. The rate of patients having an E. coli strain resistant to fluoroquinolone-based antibiotics was reasonably high: 12%. Infectious complications were rare, although 11% of the patients had an E. coli strain resistant to the given antibiotic prophylaxis. Prevalence and risk factors for having an intestinal fluoroquinolone resistant E. coli strain in this study population is analyzed in more detail in Study II of this doctoral thesis. Rectal bleeding or hematuria occurred in eight (2.7%) patients. Three patients (1.0%) experienced an urinary retention.

Almost all patients in our study (97%) had a fluoroquinolone-based antibiotic as a prophylactic regimen which is in line with the recommendations of the EAU guidelines, however, due to new regulations by the European Commission concerning fluoroquinolone use, the guidelines will be changed in the near future (Bonkat et al., 2019; Mottet et al., 2018). A Cochrane review article analyzed antibiotic prophylaxis for reducing infectious complications (bacteriuria, bacteremia, fever, urinary tract infection, hospitalization) after TRUS-Bx (Zani et al., 2011). In the analysis of antibiotics versus placebo or no treatment, all outcomes significantly favored antibiotics (Zani et al., 2011). Although the fluoroquinolones were the most analyzed antibiotic regimen in the Cochrane review article, there was no difference between fluoroquinolones and 'other classes of antibiotics' (sulfonamides, piperacillin tazobactam and ceftriaxone) (Zani et al., 2011). The most recent studies included in the Cochrane analysis were from 2009 (Zani et al., 2011). Therefore, the considerably increased fluoroquinolone resistance rate of E. coli strains worldwide and also in Finland during the last decades makes the outcome slightly outdated (CDDEP, 2019; Hakanen et al., 2016).

An increasing fluoroquinolone resistance rate in E. coli strains and serious infectious complications after TRUS-Bx is of major concern. It has been suspected that the increased number of serious infectious complications after TRUS-Bx results from the increased fluoroquinolone resistance rate of E. coli strains (Borghesi et al., 2016; Loeb et al., 2013; Pinkhasov et al., 2012; Wagenlehner et al., 2013). On the other hand, the colonization of organisms resistant to prophylactic antibiotics does not necessarily translate into a clinical infection, which was also the outcome in this study (Liss et al., 2011; Liss et al., 2014). Additionally, when determining an organism's ability to cause post-TRUS-Bx infection, fluoroquinolone resistance should be considered more as a continuum of increasing resistance for prophylactic antibiotic regimen, not as a binary value (Kalalahti et al., 2018).

The exceptionally low rate of infectious complications and the absence of septic complications in our study may be also partly explained by the prospective nature of

the study and the limitations followed by the study protocol. This can be observed in the varying practice of antibiotic prophylaxis: fosfomycin was used in patients with previous traveling history, especially in the Helsinki study center where the fluoroquinolone resistance rate in E. coli is significantly higher. Duplessis et al. presented other possible explanations for the disparity between the fluoroquinolone resistance rate and the rate of infectious complications: It is possible that the concentration of ciprofloxacin in feces may exceed the minimal inhibitory concentration of the E. coli strains in the rectum (Duplessis et al., 2012). It is also possible that there are only such small numbers of resistant strains in some patients' rectum that those were not inoculated into the tissue (Duplessis et al., 2012). Finally, not all strains have the same virulence potential (Duplessis et al., 2012).

Several means for reducing infectious complications of TRUS-Bx have been presented. These include alternative antibiotics in prophylaxis and giving targeted prophylactic antibiotic guided by preprocedural rectal swab sample (Adibi et al., 2013; Duplessis et al., 2012; Fahmy, Kotb, et al., 2016; Fahmy, Rhashad, et al., 2016; Lorber et al., 2013; Sen et al., 2015; Taylor et al., 2012). Infection after TRUS-Bx is always an iatrogenic complication, and by taking biopsies using the transperineal approach this risk can be minimized. However, this method is not easily performed in a high-volume outpatient setting but can be considered as an option in a risk patient. Another method to minimize the risk is to improve pre-biopsy diagnostics developing novel imaging modalities to avoid unnecessary biopsies.

Also non-infectious complications were rare in this study and the rate of hospitalization and emergency department visits after TRUS-Bx due to a non-infectious complication was consistent with the previous studies (see chapter 2.2.3.3: Biopsy complications). However, bleeding complications occurred slightly more often in our study, but the total number of bleeding complications was low. Contrary to the study by Pinkhasov et al., (see chapter 2.2.3.3: Biopsy complications), we made the TRUS-Bx without interrupting anticoagulation or antiplatelet therapy if the international normalized ratio (INR) was in the treatment level, as the current EAU guidelines recommend (Mottet et al., 2018; Pinkhasov et al., 2012).

The limitations of this study include the non-standardized prophylaxis protocol and the varying number of the biopsy cores. Patients known to have an increased risk were potentially treated differently and their follow-up may also have been more intense. On the other hand, the study describes quite well the prevailing clinical practice of antibiotic prophylaxis in study centers. However, varying practice makes it difficult to evaluate the prophylaxis protocol underlying the results.

6.1.3 Study II

In our prospective study, we found fluoroquinolone resistant strains in 13% and 3CEF resistant E. coli strains in 8% of the rectal swab samples of the study population taken

prior TRUS-Bx. Of the 3CEF resistant E. coli strains, 62% proved to be ESBL-producers. In multivariate risk factor analysis, international traveling during the preceding year significantly increased the risk for having a fluoroquinolone resistant E. coli strain. Surprisingly, the use of any antibiotics during the preceding year decreased the risk. We did not find any significant risk factors for having 3CEF resistant E. coli.

Previously, it has been reported that fluoroquinolone resistant coliforms can be found in 10.6–40.4% and ESBL producing organisms in 1.3–41.0% of rectal swab cultures taken prior TRUS-Bx (Batura et al., 2010; Duplessis et al., 2012; Liss et al., 2014; Taylor et al., 2013; Tsu et al., 2015). In these studies, E. coli was the most common fluoroquinolone resistant and ESBL-producing gram negative organism. In addition, it has been reported that E. coli is the most common pathogen causing infectious complications after TRUS-Bx (Loeb et al., 2012). The wide range in rates of fluoroquinolone resistant and ESBL producing microorganisms may have several explanations. The variation may be caused by territorial differences in antimicrobial susceptibility or the timing of the publications. Since these studies were published during the last nine years, the globally increasing trend of antimicrobial resistance could also slightly affect the results (CDDEP, 2019; Fasugba et al., 2015). Finally, the definitions and protocols of measuring antimicrobial susceptibility varies between the studies.

In the present study, international traveling clearly associated with the risk to have a fluoroquinolone resistant E. coli strain. We also identified a similar trend concerning the 3CEF resistant E. coli strains. The patients' traveling history and fluoroquinolone resistance of E. coli in rectal swab sample also correlate quite well with the prevailing global fluoroquinolone resistance rates (CDDEP, 2019). The correlation between international traveling and fluoroquinolone resistance has also been noted in previous studies. International traveling, and traveling to regions with high prevalence of antimicrobial resistance in particular, appears an indisputable risk factor for having fluoroquinolone resistant or ESBL-producing E. coli strains.

Variation in resistance rates between the four study centers in Finland were remarkable, especially the high rate of fluoroquinolone resistant and 3CEF resistant strains in the Helsinki study center. This finding may also relate to presumably different traveling habits and more international population in the capital of Finland.

Surprisingly, our results indicate that the use of antibiotics during the preceding year reduce the risk for having a fluoroquinolone resistant E. coli strain. Although Kamei et al. and Duplessis et al. found no difference in fluoroquinolone resistance between study groups with prior usage of fluoroquinolone or other antibiotic therapy during the preceding three months and one year, there are several reports arguing otherwise (Duplessis et al., 2012; Kamei et al., 2017). Taylor et al. presented an increased risk for fluoroquinolone resistant coliform in patients with ciprofloxacin usage during the last three months (Taylor et al., 2013). In addition, Steensels et al.

found an increased risk for having a fluoroquinolone resistant E. coli strain in patients with fluoroquinolone usage within six months prior TRUS-Bx (Steensels et al., 2012). Tsu et al. presented that antimicrobial usage during the preceding five years is a significant risk factor for colonization of fluoroquinolone resistant or ESBL-producing organisms (Tsu et al., 2015). In our study, the data regarding the use of antibiotics during the preceding year was collected with a questionnaire. Most of the patients did not recall the specific name of the antibiotic used. As the study consent did not allow us to review study patients' antimicrobial prescriptions, the data on antimicrobial usage was based on the patients' recollections of their previous antimicrobial courses. Therefore, the previous use of antibiotics was analyzed without dividing the antibiotics into different classes. Since patients who have had previous biopsies during the last 6 months and/or had an acute prostatitis were excluded from the study, fluoroquinolone exposure in other common indications (e.g. normal respiratory tract or soft tissue infections) among the study population can be assumed to be relatively low. Thus, the use of antibiotics in general, without dividing the antibiotics to different classes, is not a very informative approach for evaluating the risk of fluoroquinolone resistance in particular. However, the reduced risk for fluoroquinolone resistance is not explainable by this study.

In this study, smoking history, age or diabetes had no significant impact on the risk of having fluoroquinolone resistant or 3CEF resistant strains. Although there are previous studies addressing this issue, the results are controversial. In rectal swab samples prior TRUS-Bx, Taylor et al. were unable to detect differences in fluoroquinolone resistance rates with age or diabetes (Taylor et al., 2013). Tsu et al. detected a statistically significant increase in E. coli antimicrobial resistance with diabetics but no correlation with age (Tsu et al., 2015). Duplessis et al. detected an increase in the fluoroquinolone resistance rate with age (Duplessis et al., 2012). These studies did not analyze smoking as a risk or preventive factor for antimicrobial resistance.

Our study has several limitations. Firstly, our only source of data on the risk factors included patient questionnaire forms is thus susceptible for recall bias. In addition, we analyzed recent antibiotic usage as a whole, without dividing it into different categories. Secondly, our study concentrates on the most common pathogen, E. coli, although it is not the only microorganism causing infectious complications following TRUS-Bx. Thirdly, there are territorial differences in antibiotic susceptibility rates and population stability. Also practices of antibiotic usage vary among countries. This study was conducted in Finnish centers which affects the generalization of the results. Finally, limitations in the inclusion/exclusion criteria may also affect the generalization of the results.

6.1.4 Study III

The object of the study is to improve patient selection to prostate biopsies, aiming to reduce unnecessary biopsies in cases of equivocal PCa suspicion in MRI and thus reduce biopsy complications and insPCa diagnoses. Boesen et al. retrospectively investigated an optimal biopsy strategy combining the bpMRI score and PSA density (Boesen et al., 2019). The current study is an external validation study for the results published by Boesen et al. using our retrospective, multicenter data (Boesen et al., 2019).

We aimed to evaluate the NPV of IMPROD bpMRI protocol combined with PSA density in men with clinical suspicion of PCa. The negative predictive value for ruling out CSPCa in suspicion levels of IMPROD bpMRI Likert 3–5 and IMPROD bpMRI Likert 4–5 were 93% and 92%, respectively, while the corresponding PPVs were 57% and 72% respectively. The optimal biopsy strategy by DCA was to restrict biopsies to men with IMPROD bpMRI Likert 4–5 lesions or IMPROD bpMRI Likert 3 lesions with PSA density ≥ 0.20 ng/ml/ml. This combination demonstrated an NPV of 93% and PPV of 67% while a total of 177 men (35%) would have avoided biopsies. Although there were minor differences with the threshold values for an optimal biopsy strategy between the current and the original Boesen et al. study, we can conclude that the results are in agreement: combining bpMRI with PSA density leads to improved PCa risk stratification compared with the use of bpMRI alone (Boesen et al., 2019). However, the additional value of PSA density was marginal.

In recent studies, PSA density seems to be a better predictor for CSPCa than PSA alone, especially in higher PSA values and re-biopsy setting (Jue et al., 2017; Nordström et al., 2017). However, the additional value of PSA density to IMPROD bpMRI Likert score was marginal in the current study, potentially due to relatively high NPV of our IMPROD bpMRI protocol compared to PSA density. As a PSA derivative, PSA density bears the same clinical issues that PSA itself carries, e.g. no cut-off value in excluding CSPCa (Thompson et al., 2004). However, high PSA density values should still be taken into account in the prostate biopsy decision in spite of MRI findings.

An optimal biopsy strategy in the Boesen et al. study was to restrict biopsies to men with a bpMRI score of ≥ 4 or PSA density ≥ 0.15 ng/ml/ml (Boesen et al., 2019). This strategy demonstrated NPV of 95% and PPV of 56% for CSPCa. Using the same strategy in our study setting, it gives NPV of 92% and PPV of 58% for detecting and ruling out CSPCa. The results are practically equal.

The optimal biopsy strategy in the current study (IMPROD bpMRI Likert score ≥ 4 or IMPROD bpMRI Likert score of 3 with PSA density ≥ 0.20 ng/ml/ml) demonstrated NPV and PPV of 91% and 61%, respectively in the study by Boesen et al. (Boesen et al., 2019). These results are similar to those of our study (NPV 93% and

PPV 67%). Unfortunately, a DCA with the above biopsy strategy was not presented in the aforementioned study (Boesen et al., 2019).

Some differences in the study cohorts should be noted. Although the PSA median was practically equal between the studies, there were differences in prostate volumes resulting in lower PSA density (0.12 ng/ml/ml) in the Boesen et al. study when compared to ours (0.18 ng/ml/ml) (Boesen et al., 2019). Higher median PSA density resulted in higher prevalence of PCa, which is clearly visible in **Table 16**. In addition, sensitivity of systematic biopsies increases in smaller prostates, but the effect of prostate volume to MRI sensitivity is difficult to estimate. As expected, a PCa detection rate was higher in the current study compared to the one by Boesen et al., 63% vs. 57%, respectively (Boesen et al., 2019). The difference in the rates of CSPCas was 35% in the aforementioned study but 46% in the current study (Boesen et al., 2019). The differences in lower PCa detection rate and also the higher median prostate volume in the Boesen et al. material may originate from differences in PCa screening in the populations, or potentially because of more men with benign prostate hyperplasia in the cohort of Boesen et al. (Boesen et al., 2019). This might also explain the higher rate of bpMRI 1–2 scores in the aforementioned study (Boesen et al., 2019). In addition, the difference in PCa detection rate limits the comparability of the results of the current and the validated study: when the cancer prevalence decreases, the NPV of MRI increases, which inevitably affects the results (Moldovan et al., 2017).

The current study has several limitations: This study is retrospectively designed from prospectively collected material; however, the setting was similar in the validated study. We used the IMPROD bpMRI Likert scoring system which is not externally validated, while Boesen et al. used (modified) PI-RADS version 2 scoring system (Boesen et al., 2019). Unfortunately, a distribution of bpMRI scores was not reported in the validated study, which complicates the comparison. Additionally, the study patient characteristics and PCa prevalence differ significantly between the current and the validated study. In the studies, TRUS-Bx was used as a reference standard, which provides only limited information about the whole prostate gland pathology; thus the true prevalence of CSPCa remains unclear. This should be taken into account when using it as a reference to interpret NPV of MRI. Finally, in the current study, cognitive biopsy targeting was used in the majority of the centers, and this could lead to lower cancer detection rate in small lesions, while in the validated study, MRI-TRUS fusion was used (Boesen et al., 2019). However, in prior studies, no significant difference between various methods of MRI targeted biopsy for CSPCa detection were found (Monda et al., 2018; Wegelin et al., 2019).

6.1.5 Study IV

In the study, we compared, in a non-randomized setting, a prospectively collected patient group having a prebiopsy IMPROD bpMRI with target biopsies used in

initial PCa diagnostics (MRI group) to a retrospectively collected control cohort in whom initial PCa diagnostics were from an era, when there was no prostate MRI in clinical use in our center (non-MRI group). All the patients had biopsies due to suspicion of PCa and were prostate biopsy naïve. The study cohorts varied significantly in mean age, mean PSA level and follow-up time. Taking this into account, we presented a quite clear and significant ($p<0.05$) difference in the rate of CSPCa in initial biopsies between the study groups: 48% in the MRI group and 34% in the non-MRI group. In addition, significantly more patients got a CSPCa diagnosed during the follow-up and an upgrading PCa histology in a prostatectomy specimen in the non-MRI group. Because of the variability between the study group's baseline characteristics and follow-up time, in addition to non-randomized study setting and retrospective collection of the control cohort, this study should be considered more as a descriptive study and therefore far-reaching conclusions should not be made.

The results of our study were as expected in the light of prior studies. In the MRI group, there were more CSPCas diagnosed in the initial biopsies. However, due to the study design, where a combination of standard and targeted biopsies in the MRI group was performed, there was no difference in the rate of insPCa between the study groups. In an analysis of the follow-up period, extra care should be taken when making conclusions due to the significantly longer follow-up and the higher mean age of the men in the non-MRI group. However, there was a trend, in which more CSPCas were diagnosed with the initial biopsies in the MRI group, and more with re-biopsies in the non-MRI group. Also, highly aggressive (GGG $\geq$ 3) PCas were missed substantially more often with the initial biopsies in the non-MRI group.

Many patients from the non-MRI group had an MRI performed during the follow-up and in that subgroup, there were substantially more CSPCa, and also aggressive PCas than in men in the group with no MRI performed during the follow-up. The selection bias might have affected the result – a remaining clinical suspicion of a non-diagnosed aggressive PCa drives more easily to novel diagnostic methods.

In general, high quality prospective, randomized studies had presented convincing results of prebiopsy MRIs' high sensitivity to CSPCa in biopsy naïve patients as presented in chapter 2.3.2: MRI in prostate cancer diagnostics. These results are not only presenting the superiority of MRI to detect CSPCa, but also a poor sensitivity of the traditional diagnostic protocol. Even if the study settings in the studies reported in chapter 2.3.2 vary from our study, the results are in line with ours.

Upgrading histologies and capsule invasive PCas in prostatectomy specimens were substantially and significantly more common in the non-MRI group. Again, differences between the study groups should be taken into account: prostatectomies were done more in the MRI group which might be explainable by the younger mean age in the MRI group. However, the higher rate of men with PCa histology upgraded in prostatectomy, and also the lower rate of prostatectomies done in the non-MRI

group, could have been influenced by a 'too benign' biopsy result, taking into account the lower rate of CSPCas diagnosed in the non-MRI group. Similar results are seen in other studies: Xu et al. retrospectively investigated prostatectomy specimens between patient groups having standard TRUS-Bx or transperineal multiparametric MRI targeted biopsies (Xu et al., 2018). In the study, upgrading histology in prostatectomy was in 26.9% of men in a prebiopsy MRI group and in 73.1% of men in a standard biopsy group (Xu et al., 2018). Also Borkowetz et al. compared prostatectomy specimens in patient groups having standard TRUS guided biopsies with and without additional multiparametric MRI guided transperineal target biopsies (Borkowetz et al., 2016). In the study, a combination biopsy group and a systematic biopsy group got upgrading histology in prostatectomy in 18% and 44% of cases, respectively (Borkowetz et al., 2016).

As a conclusion, the results of the current study fortify the view that MRI with target biopsies gives a great additional value to systematic biopsies in the initial diagnostics of CSPCa, comparing it to the traditional initial diagnostic protocol which includes only systematic biopsies. It presented superiority with almost all the measured variables. The diagnostic delay to correct the histological diagnosis is important due to a significant role of correct histology in treatment planning: a delayed diagnosis gives time for progression to undiagnosed CSPCa and suspicion of CSPCa could drive to unnecessary treatments for insPCa. More importantly, a prevailing suspicion of CSPCa after negative biopsies is not only unpleasant for the patient, but it is also a burden to the healthcare system. Nevertheless, the combination gives no solution to another diagnostic issue; diagnosing insPCas, as seen also in the current study: no difference was seen in the rates of diagnosed GGG 1 PCas between the study groups. The solution to the issue could be in taking only lesion-targeted biopsies which have been investigated with promising results in the PRECISION trial described earlier (Kasivisvanathan et al., 2018). However, we are not aware about the true prevalence of CSPCa in the MRI-targeted biopsy group, so we are looking forward to the follow-up data of the PRECISION trial. Meanwhile, additional parameters are needed to estimate the risk for CSPCa when insPCa is diagnosed with or without an MRI susceptible lesion present.

6.1.6 Study V

In this pooled data analysis of 673 patients from four consecutive, prospective and registered studies, we investigated the feasibility of bpMRI with an IMPROD bpMRI protocol and IMPROD bpMRI Likert scoring system in the detection of CSPCa in patients with suspicion of PCa without a prior PCa diagnosis. We demonstrated an AUC of 0.863 to the IMPROD bpMRI Likert scoring system in diagnostics of CSPCa in the study biopsies. The overall NPVs for ruling out CSPCa in the IMPROD bpMRI Likert score groups 1–2 and 1–3 were 0.932 and 0.914, respectively. In the subgroup

analysis, no clear outlier cohorts with a higher NPV were present, which can be explained by the high NPV of IMPROD bpMRI for ruling out CSPCa in the whole study population. However, IMPROD bpMRI seems not to be as accurate in ruling out CSPCa in a subgroup of patients with PSA density ≥0.25 ng/ml/ml. The low NPV in this subgroup could be explained by a major difference in the prevalence of CSPCa comparing it to a subgroup with PSA density <0.25 ng/ml/ml (69% vs. 38%), which affects the NPV.

The NPV of prostate MRI is discussed in detail in chapter 2.3.2: MRI in prostate cancer diagnostics. Because of the differences between the study settings, only very limited conclusions in comparison to the other studies can be made. However, comparing the outcomes of our study with the prior prostate prebiopsy MRI studies with the mpMRI protocol, our IMPROD bpMRI has favorable results in the exclusion of CSPCa. Moreover, there is some evidence that an additional value given by contrast medium for sensitivity of MRI in diagnostics of CSPCa could be minimal (see chapter 2.3.3: Biparametric MRI protocol). However, the benefits of our (bpMRI) protocol were the quickness of the MRI scan due to the lack of contrast medium and usage of transrectal biopsy approach which is more convenient for outpatient use.

To our knowledge, only a few studies with systematical analysis of additional parameters aiming to increase feasibility of prostate prebiopsy MRI have been conducted (see chapter 2.3.4: Diagnostic performance of MRI combined with additional parameters). More importantly, biopsies in these studies were mostly performed via the transperineal approach and with an mpMRI. Again, because of the great variation in the study settings and definitions, as well as the lack of prospective and systematic studies on bpMRI with additional parameters, direct comparison to our study outcomes cannot be done.

A key finding in our study is that, for patients with suspected PCa, IMPROD bpMRI Likert score 1–2 finding in the IMPROD bpMRI ruled out the CSPCa in the study biopsies with almost 93% certainty. In addition, no highly aggressive (Gleason score >7) PCas were missed. Therefore, using prebiopsy IMPROD bpMRI, 20% of prostate biopsies in our study material could have been avoided.

Our study has several limitations. The most important limitation is that prostate biopsies gives only limited information on the prostate gland as a whole and that should be taken into account when using the biopsies as a reference to interpret NPV of MRI. However, it should be noted that transperineal mapping biopsies is not a feasible method in normal clinical practice. Also, it should be stated that we used mainly the cognitive MRI fusion method to target suspicious lesions. Moreover, inter-observer variability in performing an MRI targeted biopsy and reporting IMPROD bpMRI have not yet been evaluated. In addition, the transrectal approach may not be optimal when taking biopsies from the anterior or apical lesions.

6.2 Implications and future perspectives

Prostate cancer is the only solid organ cancer where diagnosis is not based on imaging or visual inspection (e.g. endoscopy) but systematic biopsies of the part of the organ where a cancer commonly – but definitely not always – is located. Even worse outcomes in accuracy are in PSA and DRE, the clinical parameters that usually rise a suspicion of PCa. Introduction of PSA dramatically increased the number of PCa diagnoses at the beginning of the 1990s, however, a corresponding decrease is not seen in PCa mortality. Discrepancy between the trends is mainly explained by an indolent nature of many PCas and also the advancing age of the male population, which increases the PCa prevalence as age is the most significant risk factor for PCa. Thus, in many cases, PCa could be considered as a chronic disease of ageing men without a need of any treatments. However, it is a major fallacy to consider PCa as one disease. It is more like a continuum of diseases from indolent neoplasms to highly aggressive cancers, while PCa is still a cancer causing second most commonly men's cancer-related deaths in Finland. Thus, in PCa diagnostics, histology is an even more substantial factor due to its significant influence on prognosis, comparing it to other common cancers.

The recent implementation of MRI into PCa diagnostics has made a shift towards the lesion-based approach; however, systematic biopsies are still a cornerstone in diagnostics and taking only lesion targeted biopsies in an initial biopsy session is not recommended by the guidelines. It is very likely that PCa diagnostics will transform to solely imaging-based diagnostics, biopsing and maybe also (focal) treatments. From today's aspect, MRI seems to be the imaging method for future diagnostics and treatment planning. However, the availability of MRI seems to be a key issue in future. Contrary to mpMRI, which is the gold standard in prostate imaging, bpMRI is faster to perform and interpret which could provide some improvement to the availability issue.

For now, as previous high-quality prospective studies have been presented, MRI targeted biopsies seem not to be sensitive enough in ruling out CSPCa – additional systematic biopsies are still needed with MRI targeted biopsies. In addition, the issue is not only in the NPV of MRI for CSPCa which was presented to be very good, but also in the accuracy in targeting biopsies. In addition, multifocality of PCa should be taken into account: the targeted (largest) lesion or lesions not always contain the most malignant histology in the gland. Additionally, a significant proportion of MRI suspicious lesions includes only insPCa histology in targeted biopsies. Is that due to a targeting error or when the clinician can rely on that the nature of the MRI lesion is established by the targeted biopsies?

Another issue is the MRI quality and variation in radiologic experience, which could vary from prospective studies performed mainly in university hospitals with experienced radiologists, high quality MRI-scanners and strictly defined MRI

protocols. This is taken into account when developing the open-access IMPROD bpMRI protocol with the IMPROD bpMRI Likert scoring system. However, further development and standardization of MRI sequences and targeting methods could change the current status.

In the current revolution of PCa diagnostics, a critical question is, what level of uncertainty is adequate to sustain in PCa diagnostics? We can compare NPVs of diagnostic protocols in e.g. breast, colorectal or lung cancer, but then, in addition to falling to ”Hume’s guillotine”, we do not take into account the very different nature of the mentioned cancers comparing to PCa and, above all, differences among various patient groups in suspicion of CSPCa. This question will lead us to the origin of DCA, the biostatistical method utilized in diagnostic studies, where we choose a certain grade of uncertainty we are able to sustain before choosing a valid diagnostics test. It is an approach that should be used more in studies concerning diagnostic tests of PCa to perform a unique diagnostic strategy for different age and risk groups. For example, there is a substantial difference of Gleason 3+4 graded PCa diagnosis in a 45- and 75-year-old man. In a 45-year-old man, even Gleason 3+3 graded PCa diagnosed with prostate biopsies is maybe something not to be totally ignored, due to a risk of sampling errors and a potential tumor progression in the course of time. However, in a 75-year-old man a Gleason 3+4 cancer is hardly lethal, and in case of multiple comorbidities, the range of treatments is much more limited.

Although the complication rates of prostate biopsies have been reported to be increasing, according to our results, biopsies seem to be quite a safe procedure and prostate biopsies should not usually be abandoned due to a risk of complications. Antibiotic prophylaxis seems to be efficient, however recent restrictions from the European Commission about fluoroquinolone usage in prophylaxis TRUS-Bx require a reassessment of the current fluoroquinolone-based prophylaxis protocol. So far, fosfomycin seems to be the most promising alternative to fluoroquinolones. Additionally, a rectal swab culture targeted prophylaxis is also a valid alternative. More high-quality prospective studies are needed to find an efficient substitute to fluoroquinolones. However, antibiotic prophylaxis should still be modified according to individual risk factors of a patient, especially the traveling history. More generally, these are the fundamentals that should also be taken into account in all modern emergency medicine, when treating (urinary tract) infections with an empiric antibiotic regimen.

Prebiopsy prostate MRI should be performed on all men when a suspicion of PCa has emerged, if the diagnosis of a PCa is not obvious e.g. due to a very high PSA level or an obviously cancerous finding in DRE. Moreover, MRI targeted biopsies find more highly aggressive PCa than systematic biopsies, and thus it is simply wrong to omit prebiopsy MRI, if available, in PCa diagnostics. Biparametric MRI protocol is sensitive enough for most cases. Based on the age and risk group, more than one approach guiding the interventions followed by the MRI result should be investigated,

preferably using DCA or a corresponding analyzing method. Firstly, when prevalence rises, NPV of MRI declines, thus men under a high risk or high level of suspicion of CSPCa should be biopsied even if the MRI was negative. Relevant factors taken into account in estimating the risk for CSPCa are a suspicious DRE finding, high PSA level and/or PSA density, family history, race, and known genetic predisposition. Additionally, suspicious results in the novel biomarker test should be taken into account in the risk assessment. Secondly, in younger men, also systematic biopsies should still be taken in addition to targeted biopsies especially to cover targeting errors and cancer multifocality. Finally, in older men and men with multiple comorbidities, it should be assessed, if only lesion-targeted biopsies are enough due to lesion size being a significant prognostic factor, and the probability for targeting error could be lower in larger lesions. In addition, in the mentioned patient group, decision to biopsy a Likert/PI-RADS 3 graded lesion, should be based on some additional parameter than MRI; so far, PSA density seems to be promising. In the future, serum and urine biomarkers might be an interesting approach to be used in combination with the MRI score in patient selection for prostate biopsies. From a point of view of an individual man, the most significant risk for an (unnecessary) biopsy could be a diagnosis of insPCa and its consequences, not a lethal complication.

Finally, we should acknowledge that the value of pre-biopsy MRI extends beyond the initial diagnostics phase. With MRI, the result of biopsies is more reliable, which helps further planning of treatment or follow-up in case of benign biopsies. Also, the prebiopsy-MRI is very valuable when treatment options and treatment execution is planned.

7 Conclusions

Fluoroquinolone resistance and/or 3CEF resistance in intestinal E. coli strains in men undergoing TRUS-Bx in Finland is remarkable. International traveling is appearing an indisputable risk factor for having an intestinal fluoroquinolone resistant E. coli strain.

The risk for having a serious infectious complication after TRUS-Bx is very low, despite the high fluoroquinolone resistance rate. Also, non-infectious complications were uncommon.

IMPROD bpMRI demonstrated a very good NPV in ruling out CSPCa and it gives a great additional value to the initial diagnostics of CSPCa, comparing to systematic biopsies. It demonstrated superiority with almost all the measured variables. IMPROD Likert score 1–2 excludes highly aggressive (Gleason score >7) PCa. No clear outlier was present when NPV of IMPROD bpMRI were analyzed in clinical subgroups. Combining PSA density with IMPROD bpMRI improved NPV mainly in men with an equivocal suspicion in IMPROD bpMRI. However, the additional value of PSA density was marginal.

Acknowledgements

This study was carried out in the Department of Urology in Turku University Hospital and in the University of Turku Doctoral Programme in Clinical Research during 2016–2020.

I hadn't planned a science career when I graduated and I was more clinically oriented in those days. However, I had a delay in my residency at 2016, and I didn't know when it would be continued. I thought that maybe I should in the meantime do some scientific research to see what it is like, and thus become better a clinician when I can read the papers more critically. However, I had no plans on a doctoral thesis in those days. I discussed it with Peter Boström and while he considered, what kind of project he will take me in on: he asked me one substantial question: "Are you a computer nerd or not?", to which I answered: "That's what I definitely am". So, I began to work with the MULTI-IMPROD project and soon Peter began to talk about the topics of my doctoral thesis – that's something that I was not prepared for. However, a science career carried me away while doing it and only thing I regret is that why did I not start it earlier.

Firstly, I want to thank my supervisor Peter for trusting my skills and taking me as a part of this great prostate MRI team. Being a part of this project provided me, as a "nobody" in a field of science, an undeserved advantage in a scientific career which I have tried to fulfil with hard work. This (in retrospect) nicely flowing project also kept me motivated to work in the often stiff and bureaucratic science world – even if Peter reminded me many times that "this is a prospective multi-institutional trial", as I got frustrated with all the delays. Later Kari Syvänen set to be as the second supervisor for my thesis, and I honestly want to thank Kari for the great practical support and above all, being always approachable as I had a thousand questions. Even a small reply to an email was of great importance to me.

Otto Ettala had maybe the most important practical impact on my thesis. Due to coincidentally having a workplace next to mine, he provided low-threshold consultation help with all the practical problems, like academic writing and statistics. I am very grateful for Otto for this help which he gave without any obligation to that. He also became a role model for me in combining the careers of a surgeon and a scientist.

My schedule with this doctoral thesis was very challenging for all, thus, I am very grateful for the reviewers, Professor Risto Vuento and Professor Henrik Thomsen for all the flexibility with my tight schedule. Special thanks to Professor Vuento for constructive reasoning about the microbiological aspects of my thesis.

I want to thank the radiologists, pathologists, IMPROD-protocol developers and study assistants of this great prostate MRI team for providing me a high quality and easily accessible study material. Especially, I want to thank Ivan Jambor. His enthusiasm about science and especially about prostate MRI is admirable and it has already shown great results. Ivan had a major impact on my thesis as he was reachable almost 24/7 and there was not any major issue that he could not solve in a day. Unfortunately, I could not perform similarly, when he asked me to do something. I think that he has to have more hours in a day than others. Also, I want to thank Pekka Taimen for being a nice guy who is easy to approach with pathologic and also other issues, and Janne Verho, especially for hours of phone calls containing valuable PhD students/residents peer support. I want to thank for Marjo Seppänen, Antti Rannikko and Jarno Riikonen, the urologists of external study centers, for being helpful in collecting the study material and being flexible with my requests. Finally, thanks for all urologists and other workmates at our clinic and Satakunta Central Hospital for all the support for my clinical career and in this project.

Microbiologists, especially Antti Hakanen and Marianne Gunell have provided me high-quality material for the microbiological part of my thesis and they also helped me, a novice, when I was publishing my first papers. I am very grateful for that and for them being always easily reachable. Thanks to Venla Autio, for having a pedantic contribution in collecting the control cohort and I am hoping that the project did not kill the spark for a scientific career. Finally, thanks to Ileana Montoya Perez for providing high quality analyses for the validation study and also for giving biostatistical expertise with an always kind and helpful attitude.

Outside of the scientific team, there is few people that I am very grateful for. Firstly, due to my impaired English skills, my sister Suvi gave major support for spellchecking the manuscripts and even writing the most important emails in short notice using the tone I wanted. And all that she did gratuitously in the middle of her hectic career and family life. I am also very grateful for my parents Pirkko and Jukka, who always gave all possible financial and non-financial support to my education from their little allowances.

This doctoral thesis is dedicated to Emilia, which tells about my gratitude to her. I hope that I am able to show her significance in my life every day, and not only in the acknowledgements of this doctoral thesis.

May 2020
Juha Knaapila

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This doctoral thesis was carried out in the Department of Urology in Turku University Hospital and in the University of Turku Doctoral Programme in Clinical Research during 2016–2020.

Juha Knaapila 2020
ISBN 978-951-29-8037-6