UiT The Arctic University of Norway

Faculty of Health Sciences

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Inflammation, hypertension, and microRNA and Prostate Cancer.

The Prostate Cancer throughout life (PROCA-*life)* study

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A dissertation for the degree of Philosophiae Doctor - March 2022

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Acknowledgements

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Abstract

Background/Aims: Prostate cancer (PCa) is the most common cancer among men in Norway as well as world-wide, and a major cause of health loss and death with 1.4 million new cases and 375 000 deaths world-wide in 2020. Biological mechanisms involved in PCa development are mainly unknown, but chronic inflammation, one hallmark of cancer development, has been questioned to play a key role in PCa development. This thesis aimed to explore whether markers that may be linked to inflammation, such as high sensitive-C-reactive protein (hs-CRP) and white blood cell count (WBC), systolic and diastolic blood pressure (BP) and miR-24-1-5p, a subtype of microRNA, may play a role in PCa development, recurrence and mortality.

Materials and methods: The Prostate Cancer Study throughout Life (PROCA-life) is a population-based cohort study, a sub study of the Tromsø Study and the present thesis includes all men, who were enrolled in the Tromsø Study between 1994 and 2016 (Tromsø 4-7). The procedures were almost identical, and assessments were done by trained research technicians. By linkage to the Cancer Registry of Norway and Norwegian Cause of Death Registry, all PCa cases and death among the participating men were identified. Detailed histopathological and medical records were obtained. Cox proportional hazard regression models were used to study the association between prediagnostic WBC and hs-CRP in serum (Paper I), prediagnostic blood pressure (Paper II) and PCa risk and prognosis. We collected prostatectomy tissue from 142 PCa patients, and we studied the influence of miR-24-1-5p regarding aggressiveness and prognosis in men diagnosed with PCa.

Results: We observed a positive dose-response relationship between hs-CRP and PCa risk. Men with an increase in hs-CRP between two measurements had a 36% increased risk of PCa, compared to men with no change or decrease in hs-CRP. Men with a high systemic inflammatory score (combination of WBC and hs-CRP) had a 68% higher risk of being diagnosed with metastatic disease compared to men with lower scores. Men (> 45 years) with a systolic BP $\geq$ 150 mmHg had a 35% increased risk of PCa compared to men with a normal systolic BP (< 130 mmHg). Among PCa cases, men with systolic BP $\geq$ 150 mmHg had a 49% increased overall mortality compared to men with a normal systolic BP. PCa patients with a high miR-24-1-5p expression in the tissue had a doubled risk of recurrence compared to patients with low miR-24-1-5p expression.

Conclusion: Our results suggest that hs-CRP alone or in combination with WBC may be a useful inflammation-related biomarker for PCa risk and prognosis, and systolic and diastolic BP may be important factors when balancing disease management in PCa patients. Moreover, a high expression of miR-24-1-5p is associated with an increased risk of recurrence of PCa after radical prostatectomy. Systemic inflammation might be the common link between these factors, but further research is needed.

Simple summary

Prostate cancer is the most common cancer among men in Norway and world-wide. Our research focuses on how factors linked to inflammation in the body affects prostate cancer development and prognosis.

The Prostate Cancer Study throughout *life* (PROCA-*life*), is a population-based cohort study, a sub study of the Tromsø Study, and includes men who were enrolled in the Tromsø Study (1994 -2016), where factors linked to inflammation were measured. Prostate cancer cases were identified through a linkage to the Cancer Registry of Norway.

Our findings suggest that men with factors associated with systemic inflammation measured in blood samples have increased risk of prostate cancer. We observed that men with high blood pressure had increased risk of prostate cancer, and that blood pressure may influence risk of dying among prostate cancer patients. We also observed that the molecule miR-24-1-5p in prostate tissue can be used to identify men with increased risk of recurrence of prostate cancer. Thus, blood pressure may be important when balancing disease management in prostate cancer patients, while our findings related to inflammatory factors and miR-24-1-5p need to be investigated in more studies.

List of abbreviations

ACC = The American College of Cardiology
ADT = androgen deprivation therapy
AHA = The American Heart Association
AJCC = American Joint Committee on Cancer
ASR = Age-standardized incidence rates
BC-RFS = biochemical-recurrence free survival
BP = blood pressure
CAPRA-S = Cancer of the Prostate Risk Assessment Postsurgical Score
CI = Confidence Interval
CRP = C-reactive protein
CSS = cancer-specific survival
CVD = cardiovascular disease
DRE = digital rectal examination
EAU = European Association of Urology
ECE = extracapsular extension
GDF = Growth Differentiation Factor
GP = General practitioner
Gy = Grey (1 Gy = the absorption of one joule of radiation energy per kilogram of matter)
H&E = Hematoxylin and Eosin staining
HbA1C = hemoglobin A1c
HDL = High-density Lipoprotein cholesterol
HR = Hazard Ratio
hs-CRP = high sensitive-C-reactive protein
IGRT = image-guided radiotherapy
IHC = immunohistochemical
IMRT = Intensity-modulated radiotherapy
ISH = in situ hybridization
ISUP = International Society of Urological Pathology
LNA = Locked Nucleic Acid probe
LNI = lymph node invasion
MetS = Metabolic syndrome
miRNA = micro-RiboNucleic Acid
miRNA = micro–RiboNucleic Acid
MLR = monocyte-to-lymphocyte ratio
mpMRI = multi-parametric magnetic resonance imaging
NE= normal epithelial cells
NLR = neutrophil to lymphocyte ratio
NT-proBNP = N-terminal pro-brain natriuretic peptide
OS = overall survival
PCa = prostate cancer
PFS = progression-free survival
PLR = platelet to lymphocyte ratio
PROCA-life = Prostate Cancer throughout life study
PSA = prostate specific antigen
PSMA = Prostate-specific membrane antigen
PET = positron-emission tomography
RAAS = renin-angiotensin-aldosterone system
RALP = robot-assisted laparoscopic prostatectomy
RCT = randomized controlled trials
REDUCE = Reduction by Dutasteride of Prostate Cancer Events study
RISC = RNA-induced silencing complex
RRP = radical retropubic prostatectomy
RT = Radiation therapy
SD = Standard deviation
SM = surgical margin
SNP = Single Nucleotide Polymorphism
SVI = seminal vesicle invasion
TE = tumor epithelial cells
TMA = Tissue microarrays
TNM = The TNM Classification of Malignant Tumors (Tumor, lymph Nodes, Metastasis)
TRUS = transrectal ultrasound
UICC = International Union Against Cancer
UNN = University Hospital of Northern Norway
WBC = White blood cell count
WHO = World Health Organization

List of papers

Paper I.

Stikbakke E, Richardsen E, Knutsen T, Wilsgaard T, Giovannucci EL, McTiernan A, Eggen AE, Haugnes HS, Thune I. *Inflammatory serum markers and risk and severity of prostate cancer: The PROCA-life study*. International journal of cancer. 2020;147(1):84-92

Paper II.

Stikbakke E, Schirmer H, Knutsen T, Støyten M, Wilsgaard T, Giovannucci EL, McTiernan A, Eggen AE, Haugnes HS, Richardsen E, Thune I. *Systolic and diastolic blood pressure, prostate cancer risk, treatment and survival. The PROCA-life Study*. Cancer Medicine doi:10.1002/ijc.32718

Paper III.

Stikbakke E, Wilsgaard T, Haugnes HS, Pedersen MI, Knutsen T, Støyten M, Giovannucci EL, Eggen AE, Thune I, Richardsen E.
Expression of microRNA miR-24-1-5p in tumor tissue influence prostate cancer recurrence. The PROCA-life Study. (*Cancer*s, *under review*)

1 INTRODUCTION

As a clinician working in the Oncology Department, I experienced a large variation in disease and response to treatment among prostate cancer patients. Patients all have their own unique and personal story, but many ask themselves the same question: *Why did I get prostate cancer*? And most often we have to answer: *We don't know.*

Prostate cancer (PCa) is today the most common cancer in Norway, and the second most common cause of cancer death. Yet still the knowledge about the causes of PCa is sparse. Moreover, prostate cancer is a heterogeneous disease. It can be a low-risk, indolent tumor localized to the prostate or a high-risk, aggressive tumor that may metastasize and be lethal if not treated. Although current advances in treatment (surgery, radiotherapy and medical treatment) have improved dramatically over the last decades, the total personal and economic burden of PCa on society is large. Several strategies to reduce this burden can be used: better understanding of the biological mechanisms involved in PCa development can potentially lead to preventive measures in the general population as well as in follow-up of the patients being diagnosed and followed for many years. In that way, we may reduce or delay the development of PCa in susceptible individuals. Moreover, advances in diagnostic and therapeutic methods may lead to earlier and more precise treatment of PCa patients. Of note here is the need for better tools to assess risk levels in the individual patient, and in that way avoid both under-treatment and over-treatment. There is a great need for better knowledge in order to answer the patient's question: *Why did I get prostate cancer*?

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1.1 The prostate and prostate cancer development

The main biological role of the prostate gland (size 30-35 gram) is to produce, temporary store and secrete prostatic fluid during ejaculation. The alkalinity of the prostate ejaculate neutralizes the acidity of the vaginal tract, and by this prolonges the lifespan of the sperm cells. The proximal part of the prostate is referred to as the base, and the distal part as the apex. Close to the prostate gland runs the dorsal vein complex and neurovascular bundle, both necessary for erectile function, and thus critical structures during prostate surgery (Figure 1).

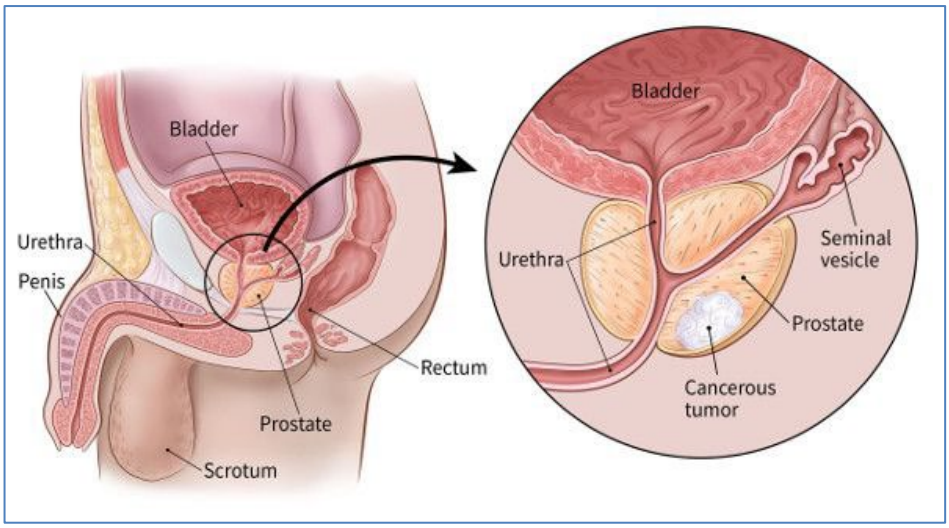

Figure 1 Illustration of the prostate location and anatomy. The prostate can be palpated in a digital rectal exam. Reprinted with permission from www.cancer.gov

The prostate gland is divided into four general zones: Peripheral zone, Central zone, Transition zone, and the Anterior Fibromuscular stroma. These zones differ in their histological composition and are sites of predilection for specific prostatic diseases. The peripheral zone account for 70% of the gland, and this is the most common origin of PCa (1) (Figure 2).

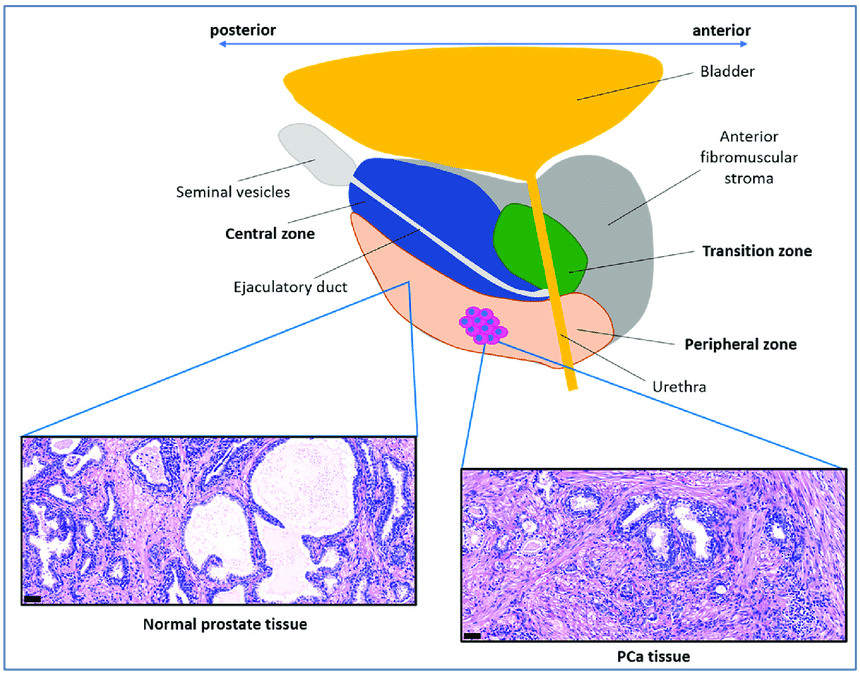

Figure 2 Prostate anatomy. Representation of the prostate anatomy oriented in the anterior-posterior body axis, with the prostatic zones highlighted in different colors. Representative histology (Hematoxylin and Eosin (H&E) staining). Normal prostate tissue (left): Epithelial cells form acinar structures surrounded by a fibromuscular stroma. PCa tissue (right): Disruption of the epithelial organization and high stroma abundance. Scale bars: 50 µm. Reprinted with permission from Bonollo (2) Creative Commons Attribution 4.0 International Public License

The prostate is made up of branched tubular-acinar glands (30 – 50) forming a convoluted pattern which is surrounded by stroma. These glands drain directly into the urethra through several ducts. The architecture of the glands is simpler in the transition zone and peripheral zone compared to the central zone, which contains large, irregular acini (1). Each acinus is organized as a lumen, surrounded by a simple columnar epithelium. The epithelium is lined by a layer of basal cells, and a small number of neuroendocrine cells resting on the basal lamina separating the acini from the surrounding stromal tissue. The surrounding stromal tissue is composed of fibroblasts, smooth muscle cells, endothelial cells, autonomic nerve cells, immune cells and extracellular matrix (3).

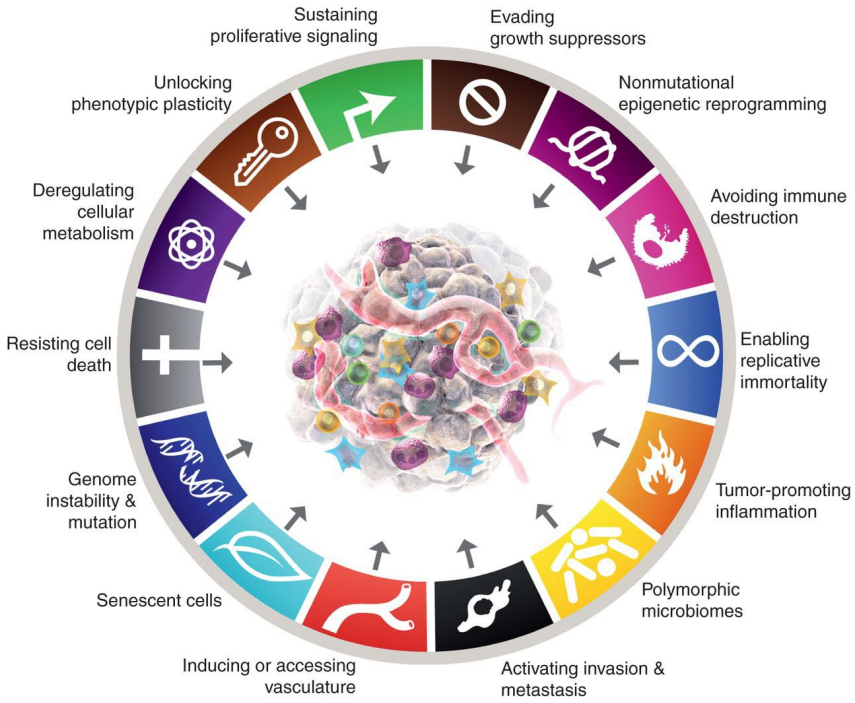

Figure 3 Hallmarks of Cancer, new additions, from Hanahan, 2022 (4). Reprint with permission from American Association for Cancer Research, Copyright © 2022

The «Hallmarks of Cancer» described for the first time by Hanahan and Weinberg in 2000, has later been updated and modified in different ways by themselves and others (4-6). These hallmarks have become a reference in our modern understanding of cancer development in general (Figure 3). The development from a normal prostate to PCa is a process with multiple steps (7). The classic hallmarks, in combination with defects in the androgen receptor signaling system, are central driving forces in this process (8). The growth and maintenance of both normal and cancerous prostatic cells is stimulated by androgens, mainly testosterone and 5α-dihydrotestosterone (9;10).

1.1.1 Prostate cancer incidence, prevalence and survival

PCa is a major cause of health loss and death, with 1.4 million new cases and 375 000 deaths world-wide in 2020. The age-standardized incidence rates for PCa are much higher in countries with a high developmental index (11). The incidence of PCa diagnosis varies widely between different geographical areas, highest in Northern Europe, closely followed by Western Europe, Caribbean and Australia/New Zealand, while the incidence is low in Eastern and South-Central Asia and Northern Africa (12). This variations in incidence rates may in part be explained by

differences in environmental factors, access to diagnostics and screening as well as variations in the aging of the population. Interestingly, migration studies have shown that when men move from low-incidence- to high-incidence areas, their risk of PCa increase considerably (13;14).

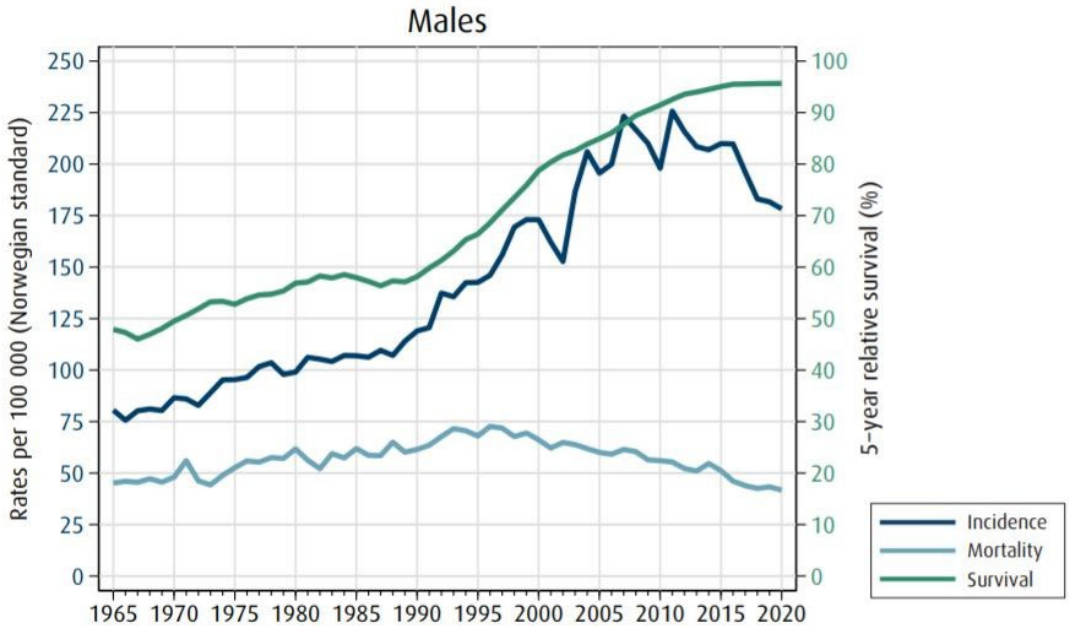

Figure 4 PCa incidence, mortality and survival rates (national standard) in Norway. Reprint from Cancer in Norway 2020, Cancer Registry of Norway, ©

The Nordic countries are among the countries in the world with the highest incidence rates of PCa, with world age-standardized incidence rates per 100 000 persons in 2020 of 95.6 in Norway, 100.4 in Sweden, and 75.6 in Denmark (15). In contrast, the world average of the age-standardized incidence rates of PCa was 30.7 per 100 000 in year 2020 (15). In Norway, PCa was the most common cancer in men in 2020, with 5 030 new PCa cases. The incidence of PCa has increased sharply from the first half of the 90s until mid-2000 (figure 4) (16). In 1980-84 the Norwegian age-standardized incidence rates of PCa was 104.4, in 2000-04 it was 176.4, and in 2016-20 it was 189.4 (per 100 000 person years).

One main cause for this rise in PCa incidence in Norway, as well as in many other countries is the aging of the population due to increased life expectancy. Almost half the cases occur in men above 74 years (17). Secondly, the increased PCa incidence may be due to the widespread use of prostate specific antigen (PSA)-test for the detection of asymptomatic PCa. Thirdly, PCa is, as many other types of cancer, a hormone and lifestyle associated disease, and the increase in

PCa incidence may be related to lifestyle and epi-genetic factors promoting PCa development. Some lifestyle and epi-genetic factors are under debate, while others are still unknown.

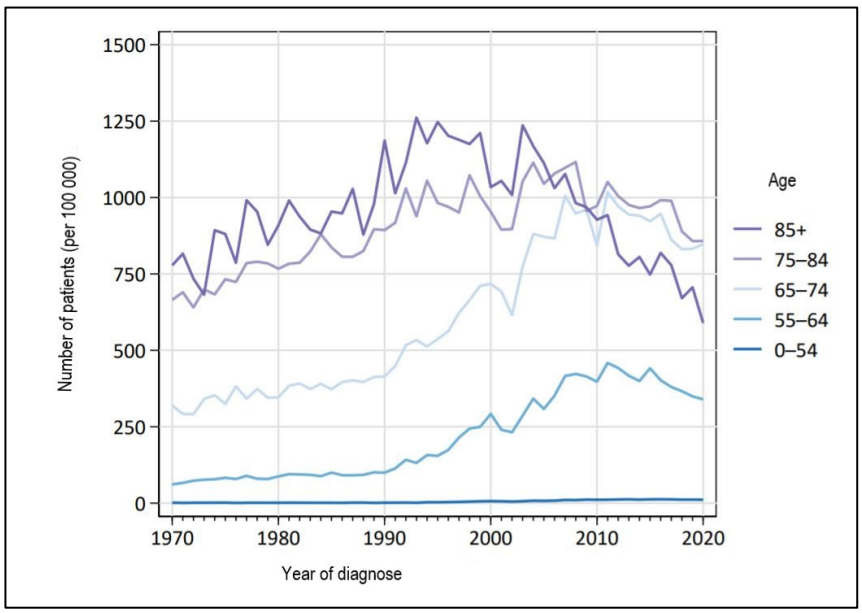

Figure 5 : Incidence rate for PCa in Norway (per 100 000 person years) by age group (1970–2020). Reprint from Annual report from Norwegian PCa Registry, Cancer Registry of Norway, 2021 (17)

PCa has traditionally been regarded as a disease of the elderly. Up until ~1990 there was a steady increase in PCa incidence in all age groups, except the youngest (age 0 - 54)(17). With the introduction of PSA-testing in Norway around 1990, and a more active effort in diagnosing the disease, there was a more marked increase in overall incidence, but also a shift towards lower age at diagnose (Figure 4 and 5). This catch-up effect might be the explanation for the subsequent reduction in incidence in the highest age-groups from around 2005 (age 75-84 and age 85+ (Figure 5). In 2020 the median age of diagnosis in Norway was 70 years. Of all cases, 52% had localized stage, 32 % had regional stage, 9% had metastatic disease, and 7% had unknown stage (17). The variation in incidence rate of PCa between the different counties of Norway have been rather small. For example in 2016-2020 it was 188.5 in Northern Norway (Troms and Finnmark County) vs the national mean of 189.4 (Norwegian standard age-standardized incidence rates per 100 000 person-years) (16).

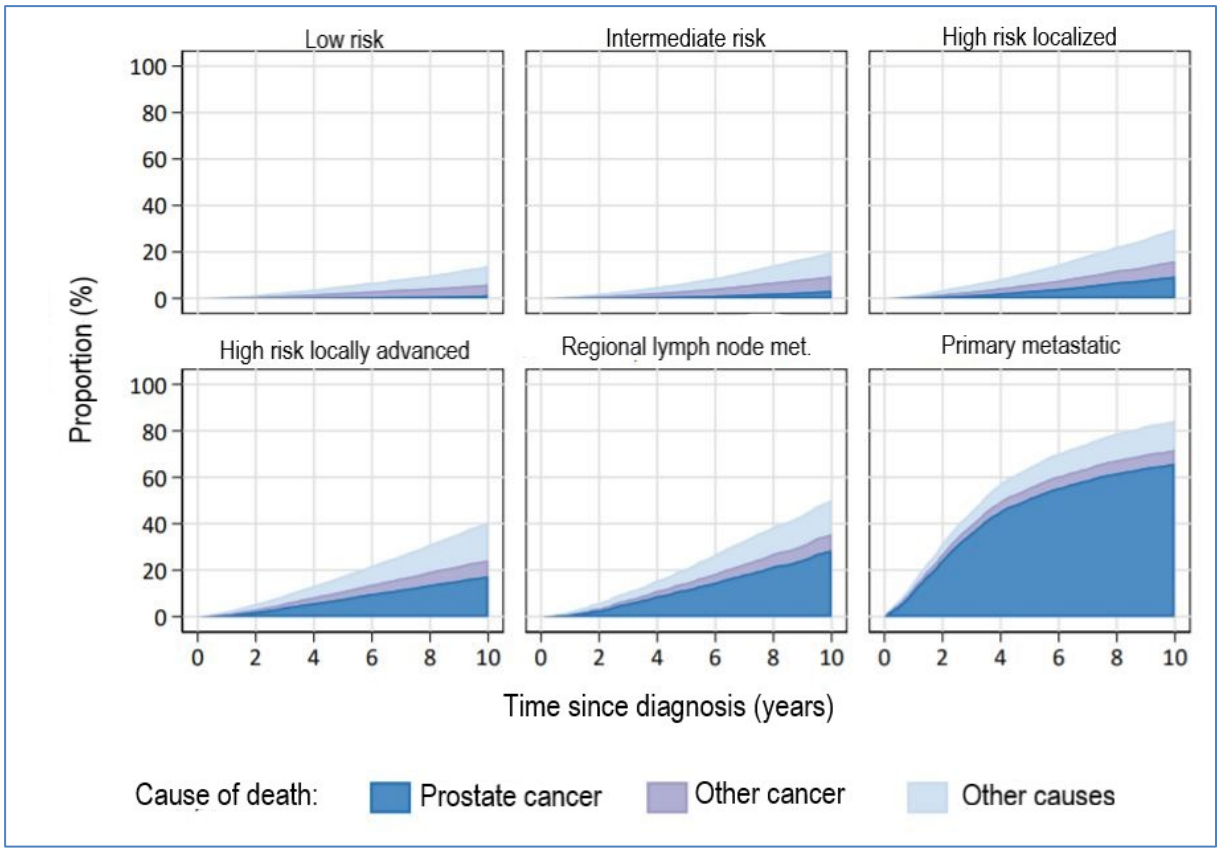

Figure 6 Mortality and cause of death by risk groups and metastatic disease, 0-10 years after diagnosis. Reprint from Annual report from Norwegian PCa Registry, Cancer Registry of Norway 2021

The survival of PCa in Norway, as in many other countries, has improved in the recent decades. In 1986-90 the 5-year relative survival of PCa was 58.1%, in 2001-2005 84.9%, and in 2016-20 95.7% (in Norway, all stages combined) (16). However, the variation in recurrence rates and mortality between subtypes of PCa is significant, pointing to that PCa is a heterogeneous disease. PCa-specific 10-year mortality was <5% among patients who received curative treatment, regardless of what sort of treatment (surgery, radiotherapy or active surveillance)(17). The mortality of PCa is highly related to the stage at diagnosis, where those with low-risk disease have a < 5% 10-year prostate-cancer specific mortality rate, compared to ~65% 10-year mortality in patients with metastatic PCa at the time of diagnosis (primary metastatic) (Figure 6).

1.1.2 Main risk factors

Family history, ethnicity and age are the most widely accepted risk factors, but a long list of other possible risk factors have been studied (18). The process where the benign prostate cells change into the malign state of PCa is most likely complex and multifactorial (19). Cancer risk factors have been identified based on a large variation in study design and study participants and include animal models, epidemiologic studies, clinical trials, and basic investigations at the biologic and the molecular biologic levels (20). A better understanding of the risk factors for PCa are of great interest for both primary and secondary prevention of PCa. Nevertheless, the risk factors associated with PCa to date lack evidence for causality, and it is thus not possible so far to suggest effective preventative strategies. Consequently, we do not know enough about the development of PCa to provide any preventive measures to the general population (21;22).

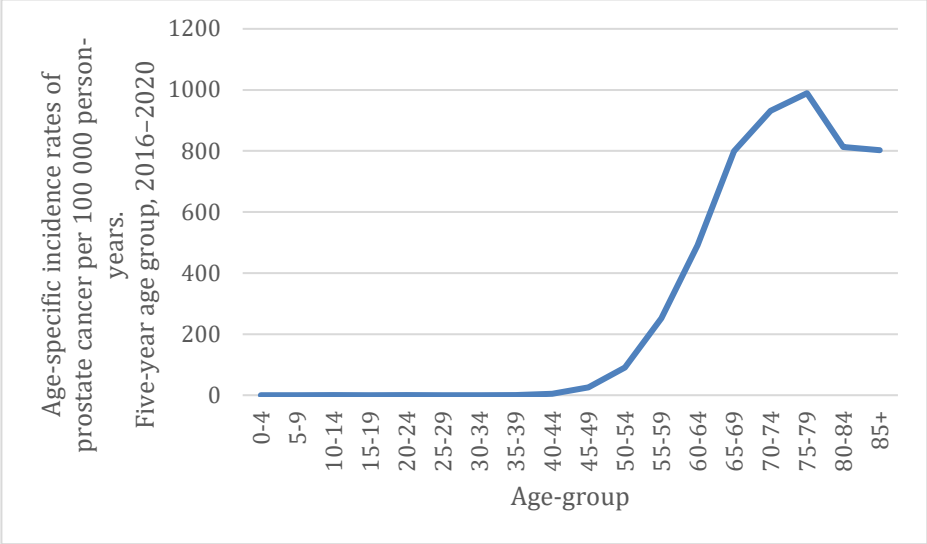

Figure 7 PCa incidence rates (per 100 000 person years) in different age-groups in Norway. Graph made with data from Cancer Registry of Norway, Cancer in Norway 2020©

1.1.2.1 Age

Age is still the most well-known risk factor for PCa. PCa diagnosed in men younger than 45 years is extremely rare, while incidence rates are rising rapidly up to age 75, before declining slightly for the oldest (Figure 7). Of note, men with PCa often die from other causes than PCa, and for some before any symptoms are clinically manifest. Incidence rates can be affected by screening programs or change in diagnostic tools, so autopsy studies have been used to quantify the reservoir of undiagnosed PCa. In a review of autopsy studies, Bell *et al* found that the estimated mean cancer prevalence at age <30 years was 5% (95% CI: 3–8%); increasing in a nonlinear fashion to 59% (95% CI: 48–71%) by age >79 years (23). It is important to bear in mind that many of the included autopsy studies were done before the PSA-era, and the results might be different today. One may also question whether lifestyle/environmental factors in combination with susceptibility may vary by age.

1.1.2.2 Genetics

Family history and ethnicity are established as important risk factors for PCa, pointing to a strong genetic component in disease development. A first-degree relative (brother or son) to a PCa patient has a 2-fold increased risk of disease compared to the general population, and the risk for men with two affected relatives increases to 3.5-fold (24;25). In a Swedish population-based study brothers of a PCa patient had a 11.4% probability of high-risk PCa at age 65 vs. a population risk of 1.4% (26). Another Nordic study among twins found the proportion of PCa variation attributed to germline genetics, to be as high as 58% (27).

Ethnicity is another established risk factor. Within the US, incidence rates vary by ethnic groups (28), as men of African-American origin get PCa at a younger age, tend to have more advanced disease and a more severe type of PCa than other men (29-31). Given the evidence that family history and ethnicity play a role in PCa development, researchers have tried to pinpoint germline mutations related to PCa, and genome-wide association studies have identified many loci potentially contributing to the risk for PCa (32-34). In a study by Giri and colleagues pathogenic variants were found mostly in the genes BRCA2, CHEK2, ATM, and BRCA1 (35). These findings have also been supported by others (36), and suggest that families at high risk of PCa may benefit from targeted genomic analysis (37). Another interesting concept in PCa

genetics is the quest to reveal genetic changes related to susceptibility, socalled susceptibility-Single Nucleotide Polymorphism (SNPs) (38). Known genetic polymorphisms (SNPs) can be used in combination with plasma biomarkers and clinical variables as a screening tool for PCa (Stockholm3) (39).

1.1.2.3 Inflammation

Chronic inflammation, one of the hallmarks of cancer development, has been questioned for playing a key role in PCa development. In the adult prostate gland, local inflammation probably has a role in formation of lesions such as proliferative inflammatory atrophy, which is proliferative glandular epithelium with morphological appearance of simple atrophy that occurs in association with inflammation (40-46). These lesions are thought to be possible precursors for PCa, and there is evidence that regenerative epithelium in response to environmental insults may precede development of prostate intraepithelial neoplasia and early carcinoma (40;42;47). Furthermore, local inflammation has been observed in 35%–100% of PCa biopsies (40;48-50). The origin of prostate inflammation is multifactorial and may include pathogens, diet, mechanical and chemical trauma. It is in many cases without symptoms, and the inflammation can be acute or chronic (Figure 8) (46;51). However, a causal relationship between inflammation and PCa development has yet to be established (19;40;43;44).

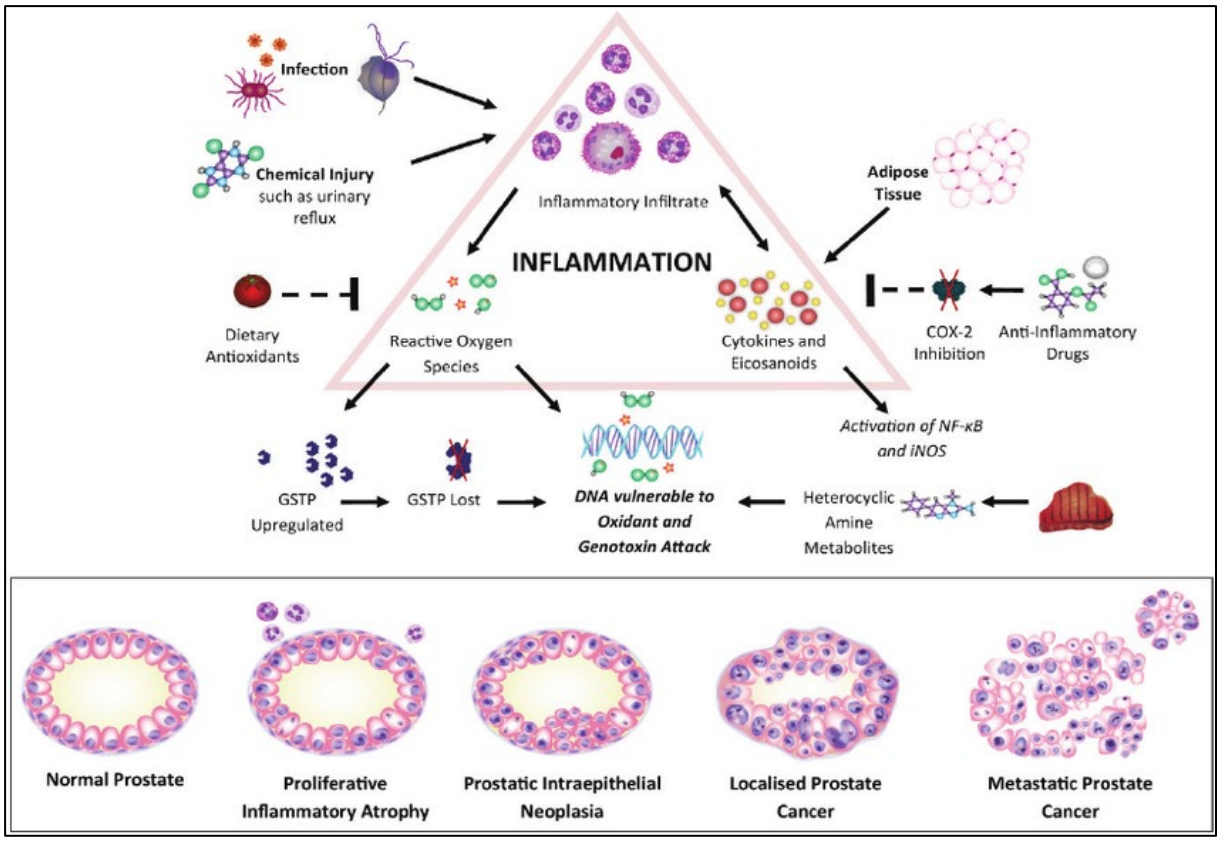

Figure 8 Inflammation in prostate carcinogenesis. Arrows: stimulates. Dashed lines: inhibits. Abbreviations: COX-2 cyclooxygenase-2, GSTP glutathione-S-transferase, NF- κ B nuclear factor- κ B, iNOS inducible nitric oxide synthase. Reprinted with permission from A Burton, 2010 (52)

Several studies have investigated how signs of inflammation affects prognosis in the PCa patient (53-60). Different markers or scoring systems have been constructed, for example the neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), or the Systemic Immune-inflammation Index , which combines the NLR and PLR, and can be used to identify PCa patients with increased risk of recurrence (53-57). Other studies have reported that elevated CRP levels are associated with a poor PCa prognosis, both in localized and metastatic disease (58;59). In order to examine whether altered genetically predicted concentration of circulating cytokines are associated with cancer development, including PCa, a total of 31,112 individuals of European descent were included in a genome-wide association-study meta-analyses of 47 circulating cytokines. No association was observed between specific inflammatory biomarker pathways in relation to PCa (61). However, much is under debate and more knowledge is needed about the details of the association between PCa and inflammation.

1.1.3 Inflammatory markers (CRP + WBC)

Blood levels of two commonly available measures, C-reactive protein (CRP) and white blood cell count (WBC), are indicators of systemic inflammation. Interesting observations suggest that these biomarkers could predict risk for PCa development and progression (59;62-64). A biomarker (biological marker) is "an objective reproducibly measurable parameter of a physiological or pathological condition that the patient cannot report her/himself " (Biomarkers Definitions Working Group 2001(65). Biomarkers can be used to diagnose diseases or predict risks of disease complications.

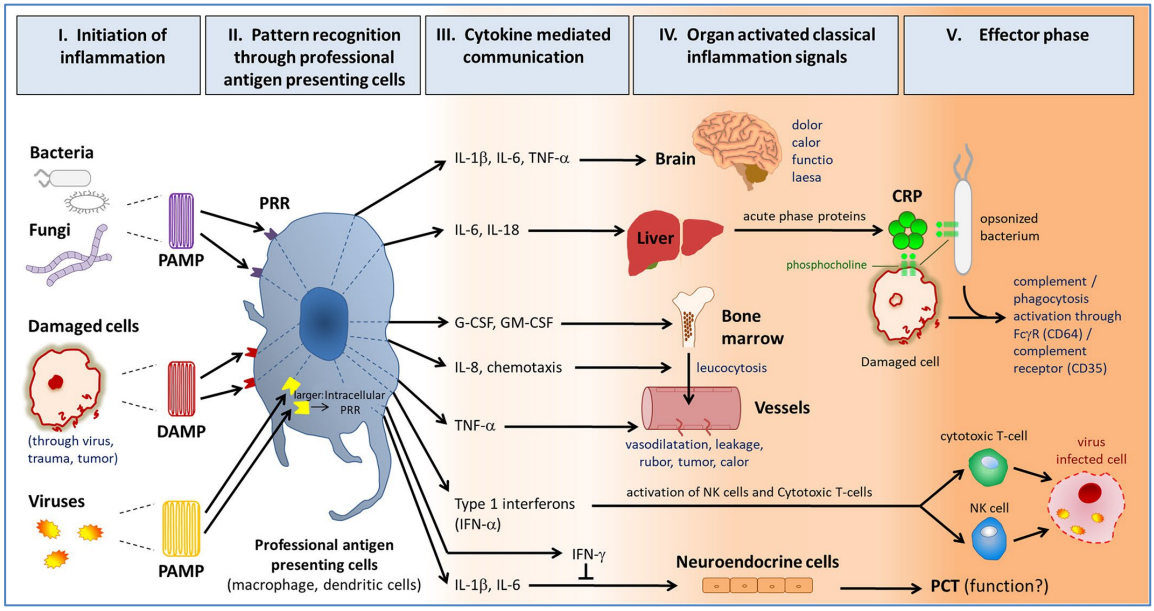

Figure 9 Physiological inflammatory response: Initiation of inflammation, pattern recognition, cytokine release, organ activation, effector phase. Figure reprinted with permission from T Niehues, 2018 (66)

CRP is an acute phase protein that reflects tissue injury and has become a widely used systemic biomarker of acute infection or inflammation in clinical practice (Figure 9). CRP is relatively stable in serial measurements in healthy individuals (67;68). Although previous data are conflicting, the inflammatory marker, hs-CRP was associated with increased PCa risk (62). However, CRP was not a sensitive marker of the acute inflammatory effects of non-metastatic PCa and treatment response with androgen ablation or radiation therapy (69). Srour and colleagues have studied the associations between with risks of cancer and cardiovascular disease (CVD), and five markers of unhealthy ageing: Growth Differentiation Factor-15 (GDF-

15), N-terminal pro-brain natriuretic peptide (NT-proBNP), glycated hemoglobin A1c (HbA1C), CRP and cystatin-C. This analysis suggests that combinations of biomarkers related to inflammation and unhealthy ageing show strong associations with cancer risk (70). In a recent meta-analysis including17,833 patient exploring the association between CRP and survival of PCa, elevated pretreatment serum CRP level was strongly correlated with worse prognosis in patients with PCa, including overall survival (OS), cancer-specific survival (CSS), progression-free survival (PFS), and biochemical-recurrence free survival (BC-RFS) (71). Thus, several studies have reported that elevated CRP levels are associated with a poor PCa prognosis, both in localized and metastatic disease (58;59;71).

White blood cells, or leukocytes, are a vital part of the body's immune system. They are produced in the bone marrow from hematopoietic stem cells, and then migrate to all parts of the body, including the blood and the lymphatic system. The different classes of WBC are grouped based on their progenitor (myeloid cells or lymphoid cells) (Figure 10). Myeloid cells (myelocytes) include neutrophils, eosinophils, mast cells, basophils, and monocytes, while lymphoid cells (lymphocytes) include T cells (subdivided into helper T cells, memory T cells, cytotoxic T cells), B cells (subdivided into plasma cells and memory B cells), and natural killer cells.

Several studies have investigated the associations between WBC and PCa, either using total white blood cell count, or by using ratios between subtypes: In a study of 458 consecutive patients who underwent TURP, BMI and WBC were found to be independent factors positively associated with the risk of incidental PCa (72). Serum neutrophil-to-lymphocyte ratio (NLR) could predict PCa in men undergoing needle biopsy (73). The monocyte fraction of WBCs was increased in patients with high Gleason score PCa (74), and human PCa cells induce inflammatory cytokine secretion by peripheral blood mononuclear cells (75). A recent study by Rundle and coworkers tested neutrophil-to-lymphocyte ratio (NLR) and monocyte-to-lymphocyte ratio (MLR) trajectories for associations with risk for PCa and found racial differences in the systemic inflammatory response to PCa (76). It is evident that the associations between the inflammation mediated through the different white blood cells and PCa are complex, and that more research is needed.

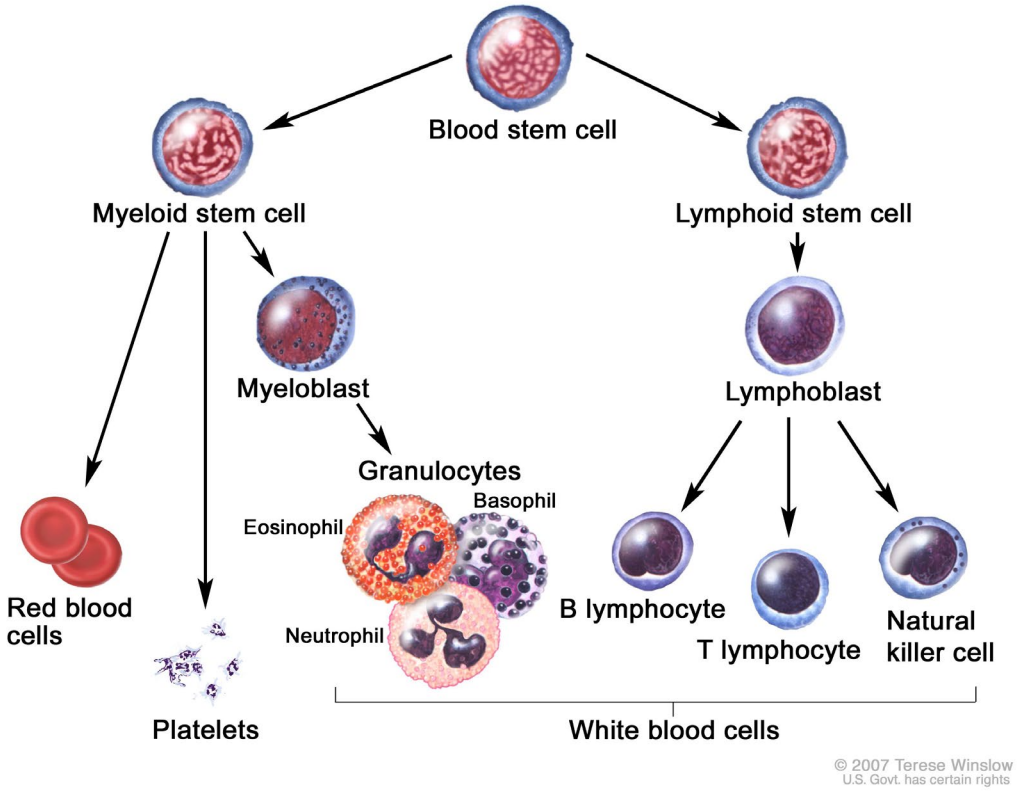

Figure 10 Blood cell development. A blood stem cell goes through several steps to become a red blood cell, platelet, or white blood cell. Reprint with permission from National Cancer Institute © 2007 Terese Winslow LLC, U.S. Govt. has certain rights.

1.1.4 Hypertension

The American College of Cardiology (ACC) and American Heart Association (AHA) recommends the BP should be categorized as normal, elevated, or stage 1 or 2 hypertension (table 1) (77). The global age-standardized prevalence of hypertension stage 2 (systolic BP ≥140 mmHg or diastolic BP ≥ 90 mmHg) in men was estimated as 24.1% in 2015 (78). Hypertension is generally classified as primary (essential) or secondary (caused by another condition or disease), with primary hypertension being far more prevalent. Systolic blood pressure (BP) above 115 mmHg is ranked as a leading risk factor for the global burden of disease (79). Hypertension is a world-wide leading risk factor for CVD and premature deaths.

Table 1 Categories of blood pressure in adults. BP indicates blood pressure (based on an average of ≥2 careful readings obtained on ≥2 occasions. Adapted from Whelton et al. 2018 (77)

BP Category	**Systolic BP**		**Diastolic BP**
Normal	<120 mm Hg	and	<80 mm Hg
Elevated	120–129 mm Hg	and	<80 mm Hg
Hypertension stage 1	130–139 mm Hg	or	80–89 mm Hg
Hypertension stage 2	≥140 mm Hg	or	≥90 mm Hg

The association between hypertension and PCa development and prognosis has been investigated in several studies with inconsistent results (80-82). Hypertension is a complex condition with numerous risk factors, including genetic and environmental factors and it is an important part of the metabolic syndrome (MetS). The pathophysiology for development of hypertension includes increased salt absorption resulting in volume expansion, impaired response of the renin-angiotensin-aldosterone system (RAAS), and increased activation of the sympathetic nervous system. This causes increased total peripheral resistance and increased afterload which in turn leads to the development of hypertension (83). Previous studies of the association between hypertension and PCa development have shown inconsistent results (80-82). Neither the European Prospective Investigation into Cancer and Nutrition (EPIC), nor a meta-analysis observed any associations between hypertension and risk of PCa (81;82). In contrast, in a longitudinal case–control study, men (aged 40-58 years at study entry) with systolic BP >150 mm Hg had an increased PCa risk compared to men with a lower systolic BP (84). Hypertension was also associated with increased risk of biochemical recurrence (BCR) after radical prostatectomy, independent of age at diagnosis and tumor pathological features (85). Whether long lasting raised diastolic hypertension influences PCa development and prognosis is not much studied. Of note, use of antihypertensive medication does not seem to have any effect on cancer risk (86). Thus, the importance of elevated BP may show variation by age at onset of hypertension, exposure time, age when diagnosed with PCa, and aggressiveness of disease (87). However, much remains unknown.

1.1.5 Other risk factors

A wide variety of exogenous/environmental factors have been discussed as being associated with the risk of developing PCa. Many of these potential risk factors hypothesized are related to the MetS, which is a cluster of risk factors for CVD and type 2 diabetes that often occur together: elevated waist circumference, elevated triglycerides, low High Density Lipoprotein (HDL) cholesterol(C), elevated blood pressure, elevated fasting glucose (88). Three out of five abnormal findings would qualify a person for the metabolic syndrome, see detailed definition in table 2 below. These factors will be briefly mentioned here.

Table 2 Criteria for clinical diagnosis of the Metabolic Syndrome. Adapted from Alberti et. al 2009 (88)

Measure	**Categorical Cut Points**
Elevated waist circumference	≥102 cm for men of European origin (Population- and country-specific definitions)
Elevated triglycerides (drug treatment for elevated triglycerides is an alternate indicator†)	≥150 mg/dL (1.7 mmol/L)
Reduced HDL-C (drug treatment for reduced HDL-C is an alternate indicator†)	<40 mg/dL (1.0 mmol/L) in males
Elevated blood pressure (antihypertensive drug treatment in a patient with a history of hypertension is an alternate indicator)	Systolic ≥130 and/or diastolic ≥85 mm Hg
Elevated fasting glucose‡ (drug treatment of elevated glucose is an alternate indicator)	≥100 mg/dL

HDL-C: high-density lipoprotein cholesterol.

†The most commonly used drugs for elevated triglycerides and reduced HDL-C are fibrates and nicotinic acid. A patient taking 1 of these drugs can be presumed to have high triglycerides and low HDL-C. High-dose ω-3 fatty acids presumes high triglycerides.

‡Most patients with type 2 diabetes mellitus will have the metabolic syndrome by the proposed criteria.

PCa incidence and mortality correlate positively with the intake of fat worldwide (89), and there is a growing concern that the increase in the obesity epidemic and diabetes cases may influence PCa development and prognosis (90;91). The data regarding metabolic syndrome is conflicting: Single components as hypertension and waist circumference have been associated with a significantly greater risk of PCa but having >3 components of MetS is associated with a reduced risk (92;93). In the Reduction by Dutasteride of Prostate Cancer Events (REDUCE)-study, metformin and statin use was tested as possible preventive agents for PCa but did not show any significant effects (94). In the REDUCE-study, obesity was associated with a lower risk of low-grade PCa, but a higher risk of high-grade PCa (95). Weight gain after being diagnosed with PCa has been associated with PCa mortality and obese men have been observed to have higher PCa mortality after radical prostatectomy (96). Thus, the association between obesity, metabolic syndrome and PCa incidence are conflicting (97-100) .

Several dietary factors have been studied with regard to risk of PCa, but the associations are mostly weak (101). High alcohol intake, high intake of dairy products, and fried food might increase risk of PCa. High intake of lycopene (from tomatoes), phytoestrogens, soy food and Vitamin E/selenium might decrease risk of PCa (101).

Higher ejaculation frequency (> 21 times a month vs. 4 to 7 times) has been associated with a 20% lower risk of PCa (102). Occupational hazards might contribute: In systematic reviews firefighters had a 12-15% increased risk of PCa, possibly linked to exposure to polycyclic aromatic hydrocarbons (103-105).

Physical activity has been suggested to reduce PCa development and may affect PCa biology through the IGF pathway, which is also linked to obesity (106), but these observations are still under debate (107;108). In addition to obesity, dyslipidemia, and type 2 diabetes mellitus, low testosterone concentrations may be an independent risk factor for hypertension in males (109;110).

1.1.6 MicroRNA

1.1.6.1 General description of microRNA

In mammals, most of the genome is transcribed into non-coding RNA, which can be classified into "housekeeping" RNA, transfer RNA, and regulatory RNA. The most studied form of regulatory RNA are small single-stranded non-coding RNA molecules (containing about 22 nucleotides) known as microRNAs (miRNAs) (111). Control of the gene expression is important for the formation and maintenance of biological structures, and miRNAs are important elements in the regulation of these processes. The miRNAs can play important gene-regulatory roles in animals and plants by pairing to the messenger RNA (mRNAs) of protein-coding genes to direct their posttranscriptional repression (112). MicroRNAs bind to the RNA-induced silencing complex (RISC) and are used to identify target mRNA transcripts (Figure 11) (113). They can prevent protein expression through cleavage of specific target mRNAs or through inhibition of their translation, and thus fulfill critical functions in developmental processes, tissue maintenance and during tumorigenesis (114). Aberrant expression of miRNA can give rise to either tumor suppressors or oncogenes in many human cancers (114).

The miRNAs are present in diverse biological fluids such as blood, cerebrospinal fluid and urine, and holds potential as both modulators of disorders and as biomarkers. Thus, they may potentially become a biomarker, a diagnostic tool as well as a marker of treatment response. Recent deep sequencing experiments have led to a dramatic increase in the number of known miRNA genes, and there are several open databases available. miRBase, the most used reference microRNA database, currently lists 1234 mouse and 1917 human mature miRNA sequences (115-117). Different miRNAs have been of interest in studies on biological mechanisms when studying various types of cancer, such as breast cancer (118), colon cancer (119), and lung cancer (120)

The role of miRNAs in PCa have been studied, but the biological mechanisms operating and types of miRNAs and their functions has not yet been clarified (114). Importantly, no real prostate-specific miRNAs have yet been identified. Our research group have previously studied the association between several miRNAs and PCa recurrence and survival (121-126). High expression of miR-205, miR-17-5p, miR-20a-5p, miR-210, and miR-141 and low expression

of miR-424 were all associated with increased risk of PCa recurrence. The miRNAs have been suggested to be associated with inflammation, however there is limited knowledge (127;128).

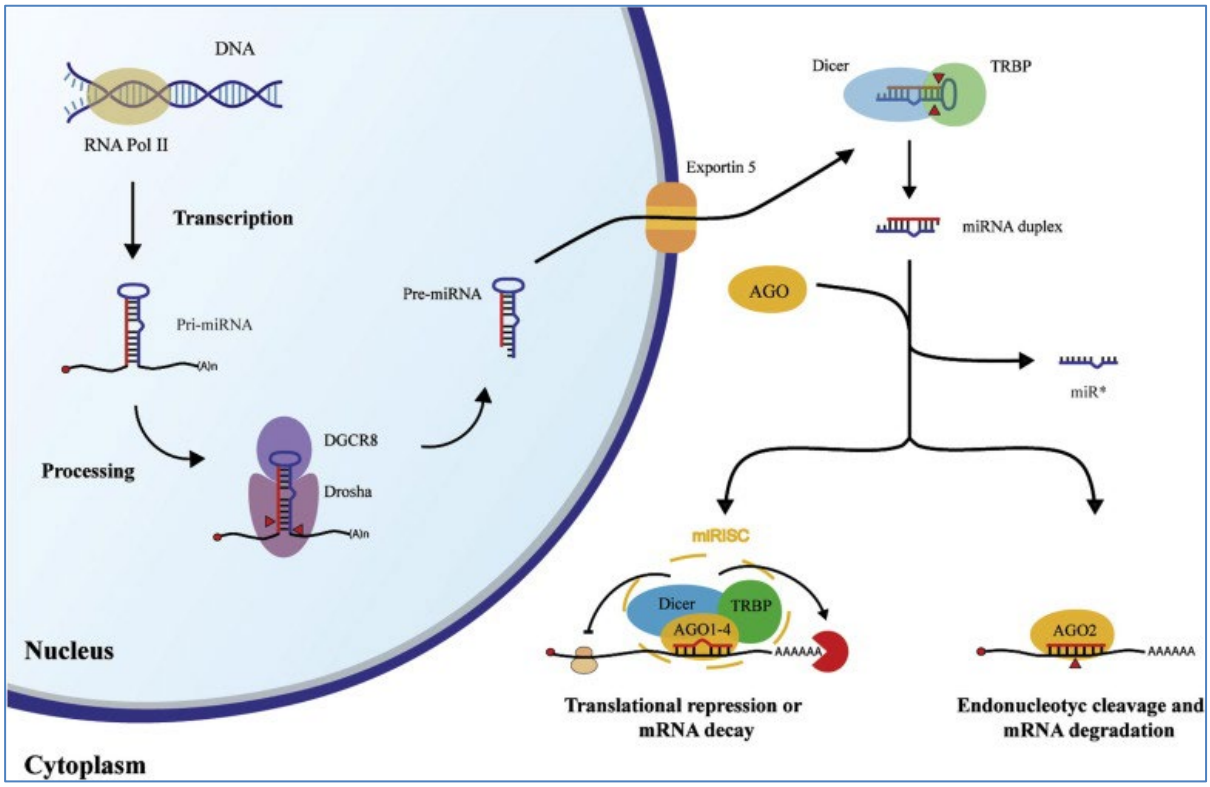

Figure 11 Schematic representation of the canonical pathway of microRNA (miRNA) biogenesis. Reprinted with permission from C Saraiva, 2017 (113)

1.1.6.2 The miR-24 in prostate cancer and other conditions including inflammation

The stem-loop sequence hsa-miR-24-1 is the processor of two mature sequences: hsa-miR-24-1-5p and hsa-miR-24-3p (117). Circulating miR-24 is elevated in diabetes, breast cancer and lung cancer, and down-regulated in PCa and hepatocellular carcinoma. It can function as an oncogenic or tumor suppressors dependent on cancer subtypes (128;129). miR-24 regulate phagocytosis in myeloid inflammatory cells (130). Others have found that miR-24 is downregulated in type-2-diabetes patients, and holds potential as biomarkers in patients with coronary heart disease and type 2 diabetes (131). miR-24 has also been linked to inflammation and cardiovascular disease (Figure 12) (127;128). In a murine model, miR-24 was a central regulator of vascular inflammation (132). In a model with primary human macrophages, miR-24 would produce anti-inflammatory action by inhibiting the production of pro-inflammatory

cytokines, and these results suggest that overexpression of miR-24 would have mostly anti-inflammatory effects (133).

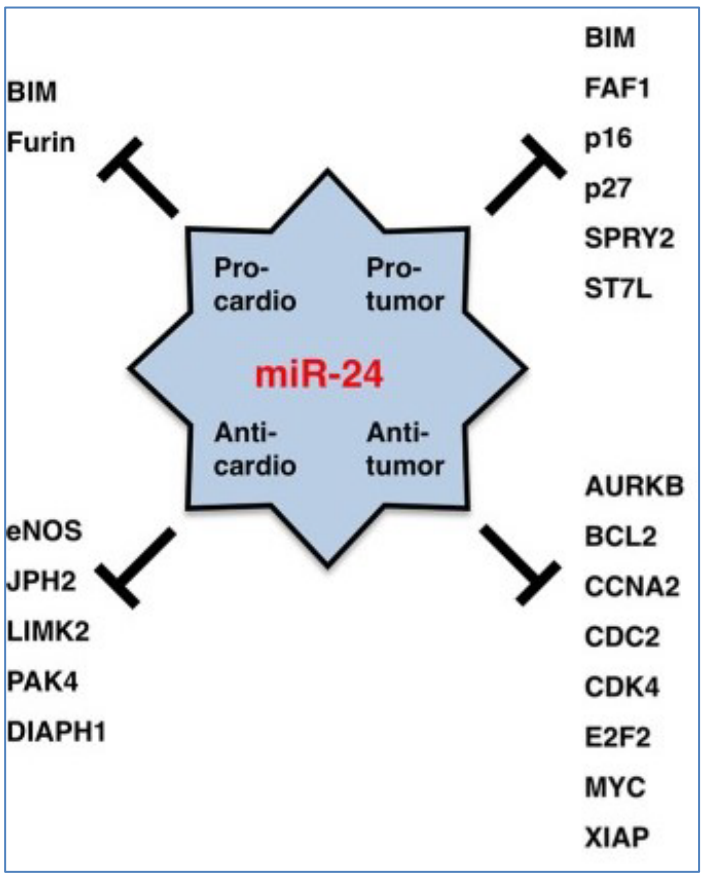

Figure 12 miR-24 in cardiology and oncology. Targets for pro-tumor, anti-tumor, pro-cardio and anti-cardio functions are shown. miR-24 function as oncogenic or tumor suppressor miRNAs dependent on cancer (sub)type. Figure adapted with permission from M Katoh.(128)

Few have investigated the association between miR-24 and PCa (134). The miRNA profile of prostate carcinoma has been obtained by deep sequencing of prostatectomy specimen and it was observed that miR-24 was downregulated compared to non-cancer prostate tissue (135). Another study, by Hashimoto *et al*. found that miR-24 was differentially expressed in African American and Caucasian American PCa patients (136). Interestingly, miR-24-3p enhanced Paclitaxel sensitivity in Paclitaxel-resistant PCa cells (137), while in xenograft cell lines, miR-24 was down-regulated in metastatic PCa, compared to non-metastatic (138). Furthermore, miR-24 expression was significantly lower in PCa cell lines compared to a normal prostate epithelial cell line. These findings suggest that miR-24 has a tumor suppressor role in PCa and

targets p27 and p16 in PCa cells (139). miR-24 is found to modulate apoptosis in the DU-145 cell lines, via targeting the coding sequence region of Fas- associated factor 1 (140). Based on this, miR-24 has been suspected to be involved in PCa progression (141).

Current knowledge about miR-24 is largely based on in vitro studies and/or mouse models. To our knowledge, previous studies have not reported which sequences of miR-24 they have used (138;139). There is a need for better and deeper understanding about the role of miR-24-1-5p in PCa.

1.2 Prostate cancer diagnosis and treatment

In Norway the diagnostic work-up of PCa most commonly starts with elevated PSA values detected in an asymptomatic patient by a general practitioner (GP). According to official Norwegian guidelines ("Pakkeforløp"), a suspicion of PCa based on an elevated PSA or urinary symptoms should lead to a general clinical examination, and digital rectal examination. If something pathological is discovered by digital rectal examination, it should lead to a direct referral regardless of PSA-levels. In the case of a normal digital rectal examination, PSA should be measured twice, with three weeks in between, and any other cause of raised PSA (urinary retention or infection) should be excluded. The patient is then referred to an urologist if the PSA levels are elevated (142).

Current guidelines recommend that the patient has a multi-parametric magnetic resonance imaging (mpMRI) of the prostate before biopsies are performed (101). Biopsies have been performed transrectal under guidance of transrectal ultrasound (TRUS), but trans-perineal biopsies emerge as a better technique with a lower frequency of infection complications, and will gradually replace transrectal biopsies (143). The standard procedure comprise a total of 10-12 systematic biopsies from the gland, and additional biopsy cores should be sampled from areas deemed suspicious by digital rectal examination, transrectal ultrasound and/or MRI (targeted biopsies) (101). Complications related to the biopsy procedure are rare, but infections can occur. To prevent this, patients receive pre-procedure antibiotics. The final diagnosis is defined by histological examination. If the histological examination of the biopsies confirms PCa, a risk stratification will be done for the patient, and this will guide further treatment.

1.2.1 Histopathological specimens

PCa is a heterogenous disease, ranging from low-grade PCa with little impact on life expectancy, to aggressive and life-threatening disease. A correct histopathological diagnosis, staging and risk-assessment of the patient is thus highly important to avoid both over and under-treatment. The first step in this risk assessment is the diagnosis performed by histological examination of the prostate tissue in the biopsies. Both cancerous and non-cancerous conditions in the prostate exists, but prostatitis, benign lesions, precancerous neoplasia, and neoplasia with uncertain malignant potential will not be described here (see table 3 for full list).

Table 3 Disease categories and histopathological classification of prostatic disease based on a table from the 2016 WHO Classification of Tumors. Reprint from Nora Ness with permission.

Disease category	Disease	Subtypes
(A)Prostatitis	Acute bacterial prostatitis Chronic bacterial prostatitis Chronic pelvic pain syndrome (CPPS)/ Chronic nonbacterial prostatitis Asymptomatic inflammatory Prostatitis	
(B) Benign lesions, precancerous neoplasia and neoplasia with uncertain malignant potential	Benign prostatic hyperplasia Atypical adenomatous hyperplasia (adenosis) Low grade intraepithelial neoplasia High grade intraepithelial neoplasia of the prostate Intraductal carcinoma (without associated invasive adenocarcinoma) Atypical small acinar proliferation Atrophic lesions	
© Malignant neoplasia	Epithelial tumors	Glandular neoplasms - Acinar adenocarcinoma (most common) *(Atrophic, pseudohyperplastic, microcystic, foamy gland, mucinous, signet ringlike cell, pleomorphic giant cell, sarcomatoid)* - Ductal adenocarcinoma *(Cribriform, papillary, solid)* - Intraductal carcinoma (Acinar or ductal) - Urothelial carcinoma (Transitional cell cancer) Squamous neoplasms - Adenosquamous carcinoma - Squamous cell carcinoma Basal cell carcinoma
	Neuroendocrine tumors	Adenocarcinoma with neuroendocrine differentiation Well-differentiated neuroendocrine tumor (carcinoid) Small cell neuroendocrine tumor Large cell neuroendocrine tumor
	Mesenchymal tumors	Different sarcomas etc.
	Haematolymphoid tumors	Different lymphomas/leukemias

Acinar adenocarcinoma is the most common form of PCa and is often simply referred to as "prostate cancer" (144). The histopathological diagnosis of PCa is based on a combination of architectural and cytological features, visible to the pathologist after the prostate specimen has been fixated in paraffine and stained with Hematoxylin and Eosin (H&E).

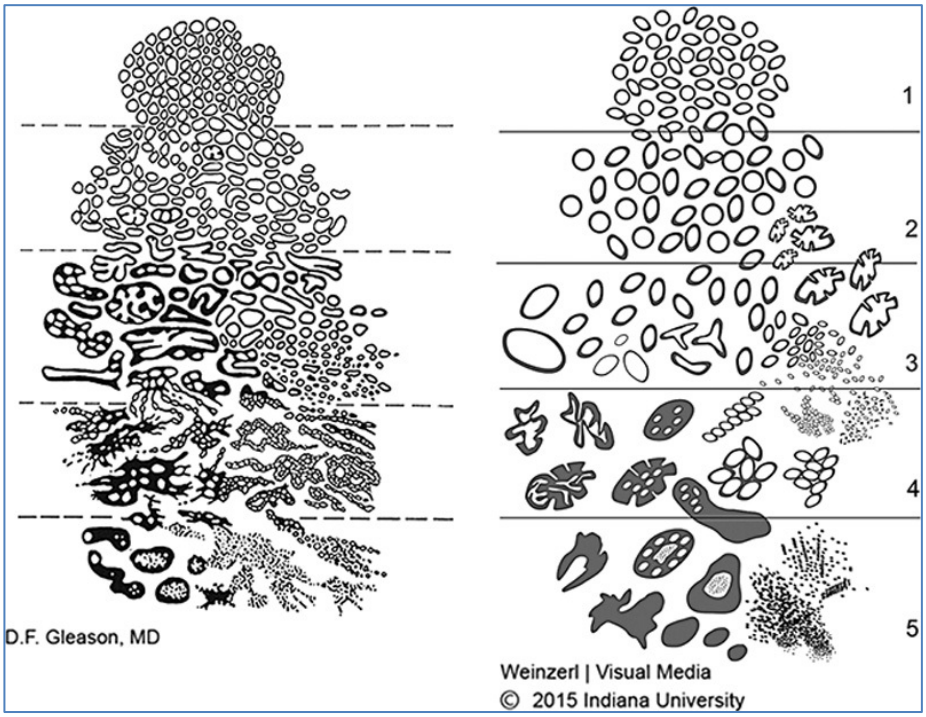

Figure 13 Prostatic adenocarcinoma histologic patterns. Original Gleason grade (left) and 2015 Modified Prostatic ISUP Gleason schematic diagrams. Reprinted with permission from Wolters Kluwer Health, Inc.

If adenocarcinoma is found in the biopsies, it is graded according to the scoring system invented by pathologist Douglas Gleason in the 50s. This system grades the histopathological patterns from well-differentiated (grade 1) to poorly differentiated (grade 5). The two most dominant Gleason grades are summed to obtain a Gleason Score. Grade 1 and 2 are not considered to be cancer and are rarely used, and this has been causing some confusion in patient communication, since Gleason Score 6 has been the lowest score/least aggressive cancer. In 2014, the International Society of Urological Pathology (ISUP) have recommended a new grading system based on the Gleason grade and score, but with a more intuitive scale (145). It is now common to report both Gleason Score and ISUP grade (table 4).

Table 4 International Society of Urological Pathology 2014 grade (group) system (145)

Gleason score	ISUP grade
2-6	1
7 (3+4)	2
7 (4+3)	3
8 (4+4 or 3+5 or 5+3)	4
9-10	5

1.2.2 Genetic risk and clinical factors in combination

The prostate-specific antigen (PSA) test is used to screen for PCa but has a high false-positive rate that causes unnecessary prostate biopsies and potential overdiagnosis of low-risk PCas. Several new models have been developed to combine individual risk factors such as clinical and genetic information to create more precise screening methods (60;146). One example of this is the Stockholm3 model, that combines plasma protein biomarkers [PSA, free PSA, intact PSA, hK2, MSMB, MIC1], genetic polymorphisms [232 SNPs], and clinical variables [age, family, history, previous prostate biopsy, prostate exam] (39). The Stockholm3 blood-test has been validated in a multi-center community cohort and can predict clinically significant cancer on biopsy (147). It has also been tested in combination with MRI-targeted biopsies with good results (148).

Recently, a Norwegian study described early experience of replacing PSA with Stockholm3 for detection of PCa in primary care (149). A majority of GP clinics started to use the test within three months. After implementation of the Stockholm3 test they observed: a 28% reduction in number of men referred for urological PCa work-up, an increase in the proportion of clinically significant cancer in performed prostate biopsies from 42 to 65%, and an estimated reduction in direct health care costs between 23 and 28% (149).

1.2.3 Staging

Risk stratification is essential to provide the best treatment for the PCa patient. The most commonly used staging system from European Association of Urology (EAU) is based upon three factors: PSA-level, ISUP grade and TNM-stage (table 5). The TNM-classification system describes the anatomical extent of disease and was developed jointly by the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) and has tables for all known cancer types. "T" describes the primary tumor, "N" describes dissemination to regional lymph nodes, and "M" describes distant metastasis. The original TNM scoring system use clinical T-stage (cT), which is based on clinical examination (i.e DRE). As the use of Mri and other imaging techniques has become more widespread, it is debated if image-based T-staging should be used instead (rT) (101;150;151). If the patient undergoes surgery and the prostate is removed and examined, a pathological T-score (pT) is also issued.

When PSA-level, ISUP-grade and TNM-stage are known, the patient can be placed in a risk group according to the EAU-guidelines. It is important to note that patients with metastatic PCa at diagnosis (primary metastatic cancer) fall outside of this risk stratification (101). All high-risk patients are submitted to either ^{99m}Tc-Bone scan or MRi of the pelvis and columna, to screen for lymph node or bone metastases (101). For those patients with so called "very-high risk", Prostate-specific membrane antigen (PSMA) positron-emission tomography (PET)/CT are now becoming the new standard of care, as this imaging modality provides a more sensitive detection of lymph node and bone metastases (152).

Table 5 European Association of Urology (EAU) risk groups for biochemical recurrence of localized and locally advanced PCa

Low-risk	Intermediate-risk	High-risk	
PSA < 10 ng/mL and GS < 7 (ISUP grade 1) and cT1-2a	PSA 10-20 ng/mL or GS 7 (ISUP grade 2/3) or cT2b	PSA >20 ng/mL or GS > 7 (ISUP grade 4/5) or cT2c	any PSA any GS (any ISUP grade) cT3-4or cN+
Localized			Locally advanced

GS = Gleason score; ISUP = International Society for Urological Pathology; PSA = prostate-specific antigen

1.2.4 Management of prostate cancer with curative intent

The choice of treatment depends on factors of the PCa, and on the age and general health of the patient. For patients with low-risk PCa, active surveillance is recommended in both Norwegian and international guidelines (101;150). Active surveillance aims to avoid unnecessary treatment in men with clinically localized PCa who do not require immediate treatment. These patients are followed with PSA-measurements every 3-6 months, and new Mri after 3-6 years. If progression to a more aggressive cancer is suspected, new biopsies are performed. If these biopsies confirm progression of the cancer, the patient is offered more definitive curative treatment. Patients who are on active surveillance should have a life expectancy of at least 10 years and be well informed before entering this program.

For patients with initial intermediate or high risk, or who progress from low risk while on active surveillance, the two main treatment options are surgery (radical prostatectomy) or radiation (external beam radiotherapy). Both treatments are well established and can provide good oncologic results for the patient, but it is still debated which treatment modality gives the best long-term survival for the patient (153;154). An ongoing large phase III Scandinavian trial (SPCG15 trial – ref clinicaltrials.gov NCT02102477) will evaluate surgery vs. radiotherapy for locally advanced disease with regard to survival and quality of life.

1.2.4.1 Surgery

Surgical removal of the prostate has been done since the 1940s, but was refined in the 1970s by Walsh, who developed the method of anatomical and physiological radical retropubic prostatectomy (RRP) (155-157). At least 3 different multi-center randomized controlled trials (RCT) have shown excellent long-term results, with cancer-specific survival ranging from 80-99% (158-160). In the last 10-15 years, robot-assisted laparoscopic prostatectomy (RALP) has been the dominating technique. Peri- and postoperative risk is low (0-1.5% mortality), but there are several significant long-term side-effects such as urinary stress incontinence, erectile dysfunction, and stricture of the vesico-urethral anastomosis. RALP is believed to cause less long-term side effects, but studies have not been able to clearly show this (161). After the prostatectomy, the removed prostate specimen is analyzed by a trained pathologist. The report should include Gleason/ISUP Grade Groups, TNM-score, and other histopathological assessments of prognostic value.

- Surgical margin: Positive if cancer cells are present in the edge of the prostatectomy specimen.
- Seminal vesicle invasion: Cancer growth into the seminal vesicles. Also equivalent to the category p3Tb in the TNM-system.
- Extracapsular extension: Defined as the presence of tumor beyond the confines of the prostate(162). Also equivalent to the category p3Ta in the TNM-system.
- Lymph node invasion: Metastases in regional node(s). Regional lymph nodes include pelvic nodes located below the bifurcation of the common iliac arteries and can be uni- or bilateral. Also equivalent to the category N1/N+ in the TNM-system.

These characteristics are combined in the Cancer of the Prostate Risk Assessment Postsurgical Score (CAPRA-S), a validated score developed to predict outcomes after radical prostatectomy (163). Points are assigned according to table 6.

Table 6 The Cancer of the Prostate Risk Assessment Postsurgical Score (CAPRA-S).

Variable	Level	Points
Pre-surgery PSA (µg/L)	0 - 6 6.01 - 10 10.01 - 20 >20	0 1 2 3
Surgical margin	Negative Positive	0 2
Seminal vesicle invasion	No Yes	0 2
Pathological Gleason score	2 – 6 7a (3+4) 7b (4+3) 8 – 10	0 1 2 3
Extracapsular extension	No Yes	0 1
Lymph node invasion	No Yes	0 1

Points are assigned for each variable: 0- 3 for prostate specific antigen (PSA) level in µg/L, 0- 3 for pathologic Gleason score, 2 each for positive surgical margin (SM) and seminal vesicle invasion (SVI), and 1 each for extracapsular extension (ECE) and lymph node invasion (LNI). Points are summed to yield the CAPRA-S score. Adapted from Cooperberg et al. 2011 (163).

1.2.4.2 Radiotherapy

Radiation therapy (RT) is the other main option for primary, curative treatment of PCa. Beams of radiation are directed to the area of interest (i.e the prostate) with the intention of creating permanent damage in the DNA of malignant cells, inducing cell death. The first reports on the use of radiation to treat localized PCa are from the beginning of the twentieth century, when radium sources were inserted into the urethra and rectum as a palliative treatment (164). This was developed further in the 1970s, to eventually become the modern-day brachytherapy. Brachytherapy is now a treatment option for localized PCa but is not much used in Norway today. When higher-energy cobalt machines that could penetrate deep tissue became available in the 1950s, therapy focused on patients with unresectable disease (164). Improvements in technology such as higher-energy accelerators, advanced radiographic and data-processing capabilities, resulted in new treatment technique.

Today, Intensity-modulated radiotherapy (IMRT) with image-guided radiotherapy (IGRT) is the preferred radiation therapy, and allows the prostate to be treated with a high dose of radiation while sparing more of the surrounding normal tissues (101). Standard radiation

fractions have been 1.8-2.2 Gray per day, up to a total of 78 Gray in intermediate and high-risk patients. Hypofractioning (increased dose per day, 2.5–3.4 Gy) seems to be equally effective, with shorter total treatment time for the patient, and might be the new standard (165;166). RT is combined with androgen deprivation therapy (ADT) treatment before, during, and after, and ADT treatment length depends on risk group. The combination of RT and ADT considerably improves overall survival vs. RT alone or ADT alone (167;168). Side effects of RT can be grouped into acute/short term (skin irritation, bowel or urinary problems) or permanent/long-term such as fecal urge, rectal bleeding, urinary symptoms and erectile dysfunction (169). There is also a small risk of secondary cancer (mainly bladder or colorectal).

1.2.5 Management of metastatic prostate cancer

If the patient has metastatic disease, curative treatment is not an option and the focus is palliative care and attempts to prolong survival. For men with metastatic PCa, the disease will eventually develop into castration resistant PCa (CRPC). Treatment choices in palliative care have previously been limited, but the field is rapidly evolving and the treatment options for metastatic PCa are increasing:

a) Watchful waiting: conservative management, until the development of local or systemic progression.
b) Primary ADT: This has been the standard care of metastatic PC for more than 50 years. The PCa is dependent on testosterone for progression, and thus removal of testosterone is an efficient brake on PCa progression. This is achieved by either surgical castration (remove testicles), chemical castration (administration of drugs that block testosterone production at the pituitary level), or administration of anti-androgen drugs (bicalutamide and others).
c) Chemotherapy: Docetaxel has several indications. It is used for patients with newly diagnosed primary metastatic PCa. An initial treatment of six, three-weekly courses of docetaxel is given in combination with life-long castration and has shown improved overall survival compared to castration alone (170) Docetaxel and the second generation taxane kabazitaxel are both used in the treatment of CRPC.
d) Second-generation androgen therapy: The effect of first-generation antiandrogens (Bicalutamide and others) is often declining after a period of use. Over the last decade, several new androgen receptor blockers have been developed with improved efficacy and

potency. Four second-generation anti-androgens are currently approved by the Food and Drug Administration (FDA); abiraterone acetate, enzalutamide, apalutamide and darolutamide (171). These drugs were first implemented in the treatment of metastatic CRPC, but their use is gradually being implemented in treatment of earlier disease stages (172;173).

e) The concept of theragnostics (therapeutic + diagnostic), where a radioligand is combined with an antigen is showing success: Lutetium-177-PSMA-617 is a radioligand therapy that delivers beta-particle radiation to PSMA-expressing cells and the surrounding microenvironment, and by this prolonges progression-free survival and overall survival (174).

f) Precision medicine, where individual genomic profiling and targeting specific cancer pathways is used to "treat the right patient with the right medicine at the right time", has also become available for PCa patients (175). One example of this is the new drug Olaparib, recently approved for PCa-patients with alteration in the genes BRCA1, BRCA2, or ATM (176).

2 AIMS OF THE THESIS

The overall aims of the thesis were to study prediagnostic systemic inflammatory markers such as high sensitive-CRP and white blood cell count (WBC), prediagnostic systolic and diastolic blood pressure, and miR-24-1-5p, a subtype of microRNA, and PCa risk, PCa aggressiveness, and PCa prognosis.

1. To study whether markers of inflammation (WBC and high sensitivity-CRP) independently or in combination were associated with risk and severity of PCa, and to study whether a change in CRP were associated with risk of PCa development and severity.

2. To study whether prediagnostic systolic and diastolic BP were associated with PCa risk, and if prediagnostic systolic and diastolic BP were associated with overall mortality among PCa patients, and if such associations vary by age and type of PCa treatment.

3. To study whether a high expression of miR-24-1-5p, regulatory RNA which are small single-stranded non-coding RNA molecules is associated with aggressiveness and prognosis in men diagnosed with PCa and treated with radical prostatectomy.

3 MATERIALS AND METHODS

3.1 Study population

3.1.1 Data acquisition

The Prostate Cancer Throughout Life (PROCA-life) study is a substudy of the Tromsø Study and includes all men who enrolled in the Tromsø Study between 1994 and 2016 (Tromsø4 – 7). The Tromsø Study, which is a population-based prospective health study started in 1974 in the municipality of Tromsø, North Norway. The Tromsø Study now consists of seven surveys (referred to as Tromsø1–7) that have been carried out in the municipality of Tromsø every 6-8 years from 1974 to 2016 (Table 7). The initial aim of the Tromsø Study was to combat the high mortality of cardiovascular diseases in the region, but over the years increasing emphasis has been put on other chronic diseases and conditions, such as atrial fibrillation, venous thromboembolism, diabetes mellitus, osteoporosis, and fractures (177). Recently there has also been an increased focus on cancer.

Table 7 The examination year, age, number of subjects (n), and attendance (%) in the 4 surveys from 1994 to 2016: The Tromsø Study

Survey	Examination years	Age (years)	Male subjects (n)	Attendance (%)
Tromsø 4	1994-1995	25-97	12865	69.6
Tromsø 5	2001-2002	30-89	3511	75.7
Tromsø 6	2007-2008	30-87	6054	62.9
Tromsø 7	2015-2016	40-99	10009	62.4

The four surveys included in the present thesis had the same general design and were based on the official population registry. Whole birth cohorts and random samples of residents of the municipality of Tromsø were invited to take part in the survey with a personal invitation by mail. The invitation leaflet included information about the survey and the examination and non-

attendees were given one reminder. Local media was used to encourage and inform the population and to ensure a high attendance rate. A sample of previous participants were invited in the next survey, and thus repeated measurements are available for many of the subjects. All surveys included questionnaires, sampling of biological specimens, and clinical measurements. From Tromsø4 and onward, whole birth cohorts and random samples of the cohort were invited back for a second visit with more extensive clinical examinations, and extended questionnaires and lab-tests.

Figure 14 Tromsø in Europe. Edited reprint from Wikipedia by user David Liuzzo, with permission

3.1.2 Clinical variables

All study participants completed a questionnaire either at home or at the study site. The questionnaire was checked for completeness and inconsistency, and included questions about medical history, lifestyle factors, and use of medication including antihypertensive drugs. Educational level was categorical (1= secondary school only, 5= college/university for 4 or

more years). Alcohol use was defined as more than 1 unit of alcohol per month, as described by others in the same cohort (178;179). We defined being physically active as: more than one hour/week of strenuous exercise, or any leisure time exercise more than 2–3 times/week.

Systolic and diastolic BP (mmHg) were measured by using an automatic device (Dinamap Vital Signs Monitor 1846; Critikon Inc., Tampa, Florida). Participants rested for 2 minutes in a sitting position, then three readings were taken on the upper right arm, separated by 1-minute intervals, and the average of the last two readings was used (180). Height and weight were measured on an electronic scale with the participants wearing light clothing and no shoes. Height was measured to the nearest 1 centimeter (cm) in Tromsø4 and nearest 0.1 cm in Tromsø5-7. Weight to the nearest 500 g in Tromsø4 and to the nearest 100 g in Tromsø5-7. Body mass Index (BMI) was calculated using the formula weight/height^2 (kg/m^2).

Blood samples were drawn by trained research assistants on attendance at each survey and were non-fasting. Analyses of serum samples were done at the Department of Laboratory Medicine, University Hospital of Northern Norway (UNN), Tromsø, Norway (181). Serum samples from men who attended the first two surveys (Tromsø4 or 5: 1994–95 and/or 2001) were kept frozen up to 12 years at –70 °C and later analyzed, while hs-CRP was assessed in fresh samples from men who attended the final survey (Tromsø6: 2007–08). Hs-CRP was analyzed by a particle-enhanced immune turbid metric assay on a Modular P auto-analyzer (Roche Diagnostics, Mannheim, Germany) with reagents from the manufacturer with a detection limit of 0.12 mg/L. For WBC counts, 5 ml of blood was collected into Vacutainer tubes containing EDTA as an anticoagulant (K3-EDTA 40 lL, 0.37 mol/L per tube), and analyzed within 12h by an automated blood cell counter (Coulter Counter, Coulter Electronics, Luton, UK and Coulter LH750, Nerliens Meszansky). Total cholesterol was analyzed by enzymatic colorimetric methods with commercially available kits (CHOD-PAP for cholesterol).

3.1.3 Characteristics of study population

All new cancer cases in Norway are registered in the Cancer Registry of Norway, by mandatory reports from all hospitals and clinics. All PCa cases among participants in the Tromsø Study were identified using the unique national 11-digit identification number through linkage with the Cancer Registry of Norway. Causes of death were identified by linkage to the Norwegian

Cause of Death Registry and dates of emigration were obtained from the Population Registry of Norway.

Paper 1 in this thesis is based on the participants with measured inflammatory markers (CRP and WBC). This included second visit of Tromsø4 and Tromsø5, and first visit of Tromsø6. Paper 2 is based on blood pressure and other clinical data from the participants in Tromsø4. Paper 3 is based on men who underwent radical prostatectomy after participation in Tromsø4, 5,6 or 7 and where the surgical specimen where available (Figure 8).

For paper 1, we included all men were inflammatory markers (CRP and WBC) had been measured. This includes men who attended the second visit in Tromsø4 or Tromsø5, and all men in Tromsø6 (n= 7 720). Measurements of pre-diagnostic hs-CRP > 20 mg/L and/or pre-diagnostic WBC > 15 x 10^9cells/L, which may mirror other acute or chronic diseases, were excluded (high hs-CRP: n= 285, high WBC: n=44). Participants with prevalent or previous cancer (n=334), or who developed cancer within the first year after the enrollment in the study (n=58) were excluded to account for the possibility that undiagnosed cancer or severe illness could influence the results, leaving a final study population of 7 270 men. Participating men with more than one measurement of hs-CRP during follow-up (n=2 210) were used in separate analysis with repeated measurements.

For paper 2, we excluded all men who had a previous history of cancer (n=382), or who emigrated, died, or were diagnosed with cancer within the first year after study entry (n=128), to account for the possibility that undiagnosed cancer or severe illness could influence our results. Participants with missing measurement of blood pressure at study entry were also excluded (n=24) leaving a final study population of 12 271 men. A total of 811 men developed PCa during follow-up between 1994 and 2018. Associations between baseline blood pressure and PCa incidence have been studied in the full cohort (n=12 271), and associations between baseline blood pressure and overall mortality have been studied in men diagnosed with PCa (n=811).

For paper 3, PCa cases during follow-up (until Dec. 31, 2018) were identified, and cases with available tissue samples after prostatectomy with curative intent were identified by cross-linkage with the archive of Department of Clinical Pathology, University Hospital of North Norway, Tromsø, Norway (n=189). Overall, 43 cases were not technically successful in the

staining process and were excluded. Furthermore, four cases were excluded because they did not have curative surgery, leaving a final study population of 142 men.

For all papers, detailed information from medical records were obtained by trained physicians (ES, TK, MS) and included PCa treatments and recurrence (see appendix). PSA measurements were done for cancer cases only, as part of clinical routine in diagnosis and follow-up (1990–1994 Stratus® PSA Fluorometric Enzyme Immunoassay, 1994–2001 AxSYM Psa Reagent Pack, Abbot®, 2001 Bayer® PSA Reagens Pack Immuno I (Prod. Nr. T01-3450-51), Technicon Immuno I). For PCa cases diagnosed or treated in other institutions, PSA values from their local laboratories were recorded. Histopathological information for the PCa cases were obtained from histopathological records and were in addition re-examined by the same specialized pathologist (ER) and classified according to the latest International Society of Urological Pathology (ISUP) guidelines on Gleason score and ISUP grade group (182). PCa cases were divided into four risk groups based on PSA level at diagnosis, highest ISUP grade group and clinical T-stage, similar to the European Association of Urology-classification (EAU) guidelines (101). Risk group 1 (low) was defined as: PSA < 10µg/L, clinical T-stage (cT-) 1, and ISUP grade group 1. Risk group 2 (intermediate) was defined as: PSA: 10–20µg/L, cT-stage 2, or ISUP grade group 2–3. Risk group 3 (high) was defined as: PSA: > 20–100µg/L, cT-stage 3, or ISUP grade group 4–5. Risk group 4 (metastatic) was defined as: PSA > 100 µg/L, or with radiological evidence of metastatic disease. ISUP grade group was reported after reclassification when available. PSA values above 100 were not included in calculation of mean or median PSA. For Paper 3, CAPRA-S score was calculated for all cases.

3.1.4 Definition of endpoints and follow-up time

For paper 1 and 2, the primary endpoint was PCa diagnosis, defined as cancers coded as C61 according to the International Classification of Diseases, 10th edition (ICD-10) (183). Endpoints were updated until December 31, 2018. For paper 1, the secondary endpoint was low, intermediate, high or metastatic PCa diagnosis. For paper 2 the secondary endpoint among the PCa cases was death of any cause.

For paper 3, the primary endpoint was defined as a composite endpoint, including any evidence of recurrent PCa after surgery: Biochemical failure (PSA-level >=0.2) and/or

clinical/radiological signs of PCa defined by the treating physician. Endpoints were updated until august 2021.

Follow-up to incidence of PCa was calculated from the date of entry into the study to the date of PCa diagnosis, date of emigration, date of death, or end of follow-up (December 31, 2018), whichever event occurred first. Follow-up to mortality after PCa diagnosis was calculated from the date of PCa diagnosis to date of death, emigration or end of follow-up (December 31, 2018). Follow-up to recurrence after PCa surgery was calculated from the date of PCa surgery to date of recurrence, date of death, emigration or end of follow-up (August 31, 2021).

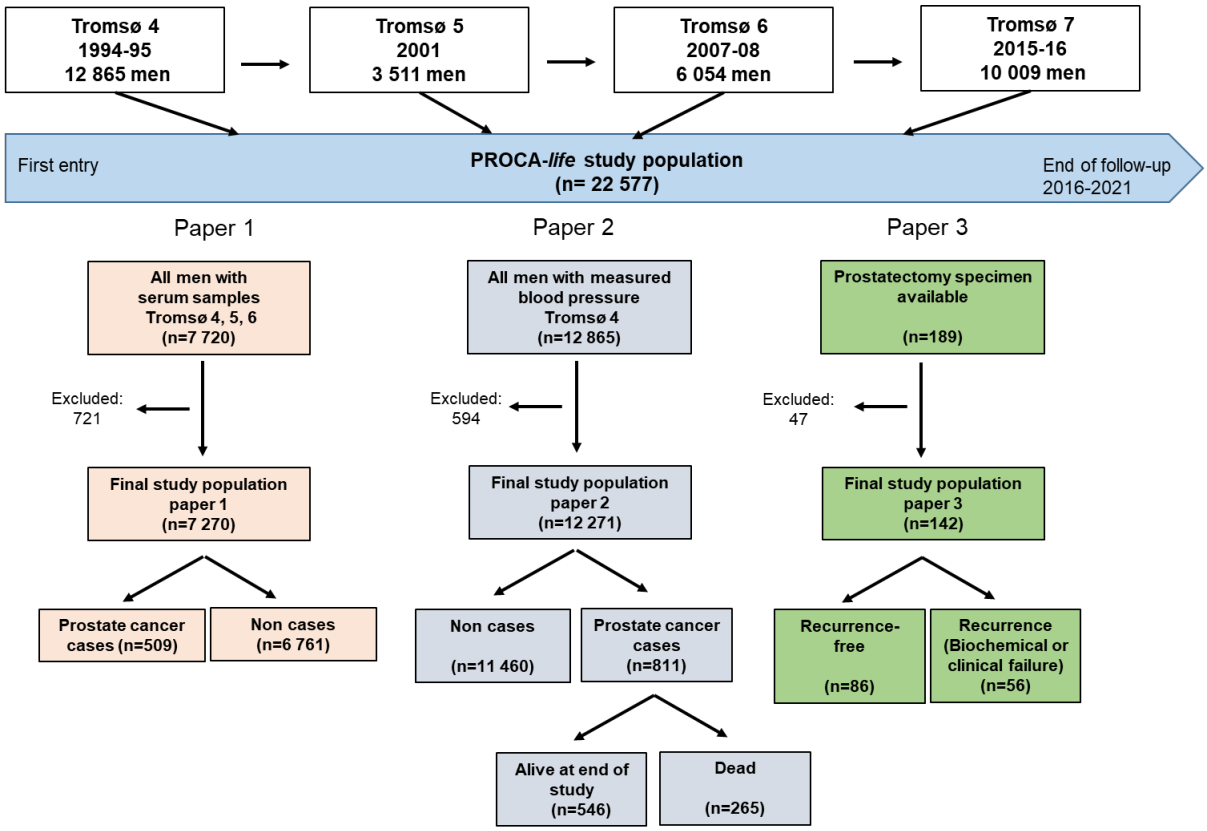

Figure 15 Flow chart of study population. See separate flowcharts in Paper I-II-III for more details

3.1.5 Ethics

The Tromsø study was approved by the Regional Committee for Medical and Health Research Ethics (REK) and the Norwegian Data Protection Authority, and was performed in accordance with the 1964 Helsinki declaration and its later amendments (181).Written informed consent was obtained from all individual participants when they enrolled in the Tromsø study (see appendix). The PROCA-life study has been approved by the Regional Committee for Medical and Health Research Ethics North (2015/1059).

3.2 Tissue preparation

3.2.1 Microarray construction

Tissue microarrays (TMAs) were constructed for the analysis of immunohistochemical (IHC) staining expression. For each case, one uropathologist (ER) identified and marked representative areas of the prostate specimens with tumor epithelial cells (TE) and normal epithelial cells (NE). From each of these areas, 0.6 mm cores were sampled from each donor block and inserted into paraffin blocks to construct TMA blocks by using a tissue-arraying instrument (Beecher Instruments, Silver Springs, MD, USA). The details of the technique have been described in detail by others, see Figure 16 (184;185).

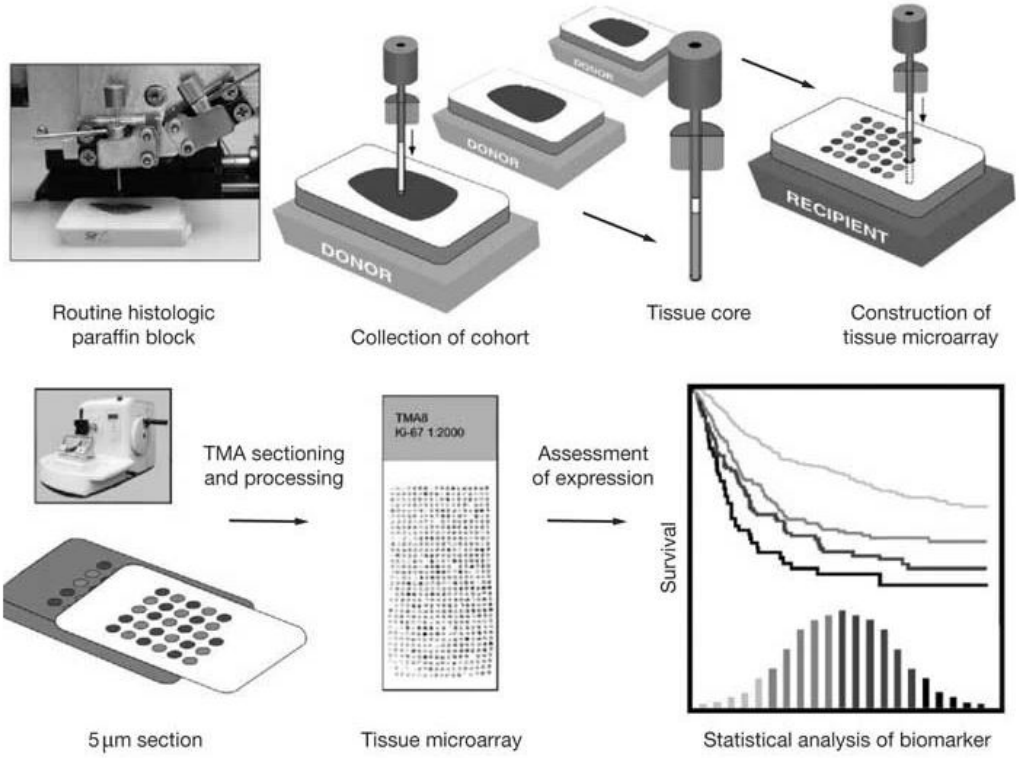

Figure 16 Overview of the steps in production and analysis of tissue with Tissue Micro Array. Reprinted with permission from Macmillan Publishers Ltd: Nature Reviews Clinical Oncology, ©2004 (185)

3.2.2 In situ hybridization (ISH)

The tissue expression of mature miR-24-1-5p in PCa was examined by in situ hybridization (ISH). The principle of the method is based on the ability of specific microRNA Locked Nucleic Acid (LNA) probes to bind to target microRNA in tissue followed by chromogenic visualization. ISH staining was done automatic in Ventana Discovery Ultra instrument. Necessary efforts to avoid RNA degradation in tissue were done in work routines and by using RNAse free buffers during the process.

Optimization and validation: LNA probe concentrations, hybridization temperatures and incubation times were optimized before staining the tissue of interest. Target retrieval treatment was adjusted to improve availability of microRNA sequence for the target and control probes. A TMA multi organ block with several normal and tumor tissues was used for optimization of ISH method and validation of miR-24-1 expression in different tissues.

We used U6snRNA probe as a positive control and to ensure the sensitivity level of the method. Strong nuclear U6snRNA staining also indicates low degree of RNA degradation of the tissue. Scramble miRNA negative control probe showed no unspecific staining (Figure 17).

Optimized ISH parameters are presented in Table 8. External validation of LNA probes was done by supplier company QIAGEN. The LNA miRNA probes were purified by HPLC (High-Performance Liquid Chromotography), analyzed by CE (Capillary Electrophoresis) or HPLC. The identity of compounds was confirmed using Mass Spectrometry.

Table 8 Optimized ISH parameters for target probe and controls.

LNA Probe	RNA Tm	Target retrieval	Dena-turation	Probe conc.	Hybrid i-zation temp.	String. Wash	Blocking	Detection	Visuali-sation	Contrast
miR-24-1-5p 1 nmol	85 °C	CC1 40 min.	90 °C 8 min	80nM	51°C	Ribo wash 51 °C 8 min	Ab block 16 min	Anti-DIG-AP 32 min.	ChromoMa Blue 120 min	Red II 4 min
Scramble-miR 25µM	87 °C	CC1 40 min	90 °C 8 min	10nM	57 °C	Ribo wash 51 °C 8 min	Ab block 16 min	Anti-DIG-AP 32 min.	ChromoMap Blue 120 min	Red II 4 min
U6 25µM	84 °C	CC1 40 min	90 °C 8 min	1.5nM	55 °C	Ribo wash 51 °C 8 min	Ab block 16 min	Anti-DIG-AP 32 min.	ChromoMap Blue 120 min	Red II 4 min

ISH procedure in short: TMA blocks were sectioned at 4 µm thickness and mounted on Superfrost Pluss glass slides. During incubation in instrument, Liquid Coverslip oil was used to protect sections from drying and ensure proper distribution of reagents.

Deparaffinization was performed at 68ºC with EZ Prep solution in three cycles. Target unmasking retrieval was done at 95ºC with CC1 buffer to improve the DIG labeled LNA probes to hybridize to the patient microRNA sequence. Sections were rinsed with Reaction Buffer between incubations.

Target microRNA 24-1-5p, positive control U6snRNA and negative control Scramble miRNA probes were diluted in microRNA ISH buffer and Elix RNAse free water to their final concentrations. To get optimal hybridization conditions probes and tissue microRNA were denaturized 8 min at 90ºC.

Hybridization of the LNA-probes was performed for 60 min. in temperatures adjusted with RNA Tm as a guideline for each probe, see Table 8. To ensure specific bindings, stringent washes were done in two cycles with RiboWash buffer. Additional blocking against unspecific bindings were done by Antibody Block solution.

For detection of tissue microRNA, anti-DIG-AP Multimer (Alkaline phosphatase (AP)-conjugated anti DIG) was incubated for 32 minutes to bind the Digoxygenin labeled probes. Blue chromogenic visualization of the AP-DIG complex was developed with NBT/BCIP from the ChromoMap Blue detection kit.

After Red II counterstain, sections were dehydrated by increasing gradients of ethanol solutions to Xylene and then mounted with Histokitt mounting medium. Ordering details of essential products used in this study are presented in Table 9.

Table 9 Ordering details of products for In Situ Hybridization

Probes and reagents	**Manufacturer**
LNA miR-24-1-5p probe	QIAGEN YD00610842-BCG
LNA Scramble miR probe	QIAGEN MiCURY LNA miRNA ISH Control set (FFPE) 1108515
LNA U6 snRNA probe	QIAGEN MiCURY LNA miRNA ISH Control set (FFPE) 1108515
Superfrost Pluss glass slides	Thermo Scientific
miRCURY LNA miRNA ISH Buffer and control	QIAGEN 339459
Liquid Coverslip oil	Roche 5264839001
EZ Prep Solution	Roche 5279755001
Discovery CC1 buffer	Roche 6414575001
Reaction buffer	Roche 5353955001
Ribo Wash	Roche 5266262001
Antibody Block	Roche 5268869001
Anti-DIG AP Multimer	Roche 7256302001
ChromoMap Blue Kit	Roche 5266661001
Red Counterstain II	Roche 5272017001

3.2.3 Scoring

The expression of miR-24-1-5p was assessed by semi-quantitative scoring by two trained independent investigators (ES, ER). The color intensity was graded as negative (0), weak (1), moderate (2), strong (3), or missing (4) (Figure 15). Two areas of TE cells and two areas of NE cells were scored for each patient. Stromal areas were not scored due to little or no positivity. Mean and median score were calculated for TE and for NE separately, and for TE+NE combined. High expression of miR-24-1-5p was defined as a score equal to or higher than the median score of the study population. Inter-observer variability was assessed by calculating linear weighted Kappa statistics and showed a moderate agreement (Kappa 0.59 (SD 0.50-0.68)).

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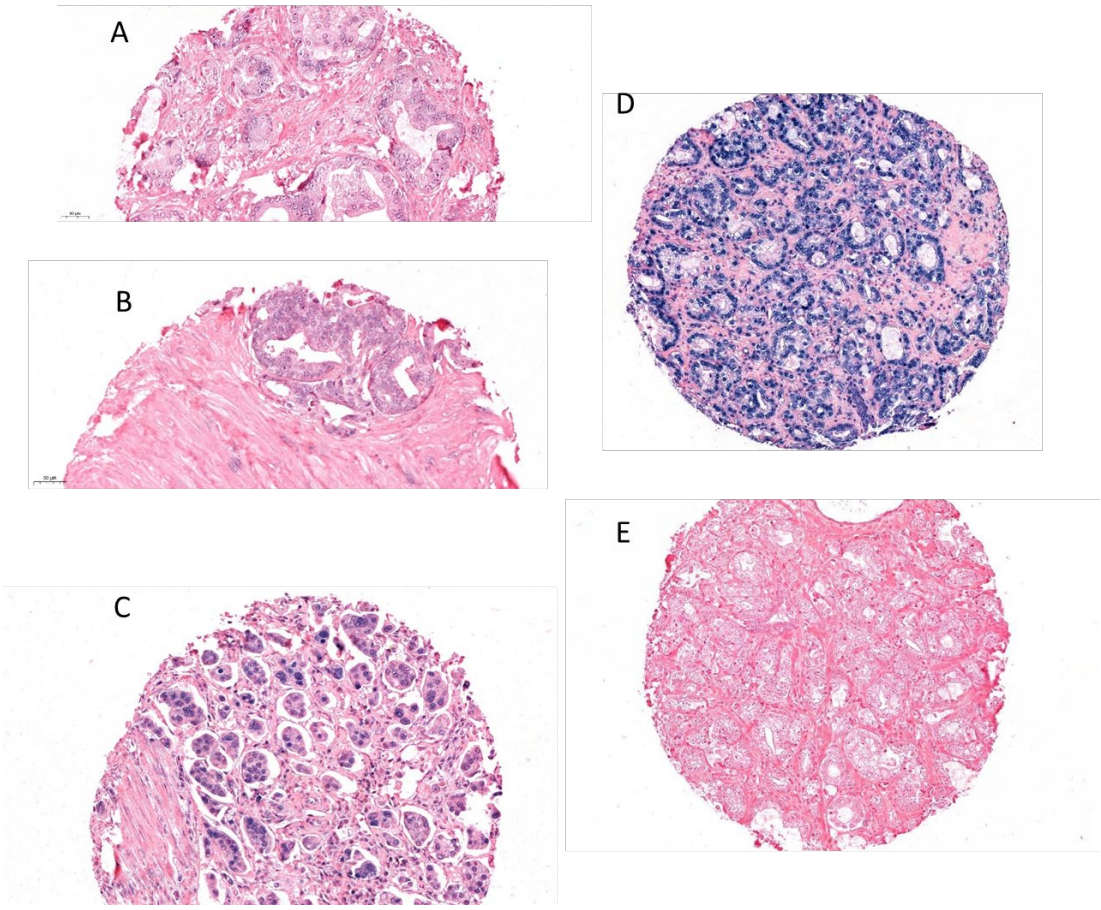

Figure 17 Panel of ISH stained cores. Representative scoring of miR-24-1-5p in tumor epithelium (TE). A) Weak expression B) Moderate expression C) Strong expression D) U6 positive control staining E) Scrambled miR negative control staining.

3.3 Statistical analysis

Descriptive characteristics of the study population were presented as means (standard deviation), median (range) or percent (numbers). Differences in the distribution of characteristics at study entry between non-PCa cases and PCa cases were assessed using t-tests for continuous variables and chi-square tests for categorical data.

Multivariable Cox proportional hazard models, with follow-up time as timescale, were used to investigate whether inflammation biomarkers (hs-CRP and WBC) or repeated assessments of hs-CRP were associated with PCa risk and severity (Paper 1), to investigate whether prediagnostic systolic or diastolic blood pressure were independently associated with PCa risk and mortality (Paper 2), and to study whether miR-24-1-5p and clinicopathological markers were independently associated with risk of PCa recurrence (Paper 3). Several variables were

assessed as potential confounders based on suggested biological mechanisms influencing PCa risk and prognosis, hypertension, and systemic inflammation. In paper 1, age at entry (continuous) and BMI (continuous), were included as covariates in the final models. In paper 2, age at entry (continuous), BMI (continuous), alcohol habits (categorical), smoking (categorical), physical activity (categorical), educational level (categorical) and diabetes (yes/no) were included as covariates in the final models. In paper 3, age at surgery (continuous), CAPRA-S (categorical), BMI (continuous), alcohol habits (categorical) and cholesterol levels (continuous) were included in the final models as covariates. The Kaplan-Meyer method was used for drawing survival plots in paper 2 and 3.

In paper 1, to study the importance of the variation in inflammation-related biomarkers in more detail, we used hs-CRP and WBC both as continuous and categorical variables, with tertile cut-points based on the distribution in the overall data set. We defined the systemic inflammatory score as the sum of tertile ranking for hs-CRP and WBC: tertile 1. Hs-CRP: ≥ 0.01 – ≤ 0.91 mg/L, WBC: ≥ 1.1 – ≤ 5.6 x 109/L/L, tertile 2. Hs-CRP: ≥ 0.92 – ≤ 2.03 mg/L, WBC: ≥ 5.7 – ≤ 7.0 x 109/L and tertile 3. Hs-CRP: ≥ 2.04 – ≤ 20 mg/L, WBC: ≥ 7. 1 – ≤ 15 x 109/L. The systemic inflammatory score ranged from 2 to 6 points; 5–6 was defined as a high score. We performed subgroup analyses by age at study entry (< 60 years vs ≥ 60 years), systolic BP (< 140 mmHg vs ≥ 140 mmHg), BMI (< 25 kg/m^2 vs ≤ 25 kg/m^2).

In paper 2, we performed separate analyses on PCa incidence in two age groups (age at entry < 45 years and age > 45 years). Furthermore, to study whether the association between prediagnostic blood pressure and mortality varied by type of PCa treatment, analyses were performed by type of treatment, curative or endocrine, within the PCa-cohort. To study the importance of the variation, prediagnostic systolic and diastolic BP were split in four levels based on international categories: Systolic BP (mmHg): <130, 130-139.9, 140-149.9, >=150 mmHg, diastolic BP (mmHg): <80, 80-89.9, 90-99.9, >=100 mmHg.

In paper 3, we performed subgroup analysis by systolic blood pressure (systolic BP ≥130mmHg). We also used the Spearman's Correlation coefficient for correlation analysis between miR-24-1-5p and clinicopathological markers. The five-year recurrence free percentage was calculated using the Kaplan–Meier survivor function, and statistical differences

between different groups (e.g ISUP grade group, EAU risk group, CAPRA-S) were tested by the log-rank test.

The proportional hazard assumption was verified by assessing the parallelism between log minus log survival curves for categories of blood pressure, tertiles of inflammatory markers, and low/high expression of miR-24-1-5p. We also performed formal tests based on Schoenfeld residuals. All statistical tests were two-sided using a significance level of $p < 0.05$ and conducted with STATA/MP version 15.1 and 16.1 (StataCorp LLC, College station, TX, USA).

4 MAIN RESULTS

4.1 Paper I

A total of 7 356 cancer-free men were included. Pre-diagnostic WBC and hs-CRP were assessed from blood collected at study entry; 2 210 participants also had a second CRP measure during follow-up. During a mean 11.8 years follow-up, 509 men developed PCa (mean age at diagnosis 71.7 years). Multivariable Cox proportional hazard regression models were used to study whether individual biomarkers (WBC, hs-CRP), a combined score based on analyte tertiles (score range 2-6) or change in CRP were associated with risk and severity of PCa. We observed a positive dose-response relationship between hs-CRP and PCa risk with a hazard ratio (HR) per mg/L of 1.3, 95% CI 1.00–1.07. Men with an increase in hs-CRP between two measurements (Δhs-CRP) of ≥ 1.00 mg/L had a 36% increased risk of PCa (HR 1.36, 95% CI 1.02 – 1.82), compared to men with no change or decrease in hs-CRP. Men with a systemic inflammatory score of 5 or 6 had a 68% higher risk of being diagnosed with metastatic disease (HR 1.68, 95% CI, 1.04–2.73) compared to men with lower scores.

4.2 Paper II

A total of 12 271 cancer were included. A total of 811 men developed PCa, and were followed for an additional 7.1 years, and we studied the association between prediagnostic BP and overall mortality among PCa patients. Men (> 45 years) with a systolic BP > 150 mmHg had a 35% increased risk of PCa compared to men with a normal systolic BP (< 130 mmHg) (HR 1.35, 95% CI 1.08-1.69). Among PCa cases, men with systolic BP > 150 mmHg had a 49% increased overall mortality compared to men with a normal systolic BP (HR 1.49, 1.06-2.01). Among PCa cases treated with curative intent, those with a high diastolic BP (> 90 mmHg) had a three-fold increase in overall mortality risk (HR 3.01, 95% CI 1.40-6.46) compared to cases with a normal diastolic BP (< 80 mmHg).

4.3 Paper III

A total of 22 577 cancer-free men were included, and a total of 947 men developed PCa during a mean follow-up time of 8.0 years. PCa patients with available tissue samples after prostatectomy with curative intent were identified (n=189) and were then followed for an additional 4.8 years. PCa prostatectomy specimens were collected from 142 patients, and detailed medical records including histology and type of treatment were obtained. The tissue expression of mature miR-24-1-5p in PCa was examined by in situ hybridization (ISH) in Tissue Micro Array (TMA) blocks. The expression of miR-24-1-5p was assessed by semi-quantitative scoring by two independent investigators in two areas of tumor epithelium (TE) and two areas of normal epithelium (NE) for each patient. Each area was scored as negative (0), weak (1), moderate (2), or strong (3). Multivariable Cox regression models were used to study the associations between miR-24-1-5p -expression and PCa recurrence. At time of prostatectomy, the PCa patients had a median age of 65.0 years (range 47-75 years), and mean prostate-specific antigen (PSA) at diagnosis was 10.5 ng/mL (SD 9.5). The tumor stage was pT2c for 47.9% of the patients, and median Cancer of the Prostate Risk Assessment Postsurgical Score (CAPRA-S) was 3. The average score for miR-24 expression was 1.60 in TE, 1.35 in NE and 1.49 in TE and NE combined. CAPRA-S group, International Society of Urological Pathology (ISUP) grade group, and European Association of Urology (EAU) Risk group were all significant prognostic factors for 5-years recurrence-free survival ($p<0.001$). PCa patients with a high miR-24-1-5p expression (≥ 1.57) in the tissue (TE+NE combined) had a doubled risk of recurrence (biochemical or clinical), compared to patients with low miR-24-1-5p- expression (HR 1.99, 95% CI 1.13-3.51).

5 DISCUSSION

5.1 General discussion

PCa is most likely caused by the interplay between genetics, lifestyle and the environment and these traits most probably influence each other through epigenetic changes. The interpretation of how, and to what extent these factors influence PCa development is challenging. However, a better understanding of the biological mechanisms involved in PCa development can potentially lead to preventive measures in the general population, and in that way reduce or delay the development of PCa in susceptible individuals. Advances in diagnostic and therapeutic methods may lead to earlier, better, and more precise treatment of PCa patients. Of note here is the need for better tools to assess risk levels in the individual patient, and in that way avoid both under-treatment and over-treatment. In this thesis we have investigated different aspects relevant to these strategies: In paper 1, we have studied if signs of systemic inflammation (i.e. increased levels of serum inflammatory markers CRP and WBC) are associated with increased risk of developing PCa later in life, and if the inflammation markers are associated with which risk group of PCa the patient falls within. In paper 2, we have shown that increased blood pressure can affect the risk of developing PCa later in life. We have also shown that prediagnostic blood pressure affects overall mortality among PCa patients. In paper 3, we have studied wether the expression of a biomarker in prostate tissue (miR-24-1-5p) is associated with risk of recurrence after prostatectomy.

5.2 Methodological considerations

5.2.1.1 Study design

This thesis is based on data included in the PROCA-life study, a sub study of the Tromsø Study, a population-based, prospective cohort study. Cohort studies are by design able to assess causality, and therefore have the potential to provide strong scientific evidence. Baseline characteristics from a large number of participants have been collected and are then followed to see if the event of interest occurs. This can be an expensive and time-consuming study design, but also allows the researchers to study the natural history of suspected risk factors. If the results from a cohort study are to be relevant for the general population, the cohort must be based on a representative sample.

The null hypothesis in an experiment or research model states that there is no association between the exposure and the outcome. If we reject the null hypothesis when it really is true, this is called a Type 1 error. On the other hand, a Type 2 error occurs if we don't reject the null hypothesis when it really is false, in other words if the study states that there is an association between the exposure and the outcome when there really isn't. Type 1 errors are considered the most serious, and the tradition in medical research is to accept a <5% probability of a Type 1 error ($p<0.05$). In Cox regression models, this translates to a 95% confidence interval that doesn't include 1.0. The statistical power of a study describes the ability to detect an association if such exists, and the goal for most studies in medical research is a power of ≥80%. This translates to a probability of making a Type 2 error of <20%.

Power calculations for the present thesis are based on the planning phase of the PROCALife-study, showing that we would need a sample of 400 PCa cases in order to have reasonable power to detect significant differences (table 10). Further on, we assumed based on recent comparable studies that the same numbers of cases were sufficient for studying the associations between inflammatory markers (paper I- 509 PCa cases) and hypertension (paper II- 811 PCa cases) and PCa. No power calculations were done for paper III, but previous work in our group have indicated that we had enough power (paper III- 189 PCa cases).

Table 10 Estimated power calculated; clinically relevant differences in variables between cases (prostate cancer) and non-cases. Number of persons without cancer (controls) are hold constant in this estimation, n = 5000.

Variable	Standardized difference	Case mean (SD)[2]	Non-casemean (SD)[2]	Power (%) # incident prostate cancer cases			
				n = 50	n = 100	n = 200	n = 400
HDL-Cholesterol	0.38	1.36 (0.42)	1.52 (0.42)	76	96	99	100
Tot Cholesterol/ HDL	0.30	(1.03)	(1.03)	56	85	99	100
Waist	0.22	80.8 (10.7)	78.3 (11.3)	33	58	86	99
Triglyceride	0.36	1.85 (1.4)	1.34 (1.4)	73	95	100	100
% sedentary	0.25	15%	25%	34	66	93	99
Heart Rate	0.6	71.3 (11.5)	64.4 (11.4)	98	99	100	100
BMI (Body Mass Index)	0.56	25.7 (3.2)	23.9 (3.2)	98	99	100	100

5.2.1.2 Study participants

The PROCA-life study, based on the Tromsø study, recruited residents of the Tromsø municipality to participate based on the official population registry and according to birth

cohorts. The high attendance proportion (mean 70%), the age range (25-97 years) in the Tromsø study and the mandatory reporting of all types of cancer including PCa reduce the risk of selection bias and increase both the internal and the external validity. Thus, the finding observed in the PROCA-Life study may be true for the population studied, and the study population may represent the general population of the Tromsø area. However, the population of Tromsø is mainly white, and shows unique characteristics of geography and lifestyle such as the high latitude and long winter one might argue that our results are not extendable to other populations of the world, and the degree of external validity of our results can therefore be debated.

5.2.1.3 Study variables

Most of the study variables used in the present thesis have either been validated or are measured and used in comparable studies.

Lifestyle variables

Questionnaires were used to collected data about the participant's lifestyle factors (e.g. education, physical activity, smoking habits, and alcohol consumption). Questionnaires were usually filled in at home and brought to the study site, where they were checked for inconsistency by trained health personnel, or they were filled in by health personnel as part of an interview at site on study entry. The questionnaires have developed and have been validated throughout the period of the Tromsø study (181).

Serum variables

Blood samples were drawn by trained research assistants on attendance at each survey and were non-fasting. Analyses of serum samples were done at the Department of Laboratory Medicine, University Hospital of Northern Norway (UNN), Tromsø, Norway (181), which is an accredited laboratory. Blood was withdrawn in a non-fasting state at the study site, and time since the last meal was recorded. Non-fasting blood sampling could lead to a non-differential misclassification.

Systolic and diastolic blood pressure

Systolic and diastolic blood pressures were measured three times with an oscillometric digital automatic device, measurements being separated by a 1-min interval after 2-min seated rest.

The average blood pressure from the lowest two values was chosen for the analysis. These standard procedures prevent systematic and random errors, ensuring accuracy, and have been validated (186;187)

miR-24

The methodology for TMA-production and in situ hybridization has been used in our laboratory for several years with different tissues and miRNA's and is well tested (121-123;188). Equipment and supplies have been validated. The scoring of miRNA-expression was semi-quantitative, with two independent observers. Inter-observer variability was assessed by calculating linear weighted Kappa statistics and showed a moderate agreement (Kappa 0.59 (SD 0.50-0.68)).

Endpoints – outcomes; PCa diagnosis, overall death, recurrence

PCa cases were identified through linkage with the Cancer Registry of Norway. All hospitals in Norway are obliged to report malignant diseases to the Cancer Registry, which, since 1952, has systematically collected all incidences of cancer for the Norwegian population. The registration in the Cancer Registry of Norway is considered to be close to complete (98.8%) (189). The accuracy and reproducibility of PCa reports depend on the uniformity of diagnosis by each pathologist and between pathologists. A majority of the pathologists in Norway are trained in a few laboratories and they participate in national and international seminars. The Cancer Registry is matched regularly against the Death Registry of Norway, to obtain information about emigration and death. In our study there is a minimal, if any, loss to follow-up and no influence on risk estimates from inadequate reporting (diagnostic bias/measurement bias).

Death among PCa patients were identified by linkage to the Death Registry of Norway, which is considered to be close to complete (98%) (190). In addition, all registrations where cross-checked and verified with the patients' medical journal. The diagnosis directly causing death or underlying diseases which may contribute to death are coded according to ICD-9 or ICD-10 classification in the Death Registry of Norway, and have been valid for patients diagnosed with cancer(191). Cause of death still can be a less accurate variable, and we chose to use only overall death in our analysis.

Information on recurrence after PCa treatment were collected from medical journals. We used objective, standard international definitions (PSA >0.2 or radiological evidence of metastasis).

Height and weight

BMI (kg/m^2) was calculated according to measured height and weight at the study site according to standard protocols to minimize any misclassification due to weight of clothes, height of shoes etc. Weight measures for all participants were performed by trained health personnel, reducing systematic errors and excluding recall bias.

5.2.1.4 Validity

Validity refers to how accurately a method measures what it is intended to measure and can be divided in external and internal validity (192).

External validity refers to how the results can be generalized to other populations or groups. The background study population for this thesis (The Tromsø Study) have included large, representative samples of the Tromsø population, with invitation of whole birth cohorts and random samples, and has an overall high attendance proportion (177). This is an argument for a high generalization to the Tromsø population, and possibly also the rest of the Norwegian population.

Internal validity refers to how a study establishes a trustworthy cause-and-effect relationship between an exposure and an outcome. By minimizing systematic errors (bias and confounding) we can assume that the results are correct and valid for the study population of interest.

5.2.1.5 Bias and confounding

Bias is systematic errors in the design or conduction of a study, that leads to results that deviate from the truth. In epidemiological studies, the majority of biases can be classified as selection bias or information bias (192).

Selection bias will occur if the selection of participants in a study is done in a skewed or non-randomized way. Participation in the Tromsø Study was based on invitation. One may speculate

that men who are more conscious about their health may be more likely to attend a health study than others, which again may be associated with higher education and socioeconomic class, thus creating a selection bias. However, approximately 70% of the invited enrolled in the study, and the study population (n=22 000) is large, so the effect of selection bias is considered small.

Information bias, or measurement bias, is caused by inaccurate measurement or definition of study variables. This misclassification can be non-differential or differential. Non-differential or random misclassification is independent of the outcome and could come from random errors in the data collection process, such as typing errors. A high degree of non-differential misclassification will generally lead to an underestimation of the associations between exposure and endpoint. In this thesis random misclassification can occur on many levels: Human errors in typing could occur in the baseline surveys (Tromsø Study) or during collection of clinical data from medical journal. However, the baseline surveys have been conducted in a professional way to minimize errors, and the clinical data were plotted in a careful and thorough way.

Differential misclassification occurs when the error rate or probability of being misclassified differs across groups of study subjects (193). All subjects in our study participated in the baseline survey before they were diagnosed with PCa. In addition, we excluded cases that were diagnosed within one year after the baseline survey, to avoid interference from sub-clinical disease. The major endpoints in our study (PCa, death) are collected from national registries with high completeness (The Cancer Registry of Norway, The Norwegian Cause of Death Registry), and have also been verified with information from the medical journals. This means that there is low risk of false endpoints included.

Systolic and diastolic blood pressures were measured by an automatic device, and mean values of the second and third measurements were used. These procedures reduce the risk of systematic and random errors. Serum sample analyses (CRP, WBC, cholesterol) were done at accredited laboratories.

Confounding is defined as the distortion of a measure of the effect of an exposure on an outcome due to the association of the exposure with other factors that influence the occurrence of the outcome (193). A confounding variable is a variable that influences both the dependent variable and independent variable. Age is an important risk factor for PCa but is also associated with increasing blood pressure and increasing CRP and is therefore a confounding factor in our

study. In paper I and II we have adjusted for age in all analyzes. Several of the lifestyle variables in this study are related, for example smoking, alcohol use, and lack of physical activity. We have therefore used multivariable analyzes to adjust for the potential confounding effect of these variables.

5.3 Discussion of the main findings

Based on plausible biological mechanisms we studied the association between prediagnostic hs-CRP and WBC, prediagnostic systolic and diastolic blood pressure and miR-24-1-5p, a subtype of microRNA, in the PROCA-life cohort study. This may enable us to discuss and sometimes generate causality. However, a set of nine criteria is often used to discuss and evaluate if causality is probable*: Strength, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment and analogy* (the Hills criteria) *(194).* These are not definite criteria (195), however because exceptions are likely to be frequent, the main results of the thesis are discussed in the light of these criteria when relevant.

PCa develops when normal cells in the prostate change into malignant cells due to a complex process (19;46), where stimulation from low-grade chronic inflammation might play an important role (4). Using the data that is collected through the population-based PROCA-life study, we have tried to assess the relation between inflammation and PCa from three different angles: Serum biomarkers (hs-CRP and WBC), clinical measurements (blood pressure), and tissue biomarkers (miR-24-1-5p).

5.3.1 Paper I

Inflammatory serum markers and risk and severity of prostate cancer. The PROCA-life study.

Serum levels of WBC and hs-CRP were assessed pre-diagnostic, and we excluded men diagnosed with any cancer < 1 year after study entry, reducing the chance that undiagnosed malignancy would affect the value of our exposure variables. Hence, the exposure precedes the outcome.

However, we had mainly a single measure point and thus changes in various clinical variables over time may have occurred. We do not know if the participating men have been living in a state of chronic inflammation for many years, or if this was a one-time incident at the baseline survey. We have tried to assess this question by using repeated measurements, but the study population with more than one measurement of hs-CRP was somewhat limited (total n=2 210, PCa cases n= 220). We calculated Δhs-CRP: the difference in hs-CRP between the first and the second measurement. We observed that Δhs-CRP was associated with risk of PCa both as a continuous variable or dichotomized as Δhs-CRP ≥ 1.00 mg/L (yes/no). Hence, the exposure precedes the outcome, and repeated assessment strengthen the overall finding of temporality.

In this study we observed that hs-CRP measured at one and two time points was associated with PCa risk in a positive dose–response manner. We designed a systemic inflammatory score (hs-CRP and WBC in combination) and observed that men with a high systemic inflammatory score had a 28% higher PCa risk and were more likely to be diagnosed with metastatic PCa compared to men having a low systemic inflammatory score. The effect size is limited and might not support causality.

The score was strongly associated with both risk for PCa risk and for severity of PCa. An inflammatory score might be a useful way of combining two or more inflammatory markers that could be used for risk classification (196), and could also increase consistency. A biologic gradient was supported because we observed a positive dose-response gradient between hs-CRP and PCa risk.

Several studies have questioned whether a systemic inflammatory score could be a valuable predictive tool for worse outcome in several types of cancers including PCa (197;198), and our results support the hypothesis that it might be valuable for PCa severity.

Both CRP and WBC are subject to rapid changes due to acute illness. We have excluded men with high levels (hs-CRP > 20 mg/L and/or WBC > 15 x 10^9cells/L) to avoid the influence of acute illness, but the inflammation markers represent a continuum, and these cut-off values are arbitrary. The specificity of our results thus might be limited. However, other studies have shown that small changes in inflammatory markers can be used for risk prediction. For example

in risk calculators for coronary heart disease, where relative risk for incident coronary heart disease was 1.58 (95% CI, 1.37 to 1.83) for CRP levels greater than 3.0 mg/L compared with levels less than 1.0 mg/L (67).

Results from previous studies of the association between hs-CRP or WBC and PCa risk have been inconsistent. Our findings that hs-CRP measured at one time point were associated with PCa risk are supported by some studies (62;196;199), but our results are also in contrast to others (200-205). A recent systematic review and meta-analysis by Michels and coworkers, published after our study, included 103 publications on inflammatory markers and cancer risk (17 papers about PCa) and observed a positive association between CRP and PCa (HR = 1.09 [95% CI 1.03–1.15]). However, they also point to the need for improvements in study quality by better verification of inflammatory status (more than one baseline measurement of one parameter), adjustment for important confounders and the need for long-term follow-up (206). To our knowledge, our study is the first study to assess the combination of hs-CRP and WBC (by using a systemic inflammatory score) in relation to PCa risk. Interestingly, our findings suggest that compared with using either WBC or hs-CRP alone, a combination of these markers may be more useful.

Inflammation, a hallmark of cancer that was recently discussed and included by Hanahan (4) as well as by others (19;207), support a role of inflammation in PCa development. Both hs-CRP and WBC may be markers of inflammation and thus biological processes that could potentiate carcinogenesis in the prostate. The observation of a relationship between inflammation and PCa has been suggested by others based on observation of local inflammation in PCa biopsies (40;48-50).

A high hs-CRP and WBC may be a marker of biological mechanisms associated with inflammation and may influence development of PCa. Furthermore, these markers may also be biomarkers of importance for optimal treatment and thus survival among PCa patients (208-210). Hence, the exposure preceding the effect in our study is in line with biological plausibility and natural history of the disease, but we cannot conclude causality between hs-CRP and WBC alone or in combination and PCa development.

5.3.2 Paper II

Systolic and diastolic blood pressure, prostate cancer risk, treatment and survival. The PROCA-life Study.

In this paper we observed a dose-response association between prediagnostic systolic BP and PCa risk in men >45 years at baseline, and a positive dose-response relationship between prediagnostic diastolic BP and risk of PCa in all men. The framework of prediagnostic exposure measured before outcome (risk and mortality) supports temporality, a necessity for evaluating the causality between blood pressure and PCa risk.

Among PCa cases we observed a positive dose response association between both systolic and diastolic BP and overall mortality. This was most evident among PCa cases treated with curative intent, where cases with a high diastolic BP (> 90 mmHg) had a three-fold increase in overall mortality risk compared to cases with a normal diastolic BP (< 80 mmHg). This strong effect size supports a possible causal relationship, but several other criteria are needed in order to conclude causality.

Several studies have hinted towards an association between hypertension and cancer incidence: (87;92;211-213). Liang and co-workers fulfilled a meta-analysis where 21 cohort and case-control studies were included. They reported 8% higher PCa risk among hypertensive people. However, they did not consider individual study design and quality (80). In contrast, other large studies such as the European Prospective Investigation into Cancer and Nutrition, or the meta-analysis by Seretis and coworkers in 2019 did not observe any associations between hypertension and risk of PCa (81;82).

As in paper 1, the exposure variables and other baseline variables were based on a single time pre-diagnostic measure. However, tracking studies from the same cohort of men as our study (the Tromsø Study) have shown that men tend to follow a trajectory of BP suggesting an accumulated lifetime exposure (180). In contrast, a Swedish study including 330 000 men that were enrolled between 1971-1993 with a mean age at entry of 34.7 years, found that both systolic and diastolic BP were associated with a decreased risk of incident PCa (99). These findings may suggest that the association between elevated blood pressure and PCa may vary by time periods due to several factors, including improved diagnostic possibility of PCa and an

aging population at risk. The frequency of PSA-testing in the population increased during the study period, which also influences the incidence of PCa, and the age at diagnosis (214). Clinical guidelines for elevated systolic and diastolic BP vary throughout different time periods, and treatment for hypertension is initiated at a lower level of diastolic and systolic BP today compared with 1970s- '80s. These settings may complicate interpretation and comparisons between studies regarding raised BP and PCa risk.

In many men diagnosed with PCa, hypertension will not be an isolated condition, but will occur together with other risk factors for cardiovascular disease as part of a metabolic syndrome, and other lifestyle factors. In our analysis we have adjusted for the potential confounders age, BMI, alcohol habits, smoking, physical activity, educational level and diabetes. However, it is possible that the interplay between these factors is more complex, and that this in part explains the differences in previous studies. Many of the risk factors for cardiovascular disease are linked to systemic inflammation, as are many of the risk factors suggested for development of PCa (215-217). More research is needed to determine whether systemic inflammation caused by both raised systolic and diastolic BP plays a role or shares common biological pathways influencing PCa development, or if pre-malignant cells cause the inflammation that causes the hypertension.

The second aim of paper II was to investigate if prediagnostic systolic and diastolic BP were associated with overall mortality among PCa patients. Recently there has been an increased interest in the interaction between cardiovascular disease and cancer, in general and including PCa. This raised interest has been triggered by the increase in number of cancer survivors, more advanced cancer treatment with cardiovascular side effects, and the development of a new discipline called "cardio-oncology". This subspecialty of cardiology focuses on "primary and secondary risk approaches through surveillance as well as interventions to stratify and diminish cardiovascular risk, to preclude cardiovascular toxicity and its progression, and to manage the adverse effects of anticancer treatments"(218) . Also "reverse cardio-oncology" has gained interest and focuses on increased cancer risk in patients with cardiovascular disease due to common risk factors, as mentioned above.

Our findings indicate that prediagnostic blood pressure influences overall mortality even after a cancer diagnosis, several years after the blood pressure was recorded. We see this as an

argument for careful monitoring of blood pressure, as it affects overall survival. The sample size was not large enough to conduct detailed subgroup analysis on cause of death, nor subgroup analysis on PCa risk group. A larger study population and repeated measurements of blood pressure over time, both pre- and post- diagnosis, is needed to better understand the interplay between blood pressure, PCa and mortality.

5.3.3 Paper III

Expression of microRNA miR-24-1-5p in tumor tissue influence prostate cancer recurrence. The PROCA-life Study.

We observed that a high expression of miR-24-1-5p was associated with an almost doubled risk of recurrence (biochemical or clinical) after radical prostatectomy, when adjusting for known histopathological risk factors. Only a few experimental studies have investigated the role of miR-24-1-5p in PCa (134-136;219), and more studies are needed (220).

To our knowledge, this is the first study to investigate whether the expression of miR-24-1-5p in PCa tissue is associated with prognosis. A recent meta-analysis studied the prognostic significance of miR-24 in various cancers and found that high miR-24 expression was associated with poor overall survival (221). The meta-analysis consisted of 17 studies, and a total of 1705 patients, of whom none had PCa. A few studies have evaluated the other mature sequences of miR-24, miR-24-3p, which has been suggested as a diagnostic biomarker for PCa in serum (222), or as a tumor suppressor in PCa cell lines (223). However, it is unclear whether these results will be valid for the association between miR-24-1-5p and PCa development.

Circulating miR-24 has been observed to be elevated in diabetes, breast cancer and lung cancer, and down-regulated in PCa and hepatocellular carcinoma. It can function as an oncogenic or tumor suppressors dependent on cancer subtypes (128). miR-24 has also been linked to inflammation (127). In a murine model, miR-24 was a central regulator of vascular inflammation (132). In a model with primary human macrophages, miR-24 would produce anti-inflammatory action by inhibiting the production of pro-inflammatory cytokines, and these results suggest that overexpression of miR-24 would have mostly anti-inflammatory effects

(133). miR-24 belongs to the miR-23~27~24 cluster and this cluster has been shown to reduce TNF-α and IL-6 production (224).

Despite the limited current knowledge, miR-24 is an interesting molecule because of its dual role in both cardiovascular diseases and cancer (128). miR-24 protects cardiomyocytes and reduces cardiac fibrosis, but also inhibits angiogenesis and worsens heart failure (128). miR-24 seems to be a multi-functional cardio-miRNA that plays good and bad roles in heart failure (128).

Our results are based on a relatively limited sample of patients with PCa prostatectomy specimen (n=142) but include detailed histopathological and medical records for all the patients. Our study uses human PCa tissue, while earlier studies have focused on murine models and cell lines. The methodology for TMA-production and in situ hybridization has been used in our laboratory for different tissues and is well established (121-123;188). However, the small sample size limited the possibility to perform sub-group analysis, and we were not able to test the expression of miR-24-1-5p in other samples such as serum or urine, or in prostate tissue form non-cancer patients. The scoring of miRNA-expression was semi-quantitative, and thus subject to variability and human errors. The patients were all from a single center, and the results have not been validated in a separate cohort.

There are proposed biological mechanisms linking miR-24-1-5p to PCa recurrence. Hence, we may propose that our results are in coherence with the biological mechanisms operating.

6 CONCLUSION

In summary, our findings from the population based PROCA-life study, a sub study of the prospective Tromsø Study, suggest that high levels of prediagnostic systemic inflammatory markers such as high sensitive-CRP and white blood cell count (WBC), prediagnostic systolic and diastolic blood pressure and miRNA -24-1-5p, a subtype of microRNA, may play a role in PCa risk, PCa aggressiveness, recurrence and mortality.

- Our findings support a positive association between prediagnostic hs-CRP, hs-CRP and WBC in combination and risk for both PCa and for metastatic PCa. Our findings contribute to understanding the relationship between inflammation and PCa development and may be useful in future research. However, larger studies are needed.

- Our findings suggest that both elevated prediagnostic systolic and diastolic BP are associated with PCa risk, and with overall mortality. Our results support that systolic and diastolic BP are important factors when balancing disease management in PCa patients.

- Our findings suggest that a high expression of miR-24-1-5p is associated with an increased risk of recurrence of PCa after radical prostatectomy pointing to a potential diagnostic and therapeutic value of detecting miR-24-1-5p in PCa patients. However, larger studies are needed.

7 IMPLICATIONS FOR FURTHER RESEARCH

PCa is a heterogeneous disease, and may be a low-risk, indolent tumor localized in the prostate or a high-risk, aggressive tumor that may metastasize and prove lethal if untreated. Furthermore, while PCa is a major cause of health loss and death world-wide, the biological mechanisms involved in prostate cancer development are mostly unknown. Findings summarized by the World Cancer Research Fund/American Institute for Cancer Research Third Expert Report Diet, Nutrition, Physical Activity and Cancer: a Global Perspective underline that beside being overweight or obese, which increase the risk of advanced prostate cancer, much remains unknown when it comes to preventive factors (225). However, migration studies have shown that when men move from low-incidence- to high-incidence areas, their risk of PCa increase considerably (13;14). This underlines the need for more studies focusing on the effect of lifestyle on prostate cancer risk.

Chronic inflammation, one of the hallmarks of cancer development, has been suspected of playing a key role in PCa development. Langston and coworkers have recently described how the concept of colliding bias can be an explanation for why the link between prostate cancer and inflammation can be observed in animal models and histopatologic observations, but are harder to discover in epidemiological studies (226). They suggest using autopsy-studies, or studies on patients that had surgery because of benign prostate hyperplasia and not PCa. It is possible that studies with this design could be conducted within the PROCA-life framework.

The global high prevalence of both hypertension and PCa have led to several studies investigating whether an association between these two conditions exists (80-82). However, the importance of elevated BP in relation to PCa shows variation by age at onset of hypertension, exposure time, age when diagnosed with prostate cancer, and aggressiveness of disease. Much remain unknown (87). Larger studies that include repeated measurements of diastolic and systolic blood pressure is needed in order to improve knowledge regarding balancing disease management in PCa patients with low-risk and high-risk PCa.

The widespread use of PSA test, a test which is not specific enough in diagnosing PCa, has led to an increase in PCa incidence and results in a high proportion of false positive as well as detection of indolent disease (227). The risk stratification based on PSA, Gleason score and TNM-stage might not be precise enough, and might have led to an overtreatment of patients

without any benefit. Several new models have been developed and combine individual risk factors such as clinical and genetic information to create more precise screening methods. The Stockholm3 model has been observed to increase the proportion of clinically significant cancer in performed prostate biopsies, and an estimated reduction in direct health care costs (149). More studies are needed in order to reduce false positive tests and detect the PCa tumors that are most aggressive and may metastasize (228).

The miRNAs are associated with both regulation of gene expression and are “fine-tuners” of the immune system. Thus, miRNAs have been studied for their potential to serve as molecular prognostic biomarkers for PCa. Differences in miRNA expression profiles between tumor and normal tissues have been observed for PCa as well as for other cancer types (229;230). In a recent systematic review, fifteen miRNAs were associated with PCa prognosis, but miR-24 was not included (230). Further research is needed in order to clarify the role of miRNAs including the role of miR-24 as a potential important clinical tool.

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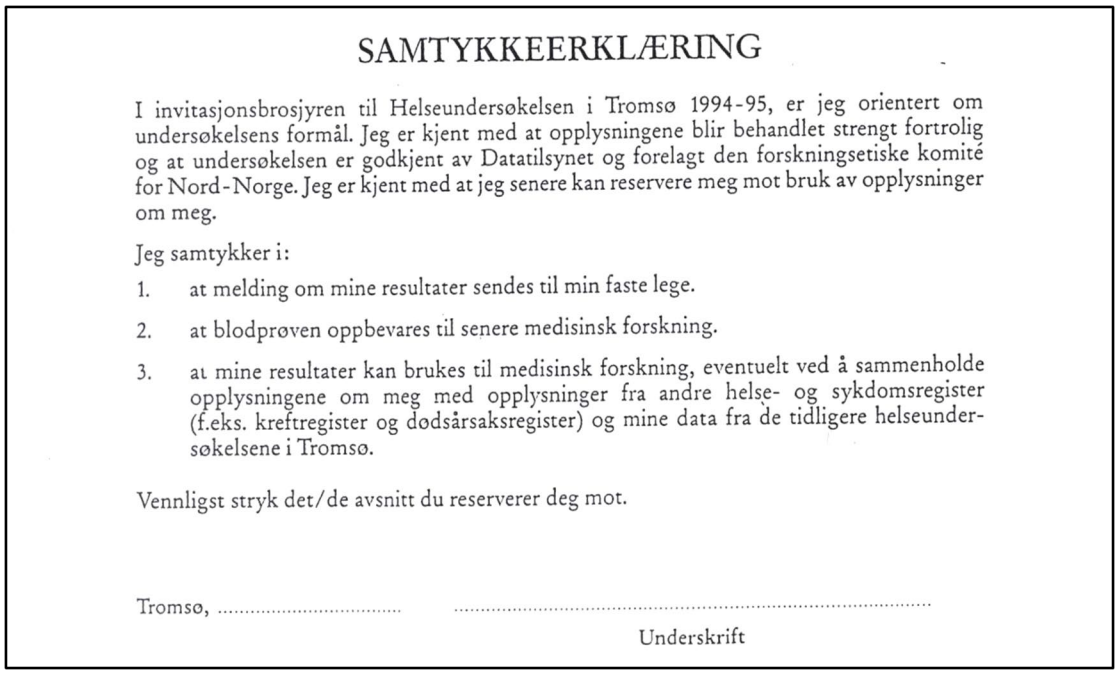
APPENDIX

Consent forms from Tromsø 4, Tromsø 5 and Tromsø 6



SAMTYKKEERKLÆRING

I invitasjonsbrosjyren til Helseundersøkelsen i Tromsø 1994-95, er jeg orientert om undersøkelsens formål. Jeg er kjent med at opplysningene blir behandlet strengt fortrolig og at undersøkelsen er godkjent av Datatilsynet og forelagt den forskningsetiske komité for Nord-Norge. Jeg er kjent med at jeg senere kan reservere meg mot bruk av opplysninger om meg.

Jeg samtykker i:

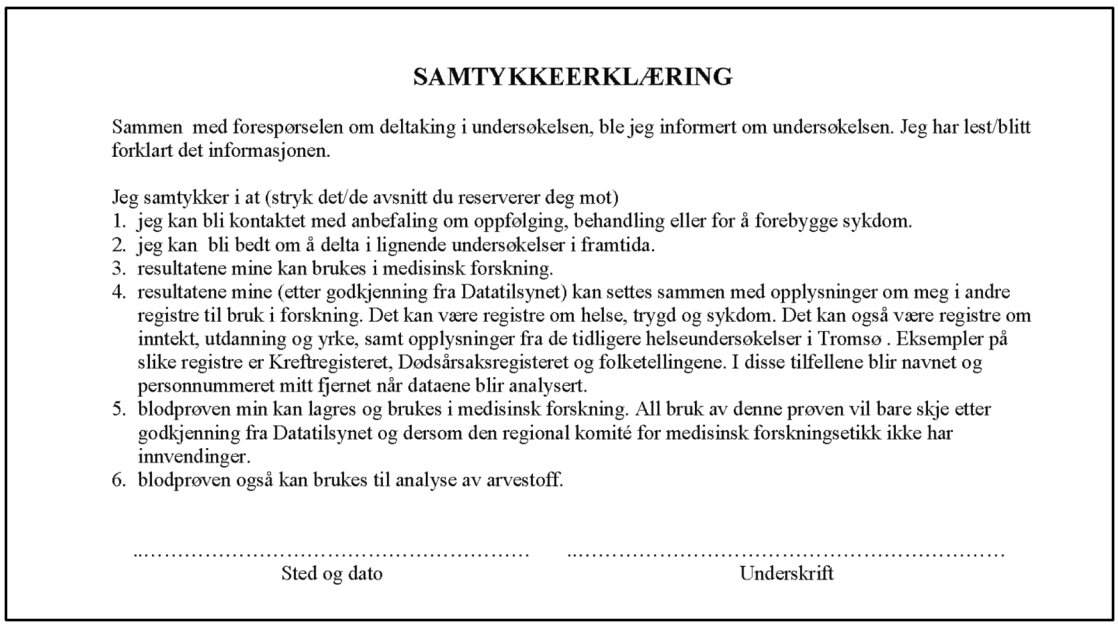
1. at melding om mine resultater sendes til min faste lege.
2. at blodprøven oppbevares til senere medisinsk forskning.
3. at mine resultater kan brukes til medisinsk forskning, eventuelt ved å sammenholde opplysningene om meg med opplysninger fra andre helse- og sykdomsregister (f.eks. kreftregister og dødsårsaksregister) og mine data fra de tidligere helseundersøkelsene i Tromsø.

Vennligst stryk det/de avsnitt du reserverer deg mot.

Tromsø, ...

Underskrift

Figure 18 Consent form Tromsø 4



SAMTYKKEERKLÆRING

Sammen med forespørselen om deltaking i undersøkelsen, ble jeg informert om undersøkelsen. Jeg har lest/blitt forklart det informasjonen.

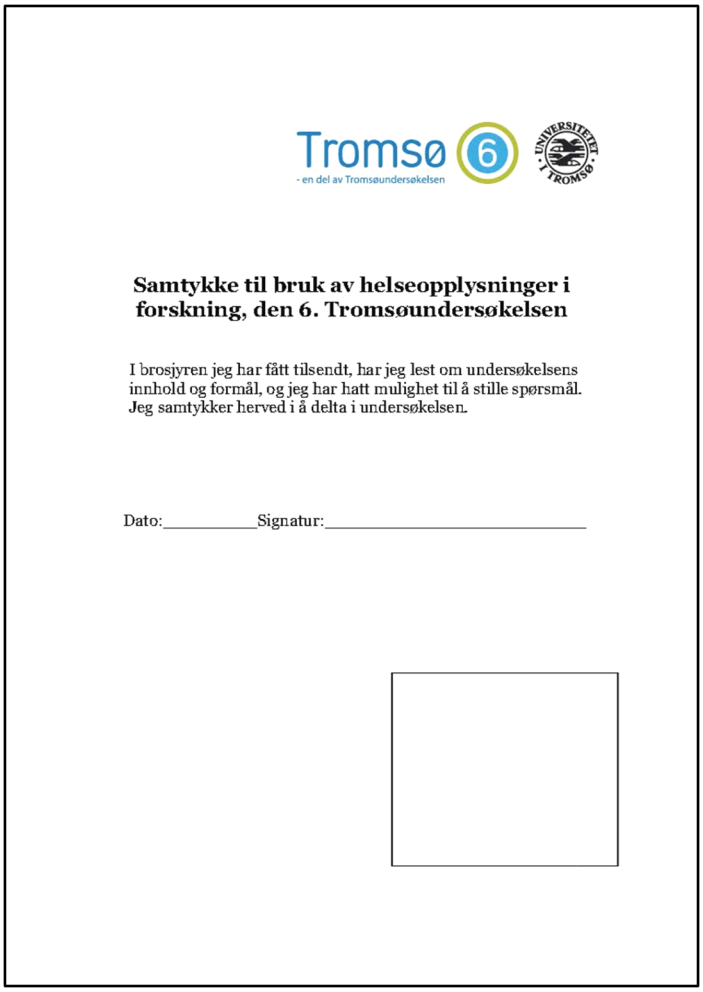
Jeg samtykker i at (stryk det/de avsnitt du reserverer deg mot)

1. jeg kan bli kontaktet med anbefaling om oppfølging, behandling eller for å forebygge sykdom.
2. jeg kan bli bedt om å delta i lignende undersøkelser i framtida.
3. resultatene mine kan brukes i medisinsk forskning.
4. resultatene mine (etter godkjenning fra Datatilsynet) kan settes sammen med opplysninger om meg i andre registre til bruk i forskning. Det kan være registre om helse, trygd og sykdom. Det kan også være registre om inntekt, utdanning og yrke, samt opplysninger fra de tidligere helseundersøkelser i Tromsø . Eksempler på slike registre er Kreftregisteret, Dødsårsaksregisteret og folketellingene. I disse tilfellene blir navnet og personnummeret mitt fjernet når dataene blir analysert.
5. blodprøven min kan lagres og brukes i medisinsk forskning. All bruk av denne prøven vil bare skje etter godkjenning fra Datatilsynet og dersom den regional komité for medisinsk forskningsetikk ikke har innvendinger.
6. blodprøven også kan brukes til analyse av arvestoff.

....

Sted og dato Underskrift

Figure 19 Consent form Tromsø 5



Tromsø 6
- en del av Tromsøundersøkelsen

Samtykke til bruk av helseopplysninger i forskning, den 6. Tromsøundersøkelsen

I brosjyren jeg har fått tilsendt, har jeg lest om undersøkelsens innhold og formål, og jeg har hatt mulighet til å stille spørsmål. Jeg samtykker herved i å delta i undersøkelsen.

Dato:_____Signatur:_____

Figure 20 Consent form Tromsø 6

Clinical variables collected from medical journals

Nr	Name	Options		Description
1	UNIKT_LOPENR			Id
2	Foedselsnummer			Foedselsnummer
3	Foedselsdato			Foedselsdato
4	Personnummer			Personnummer
5	TromsoeID			ID-nummer i Tromsøundersøkelsen
6	Seksuell_funksjon_pre	,00	Normal	Seksuell funksjon ved diagnosetidspunkt
		1,00	Nedsatt	
		9,00	mangler informasjon	
7	Aarsak_utredn_PSA	,00	Nei	Var forhøyet PSA årsak til utredning av kreften?
		1,00	Ja	
		9,00	vet ikke	
8	Aarsak_utredn_urinsympt	,00	Nei	Var urinveissymptomer årsak til utredning?
		1,00	Ja	
		9,00	vet ikke	
9	Aarsak_utredn_palpasjon	,00	Nei	Var palpasjonsfunn årsak til utredning?
		1,00	Ja	
10	Aarsak_utred_metastase	,00	Nei	Var mistanke om metastaser årsak til utredning?
		1,00	Ja	
11	Aarsak_utredn_TURP	,00	Nei	Ble kreften tilfeldig påvist ved TUR-P (eller andre kir. inngrep)?
		1,00	Ja	
		9,00	vet ikke	
12	Klinisk_diagnosetid			Tidspunkt for klinisk diagnose
13	PSA_diagnose			PSA-verdi ved diagnose
14	Histologi	,00	Adenocarcinom	Histologisk type
		1,00	Annen histologi	
		2,00	Ingen histologi	
		9,00	Ikke angitt	
15	Histologi_annen			Om annen histologi enn adenocarcinom, spesifiseres her
16	Gleason_1			Gleason grade primary (høyeste hvis flere bx)
17	Gleason_2			Gleason grade sec (fra samme bx som Gleason_1)
18	Biopsier_antall			Totalt antall biopsier som ble tatt
19	Biopsier_ant_pos			Antallet biopsier som var positive (inneholdt kreft)
20	Biopsier_mengde_vev			Hvor mye vev (i mm) ble fjernet ved biopsitaking?
21	Biopsier_mengdevev_positive			Hvor mye av fjernet vev ved biopsitaking inneholdt kreft (i mm)?
22	TUR_spon_totalt			Hvor mye vev fra TUR-P totalt?
23	TUR_spon_positiv			Hvor mye inneholder kreft?
24	Biopsi_kommentarer			Kommentarer til biopsi/diagnose
25	Tstad_klinisk	1,00	cT1	Klinisk T-stadium
		2,00	cT2	

		2,50	cT3	
		3,00	cT3A	
		4,00	cT3B	
		5,00	cT4	
		9,00	cTX	
26	Radiologi_bekken	,00	ingen radiologi	Ble det tatt CT eller MR i forbindelse med utredning, for T og N stadium?
		1,00	CT bekken	
		2,00	MR prostata	
		3,00	MR bekken	
		4,00	Både MR prostata og bekken	
27	Radiologi_skjelett	,00	Ingen	Ble det tatt CT, MR eller skjelettscintigrafi for vurdering av skjelettet?
		1,00	Skjelettscintigrafi	
		2,00	MR axialskjelett	
		3,00	Både skjelettscintigrafi og MR	
		4,00	PSMA-pet	
28	Tstad_radiologisk	1,00	T1	Radiologisk T-stadium
		2,00	T2	
		3,00	T3A	
		4,00	T3B	
		5,00	T4	
		9,00	TX	
29	Nstad_radiologisk	,00	N0	Radiologisk N-stadium
		1,00	N1	
		9,00	Nx	
30	Mstad_radiologisk	,00	M0 (ingen fjernmetastaser)	Radiologisk M-stadium
		1,00	M1a (ikke-regionale lymfeknuter)	
		2,00	M1b (skjelettmetastaser)	
		3,00	M1c (andre metastaser enn skjelett)	
31	M1c_lokalisasjon	1,00	Lever	Dersom M1c, angi lokalisasjon
		2,00	Lunge	
32	Risikogruppe	1,00	lav riskogruppe	Angis for pasienter som er M0 (ingen fjernmetastaser)
		2,00	intermediær riskogruppe	
		3,00	høy risikogruppe	
33	Vekt_diagnose			Vekt ved diagnosetidspunkt, i kg (se anestesijournal)
34	Hoeyde_diagnose			Høyde ved diagnosetidspunkt, i cm (se anestesijournal)
35	Behandlingsintensjon	,000	palliativ behandling	Behandlingsintensjon ved diagnosetidspunkt
		1,000	kurativ behandling	
		9,000	mangler informasjon	
36	Aktiv_overvaakning	,00	Nei	Om kurativ intensjon, ble aktiv overvåking brukt?
		1,00	Ja	

37	Aktiv_overv_startdato			Dersom aktiv overvåkning, oppgi dato for oppstart/beslutning om aktiv overvåkning
38	Aktiv_overv_sluttdato			Dersom aktiv overvåkning, oppgi dato for slutt aktiv overvåkning
39	Aarsak_aktivov_slutt	,00	sykdomsprogresjon	Årsak til at aktiv overvåking avsluttes
		1,00	Død	
		3,00	andre årsaker	
		9,00	Ikke avsluttet	
40	Aktiv_overv_nyebiopsier_beh			Angi resultatet av PSA og Gleason score i nye biopsier som medfører behandling etter tidligere aktiv overvåking
41	Kirurgi	,00	Nei	Kirurgisk behandling, kurativ intensjon?
		1,00	Ja	
42	Kirurgi_dato			dato for kirurgisk inngrep
43	Kirurgi_type	1,00	åpen prostatectomi, retropubisk	Type inngrep, prostatakjertelen
		2,00	åpen prostatektomi, perineal	
		3,00	laparaskopisk prostatektomi	
		4,00	RALP (robot-assistert laparskopisk prostatektomi)	
		5,00	kun diagnostisk glandeltoilette	
		9,00	Annen kirurgi	
44	Kirurgi_nervesparende	,00	Nei	Ble inngrepet gjort nervesparende?
		1,00	ja, ikke nærmere presisert	
		2,00	Ensidig nervesparende	
		3,00	Bilateral nervesparende	
		9,00	vet ikke	
45	Kirurgi_glandeltoilette	,00	Nei	Ble det utført et glandeltoilette?
		1,00	Ja	
46	Glandeltoilette_type	1,00	Obturatortoilette	Type glandeltoilette
		2,00	utvidet toilette	
47	Kommentar_kirurgi			Kommenttar til kirurgisk beh
48	Tstad_patologisk	1,00	pT2a	T-stadium etter prostatektomi
		2,00	pT2b	
		3,00	pT2c	
		4,00	pT3A	
		5,00	pT3B	
		6,00	pT4	
49	Nstad_patologisk	,00	N0	N-stadium etter glandeltoilette
		1,00	N1	
		9,00	NX	
50	Lymfeknuter_antall			Totalt antall lymfeknuter fjernet
51	Lymfeknuter_antall_pat			Antallet lymfeknuter med metastaser
52	Prostata_vekt			Prostatas vekt (gram)
53	Gleason_preparat1			Primær gleason grad i operasjonspreparat
54	Gleason_preparat2			Sekundær gleason grad i operasjonspreparat

55	Perineural_infiltrasjon	,0	Nei	Forelå det perineural infiltrasjon? se patologirapport etter prostatektomi
		1,0	Ja	
		9,0	ikke angitt	
56	Vesikkelinfiltrasjon	,00	Nei	Forelå det infiltrasjon i vesiklene? se patologirapport etter prostatektomi
		1,00	Ja	
		9,00	ikke angitt	
57	Tumorinfiltrasjon_kar	,00	Nei	Forelå det tumorinfiltrasjon i kar?
		1,00	Ja	
		9,00	ikke angitt	
58	Ekstraprostatisk_vekst	,00	Nei	Forelå det ekstraprostatisk vekst?
		1,00	Ja	
		9,00	ikke angitt	
59	Ekstrasprostatisk_utbredelse	1,00	Fokal	Dersom ekstraprostatisk vekst, angi utbredelse
		2,00	bred front	
		9,00	ikke angitt	
60	normalvev_Sirkumferent_rand	,00	Nei	Forelå det normalt prostatavev i sirkumferent reseksjonsrand?
		1,00	ja	
		9,00	Ikke angitt	
61	Ufrie_marginer	,00	nei	Forelå det ufrie kirurgiske render? se patologirapport etter prostatektomi
		1,00	ja	
		9,00	ikke angitt	
62	Ufrie_marginer_lokalisasjon	1,00	posterolateralt	hvis ufrie marginer, angi lokalisasjon
		2,00	apex	
		3,00	basis	
		4,00	blærehals	
		5,00	flere lokalisasjoner/ utbredt	
		6,00	sirkumferent	
		9,00	ikke angitt	
63	Straaleterapi	,00	nei	Ble det gitt stråleterapi med kurativ intensjon?
		1,00	ja	
64	Straaleterapi_dato			dato for oppstart stråleterapi
65	Straaleterapi_intensjon	1,00	primær stråleterapi (ikke kirurgi)	Hva var intensjonen ved å gi stråleterapi?
		2,00	postoperativ stråleterapi, ufrie marginer	
		3,00	postoperativ stråleterapi, andre årsaker	
		4,00	salvage stråleterapi (PSA-residiv)	
66	Straaleterapi_type	1,00	Ekstern stråleterapi	Hva slags type stråleterapi ble anvendt?
		2,00	Brachyterapi	
		3,00	Kombinasjon av ekstern og brachyterapi	
67	Straaleterapi_plan	1,00	ved hjelp av simulator/vanlige rtg bilder	Hvordan ble strålebehandlingen planlagt?

		2,00 3,00	CT for doseplan, boksteknikk (4-felt) CT for doseplan og IMRT/VMAT	
68	Straaleterapi_IGRT	1,00 2,00 3,00	skjelett (standard før i tiden) Beam-cath gullmarkører	Hva ble brukt for å matche på under strålebehandling (IGRT)?
69	Straaleterapi_målvolum	1,00 2,00	prostata prostataseng	Hva slags målvolum ble tegnet inn?
70	Straaleterapi_bekken	,00 1,00	nei ja	Ble det gitt elektivt felt mot lymfekuter i bekkenet?
71	Straaleterapi_frdoseprostata			Hva slags fraksjonsdose ble gitt mot prostata/seng?
72	Straaleterapi_totaldoseprostata			Hva slags totaldose ble gitt mot prostata/seng?
73	Straaleterapi_totaldosebekken			Hva slags totaldose ble gitt mot lymfeknuter i bekkenet?
74	Straaleterapi_mamiller	,00 1,00	nei Ja	Ble det gitt stråleterapi mot mamiller?
75	PSA_Nadir			Hva var nadirverdien for PSA etter kurativ behandling?
76	Straaleterapi_mamiller_dose			Hva slags totaldose ble gitt mot mamillene?
77	Pall_RT	,00 1,00	nei ja	Har pasienten fått palliativ strålebehandling?
78	Pall_RT_dato			Hvis ja, når ble palliativ strålebeh. gitt første gang?
79	Pall_RT_lokalisasjon	1,00 2,00 3,00 4,00 6,00	Prostata Skjelett Glandler Andre områder Kombinasjon av flere områder	Hvis ja, hvilke områder ble strålebehandlet
80	Endokrin_behandling	,00 1,00	nei ja	Har pasienten noen gang mottatt endokrin terapi?
81	Endokrinbeh_indikasjon	1,00 2,00 3,00	neoadjuvant/konkomitant/adjuvant ved stråleterapi adjuvant til kirurgisk behandling palliativ behandling	Hva var årsaken til endokrin terapi?
82	Endokrinbeh_Startdato			dato for oppstart endokrin terapi
83	Endokrinbeh_Sluttdato			dato for slutt endokrin beh
84	Endokrinbeh_tid_mnd			varighet av endokrin terapi der sluttdato mangler (som skissert i notat onkolog..)
85	Endokrinbeh_antiandrogen	,00 1,00	nei ja	Fikk pasienten endokrin behandling i form av antiandrogener (ex bicalutamid, Casodex)?
86	Endokrinbeh_LHRHagonist	,00 1,00	nei ja	Fikk pasienten endokrin behandling i form av LHRH agonist (ex Zoladex, Eligard))?
87	Endokrinbeh_LHRHantagonist	,00 1,00	nei ja	Fikk pasienten endokrin behandling i form av LHRH antagonist (ex Degarelix/Firmagon)?
88	Endokrinbeh_kir_kastrasjon	,00 1,00	nei ja	Fikk pasienten endokrin behandling i form av kirurgisk kastrasjon (bilat orkiektomi)?

89	Annen_kurativ_behandling			Har pasienten fått annen kurativ beh?
90	Residiv	,00 1,00	nei ja	Har pasienten noen gang hatt residiv?
91	Residiv_dato			Dersom residiv, dato for residiv (hentes fra lab-journal)
92	Residiv_dato_klinisk			Dersom residiv, når ble dette erkjent klinisk
93	PSA_residiv			PSA verdi ved påvist residiv (gjelder også dersom kun biokjemisk residiv)
94	PSA_doblingstid_mnd			PSA doblingstid (mnd) ved residiv (evt legge inn flere verdier per apsient i excel ark, bruke formel)
95	Residiv_lokalisasjon	1,00 2,00 3,00 4,00 5,00 6,00	biokjemisk (kun forhøyet PSA) lokalt residiv (påvist radiologisk el biopsi) lymfeknuter skjelettmetastaser både skjelett og lymfeknuter andre lokalisasjoner	Lokalisasjon residiv, første gang
96	Lokalisasjon_Annet			
97	Residiv_behandling	1,00 2,00 3,00 4,00 5,00 6,00	salvage kirurgi salvage ekstern stråleterapi Brachyterapi HIFU Endokrin terapi Observasjon, ingen videre behandling	Hva slags behandling ved gitt ved residiv?
98	Residiv_beh_kommentar			utdypende, dersom annen beh eller flere typer residivbeh
99	Residiv_etter_salvage	,00 1,00 9,00	nei ja Vet ikke	Har pasienten utviklet residiv etter salvage-behandling?
100	Skjelettbiopsi	,00 1,00 2,00	nei ja Ukjent	Er det gjort skjelettbiopsi?
101	Dato_skjelettbiopsi			Dato første skjelettbiopsi
102	Histologi_skjelett	1,00 2,00 3,00 4,00	Benign Metastase prostatacancer Annen cancer Ukjent	Histologi skjelettbiopsi
103	Kommentar_skjelett			Kommentar skjelett
104	Primar_metastase	,00 1,00 9,00	nei ja vet ikke	Har pasienten metastatisk sykdom på diagnosetidspunkt?
105	Tidlig_kjemo	1,00 2,00 3,00 4,00	ja nei, medisinsk kontraindikasjon nei, pasienten ønsket ikke nei, ikke nærmere spesifisert	Fikk pasienten tidlig kjemoterapi (oppstart innen 3 mnd etter diagnose/endokrin beh.)?

		9,00	vet ikke	
106	Tidligkjemo_type	1,00 9,00	Taxotere/docetaxel Ikke angitt	Hvilken type kjemoterapi fikk pasienten i tidlig fase?
107				Dato for første kur kjemoterapi
108	Tidligkjemo_antall	,00 1,00 3,00 5,00 7,00 9,00	Ingen 1-2 3-4 5-6 Mer enn 6 Vet ikke	Hvor mange kurer gjennomførte pasienten? (vanligvis 6)
109	Kommentar_tidligkjemo			Kommentarer tidlig kjemoterapi
110	Kastrasjonsresistens	,00 1,00	nei ja	Har pasienten utviklet kastrasjonsresistent sykdom?
111	Kastrasjonsres_dato			dato for påvisning av kastrasjonsresistent sykdom
112	PSA_kastrasjonsres			PSA verdi ved påvisning av kastrasjonsresistent sykdom
113	kastrasjonsresistens_bakgrunn	1,00 2,00 3,00	kun stigende PSA Radiologisk progresjon Både stigende PSA og radiologisk progresjon	Bakgrunn for at pas er blitt kastrasjonsresistent
114	kastrasjonsres_Nstad	,00 1,00 9,00	N0 N1 Nx	N-stadium ved påvisning av kastrasjonsresistent sykdom
115	kastrasjonsres_Mstad	,00 1,00 2,00 3,00	M0 M1a M1b M1c	M-stadium ved påvisning av kastrasjonsresistent sykdom
116	M1cstad_kastrasjon			Angi lokalisasjon dersom M1c stadium
117	kastrasjonsres_beh1.linje	1,00 2,00 3,00 4,00 5,00 9,00	kjemoterapi, docetaxel (Taxotere) kjemoterapi, annen Abiraterone (Zytiga) Enzalutamide (Xtandi) Radium 223 (Xofigo) Ingen systemisk behandling	1. linjes behandling ved kastrasjonsresistent sykdom
118	Beh_1linje_start			Startdato for 1. linjes behandling ved kastrasjonsresist sykdom
119	Beh_1linje_slutt			Sluttdato for 1. linjes behandling ved kastrasjonsresist sykdom
120	Aarsakslutt_beh1linje	1,00 2,00 3,00 4,00 5,00 9,00	sykdomsprogresjon bivirkninger kombinasjon av 1 og 2 pasientens ønske andre årsaker vet ikke	årsak til at 1. linjes behandling ble seponert

121	kastrasjonsres_beh2linje	1,00 2,00 3,00 4,00 5,00 9,00	kjemoterapi, docetaxel (Taxotere) kjemoterapi, annen Abiraterone (Zytiga) Enzalutamide (Xtandi) Radium 223 (Xofigo) Ingen systemisk behandling	2. linjes behandling ved kastrasjonsresist sykdom
122	Beh_2linje_start			Startdato for 2. linjes behandling ved kastrasjonsresist sykdom
123	Beh_2linje_slutt			Sluttdato for 2. linjes behandling ved kastrasjonsresist sykdom
124	Aarsakslutt_beh_2linje	1,00 2,00 3,00 4,00 5,00 9,00	sykdomsprogresjon bivirkninger kombinasjon av 1 og 2 pasientens ønske andre årsaker vet ikke	årsak til at 2. linjes behandling ble seponert
125	kastrasjonsres_beh_3linje	1,00 2,00 3,00 4,00 5,00	kjemoterapi, docetaxel (Taxotere) kjemoterapi, annen Abiraterone (Zytiga) Enzalutamide (Xtandi) Radium 223 (Xofigo)	3. linjes behandling ved kastrasjonsresist sykdom
126	Beh_3linje_start			Startdato for 3. linjes behandling ved kastrasjonsresist sykdom
127	Beh_3linje_slutt			Sluttdato for 3. linjes behandling ved kastrasjonsresist sykdom
128	Aarsakslutt_beh_3linje	1,00 2,00 3,00 4,00 5,00 9,00	sykdomsprogresjon bivirkninger kombinasjon av 1 og 2 pasientens ønske andre årsaker vet ikke	årsak til at 3. linjes behandling ble seponert
129	kastrasjonsres_beh_4linje	1,00 2,00 3,00 4,00 5,00	kjemoterapi, docetaxel (Taxotere) kjemoterapi, annen Abiraterone (Zytiga) Enzalutamide (Xtandi) Radium 223 (Xofigo)	4. linjes behandling ved kastrasjonsresist sykdom
130	Beh_4linje_start			Startdato for 4. linjes behandling ved kastrasjonsresist sykdom
131	Beh_4linje_slutt			Sluttdato for 4. linjes behandling ved kastrasjonsresist sykdom
132	Aarsakslutt_beh_4linje	1,00 2,00 3,00 4,00 5,00 9,00	sykdomsprogresjon bivirkninger kombinasjon av 1 og 2 pasientens ønske andre årsaker vet ikke	årsak til at 4. linjes behandling ble seponert

133	Dato_sistekontakt_UNN			dato for siste kontakt ved UNN (siste PSA og/eller klinisk kontroll)
134	Dato_sistekontakt_andre			Dato for siste kontakt andre sykehus/behandler
135	Doedsdato			Dødsdato
136	gleason_grade_group			Gleason Grade Group
137	pnr_A			Fødselsnummer
139	datokr_A			Dato kreftdiagnose (fra Kreftregisteret)
141	aarkr			År kreftdiagnose
143	datobl_A			Dato første oppmøte TUS
145	aarTUS			År første oppmøte TUS
146	sykehus			Sykehuskode diagnosested (fra Kreftregisteret)
148	SHNavn			Sykehusnavn diagnosested
149	Papirjournal			Papirjournal
150	Paabegynt			Journalgjennomgang påbegynt
151	Fullfoert			Journalgjennomgang fullført
152	JournalutenforUNN			Journal utenfor UNN
153	Nyadresse			Ny adresse
154	Kommentarer			Kommentar til journalgjennomgang
155	Preparatnr_naalebiopsi			Preparatnr nålebiopsi
156	Tilstede_b1	1,00 2,00 3,00 9,00	tilstede multiple blokker se kommentar feil	Preparat tilstede biopsi
157	Revurderd_gleason1_biopsi			Revurdert primær gleason grad biopsi
158	Revurdert_gleason2_biopsi			Revurdert sekundær gleason grad biopsi
159	Preparatnr_naalebiopsi_2			Preparatnr nålebiopsi 2
160	Tilstede_b2	1,00 2,00 3,00 9,00	tilstede multiple blokker se kommentar feil	Preparat tilstede biopsi
161	Preparatnr_andre			Preparatnr andre
162	Tilstede_andre	1,00 2,00 3,00 9,00	tilstede multiple blokker se kommentar Feil	Preparat tilstede andre preparat
163	Revurdert_gleason1_andre			Revurdert primær gleason grad andre preparat
164	Revurdert_gleason2_andre			Revurdert sekundær gleason grad andre preparat
165	Preparatnr_TURP			Preparatnr TURP
166	Tilstede_turp	1,00 2,00 3,00 9,00	Tilstede multiple blokker se kommentar feil	Preparat tilstede TURP
167	Reurdert_gleason1_TURP			Revurdert primær gleason grad TURP
168	Revurdert_gleason2_TURP			Revurdert sekundær gleason grad TURP

169	Preparatnr_prostatektomi			Preparatnr prostatektomi
170	Tilstede_opr	1,00 2,00 3,00 4,00 9,00	tilstede multiple blokker se kommentar operasjons-kohorte feil	Preparat tilstede prostatektomi
171	Revurdert_gleason1_prostatektomi			Revurdert primær gleason grad operasjonspreparat
172	Revurdert_gleason2_prostatektomi			Revurdert sekundær gleason grad operasjonspreparat
173	Eksklusjon	1 2 3	Manglende journalopplysninger Usikker diagnose Reservasjon	Ekskludert fra klinisk datasett, årsak
174	Patolog_biopsi			Hvilken patolog har gjort primærvurdering av biopsi
175	Patolog_TURP			Hvilken patolog har gjort primærvurdering av TUR-P
176	Patolog_prostatektomi			Hvilken patolog har gjort primærvurdering av operasjonspreparat

Questionnaire from the Tromsø Study

Example of questionnaire from first visit, Tromsø 4. A full list of all questionnaires can be found at https://uit.no/research/tromsoundersokelsen

Hovedformålet med Tromsøundersøkelsene er å skaffe ny kunnskap om hjerte-karsykdommer for å kunne forebygge dem. I tillegg skal undersøkelsen øke kunnskapen om kreftsykdommer og andre alminnelige plager som f.eks. allergier, smerter i muskulatur og nervøse lidelser. Vi ber deg derfor svare på noen spørsmål om forhold som kan ha betydning for risikoen for disse og andre sykdommer.

Skjemaet er en del av Helseundersøkelsen som er godkjent av Datatilsynet og av Regional komite for medisinsk forskningsetikk. Svarene brukes bare til forskning og behandles strengt fortrolig. Opplysningene kan senere bli sammenholdt med informasjon fra andre offentlige helseregistre etter de regler som Datatilsynet og Regional komite for medisinsk forskningsetikk gir.

Hvis du er i tvil om hva du skal svare, sett kryss i den ruten som du synes passer best.

Det utfylte skjema sendes i vedlagte svarkonvolutt. Portoen er betalt.

På forhånd takk for hjelpen!

Med vennlig hilsen

Fagområdet medisin
Universitetet i Tromsø **Statens helseundersøkelser**

Hvis du ikke ønsker å besvare spørreskjemaet, sett kryss i ruten under og returner skjemaet. Da slipper du purring.

Jeg ønsker ikke å besvare spørreskjemaet ..... 17 ❑

Dag Mnd År

Dato for utfylling av skjema: ..... 18 ...../...../.....

OPPVEKST

I hvilken kommune bodde du da du fylte 1 år?

..... 24 - 28

Hvis du ikke bodde i Norge, oppgi land i stedet for kommune.

Hvordan var de økonomiske forhold i familien under din oppvekst?
- Meget gode ..... 29 ❑
- Gode ❑
- Vanskelige ❑
- Meget vanskelige ❑

Hvor mange av de første 3 årene av ditt liv
- bodde du i by? ..... 30 _____ år
- hadde dere katt eller hund i hjemmet? ..... 31 _____ år

Hvor mange av de første 15 årene av ditt liv
- bodde du i by? ..... 32 _____ år
- hadde dere katt eller hund i hjemmet? ..... 34 _____ år

BOLIG

Hvem bor du sammen med?
Sett ett kryss for hvert spørsmål og angi antall.

	Ja	Nei	Antall
Ektefelle/samboer 36	❑	❑	
Andre personer over 18 år 37	❑	❑	_____
Personer under 18 år 40	❑	❑	_____

Hvor mange av barna har plass i barnehage? ..... 43 _____

Hvilken type bolig bor du i?
- Enebolig/villa ..... 45 ❑ 1
- Gårdsbruk ❑ 2
- Blokk/terrasseleilighet ❑ 3
- Rekkehus/2-4 mannsbolig ❑ 4
- Annen bolig ❑ 5

Hvor stor er din boenhet? ..... 46 _____ m^2

I omtrent hvilket år ble boligen bygget? ..... 49 _____

	Ja	Nei
Er boligen isolert etter 1970? 53	❑	❑
Bor du i underetasje/kjeller? 54	❑	❑
Hvis "Ja", er gulvbelegget lagt på betong? 55	❑	❑

Hvordan er boligen hovedsakelig oppvarmet?
- Elektrisk oppvarming ..... 56 ❑
- Vedfyring ❑
- Sentralvarmeanlegg oppvarmet med:
  - Parafin ❑
  - Elektrisitet ❑

	Ja	Nei
Er det heldekkende tepper i stua? 60	❑	❑
Er det katt i boligen? 61	❑	❑
Er det hund i boligen? 62	❑	❑

ARBEID

Hvis du er i lønnet eller ulønnet arbeid, hvordan vil du beskrive ditt arbeid?
- For det meste stillesittende arbeid? ..... 63 ❑ 1
  (f.eks. skrivebordsarbeid, montering)
- Arbeid som krever at du går mye? ❑ 2
  (f.eks. ekspeditørarb., lett industriarb., undervisning)
- Arbeid hvor du går og løfter mye? ❑ 3
  (f.eks. postbud, pleier, bygningsarbeid)
- Tungt kroppsarbeid? ❑ 4
  (f.eks. skogsarb., tungt jordbruksarb., tungt bygn.arb.)

Kan du selv bestemme hvordan arbeidet ditt skal legges opp?
- Nei, ikke i det hele tatt ..... 64 ❑ 1
- I liten grad ❑ 2
- Ja, i stor grad ❑ 3
- Ja, det bestemmer jeg selv ❑ 4

	Ja	Nei
Har du skiftarbeid, nattarbeid eller går vakter? 65	❑	❑

Har du noen av følgende yrker (heltid eller deltid)?
Sett ett kryss for hvert spørsmål.

	Ja	Nei
Sjåfør 66	❑	❑
Bonde/gårdbruker	❑	❑
Fisker	❑	❑

EGNE SYKDOMMER

Har du noen gang hatt:
Sett ett kryss for hvert spørsmål. Oppgi alderen ved hendelsen.
*Hvis det har skjedd flere ganger, hvor gammel var du **siste** gang?*

	Ja	Nei	Alder
Lårhalsbrudd 69	❑	❑	_____
Brudd ved håndledd/underarm 72	❑	❑	_____
Nakkesleng (whiplash) 75	❑	❑	_____
Skade som førte til sykehusinnleggelse 78	❑	❑	_____
Sår på magesekken 81	❑	❑	_____
Sår på tolvfingertarmen 84	❑	❑	_____
Magesår-operasjon 87	❑	❑	_____
Operasjon på halsen 90	❑	❑	_____

Har du eller har du hatt:
Sett ett kryss for hvert spørsmål.

	Ja	Nei
Kreftsykdom 93	❑	❑
Epilepsi (fallesyke)	❑	❑
Migrene	❑	❑
Kronisk bronkitt	❑	❑
Psoriasis	❑	❑
Benskjørhet (osteoporose) 96	❑	❑
Fibromyalgi/fibrositt/kronisk smertesyndrom	❑	❑
Psykiske plager som du har søkt hjelp for	❑	❑
Stoffskiftesykdom (skjoldbruskkjertel)	❑	❑
Sykdom i leveren	❑	❑
Nyrestein 100	❑	❑
Blindtarmsoperasjon	❑	❑
Allergi og overfølsomhet		
Atopisk eksem (f.eks. barneeksem)	❑	❑
Høndeksem	❑	❑
Høysnue	❑	❑
Matvareallergi 108	❑	❑
Annen overfølsomhet (ikke allergi)	❑	❑

Hvor mange ganger har du hatt forkjølelse, influensa, "ræksjuka" og lignende siste halvår? 110 _____ ganger

	Ja	Nei
Har du hatt dette siste 14 dager? 112	❑	❑

SYKDOM I FAMILIEN

Kryss av for de slektningene som har eller har hatt noen av sykdommene:
Kryss av for "Ingen" hvis ingen av slektningene har hatt sykdommen.

	Mor	Far	Bror	Søster	Barn	Ingen
Hjerneslag eller hjerneblødning 113	❑	❑	❑	❑	❑	❑
Hjerteinfarkt før 60 års alder 119	❑	❑	❑	❑	❑	❑
Kreftsykdom 125	❑	❑	❑	❑	❑	❑
Astma 131	❑	❑	❑	❑	❑	❑
Mage/tolvfingertarm-sår 137	❑	❑	❑	❑	❑	❑
Benskjørhet (osteoporose) 143	❑	❑	❑	❑	❑	❑
Psykiske plager 149	❑	❑	❑	❑	❑	❑
Allergi 155	❑	❑	❑	❑	❑	❑
Diabetes (sukkersyke) 161	❑	❑	❑	❑	❑	❑
– alder da de fikk diabetes 167	___	___	___	___	___	

SYMPTOMER

	Ja	Nei
Hoster du omtrent daglig i perioder av året? 177	❑	❑
Hvis "Ja":		
Er hosten vanligvis ledsaget av oppspytt? 178	❑	❑
Har du hatt slik hoste så lenge som i en 3 måneders periode i begge de to siste år? 179	❑	❑
Har du hatt episoder med piping i brystet? 180	❑	❑
Hvis "Ja", har dette oppstått:		
Sett ett kryss for hvert spørsmål.		
Om natten 181	❑	❑
Ved luftveisinfeksjoner	❑	❑
Ved fysiske anstrengelser	❑	❑
Ved sterk kulde	❑	❑
Har du merket anfall med plutselig endring i pulsen eller hjerterytmen siste år? 185	❑	❑

Hvor ofte er du plaget av søvnløshet?

Aldri, eller noen få ganger i året 186	❑ 1
1-2 ganger i måneden	❑ 2
Omtrent en gang i uken	❑ 3
Mer enn en gang i uken	❑ 4

Hvis du er plaget av søvnløshet i perioder, når på året er du mest plaget?

Ingen spesiell tid 187	❑ 1
Særlig i mørketiden	❑ 2
Særlig i midnattsoltiden	❑ 3
Særlig vår og høst	❑ 4

	Ja	Nei
Har du det siste året vært plaget av søvnløshet slik at det har gått ut over arbeidsevnen? 188	❑	❑

Hvor ofte er du plaget av hodepine?

Sjelden eller aldri 189	❑ 1
En eller flere ganger i måneden	❑ 2
En eller flere ganger i uken	❑ 3
Daglig	❑ 4

Hender det at tanken på å få alvorlig sykdom bekymrer deg?

Ikke i det hele tatt 190	❑ 1
Bare i liten grad	❑ 2
En del	❑ 3
Ganske mye	❑ 4

BRUK AV HELSEVESENET

Hvor mange ganger har du siste året, på grunn av egen helse eller sykdom, vært:
*Sett **0** hvis du **ikke** har hatt slik kontakt.*

	Antall ganger siste år
Hos vanlig lege/legevakt 191	_____
Hos psykolog eller psykiater	_____
Hos annen legespesialist utenfor sykehus	_____
På poliklinikk 197	_____
Innlagt i sykehus	_____
Hos bedriftslege	_____
Hos fysioterapeut 203	_____
Hos kiropraktor	_____
Hos akupunktør	_____
Hos tannlege 209	_____
Hos naturmedisiner (homøopat, soneterapeut o.l.)	_____
Hos håndspålegger, synsk eller "leser"	_____

LEGEMIDLER OG KOSTTILSKUDD

Har du det siste året periodevis brukt noen av de følgende midler daglig eller nesten daglig?
Angi hvor mange måneder du brukte dem.
*Sett **0** hvis du **ikke** har brukt midlene.*

Legemidler
- Smertestillende (215) ____ mnd.
- Sovemedisin ____ mnd.
- Beroligende midler ____ mnd.
- Medisin mot depresjon (221) ____ mnd.
- Allergimedisin ____ mnd.
- Astmamedisin ____ mnd.

Kosttilskudd
- Jerntabletter (227) ____ mnd.
- Kalktabletter eller benmel ____ mnd.
- Vitamin D-tilskudd ____ mnd.
- Andre vitamintilskudd (233) ____ mnd.
- Tran eller fiskeoljekapsler ____ mnd.

Har du de siste 14 dager brukt følgende legemidler eller kosttilskudd?
*Sett **ett** kryss for **hvert** spørsmål.*

	Ja	Nei
Legemidler		
Smertestillende medisin (237)	❑	❑
Febersenkende medisin	❑	❑
Migrenemedisin	❑	❑
Eksemsalve	❑	❑
Hjertemedisin (ikke blodtrykksmedisin)	❑	❑
Kolesterolsenkende medisin (242)	❑	❑
Sovemedisin	❑	❑
Beroligende medisin	❑	❑
Medisin mot depresjon	❑	❑
Annen nervemedisin	❑	❑
Syrenøytraliserende midler (247)	❑	❑
Magesårsmedisin	❑	❑
Insulin	❑	❑
Tabletter mot diabetes (sukkersyke)	❑	❑
Tabletter mot lavt stoffskifte (thyroxin)	❑	❑
Kortisontabletter (252)	❑	❑
Annen medisin	❑	❑
Kosttilskudd		
Jerntabletter	❑	❑
Kalktabletter eller benmel	❑	❑
Vitamin D-tilskudd	❑	❑
Andre vitamintilskudd (257)	❑	❑
Tran eller fiskeoljekapsler	❑	❑

VENNER

Hvor mange gode venner har du som du kan snakke fortrolig med og gi deg hjelp når du trenger det? (259) ____ gode venner
Tell ikke med de du bor sammen med, men ta med andre slektninger!

Hvor mange av disse gode vennene har du kontakt med minst en gang i måneden? (261) ____

	Ja	Nei
Føler du at du har nok gode venner? (263)	❑	❑

Hvor ofte tar du vanligvis del i foreningsvirksomhet som f.eks. syklubb, idrettslag, politiske lag, religiøse eller andre foreninger?
- Aldri, eller noen få ganger i året (264) ❑ 1
- 1-2 ganger i måneden ❑ 2
- Omtrent en gang i uken ❑ 3
- Mer enn en gang i uken ❑ 4

KOSTVANER

Hvis du bruker smør eller margarin på brødet, hvor mange skiver rekker en liten porsjonspakning vanligvis til? Vi tenker på slik porsjonspakning som du får på fly, på kafé o.l. (10-12 gram).

Den rekker til omtrent (265) ____ skiver

Hva slags fett blir vanligvis brukt til **matlaging** (ikke på brødet) i din husholdning?
- Meierismør (266) ❑
- Hard margarin ❑
- Bløt (Soft) margarin ❑
- Smør/margarin blanding ❑
- Oljer (270) ❑

Hva slags type brød (kjøpt eller hjemmebakt) spiser du vanligvis?
Sett ett eller to kryss!

	Loff	Fint brød	Kneip-brød	Grov-brød	Knekke-brød
Brødtypen ligner mest på:	❑ (271)	❑	❑	❑	❑ (275)

Hvor mye (i **antall** glass, kopper, poteter eller brødskiver) spiser eller drikker du vanligvis **daglig** av følgende matvarer?
*Kryss av for **alle** matvarene.*

	0	Færre enn 1	1-2	3-4	5-6	Mer enn 6
Helmelk (søt eller sur) (glass) (276)	❑	❑	❑	❑	❑	❑
Lettmelk (søt eller sur) (glass)	❑	❑	❑	❑	❑	❑
Skummet melk (søt eller sur) (glass)	❑	❑	❑	❑	❑	❑
Te (kopper)	❑	❑	❑	❑	❑	❑
Appelsinjuice (glass)	❑	❑	❑	❑	❑	❑
Poteter (281)	❑	❑	❑	❑	❑	❑
Brødskiver totalt (inkl. knekkebrød)	❑	❑	❑	❑	❑	❑
Brødskiver med						
– fiskepålegg (f.eks. makrell i tomat)	❑	❑	❑	❑	❑	❑
– magert kjøttpålegg (f.eks. skinke)	❑	❑	❑	❑	❑	❑
– fetere kjøttpålegg (f.eks. salami)	❑	❑	❑	❑	❑	❑
– gulost (286)	❑	❑	❑	❑	❑	❑
– brunost	❑	❑	❑	❑	❑	❑
– kaviar	❑	❑	❑	❑	❑	❑
– syltetøy og annet søtt pålegg	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	❑ 6

Hvor mange **ganger i uka** spiser du vanligvis følgende matvarer?
*Kryss av for **alle** matvarene.*

	Aldri	Færre enn 1	1	2-3	4-5	Omtrent daglig
Yoghurt (290)	❑	❑	❑	❑	❑	❑
Kokt eller stekt egg	❑	❑	❑	❑	❑	❑
Frokostblanding/havregryn o.l.	❑	❑	❑	❑	❑	❑
Middag med						
– rent kjøtt	❑	❑	❑	❑	❑	❑
– pølser/kjøttpudding/-kaker	❑	❑	❑	❑	❑	❑
– feit fisk (f.eks. laks/uer) (295)	❑	❑	❑	❑	❑	❑
– mager fisk (f.eks. torsk)	❑	❑	❑	❑	❑	❑
– fiskeboller/-pudding/-kaker	❑	❑	❑	❑	❑	❑
– grønnsaker	❑	❑	❑	❑	❑	❑
Majones, remulade o.l.	❑	❑	❑	❑	❑	❑
Gulrøtter (300)	❑	❑	❑	❑	❑	❑
Blomkål/kål/brokkoli	❑	❑	❑	❑	❑	❑
Epler/pærer	❑	❑	❑	❑	❑	❑
Appelsiner, mandariner o.l.	❑	❑	❑	❑	❑	❑
Sukkerholdige leskedrikker	❑	❑	❑	❑	❑	❑
Sukkerfrie («Light») leskedrikker	❑	❑	❑	❑	❑	❑
Sjokolade	❑	❑	❑	❑	❑	❑
Vafler, kaker o.l. (307)	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	❑ 6

Paper I

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Cancer Epidemiology

Inflammatory serum markers and risk and severity of prostate cancer: The PROCA-*life* study

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Whether chronic inflammation mirrored by high levels of systemic inflammatory markers such as high sensitive-CRP (hs-CRP) and white blood cell count (WBC) are associated with prostate cancer development remains unclear. In the Prostate Cancer Study throughout Life (PROCA-*life*), a prospective population-based cohort study, 7,356 men were included. Prediagnostic WBC and hs-CRP were assessed from blood collected at study entry; 2,210 participants also had a second CRP measure during follow-up. During a mean 11.8 years follow-up, 509 men developed prostate cancer (mean age at diagnosis 71.7 years). Multivariable Cox proportional hazard regression models were used to study whether individual biomarkers (WBC, hs-CRP), a combined score based on analyte tertiles (score range 2–6), or change in CRP were associated with risk and severity of prostate cancer. We observed a positive dose–response relationship between hs-CRP and prostate cancer risk with a Hazard Ratio (HR) per mg/l of 1.3, 95% CI 1.00–1.07. Men with an increase in hs-CRP between two measurements (Δhs-CRP) of ≥1.00 mg/l had a 36% increased risk of prostate cancer (HR 1.36, 95% CI 1.02–1.82), compared to men with no change or decrease in hs-CRP. Men with a systemic inflammatory score of 5 or 6 had a 68% higher risk of being diagnosed with metastatic disease (HR 1.68, 95% CI, 1.04–2.73) compared to men with lower scores. Our study supports that hs-CRP including repeated measurements alone or in combination with WBC may be a useful inflammation-related biomarker for prostate cancer risk and prognosis.

Additional Supporting Information may be found in the online version of this article.

Key words: prediagnostic inflammatory markers, repeated assessments, prostate cancer, white blood cells, hs-CRP, incidence

Abbreviations: BMI: body mass index; CI: confidence interval; HR: hazard ratio; Hs-CRP: high sensitivity C-reactive protein; ISUP: International Society of Urological Pathology; n: numbers; PSA: prostate-specific antigen; WBC: white blood cell count; Δhs-CRP: change in hs-CRP across two measurements

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What's new?

Although chronic inflammation likely influences prostate cancer development, a clear association is yet to be established. In particular, uncertainties remain regarding the relationship between systemic inflammatory markers and prostate cancer. In this investigation of data for more than 7,350 men, pre-diagnostic levels of C-reactive protein (CRP), measured *via* high-sensitivity CRP (hs-CRP) testing at study entry and at follow-up, were associated with a dose-response increase in prostate cancer risk. Risk and disease severity were further associated with a combined score incorporating both hs-CRP and white blood cell count, highlighting the relevance of inflammation in prostate cancer development and prognosis.

Introduction

Chronic inflammation, one hallmark of cancer development[1] has been questioned as playing a key role in prostate cancer development. The suggested hypothesis is partly based on observations of inflammatory cells in the prostate microenvironment of adult men, and on inflammation being associated with precursor lesions in the prostate gland, termed proliferative inflammatory atrophy. However, a causal relationship between inflammation and prostate cancer development—one of the most common invasive cancers among men globally—has yet to be established.[2–4]

Currently, prostate-specific antigen (PSA) is the only non-invasive biomarker in clinical use to detect and evaluate efficacy of prostate cancer treatment, but has a low sensitivity in prostate cancer diagnosis.[5] However, PSA testing has led to a dramatic increase in incidence of prostate cancer, and the majority of prostate cancer cases have been localized disease.[6] Key challenges in diagnostics of prostate cancer are to develop better tools to identify individuals at high risk for prostate cancer, and to distinguish between tumors with a low malignant potential that are unlikely to require therapeutic intervention compared to tumors that should be treated.

Blood levels of two commonly available measures—C-reactive protein (CRP) and white blood cell count (WBC) are indicators of systemic inflammation. Interesting observations suggest that these biomarkers could predict risk for prostate cancer development and progression.[7–10] CRP is an acute phase protein that reflects tissue injury and has become a widely used systemic biomarker of acute infection or inflammation in clinical practice. CRP is relatively stable in serial measurements in healthy individuals.[11,12] Furthermore, local inflammation has been observed in 35–100% of prostate cancer biopsies.[2,13–15]

Previous studies investigating the association between CRP and risk for prostate cancer development have shown conflicting results, as some studies found positive associations between level of inflammation-related biomarkers and risk of prostate cancer[7,16,17]; others have not.[16–24] However, most studies have included only one single measurement of CRP, with a limited number of prostate cancer cases and short follow-up time.[7,16–24]

The aim of the present study was to investigate associations between the inflammation-related biomarkers CRP and WBC and risk of prostate cancer development and severity. A second aim is to determine whether markers of inflammation (WBC and high sensitivity-CRP, hs-CRP) independently or in combination were associated with risk and severity of prostate cancer, and to look at change in CRP and risk of prostate cancer development and severity. The Prostate Cancer Study throughout life (PROCA-*life*) study includes a subset of men included in the population-based Tromsø Study, who had available measures of CRP and WBC.

Methods

Study population

The PROCA-*life* study includes all men, age > 25 years who enrolled in the population-based, prospective cohort Tromsø study between 1994 and 2008 (Tromsø 4, 1994–1995, Tromsø 5, 2001, Tromsø 6, 2007–2008).[25,26] The procedures were almost identical and assessments were done by trained research technicians. All age-eligible men in the Tromsø geographic area were invited to participate *via* a personal written invitation, and non-respondents were given one reminder. Once enrolled, all participants were invited to participate in the regular next follow-up survey (second measurement). The attendance rate for men was on average 67% in the three health surveys.[26] For the present study, only men who attended the second visit in Tromsø 4 or Tromsø 5, and all men in Tromsø 6, were eligible (n = 7,720). Measurements of prediagnostic hs-CRP > 20 mg/l and/or prediagnostic WBC > 15 x 10^9cells/l, which may mirror other acute or chronic diseases, were excluded (high hs-CRP: n = 285, high WBC: n = 44). Participants with prevalent or previous cancer (n = 334), or who developed cancer within the first year after the enrollment in the study (n = 58) were excluded to account for the possibility that undiagnosed cancer or severe illness could influence the results (Fig. 1). All men completed questionnaires, blood draws and basic clinical measurements. The PROCA-*life* study has been approved by the Regional Committee for Medical and Health Research Ethics North (REK) (2015/1059). All participants have signed consent declarations when enrolled in the Tromsø Study.

Questionnaires and clinical assessments

Information about medical history, lifestyle factors, dietary factors, medication, smoking history, and level of physical activity were obtained from the questionnaires. We defined being physical active as: more than 1 hour/week of strenuous exercise, or any leisure time exercise more than two to three times/week.

Height and weight were measured on an electronic scale with the participants wearing light clothing and no shoes. Height was

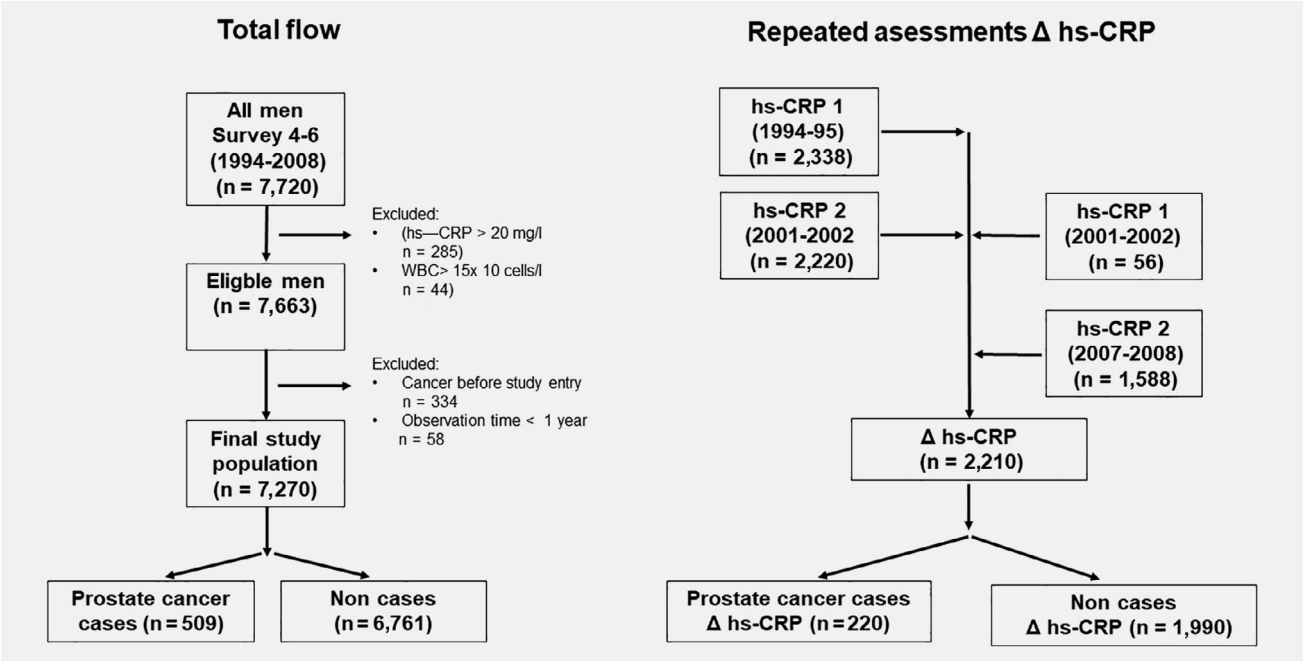

Figure 1. Flow chart for the population included in the PROCA-life study between 1994 and 2008.

measured to the nearest centimeter (cm) and weight to the nearest kilogram. BMI was calculated using the formula weight/height^2 (kg/m^2).[25] Blood pressure was measured three times in a resting position, and the mean of the two last measurements were used.

Assessment of serum inflammation-related biomarkers and other serum markers

Blood samples were drawn by trained research assistants on attendance at each survey, and were nonfasting. Analyses of serum samples were done at the Department of Laboratory Medicine, University Hospital of Northern Norway (UNN), Tromsø, Norway.[25] Serum samples from men who attended the first two surveys (Tromsø 4 or 5: 1994–95 and/or 2001) were kept frozen up to 12 years at −70°C and later analyzed, while hs-CRP was assessed in fresh samples from men who attended the final survey (Tromsø 6: 2007–08). Hs-CRP was analyzed by a particle-enhanced immune turbid metric assay on a Modular P auto-analyzer (Roche Diagnostics, Mannheim, Germany) with reagents from the manufacturer with a detection limit of 0.12 mg/l. For WBC counts, 5 ml of blood was collected into Vacutainer tubes containing EDTA as an anticoagulant (K3-EDTA 40 ll, 0.37 mol/l per tube), and analyzed within 12 hr by an automated blood cell counter (Coulter CounterÒ, Coulter Electronics, Luton, UK and Coulter LH750, Nerliens Meszansky). Total cholesterol was analyzed by enzymatic colorimetric methods with commercially available kits (CHOD-PAP for cholesterol). PSA measurements were done for cancer cases only, as part of clinical routine in diagnosis and follow-up (1990–1994 Stratus® PSA Fluorometric Enzyme Immunoassay, 1994–2001 AxSYM Psa Reagent Pack, Abbot®, 2001 Bayer® PSA Reagens Pack Immuno I (Prod. Nr.T01-3450-51), Technicon Immuno I). For prostate cancer cases diagnosed or treated in other institutions (n = 21), PSA values from their local laboratories were recorded.

Identification of prostate cancer cases during follow-up

Prostate cancer cases during follow-up (until December 31, 2016) were identified by using the unique national 11-digit identification number through linkage with the Cancer Registry of Norway. Among 7,270 men that were included in our study, 509 men were diagnosed with verified invasive prostate cancer during follow-up, and there was no ongoing screening programs for prostate cancer in Tromsø during the study period. Follow-up time was calculated from date of entry into the study, to the date of censoring (prostate cancer diagnosis, emigration, death, or end of follow-up [December 31, 2016]).

Detailed clinical information for the prostate cancer cases was obtained from the medical records (e.g., disease stage, treatments, recurrence) by trained physicians (TK and ES). All histopathological specimens were reexamined by the same uropathologist (ER) and classified according to the latest International Society of Urological Pathology (ISUP) guidelines on Gleason score and ISUP grade group.[27]

Prostate cancer cases were divided into four risk groups based on PSA level at diagnosis, highest ISUP grade group and clinical T-stage, according to the EAU guidelines.[28] Risk group 1 (low) was defined as: PSA < 10 μg/l, clinical T-stage (cT-) 1, and ISUP grade group 1. Risk group 2 (intermediate) was defined as: PSA: 10–20 μg/l, cT-stage 2, or ISUP grade

group 2–3. Risk group 3 (high) was defined as: PSA: > 20–100 μg/l, cT-stage 3, or ISUP grade group 4–5. Risk group 4 (metastatic) was defined as: PSA > 100 μg/l, or with radiological evidence of metastatic disease. ISUP grade group were reported after reclassification when available. PSA values above 100 were not included in calculation of mean or median PSA.

Statistical methods

Descriptive characteristics of the study population were presented as means (standard deviation) or percent (numbers). Differences in the distribution of characteristics at study entry between nonprostate cancer cases and prostate cancer cases were assessed using *t*-tests for continuous variables and Chi-square tests for categorical data. No large differences was observed

Table 1. Distribution of selected characteristics for men with prostate cancer (cases) and without prostate cancer (noncases) in the PROCA-*life* Study (1994–2008)

Characteristics	Overall (*n* = 7,270)	Noncases (*n* = 6,761)	Prostate cancer cases (*n* = 509)
Age at first attendance (years)	56.9 (10.5)	56.5 (10.6)	66.8 (7.6.)
Observation time (years)	11.8 (6.0)	11.9 (6.0)	9.9 (5.9)
Observation time ≤5 years (%)	5.8	4.3	24.8
Observation time 5.1–10 years (%)	59.9	61.9	33.2
Observation time › 10 years (%)	34.3	33.8	42.0
Systolic blood pressure (mm Hg)	137.7 (19.8)	137.2 (19.6)	143.3 (20.5)
Body mass index (kg/m^2)	26.8 (3.67)	26.8 (3.7)	26.6 (3.6)
Serum samples at study entry			
Total cholesterol (mmol/l)	5.98 (1.21)	5.96 (1.21)	6.19 (1.24)
Hs-CRP (mg/l)	2.10 (2.46)	2.10 (2.46)	2.17 (2.47)
Hs-CRP (mg/l) median (interquartile range)	1.28 (0.70–2.45)	1.27 (0.69–2.44)	1.36 (0.78–2.61)
White blood cells (x10^9/l)	6.62 (1.79)	6.61 (1.80)	6.75 (1.68)
White blood cells (x10^9/l) median (interqu. range)	6.3 (5.3–7.6)	6.3 (5.3–7.6)	6.4 (5.6–7.7)
Lifestyle factors at study entry			
Lipid-lowering drugs, current use (%)	8.5	8.5	8.3
Current smokers (%)	26.7	26.6	28.5
Physically active (%)	41.0	41.1	39.2
Characteristics among prostate cancer cases			
Age at diagnosis (years)			71.7 (7.5)
Cancer specific mortality (%)			8.8
PSA at diagnosis (μg/l)[1]			14.3 (14.3)
PSA at diagnosis, median (μg/l)[1]			9.9
Time from last blood sample to diagnosis (years)			5.4 (3.2)
Tumor characteristics			
T-stage			
T1 + T2 (%)			74.1
T3 + T4 (%)			22.0
Tx (%)			3.9
ISUP grade group			
1–3 (Gleason score 6–7) (%)			72.7
4–5 (Gleason score 8–10) (%)			18.3
ISUP missing (%)			9.0
Risk group			
Low (%)			16.1
Intermediate (%)			41.9
High (%)			24.2
Metastatic (%)			12.1
Unknown (%)			5.5

Numbers may vary due to missing information. Values are mean (standard deviation) unless otherwise specified.
Abbreviations: Hs-CRP, high sensitivity C-reactive protein; ISUP, International Society of Urological Pathology; PSA, prostate-specific antigen.
[1]PSA values above 100 are excluded from calculation of mean and median.

between cases and noncases and therefore not shown in text or tables. Multivariable Cox proportional hazard models were used to investigate whether inflammation biomarkers (hs-CRP and WBC) or repeated assessments of hs-CRP (Fig. 1) were associated with prostate cancer risk and severity, presented with hazard ratio (HR) and 95% confidence interval (CI). The inflammatory markers (hs-CRP and WBC) were not normally distributed, and log-transformation was tested, but did not influence results.

To study the importance of the variation in inflammation-related biomarkers in more detail, we used hs-CRP and WBC both as continuous and categorical variables, with tertile cut-points based on the distribution in the overall data set. Continuous variables are presented as HR per unit increase. We defined the systemic inflammatory score as the sum of tertile ranking for hs-CRP and WBC: tertile 1. hs-CRP: ≥0.01 – ≤0.91 mg/l, WBC: ≥1.1 – ≤5.6 × 10^9/l/l, tertile 2. hs-CRP: ≥0.92 – ≤2.03 mg/l, WBC: ≥5.7 – ≤7.0 × 10^9/l and tertile 3. hs-CRP: ≥2.04 – ≤20 mg/l, WBC: ≥7.1 – ≤15 × 10^9/l. The systemic inflammatory score ranged from 2 to 6 points; 5–6 were defined as a high score. The endpoints in the study were prostate cancer overall (Table 2), or prostate cancer split into risk groups as separate endpoints (Table 3). When using prostate cancer of a specific risk group as endpoint, prostate cancer cases in other or unknown risk group were excluded from the analysis.

Participating men with more than one measurement of hs-CRP during follow-up (n = 2,210) were included in the data set by using the "reshape" command in STATA, thus updating the measured levels of inflammation-related biomarkers for the next period at risk. We then calculated Δhs-CRP: the difference in hs-CRP between the first and the second measurement. In separate models, Δhs-CRP was included as a continuous variable or dichotomized as Δhs-CRP ≥1.00 mg/l (yes/no).

Based on suggested biological mechanisms influencing our inflammation-related biomarkers, and/or prostate cancer risk, several variables were assessed as potential confounders. Age at entry (continuous) and BMI (continuous), were included as covariates in the final models. Lipid-lowering drugs (categorical), alcohol habits (categorical), and physical activity (categorical) did not influence our results and were not included. The analyses with Δhs-CRP as an explanatory variable were also adjusted for hs-CRP at baseline. We performed stratified analyses by age at study entry (<60 years *vs.* ≥60 years), systolic BP (<140 mm Hg *vs.* ≥140 mm Hg), BMI (<25 kg/m^2 *vs.* ≤25 kg/m^2).

The proportional hazard assumption was verified by visual inspection of log minus log survival curves in tertiles of hs-CRP and WBC and in groups according to Δhs-CRP or systemic inflammation score. All statistical tests were two-sided using a significance level of $p < 0.05$, and conducted with STATA/MP version 15.1 (StataCorp LLC, College station, TX).

Data availability

The data set used in our study is available upon request, pending permission from the Tromsø Study (www.tromsoundersokelsen.no).

Results

The cohort of 7,270 participating men had the following means: age at entry 56.9 years, hs-CRP 2.10 mg/l, and WBC 6.62 (×10^9cells/l) (Table 1). A total of 509 men developed prostate cancer during 11.8 years of follow-up. Men with one measurement of inflammatory markers compared to men with two measurements had a mean follow up and incidence rate of 8.4 years and 60.9/1000 men, and 18.3 years and 124/1000 men, respectively (not presented in tables). The prostate cancer cases with a mean age at diagnosis of 71.7 years had a mean PSA at diagnosis of 14.3 μg/l. Among prostate cancer cases, 16.1% were in the low-risk group, 41.9% were in the intermediate-risk group,

Table 2. Hazard ratios (HR) for risk of prostate cancer by prediagnostic hs-CRP, WBC or by a combination of hs-CRP and WBC (systemic inflammatory score). The PROCA-*life* study (1994–2008)

Inflammatory markers	Cases *N*	Age-adjusted (*n* = 7,270) HR (95% CI)	Multivariable[1] (*n* = 7,270) HR (95% CI)
Hs-CRP			
Continuous, mg/l	509	**1.04 (1.01–1.07)**	**1.03 (1.00–1.07)**
Continuous, 1 SD	509	**1.09 (1.01–1.17)**	**1.09 (1.01–1.17)**
Tertiles			
‹0.91 mg/l	131	1.00 (reference)	1.00 (reference)
0.92–2.03 mg/l	174	1.16 (0.93–1.46)	1.16 (0.92–1.46)
›2.04 mg/l	204	**1.31 (1.05–1.63)**	**1.30 (1.04–1.63)**
WBC			
Continuous, x 10^9/l	490	1.04 (0.98–1.09)	1.04 (0.98–1.09)
Continuous, 1 SD	490	1.06 (0.97–1.17)	1.06 (0.97–1.17)
Tertiles			
≤5.6 x 10^9/l	147	1.00 (reference)	1.00 (reference)
5.7– ≤7.0 x 10^9/l	196	**1.46 (1.18–1.80)**	**1.46 (1.17–1.80)**
≥7.1 x 10^9/l	147	1.23 (0.98–1.55)	1.23 (0.98–1.55)
Systemic inflammatory score (SIS)			
Continuous per 1 point	490	**1.09 (1.02–1.17)**	**1.09 (1.02–1.17)**
SIS low[2–4]	285	1.00 (reference)	1.00 (reference)
SIS high[5,6]	205	**1.28 (1.07–1.53)**	**1.28 (1.06–1.53)**
Δhs-CRP[2]			
Continuous, mg/l	220	1.04 (0.99–1.09)	**1.05 (1.01–1.10)**
Positive change			
‹1.00 mg/l	155	1.00 (reference)	1.00 (reference)
≥1.00 mg/l	65	**1.35 (1.01–1.80)**	**1.36 (1.02–1.82)**

Statistically significant (p value ‹0.05) hazard ratios are marked in bold letters. The systemic inflammatory score ranged from two to six points; high systemic inflammatory score: 5–6 were defined as a high systemic inflammatory score-score. Low systemic inflammatory score: Systemic inflammatory score = 2, 3 and 4.

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; Hs-CRP, high sensitivity C-reactive protein; *n*, numbers; WBC, white blood cell count.

[1]Adjusted for age at entry and BMI. Analyses with Δhs-CRP also adjusted for hs-CRP at baseline.

[2]Δhs-CRP: Change in hs-CRP across two measurements. Analyzed in subgroup with repeated measurements available.

Table 3. Age-adjusted hazard ratios (HR) for different risk groups of prostate cancer, by prediagnostic hs-CRP, WBC or by a combination of hs-CRP and WBC (systemic inflammatory score). The PROCA-*life* study (1994–2008)

	Low-risk prostate cancer (N_{total}: 7,023 [1])		Intermediate-risk prostate cancer (N_{total}: 7,112 [1])		High-risk prostate cancer (N_{total}:: 7,064 [1])		Metastatic prostate cancer (N_{total}: 7,031 [1])	
Inflammatory markers	**Cases**	**HR (95% CI)**	**Cases**	**HR (95% CI)**	**Cases**	**HR (95% CI)**	**Cases**	**HR (95% CI)**
Hs-CRP								
Continuous, mg/l	80	1.00 (0.92–1.09)	195	**1.07 (1.03–1.22)**	132	1.01 (0.95–1.08)	72	1.00 (0.92–1.09)
Continuous, 1 SD	80	1.00 (0.81–1.25)	195	**1.20 (1.08–1.33)**	132	1.04 (0.89–1.21)	72	1.00 (0.81–1.24)
Tertiles								
‹0.91 mg/l	24	1.00 (ref.)	44	1.00 (ref.)	39	1.00 (ref.)	16	1.00 (ref.)
0.92–2.03 mg/l	30	1.12 (0.66–1.92)	65	1.31 (0.90–1.93)	44	0.99 (0.64–1.53)	25	1.30 (0.69–2.43)
›2.04 mg/l	26	0.99 (0.56–1.73)	86	**1.72 (1.19–2.48)**	49	1.04 (0.68–1.60)	31	1.44 (0.79–2.65)
WBC								
Continuous, x 10^9/l	77	1.01 (0.89–1.15)	192	1.03 (0.94–1.11)	126	1.00 (0.90–1.11)	66	1.11 (0.97–1.28)
Continuous, 1 SD	77	1.02 (0.80–1.29)	192	1.05 (0.90–1.21)	126	1.00 (0.83–1.21)	66	1.21 (0.95–1.55)
Tertiles								
≤5.6 x 10^9/l	28	1.00 (ref.)	59	1.00 (ref.)	38	1.00 (ref.)	17	1.00 (ref.)
5.7– ≤7.0 x 10^9/l	30	1.22 (0.73–2.04)	76	**1.44 (1.03–2.03)**	54	**1.54 (1.02–2.33)**	22	1.41 (0.75–2.66)
≥7.1 x 10^9/l	19	0.88 (0.49–1.58)	57	1.23 (0.85–1.77)	34	1.07 (0.67–1.70)	27	**1.91 (1.03–3.52)**
Systemic inflammatory score								
Continuous per 1 point	77	0.96 (0.80–1.14)	192	**1.16 (1.04–1.30)**	126	1.06 (0.88–1.17)	66	**1.25 (1.02–1.51)**
Low[2–4]	51	1.00 (ref.)	108	1.00 (ref.)	77	1.00 (ref.)	33	1.00 (ref.)
High[5,6]	26	0.96 (0.60–1.54)	84	**1.43 (1.07–1.90)**	49	1.11 (0.78–1.60)	33	**1.68 (1.04–2.73)**

Statistically significant (p value ‹0.05) hazard ratios are marked in bold letters. Values given are hazard ratios with 95% confidence interval. Numbers may vary due to missing information. The systemic inflammatory score ranged from 2 to 6 points; High Systemic inflammatory score: 5–6 were defined as a high systemic inflammatory score. Low systemic inflammatory score: Systemic inflammatory Score = 2, 3 and 4.
Abbreviations: CI, confidence interval; HR, hazard ratio; Hs-CRP, high sensitivity C-reactive protein; *n*, numbers; WBC, white blood cell count.
[1]Prostate cancer cases in other risk groups or unknown risk group were excluded from the analysis.

24.2% in the high-risk group, and 12.1% had metastatic disease at the time at diagnosis.

Inflammation-related biomarkers and prostate cancer risk

We observed a positive dose–response relationship between hs-CRP and prostate cancer risk (HR per unit 1.03, 95% CI 1.00–1.07) after adjustments for potential confounding factors (Table 2). Men in the upper tertile of hs-CRP (>2.04 mg/l) had a 30% increased prostate cancer risk (HR 1.30, 95% CI 1.04–1.63) compared to men in the lower tertile of hs-CRP (<0.91 mg/l). We also observed that an increase in hs-CRP between two measurements (Δhs-CRP) of more than 1.00 mg/l increased the risk of prostate cancer by 36% (HR 1.36, 95% CI 1.02–1.82), compared to those men who had a small increase or a decrease in hs-CRP level between two measurements (Table 2). Time between two measurements did not influence these observed results, and mean time between measurements was 6.7 years (range 5.7–14.1 years) (results not presented in table). A similar dose–response relationship was observed between WBC and prostate cancer risk, but the results were not statistically significant.

When the levels of hs-CRP and WBC were combined in a systemic inflammatory score (range 2–6), a positive dose–response association was observed between systemic inflammatory score and prostate cancer risk (HR per unit 1.09, 95% CI 1.02–1.17). Men with a high systemic inflammatory score[5,6] had a 28% increased risk of prostate cancer (HR 1.28, 95% CI 1.07–1.53) when compared to men with a lower systemic inflammatory score.[2–4] When stratified by age at study entry (<60 years *vs.* ≥60 years at study entry), we observed a positive dose–response relationship between systemic inflammation score and prostate cancer risk (HR 1.08, 95% CI 1.00–1.17) only among men who were ≥60 years at study entry, but interaction terms between groups were not significant (Supporting Information Table S1). Among those with a prediagnostic BMI ≥25 kg/m^2 we observed a 1.27 times increased risk (95% CI 1.03–1.58) of prostate cancer for men with an high systemic inflammatory score when compared to men with a low score (Supporting Information Table S2). When stratified by systolic blood pressure (<140 mm Hg *vs.* ≥140 mm Hg), we observed a positive dose–response relationship between hs-CRP and prostate cancer risk (HR 1.06, 95% CI 1.02–1.11) among men who had a systolic blood pressure < 140 mm Hg. Interaction terms between groups were not significant (Supporting Information Table S3).

Inflammation-related biomarkers and severity of prostate cancer

Men with a WBC count in the upper tertile (≥7.1 x 10^9cells/l), had a 1.91 (95% CI 1.03–3.52) times increased risk of metastatic

prostate cancer when compared to men with the lowest tertile of WBC ($\leq 5.6 \times 10^9$/l).

We observed a dose–response association between systemic inflammatory score and both being diagnosed within an intermediate prostate cancer risk group (HR 1.16, 95% CI 1.04–1.30) and being diagnosed with metastatic disease, (HR 1.25, 95% CI 1.02–1.51). Men with a high systemic inflammatory score[5,6] had a 43% increased risk for intermediate risk prostate cancer (HR 1.43, 95% CI 1.07–1.90), and a 68% increased risk of metastatic prostate cancer (HR 1.68, 95% CI 1.04–2.73) when compared to men having a systemic inflammatory score between 2 and 4 (Table 3).

Discussion

In this population-based prospective study with repeated measurements of prediagnostic inflammatory markers, we observed that hs-CRP measured at one and two time points was associated with prostate cancer risk in a positive dose–response manner; among men with an increase in hs-CRP between two measurements (≥1.00 mg/l), we observed a 36% higher prostate cancer risk compared to those who had small increase or a decrease in hs-CRP level. Men with a high systemic inflammatory score (hs-CRP and WBC in combination) had a 28% higher prostate cancer risk, and were more likely to be diagnosed with metastatic prostate cancer compared to men having a low systemic inflammatory score (2–4).

Results from previous studies of the association between hs-CRP or WBC and prostate cancer risk have been inconsistent. Our findings that hs-CRP measured at one time point were associated with prostate cancer risk are supported by some studies,[7,17,24] but our results are also in contrast to others.[16,18,19,21–23] In a nested case–control study including 622 prostate cancer cases, a positive association was observed between prediagnostic CRP and prostate cancer risk among men with BMI <25 kg/m^2, even when CRP was measured several years before the diagnosis.[21] In the present study, we did not observe any clear pattern of variation in the associations studied between inflammatory markers and prostate cancer when stratified by BMI (BMI <25 kg/m^2 *vs.* BMI ≥25 kg/m^2). However, among those with a prediagnostic BMI ≥25 kg/m^2 we observed a 1.27 times increased risk (95% CI 1.03–1.58) of prostate cancer for men with a high systemic inflammatory score when compared to men with a low score. These findings support that excess weight may mirror a low grade inflammation by resulting in a higher systemic inflammatory score not observed among the leaner men (BMI <25 kg/m^2).

Our findings suggesting that both hs-CRP and an increase in hs-CRP during follow-up were associated with risk of metastatic prostate cancer are partly in line with the Swedish AMORIS study.[17] In the AMORIS study,[17] CRP was dichotomized into low (<10 mg/ml) and high (≥10 mg/ml) and it was observed that CRP levels assessed on average 14 years before being diagnosed with prostate cancer predicted worse outcome (high-risk prostate cancer and metastatic prostate cancer). A positive association between hs-CRP and advanced prostate cancer is also supported by others.[29–31]

However, to our knowledge, this is the first study to assess the combination of hs-CRP and WBC creating a systemic inflammatory score in relation to both prostate cancer risk and severity. Interestingly, our findings suggest that compared to using either WBC or hs-CRP alone, a combination of these markers may be more useful. The score was strongly associated with both risk for prostate cancer and for severity of prostate cancer. Thus, an inflammatory score might be a useful way of combining two or more inflammatory markers that could be used for risk classification.[24] In a large population-based study by Morrison *et al.*, CRP and WBC were combined into a Z-score.[32] They found an association between the inflammation Z-score and risk for overall cancer, including prostate cancer. In contrast, in another study, a high score based on three inflammatory biomarkers (CRP, WBC and fibrinogen) was not associated with prostate cancer risk.[33] Additionally, several studies have questioned whether a systemic inflammatory score could be a valuable predictive tool for worse outcome in several types of cancers including prostate cancer,[34,35] and our results support the hypothesis that it might be valuable for prostate cancer severity.

Published studies suggest a dual effect of obesity: an increased risk for advanced prostate cancer,[36] low-grade systemic inflammation,[37] severity of prostate cancer and a decreased risk of localized prostate cancer.[38] In our study, we did not find any variation by measured BMI (kg/m^2), in contrast to others,[39] but we included only one BMI measurement. Wang *et al.* found that men with an increase in BMI from normal to an overweight or obese condition experienced increased risk of prostate cancer compared to men with persistently normal BMI, and that this was most pronounced for men with ISUP grade group ≥7. The biological explanation is not fully understood, but there is evidence suggesting that substantial crosstalk occurs between molecular pathways involved in inflammation and obesity. Studies have investigated the association between inflammatory markers and hypertension,[40,41] where low-grade systemic inflammation might be a common cause. We stratified our results by systolic BP ($\lessgtr$140 mm Hg), but did not observe any significant association between the systemic inflammatory score and risk of prostate cancer (Supporting Information Table S3).

Inflammation is one of the hallmarks of cancer development,[33] and CRP is found in blood plasma, with rising levels in response to factors released by inflammatory associated cells as macrophages and fat cells.[42] Chronic inflammation is evident in the adult prostate and probably has a role in formation of lesions such as proliferative inflammatory atrophy, which is proliferative glandular epithelium with morphological appearance of simple atrophy that occurs in association with inflammation.[2,43,44] These lesions are thought to be possible precursors for prostate cancer.[2,45] Further, there is evidence that regenerative epithelium in response to environmental insults may precede development of prostate intraepithelial neoplasia and early carcinoma.[44,45] The origin of prostate inflammation is multifactorial

and in many cases without symptoms. The inflammation could be either acute or chronic.[46]

The strengths of our study include its prospective and population-based design and the high attendance rate (65.7–78.5%[25]), which lessens the chance of biased observations. In addition, a high completeness rate of identification of prostate cancer cases (Cancer Registry of Norway) at 98.8% is another strength.[47] Furthermore, the rather long follow-up time, broad information about baseline characteristics and repeated measurements of hs-CRP strengthen the results observed. All medical records for the prostate patients were carefully reviewed by trained physicians with systematic abstraction of histopathology and clinical characteristics. The study was able to control for several potential confounding factors, and to address effect modification, such as age, body mass index, smoking habits, and physical activity.

However, our study also has some limitations. The population in Tromsø is mainly Caucasian, and the results may therefore not be relevant for populations including other ethnicities. Repeated assessments of inflammation-related biomarkers were only assessed among a subgroup of men, thus limiting the sample size. A limitation of our study is the long time between exposure measurement and diagnosis. Thus, changes in various clinical variables over time may have occurred, and two measurements of variables may only in part account for the cumulative effect of the markers on risk of prostate cancer. The levels of these exposures may be affected by various factors over the life-course and may tend to fluctuate. However, measurements of BMI made earlier in life have been found to be strongly related to measurements later in life.[48,49] Moreover, adjustment for time between measurement and diagnosis did not change our results. Information regarding family history of prostate cancer was not available and could therefore not be included in the analysis.

Conclusion

Our study supports a positive association between hs-CRP, hs-CRP and WBC in combination and risk for both prostate cancer and for metastatic prostate cancer. Importantly, hs-CRP and WBC are often used in routine clinical practice, and thus easily accessible. Our findings contribute to understanding the relationship between inflammation and prostate cancer development, and may be useful in future research on prostate cancer etiology and possibly prevention. However, our results are based on a relatively small sample size and should be interpreted with caution.

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Paper II

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RESEARCH ARTICLE

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Systolic and diastolic blood pressure, prostate cancer risk, treatment, and survival. The PROCA-*life* study

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Abstract

Background: Inflammation has been linked to prostate cancer and hypertension, but it remains equivocal whether elevated blood pressure (BP) influence prostate cancer risk and survival.

Method: Using Cox regression models, we examined the association between prediagnostic BP and prostate cancer risk among 12,271 men participating in the Prostate Cancer throughout life (PROCA-life) study. Systolic and diastolic BP were measured. A total of 811 men developed prostate cancer, and followed for additional 7.1 years, and we studied the association between prediagnostic BP and overall mortality among patients with prostate cancer.

Results: Men (>45 years) with a systolic BP >150 mmHg had a 35% increased risk of prostate cancer compared with men with a normal systolic BP (<130 mmHg) (HR 1.35, 95% CI 1.08–1.69). Among patients with prostate cancer, men with

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systolic BP >150 mmHg had a 49% increased overall mortality compared with men with a normal systolic BP (HR 1.49, 1.06–2.01). Among patients with prostate cancer treated with curative intent, those with a high diastolic BP (>90 mmHg) had a threefold increase in overall mortality risk (HR 3.01, 95% CI 1.40–6.46) compared with patients with a normal diastolic BP (<80 mmHg).

Conclusion: Our results support that systolic and diastolic BP are important factors when balancing disease management in patients with prostate cancer.

KEYWORDS

hypertension, inflammation, mortality, prostate cancer, risk

1 | INTRODUCTION

Prostate cancer and hypertension are both common and complex conditions among men world-wide. While prostate cancer is one of the most common cancers in men and its incidence continues to rise, systolic blood pressure (BP) above 115 mmHg is ranked as a leading risk factor for the global burden of disease.[1] The global age-standardized prevalence of elevated BP (systolic BP ≥140 mmHg or diastolic BP ≥90 mmHg) in men was estimated as ≥20% in 2015.[2] Of note, high BP may last for several decades ahead of any disease development, reflecting a long-lasting cumulative exposure and exposure time of interest in an ageing-related disease as prostate cancer.

Hypertension has been linked to inflammation, and inflammation is one of the hallmarks of cancer development.[3] Inflammatory cells in the prostate microenvironment associated with precursor lesions for prostate cancer in the prostate gland, termed proliferative inflammatory atrophy, have been observed.[4] Recently, we observed that systemic prediagnostic inflammatory biomarkers including high sensitive C-reactive protein (hs-CRP) and white blood cells were associated with prostate cancer development, and our results are supported by others linking systemic inflammatory biomarkers to prostate cancer development.[5]

Results from previous studies of the association between hypertension and prostate cancer development have been inconsistent.[6–8] Neither the European Prospective Investigation into Cancer and Nutrition (EPIC) nor a meta-analysis observed any association between hypertension and risk of prostate cancer.[7,8] However, a meta-analysis including case–control and cohort studies support that hypertension may increase prostate cancer risk.[6] Moreover, in a longitudinal case–control study, men (aged 40–58 years at study entry) in the highest quartile of systolic BP (>150 mmHg) had an increased prostate cancer risk.[9] Hypertension was also associated with increased risk of biochemical recurrence after radical prostatectomy, independent of age at diagnosis and tumor pathological features.[10] Whether long-lasting, raised diastolic hypertension influences prostate cancer development and prognosis has not been much studied. Use of antihypertensive medication does not seem to have any effect on cancer risk.[11] Thus, the importance of elevated BP may show variation by age at onset of hypertension, exposure time, age when diagnosed with prostate cancer, and aggressiveness of disease.[12]

Whether long-lasting, modern, prostate cancer treatments interact with systolic and diastolic BP of importance for survival has not been much studied.[13] Androgen deprivation therapy (ADT) has a key role in adjuvant prostate cancer treatment combined with radiation therapy, as well as in the lifelong treatment of metastatic prostate cancer.[14,15] However, important side effects from ADT include a higher risk of later cardiovascular disease (CVD).[16] Men with prostate cancer, aged ≥40 years, who underwent ADT, were observed to have a higher risk of developing hypertension.[17] However, there is a knowledge gap regarding elevated BP before, during, and after prostate cancer treatment. Furthermore, we lack information about the importance of a pre-existing hypertension on the risk for future CVD events after initiating ADT among patients with prostate cancer.

The aim of the present study was, therefore, to study whether prediagnostic systolic and diastolic BP were associated with prostate cancer risk, if prediagnostic systolic and diastolic BP were associated with overall mortality among patients with prostate cancer, and if such associations vary by age and type of prostate cancer treatment.

2 | METHOD

2.1 | Study design, settings, and participants

The Prostate Cancer Study throughout life (PROCA-life) includes all men older than 25 years at study entry who

were enrolled in the population-based Tromsø Study in 1994/1995 (Tromsø 4).[18,19] The procedures and assessments were performed by trained research technicians at one study site. All age-eligible men in the Tromsø municipality were invited to participate with a personal written invitation, and nonresponders were given one reminder. The attendance proportion for men included in the present study was 69.6% of those invited.[19]

2.2 | Questionnaire and assessments of lifestyle factors

The questionnaire was checked for completeness and inconsistency and included questions about medical history, lifestyle factors, and use of medication including antihypertensive drugs. Educational level was categorical (1 = secondary school only, 5 = college/university for 4 or more years). Alcohol use was defined as more than 1 unit of alcohol per month, defined by others in this cohort.[20,21] We defined being physically active as more than 1 h/week of strenuous exercise, or any leisure time exercise more than 2–3 times/week.

2.3 | Assessments of systolic and diastolic blood pressure and clinical assessments

Systolic and diastolic BP (mmHg) were measured by using an automatic device (Dinamap Vital Signs Monitor 1846; Critikon Inc.). Participants rested for 2 min in a sitting position, then three readings were taken on the upper right arm, separated by 1-min intervals, and the average of the last two readings was used.[22]

Height and weight were measured on a regularly calibrated electronic scale with the participants wearing light clothing and no shoes. Height was measured to the nearest centimeter (cm) and weight to the nearest kilogram (kg). Body mass index (BMI) was calculated using the formula weight/height^2 (kg/m^2).

2.4 | Assessment of serum samples

Blood samples (nonfasting) were drawn by trained research assistants on attendance. Analyses of serum samples were done at the Department of Laboratory Medicine, University Hospital of Northern Norway (UNN).[18] Prostate-specific antigen (PSA) measurements were done for cancer cases only, as part of clinical routine in diagnosis and follow-up (1990–1994 Stratus® PSA Fluorometric Enzyme Immunoassay, 1994–2001 AxSYM Psa Reagent Pack, Abbot®, 2001 Bayer® PSA Reagens Pack Immuno I [Prod. Nr.T01-3450-51], Technicon Immuno I). For patients with prostate cancer diagnosed or treated in other institutions ($n = 21$), PSA values from their local laboratories were recorded.

2.5 | Identification of patients with prostate cancer during follow-up

Patients with prostate cancer diagnosed during follow-up (until December 31, 2018) were identified by using the unique national 11-digit identification number through linkage with the Cancer Registry of Norway. We excluded all men who had a previous history of cancer ($n = 382$), or who emigrated, died, or were diagnosed with cancer within the first year after study entry ($n = 128$), to account for the possibility that undiagnosed cancer or severe illness could influence our results. Participants with missing measurement of BP at study entry were also excluded ($n = 24$) leaving a final study population of 12,271 men (Figure S1).

A total of 811 men developed prostate cancer during follow-up between 1994 and 2018. There were no ongoing screening programs for prostate cancer in Norway during the study period. Causes of death were identified by linkage to the Norwegian Cause of Death Registry, and dates of emigration were obtained from the Population Registry of Norway.

Detailed information from medical records were obtained by trained physicians (TK, MS, and ES) and included prostate cancer treatments and recurrence. A total of 153 patients with prostate cancer had missing data in treatment details or follow-up but were still included if baseline data; data about diagnosis and data on cause of death were complete (Figure S1).

Histopathological information for the patients with prostate cancer was obtained from histopathological records and were in addition re-examined by the same specialized pathologist (ER) and classified according to the latest International Society of Urological Pathology (ISUP) guidelines on Gleason score and ISUP grade group.[23] Patients with prostate cancer were divided into four risk groups based on PSA level at diagnosis, highest ISUP grade group and clinical T-stage, similar to the European Association of Urology-classification (EAU) guidelines.[14] Risk group 1 (low) was defined as PSA <10 μg/L, clinical T-stage (cT-) 1, and ISUP grade group 1. Risk group 2 (intermediate) was defined as PSA: 10–20 μg/L, cT-stage 2, or ISUP grade group 2–3. Risk group 3 (high) was defined as PSA: >20–100 μg/L, cT-stage 3, or ISUP grade group 4–5. Risk group 4 (metastatic) was defined as PSA >100 μg/L, or with radiological evidence of metastatic disease. ISUP grade group was reported

after reclassification when available. PSA values above 100 were not included in calculation of mean or median PSA.

2.6 | Statistical methods

Descriptive characteristics of the study population were presented as means (standard deviation) or percent (numbers). Multivariable Cox proportional hazard models, with follow-up time as timescale, were used to investigate whether prediagnostic systolic or diastolic BP were independently associated with prostate cancer risk and mortality. To study the importance of the variation, prediagnostic systolic and diastolic BP were split in four levels based on international categories: systolic BP (mmHg): <130, 130–139.9, 140–149.9, ≥150 mmHg, diastolic BP (mmHg): <80, 80–89.9, 90–99.9, ≥100 mmHg.

Associations between baseline BP and prostate cancer incidence have been studied in the full cohort ($n = 12{,}271$), and associations between baseline BP and overall mortality have been studied in men diagnosed with prostate cancer (the PCa-cohort, $n = 811$). Follow-up to incidence of prostate cancer was calculated from the date of entry into the study to the date of prostate cancer diagnosis, date of emigration, date of death, or end of follow-up (December 31, 2018), whichever event occurred first. Follow-up to mortality after prostate cancer diagnosis was calculated from the date of prostate cancer diagnosis to date of death, emigration, or end of follow-up (December 31, 2018). Based on biological mechanisms hypothesized and previous observations suggesting that risk factors for prostate cancer may vary by time period during lifetime and by length of exposure,[24] separate analyses on prostate cancer incidence were performed in two age groups (age at entry <45 years and age >45 years). Furthermore, to study whether the association between prediagnostic BP and mortality varied by the type of prostate cancer treatment, analyses were performed by type of treatment, curative or endocrine, within the PCa-cohort.

Several variables were assessed as potential confounders based on suggested biological mechanisms influencing systolic and diastolic BP and/or prostate cancer risk and prognosis. Age at entry (continuous), BMI (continuous), alcohol habits (categorical), smoking (categorical), physical activity (categorical), educational level (categorical), and diabetes (yes/no) were included as covariates in the final models. Use of lipid-lowering and/or antihypertensive medication were included but did not influence the results and were excluded in the final models.

Kaplan–Meier survival curves of prostate cancer incidence and of total mortality were presented for the full cohort and for the PCa cohort, respectively. The proportional hazard assumption was verified by assessing the parallelism between log minus log survival curves for categories of BP and also formal tests based on Schoenfeld residuals. All statistical tests were two-sided using a significance level of $p < 0.05$ and conducted with STATA/MP version 16 (StataCorp LLC).

3 | RESULTS

At study entry, the cohort participants had the following means: age at entry 45.6 years (SD 14.2), prediagnostic systolic BP 134.1 mmHg and prediagnostic diastolic BP 77.5 mmHg (Table 1). During follow-up, a total of 811 men developed prostate cancer with a mean age at diagnosis of 69.4 years. A total of 18.0% of the patients with prostate cancer were in the low-risk group, and 21.7% were in the high-risk group at the time of diagnosis. A total of 265 patients with prostate cancer (32.7%) died during 7.1 years of follow-up, of whom 41.9% ($n = 111$) were classified as prostate cancer death, 12.5% ($n = 33$) as cardiovascular death and 45.7% ($n = 121$) other causes of death (Table 1, Table S2).

3.1 | Prediagnostic systolic and diastolic blood pressure and prostate cancer risk

We observed an increased incidence of prostate cancer among men in the upper level of both systolic and diastolic BP (systolic BP ≥150 mmHg, diastolic BP ≥100 mmHg) in crude data (Figure 1). Among men aged >45 years at study entry, we observed, when adjusted for potential confounding factors, a positive dose–response association between prediagnostic systolic BP and prostate cancer risk (HR 1.07 per SD increase, 95% CI 1.00–1.16). Furthermore, men with a prediagnostic systolic BP >150 mmHg had a 35% increased risk of prostate cancer compared with men with prediagnostic systolic BP <130 mmHg (HR 1.35, 95% CI 1.08 −1.69). We observed an overall positive dose–response relationship between prediagnostic diastolic BP and risk of prostate cancer (HR 1.08 per SD increase, 95% CI 1.01–1.17) (Table 2, Figure 1). Associations between BP and incidence of different risk-groups of prostate cancer has been tested but did not provide statistically significant results.

3.2 | Prediagnostic systolic and diastolic blood pressure and survival

After 7.1 years of follow-up after being diagnosed with prostate cancer, there was among patients with prostate

TABLE 1 Distribution of selected prediagnostic characteristics for men with prostate cancer (cases) and without prostate cancer (non-cases) in the PROCA-life Study (1994–2018)

Characteristics	Non-cases (n = 11,460)	Prostate cancer cases (n = 811)
Age at entry (years)	45.6 (14.2)	54.4 (10.8)
Observation time (years)	21.0 (6.0)	14.0 (6.1)
Clinical variables, mean (SD)		
Systolic blood pressure (mmHg)	134.1 (16.8)	137.9 (18.9)
Diastolic blood pressure (mmHg)	77.5 (11.6)	80.8 (11.7)
Body mass index (kg/m^2)	25.6 (3.3)	25.9 (3.2)
Serum samples at study entry mean (SD)		
Total cholesterol (mmol/L)	6.02 (1.2)	6.32 (1.2)
Hs-CRP (mg/L)[a]	2.97 (7.4)	2.57 (4.7)
White blood cells ($\times 10^9$/L)	7.07 (2.0)	6.98 (1.8)
Lifestyle factors (%)		
Lipid-lowering drugs, current use	1.0	1.4
User of blood pressure–lowering medication	7.2	9.3
Current smokers	36.8	31.0
Physically active	37.6	36.0
Alcohol user	66.5bn	66.8
Characteristics among patients with prostate cancer		
Age at diagnosis, mean (SD) (years)		69.4 (9.0)
PSA at diagnosis, median (μg/L)[b]		10.9
Observation time after diagnosis (years)		7.1
Cancer-specific mortality, % of all death (n)		41.9 (111)
Cardiovascular death, % of all death (n)		12.5 (33)
Other causes, % of all death (n)		45.7 (121)
Tumor characteristics		
T-stage, % (n)		
T1		42.4 (344)
T2		24.4 (198)
T3		13.1 (106)
T4		3.8 (31)
Tx		16.2 (132)
ISUP Grade Group, % (n)		
1 (Gleason 3+3)		39.1 (317)
2 (Gleason 3+4)		19.5 (158)
3 (Gleason 4+3)		8.5 (69)
4 (Gleason 4+4)		6.9 (56)
5 (Gleason 4+5/5+4/5+5)		7.4 (60)
ISUP missing		16.8 (151)
Risk group, % (n)		
Low		18.0 (146)
Intermediate		32.9 (267)
High		21.7 (176)
Metastatic		9.0 (73)
Unknown		18.4 (149)

(Continues)

TABLE 1 (Continued)

Characteristics	Non-cases (n = 11,460)	Prostate cancer cases (n = 811)
Prostate cancer treatment characteristics, % (n)		
Curative intended treatment		58.7 (476)
Endocrine treatment, overall		36.0 (292)
Endocrine treatment, curative		19.2 (156)

Numbers may vary due to missing information. Values are mean (standard deviation) unless otherwise specified.

Prostate cancer risk group definitions: Low: PSA <10 μg/L, clinical T-stage (cT-) 1, and ISUP grade group 1. Intermediate: PSA: 10–20 μg/L, cT-stage 2, or ISUP grade group 2–3. High: PSA: >20–100 μg/L, cT-stage 3, or ISUP grade group 4–5. Metastatic: PSA >100 μg/L, or with radiological evidence of metastatic disease.

Abbreviations: Hs-CRP, high-sensitivity C-reactive protein; PSA, prostate-specific antigen; ISUP, International Society of Urological Pathology.

[a]CRP measured only in 2781 men.

[b]PSA values above 100 were not included in calculation of mean or median PSA.

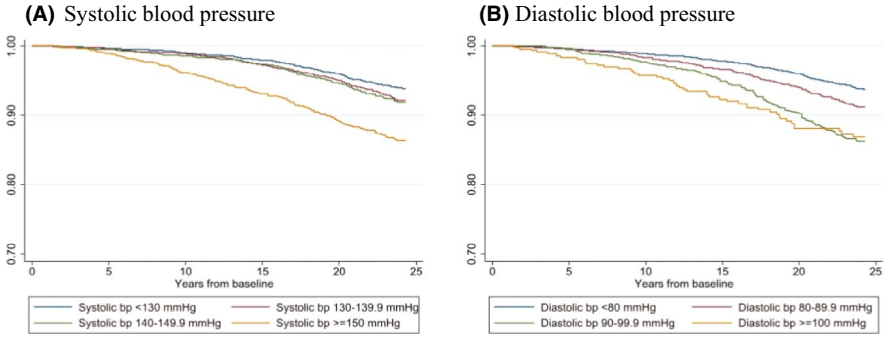

FIGURE 1 Kaplan–Meier survival curves of prostate cancer incidence according to prediagnostic systolic (A) and diastolic (B) blood pressure (bp)

cancer a positive dose–response association between prediagnostic systolic BP and overall mortality (HR 1.14 per SD increase, 95% CI 1.03–1.27) and prediagnostic diastolic BP and overall mortality (HR 1.17 per SD increase, 95% CI 1.03–1.32). Patients with prostate cancer with a prediagnostic diastolic BP ≥100 mmHg, had an 85% increased overall mortality compared with patients with prostate cancer with diastolic BP <80 mmHg (HR 1.85, 95% CI 1.22–2.82). Patients with prostate cancer treated with curative intention and with a high prediagnostic diastolic BP (≥100 mmHg) had a threefold higher overall mortality risk compared with the patients with prostate cancer with a prediagnostic diastolic BP <80 mmHg (HR 3.05, 95% CI 1.42–6.55). Among patients with prostate cancer receiving endocrine treatment, those with a high prediagnostic diastolic BP (≥100 mmHg) at study entry had a twofold increase in overall mortality risk compared with those with a prediagnostic diastolic BP <80 mmHg (HR 2.15, 95% CI 1.25–3.69) (Table 3).

After 10 years of follow-up, we observed that among patients with prostate cancer, 49% of those with a prediagnostic systolic BP ≥150 mmHg were alive, compared with 66% of patients with prostate cancer with a normal prediagnostic systolic BP (<130 mmHg). Among those with a prediagnostic diastolic BP ≥100 mmHg, 33% were alive, compared with 61% of the patients with prostate cancer with a normal prediagnostic diastolic BP (<80 mmHg). (Figure 2).

This association was even more pronounced among men >45 years at entry compared with overall, where the patients with prostate cancer with a high prediagnostic diastolic BP (≥100 mmHg) at study entry had a nearly doubled overall mortality risk compared with those with a prediagnostic diastolic BP <80 mmHg (HR 1.99, 95% CI 1.30–3.04), and a positive dose–response association was observed between prediagnostic BP and overall mortality (p-trend = 0.002) (Table 3, Table S1).

4 | DISCUSSION

In this prospective study, we observed a dose–response association between prediagnostic systolic and diastolic BP and prostate cancer risk and overall survival. Additionally, among prostate cancer patients treated with curative intention and patients receiving endocrine treatment a high prediagnostic diastolic BP (≥100 mmHg) was associated with a threefold and twofold increased increased overall mortality risk, respectively, compared with those with a prediagnostic diastolic BP below 80 mmHg.

Our results extend previous results but are also in part supported by others who have observed that elevated

TABLE 2 Multivariable adjusted[a] hazard ratios (HRs) for incident prostate cancer according to the levels of prediagnostic systolic and diastolic blood pressure by age-group (≤/>45 years). The PROCA-*life* study (1994–2018)

	All age (patients with prostate cancer $n = 811$)		≤45 years at baseline (patients with prostate cancer $n = 183$)		>45 years at baseline (patients with prostate cancer $n = 628$)	
	HR (95%CI)			HR (95%CI)		HR (95%CI)
	Number of cases	Multivariable[a]	Number of cases	Multivariable[a]	Number of cases	Multivariable[a]
Systolic blood pressure (mmHg)						
<130	296	1.00 (ref.)	95	1.00 (ref.)	201	1.00 (ref.)
130–139.9	221	**1.20 (1.01–1.43)**	56	1.13 (0.81–1.58)	165	**1.28 (1.04–1.58)**
140–149.9	121	0.99 (0.80–1.23)	23	1.03 (0.65–1.64)	98	1.08 (0.84–1.38)
≥150	173	1.13 (0.92–1.39)	9	0.87 (0.43–1.74)	164	**1.35 (1.08–1.69)**
p for trend[b]		*0.41*		*0.967*		***0.025***
Per SD increase		1.00 (0.93–1.08)		0.94 (0.76–1.16)		**1.07 (1.00–1.16)**
Diastolic blood pressure (mmHg)						
<80	404	1.00 (ref.)	132	1.00 (ref.)	272	1.00 (ref.)
80–89.9	227	0.99 (0.83–1.16)	37	0.80 (0.55–1.15)	190	0.93 (0.77–1.13)
90–99.9	132	**1.25 (1.02–1.54)**	11	0.79 (0.42–1.49)	121	1.20 (0.96–1.50)
≥100	48	1.20 (0.88–1.64)	3	0.76 (0.24–2.40)	45	1.15 (0.83–1.59)
p for trend[b]		*0.056*		*0.223*		*0.165*
Per SD increase		**1.08 (1.01–1.17)**		0.88 (0.74–1.06)		1.05 (0.97–1.15)

Statistically significant (p-value < 0.05) hazard ratios are marked in bold letters. p-value for linear trend in blood pressure categories are marked in italic letters.

[a]Adjusted for age at baseline, body mass index (BMI, kg/m^2), smoking, alcohol use, physical activity, diabetes, and education level.

[b]p-value for linear trend in blood pressure categories.

systolic BP is associated with increased incidence of prostate cancer.[25–28] Interestingly, hypertension was associated with higher prostate cancer risk, with the strongest association for fatal prostate cancer.[12] In contrast, neither the EPIC-study nor a meta-analysis observed any associations between hypertension and risk of prostate cancer.[7,8] Our findings that elevated prediagnostic systolic BP might be a risk factor only in men above 45 years may be an observation only by chance or may suggest variation by age groups and a reason for the inconsistent findings observed in previous studies. Of note, in a Swedish study including 330,000 men that were enrolled into the study between 1971 and 1993 with a mean age at entry of 34.7 years, both systolic and diastolic BP were associated with a decreased risk of incident prostate cancer.[29] These findings may suggest that the association between elevated BP and prostate cancer may vary by time periods due to several factors, including improved diagnostic possibility of prostate cancer and an aging population at risk. Importantly, biological mechanism risk factors including chronic inflammation initiating raised systolic and diastolic BP may also vary throughout different time periods, and treatment for hypertension is initiated at a lower level of diastolic and systolic BP today compared with 1970s- '80s. These settings may complicate interpretation and comparisons between studies regarding raised BP and prostate cancer risk and survival throughout time periods, even if tracking of BP is high.[22] Furthermore, the age at onset of hypertension and the cumulative exposure of hypertension during lifetime may complicate the interpretation of any association between elevated BP and prostate cancer during long-term follow-up. Of note, all our participants have measured BP at study entry.

Few studies have looked at the isolated effect of diastolic BP on prostate cancer development, but among patients with prostate cancer with a mean age at diagnosis of 70 years, high levels of PSA were associated with high levels of systolic and diastolic BP.[30] In another study, a positive association between PSA and diastolic BP was observed when adjusting for age and other clinical and socioeconomic factors,[31] and a 5% increased risk for prostate cancer for each 11.4 mmHg increase in prediagnostic diastolic BP has been observed by others.[32] These findings support our findings suggesting that elevated diastolic BP may play a role in relation to prostate cancer development.

To our knowledge, we are the first to investigate the effect of prediagnostic diastolic BP by treatment details (curative intent, endocrine treatment). However, our

TABLE 3 Multivariable adjusted[a] hazard ratios (HRs) for all-cause mortality according to prediagnostic systolic and diastolic blood pressure among patients with prostate cancer by the type of treatment (curative and endocrine prostate cancer treatment). The PROCA-*life* study (1994–2018)

		All prostate cancer		Curative treatment		Endocrine treatment
	Number of deaths/cases	265/798	Number of deaths/cases	86/476	Number of deaths/cases	168/292
		HR (95% CI)		HR (95% CI)		HR (95% CI)
Systolic blood pressure (mmHg)						
<130	67/296	1.00 (reference)	22/196	1.00 (reference)	44/94	1.00 (reference)
130–139.9	60/221	1.08 (0.75–1.55)	21/112	1.11 (0.59–2.08)	40/72	0.87 (0.55–1.36)
140–149.9	46/121	0.97 (0.65–1.47)	17/70	1.58 (0.81–3.10)	30/48	0.91 (0.55–1.51)
≥150	92/173	1.35 (0.96–1.90)	26/82	1.83 (0.99–3.40)	54/78	1.11 (0.73–1.71)
p for trend[b]		*0.091*		***0.029***		*0.51*
Per SD increase		**1.14 (1.03–1.27)**		**1.26 (1.03–1.55)**		1.14 (0.99–1.31)
Diastolic blood pressure (mmHg)						
<80	110/404	1.00 (reference)	32/238	1.00 (reference)	74/125	1.00 (reference)
80–89.9	75/227	1.08 (0.80–1.45)	24/134	1.10 (0.64–1.88)	48/94	0.98 (0.67–1.42)
90–99.9	50/132	1.24 (0.87–1.75)	20/80	1.75 (0.97–3.14)	26/49	0.91 (0.57–1.45)
≥100	30/48	**1.85 (1.22–2.82)**	10/24	**3.05 (1.42–6.55)**	20/24	**2.15 (1.25–3.69)**
p for trend[b]		***0.009***		***0.004***		*0.13*
Per SD increase		**1.17 (1.03–1.32)**		**1.43 (1.17–1.75)**		1.12 (0.97–1.30)

Statistically significant (p-value < 0.05) hazard ratios are marked in bold letters. p-value for linear trend in blood pressure categories are marked in italic letters.

[a]Adjusted for age at baseline, body mass index (BMI, kg/m^2), smoking, alcohol use, physical activity, diabetes, and education level.

[b]p-value for linear trend in blood pressure categories.

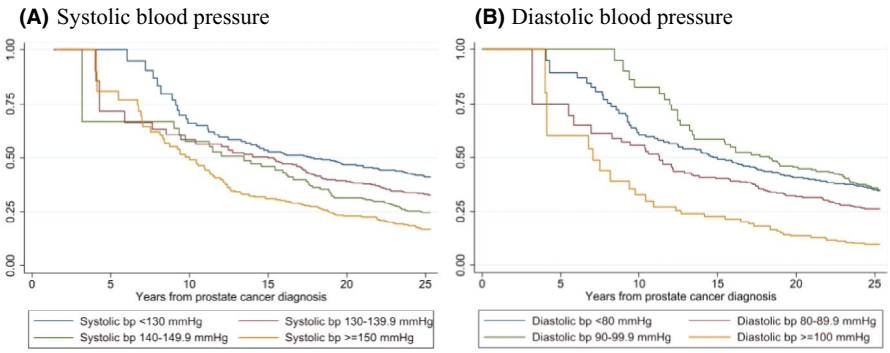

FIGURE 2 Kaplan–Meier survival curves of overall mortality among prostate cancer cases ($n = 811$) according to prediagnostic systolic (A) and diastolic (B) blood pressure (bp)

findings of a threefold increased mortality risk among patients with prostate cancer receiving curative treatment with a prediagnostic diastolic BP >100 mmHg compared with patients with prostate cancer with diastolic BP <80 mmHg are in part supported. Moustsen et al. observed that men who received first-line palliative treatment had higher rates of ischemic stroke or heart failure, compared with prostate cancer–free men.[33] These findings are also in line with our observation that men with prostate cancer die at an earlier age than prostate cancer–free men (Table S2). In addition, in a retrospective cohort study with 1900 patients with nonmetastatic prostate cancer, 10 years after diagnosis the cumulative probability of prostate cancer mortality and CVD mortality was 16.4% and 10.0%, respectively.[34] These findings support our findings as we observed that patients with prostate cancer died at an earlier age if they died due to prostate cancer than if they died of CVDs. Furthermore, pre-existing hypertension, hyperglycemia, and overweight were associated

with poor prostate cancer prognosis.[35] Of note, in our study, diabetes and body composition were included as covariates in our final model, as they influenced our risk estimates.

Recently, cardiovascular health, including optimal BP, is suggested to be an important factor when balancing disease management and monitoring cardiovascular health in patients with prostate cancer. The importance of including optimal BP treatment among patients with prostate cancer was underlined in a recent study, as men who received first-line palliative treatment had higher rates of heart failure and ischemic stroke.[33]

Systemic inflammation is among the potential biological mechanisms operating to explain the observed association between hypertension and prostate cancer.[36–38] Inflammation is one of the hallmarks of prostate cancer development,[3] and inflammatory cells associated with precursor lesions for prostate cancer in the prostate gland, have been observed.[4] Interestingly, our results suggest that elevated diastolic BP is a stronger risk factor than elevated systolic BP for prostate cancer development, and in particular for mortality risk. Whether diastolic BP rather than systolic BP is more linked to chronic inflammation is not much studied.[39] However, the main determinants of the systemic arterial BP is cardiac output, systemic vascular resistance, and a critical closing pressure at the level of the arterioles.[40] Raised BP may downregulate IGF-binding protein-1 (IGFBP-1), and this might increase the risk of prostate cancer by increasing IGF-1 activity.[32] More research is needed to determine whether systemic inflammation caused by both raised systolic and diastolic BP play a role or share common biological pathways influencing prostate cancer development, or if premalignant cells cause the inflammation that causes the hypertension.

The strengths of our study include the measured BP, its population based and prospective design with high attendance rate, and a high completeness rate of identification of patients with prostate cancer (98.8%).[41] Furthermore, the rather long, follow-up time, which may result in long exposure time of elevated BP, the broad information about baseline characteristics and precise measurements of risk factors strengthens the results observed. All medical records for the patients with prostate were carefully reviewed by trained physicians with systematic abstraction of histopathology and clinical characteristics. The study was able to control for several potential confounding factors, and to address effect modification, such as age, BMI, smoking habits, diabetes, and physical activity.

Our study also has some limitations. The exposure variables and other baseline variables were based on a single-time, prediagnostic measure. However, tracking studies from the same cohort of men have shown that men tend to follow a trajectory of BP suggesting an accumulated lifetime exposure.[22] The associations between all-cause mortality and baseline BP among patients with prostate cancer (Table 3) are based on few events within each category, and results should be interpreted with care. The frequency of PSA-testing in the population increased during the study period, which also influences the incidence of prostate cancer and the age at diagnosis.[42] The year of prostate cancer diagnosis varies from 1996 to 2018 (median 2011). In the group aged <45 years at baseline ($n = 161$) the year of diagnosis varies from 1999 to 2018 (median 2015). In the group aged ≥45 at baseline ($n = 650$) the year of diagnosis varies from 1996 to 2018 (median 2010). The increase in PSA testing has been prominent regardless of age, and it seems less likely that this would affect our results[42]

The sample size was not large enough to conduct detailed subgroup analysis on the cause of death, and information regarding family history of prostate cancer was not available. We did not have access to serum testosterone levels at baseline and was not able to control for this factor in our analyze. Low testosterone concentrations may be an independent risk factor for hypertension in males.[43,44] Although ADT is a cornerstone in the treatment of metastatic prostate cancer, there is no solid evidence regarding the testosterone level and risk of prostate cancer,[45] but testosterone levels might influence both BP and prostate cancer development and could be an important factor. We did not have access to genetic analyses, in particular polygenic hazard scores, which might be an up-and-coming tool for prostate cancer risk stratification.

In conclusion, our study supports that both elevated prediagnostic systolic and diastolic BP are associated with prostate risk, and with overall mortality among patients with prostate cancer. These findings underline that both systolic and diastolic BP are important factors when balancing disease management and monitoring cardiovascular health in patients with prostate cancer. Our results are based on a single data point of BP, and should be interpreted with caution, and further studies are needed. Nevertheless, the present study supports the view that clinical follow-up visits of patients with prostate cancer should include measuring BP and initiate hypertensive treatment when appropriate, to balance and optimize the management of patients with prostate cancer.

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CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

AUTHORS' CONTRIBUTION

Conceived the study: ES, ER, HSH, and IT. Interpretation of data from the Tromsø study: TW, AEE, ES, ER, IT, HSH, and HS. Constructed the clinical database: ES, TK, MS, IT, HSH, and ER. Performed histological examination: ER. Performed statistical analyses and drafted the manuscript: ES, TW, ER, HSH, EG, AM, and IT. Critically reviewed the manuscript: all authors. Approved the final manuscript: all authors.

ETHICS

This study has been approved by the Regional Committee for Medical and Health Research Ethics North (REK) (2015/1059) and was performed in accordance with the 1964 Helsinki Declaration and its later amendments. Informed consent was obtained from all individual participants included in the study.

DATA AVAILABILITY STATEMENT

The data set used in our study is available upon request, pending permission from the Tromsø Study (www.troms oundersokelsen.no).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Paper III

Article

Expression of microRNA miR-24-1-5p in tumor tissue influence prostate cancer recurrence. The PROCA-*life* Study

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Simple Summary: Prostate cancer is a major cause of health loss and death world-wide, and we need better tools to assess risk levels in the individual patient in order to optimize treatment. MicroRNAs (miRNAs) are small molecules with critical regulatory roles in cell functions and are also involved in prostate cancer development. The aim for this study was to investigate the role of miR-24-1-5p in prostate cancer tissue. We collected prostate cancer tissue from 142 men already enrolled in a population-based cohort study, who underwent prostatectomy. We examined the tissue expression of miR-24-1-5p in prostate cancer using in situ hybridization (ISH) and semi-quantitative scoring. We found that a high miR-24-1-5p expression was associated with a doubled risk of recurrence of prostate cancer.

Abstract: The role of miR-24-1-5p, and its prognostic implications associated with prostate cancer is mainly unknown. In a population-based cohort, the Prostate Cancer Study throughout life (PROCA-*life)* all men had a general health examination at study entry and were followed between 1994 and 2016. Patients with available tissue samples after prostatectomy with curative intent were identified (n=189). The tissue expression of miR-24-1-5p in prostate cancer was examined by in situ hybridization (ISH) in Tissue Micro Array (TMA) blocks by semi-quantitative scoring by two independent investigators. Multivariable Cox regression models were used to study the associations between miR-24-1-5p -expression and prostate cancer recurrence. The prostate cancer patients had a median age of 65.0 years (range 47-75 years). Cancer of the Prostate Risk Assessment Postsurgical Score, International Society of Urological Pathology grade group, and European Association of Urology Risk group were all significant prognostic factors for 5-years recurrence-free survival ($p<0.001$). Prostate cancer patients with a high miR-24-1-5p expression (≥ 1.57) in the tissue had a doubled risk of recurrence compared to patients with low expression (HR 1.99, 95% CI 1.13-3.51). Our study suggests that a high expression of miR-24-1-5p is associated with an increased risk of recurrence of prostate cancer after radical prostatectomy pointing to a potential diagnostic and therapeutic value of detecting miR-24-1-5p in prostate cancer cases.

Keywords: prostate cancer; microRNA; miR-24-1-5p; radical prostatectomy; biomarker; population study

1. Introduction

Prostate cancer (PCa) is a major cause of health loss and death world-wide, and it is a heterogeneous disease (1, 2) Compared with localized low-risk PCa that can be actively surveyed without management, the treatment of aggressive high-risk PCa most often require systemic and complex treatment. We need valid prognostic biomarkers to distinguish low-risk indolent PCa from aggressive PCa.

The microRNAs (miRNAs) are a class of endogenous non-coding small RNA molecules associated with both regulation of gene expression, and are "fine-tuners" of the immune system (3). The miRNAs have been studied for their potential to serve as molecular prognostic biomarkers for cancer including PCa (4). In particular, differential miRNAs expression profiles between tumour and normal tissues have been observed for PCa as well as for other other cancer types (4, 5). In a recent systematic review, fifteen miRNAs were associated with PCa prognosis (4). The miRNAs are transcribed as ~70 nucleotide precursors in a stem-loop sequence and subsequently processed by the Dicer enzyme to give two mature ~22 nucleotide products. miRNAs bind to the RNA-induced silencing complex (RISC) and are used to identify target messenger RNA (mRNA) transcripts. They can prevent protein expression through cleavage of specific target mRNAs or through inhibition of their translation, and thus influence developmental processes, tissue housekeeping and tumorigenesis (6). Aberrant expression of miRNA can influence activity of tumor suppressors or oncogenes in many human cancers (6), including prostate cancer (7).

The miRNAs have also been associated with the tumor-micro environment and PD-L1 and STAT3 signaling in prostate cancer cells supporting a role of miRNAs linked to inflammation (8). Most prostate tumors contains immune cells, and chronic inflammation, one of the hallmarks of cancer development (9), has been proposed as a key factor in prostate cancer development(10-12). The suggested hypothesis is partly based on observations of inflammatory cells in the prostate microenvironment of adult men, and by the observation that this inflammation has been associated with precursor lesions in the prostate gland, termed proliferative inflammatory atrophy (13-16). However, much remains unknown regarding possible biological mechanisms operating in relation to prostate cancer development and systemic and local inflammation, and several mechanisms including miRNAs and factors related to the immune system have been studied (3, 17).

The effects of miRNAs in prostate cancer have been studied, but the biological mechanisms operating and type of miRNAs and their function have not yet been clarified (6, 18, 19). Importantly, no prostate-specific miRNAs have yet been definitively identified. We have previously studied the association between several miRNAs and prostate cancer recurrence and survival (20-25). High expression of miR-205, miR-17-5p, miR-20a-5p, miR-210, and miR-141 and low expression of miR-424 were all associated with increased risk of prostate cancer recurrence. These miRNAs have been suggested to be associated with inflammation; however, there is limited knowledge (3). Furthermore, few have investigated the association between miR-24 and prostate cancer (7). Through deep sequencing of prostatectomy specimens, it was observed that miR-24 was downregulated compared to non-cancer prostate tissue (26). Another study, by Hashimoto *et al*. found that miR-24 was differentially expressed in African American and Caucasian American prostate cancer patients (27). Interestingly, miR-24-3p enhanced Paclitaxel sensitivity in Paclitaxel-resistant prostate cancer cells (28), while in xenograft cell lines, miR-24 was down-regulated in metastatic compared to non-metastatic prostate cancer (29). Furthermore, miR-24 expression was significantly lower in prostate cancer cell lines compared to

a normal prostate epithelial cell line. These findings suggest that miR-24 has a tumor suppressor role in prostate cancer and targets p27 and p16 in prostate cancer cells (30). Current knowledge about miR-24 is largely based on in vitro studies and/or mouse models. The stem-loop sequence hsa-miR-24-1 is the processor of two mature sequences: hsa-miR-24-1-5p and hsa-miR-24-3p (31). To our knowledge, previous studies have not reported which sequences of miR-24 they have used (29, 30).

The present study is based on men participating in the Tromsø Study, a population-based cohort study, which has a high attendance proportion and long follow-up time. Complete information on prostate cancer cases, including detailed medical and pathological records, has been obtained in a sub study, The Prostate Cancer Study throughout life (PROCA-*life*). The role of miR-24s, including the different types of miR-24 and their prognostic implications is still under debate, and their potential diagnostic and therapeutic value are not clarified. Therefore, the main aim of the present study was to analyze the influence of miR-24-1-5p regarding aggressiveness and prognosis in men diagnosed with prostate cancer and treated with radical prostatectomy.

2. Materials and Methods

2.1. Study sample

The present study cohort, PROCA-*life* study, is based on all men aged ≥ 25 years who were enrolled in the population-based Tromsø Study in 1994 to 2016 (Tromsø 4, 1994-95, Tromsø 5, 2001, Tromsø 6, 2007-2008, Tromsø 7, 2015-2016) (32). The procedures of invitations, screening and examinations were almost identical in all three surveys. Moreover, all data collection was done by trained research technicians at one study site. Age-eligible men were invited to participate by a personal invitation (32, 33). In total 75.6% of invited men attended and completed questionnaires and provided biological specimen samples and clinical measurements.

2.2. Questionnaires, clinical assessments, and assessment of lipids and PSA

Height and weight were measured on an electronic scale with the participants wearing light clothing and no shoes. Height was measured to the nearest 1 centimeter (cm) in Tromsø 4 and nearest 0.1 cm in Tromsø 5-7. Weight to the nearest 500 g in Tromsø 4 and to the nearest 100 g in Tromsø 5-7. Body mass Index (BMI) was calculated using the formula weight/height^2 (kg/m^2). Blood pressure (BP) was measured on the right arm three times at one-minute intervals after two minutes seated rest, and the mean of the two last measurements were used. Information about lifestyle factors were obtained from the questionnaires. Alcohol consumption was defined as more than 1 unit (drink) of alcohol per month, as described by others in the same cohort (34, 35).

Blood samples were drawn by trained research assistants on attendance at each survey and were non-fasting. Analyses of serum samples were done at the Department of Laboratory Medicine, University Hospital of Northern Norway (UNN), Tromsø, Norway (33). For white blood cell count (WBC), 5 ml of blood was collected into Vacutainer tubes containing K3-EDTA 40 lL, 0.37 mol/L per tube, and analyzed within 12 h by an automated blood cell counter (Coulter CounterÒ, Coulter Electronics, Luton, UK, and Coulter LH750, Nerliens Meszansky). Total cholesterol and triglyceride levels was analyzed by enzymatic colorimetric methods with commercially available kits (CHOD-PAP for cholesterol). Prostate Specific Antigen (PSA) measurements were done for prostate cancer cases only, as part of clinical routine in diagnosis and follow-up (1990–1994 Stratus® PSA Fluorometric Enzyme Immunoassay, 1994–2001 AxSYM Psa Reagent Pack, Abbot®, 2001-2020 Bayer® PSA Reagens Pack Immuno I (Prod. Nr. T01-3450-51), Technicon Immuno I).

2.3. Identification of prostate cancer cases and detailed medical history during follow-up

Prostate cancer cases during follow-up (until Dec. 31, 2018) were identified by using the unique national 11-digit identification number through linkage with the Cancer Registry of Norway (n=947, supplementary figure 1). Cases with available tissue samples after prostatectomy with curative intent were identified by cross-linkage with the archive of Department of Clinical Pathology, University Hospital of North Norway, Tromsø, Norway (N=189), and these constituted the eligible study population in the current study. Overall, 43 cases were not technically successful in the in-situ hybridization (ISH) staining process and were excluded. Furthermore, four cases were excluded because they did not have curative surgery, leaving a final study population of 142 men (Supplementary figure A1).

Detailed clinical information was obtained by trained physicians (MS, TK, and ES) and included prostate cancer treatments and recurrence. Cause of death was obtained through linkage with the Norwegian Death Registry by use of the unique personal identification number. Most of the prostate cancer patients (88.7%) underwent prostatectomy a few months after being diagnosed, the remaining of the study population (11.2%) underwent active surveillance until their prostate cancer showed signs of increasing aggressiveness. Date of prostatectomy was used for calculation of age and follow-up time. The current study is based on the Tromsø Study survey closest to the date of prostatectomy for baseline data such as height, weight, blood pressure, triglyceride levels, and alcohol use.

Histopathological information was obtained from medical records, but all histopathological specimens were re-examined by one specialized uropathologist (ER) and classified according to the latest International Society of Urological Pathology (ISUP) guidelines on Gleason score and ISUP grade group (36). Prostate cancer cases were divided into three risk groups based on PSA level at diagnosis, highest ISUP grade group and clinical T-stage, according to the European Association of Urology-classification (EAU) guidelines (37). Risk group 1 (low) was defined as: PSA < 10 μg/L, clinical T-stage (cT-) 1, and ISUP grade group 1. Risk group 2 (intermediate) was defined as: PSA: 10–20 μg/L, cT-stage 2, or ISUP grade group 2–3. Risk group 3 (high) was defined as: PSA: > 20–100 μg/L, cT-stage 3, or ISUP grade group 4–5. ISUP grade groups were reported after reclassification when available. Cancer of the Prostate Risk Assessment Postsurgical Score (CAPRA-S Score), a validated score developed to predict outcomes after radical prostatectomy, was also used to classify patients in risk groups (38). This score is based on surgical margin, seminal vesicle invasion, extracapsular extension, and lymph node invasion, PSA value and Gleason/ISUP Grade Groups.

2.4. Microarray construction

Tissue microarrays (TMAs) were constructed for the analysis of ISH staining expression. For each case, one uropathologist (ER) identified and marked representative areas of the prostate specimens with tumor epithelial cells (TE) and normal epithelial cells (NE). From each of these areas, 0.6 mm cores were sampled from each donor block and inserted into paraffin blocks to construct TMA blocks by using a tissue-arraying instrument (Beecher Instruments, Silver Springs, MD, USA). The details of the technique have been described earlier (39).

2.5. In situ hybridization (ISH)

The tissue expression of mature miR-24-1-5p in prostate cancer was examined by in situ hybridization (ISH). The principle of the method is based on the ability of specific microRNA locked nucleic acid (LNA) probes to bind to target microRNA in tissue followed by chromogenic visualization. ISH staining was done automatically in a Ventana

Discovery Ultra instrument. Necessary efforts to avoid RNA degradation in tissue were done in work routines and by using RNAse-free buffers during the process.

2.6. Optimization and validation

LNA probe concentrations, hybridization temperatures and incubation times were optimized before staining the tissue of interest. Target retrieval treatment was adjusted to improve availability of microRNA sequence for the target and control probes. A TMA multi organ block with several normal and tumor tissues was used for optimization of the ISH method and validation of miR-24-1 expression in different tissues. We used U6snRNA probe as a positive control and to ensure the sensitivity level of the method. Strong nuclear U6snRNA staining also indicate low degree of RNA degradation of the tissue. Scramble miRNA negative control probe showed no unspecific staining. Optimized ISH parameters are presented in Supplementary table A1 and ordering details of products are presented in Supplementary table A2.

External validation of LNA probes was done by supplier company QIAGEN. The LNA miRNA probes were purified by HPLC (High-Performance Liquid Chromatography) and analyzed by Capillary Electrophoresis or HPLC. The identity of compounds was confirmed by using Mass Spectrometry. For more details on the ISH procedure, see appendix.

2.7. Scoring

The expression of miR-24-1-5p was assessed by semi-quantitative scoring by two trained independent investigators (ES, ER). The color intensity was graded as negative (0), weak (1), moderate (2), strong (3), or missing (4) (figure 1). Two areas of TE cells and two areas of NE cells were scored for each patient. Stromal areas were not scored due to little positivity. Mean and median score was calculated for TE and for NE separately, and for TE+NE combined. High expression of miR-24-1-5p was defined as a score equal to or higher than the median score of the study population. Inter-observer variability was assessed by calculating linear weighted Kappa statistics and showed a moderate agreement (Kappa 0.59 (SD 0.50-0.68))

The primary endpoint was defined as a composite endpoint, including any evidence of recurrent prostate cancer after surgery: Biochemical failure (PSA-level >=0.2) and/or clinical/radiological signs of prostate cancer defined by the treating physician. Endpoints were updated until August 2021.

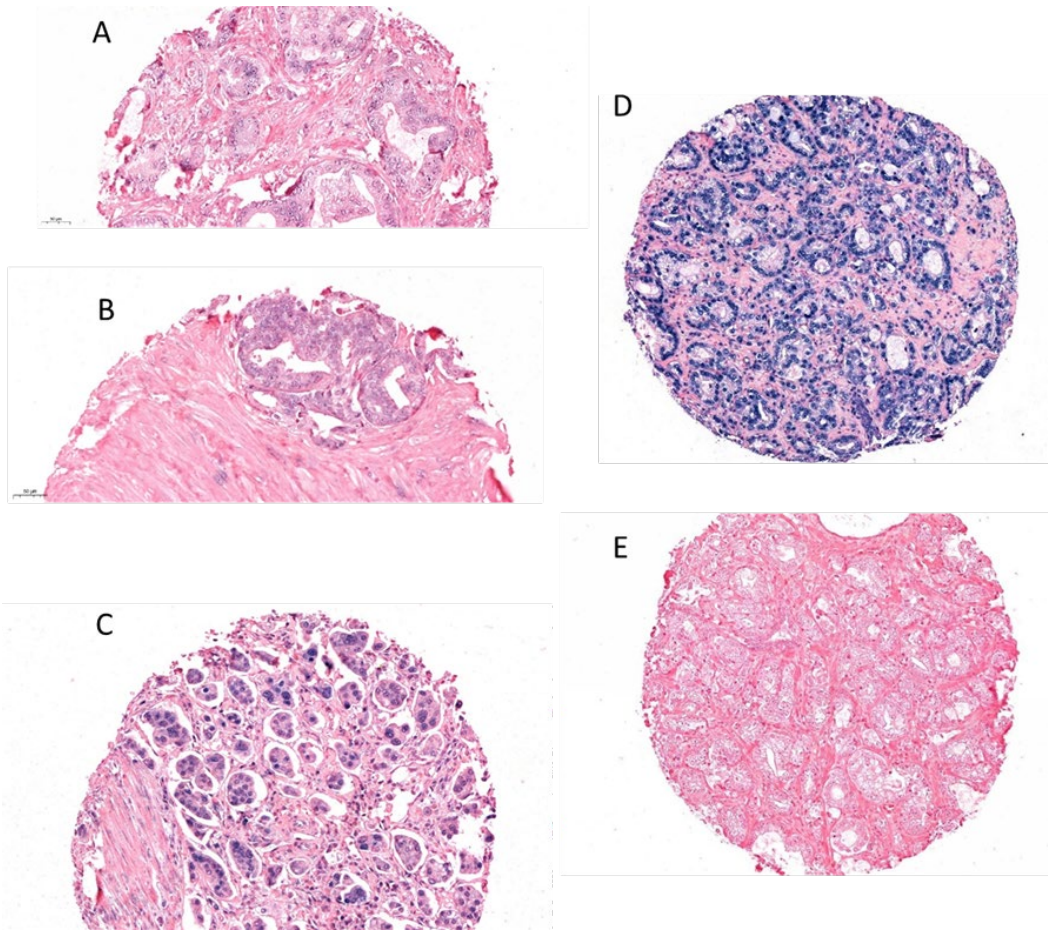

Figure 1. Panel of ISH stained cores. Representative scoring of miR-24-1-5p in tumor epithelium (TE). A) Weak expression B) Moderate expression C) Strong expression D) U6 positive control staining E) Scrambled miR negative control staining. The PROCA-life study.

2.8. Statistical methods

Selected characteristics that describe the study population are presented as means (standard deviation), median (range) or percent (numbers). Spearman's Correlation coefficient was used for correlation analysis between miR-24-1-5p and clinicopathological markers. The five-year recurrence free percentage was calculated using the Kaplan–Meier survivor function, and statistical differences between different groups (e.g., ISUP grade group, EAU risk group, CAPRA-S) were tested by using log-rank test.

Multivariable Cox proportional hazard models, with time after surgery as timescale, were used to study whether miR-24-1-5p and clinicopathological markers were independently associated with risk of prostate cancer recurrence. Several variables were assessed as potential confounders based on suggested biological mechanisms and/or significant associations in unadjusted models. Age at surgery (continuous), CAPRA-S(categorical), BMI (continuous), alcohol habits (categorical) and cholesterol levels (continuous) were included in the final models as covariates. We performed stratified analysis by systolic blood pressure based on previous observations suggesting that elevated systolic blood pressure is associated with prostate cancer risk (40). The proportional hazard assumption was assessed by visually controlling that the log minus log survival curves were parallel. The Kaplan-Meyer method was used for drawing survival plots for high vs low expression of miR-24-1-5p. We conducted all statistical tests with STATA/MP version 16 (StataCorp LLC, College station, TX, USA), and used a two-sided significance level of $p < 0.05$.

2.9. Ethics

All participants gave written informed consent on first entry in the Tromsø Study, including permission to linkage to other health and medical registers. The PROCA-*life* study was approved by the Regional Committees for Medical Health Research Ethics (REK Nord), reference no: 2015/1059.

3. Results

3.1. Patient characteristics

The 142 men that constituted the study population entered the Tromsø Study on average 8.0 years before prostatectomy. The median age at prostate cancer diagnosis was 64 years (range 46-74 years), median age at prostatectomy 65 years (47-75 years), and prostatectomy was performed between 2001 and 2018 (Table 1). The prostate cancer patients had an average BMI of 27.1 kg/m^2, systolic BP of 134.9 mmHg (SD 16.8), and diastolic BP of 80.4 mmHg (SD 9.4) at study entry. A total of 61.3 % of the prostate cancer patients had a systolic blood pressure higher than 130 mmHg. Mean level of white blood cells was 6.60 x10^9/L (SD 1.67), total cholesterol 5.78 mmol/L (SD 1.12), triglyceride level 1.70 mmol/L (SD 0.90), and 46.1 % were alcohol users.

Surgical technique changed during the study period: 47.2% of the patients had open (retropubic or perineal) prostatectomy, mostly before year 2012, while 52.8% had laparoscopic prostatectomy (manual or robot-assisted). Lymph node dissection was performed in 36.6% of the patients. Mean PSA at prostate cancer diagnosis was 10.5 ng/mL (SD 9.5). The histopathologic tumor stage was pT2c for 47.9% of the patients, while 26.1% had pT3, and the ISUP grade group was 1 or 2 for 73.8% of the patients. The median CAPRA-S score was 3 (39.4% Capra-S Low (0-2), 46.5% Capra-S Intermediate (3-5), 14.1% Capra S High (6-12). Positive surgical margins were found in 30.5 % of the cases. Overall, 26.9% of the prostate cancer patients had a relapse after prostatectomy during follow-up (until august 2021).

Table 1. Distribution of selected characteristics among the prostate cancer patients who received prostatectomy in the PROCA-life Study (1994–2018).

Characteristics	Prostatectomy cases (n=142)
Age at study entry, median, range (years)	58.5 (34-73)
Birth year median, range (year)	1947 (1934-1967)
Age at surgery, median, range (years)	65.0 (47-75)
Observation time from study entry to surgery (years)	8.0 (6.6)
Observation time from surgery to end of follow-up (years)	4.8 (3.4)
Clinical Assessments at study entry	
Body Mass Index (kg/m^2)	27.1 (3.15)
Systolic Blood Pressure (mmHg)	134.9 (16.8)
Diastolic Blood Pressure (mmHg)	80.4 (9.4)
White blood cells (x10^9/L)	6.60 (1.67)
Total cholesterol (mmol/L)	5.78 (1.12)
Triglyceride (mmol/L)	1.70 (0.90)
Alcohol intake (> 1 unit of alcohol per month), % *(n)*	46.1 (65)
Surgical technique, % (n)	
Open prostatectomy, retropubic	38.0 (54)
Open prostatectomy, perineal	9.2 (13)
Laparoscopic prostatectomy	6.3 (9)
Robotic-assisted laparoscopic prostatectomy (RALP)	46.5 (66)
Lymph node dissection performed, % (n)	36.6 (52)
Histopathological stage, % (n)	
pT2a	17.0 (24)
pT2b	8.5 (12)
pT2c	48.2 (68)
pT3a	16.3 (23)
pT3b	9.9 (14)
PSA at diagnosis (μg/L)	10.5 (9.5)
ISUP Grade Group, % (n)	
1 (Gleason 3+3)	29.1 (41)
2 (Gleason 3+4)	44.7 (63)
3 (Gleason 4+3)	18.4 (26)
4 (Gleason 4+4)	6.4 (9)
5 (Gleason 4+5/5+4/5+5)	1.4 (2)
Risk group, % (n)	
Low	25.5% (36)
Intermediate	56.0% (79)
High	18.4% (26)
Other histopathological characteristics, % (n)	
Positive lymph nodes (N+)	3.6 *
Perineural infiltration	21.3 (30)
Extraprostatic growth	22.7 (32)
Normal tissue in surgical margin	15.6 (22)
Positive surgical margin	30.5 (43)
Relapse rate (biochemical + clinical), % (n)	26.9 (38)

* (5 in 52 patients with lymph node dissection). Numbers may vary due to missing information. Values are mean (standard deviation) unless otherwise specified. Abbreviations: PSA; Prostate-specific antigen, ISUP: International Society of Urological Pathology. Prostate cancer risk group definitions: Low: PSA < 10μg/L, clinical T-stage (cT-) 1, and ISUP grade group 1. Intermediate: PSA: 10–20μg/L, cT-stage 2, or ISUP grade group 2–3. High: PSA: > 20–100μg/L, cT-stage 3, or ISUP grade group 4–5.

3.2. miR-24-1-5p expression

The mean score for miR-24-1-5p expression was 1.60 in TE cells, 1.35 in NE cells and 1.49 in TE and NE cells combined (Table 2). The median value was used as cut-off value for high miR-24 -1-5p expression, and was ≥1.67 in TE, and ≥1.50 for NE. The cut-of value for high TE+NE combined was ≥ 1.57. In the total population, 43.7% had high TE+NE, 43.7% had high TE, and 45.1% had high NE.

Table 2. Distribution and mean score (SD) of miR-24-1-5p expression in prostate cancer tissue by selected characteristics and their subgroups. The PROCA-life study (1994-2018).

Group	N	Tumor-epithelium (TE)	Normal epithelium (NE)	Tumor- + Normal- epithelium (TE+NE)
All cases	142			
Mean score miR-24-1-5p (SD)		1.60 (0.73)	1.35 (0.68)	1.49 (0.53)
Distribution				
0-0.49 Negative % (n)		3.5 (5)	7.0 (10)	2.8 (4)
0.5-1.49 Weak % (n)		30.3 (43)	40.1 (57)	39.4 (56)
1.5-2.49 Moderate % (n)		43.0 (61)	38.0 (54)	53.5 (76)
2.5-3 Strong % (n)		13.4 (19)	7.0 (10	4.2 (6)
Missing % (n)		9.9 (14)	7.8 (11)	-
Age at surgery				
<65 year	69	1.63 (0.67)	1.45 (0.64)	1.59 (0.50)
≥65 year	73	1.58 (0.79)	1.25 (0.71)	1.40 (0.55)
Capra-S				
Low (0-2)	56	1.46 (0.69)	1.42 (0.76)	1.44 (0.53)
Intermediate (3-5)	66	1.69 (0.75)	1.35 (0.61)	1.57 (0.49)
High (6-12)	20	1.75 (0.73)	1.11 (0.67)	1.40 (0.67)
Systolic blood pressure				
<130 mmHg	55	1.79 (0.63)	1.38 (0.60)	1.62 (0.49)
≥130 mmHg	77	1.48 (0.77)	1.33 (0.73)	1.41 (0.55)

Numbers may vary due to missing information. Values are mean (standard deviation) unless otherwise specified. Abbreviations: CAPRA-S: Cancer of the Prostate Risk Assessment Postsurgical Score.

3.3. miR-24-1-5p correlations

The level of white blood cells at study entry (pre-diagnostic) correlated with miR-24-1-5p expression in both TE and NE (r= 0.21, p=0.02, and r=-0.21, p=0.01 respectively). Furthermore, BMI and triglyceride levels at study entry correlated with miR-24-1-5p expression in NE (r=-0.27, p=0.01, r=-0.24, p=0.006). Positive surgical margin correlated with miR-24 -1-5p expression in TE (r=0.19, p=0.029). CAPRA-S correlated with miR-24-1-5p expression in TE (r=0.21, p=0.020) (results not presented in table).

3.4. Recurrence-free survival

Age at surgery was not associated with recurrence-free survival (Table 3). Increasing CAPRA-S score, ISUP grade group, and EAU risk group were all significant prognostic factors for decreasing five-year recurrence-free survival (p<0.001). Our data suggested a higher number of recurrences in the group with high expression of miR-24-1-5p, but of borderline significance (p= 0.098) (Figure 2). In the subgroup of prostate cancer patients with high pre-diagnostic systolic blood pressure (≥130 mmHg), high expression of miR-24-1-5p was a prognostic factor for recurrence.

Table 3. Five-year recurrence free survival (%) for prostate cancer patients after prostatectomy by selected characteristics for all cases and by a subgroup with systolic BP ≥130mmHg. The PROCA-life study (1994-2018).

Characteristics	**All cases**			Cases with pre-diagnostic Systolic BP ≥130mmHg		
	N	**Five-year recurrence free survival, % (95% C.I.)**	**P ***	n	Five-year recurrence free survival, % (95% C.I.)	P *
Age at surgery			*0.59*			*0.27*
<65 year	69	69.3 (56.9-78.8)		37	72.8 (55.4-84.4)	
≥65 year	73	65.0 (52.7-74.8)		50	64.9 (49.7-76.5)	
ISUP Grade Group			*<0.001*			*<0.001*
1 (Gleason 3+3)	33	81.4 (63.1-91.2)		15	85.6 (53.3-96.2)	
2 (Gleason 3+4)	66	77.2 (65.1-85.6)		44	77.3 (61.9-87.1)	
3 (Gleason 4+3)	28	41.3 (22.8-59.0)		21	47.1 (25.1-66.4)	
4 (Gleason 4+4)	9	50.8 (15.7-78.1)		4	66.7 (5.4-94.5)	
5 (Gleason 4+5/5+4/5+5)	6	16.7 (0.8-51.7)		3	N.a	
Risk group			*<0.001*			*0.0003*
Low	36	85.7 (68.9-93.8)		18	88.2 (60.2-96.9)	
Intermediate	80	70.8 (59.3-79.5)		54	71.8 (57.7-82.0)	
High	26	30.8 (14.6-48.6)		15	33.3 (12.1-56.4)	
Capra-S			*<0.001*			*<0.001*
Low (0-2)	56	89.2 (77.6-95.0)		33	93.9 (77.9-98.4)	
Intermediate (3-5)	66	61.2 (48.1-71.2)		41	62.6 (45.8-75.5)	
High (6-12)	20	25.0 (9.1-44.9)		13	23.1 (5.6-47.5)	
miR-24-1-5p			*0.098*			*0.026*
TE+NE low	80	71.6 (60.1-80.3)		55	75.5 (61.5-85.0)	
TE+NE high	62	61.1 (47.7-72.0)		32	55.8 (37.0-71.0)	

* Log rank test for difference between groups during follow-up until study end. Numbers may vary due to missing information. miR-24-1-5p: Low score was defined as <1.57 and high score ≥1.57. Prostate cancer risk group definitions: Low: PSA < 10μg/L, clinical T-stage (cT-) 1, and ISUP grade group 1. Intermediate: PSA: 10–20μg/L, cT-stage 2, or ISUP grade group 2–3. High: PSA: > 20–100μg/L, cT-stage 3, or ISUP grade group 4–5. Abbreviations: BP: blood pressure. CAPRA-S: Cancer of the Prostate Risk Assessment Postsurgical Score. ISUP: International Society of Urological Pathology. CI: Confidence Interval. TE: tumor epithelium. NE: normal epithelium.

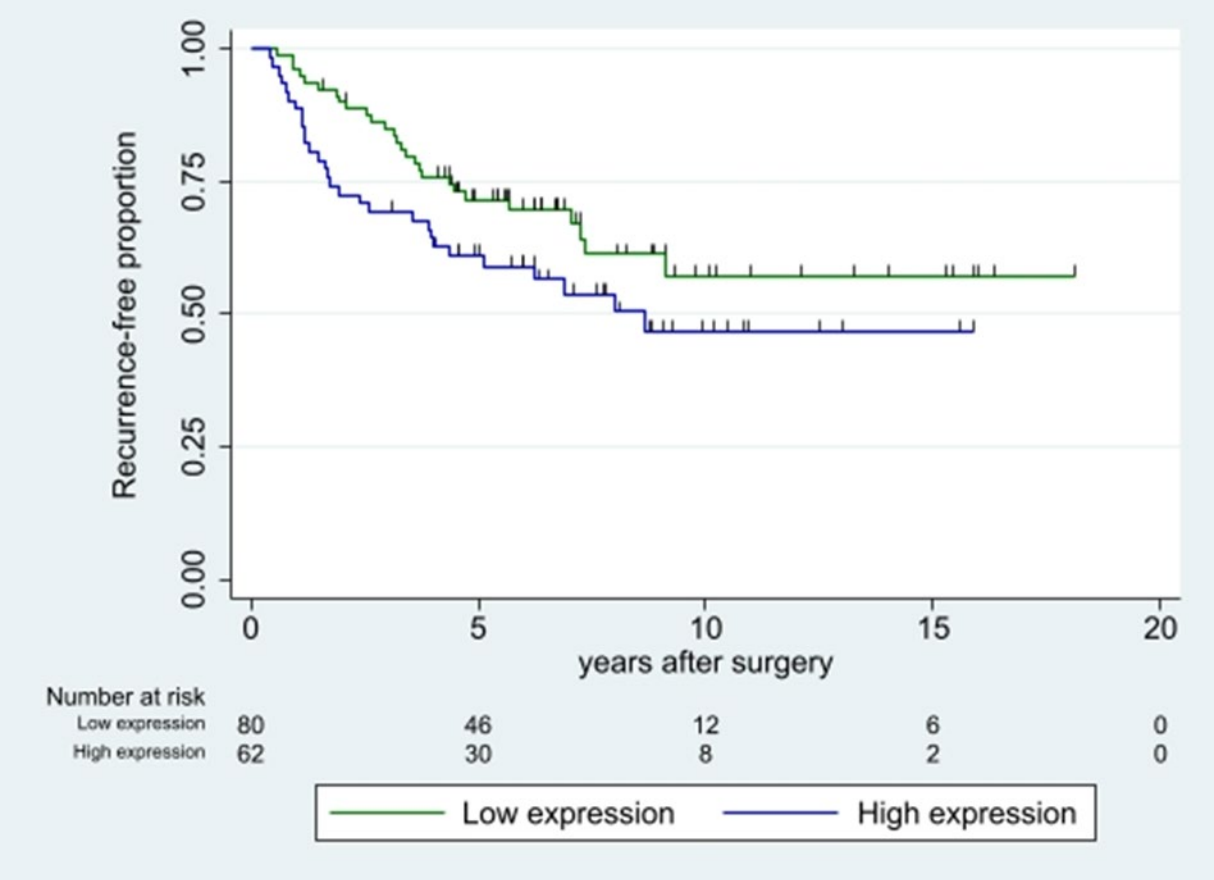

Figure 2. Recurrence-free proportion of prostate cancer after prostatectomy, dichotomized into high vs low expression of miR-24-1-5p in prostate cancer tissue (tumor epithelium and normal epithelium combined (TE+NE)). Low score was defined as <1.57 and high score.

3.5. Multivariable analyses

In our multivariable model we adjusted for age, Capra-S group, BMI, cholesterol level and alcohol use, based on suggested biological mechanisms. A high miR-24 expression in the tissue (TE+NE) was associated with an almost doubled risk of recurrence of prostate cancer, compared to those with low miR-24 -1-5p expression (HR 1.99, 95% CI 1.13-3.51) (Table 4). The results were also observed in the subgroup of prostate cancer patients with high pre-diagnostic systolic blood pressure. There was no significant interaction between miR-24 expression and blood pressure, nor between miR-24 expression and follow-up time.

Table 4. Multivariable adjusted* hazard ratio of recurrence of prostate cancer after radical prostatectomy, by all cases and by a subgroup of systolic BP ≥130mmHg. The PROCA-life study (1994-2018).

	All cases			Cases with pre-diagnostic hypertension Systolic BP ≥130mmHg		
	n	**Hazard Ratio (95% C.I.)**	**p**	n	Hazard Ratio (95% C.I.)	P
Age per 10 years	142	1.13 (0.69-1.82)	0.63	87	1.17 (0.58-2.36)	0.66
Capra-S						
Low (0-2)	58	1 (reference)		33	1 (reference)	
Intermediate (3-5)	66	3.75 (1.17-8.27)	0.001	41	6.25 (1.41-27.3)	0.015
High (6-12)	21	16.0 (6.59-39.2)	<0.001	13	31.9 (6.50-156.5)	<0.001
miR-24-1-5p						
TE+NE low	82	1 (reference)		55	1 (reference)	
TE+NE high	63	1.99 (1.13-3.51)	0.017	32	2.85 (1.25-6.47)	0.013

* Adjusted for age, Capra-S group, MiR-24 expression, BMI, kg/m², cholesterol and alcohol use. miR-24-1-5p: Low score <1.57 and high score ≥1.57. Abbreviations: Sys Systolic. BP blood pressure. CAPRA-S: Cancer of the Prostate Risk Assessment Postsurgical Score. CI Confidence interval. TE: tumor epithelium. NE: normal epithelium.

4. Discussion

We found that a high expression of miR-24-1-5p was associated with an almost doubled risk of recurrence (biochemical or clinical) after radical prostatectomy, when adjusting for known histopathological risk factors. We were also able to adjust for known lifestyle risk factors, due to the pre-diagnostic information assessed at the study entry. Of note was also the observation that positive surgical margins and CAPRA-S correlated with miR-24 -1-5p expression. Prostate cancer is a heterogeneous condition, ranging from indolent to life-threatening, and we need better tools for disease stratification. Development of biomarkers for risk stratification, personalized treatment and follow-up and is needed. Other miRNAs have shown good correlation between levels in tissue and in blood or urine, and development of liquid biomarkers would be a great advantage for the patient by limiting the need for invasive tissue biopsies (41).

To our knowledge, this is the first study to investigate whether the expression of miR-24-1-5p in prostate cancer tissue is associated with prognosis. Our findings are in part supported by others, although few studies have investigated the role of miR-24-1-5p in prostate cancer. Most of these studies have been experimental. A recent meta-analysis studied the prognostic significance of miR-24 in various cancers and found that high miR-24 expression was associated with poor overall survival (42). The meta-analysis consisted of 17 studies, and a total of 1705 patients, of whom none had prostate cancer. Another recent study observed that the expression of miR-24-1-5p decreased 16-fold after radiotherapy doses of 6 and 7 Gy in prostate cancer cell lines treated with radiation, suggesting that expression of miR-24-1-5p may impact efficacy of important treatment modalities as radiation therapy (43). Further studies are needed to explore the causal implication of this observation.

A few studies have evaluated the other mature sequences of miR-24, miR-24-3p, which has been suggested as a diagnostic biomarker for prostate cancer in serum (41). circRNA protein kinase C-iota has been suggested to influence tumor development, and a study found this molecule to trigger growth and metastasis in prostate cancer by down-regulation of miR-24-3p (44). However, it is unclear whether these results will be valid for the association between miR-24-1-5p and prostate cancer development.

The relationship between prostate cancer and inflammation has been subject to several studies. Inflammation is one of the classic hallmarks of cancer (9), and inflammatory cells associated with precursor lesions for prostate cancer in the prostate gland have been observed (10). We have previously discovered that systemic pre-diagnostic inflammatory biomarkers were associated with prostate cancer development (45). miR-24 have also been

linked to inflammation (3). In a murine model, miR-24 was a central regulator of vascular inflammation (46). In a model with primary human macrophages, miR-24 would produce anti-inflammatory action by inhibiting the production of pro-inflammatory cytokines, and these results suggest that overexpression of miR-24 would have mostly anti-inflammatory effects (47). miR-24 belongs to the miR-23~27~24 cluster and this cluster has been shown to reduce TNF-α and IL-6 production (48). Our observation that an association between miR-24-1-5p and prostate cancer recurrence was suggestively more pronounced among the prostate cancer patients with high pre-diagnostic systolic blood pressure supports a role associated with low-grade systemic inflammation.

The strengths of our study include the broad pre-diagnostic information about the participating prostate cancer patients, a relatively large sample of patients with prostate cancer prostatectomy specimen (n=142), and detailed histopathological and medical records for all the patients. The methodology for TMA-production and in situ hybridization has been used in our lab for several tissues and is well tested (20-22, 49). Scoring of miR-24-1-5p was done by two independent observers and showed a moderate inter-observer variability. Earlier studies have focused on murine models and cell lines, while our study uses human prostate cancer tissue which is in line with future clinical studies. Our study also had some weaknesses. The sample size was not large enough for sub-group analysis, and 42 samples were lost due to technical problems in the ISH-process. The scoring of miRNA-expression was semi-quantitative, and thus subject to variability. We only had prostate tissue available and were not able to test the expression of miR-24-1-5p in other samples such as serum or urine.

5. Conclusions

Our study suggests that a high expression of miR-24-1-5p is associated with an increased risk of failure after radical prostatectomy, also when adjusting for known histopathological risk factors. The results are experimental, based on a relatively small sample size, and should be interpreted with caution. Nevertheless, this could be the steppingstone to further research about the role of miR-24 in prostate cancer, and possibly a future tool for better risk stratification.

Author Contributions: Conceived the study: ES, ER, HSH, IT. HS. Constructed the clinical database: ES, TK, MS, IT, HSH, ER. Performed histological examination: ER. Performed TMA-construction, in situ hybridization and scoring: MIP, ER, ES. Performed statistical analyses and drafted the manuscript: ES, ER, IT. Critically reviewed the manuscript: all authors. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The PROCA-*life* study was approved by the Regional Committees for Medical Health Research Ethics (REK Nord), reference no: 2015/1059. The study was conducted according to the guidelines of the Declaration of Helsinki

Informed Consent Statement: All participants gave written informed consent on first entry in the Tromsø Study, including permission to linkage to other health and medical registers.

Data Availability Statement: Dataset available pending permission from the Tromsø Study. Please send request to first author.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

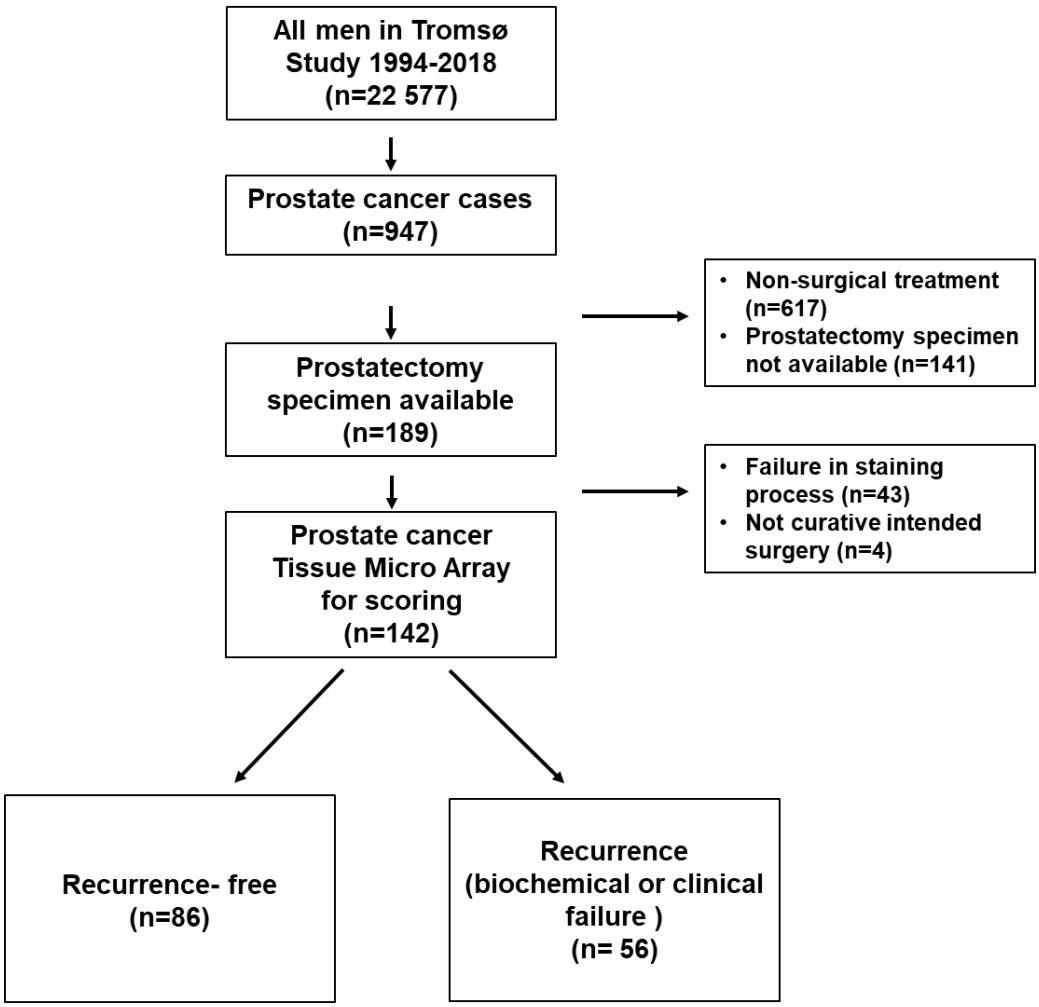

Figure A1. Flow chart of prostate cancer cases. The PROCA-life study (1994-2018).

Supplementary method:

ISH procedure in short:

TMA blocks were sectioned at 4 μm thickness and mounted on Superfrost Pluss glass slides. During incubations in instrument, Liquid Coverslip oil was used to protect sections from drying and ensure proper distribution of reagents.

Deparaffinization was performed at 68°C with EZ Prep solution in three cycles. Target unmasking retrieval was done at 95°C with CC1 buffer to improve the DIG labeled LNA probes to hybridize to the patient microRNA sequence. Sections were rinsed with Reaction Buffer between incubations.

Target microRNA 24-1-5p: positive control U6snRNA and negative control Scramble miR probes were diluted in microRNA ISH buffer and Elix RNAse free water to their final concentrations. To get optimal hybridization conditions probes and tissue microRNA was denaturated 8 min at 90°C.

Hybridization of the LNA-probes was performed for 60 min. in temperatures adjusted with RNA Tm as a guideline for each probe, (Supplementary Table A1). To ensure specific bindings, stringent washes was done in two cycles with RiboWash buffer. Additional blocking against unspecific bindings were done by Antibody Block solution.

For detection of tissue microRNA, anti-DIG-AP Multimer (Alkaline phosphatase (AP)-conjugated anti DIG) was incubated for 32 minutes to bind the Digoxygenin labeled probes. Blue chromogenic visualization of the AP-DIG complex was developed with NBT/BCIP from the ChromoMap Blue detection kit.

After Red II counterstain, sections were dehydrated by increasing gradients of ethanol solutions to Xylene and then mounted with Histokitt mounting medium. Ordering details of essenziell products used in this study are presented in Supplementary Table A2.

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