Development and evaluation of prostate cancer risk prediction models for use in the community

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Abbreviation

AMACR	Alpha-methylacyl-CoA racemase
ANNs	Artificial neural networks
ANOVA	Analysis of variance
AUC	Area under the curve
BMI	Body mass index
BPH	Benign prostate hypertrophy
CI	Confidence interval
cPSA	Complexed prostate-specific antigen
CRP	C-reactive protein
DII	Dietary inflammatory index
DRE	Digital rectal examination
EPCA	Early prostate cancer antigen
ERSPC	European Randomised Study of Screening for Prostate Cancer
FDA	Food and Drug Administration
fPSA	Free prostate-specific antigen
GI	Glycaemic index
GL	Glycaemic load
GOLPH2	Golgi phosphoprotein 2
GWAS	Genome-wide association study
HGB	Haemoglobin
HR	Hazard ratio
IGF	Insulin-like growth factor

IGFBP	Insulin-like growth factor binding proteins
LUTS	Urinary tract symptoms
MCV	Mean corpuscular volume
MRI	Magnetic resonance imaging
NHS	The National Health Service
NICE	National Institute for Health and Excellence
NPV	Negative predictive value
NSAIDs	Non-steroidal anti-inflammatory drugs
OR	Odds ratio
P2PSA	Precursor of prostate-specific antigen
PCA3	Prostate cancer antigen 3
PCR	Polymerase chain reaction
PHI	Prostate Health Index
PIA	Proliferative inflammatory atrophy
PIN	Intraepithelial neoplasia
PPV	Positive predictive value
PRACTICAL	Prostate Cancer Association Group To Investigate Cancer Associated Alterations in the genome
proPSA	Inactive precursor enzyme prostate-specific antigen
PSA	Prostate-specific antigen
PSAD	Prostate-specific antigen density
PSAD-TZ	Prostate-specific antigen transition zone density
PSGR	Prostate-specific G protein coupled receptor
PSMA	Prostate-specific membrane antigen

RBC	Red blood cell count
ROC	The receiver operating characteristic
RR	Relative risk
SD	Standard deviation
SNPs	Single nucleotide polymorphisms
STDs	Sexually transmitted diseases
TFGβ	Transforming growth factors beta
TMPRSS2	Transmembrane protease serine 2
TNM	Tumour, Node, and Metastasis staging system
TRUS	Transrectal ultrasonography
WC	Waist circumference
WHR	Waist-to-hip ratio
%fPSA	Free to total prostate-specific antigen ratio

Abstract

Prostate cancer is one of the most common cancers in men, and the incidence is increasing around the world. Unlike breast cancer in women, there are no effective early detection programs such as screening. This is partly due to lack of an adequate biomarker with ability to detect clinically significant prostate cancer and to be specific to it. The Prostate-specific antigen test has been used in addition to the digital rectal examination (DRE) to determine prostate cancer risk. These tests can produce false positive or false negative results.

This challenging issue of inaccuracy in detecting prostate cancer can be improved by using a risk prediction model. Many researchers have tried to develop a predictive model to improve the performance of prostate cancer detection by combining several factors and tests that are related to prostate cancer. However, the majority of the existing risk prediction models are not suitable to be implemented in primary care settings either due to incorporating inappropriate invasive tests or issues relating to the study design and methodology at the development stage. Therefore, there is a need to develop a risk prediction model for prostate cancer that consists of readily available, easy to measure, and low-cost so that it can be implemented in primary care and community settings.

In this thesis, I investigated the existing risk models for prostate cancer that can be implemented in primary care by conducting a systematic review. The findings suggested that there is a paucity of such models.

I also reviewed the literature on prostate cancer risk factors. To date, there is some emerging evidence that suggests causal relationships with the disease such as physical activity and some genetic factors, in particular single nucleotide polymorphism. This new knowledge can help advance cancer prevention.

I examined the relationship between body size and body shape and the risk of prostate cancer. The findings suggest an “apple” body shape indicative of central obesity was protective against prostate cancer. The changes in body size did not show any association.

Next, I developed a risk prediction model that consists of age, prostate-specific antigen (PSA), and free-to-total PSA ratio (%fPSA) using multivariate logistic regression analysis. I compared the results in two different outcomes. The first outcome was prostate cancer detected by multiparametric reasoning imaging targeting biopsy, and the second outcome was by the conventional biopsy needle. The results demonstrate the usefulness of our model in detecting prostate cancer which outperformed PSA alone in detecting prostate cancer. Also, it shows that by using the model, fewer clinically significant cases are missed. Furthermore, my results also showed that there is a demand for testing by men to increase their awareness and knowledge of their risk of having the disease so they can treat it as early as possible.

Lastly, I explored men’s perspectives on home testing for prostate cancer using a PSA kit. The findings suggested men were happy with the home testing kit.

In sum, prostate cancer incidence is increasing worldwide. A good risk prediction model offers a way forward to aid the early detection of prostate cancer as its performance is better than the PSA test alone. Risk prediction model can be applied to detect prostate cancer either with conventional needle biopsy or MRI-guided biopsy. PSA home testing kit is considered as a future proof to enhance the number of men taking the test, in particular, men with ethnic minority and harder to reach groups.

Declaration

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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http://www.manchester.ac.uk/library/aboutus/regulations) and in The University's policy on Presentation of Theses.

Dedication

To Abdulhadi & Amsha my lovely parents, Abdulhadi, Abdullah and Nayef my wonderful sons

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In the name of Allah, the Most Beneficent and Merciful. First and foremost, I am indebted to Almighty Allah for his blessings and for providing me with the ability and guidance necessary to successfully complete this thesis.

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I would like to thank all friends and kind people I met and shared beautiful moments with.

Although my PhD journey was very difficult and challenging, I am grateful for the strengths I have gained, the skills I have acquired, the relationships I have made, the places I have visited, and the moments I enjoyed.

Chapter 1 Introduction

1.1 Background and motivation

Prostate cancer is the second most common cancer and the fifth cancer-site leading mortality among men, with an estimated 1,414,259 new cases in 2020 worldwide [1]. In the United Kingdom (UK), around 52,000 new cases are diagnosed every year [2]. Despite these statistics and the projected increase in the number of cases, there are no prostate cancer screening programs in many countries, including the UK.

Screening is a method of detecting cancer in asymptomatic people, which can help in detecting malignancies at an early stage when they are easier to manage and treat. Prostate-specific antigen (PSA) levels in men's serum and digital rectal examination (DRE) are methods used for early testing and screening for prostate cancer. Nevertheless, the introduction of screening for prostate cancer is still highly debated as it is unclear whether the benefits of prostate cancer screening outweigh potential harms; for example, issues related to current methods of prostate cancer screening suggest it is inaccurate in detecting the cancer and could produce, in many situations, unclear results. Furthermore, such a lack of accuracy and specificity of the tests could lead to over diagnosis and inappropriate clinical interventions where prostate cancer is not causing symptoms or is life-threatening. In many of these indolent cancer cases treatment could potentially lead to other problems that could have serious consequences for normal urinary or bowel function that impact men's quality of life.

1.2 Scope

Several attempts have been made to overcome such issues with prostate cancer screening. These have included building a risk prediction model by using mathematical and statistical approaches and which have incorporated various factors related to the

disease. A variety of predictive approaches have been taken, ranging from simple ones such as linear regression to more complicated ones like artificial neural networks. Additionally, there are models that predict the presence of prostate cancer from initial biopsy, models that predict prostate cancer on repeat biopsy, pre-treatment models that predict recurrence, models for tumour staging, models for progression, models for predicting survival, and models for predicting life expectancy.

Many prediction models that target identification of prostate cancer at initial biopsy exist; the vast majority include either clinical or genetic tests that are expensive and not routinely available. To address these limitations, my focus has been to establish a logistic regression model that is based on the initial biopsy and is a risk prediction model for prostate cancer that incorporates low-cost, easy-to-obtain, non-clinical and non-genetic variables and can be used in primary care or community settings.

1.3 Aims and research questions

The aim of this research was to develop a risk prediction model for prostate cancer that can be used in primary care or community settings and which helps stratify men according to their potential risk of having the disease as well as suggesting the need for further examinations. In so doing, the objectives are:

A. To review risk factors associated with prostate cancer.
B. To assess the value of anthropometric measures in terms of body size and body shape and their relationship to prostate cancer.
C. To systematically review published risk prediction models for prostate cancer that do not incorporate invasive clinical tests or genetic profiles.

D. To develop and internally validate a risk prediction model for prostate cancer using easy, low-cost, readily available tests and evaluate/compare the model performance in two cohorts with different outcome yield methods.
E. To explore men's perspectives and views on home-based PSA testing and screening for prostate cancer.

1.4 Overview of this thesis

With permission from the Faculty of Biology, Medicine and Health, this thesis is submitted in an alternative format. As a result, the key chapters within this thesis (Chapters 3 to 6) are presented in the form of research papers.

The content of each chapter and the corresponding publication are listed below:

Chapter 2 provides a literature review of prostate cancer and its epidemiology. It also covers diagnosis and management practices/guidelines for prostate cancer screening and more importantly, risk factors associated with the disease.

Chapter 3 explores the relationship between body size and shape with risk of prostate cancer. The content of this chapter is adapted from:

Aladwani, M., Lophatananon, A., Robinson, F., Rahman, A., Ollier, W., Kote-Jarai, Z., Dearnaley, D., Koveela, G., Hussain, N., Rageevakumar, R. and Keating, D., 2020. Relationship of self-reported body size and shape with risk for prostate cancer: A UK case-control study. PloS one, 15(9), p.e0238928.

Chapter 4 presents a systematic review of existing prostate cancer models that do not incorporate clinical and/or genetic tests. The review compares the performance of the identified models and discusses their limitations. The content of this chapter is adapted from:

Aladwani, M., Lophatananon, A., Ollier, W. and Muir, K., 2020. Prediction models for prostate cancer to be used in the primary care setting: a systematic review. BMJ open, 10(7), p.e034661.

Chapter 5 covers the development of a risk prediction model for prostate cancer (RISKMAN). The purpose of this model is to stratify men according to their risk based on simple, yet established factors and filter out individuals who might need further investigations. The model was built using two cohorts with different methods of outcome yield; one cohort is a traditional systematic biopsy, and the other is magnetic resonance imaging (MRI) based targeted biopsy.

Chapter 6 investigates men's opinions and views on home-based testing for PSA and prostate cancer screening. The content of this chapter was adapted to generate a publication as reference below:

Aladwani, M., Lophatananon, A., Fulford, G., Young, J., Hart, S. and Muir, K., 2021. The PSA home testing kit survey. This paper was submitted to Scientific Reports.

Chapter 7 provides an overall conclusions section by summarising the key findings of this thesis and listing its potential strengths and limitations. This also identifies possible implications for the public, along with discussing directions for future work.

1.5 Contributions

The contributions related to the publications in this thesis are outlined below:

1- **Aladwani, M.,** Lophatananon, A., Robinson, F., Rahman, A., Ollier, W., Kote-Jarai, Z., Dearnaley, D., Koveela, G., Hussain, N., Rageevakumar, R. and Keating, D., 2020. Relationship of self-reported body size and shape with risk

for prostate cancer: A UK case-control study **(Published: 17th September 2020, Journal: PLOS ONE)**

- **Candidate's role:** Data control quality, data curation, results analysis and interpretation, draft manuscript, writing review and editing.

2- **Aladwani, M**., Lophatananon, A., Ollier, W. and Muir, K., 2020. Prediction models for prostate cancer to be used in the primary care setting: a systematic review **(Published 19th July 2020, Journal: BMJ open)**

- **Candidate's role:** Idea conceptualisation, data collection, literature review, results interpretation, writing original draft, critical revision and editing of the manuscript.

3- **Aladwani, M.,** Lophatananon, A., and Muir, K. Development and internal validation of risk prediction model for prostate cancer for primary care settings **(Written and to be modified for submission)**

- **Candidate's role:** Data acquisition, data analysis, results interpretation, results validation, writing original draft, critical revision and editing of the manuscript.

4- **Aladwani, M.,** Lophatananon, A., Fulford, G., Young, J., Hart, S., and Muir, K., 2021. The PSA home testing kit survey **(Submitted to Scientific reports Journal)**

- **Candidate's role:** Data quality control, data analysis and interpretation, drafting the original manuscript, critical revision of the manuscript.

Chapter 2 Literature review

2.1 Introduction

This review was conducted to give an overview of the clinical features of prostate cancer and the current practice used for its diagnosis, treatment, and identification. It also includes prostate cancer epidemiology and the UK national guidelines for prostate cancer screening. A literature review of prostate cancer risk factors is described together with serum biomarkers and genetic biomarkers previously linked with prostate cancer.

2.2 Methods

An extensive search was carried out to retrieve relevant studies by using a variety of truncation keywords and alternative terms. The search engines included (but not exclusively); Google Scholar, PubMed, Medline Database and manual searches from relevant journals. The bibliography of relevant studies was reviewed.

The inclusion criteria for the review were (a) evidence from various types of studies including; mendelian randomisation studies where genetic instruments were used as a proxy risk factor to explore the causal effect between exposure and disease outcome; systematic reviews; meta-analyses; cohort studies; and case-control studies (b) peer-reviewed studies with the availability of either full-text or an abstract in the English language. The time span of publications was unrestricted but with a preference for those published since 2010; this was to gather the most recent evidence, as prostate cancer has been most extensively examined in the last two decades. Any publications related to prostate cancer recurrence, prognosis or site tumour were excluded as it was beyond the scope of this study.

2.3 Prostate cancer clinical features

The prostate is a walnut size gland that is part of the male reproductive system surrounding the urethra and located under the bladder. It is responsible for the production of a component of seminal fluid [3]. Cancer starts when mutations occur in the cell's DNA, triggering cells to grow in an uncontrolled way [4, 5]. Typically, the size of the prostate gland becomes larger when men get older. Hence, older men are at greater risk of prostate diseases/problems such as prostatitis (which is caused by inflammation), benign prostatic hyperplasia (BPH), and prostate cancer [6]. Other prostate conditions are prostatic intraepithelial neoplasia (PIN), where there is a change in the appearance of prostate gland cells when viewed under a microscope, and proliferative inflammatory atrophy (PIA), where prostate cells appear smaller than normal [4]. PIN has been established as being a prostate cancer precursor [7]; PIA has been considered to be an indirect cause of cancer by developing into high-grade PIN [8]. In regard to prostate cancer, adenocarcinoma is the most common type of cancer, which constitutes 95% of all prostate malignancies [9]. Other prostate cancers include; small cell carcinomas, transitional cell carcinomas, neuroendocrine tumours, and sarcomas [4]. Figure 2.1 illustrates normal and cancerous prostate.

2.4 Staging and grading of prostate cancer

Prostate cancer can be classified according to either its stage or its grade. Prostate tumours are staged using the TNM system (TNM stands for Tumour, Node, and Metastasis). This standardised TNM system can be used to inform how far cancer has spread [10]. There are four stages of prostate cancer ranging from T1 to T4. T1 indicates the cancer is too small and cannot be detected by digital rectal examination (DRE). T1

are also sub-classified into T1a, T1b, and T1c. T1a is when the cancer is found incidentally during other surgery where the cancer is present in less than 5% of the removed tissue.

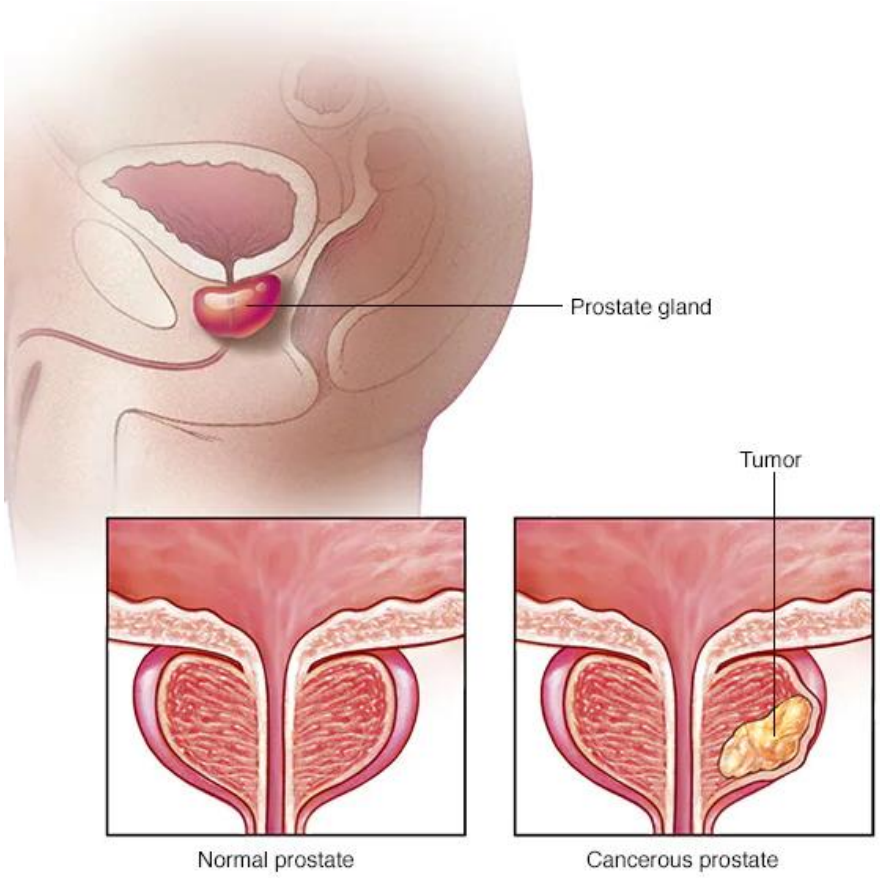

Figure 2.1: Diagramme of thre prostate gland and comparison of normal and cancerous prostate. Available from www.mayoclinic.org/diseases-conditions/prostate-cancer

T1b is where the cancer is found in 5% or more of the tissue removed. T1c represents cancer found by biopsy after a raised prostate-specific antigen (PSA) level. T2 tumours are completely confined inside the prostate gland and can be detected by DRE. T2 is also further classified as; T2a, T2b, and T2c. T2a indicates that the tumour is in one half of only one side of the prostate gland. T2b indicates that the tumour is in more than half of one side of the prostate gland; T2c is when the tumour is in both sides of the

prostate gland. The T3 stage indicates that tumours have spread outside the prostate and are classified into T3a and T3b. In T3a, cancer spreads to the covering capsule of the prostate gland, and in T3b, cancer has spread to seminal vesicles. T4 stage means that cancer has spread into nearby body organs such as the bladder or the pelvis.

The N stage informs whether cancer has spread to the regional lymph nodes; N1 indicates the lymph node contains cancer cells, and N0 indicates it does not contain cancer cells. The M stage informs whether cancer has spread to distant parts of the body or not. M0 means the cancer is confined to the regional lymph nodes, and M1 means cancer has spread to other organs, and it is classified as M1a, M1b and M1c. M1a is where the cancer is found in lymph nodes outside the pelvis. M1b where there are cancer cells in the bone, and M1c indicates that cancer has spread to other organs [11-13].

An alternative prostate cancer staging system is the Gleason system which was created in the 1960s and based on tumour architectural patterns [14]; it is considered one of the most important prognostic factors for prostate cancer patients [15-18]. This system assesses cancer cells obtained from a biopsy sample under the microscope and grades them on a scale of 1 to 5 according to tumour differentiation and growth aggressiveness. Grade 1 indicates normal prostate tissue, and grade 5 represents poorly differentiated disease [19]. The pathologist assigns one Gleason grade to the most common pattern in the biopsy and a second grade to the second most common pattern. The sum of these two grades determines the Gleason score, which can range from 2-10 [16, 20]. Several limitations have been reported with the original system. For instance, there is poor concordance between the Gleason score of the biopsy core and the radical prostatectomy specimen [21-23]. This discordance between Gleason scores might lead

to inappropriate treatment recommendations [24]. Previously thick-gauge needles were used for prostate biopsies [25], then 18-gauge needles and multiple core biopsies such as sextant biopsy were introduced in the 1980s [26]. With these changes, pathologists faced challenges in grading multiple cores of prostate cancer as well as grading small foci of cancer [25, 27, 28]. As a consequence, the Gleason system has been revised twice, first in 2005 and then subsequently in 2014 [14, 29]. The current application of Gleason grading does not assign Gleason scores 2-5 on needle core biopsy due to poor concordance with radical prostatectomy specimens [16, 30]. Instead, it was recommended that a Gleason score of 6 should be the lowest grade, 7 –be an intermediate grade, and 8-10 as the highest-grade. Furthermore, a five-grade group system was applied in the 2014 revision; grade group 1 has a Gleason score of <=6, grade group 2 has a Gleason score of 7 (3+4), grade group 3 has a Gleason score of 7 (4+3), grade group 4 has Gleason score 8, and grade group 5 has Gleason score 9-10. With this grade group system, overtreatment of low-grade prostate cancer could be reduced [16, 28].

2.5 Prostate cancer symptoms

Most prostate cancers grow slowly and arise in the peripheral zone and are confined to the prostate gland [31-33]. Therefore, usually there are no symptoms or signs in the early stages of prostate cancer [34]. However, since the prostate gland is close to the urethra and bladder, prostate cancer may cause a variety of urinary symptoms. Men may experience symptoms if cancer grows and the tumour is large enough to press against the urethra [12, 34].

Some of the urinary symptoms include; frequent urination or hesitancy, weak or interrupted flow, dribbling, difficulty in emptying the bladder, blood in urine or semen, erectile dysfunction, and in some advance cases there may be a back or hips pain and unexplained weight loss. Nevertheless, these symptoms do not necessarily indicate the presence of prostate cancer as these symptoms can also be caused by other prostate conditions such as BPH or prostatitis [12, 34, 35]. These urinary symptoms are called lower urinary tract symptoms (LUTS) [36].

Many men have the perception that having LUTS will increase their risk of prostate cancer compared to a non-symptomatic man [37, 38]. In practice, most clinical guidelines recommend doing a PSA test and/or DRE for men with LUTS for suspicious prostate cancer [39-42]. Existing evidence regarding the association of LUTS with the risk of prostate cancer is controversial. Several studies from western countries have found that in men with raised PSA levels and LUTS, their risk of prostate cancer is reduced and that LUTS were more to be found in patients with benign conditions than prostate cancer [43-46]. In a Norwegian cohort study based on 21,159 men, they found that LUTS, except for advanced and aggressive, are positively correlated with localised prostate cancer, suggesting LUTS are not caused by prostate cancer and screening based on this criterion may not be justified [47]. Similarly, in a Japanese study, they found that men with absent or mild LUTS are at increased risk of prostate cancer and high-grade stage tumours [37]. Another prospective Japanese study concluded that men with LUTS are not at higher risk of prostate cancer compared with asymptomatic men, regardless of PSA levels [48]. Moreover, several studies have reported that race might have an impact on LUTS prevalence [49-51], and hence, the results of studies in

western populations are not typical. Therefore, whether LUTS is associated with prostate cancer remains uncertain [52].

2.6 Diagnosis

The most common tests undertaken for prostate cancer are PSA and/or DRE. However, neither of these tests is cancer-specific. Since there is no specific test that would determine the presence of prostate cancer definitively, a physician may recommend further tests on whether a patient has prostate cancer [53]. Such tests include trans-rectal ultrasound where a small probe is inserted into the rectum and creates a picture of the prostate gland using sound waves. Another method of testing is magnetic resonance imaging (MRI), which creates a more detailed picture of the gland. Nevertheless, the only definitive way of diagnosing prostate cancer is by taking a sample tissue of the prostate gland i.e., needle biopsy, and the sample examined by a pathologist to determine the presence of cancer cells in the prostate [54].

Both trans-rectal ultrasonography (TRUS) and MRI can help direct prostate biopsy, with TRUS being widely used as a standard investigation in the diagnosis of Prostate cancer [55-57]. However, TRUS-guided biopsy is mainly sampling the peripheral of the prostate gland; hence, there may be misclassification or under diagnosis of prostate diseases [58]. Recent growing evidence supports using MRI in the diagnostic pathway for prostate cancer [59, 60], and it has become the method of choice for diagnosing prostate cancer over the last decade rather than conventional systematic biopsy [61]. The usage of MRI prior to any prostate biopsy is also supported by the European prostate cancer diagnosis guidelines [57, 62]. It has been found that MRI-directed biopsy has the potential to reduce the risks and harms, and address the issues such as

over diagnosis of clinically insignificant prostate cancer and under diagnosis of clinically significant prostate cancer that are associated with systematic biopsy [61].

A recent systematic review and meta-analysis reported that targeted MRI biopsy improved the detection of clinically significant prostate cancer. It also limited the number of biopsy cores needed and reduced unnecessary biopsy and adverse effects associated with this procedure [59], making it a more favoured investigation for men [63]. Recently in the UK, pre-biopsy-MRI has been recommended for biopsy-naïve patients with suspected prostate cancer as a standard practice [64]. The only obstacle to implementing MRI as a standard diagnostic pathway for prostate cancer is its availability in health care settings, along with trained urologists and radiologists using it [59].

2.7 Current practice for initial prostate cancer identification

2.7.1 Prostate-Specific antigen (PSA) screening

PSA is a glycoprotein produced by epithelial cells in both normal and malignant prostate glands [65, 66]. PSA level also increases in non-malignant conditions such as prostatic inflammation and particularly BPH [67-69]. In contrast, several drugs like dutasteride and finasteride that are used in BPH treatment have been documented to decrease PSA levels [70]. However, it has been observed that PSA is produced significantly more in serum in prostate cancer compared with BPH or normal tissues [65]. Other factors that can affect PSA level are age and ethnicity [71, 72], in addition to issues related to sample freezing, thawing, and handling [73]. Moreover, evidence showed that even with low PSA concentrations, a large number of men could be diagnosed with prostate cancer following performed a biopsy [74, 75].

These limitations and overlaps in PSA levels make the PSA test as a screening method a subject of debate as it has poor specificity and sensitivity to discriminate prostate cancer from other benign conditions [65, 67, 76, 77]. The most problematic range where the specificity of the PSA test is lacking is between 3-10 ng/ml, where half of the detected cancers could be indolent that are improbable to result in future health problems even when not treated [78, 79]. Also, a negative biopsy can be found in up to 80% [67, 80, 81]. This means most men with elevated PSA did not have prostate cancer but had an unnecessary biopsy. These, therefore, could cause anxiety, bleeding and potential infections [67]. Moreover, men with indolent cancers who had radical prostatectomy or radiotherapy could potentially have bowel, urinary and sexual side effects [82, 83]. Utilising PSA as a screening tool, therefore, can result in over diagnosis and over treatment. The PSA value range needs to be lowered prior to recommending the application of a PSA test at the population level [83-85]. So far, evidence shows that the PSA test lacks the essential criteria to be applied as a wide-spread screening method for prostate cancer [86, 87].

Despite that, the PSA test remains the most common method of prostate cancer screening worldwide as it is the only approach to detecting men with asymptomatic prostate cancer [88-90]. It has the potential to predict the risk of high-grade prostate cancer and metastasis many years in advance [67, 91]. Also, emerging evidence shows that using the PSA test for early detection could detect localised prostate cancer that was confined to the prostate gland, consequently decreasing prostate-specific mortality and morbidity [66, 92-95]. For example, In the United States, PSA screening has contributed to a decreased prostate-specific mortality of more than 30% [76, 96]. The European Randomised Study of Screening for Prostate Cancer (ERSPC) revealed that

PSA screening could minimize prostate-specific mortality by around 21% within 13 years [95, 97]. Nevertheless, this benefit comes at the cost of over diagnosis and accompanying negative effects from treatment [98]. For instance, an estimation of 1055 men need to be screened, and around 40 cancers need to be diagnosed to avoid one death [93]. Furthermore, the actual benefit from PSA screening needs several years to accrue [99, 100]. Therefore, men with an estimated life expectancy of less than ten years need to be well informed that screening for prostate cancer is unlikely to be useful, provided the harms involved with screening [79].

In the UK, although the National Screening Committee does not recommend population-based screening for prostate cancer [101], the Prostate Cancer Risk Management Programme (PCRMP) provides information for men above 50 years of age who want to have the test as well as for general practitioners to help them discuss the complex issues with such men [102]. A consensus document was released comprising of a set of thirteen statements to aid healthcare providers in using the PSA test more effectively in asymptomatic men [102]. Unsurprisingly, these statements are in line with what was mentioned above.

2.7.2 Digital rectal examination

The function of DRE as a screening tool remains dubious [83]. Few reports have shown that the DRE test has the potential to help in identifying advanced cancers in men with PSA values less than 2.5 ng/ml [103]. However, it can reduce over diagnosis by detecting grown tumours by physical examination [79]. It has been found in the ERSPC that men with a PSA value greater than 3 ng/ml and abnormal DRE results were more prone to be diagnosed with prostate cancer than those with only a PSA level elevated

more than 3 ng/ml [104]. Therefore, DRE should be considered before performing a biopsy in men within that range [105].

However, several studies have demonstrated that DRE has poor specificity, sensitivity, and reliability [106-108]. As a result, it has an extremely low predictive value in men with PSA levels below the biopsy threshold [104]. For example, it has been found in the Prostate Cancer Prevention Trial that DRE provided a modest value to prostate screening as the absolute difference in the area under the curve (AUC) was just 0.02 for diagnosing prostate cancer compared to PSA alone [75]. Therefore, it should be used, if necessary, along with the PSA test in men with low PSA levels, as it has the potential to identify aggressive cancer at that level [109].

2.8 Studies with and against screening

There are several national guidelines from different countries, and their recommendations vary considerably. Most of them do not recommend population-based screening for prostate cancer using PSA, DRE or biopsy either due to the uncertainty of its benefits or the high risks and harms relating to over diagnosis and overtreatment. Others suggest screening only for those who are well informed about the benefits and risks associated with screening and who can make their own decisions based on discussion with their healthcare provider. Table 2.1 shows the available guidelines and their recommendations.

Table 2.1: National guidelines for prostate cancer screening.

Reference	Guideline	Publication year/ update	Data included	Statement
[110]	The National Screening Committee, United Kingdom	2009	PLCO* and ERSPC	Due to insufficient evidence, the Committee does not recommend PSA screening
[111]	The European Association of Urology Clinical Practice Guidelines	2010	PLCO, ERSPC, and Quebec	Initial PSA test at age 40 years help risk-stratification PSA screening is not recommended for men older than 75 years
[112]	The U.S Preventive Services Task Force	2012	PLCO and ERSPC and other reviews	PSA screening is not recommended (grade D recommendation)
[113]	The American Cancer Society practice guidelines	2010	PLCO and ERSPC	Men with at least 10-year life expectancy should be informed about risks and benefits before screening
[114]	The Japanese Guideline for Prostate Cancer Screening	2009	Only preliminary data from ERSPC	Did not recommend screening for prostate cancer and patients who request screening should be informed about the benefits and harms
[115]	The Japanese Urological Association	2010	PLCO and ERSPC	Recommends PSA testing for men at risk
[116]	The American Urological Association	2009	PLCO and ERSPC	Recommends PSA testing for patients requesting to pursue after they informed about the risks and benefits
[117]	The Royal Australian College of General Practitioners	2016	PLCO and ERSPC	Does not recommend routine prostate screening and patients should make their own decision after being informed
[118]	The Canadian Task Force on Preventive Health	2014	Systematic review of literature	Does not recommend screening with PSA test
[119]	The American College of Preventive Medicine	2008	Preliminary data from ERSPC	Does not recommend routine screening with PSA due to insufficient evidence

* The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial

2.9 Treatments for prostate cancer

There are several types of treatment for prostate cancer and deciding which one is a better choice depends on many factors such as the aggressiveness of cancer, how far it has spread, overall health and life expectations of the patient.

Active surveillance by monitoring cancer closely and performing PSA and DRE tests regularly is an alternative choice for those who have a long-life expectation that they would benefit from other treatment options if cancer becomes more aggressive or spreads over time [120, 121]. Also, the concept is based on the fact that low-risk cancer is unlikely to cause any harm or even lower life expectancy [120].

Observation or (watchful waiting) on the other hand, is an option with less follow-up and fewer tests where treatment is provided only if a patient develops symptoms. Observation is usually recommended for older men for whom the cancer is unlikely to affect their lifespan or have other health conditions that prevent them from undergoing other treatment methods [121]. Some men do not prefer these treatments because they fear cancer might grow and spread [122].

A radical prostatectomy is a surgery that involves removing the prostate gland. It is a common option if the cancer is confined to the prostate gland and has not spread. For men with advanced stage, the prostate can be removed along with the surrounding tissues and a few lymph nodes in combination with other treatments [120, 123]. However, radical prostatectomy carries some risks. A randomised trial study has shown the long-term effects of radical prostatectomy on quality of life and found that erectile dysfunction and urinary leakage were common negative side-effects of the surgery [124].

Another treatment method for localised or locally advanced prostate cancer is *radiation therapy* by using high-powered rays to destroy cancer cells. The radiation can come from outside the body (external beam) or inside the body by placing radiation seeds in the prostate tissue near the tumour (Brachytherapy) [125]. The latter is usually used for men with low or intermediate risk [120]. Similar side-effects to the surgery have been reported after radiation therapy, however, since the operation is less invasive compared to the surgery, the magnitude of these side effects was much slower and lower [120].

Other treatment options include; hormone therapy, chemotherapy, immunotherapy, and cryosurgery [126].

2.10 Prostate cancer epidemiology

Prostate cancer is the second most common type of malignancy and the fifth leading cause of mortality and morbidity in men around the world, with 1,414,259 new cases estimated in 2020, of which 375,304 were fatal [127]. In the UK, prostate cancer has overtaken breast cancer and became the most commonly diagnosed cancer in 2018 [128]. In 2020 in the UK, the estimated number of prostate cancer cases was 56,780, with a mortality rate of approximately 23% (13,168) [127].

2.10.1 Incidence

The incidence of prostate cancer varies across the world based on the geographic region [129, 130]. The highest incidence rate is seen in western and developed countries such as the United States, UK, New Zealand, Australia and Europe. The lowest rates of incidence are found in Asian and African countries [130, 131]. It has been established that prostate cancer is correlated positively with age. The highest age-standardised rate

of incidence is in Northern Europe (83.4) per 100,000, followed by Western Europe (77.6), and (73.0) for Northern America, whereas Northern Africa and South-Central Asia are among the lowest (16.6), (6.3) respectively [127]. It has been found that in men above the age of 65 years, the incidence rate could reach 60% [132]. The reason behind these discrepancies is not clear, but it can be attributed to the widespread use of PSA tests, particularly in developed countries [130, 133, 134]. Figure 2.2 shows age-standardised rates for prostate cancer worldwide from 2020.

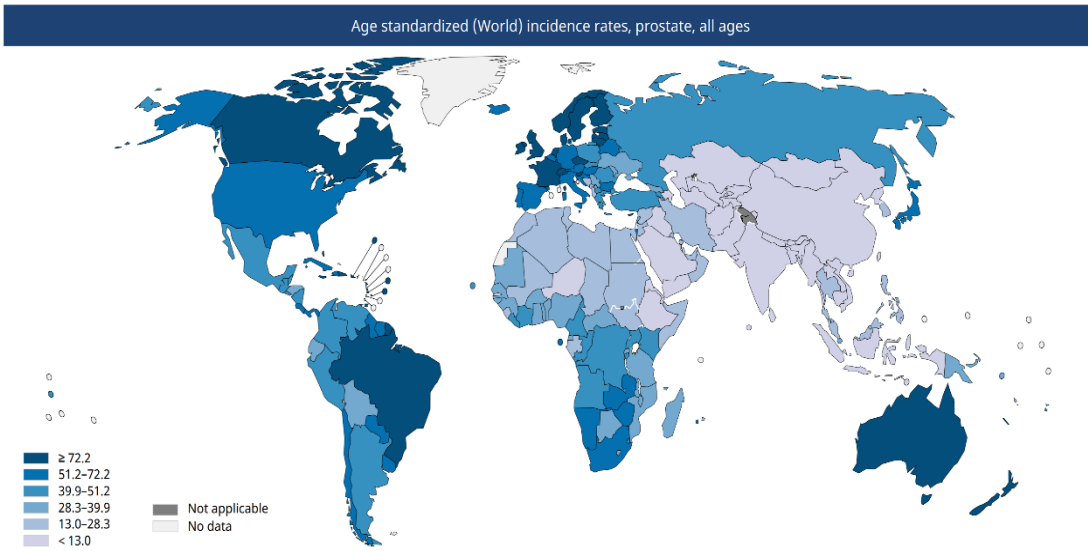

Figure 2.2: Prostate cancer age-standardised incidence rates from 2020 world statistics presented by the International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today/home

Evidence has shown that the highest incidence of prostate cancer worldwide is found among African-Americans, and they are more likely to develop the cancer much earlier compared to other ethnicities [135]. This is also reflected in men of African decent living in Europe and in the Caribbean, as they may share genetic backgrounds making them more susceptible to developing the cancer [131]. However, environmental and lifestyle factors play an important role in the variation of prostate cancer incidence. For example, Afro-American men who live in the United States have 40 times a higher

incidence compared to men in Africa [136]. Furthermore, the prostate cancer incidence rate increases among migrants who left low-risk regions to the high-risk region compared to the men who lived in their native countries [137, 138]. Figure 2.3 below shows the age-specific incidence rate for prostate cancer per 100,000 men in the UK between 2016 and 2018.

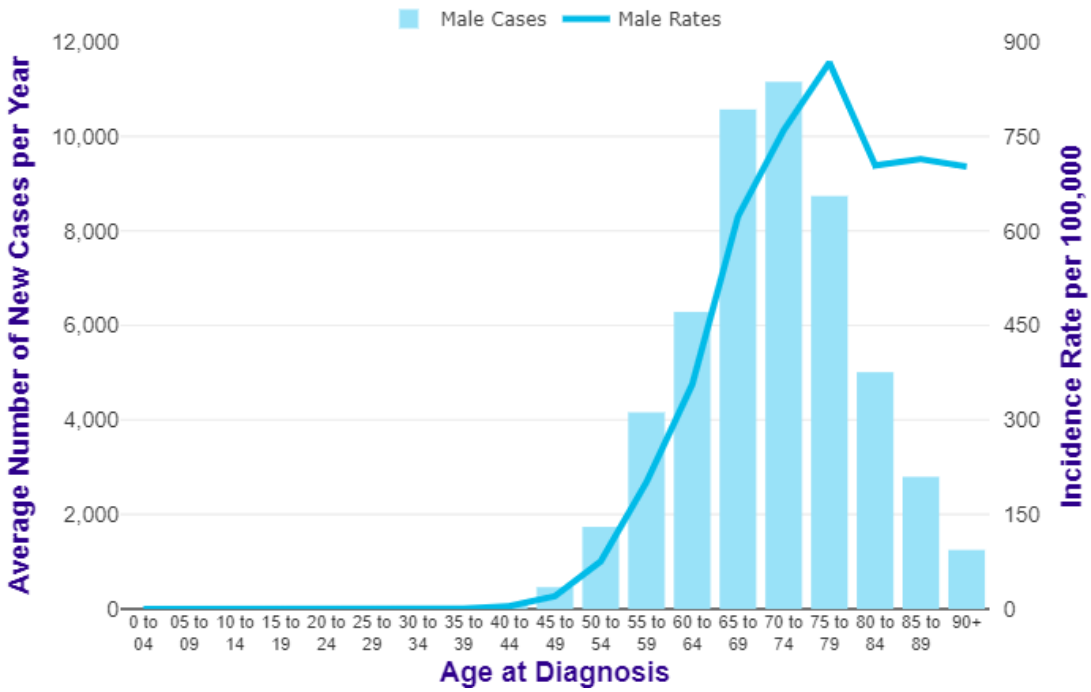

Figure 2.3: Age-specific prostate cancer incidence rates per 100,000 males in the UK between 2016 and 2018. Available from: https://www.cancerresearchuk.org/

2.10.2 Mortality

Prostate cancer is the fifth cancer-specific cause of death in men globally. Similar to incidence, there is a discrepancy in mortality rate between regions and countries. In 2020, the highest age-standardised mortality rate was in the Caribbean with 27.9 per 100,000, followed by Central Africa at 24.8, whereas the lowest rates were reported in Eastern and South-Central Asia at 4.6 and 3.1, respectively [127]. In Europe, Estonia has the highest mortality rate with 21.8, and Italy was the lowest with 5.9, whereas in

the UK the mortality rate was 12.4. Likewise, the United States has a mortality rate of 8.2 [127]. The reduction in mortality rate in western and developed countries could be a result of the intensity of PSA diagnostic testing as well as their effective treatment plans, especially at the early stages of the disease [139]. As with incidence, the mortality rate increases with age, and approximately 55% of all mortality occur in men with age 65 and over [127].

Of note, African-Americans have a mortality rate that is approximately twice that of American Caucasian decent [140]. This may illustrate that this ethnic group not only possess different genetic risk backgrounds that may alter susceptible to the disease but also make them prone to developing more aggressive cancers [131]. Overall, it is notable that while the incidence rates are much higher in developed regions, the mortality rates are higher in less-developed regions [141]. Figure 2.4 shows age-standardised rates for prostate cancer mortality worldwide from 2020.

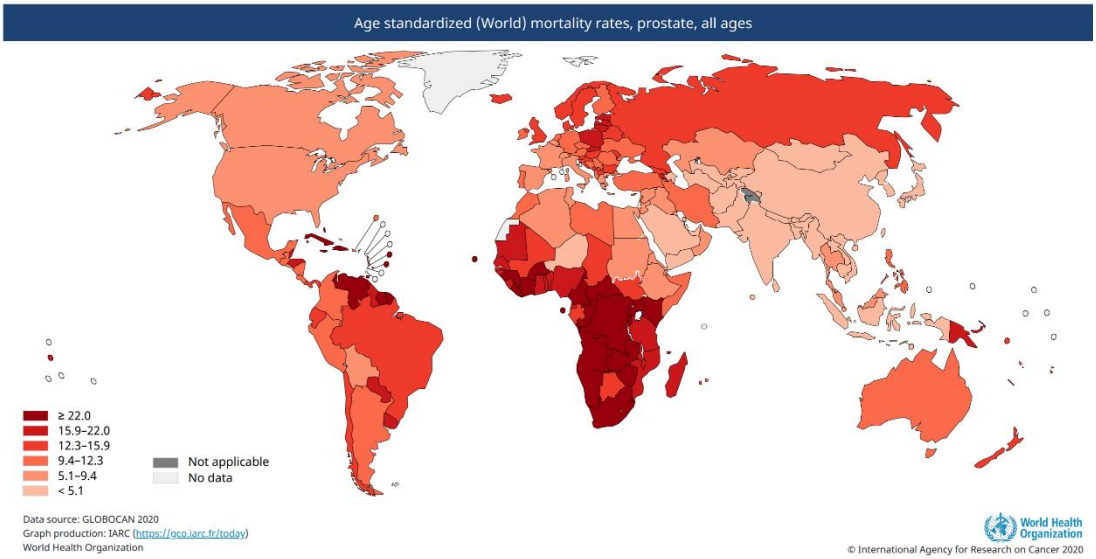

Figure 2.4: Prostate cancer age-standardised mortality rates from 2020 world statistics presented by the International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today/home

2.10.3 Trends

Several studies have reported a substantial increase in the incidence of prostate cancer over the last few decades [142-144]. Incidence rates in the United States, however, are now decreasing after rates peaked in the 1990s following the introduction of PSA testing. The decline in rates is thought to be a result of the rapid dissemination of PSA tests [145, 146]. On the contrary, incidence rates in Europe have increased slightly due to increased awareness and adoption of PSA screening [147]. The age-standardised incidence rate of prostate cancer has increased in the UK by 8% over the last decade (Figure 2.5).

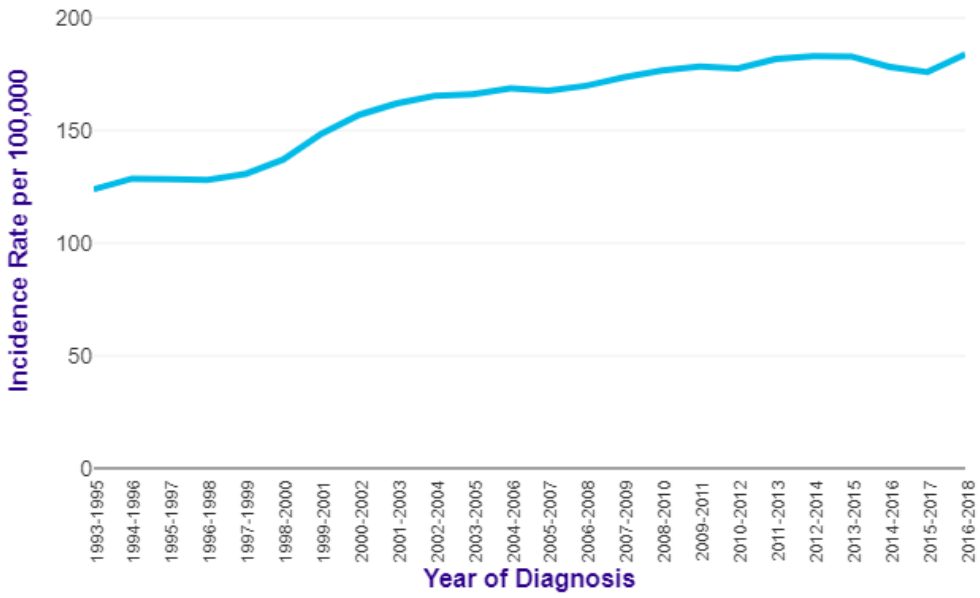

Figure 2.5: Age-Standardised incidence rate of prostate cancer in the UK from 1993 to 2018. Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/

Worldwide, it is estimated that there will be almost 2.5 million new cases by 2040, an increase of 1,200,000 new cases from 2020, with an overall change of 72.34% [148]. The highest estimated incidence will be in Africa (+108.4%), followed by Latin

America and the Caribbean (+81.3%) and Asia (+74.4%). The lowest incidence will be in Northern America (+23.8) and Europe (+27.7). The trend of increasing incidence seems to be due to an increase in life expectancy in the world [149-151] and improved access to healthcare in developing countries [131]. It is notable that in some areas where PSA testing is not recommended in clinical practice, the increase in incidence suggests that lifestyle or environmental factors may influence prostate cancer incidence [152].

The highest mortality rate is expected to be in Africa (+108.4), followed by Asia (+115.1) and Latin America and the Caribbean (+109.8). The lowest mortality rate will be in Europe (+54.6) and Northern America (+83.6). Prostate cancer mortality rates in Northern America and Europe are constantly decreasing [145, 152]. The reasons behind the decline in mortality rates in these regions are not clear. However, it may be a consequence of early detection and enhanced treatments [119, 153, 154]. In contrast, poorer access to healthcare and limited resources in developing regions could explain the high mortality rate despite the lower incidence [131].

2.11 Risk factors

The aetiology of prostate cancer, like many other cancers, is still unclear. Therefore, scientists have searched for possible risk factors that could be associated with prostate cancer in an attempt to better understand the disease and to provide health measures to patients and men who are at higher risk of developing the disease. Risk factors could potentially be an exposure to certain substances or chemicals, as well as certain lifestyles and behaviours. Some of these risk factors cannot be modified, such as age, family history, and ethnicity, while others are modifiable (e.g. tobacco use and alcohol consumption). In addition, risk factors could increase the likelihood of a subject

developing a disease or lower the chances of contracting a disease (protective factors). It is worth noting that both risk and protective factors are co-relational, as with most risk factors, they do not directly cause a disease. Although epidemiological studies cannot prove a causal relationship between a risk factor and an outcome, when several robust studies point to a similar correlation between a risk (and protective) factor with cancer, that correlation becomes stronger and should be taken into consideration. In prostate cancer the established risk factors are age, family history, and ethnicity. In this section, a detailed discussion about these established risk factors is provided. Other risk factors are presented in Table 2.2 with a summary of evidence.

2.11.1 Age

Prostate cancer risk increases significantly with age. Parkin et al. found that around 75% of prostate cancers are detected in men in their mid-60s and over [155]. There is some disagreement about the right age group that needs to be screened. Concerning the long-term predictive value of PSA, some doctors support starting screening for men in their early 40's as it has been found that PSA testing can predict prostate cancer diagnosis and metastases more than 25 years later [76, 91, 156] and those in early mid-life will benefit the most from the screening [67]. Moreover, unlike older men, younger men are less likely to be diagnosed with an incurable disease at their initial screening and can take advantage of regular screenings [93].

In addition, the elevation of PSA in men in their mid-40s is associated with a diagnosis of prostate cancer more than an elevation in men aged 60 or above, where BPH becomes more common [72, 157]. Therefore, although PSA screening in men between 40-50 has not improved prostate cancer mortality within ten years [158], the ultimate goal of

screening men in their mid-life is not to detect the cancer but to aid in stratifying the risk earlier [56, 83, 157].

Results from a European trial showed that screening men aged 60-69 reduce mortality [92]. However, screening men at age 60 with low PSA shows no mortality reduction but rather causes a substantial risk of over diagnosis [90, 94]. PSA value at age 60, however, predicts the risk of death from prostate cancer 25 years later [159]. Furthermore, there is growing evidence that suggests men aged 60 with a PSA value below 1 ng/ml can be exempted from further screening, even if they might have cancer which in their case is less likely to become life-threatening. Restricting screening for those aged 60 and older with PSA levels greater than 1 ng/ml would improve the ratio of benefits to harm of prostate cancer screening [82, 90, 94, 160].

Although guidelines vary over the best age to start and terminate screening, there is an agreement that screening men older than 70 years must stop due to a higher risk of over diagnosis and overtreatment, and only those in good health with a life expectancy more than ten years and a PSA above average should be screened [84, 92, 161-164]. A micro-simulation study shows that terminating screening at age 69 instead of 74 would result in a 50% reduction of over diagnosis [165].

2.11.2 Family history

Prostate cancer is one of the most heritable cancers due to genetic factors [166, 167]. The hereditary factor to the risk of prostate cancer has been acknowledged since 1960 [168]. Following that, many studies investigated the association between family history and the risk of prostate cancer and reported a positive association. Thus, family history is considered to be a well-established risk factor for prostate cancer. Furthermore, evidence has shown that the magnitude of the risk varies based on the degree of

relationship and age of onset of the cancer [169]. In 1990, Steinberg et al. conducted a case-control study which included 691 cases and 640 controls, where they found having a first-degree relative (father or brother) diagnosed with prostate cancer increased the risk of developing prostate cancer twice compared to those with no diagnosed relatives [170]. Also, they reported an increased risk between 5-11-fold in men with more than two first-degree relatives diagnosed with prostate cancer and the risk increase as the number of affected relatives increase [170]. Similar results have been reported in other studies [171, 172]. Specifically, some studies found that men with their fathers diagnosed with prostate cancer have more than a two-fold greater risk of prostate cancer; for those with affected brothers, the risk is even higher [173-177]. Furthermore, Chen et al. found the risk of developing prostate cancer is increased when relatives are diagnosed at an early age. They also showed in their study that a family history of prostate cancer elevates men's risk of developing aggressive prostate cancer [175].

A systematic review and meta-analysis study reported a pooled relative risk (RR) of 2.5 (95% confidence interval (CI)= 2.2-2.8) of having first-degree relatives with prostate cancer, and the highest risk is in men with relatives diagnosed before the age of 60. In the same study, they reported a 3-5 fold (95% CI = 2.6-4.8) increased risk in men with two affected relatives [178]. Another systematic review and meta-analysis study have shown similar results [169].

2.11.3 Ethnicity

Prostate cancer incidence and mortality vary among ethnic groups. Worldwide, the greater incidence of prostate cancer for men of African origin is well established. African-Americans, in particular, have the highest incidence of prostate cancer in the world [179]. Moreover, African-Americans have a higher risk of having advanced-

stage prostate cancer than other ethnicities. A large cohort study in 2001 found that aggressive prostate cancer (defined by Gleason Score >=8) is presented in about 17% of African-Americans, whereas it was observed in ~13% and 11% of Hispanics and non-Hispanics whites, respectively [180]. The same study also showed the highest percentage of advanced-cancer in African-Americans (12.5%), followed by Hispanics and non-Hispanic whites 10.5% and 6.3%, respectively [180]. Interestingly, a study found that African-Americans perceived their risk of developing prostate cancer less than Hispanic men, but not non-Hispanic whites [181]. Furthermore, the incidence rate per 100,000 for patients under the age of 50, 50-64, and above 65 years was the highest in Africans and the lowest in Native Americans and Asians. Similarly, the age-adjusted mortality rate was the highest among African-Americans (37.9 per 100,000) compared to white (17.9), Hispanics (15.8), and Asians (8.6) [182].

In the UK, several studied have investigated the differences in incidence and mortality rates between ethnicity groups. A cohort study in 2007 reported a higher age-adjusted rate in men of African descent, which ranged from 151 to 180 per 100,000, followed by Caucasians who ranged from 53.3 to 59.5. The study also confirmed a higher risk in younger age groups among Africans compared to Caucasian men in the same age groups [183]. Likewise, in England, men of African and Afro-Caribbean descent have three times the age-adjusted rate of diagnosed prostate cancer relative to their UK Caucasian counterparts [184]. Using 2008-2010 data in England, Llyod et al. found that the lifetime risk of being diagnosed with prostate cancer was 13.3% in Caucasian men, while it was higher in UK men of African descent (29.3%) and lower in Asian men (7.9%). Also, they have shown the lifetime risk of dying in men of African heritage is twice that in Caucasian men, whereas it is lower in Asian men [185].

Other less established risk factors are summarised in Table 2.2.

Table 2.2: Other prostate cancer risk factors.

Risk factor	Description	References
Body features		
Tall height	The association between height and total prostate cancer is controversy. Some studies report a positive and significant association, while others found no association. However, it seems that height is more associated with a high-grade prostate cancer hazard ratio (HR) of 1.54 (95% CI = 1.18-2.03), as well as with the risk of dying due to prostate cancer HR 1.43 (95%CI= 1.43-1.80). A meta-analysis study included 1,357 cases and 7,990 controls report a strong association between height and high-grade prostate cancer odds ratio (OR) 1.23 (95%CI= 1.06-1.43), but not with low-grade cancer OR 0.99(95%CI= 0.90-1.10). Also, evidence shows that for a 10-cm increase in height, the positive risk ranges from 5% to 12%.	[186-190]
Obesity/BMI	Growing evidence suggests that obesity, measured by body mass index (BMI), is linked to a higher risk of cancer. The relationship of this association, however, remains unclear. A recent study found that a 5 kg/m2 rise in BMI was inversely correlated with a change in PSA of about -6%. Another study observed a significant inverse correlation between BMI and localised prostate cancer (RR=0.97, 95%CI= 0.95-0.99). Whereas meta-analysis study investigated the association between adiposity and different type of cancers, reported no relation between adiposity and prostate cancer. Obesity has been linked to fatal prostate cancer and mortality. A meta-analysis in 2011 found that for each 5 kg/m2 increase in BMI, the	[191-195]

Risk factor	Description	References
	risk of dying of prostate cancer was increased by 15% (RR=1.15, 95% CI=1.06-1.25).	
Waist circumference/central adiposity	A study that included 46,094 men (5,711 cases) investigated the association between waist circumference (WC) and prostate cancer. They found no association between WC with total and high-grade (Gleason score >=8) prostate cancer incidence or mortality even in the adjusted model. However, an inverse association with low-grade prostate cancer (Gleason score <8) was observed, but this association was not significant after adjustment for BMI. The results were consistent with a previous study where they reported a weak association for each 10 cm increase in WC with prostate cancer RR 1.03 (95% CI = 0.99-1.07). Also, WC is not significantly correlated with prostate cancer mortality.	[189, 196]
Weight change	Several studies examined the associations between weight change and prostate cancer, but the findings are inconsistent. A meta-analysis study investigated the association of weight loss in different cancer sites and found a positive association with prostate cancer with a positive predictive value of 3.3%. Another study included 497,634 men, of whom 22,338 cases from nine trials. A favourable association with adult weight gain was observed for total prostate cancer before the weight gain increased to more than 30kg. A similar association was found for low-intermediate prostate cancer until weight gain rose to >15 kg. Also, a positive linear correlation with every 5 kg increase in adult weight gain with high-risk prostate cancer RR 1.02 (95% CI= 1.00-1.04), whereas for fatal prostate cancer, it was RR 1.12 (95%	[197-199]

Risk factor	Description	References
	CI=1.05-1.19). A previous study found that weight change was not associated with the incidence of all prostate cancer. However, they reported a positive association between weight gain and prostate cancer mortality for each 5 kg increase in weight HR= 1.13 (95% CI = 1.02-1.26).	
Waist-to-hips-ratio	Waist-to-hip ratio (WHR) is a method to measure central adiposity. A recent pooled analysis study included 830,772 men of whom 51,734 cases, revealed that WHR was associated with a substantial increase in the risk of high-grade (Gleason score>=8) prostate cancer (up to 16%) and between 20% to 39% increase in the risk of prostate cancer mortality. Another study confirmed the results where they found, after adjusting for race, WHR > 0.98 was associated with high aggressive cancer (Gleason score>=8 or PSA >20ng/ml) with OR = 1.42 (95% CI= 1.00-2.00) compared with WHR < 0.90. However, a case-control study included 960 cases and 4156 controls found no association between WHR with total prostate cancer OR=0.93 (95% CI = 0.77-1.11). A mendelian randomisation study examined the association of adiposity-related measurement with many cancers and consisted of 51,537 cancer cases and 61,600 controls, where they found no clear evidence between WHR or other measurements and prostate cancer.	[200-203]
Diet-related		
Intakes of processed food/meat	The findings from epidemiological studies that examined the association of processed food with the risk of prostate cancer are inconsistent. A case-control study in Uruguay included 464 cases and 472 controls found that processed meat increases the risk of prostate cancer	[204-210]

Risk factor	Description	References
	OR=1.78 (95% CI= 1.22-2.59). Another prospective study observed an increased risk of metastatic prostate cancer with processed meats. Likewise, a case-control study in Canada included 1919 cases and 1991 controls revealed that consumption of unprocessed or minimally processed foods has an inverse, but a small association with prostate cancer OR=0.86 (95%CI= 0.70-1.07), while processed foods were associated with higher risk OR=1.29 (95%CI= 1.05-1.59), whereas there is no association with ultra-processed foods or drinks. Conversely, several other studies reported no association between processed foods/meat with prostate cancer. The latter result was confirmed by a pooled analysis that examined 15 prospective cohort studies.	
Meat	A recent prospective analysis using UK Biobank data consisting of 474,996 individuals, of whom 46% were men, analysed the association between meat intake and risk of common cancers. The study reported a positive association between consuming red meat and prostate cancer HR=1.13 (95%CI= 1.01-1.27). However, since the red meat definition may sometime include both processed and unprocessed meat, the researcher then combined red meat and processed meat together, in this case, the association was positive but not significant HR 1.04 (95%CI= 0.93-1.17). A meta-analysis study that included over 700,000 participants found no significant association between total red meat or fresh red meat with prostate cancer, summary relative risk estimates were 1.02 (95%CI=0.92-1.12) and 1.06 (95%CI=0.97-1.16), respectively.	[211, 212]

Risk factor	Description	References
Fish	Several studies have suggested a protective role of fish and fish oils on cancer. However, its association with cancer remains controversial. A systematic review of 37 studies showed that 10 studies reported a significant inverse association between fish and fish-oil intake with the risk of prostate cancer, whereas only three studies examined the relationship with aggressiveness and reported higher intake of total fish significantly reduced the risk of aggressive cancer OR=0.56 (95%CI=0.37-0.86). Moreover, three studies reported a reduced risk of prostate cancer mortality at the highest fish intakes. A previous meta-analysis included 12 case-control studies and 12 cohort studies found no association between fish intake and reduced prostate cancer risk. In the case-control studies, OR was 0.85 (95%CI=0.72-1.00) whereas the RR was 1.01 (95%CI=0.90-1.14) in cohort studies. Moreover, analyses of four cohort studies revealed a significant reduction in prostate cancer mortality RR= 0.37 (95%CI=0.18-0.74). A more recent cohort study, however, reported a higher intake of fatty fish was correlated with a higher prostate-specific mortality rate (1.27; 95%CI= 1.04-1.55). In addition, they found no association between fish intake and the risk of overall or high-grade prostate cancer.	[213-215]
Intakes of tomatoes/lycopene	Tomatoes and its products that contain high levels of lycopene act as an antioxidant agent and are thought to have a protective effect against prostate cancer. Several studies have reported an inverse association of lycopene in relation to prostate cancer risk, while others reported null findings. A meta-analysis in 2004 showed a reduced risk of prostate cancer	[216-220]

Risk factor	Description	References
	among high consumers (5th quintile) of raw tomato RR=0.89 (95%CI=0.80-1.00) compared to those who do not consume tomatoes frequently (1st quintile). However, the study found a significant inverse association for high intake of cooked tomato products RR=0.81 (95%CI=0.71-0.92). A subsequent meta-analysis in 2013 included 11 nested case-control studies and six cohort studies reported a non-significant inverse association of higher raw tomato, cooked tomato, and higher lycopene intake. Another meta-analysis conducted in 2017 and included 692,012 participants of whom 43,851 were cases, found that dietary intake and circulating levels of lycopene were significantly associated with reduced risk of prostate cancer, RR= 0.88 (95%CI=0.78-0.98). In this study, lycopene however, was not associated with advanced prostate cancer. More recent dose-response analysis which included 260,461 men of whom 24,222 cases, showed that higher consumption of total tomatoes could reduce the risk of prostate cancer by 19%. (RR=0.81, 95%CI= 0.71-0.92), but they did not find an association with raw tomatoes. The above results indicate that tomato/lycopene may have a modest reduction effect on prostate cancer risk, however, more research to demonstrate causality is needed.	
Cruciferous vegetables	There is limited and contradictory evidence of an inverse correlation between cruciferous vegetable consumption and prostate cancer. Only one meta-analysis was conducted to evaluate the association between cruciferous vegetables and the risk of prostate cancer. The study included six case-control and seven cohort studies. The overall analysis showed a significant	[221, 222]

Risk factor	Description	References
	reduction in the risk of prostate cancer RR= 0.90 (95%CI= 0.85-0.96). The subgroup analysis was also significant in case-control studies RR=0.79(95%CI= 0.69-0.89), but not in cohort studies RR= 0.95(95%CI= 0.88-1.02). More prospective studies are needed to examine the protective role of cruciferous vegetables on prostate cancer.	
Soy foods/ Isoflavone	Soy foods which contain Isoflavone have been suggested to have a preventive role against prostate cancer. A meta-analysis that included six case-control and two cohort studies has evaluated the relationship between soy foods, excluding fermented soy foods, and the risk of prostate cancer. The analysis showed a reduced risk of prostate cancer, RR= 0.70 (95%CI= 0.59-0.83). Another study investigated non-fermented soy foods and prostate cancer risk. Result showed a non-statistical association (RR= 1.02 (95%CI= 0.73-1.42). The study also observed an inverse relation with isoflavones, but it was not significant RR= 0.88 (95%CI= 0.76-1.02). A significant reduction in prostate cancer risk associated with soy foods and/or isoflavones was reported in two subsequent meta-analysis studies. The estimated risks ranged between 0.49 and 0.75.	[223-226]
Dairy products	The increased consumption of dairy products has been related to an increased risk of developing prostate cancer. A meta-analysis which included 32 cohort studies found that consumption of 400 grams per day of total dairy products was associated with an increased risk of prostate cancer (RR=1.07, (95%CI= 1.02-1.12), consumption of 200 grams per day of total milk (RR=1.03,95%CI= 1.00-1.07), 200 gram per day of low-fat milk	[227-229]

Risk factor	Description	References
	(RR=1.06, 95%CI= 1.01-1.11), and 50 gram per day of cheese (RR= 1.09 ,95%CI= 1.02-1.18). A recent systematic review of meta-analyses studies concluded that although evidence showed a higher intake of dairy products may increase the risk of prostate cancer, yet these results are not consistent.	
Fruit and vegetable intake	High fruit and vegetable consumption is widely associated with a decreased risk of various human cancers, including prostate cancer. It is unclear which compound in these dietary has the protective effect, nonetheless, the flavonoid is the most substance that has been investigated. A meta-analysis included 16 cohort studies found no association between both fruit and vegetables with the risk of prostate cancer. The RR of pooled analysis was 0.97 (95%CI= 0.93-1.01) for vegetables and 1.02 (95%CI= 0.98-1.07) for fruit. A recent systematic review, however, evaluated the association between dried fruits with multiple human cancers. They found that more than three servings of dried fruits per week were associated significantly with a reduction of prostate cancer by 49%.	[230-232]
Tea and coffee	Several epidemiological research on the association between coffee/tea intake and prostate cancer risk have been published, but with controversial findings. A case-control study involving 892 cases and 863 controls found tea intake was associated with a decreased overall risk of prostate cancer in the highest versus lowest tea intake group, OR=0.63 (95%CI=0.45-0.90). Relative risk by Gleason grade or cancer stage did not differ significantly. Coffee intake was not associated with the risk of overall prostate cancer or tumour grade and cancer stage. A recent large	[233-238]

Risk factor	Description	References
	cohort study included 142,196 participants, of whom 7,036 were prostate cancer cases. The study assessed the association between tea and coffee consumption and prostate cancer risk and found no association with total prostate cancer or cancer grade, stage and fatality. Another cohort study showed that consuming ≥3 cups of coffee per day was associated with a 55% decreased risk of high-grade cancer (Gleason score ≥8), HR=0.45 (95%CI=0.23-0.90). Meta-analyses studies have also shown inconsistent results. Further research is needed on the type and concentration of tea and coffee and their effect on prostate cancer.	
Total energy consumption	There is limited evidence on the association of total energy consumption, measured as kcal, with prostate cancer. One case-control study included 605 cases and 592 controls examined the association of total energy and found that it was associated with an increased risk of localised and distant prostate cancer. Compared to men in the lower quintile of energy intake, those in the upper quintile had three times the risk of developing localised and regional prostate cancer, the adjusted OR for localised cancer was 2.15 (95%CI= 1.35-3.43), whereas for regional cancer was 1.96 (95%CI= 1.08-3.56).	[239]
Cholesterol levels in the blood	Studies that evaluated the association between cholesterol levels and prostate cancer produced inconsistent findings. A cohort study included 12,926, of whom 650 men diagnosed with prostate cancer after a follow-up period of 37 years, found that higher cholesterol levels were associated with an increased risk of high-grade prostate cancer (Gleason score ≥ 8). The association was stronger among men	[240-243]

Risk factor	Description	References
	in the second-quintile for cholesterol level compared to baseline (6.1-6.69 mmol/I, and <5.05 mmol/I), HR =2.28 (95%CI= 1.27-4.10). Contradicting results were found in another cohort where they reported that low cholesterol was associated with an increased risk of prostate cancer. Compared to the 4th quartile for cholesterol, men in the 1st quartile had a two-fold risk of developing prostate cancer, sub-hazard ratio = 2.2 (95%CI= 1.4-3.2). However, a meta-analysis included 14 prospective studies found that total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were not correlated with overall or high-grade prostate cancer. The latter results were confirmed in a subsequent mendelian randomisation analysis which suggested that cholesterol and its derivatives were not a causal factor for prostate cancer.	
Dietary inflammatory index	The dietary inflammatory index (DII) is an instrument that measures the inflammatory potential of diet and emerging studies have linked it to cancer risks. A meta-analysis included 13 studies reported an increased risk of prostate cancer among men in the highest DII group compared to the lowest DII group, OR= 1.31 (95%CI= 1.04-1.57). A positive association was observed in the other two independent meta-analyses, where they also found for each one increment increase in DII, the risk of prostate cancer was higher by ~10%.	[244-246]
Glycaemic index/ glycaemic load	Glycaemic load (GL) is a measure that estimates the amount of carbohydrate in food which increases the level of blood glucose after eating it, whereas glycaemic index (GI) assigns a score between 0 to 100 on how fast it raises the blood	[247-249]

Risk factor	Description	References
	glucose. GL and GI have been investigated as risk factors for cancers. A meta-analysis in 2015 included 27 observational studies reported an insignificant association between GL and GI with prostate cancer. The pooled RR for GI was 1.06 (95%CI= 0.96-1.18) and for GL was 1.04 (95%CI=0.91-1.18). Two other meta-analyses also observed no association between GL or GI with prostate cancer.	
Diet pattern, Western/Mediterranean	The role of dietary patterns in cancer risk has been examined by multiple studies, however the results were inconsistent. A recent meta-analysis included 10 studies reported no association between Mediterranean diet pattern with risk of overall, advanced, localised, and fatal prostate cancer. Another meta-analysis included 12 observational studies found that Western diet pattern was significantly associated with an increased risk of prostate cancer OR= 1.34 (95%CI= 1.08-1.65).	[250, 251]
Other Risk Factors		
Diabetes	A meta-analysis including 19 studies in 2006 observed an inverse relation between diabetes mellitus and prostate cancer, RR=0.84 (95%CI= 0.76-0.93). Similar findings were reported in a subsequent meta-analysis that included nine studies. The latter study has found an inverse relation with different stages or grades of prostate cancer. However, a mendelian randomisation study used The Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) data in 2019 found no association between type 2 diabetes with prostate cancer OR 1.02 (95%CI= 0.97-1.07).	[252-254]

Risk factor	Description	References
Metabolic syndrome	Several studies investigated the association between metabolic syndrome (MetS) and prostate cancer. Some of these studies reported a positive, negative, and null association. The overall analyses of a meta-analysis that included 19 studies showed no correlation between MetS and the risk of prostate cancer, while MetS was associated with high-grade and advanced prostate cancer, OR=1.44 (95%CI= 1.20-1.72) and 1.37 (95%CI= 1.12-1.68), respectively. Another meta-analysis reported similar results, where they found a borderline association with prostate cancer incidence and a significant association with high-grade cancer. However, in that study, authors reported a statistically heterogeneous between studies included. The non-significant association was also reported in a different meta-analysis that included 14 studies with combined prostate cancer cases of 4,728.	[255-258]
Alcohol consumption	Alcohol is associated with the risk of various cancer sites. A large meta-analysis that investigated the association of alcohol consumption with a different type of cancer and included 43 studies related to prostate cancer showed that light (<=12.5g/day), and moderate (<=50g/day), but not heavy drinking (>50g/day), were significantly associated with increased risk of prostate cancer. The RR for light, moderate, heavy drinking were 1.04 (95%CI=1.01-1.08), 1.06 (95%CI=1.01-1.11), and 1.09 (95%CI=0.98-121). Similar findings of statistically significant increased risk in all drinking volume categories were reported in another meta-analysis that included 27 studies. However, a recent mendelian randomisation study that analysed UK Biobank data found no causality between	[259-261]

Risk factor	Description	References
	alcohol consumption and prostate cancer risk.	
Physical activity/ Sedentary	A meta-analysis study included 30,810 cases from 12 cohort studies found that sedentary behaviour was not associated with prostate cancer RR 1.07(95%CI= 0.99-1.16). However, they observed a statistically strong association with aggressive cancer if it was not adjusted to BMI (RR 1.21, 95%CI= 1.03-1.43), whereas this association became insignificant when adjusted to BMI. This indicated that sedentary behaviour was not correlated independently to prostate cancer. On the other hand, numerous studies have investigated the correlation between physical activity and prostate cancer but yielded inconsistent findings. One meta-analysis that included 88,294 cases from 19 cohorts and 24 case-control studies found that when data from cohort and case-control studies were combined, total physical activity reduced the risk of prostate cancer significantly with an RR of 0.90 (95%CI= 0.84-0.95). A significant reduction in the risk of prostate cancer was also observed with occupational physical activity, while with recreational physical activity, there was a borderline association of decreased risk. However, a more recent and larger meta-analysis that included 151,748 cases from 72 observational studies reported a null association between total physical activity and risk of prostate cancer, as well as advanced and non-advanced prostate cancer.	[262-264]
Smoking	Evidence suggested an association between smoking and several cancers and diseases. A meta-analysis of 24 cohort studies with 21,579 cases revealed that current smokers had no additional risk of developing prostate cancer, but they had a	[261, 265, 266]

Risk factor	Description	References
	higher risk of fatal prostate cancer RR=1.14 (95%CI= 1.06-1.19). The study also showed that former smokers had an elevated risk RR=1.09 (95%CI= 1.02-1.16). In contrast, another meta-analysis including 51 studies, found that current smoking was associated with a reduced risk of prostate cancer RR=0.90 (95%CI= 0.85-0.96). In that study, current smoking was also associated with an increased risk of prostate cancer mortality RR= 1.24 (95%CI= 1.18-1.31), however the authors reported different levels of heterogeneity in the results. The inverse relationship between smoking and prostate cancer was also found in the mendelian randomisation study, however, the association was not statistically significant.	
Aspirin/non-steroidal anti-inflammatory drugs	Regular use of non-steroidal anti-inflammatory drugs (NSAIDs) was suggested by several studies to reduce the risk of prostate cancer. A meta-analysis comprised of 24 observational studies with 24,230 cases found that the use of aspirin was inversely correlated with the risk of total prostate cancer (OR=0.83,95%CI= 0.77-0.89), while advanced prostate cancer had OR of 0.81 (95%CI= 0.72-0.92). Similar findings were also reported in a subsequent meta-analysis. The latter study has found that the inverse association between aspirin use and overall and advanced prostate cancer was stronger with long-term use (≥4 years). Another meta-analysis included 43 studies also reported an inverse association of aspirin, however, they observed no significant association between non-aspirin NSAIDs with overall, advanced, or Gleason score ≥7 prostate cancer. Interestingly, a meta-analysis study showed that all-NSAIDs intake was associated with an increased	[267-270]

Risk factor	Description	References
	risk of prostate cancer (OR= 1.18, 95%CI= 1.15-1.22).	
Sexually transmitted diseases	Evidence suggests that inflammation caused by infectious diseases could be a risk factor for prostate cancer. A meta-analysis included 13,342 participants, of whom 6,022 cases from 29 case-control studies assessed the association between sexually transmitted diseases (STDs) and prostate cancer. The study revealed that gonorrhoea and human papillomavirus were associated with prostate cancer. The OR for gonorrhoea and human papillomavirus was 1.35 (95%CI = 1.05-1.83) and 1.39 (95%CI= 1.12-2.06), respectively, whereas for any STDs it was 1.48 (95%CI= 1.26-1.73). Another meta-analysis has also reported a significantly increased risk with gonorrhoea RR= 1.20 (95% CI= 1.05-1.37) but found no association with other STDs. The positive association between gonorrhoea with prostate cancer was also confirmed in a larger meta-analysis that included 21 observational studies, especially among African-American men.	[271-273]
Sexual activity/testosterone levels	Sexual activity is thought to play a role in prostate cancer risk. A meta-analysis included 22 observational studies (21 case-control) concluded that men who had more female partners (an increment of 10) had an increased risk of prostate cancer OR= 1.10 (95%CI= 1.01-1.21), and those who were older when they had first intercourse (every 5 years delay) had a reduced risk OR=0.96 (95%CI = 0.92-0.99), while moderate ejaculation frequency (2-4 times/week) was also associated with risk reduction OR=0.91 (95%CI= 0.87-0.96). However, a previous meta-analysis found no strong association between sexual activity and prostate	[274-277]

Risk factor	Description	References
	cancer risk. In regard to testosterone levels in relation to the risk of prostate cancer, the evidence is unclear and inconclusive. One meta-analysis included 6,933 cases from 20 observational studies observed a lower risk in men with the lowest free testosterone level compared to men in the highest group, OR= 0.77 (95%CI= 0.69-0.86). Nevertheless, the authors stated that heterogeneity was present and the results could be due to detection bias. The high heterogeneity in studies that examined the association between total and free testosterone with prostate cancer was also reported by a systematic review of 124 publications.	
Vasectomy	Several studies investigated the association between vasectomy with the risk of prostate cancer, however, these studies produced mixed results. A meta-analysis of 15 cohort studies published in 2021 found that vasectomy increased the risk of prostate cancer RR= 1.09 (95%CI= 1.04-1.13) and advanced cancer RR= 1.07 (95%CI= 1.02-1.13). However, heterogeneity was present in the analysis. Furthermore, four previous meta-analyses studies found no association between vasectomy and prostate cancer risk.	[278-282]
Male pattern baldness/alopecia	A number of epidemiological studies have hypothesised the association between male pattern baldness and androgenic alopecia with prostate cancer risk. A meta-analysis of 7 case-control studies that included about 9000 participants, of whom 4078 were cases, found vertex baldness was significantly associated with increased risk of prostate cancer OR =1.25 (95%CI= 1.09-1.44), but they did not find an association between any pattern of androgenic alopecia with prostate cancer OR=1.03 (95%CI= 0.93-1.13). However,	[283-286]

Risk factor	Description	References
	since the analysis only included case-control studies, the results were prone to bias. Vertex baldness was also reported to be significantly associated with prostate cancer in a subsequent meta-analysis of 17 studies, OR 1.18 (95%CI= 1.05-1.32). The latter study has also observed a strong association between male pattern baldness and risk of aggressive prostate cancer OR=1.59 (95%CI= 1.36-1.86). Moreover, another meta-analysis comprised of 5 cohorts and 15 case-control studies showed that vertex baldness significantly increased the risk of high-grade prostate cancer OR= 1.42 (95%CI=1.02-1.99). A positive correlation between vertex baldness with prostate cancer was also reported in a different meta-analysis, but not with other types of baldness.	
Hand patterns/Right-hand digit (2D:4D)	Few studies have linked the second to fourth digit ratio (2D:4D) to prostate cancer. A small study that included 100 cases and 100 controls found that men who were diagnosed with prostate cancer had lower left 2D:4D (P=0.002) and right 2D:4D (P=0.001). Furthermore, the study did not find an association between digit ratio and aggressive prostate cancer. A cross-sectional study of 238 participants found that, compared to Caucasian cases, African-American cases were 3.70 times more likely to have low 2D:4D (95%CI= 1.98-6.92). The study also confirmed no correlation between digit ratio and Gleason score or metastasis cancer. However, one systematic review and meta-analysis that assessed the association between digit ratio and several cancers which included 9 studies related to prostate cancer found that the majority of studies reported no association between digit ratio with prostate cancer.	[287-289]

Risk factor	Description	References
Acne in adolescence	The relationship between acne in adolescence and the risk of prostate cancer has not been researched extensively and the results of existing evidence are inconclusive. One meta-analysis that included 10,145 cases from 3 cohorts and 4 case-control studies showed that in the overall analysis of combined study types, there was no significant association between acne and prostate cancer OR=1.08 (95%CI= 0.93-1.25). However, they observed a significantly increased risk in cohort studies but not in case-control studies. The OR for cohort studies was 1.51 (95%CI= 1.19-1.93), while in the case-control studies, OR was 0.98 (95%CI= 0.86-1.12). The difference in the association in the subgroup analysis by study type was due to the significant heterogeneity that is present across studies.	[290]
C-reactive protein	The majority of studies have examined the association between C-reactive protein (CRP) and survival among patients diagnosed with prostate cancer. However, one meta-analysis that investigated the association between CRP and the risk of many cancers found no association between CRP and the risk of prostate cancer HR=1.06 (95%CI= 0.96-1.16).	[291]

In recent decades, many attempts have been made to identify biomarkers that can perform better than PSA. The review here focuses on potential biomarkers that can be used in prostate cancer early detection or diagnosis.

Biomarkers for prostate cancer might be found in serum, urine, prostatic fluid, or prostate tissue. The method of use varies from detection of DNA, mRNA, protein or proliferation assays, among others [292]. An ideal biomarker is one that can be measured using simple, low-cost, less invasive tests and that has high sensitivity and specificity [293].

2.12 Prostate cancer biomarkers

The rationale for using biomarkers in cancers is its potential benefit in predicting early detection, staging, treatment response, risk stratification, or the reduction of over-diagnosis [294]. A biomarker is usually classified by its application i.e. predictive, diagnostic or prognostic. Predictive biomarkers are often used in screening to assess the likelihood of developing prostate cancer. Diagnostic biomarkers help to differentiate those who have cancerous cells from normal cells [295], while prognostic biomarkers predict the course and outcome of prostate cancer [296]. Furthermore, biomarkers can also be classified by their molecule type e.g. proteomic, epigenetic, and genetic. Also, a biomarker can be categorised according to the type of molecule or test used e.g. serum-based, urine, and tissue-based. Proteomic biomarkers are proteins associated with prostate cancer. Previous studies have demonstrated the importance of serum protein in prostate cancer stratification [297]. Epigenetic biomarkers are involved in the alteration of the genome without any modification in DNA sequence [298]. These alterations are represented in DNA methylation, histone modification,

non-coding micro-RNA, and chromatin remodelling. [299-301]. Genetic biomarkers are used to identify changes or mutations in chromosomes, proteins, or genes that are correlated with disorders [302]. Each of these types of biomarkers can be used in the screening, diagnosis, or prognosis of a disease.

A number of biomarkers have been discovered for use in the field of oncology. However, not all biomarkers are effective. Ideal biomarkers share key characteristics; they are inexpensive, convenient, reliable, easily measured, consistent, and most importantly have high specificity and sensitivity [294, 303]. Specificity and sensitivity are important for prostate cancer so as to reduce the rate of false positives and also correctly rule out those without the disease [294, 296, 299].

2.12.1 PSA levels

PSA is the most common biomarker for testing prostate cancer. The following review, therefore, describes in detail this particular biomarker relating to its chemistry, its derivatives, clinical features and its utility for use in prostate cancer.

Normal PSA level in the past was set to be below 4.0ng/ml in men aged between 50 to 80 years, indicative of no prostate cancer. However, this is no longer recognised with regard to the risk of prostate cancer [67, 304]. For example, results from the Prostate Cancer Prevention Trial (PCPT) have found choosing a PSA level of 4 ng/ml as a cut-off to recommend a biopsy is irrelevant [305]. No single PSA cut-off level has been proven to successfully stratify men with a high risk of prostate cancer and low-risk cancer [71, 76, 306]. Setting the PSA cut-off too high may increase the diagnostic specificity, but many cases of high-grade prostate cancer could be missed. Alternatively, setting the PSA cut-off too low may increase detection rates, but it decreases the specificity and might expose a large number of men without prostate

cancer to a pointless biopsy and harms associated with this unpleasant approach [67, 85, 157, 307].

Moreover, there is no low PSA level in which prostate cancer cannot be detected at biopsy among men above 60 years [65, 69, 88, 308]. Several studies revealed that a minimal increase in PSA level above the median is associated with increased prostate cancer risk [72, 309-311]. Another study found that even a PSA level below the median (0.7 ng/ml) increased the risk of prostate cancer by 6.6%, and lowering the PSA cut-off could benefit many men at risk of prostate cancer in the long term [76]. Therefore, the PSA level at which a biopsy is recommended is controversial [66, 67, 89, 312]. For instance, the first threshold indicated in the early 1990s was 4 ng/ml by Catalona and his colleagues [304]. The specificity of PSA at this level is estimated between 60-70% and only a quarter of detected cases are positive on biopsy [313]. However, they later suggested 2.5 ng/ml [314]. After that, Schröder et al. from the ERSPC determined that a cut-off of 3.0ng/ml was more ideal [315]. Their study showed that only 23% of detected cancers had been confirmed on biopsy using a PSA cut-off of 3 ng/ml [95]. Furthermore, different organisations use different cut-offs. The American Cancer Society recommends men with PSA > 4ng/ml do a biopsy for more evaluation [113]. Whereas various other organisations in the United States and the world applied a PSA range of 2.5-3.0 ng/ml as a cut-off to perform a biopsy [69]. A threshold of 3.0 ng/ml was also used in the Prostate Testing for Cancer and Treatment (ProtecT) trial in the UK, and among men who performed a biopsy, only 39% were confirmed to have prostate cancer [316, 317]. In general, the lowest acceptable PSA threshold is 2.5 ng/ml, where the approximate risk of prostate cancer is 24% at this level [308].

Nevertheless, increasing PSA levels is a powerful biomarker that can predict future risk of prostate cancer diagnosis and mortality [91, 318-320]. Carlsson et al. estimated that 90% of mortality was found to be among men with PSA levels >2 ng/ml (the top 25%) [90]. Similarly, Vickers et al. found 95% in men with >1 ng/ml and 66% in men with >3.4 (the top 10%) [159]. Moreover, prostate-specific mortality was found to be extremely rare in men with PSA levels less than 1 ng/ml, with an estimation between 0.04% to 0.2% [311, 321]. Accordingly, emerging evidence found that men at age 60 years with PSA levels < 1ng/ml, which constitute almost half of the population, can be exempted to do further screening. Although they might harbour prostate cancer which is deemed as indolent prostate cancer hence unlikely to affect their life quality or become life-threatening. Therefore, screening should be restricted to men at 60 years or older with PSA >1 ng/ml [90, 159, 160]. Those with PSA 1-3 ng/ml are at intermediate risk and could do another screening every 1-4 years [156]. In comparison, those with modestly elevated PSA (4-10 ng/ml) should repeat the PSA and combine it with other tests like free-to-total ratio [322], as it has been found that 60-70% of men in this range do not have prostate cancer and therefore underwent unnecessary biopsy [65, 74]. The current threshold recommended in the UK is a PSA level of 3 ng/ml or higher for men aged 50-69 years who are suspected of prostate cancer and advised for further investigations by a specialist [323].

2.12.2 PSA test intervals

One way to enhance the risk prediction of prostate cancer is by repeating the PSA test [89]. This is mainly based on the fact that PSA levels can change over time due to several factors, such as BPH which increases PSA in men above 50 [157], and that elevating PSA levels over a short time is not necessarily an indication of high risk as it

is with stable PSA [88]. Although there is evidence that is repeating PSA tests improves the accuracy of risk prediction and should be recommended before performing biopsy directly [89, 324], however, there are some disagreements about tailoring it according to initial PSA level and age [83].

The European Association of Urology advised taking a second PSA test within a few weeks before deciding on a biopsy [56]. Likewise, participants in the Stockholm study who had a PSA value between 3-10 ng/ml performed a second test after eight weeks prior to the biopsy [88, 325]. Other organisations and research communities recommend annual screening. The American Cancer Society (ACS) suggests that men with PSA > 2.5 ng/ml should be screened every year and those with PSA value below that do it biennially [113]. Similar recommendations were found in various guidelines [326, 327]. Previously, Etzioni and associates found that biennial screening could reduce the rates of false-positive outcomes and screen frequency up to 50% [328]. However, more recently, many studies reported that screening annually or biennial might reduce the incidence rate of advanced prostate cancer in men over 50 years and could reduce prostate cancer mortality by almost 30%, but it is also reducing quality-adjusted life-year (QALYs) by 23% due to over diagnosis and long-term harm from treatment [329, 330]. Moreover, results from randomised controlled trials show that screening annually is unlikely to improve survival benefit compared to screening biennially [83].

On the other hand, Cuzick et al. also found extending the PSA test interval to 2-4 years, as reported in the ERSPC trial, may substantially reduce the adverse effects of overdiagnosis without a significant effect on survival rates [84, 156]. Several modelling studies demonstrated that men who have a PSA value less than 1 ng/ml could be

rescreened at three-year intervals [331] or even eight-year intervals, according to the European Association of Urology [56, 79]. Supporting results came from the Rotterdam arm of the ERSPC, where they found only 3% of men with a PSA baseline of less than 1 ng/ml increased to PSA greater than 3 ng/ml within eight years of follow-up [332].

2.12.3 PSA velocity

Other efforts to enhance the specificity and accuracy of the PSA test include PSA velocity, which measures the speed of alteration in PSA level over time [65, 66]. Many studies have demonstrated that a rapid increase in PSA velocity in men puts them at a high risk of developing prostate cancer compared to those with relatively low PSA velocity [333-335]. Moreover, Carter et al. have reported that PSA velocity can predict the risk of prostate cancer within a decade before cancer diagnosis [336]. However, most of these studies had weaknesses [337]. In fact, more recent studies have shown that PSA velocity has a very low predictive value [338], and no significant evidence shows that using PSA velocity has contributed to reducing unnecessary biopsies or improving early detection [66, 116]. Hence, applying this strategy for population-based screening appears to be of limited value [318, 338].

2.12.4 Free PSA

PSA can be found in the serum in two forms; bounded to other proteins or unbounded (Free PSA “fPSA”). The free PSA test measures unbound PSA as a percentage (%fPSA) [339]. It was approved by the Food and Drug Administration (FDA) in the late 1990s to aid the detection of prostate cancer among men with PSA levels of 4-10 ng/ml [69]. fPSA is found in the blood in an approximate ratio of 10-30% [65] and is associated with prostate malignancy more than total PSA [340]. Although %fPSA as

PSA has no optimal cut-off, instead, it is an accumulative risk [65]. The lower ratio in men implies a greater probability that elevated PSA between 3-10 ng/ml is caused by cancer rather than BPH [341, 342].

Several studies have shown that utilising free PSA ratios improve the accuracy and specificity of prostate cancer detection [157, 322, 343-345], particularly in the grey area between 4-10 ng/ml [343, 346], and could reduce 25-40% of unnecessary biopsies in men at that range [322, 344, 347]. For example, in a large study, Catalona et al. showed that 56% of men with a free-to-total ratio below 10% had been diagnosed with prostate cancer, compared with 8% of men with a ratio of 25% [348]. This promising outcome along with being adequately evaluated, justified its clinical use [157] and has encouraged several organisations to recommend using %fPSA in their guidelines to distinguish BPH from prostate cancer [65, 349].

2.12.5 Intact PSA

Intact PSA is a subfraction of free PSA that has been associated with prostate cancer. There is no difference in intact PSA levels between men with or without cancer. Nonetheless, previous studies showed that intact PSA is useful in discriminating benign from malignant cases, especially if it is used as a ratio of intact-to-free PSA [350, 351]. Intact PSA is often incorporated into a panel or multi-parametric model with other measurements. For instance, Vickers et al. investigated the role of different Kallikrein assays, including intact PSA, and found that intact PSA only has value if it is included in the model with free PSA [352]. However, unlike PSA and free PSA, intact PSA requires a more sophisticated assay [353, 354]. Therefore, the clinical value of any panel that includes intact PSA is still debatable if such data cannot be readily available to urologists [355, 356].

2.12.6 Complexed PSA

The complexed PSA (cPSA) assay detects an isoform of PSA that is bound to other proteins. This provides a promising approach which increases the specificity in screening for prostate cancer [357, 358]. In a multi-centre cohort study that included 831 men, Wolfgang and colleagues evaluated the clinical value of cPSA for early detection of prostate cancer in men with a PSA level between 2-4 ng/ml in comparison to other PSA derivate markers. They found that the AUC of cPSA was statistically significantly greater than for PSA (0.64 compared to 0.57; $p < 0.0001$). Also, by using a cut-off point of 2.1 ng/ml for cPSA and 2.5 mg/ml for PSA, their specificity was 34.2% and 20.3%, respectively, with a sensitivity of 86% [359]. Moreover, the use of cPSA may reduce the number of unnecessary biopsies in men with normal PSA levels [360]. Similar results have been reported in another study as well in men with PSA levels of 4-10 ng/ml (the grey area) [361]. Another feature of cPSA is that it has better stability than PSA, which is important for storing it in clinical laboratories for short or long periods [362, 363].

In contrast, other studies reported that cPSA did not have clinical benefit or advantage over PSA in discriminating patients with prostate cancer from those with a benign disease of the prostate [364, 365]. Although cPSA as a single test provided better specificity in PSA ranges between 2-10 ng/ml compared to PSA [366], the clinical value of cPSA in higher PSA levels remains unclear.

2.12.7 PSA density

PSA density (PSAD) is calculated as a ratio between the value of PSA and the prostate volume and was first introduced in 1992 as a method of increasing PSA testing accuracy

[367]. The suggested cut-off point for PSAD is 0.15 ng/ml/cc [367]. Castro et al. utilised this cut-off to evaluate the contribution of PSAD in predicting prostate cancer in 1,282 men with PSA levels between 2.6 and 10 ng/ml; the AUC was 0.72 with specificity and sensitivity of 74% and 70, respectively. They also found that by using PSAD, the percentage of unnecessary biopsies that could be reduced was less than 30% [368]. However, Catalona et al. in a multi-centre study comprised of 773 patients, recommended that the PSAD cut-off should be lowered to 0.078 ng/ml/cc. At this value, they found that 95% of cancer would be detected [348]. Another application of PSAD is that it can be used to determine the aggressiveness of prostate cancer [369]. Nevertheless, PSAD at best offered comparable results to %fPSA, and therefore, it can be replaced by %fPSA for biopsy decisions and in prediction models since it does not require ultrasound as with PSAD [348].

2.12.8 Precursor forms of PSA

PSA contain 17 amino acid when it is first produced, then these amino acid produce an inactive precursor enzyme (proPSA) with seven amino acid pro-leader peptide after it is cleaved by human kallikrein 2 (hK2) [370, 371]. Unlike other PSA derivatives, proPSA is expressed mostly in the peripheral zone of the prostate gland, an area where the cancer often occurs [372]. proPSA could be found in different versions according to the truncated leader sequences of the amino acid it contains, i.e. [-4, -5, -7] proPSA [373, 374]. However, it has been shown that proPSA is primarily consisting of a truncated form of proPSA that contains only two pro-leader peptides [-2]proPSA instead of the common seven peptides [-7]proPSA [375].

Precursor forms of PSA, especially [-2]proPSA, have emerged as a marker for early detection and management of prostate cancer. Several studies have reported that

proPSA is increased in patients with prostate cancer [372, 376]. Other studies have demonstrated that the ratio of proPSA to free PSA (%proPSA) performed better than PSA and %fPSA in detecting prostate cancer in selected patients with PSA levels between 2-10 ng/ml [377, 378]. Moreover, the [-2]proPSA is part of the Beckman Coulter Prostate Health Index (phi) [379, 380], which showed that it outperformed both PSA and %fPSA in detecting prostate cancer [380]. Furthermore, both proPSA and %[-2]proPSA have been associated with the aggressiveness of prostate cancer on biopsy [381, 382]. However, in a prospective multi-centre study, Sokoll et al. found that %[-2]proPSA has a comparative prediction ability to PSA and %fPSA in detecting the cancer when it is used in an entire study population where PSA levels ranged from 0.29 to 310.6 ng/ml. However, it performed better in subgroup patients with PSA levels of 2-4 ng/ml [382]. Also, [-2]proPSA produced promising results in detecting prostate cancer within the grey zone of 2-10 ng/ml, and it is even enhanced if it is incorporated into mathematical models [382, 383].

2.12.9 Prostate-specific membrane antigen

Prostate-specific membrane antigen (PSMA) is a protein with enzymatic functions. It is overexpressed on prostate tumour cells and may play a role in tumour progression. Its expression has also been found to be correlated with tumour grade and advanced stage of cancer [384]. Bostwick et al. reported 82% positive rates in the primary tumour of the prostate [385]. PSMA is generally detected in prostate tissue, circulating cancer cells, and serum [386], and its level increases with age [296]. Moreover, evidence suggests that PSMA expression does not overlap between BPH and prostate cancer [387]. Another study by Xiao et al. found that serum levels of PSMA in men with prostate cancer are significantly higher than those with BPH or a normal prostate [388].

However, the results of serum PSMA in relation to the detection or aggressiveness of the disease are inconclusive and the evidence is not concrete [296, 384].

More recently, PSMA has been assessed as a urinary biomarker for prostate cancer. Using quantitative polymerase chain reaction (PCR) analysis, Rigau et al. evaluated PSMA, among other urine markers, for detecting prostate cancer in 154 urine samples measured after prostate massage in men with PSA levels between 4-10ng/ml. Their results revealed the highest sensitivity for PSMA (64%) compared to prostate cancer antigen 3 (PCA3) (46%) at 70% specificity [389].

However, PSMA is not prostate-specific. It is also found in different types of tumours such as colon, renal, and bladder. Hence, using it as a marker to differentiate prostate diseases is not encouraging [390]. Further, the immunoassay for PSMA lacks sensitivity, which has reduced its clinical utility in diagnosing prostate cancer [391]. As a result, PSMA is still not fully recognised as an effective marker for prostate cancer [392, 393].

2.12.10 Transforming growth factor-beta

Transforming growth factors beta (TGFβ) are cytokines that play an important role in the regulation of cellular proliferation, differentiation and immune response [394-396]. TGFβ is shown to have a dual role in prostate tumour development. In the early stages of tumour development, it suppresses tumour growth by inhibiting proliferation and inducing apoptosis, whereas in the late stages, it promotes tumour progression, invasion and metastasis [396-398].

There are three isoforms of TGFβ; TGFβ1, TGFβ2 and TGFβ3 [399]. All of these isoforms are expressed in prostate cells; TGFβ1 is expressed at higher levels than the others [400] and is involved in prostate cancer tumorigenesis [397, 401]. Also, higher

levels of serum TGFβ1 are found to be associated with more aggressive and metastatic cancers [396, 402]. Nonetheless, TGFβ1 does not have the ability to differentiate between men who have cancer from those who are healthy [403]. Moreover, there is a need to validate TGFβ in general prior to it being regarded as a prostate cancer biomarker [402].

2.12.11 Alpha-methylacyl-CoA racemase

Alpha-methylacyl-CoA racemase (AMACR) is an enzyme that is involved in fat metabolism [303]. AMACR has been found to be consistently overexpressed in prostate cancer epithelium and has shown a promising result in distinguishing prostate cancer cells from benign and healthy ones, making it a specific biomarker for prostate cancer [314, 404]. The overexpression was found in approximately 80% of prostate cancers after biopsy [405, 406]. A previous study reported that AMACR has been detected in 137 patients with prostate cancer and achieved 100% accuracy [407]. Another small study also reported a 100% accuracy when using a cut-off of AMACR between 0.08 and 0.9 [408]. AMACR was initially used as a tissue marker and analysed by immunohistochemistry to diagnose prostate cancer. The high sensitivity of AMACR in cancer tissue has led scientists to investigate its potential use of AMACR as a serum and urine marker for diagnosing prostate cancer using reverse transcription-polymerase chain reaction (RT-PCR) [409]. Rogers et al. evaluated the accuracy of urine AMACR to detect prostate cancer in men who underwent prostate biopsy and reported 100% sensitivity and 58% specificity [410]. A similar study has reported an 87.5% sensitivity and 100% specificity [411]. A meta-analysis study included 22 studies and comprised 4,385 patients reported a significant correlation and increased diagnosis of prostate cancer by immunohistochemistry AMACR with OR of 76 ($P<0.00001$), whereas

AMACR by PCR was associated with an increased risk of prostate cancer with OR of 33.60 (P<0.00001) [412]. However, the results of this study were not adjusted to other variables that might confound such results. Moreover, AMACR was detectable in urine after patient underwent prostate biopsy, which might increase the proteins produced by the prostate gland. The variation of AMACR assays also raises a question regarding its clinical utility as a biomarker [408, 413]. Furthermore, AMACR is not only specific to prostate cancer and can be found in other malignancies [414]. Its usefulness in prostate cancer screening and diagnosis depends on the method of detection and analysis in clinical practice. Therefore, future prospective studies are needed to validate the current method and use of AMACR as a biomarker and screening tool for prostate cancer.

2.12.12 Prostate specific G protein coupled receptor

Prostate specific G protein coupled receptor (PSGR) has been found to have restricted expression in prostate epithelial cells [415]. That expression also has been reported to be increased in prostate cancer [416], suggesting that PSGR may also be involved in primary prostate cancer and progression. Although its role in cancer progression is still not clear, it might have the potential to act as a detection biomarker for prostate cancer [402]. PSGR has been analysed and evaluated as both a tissue marker [417] and a urine marker for prostate cancer [418]. Rigau et al. used urine samples after a prostate massage from 215 patients and found that PSGR is a significant predictor of prostate cancer with an AUC of (0.681) compared to PSA (0.602) and PCA3(0.656). However, at 95% sensitivity, the specificity for PSGR was 15% and 17% for PCA3 [418]. The same author in another study analysed samples from 154 patients with elevated PSA (>4ng/ml) and/or abnormal DRE. They reported that at 70% specificity, the sensitivity of PSGR was 61%, whereas it was 46% and 64% for PCA3 and PSMA, respectively.

Also, the AUC for PSGR was 0.65 and for PSMA and PCA3 was 0.62 and 0.60, respectively [389].

2.12.13 Insulin-like growth factors

Insulin-like growth factors (IGF-I, IGF-II) and binding proteins (IGFBP1-6) are the major molecules of the IGF system that are involved in cellular metabolism, proliferation, differentiation and apoptosis of both normal development and neoplastic cell growth [419]. IGF-I, in particular, has a key role in the stimulation of normal and malignant cell growth [420], with IGFBP-3 being the main binding protein that can reduce the effects of IGF-I in stimulating growth as well as suppress cell proliferation in prostate cancer [421, 422].

Several studies have linked IGF-I and IGFBP-3 with increased risk of prostate cancer. A Swedish case-control study comprised of 210 patients [423] found that the mean serum of IGF-I level was significantly higher in cases than in controls (158.4 ng/ml VS 147.4 ng/ml, p= 0.02), with OR of 1.51 (95% CI = 1.0-2.26 per 100 ng/ml increment), suggesting a moderately strong positive association with risk of prostate cancer (P=0.04). However, the author did not find an association between IGFBP-3 and with increased risk of prostate cancer [423]. Another study used a case-control nested approach from the European Prospective Investigation into Cancer and Nutrition (EPIC), which reported that high levels of IGF-I were positively associated with the risk of prostate cancer with an OR between highest versus lowest quartile of 1.69 (95% CI = 1.35-2.13; P_{trend} = 0.0002) [424]. Moreover, a prospective study showed increases in prostate cancer risk with IGFBP-3 but not with IGFBP-1, IGFBP-2, or insulin [425]. A meta-analysis consisting of 14 case-control studies confirmed similar results [426].

Notably, a study in Japan found no association between serum levels of IGF-I, IGF-II, or IGFBP-3 and with risk of prostate cancer [427]. Furthermore, Mehta et al. [428] reported an inverse correlation between IGFBP-3 and an increased risk of aggressive and metastatic prostate cancer. Similar findings have been reported previously by another prospective study [429]. Therefore, IGFs values did not improve significantly compared with PSA performance in detecting prostate cancer [403]. Further research is needed to investigate the relationship between ethnicity and IGFs levels and their role in prostate cancer.

2.12.14 Early prostate cancer antigen

Early prostate cancer antigen (EPCA) is a nuclear matrix protein that was discovered in 2004 and was found to be expressed in prostate tissue of prostate cancer patients but not in those without cancer [430]. EPCA was rarely identified in benign glands nor in BPH samples of organ donors, with a sensitivity of 84% and a specificity of 85% [430, 431]. This encouraged scientists to further investigate EPCA as a specific biomarker for use in the diagnosis of prostate cancer. Paul et al. used enzyme linked immunosorbent assays (ELISA) to assess plasma samples obtained from 12 men with prostate cancer, 16 healthy donors, and 18 patients with other benign and malignant conditions. The authors found that plasma levels of EPCA were significantly higher in prostate cancer patients compared to other groups. They also reported a sensitivity of 92% for this immunoassay, with a specificity of 100% for healthy donors and 94% for the entire control groups, at a cut-off of 1.7 [432]. Another study found that high levels of serum EPCA were positively correlated with Gleason scores and advanced-stage cancers [433].

EPCA-2 is a subtype of a nuclear protein that was identified and this has also been found at elevated levels in sera of patients with prostate cancer, but not in healthy men. It has been reported that EPCA-2 can accurately differentiate between localised and non-localised diseases with high levels of sensitivity and specificity [434]. However, this study was later retracted from publication following criticism for representing a promising but false discovery. It is apparent that such studies used a relatively small population to analyse EPCA utility. Analysis using much larger and prospective studies is warranted to evaluate this biomarker for its clinical utility in prostate cancer.

2.13 Epigenetic biomarkers

Epigenetics is the study of heritable changes that affect gene expression and chromatin without altering DNA nucleotide sequences [435, 436]. Epigenetic modifications, including histone modification and DNA methylation, are often established during early development and are maintained during cell division [437]. Normal DNA methylation is important for the function of healthy cells [438, 439]. In cancer, in addition to genetic changes, epigenetic patterns are also altered and disrupted. For instance, there is simultaneous genome-wide hypo-methylation and hyper-methylation of promoter regions that lead to alterations in gene expression. Specifically, DNA hyper-methylation of promoter regions is associated with silenced genes, including the silencing of tumour suppressor genes [440]. In contrast, gene promoter hypo-methylation is associated with the activation of genes, including the activation of oncogenes [437]. Altered gene expression of tumour suppressor genes and oncogenes is present in all tumours [441], which increases cell proliferation, degradation, motility and tumour growth.

Recently, studies have investigated the use of DNA methylation patterns as a source of biomarkers in individual cancer patients and assessed their utility as markers for early detection and diagnosis, monitoring tumour progression, and targeting therapy [442]. Epigenetic changes, including hyper-methylation of promoter regions of tumour suppressor genes, occur early in tumour growth [443-445]. In addition to the analysis of tumour tissue DNA, many tumours shed cancer cells and cancer DNA into the bloodstream and other bodily fluids including urine [446, 447]. Body fluids, therefore, provide a non-invasive source of cancer DNA that could be potentially used for the early detection of cancer by assessing aberrant DNA methylation biomarkers [448]. In prostate cancer, DNA hyper-methylation is the most common epigenetic modification [409]. Table 2.3 below summarises the most common epigenetic biomarkers that have been investigated for the detection, diagnosis, and prognosis of prostate cancer.

Table 2.3: Epigenetic biomarkers for prostate cancer.

Biomarker	Description	References
GSTP1	It is the most common gene alteration in prostate cancer. Methylation of GSTP1 in urine was found to have 75% sensitivity and 98% specificity. Another study reported a detection rate of 50%. A meta-analysis study found that the pooled specificity of GSTP1 methylation in serum, plasma, and urine is higher than PSA but not sensitivity.	[449-451]
RASSF1A	A tumour suppressor gene and hyper-methylation of this gene is frequent in many solid tumours. A meta-analysis study shows an odds ratio (OR) of 14.73 (95% CI 7.58-28.61) in prostate cancer cases compared to control. Also, it is associated with the Gleason score with an OR of 2.35 (95% CI 1.56-3.53). The pooled specificity was %87, and sensitivity was %76.	[452]
PDLIM4	A tumour suppressor gene that is found to be down-regulated in prostate cancer tissues with a specificity of %90.5 and sensitivity of %94.7. Hyper-methylation of this gene may be useful in detecting prostate tumorigenesis.	[453, 454]
DLC1	A tumour suppressor gene in many cancers. Inactivation of this gene increases in prostates of older men.	[455, 456]
PcG proteins	Complex proteins that involved in regulating developmental and physiological processes in the cells. EZH2, a polycomb protein, is overexpressed in prostate cancer. Modification in PcG could serve as a marker for aggressive prostate cancer.	[457-460]
ASC/ TMS1 (PYCARD)	Gene that regulates immune response and apoptosis. Hyper-methylation of PYCARD	[461-463]

Biomarker	Description	References
	is frequent in prostate cancer. One study found methylation of ASC is present in 65% of prostate cancer tissue.	
EPB41L3	A cortical cytoskeleton gene. Hyper-methylation of this gene is found in 70% of prostate cancer cases.	[456]
CDKN1C	Potential tumour suppressor in several human cancers. Hyper-methylation of this gene leads to the inactivation of the promoter region in prostate cancer.	[456, 464]
JMJD3	Histone demethylase that is associated with the prognosis of many cancers. It is upregulated in prostate cancer.	[456, 465, 466]
HDAC1	Histone deacetylase 1 is involved in cell development and proliferation control. It is found to be overexpressed in prostate cancer higher than other prostate conditions and contributes to poor prognosis in prostate cancer.	[467-470]
RNASEL	A candidate gene for hereditary prostate cancer (HPC1).	[471]
H3K4 / H3K18	The tri-methylation of histone 3 at lysine 4 (H3K4) and at lysine 18 (H3K18) is found to be correlated with a poor prognosis of prostate cancer.	[456, 472]
IGF2	Methylation of this gene is accompanied by loss of imprinting and reduces IGF2 expression in prostate cancer. This alteration in IGF2 regulation is increased in the ageing prostate.	[456, 473, 474]

2.14 Genetic biomarkers

Multiple mutations in genes such as tumour-suppressor genes or oncogenes result in the development and progression of cancer by affecting cell growth and differentiation [475]. It is unlikely that a single genetic variation could initiate the progression of prostate cancer. Instead, it is thought to be an accumulation of mutations to different degrees [303]. Genetic mutations could be somatic, where they occur in tumour tissue, or heritable when passed through the germline DNA of parents [475]. Activation of cancer-signalling pathways due to either silencing of tumour-suppressor genes and/or activating oncogenes is accompanied by secretion of specific protein signatures that could be measured in bodily fluids and used in detecting cancer at an early stage and grading of the tumour [476]. Moreover, in molecular biomarkers, genetic markers stand out for their accessibility at any age and because they do not fluctuate over time [477]. Consequently, genetics biomarkers are considered to provide information about the aetiology of the disease and thus play a key role in clinical oncology [391, 476]. Over the last two decades, several genetic mutations occurring during prostate cancer development and progression have been reported. However, it is still debated which precise genes on each chromosome are associated with prostate cancer [303].

2.14.1 TMPRSS2-ERG gene Fusion

The fusion of the transmembrane protease serine 2 (TMPRSS2) gene with the oncogene homolog (ERG) gene, referred to as TMPRSS2:ERG, has been linked to prostate cancer development. The protein product of this fusion cannot be detected in serum, although it can be measured using urine samples after DRE [478]. One study found that TMPRSS2:ERG expression is detected in 42% of patients [479]. Another study

reported overexpression of TMPRSS2:ERG in more than 50% of screened prostate cancers [478]. Evidence shows that TMPRSS2:ERG fusion protein in prostate cancer pathogenesis occurs early and frequently and can be indicative of cancer aggressiveness [480].

TMPRSS2:ERG, as a single marker has a low sensitivity of approximately ~37% [481], however, it has a high specificity that is over 87%. The discrimination value of TMPRSS2:ERG (AUC) is 0.77. This is better than PSA alone 0.72 and PCA3 (another biomarker) 0.65 [482]. Using TMPRSS2:ERG along with PCA3 and serum PSA in an algorithm can enhance prostate cancer prediction significantly [482].

2.14.2 PCA3

Prostate cancer antigen 3 (PCA3) is a prostate-specific non-coding mRNA and has been found to be highly over-expressed in more than 95% of primary prostate cancer and upregulated 66 times more than surrounding normal tissues [483]. Furthermore, the expression of PCA3 is almost limited to the prostate[484]. A PCA3 test can be performed on urine, and it was evaluated in 108 men who underwent biopsy based on a PSA level of >3ng/ml. Using prostate biopsy as being indicative of the presence of a tumour, this test had 67% sensitivity, 83% specificity, and a negative predictive value of 90% [485]. The high specificity of PCA3 indicates that, unlike PSA, this biomarker can differentiate between prostate cancer and other benign conditions. Although several studies have reported a correlation between PCA3 level and prostate cancer aggressiveness [486, 487], other studies found no correlation [483, 488-491]. The PCA3 test achieved European compliance in 2006 and gained FDA approval for clinical use in 2012 [492].

2.14.3 GOLPH2

Golgi phosphoprotein 2 (GOLPH2) is a gene that encodes for GOLPH2/GP73, a type II Golgi membrane antigen. The gene is normally expressed in different epithelial cells. A higher level of GOLPH2 was found in prostate cancer cells, which indicates using GOLPH2 as a biomarker could aid in distinguishing between normal cells and cancer cells [493]. In comparison with normal tissue, GOLPH2 antigen expression was found to be significantly higher in prostate cancer tissues, and it was upregulated in 90% of cases [494]. Another study examined GOLPH2, along with other markers, and found that higher levels of GOLPH2 can predict prostate cancer. Furthermore, it was better than PSA or PCA3 alone [495]. GOLPH2 can be analysed in tissue using microarrays [496], and it can be assayed in urine [303].

2.14.4 P53 gene

The p53 gene is a tumour suppressor gene which has an important role in the cell's stress response. Evidence shows that the p53 gene is mutated in 50% of all human cancers [497, 498]. Kluth et al. analysed p53 alteration using 11,152 prostate tissues collected from patients after surgery. They found high levels of p53 in 77% of cases (17 of 22). They also found the tp53 deletion in almost 15% of tumours [499]. Similarly, Verma et al. reported an expression of p53 in 76% of cases (38 of 50), whereas in BPH it was expressed only in 20% (2 of 10). They also found that p53 is strongly correlated with the Gleason score [500].

2.14.5 PIM1

PIM1 is an oncogene of serine/threonine kinase that is involved in cell cycle progression and apoptosis. It is implicated in various human cancers, including prostate

cancer, where its expression is dysregulated [501-504]. In a previous study, PIM1 overexpression was found in ~50% of prostate cancers [505]. Another study revealed an association between PIM1 expression and tumour grade. They found higher levels of PIM1 expression in prostate cancer with a Gleason score of 7 or above (76%), compared to lower Gleason score tumours where it was 58%. They also found in their study that expression of PIM1 in high-grade prostatic intraepithelial neoplasia could be an early sign of prostate cancer development [503]. More recently, a study has reported a correlation between overexpression of PIM1 with higher Gleason grades [506].

2.14.6 Hepsin

Hepsin is a gene that encodes a type II trans-membrane serine protease and is involved in cell migration and invasion [507-509]. Hepsin has been considered to be one of the most upregulated genes, which results in its over-expression in prostate cancer tissue. It has been found to be over-expressed and much higher in prostate tumours compared to adjacent non-cancerous prostate tissues [510]. Goel et al. reported a 100% expression of Hepsin in prostate cancer, compared to ~12% in BPH. They also found, in high-grade cancers, that Hepsin is highly expressed compared to low-grade cancers [507]. Similar results have been reported in another study [511]. Other studies have reported an over-expression of Hepsin in up to 90% of prostate cancer tissues and its correlation with cancer aggressiveness [512, 513].

2.14.7 NKX3A

NKX3A is a prostate-specific gene that encodes a transcription factor and it is almost exclusively expressed in the prostate [514, 515]. It is expressed in the adult prostate at

a higher level, but its expression in prostate tumour cells is decreased. Deletion or losses in a region containing NKX3A is frequent in prostate cancer [515].

2.14.8 PTEN

This tumour suppressor gene is implicated in many tumours. Mutation or deletion of PTEN results in rapid cell growth and cell division. The frequency of PTEN mutations is higher in metastases prostate cancer than in localised cancer, indicating its association is with higher tumour grade and cancer progression [514-516].

2.15 Genetic predisposition

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation among people [517]. They are located in either coding sequences of genes or non-coding regions of genes. However, the most common variations are found in the DNA between genes. There are around 10 million SNPs in the human genome, which account for many of the genetic differences between individuals [518, 519]. While most SNPs do not have an effect on health or physical appearance, some of these SNPs can affect disease development and determine the response to drug treatment [520]. When a SNP occurs inside a gene or close to it, it may have an important role in developing diseases if it impacts gene functionality or the transcription and translation processes which ultimately lead to producing different proteins that function atypically. In such cases, it could be considered as being a biological marker associated with the disease.

Genome-wide association studies (GWAS) are a strategy used to search in an *a priori* way for genetic variants and investigate their association with particular diseases [521, 522]. For prostate cancer, the first GWAS was carried out in 2006 and this identified associated SNPs on chromosome 8q244 [523]. In 2008, Eeles et al. conducted a GWAS

and analysed 541,129 SNPs, where they identified seven variants associated with prostate cancer [524]. Subsequent GWAS, in collaboration with the PRACTICAL consortium, used a blood DNA sample from around 25,000 cases and 24,000 controls and identified 23 new prostate cancer susceptibility loci. In the same study, they identified 16 SNPs that correlated with aggressive prostate cancer. However, these SNPs were also correlated with non-aggressive prostate cancer [525] and thus would not be of clinical value in identifying severe cases of prostate cancer. Currently, more than 40 GWAS for prostate cancer has been conducted, with around 170 variants identified [526]. Each of these SNPs conveys a small risk; however, when used in aggregation as a composite score, it can be a powerful tool to identify individuals with genetic predisposition risk. These SNPs are often found in sporadic cases, which encompass about 90% of prostate cancer cases. Therefore, genetic predisposition has potential to be used to stratify men and identify those requiring further investigation. Recent studies demonstrate that using genetic biomarkers in risk stratification is promising approach and is effective for multi-ethnic groups. Studies have revealed that the polygenic hazard ratio score for prostate cancer is 5.54, 4.49, and 2.54 for European, Asian, and African men, respectively [527]. The evidence regarding most biomarkers and risk factors related to prostate cancer is still not definitive.

In this chapter, I discussed and provided a detailed overview of the characteristics and epidemiology of prostate cancer. Also, I investigated several risk factors and biomarkers associated with the disease and highlighted the contradicting results between those studies. Some of these risk factors are not modifiable such as age and family history, while others are potentially modifiable such as obesity and smoking. Those modifiable risk factors are generally easy to measure and obtain, making them

one of the go-to variables to be included in a risk stratification model for any disease. Hence, further studying those modifiable factors to prevent their effect and reduce prostate cancer risk is crucial.

In the next chapter, I investigated the relationship between self-reported obesity as an indicative of body size and shape with risk of prostate cancer. Although the principal aim of my thesis was to investigate available risk prediction models for prostate cancer and to develop a new model for use in the community, during my first year, I did an extensive literature review on prostate cancer risk factors. It was clear that further to age, family history and ethnicity, no other concrete risk factors have been documented. Our research group has been conducting the largest case-control study in the UK therefore this provided an opportunity to explore a suggested aetiological factor that could potentially be incorporated into the model. The findings could potentially expand the number of readily available factors to include in the prediction model and could also have an application in prostate cancer prevention. The work in the next chapter also provided me with an opportunity to use a wider range of analysing epidemiological data.

Chapter 3 Relationship of self-reported body size and shape with risk for prostate cancer: a UK case-control study

The work within this Chapter is published in the PLOS ONE Journal (Appendix D)

Relationship of self-reported body size and shape with risk for prostate cancer: A UK case-control study

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Abstract

Introduction

Previous evidence has suggested a relationship between male self-reported body size and the risk of developing prostate cancer. In this UK-wide case-control study, we have explored the possible association of prostate cancer risk with male self-reported body size. We also investigated body shape as a surrogate marker for fat deposition around the body. As obesity and excessive adiposity have been linked with increased risk for developing a number of different cancers, further investigation of self-reported body size and shape and their potential relationship with prostate cancer was considered to be appropriate.

Objective

The study objective was to investigate whether underlying associations exist between prostate cancer risk and male self-reported body size and shape.

Methods

Data were collected from a large case-control study of men (1928 cases and 2043 controls) using self-administered questionnaires. Data from self-reported pictograms of perceived body size relating to three decades of life (20's, 30's and 40's) were recorded and analysed, including the pattern of change. The associations of self-identified body shape with prostate cancer risk were also explored.

Results

Self-reported body size for men in their 20's, 30's and 40's did not appear to be associated with prostate cancer risk. More than half of the subjects reported an increase in self-reported body size throughout these three decades of life. Furthermore, no association was observed between self-

reported body size changes and prostate cancer risk. Using a ‘symmetrical’ body shape as a reference group, subjects with an ‘apple’ shape showed a significant 27% reduction in risk (Odds ratio = 0.73, 95% C.I. 0.57-0.92).

Conclusions

Change in self-reported body size throughout early to mid-adulthood in males is not a significant risk factor for the development of prostate cancer. Body shape indicative of body fat distribution suggested that an ‘apple’ body shape was protective and inversely associated with prostate cancer risk when compared with a ‘symmetrical’ shape. Further studies which investigate prostate cancer risk and possible relationships with genetic factors known to influence body shape may shed further light on any underlying associations.

3.1 Introduction

Prostate cancer is the most prevalent cancer in men [528]. It is also the third most common cancer-specific cause of death for men living in Europe [529, 530]. In 2016, it accounted for approximately one quarter of all cancers diagnosed in men within the United Kingdom (UK) [531]. Apart from the established cancer risk factors, such as age, ethnicity and family history of prostate cancer in first-degree relatives, other potential risk factors include height, obesity/high body mass index (BMI) and levels of insulin-like growth factor-I [532-534].

Over the last few decades, obesity has increased by approximately 30% in European men [535, 536]. This has been linked to an increased risk of developing several chronic diseases and cancers [537]. Extensive studies have investigated the association of both obesity and body size with prostate cancer risk. However, this relationship remains inconclusive [538-542]. Anthropometrics that have been used to measure obesity and body adiposity include waist circumference, waist-hip ratio and BMI [543]. The majority of epidemiologic studies investigating prostate cancer risk have used BMI to evaluate obesity rather than body fat distribution [530]. Previous studies have suggested that high BMI is associated with increased risks for advanced, aggressive and fatal prostate cancer [540, 542, 544-549]. In contrast, other studies have observed a decreased risk of localised/indolent cancer [540, 542, 550-552]. A large meta-analysis consisting of 27 prospective studies of prostate cancer observed no or weak association between BMI and total prostate cancer [553]. Similar findings have come from another systematic review examining the exposure in early adult life [554]. These conflicting results may, in part, be due to the fact that BMI has been criticised for its inaccuracy in measuring obesity and its ability to differentiate adipose and non-

adipose tissues [555, 556]. This suggests that any association could be dependent on particular disease subtypes and the age of exposure [533, 539, 540, 557].

Both body shape and body size have often been used to describe the characteristics of the human body in health-related research. Defining obesity or adiposity through the use of clinical judgement, including a consideration of body size appearance, provides an alternative approach for determining the wider distribution of fat tissue over time.

The issue of whether weight change during adulthood is more strongly associated with prostate cancer than cross-sectional 'current' adiposity has not as yet been fully explored [558, 559]. Prostate cancer is characterised as being a slow developing disease. Thus the age that obesity develops in early adult life may be an important factor within the aetiology of this cancer [554, 560-562]. Moreover, early changes in prostate tissue have been seen in men during their early adulthood, suggesting that body size over a lifetime is important [560, 563]. Adult weight change is a dynamic measure that could reflect imbalances in weight over time and it is thought to be more accurate than a static measure of adiposity such as BMI [546, 564]. However, these studies have reported inconsistent results [546, 558, 559]. Some studies found positive associations between weight gain and prostate cancer [565], whereas others have found an inverse association [566] or no association at all [541, 548]. In this study, we specifically address the issues of whether male self-reported body size and overall body shape and self-reported body size and its change across three decades of life are associated with prostate cancer risk.

3.2 Methods

The 'Prostate Cancer Study on Gene-Environment Interactions' is a large scale case-control study identifying and investigating potential risk factors for the development of prostate cancer in the

UK. The study used a self-administered questionnaire and written informed consent was obtained from each participant. Cases comprised adult men >36 years at diagnosis with histologically confirmed prostate cancer. Male adult controls were selected from the same general practices as cases. Eligible controls were men without a history of prostate cancer and were within an age range of ±5 years of cases. This study received ethical committee approval MREC/99/4/013 (Trent Research Ethics Committee), 07/MRE04/29 (Nottinghamshire County Teaching and Primary Care Trust).

Epidemiological data were collected for two time periods; the first between 1997-2004 and the second between 2007-2009. In the second period, some additional questions were added and other questions were expanded within the questionnaire to provide more in-depth information, including information on body shape. This was done following a preliminary analysis of data collected from the first period. Data collection from the two time periods involved different subjects and no repeated measurements were performed. Individuals did not contribute their data more than once. Data on education was based on the UK educational system and social class was based on the UK occupational social class classification. Data on self-reported body size were available from both periods, but data on body shape were only available from the second period of data collection. Self-reported body size at different ages was assessed using a pictogram (Figure 3.1) with drawings of body silhouettes of nine different sizes ranging from 1 (very thin) to 9 (severely obese) [567]. Subjects were asked to recall information relating to their self-reported body size during their 20's, 30's and 40's. Cases and controls were asked to rate their perceived body size for the last 5 years period prior to diagnosis in the case group and for the last 5 years prior to receiving the questionnaire in the control group. Participants were excluded from the analysis if there were

incomplete data (i.e. missing data for any decade). This was done to ensure each participant had data to investigate self-reported body size changes throughout the decades. 1928 cases and 2043 controls were available for the analysis of self-reported body size in the 20's and 30's. Six subjects were younger than 40 years of age at the time data were collected; hence the number of cases and controls eligible for self-reported body size analysis in the 40's were 1924 and 2041, respectively. Ordinal scale data (scale of 1 to 9) for self-reported body size at age 20's, 30's, and 40's were grouped into 'thin' (scale 1-3), 'medium' (scale 4-6) and 'large' (scale 7-9).

To explore the effect of self-reported body size increase during adulthood on prostate cancer risk, we restricted our analysis to include only subjects whose self-reported body size remained as medium size from 20's to 40's as our reference group and subjects whose self-reported body size was medium both in their 20s and 30s but increased to large in their 40's as our exposed group (Figure 3.1). There are 1057 cases and 1099 controls.

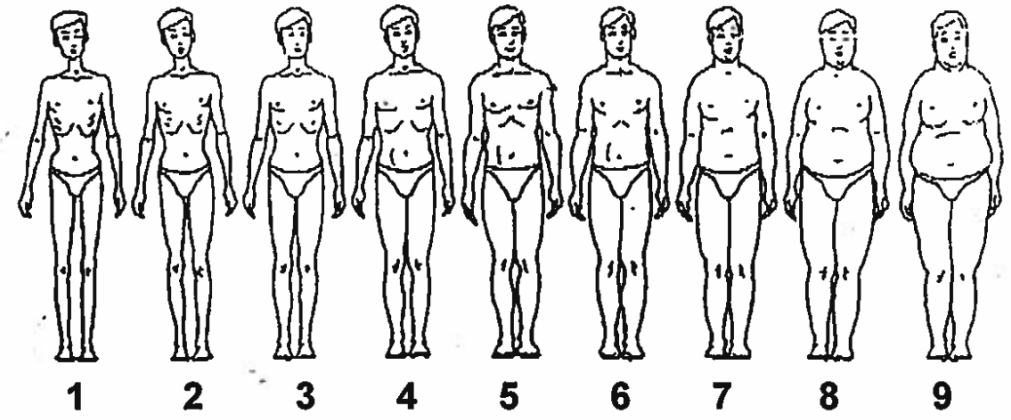

Figure 3.1: Pictogram with silhouette drawings used for recalling self-reported body size at each decade 20s, 30s, and 40s (taken from Stunkard et al., 1983).

For body shape, participants were asked to select their body shape in four different forms (apple, pear, oval and symmetrical) that best represented their body shape throughout their life. Description of each body shape type was provided to aid subject's understanding of its meaning ('Apple' shape is where body fat is distributed mainly around the central abdominal area; 'Pear' shape is where body fat is distributed mainly around the hip and thigh; 'Oval' shape is where body fat is distributed around the neck, chest, abdominal area and thigh; 'Symmetrical' shape is where the person has a lean body with no fat). The numbers of subjects included in this particular analysis were 1329 cases and 812 controls.

3.2.1 Statistical analysis

Logistic regression analysis was performed on the data using Stata version 15.0 [568]. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for total prostate cancer risk. Forward stepwise logistic regression was performed on demographic factors to identify potential confounders. The final multivariate logistic regression model included education, ethnicity, study phase (I and II) and family history of prostate cancer in first-degree relatives. Multivariable logistic regression was fitted with all confounders. Age was also included as an *a-priori* variable in all regression models. For self-reported body size, medium size was used as reference category and for pattern of change, no change from 20s to 40s was used as a reference group. In the multivariate model, self-reported body size at age 30's and 40's were adjusted further to self-reported body size at age 20's to minimise the effect of correlation between self-reported body size at age 20's to age 30's and 40's. For body shape, the symmetrical shape was used as a reference category. Estimated risks were obtained from multivariate logistic regression models. A significant odds ratio is considered when 95% CI does not include 1.

3.2.2 Sensitivity analysis

We collected data on current BMI from both periods. A sensitivity analysis was performed to explore if self-reported body size can be used as a proxy marker for BMI. We used the self-reported body size and BMI reported during the last 5 years prior to completing the questionnaire only in the controls due to the fact that prostate cancer may have affected current BMI in cases. Data were available in 766 controls. BMI as a continuous variable was normally distributed hence we applied Analysis of Variance (ANOVA) to explore the differences among group means. A finding was deemed to be statistically significant when the P-value was less than 0.05.

3.2.3 Study power

As there are no previous studies on body shape and prostate cancer, we computed our study power based on exposure in our study. Our study of 1329 cases and 812 controls with a probability of exposure (apple body shape) among controls of 0.62, had a 95% study power to detect odds ratios for the disease of 0.72 or 1.41 [569].

3.3 Results

The overall study response rates after initial consent to complete the questionnaire were 85.0% for cases and 74.4% for controls. Table 3.1 shows the study population characteristics. The median age for both case and control subjects was 60 and 59 years, respectively. The vast majority of study subjects described themselves as white (98%).

Table 3.1: Demographic and social characteristics of participants in the Prostate Cancer Study on Gene-Environment Interactions.

Characteristics	Cases (n=1,928)	Controls (n=2,043)	OR of prostate cancer	(95% CI)
Age (years)	60 (range 36-84)	59 (range 36-76)		
	n (%)	n (%)		
Marital Status				
Married or partnership	1,585 (82.2%)	1,691 (82.8%)	-Ref-	
Divorced, separated or widowed	227 (11.8%)	260 (12.7%)	0.93	0.77 – 1.13
Single	89 (4.6%)	68 (3.3%)	1.39	1.01 – 1.93
Missing	27 (1.4%)	24 (1.2%)		
Education				
No qualifications	433 (22.5%)	558 (27.31%)	-Ref-	
GCSE, O levels or equivalent	357(18.5%)	342 (16.74%)	1.35	1.11 – 1.64
A levels, higher or equivalent	132 (7.0%)	148 (7.24%)	1.16	0.89 – 1.51
Higher or professional qualification e.g. degree, HND	716 (37.0%)	742 (36.32%)	1.25	1.06 – 1.47
Others	252 (13.0%)	229 (11.21%)	1.42	1.14 – 1.76
Missing	38 (2.0%)	24 (1.17%)		
Ethnicity				
White	1,832 (95.0%)	2,000 (97.9%)	-Ref-	
Black	29 (1.5%)	4 (0.2%)	8.1	2.84 – 23.12
Asian	13 (0.7%)	7 (0.34%)	1.99	0.79 – 5.02
Other	26 (1.4%)	13 (0.64%)	2.19	1.12 – 4.29
Missing	28 (1.4%)	19 (0.93%)		
Social class				
I	236 (12.2%)	224 (11%)	-Ref-	
II	797 (41.3%)	851 (41.7%)	0.89	0.72 – 1.10
IIIN	193 (10.0%)	208 (10.2%)	0.88	0.67 – 1.15
IIIM	499 (26.0%)	528 (25.8%)	0.90	0.73 – 1.13
IV	108 (5.6%)	111 (5.4%)	0.93	0.67 – 1.28
V	18 (0.9%)	31 (1.5%)	0.56	0.30 – 1.02
Missing	77 (4.0%)	90 (4.4%)		
Family history of prostate cancer				
No	1,312 (68.0%)	1,880 (92.0%)	-Ref-	
Yes	533 (27.7%)	100 (4.9%)	7.61	6.08 – 9.54
Missing	83 (4.3%)	63 (3.1%)		
*Unadjusted OR. The rest of ORs were adjusted for age.				

Table 3.2 summarises the number of subjects and their self-reported body size at each of the three decades of their life. The majority of participants were medium across all three decades in both case and control groups.

Table 3.2: Self-reported body sizes at each decade among cases and controls.

Body size at 20's	**Cases**	**Controls**	**OR of prostate cancer[a]**	**OR of prostate cancer[b]**
Medium	1,159 (60.1%)	1,208 (59.1%)	-Ref-	-Ref-
Thin	690 (35.8%)	736 (36.0%)	0.97 (0.85-1.11)	1.10 (0.95-1.28)
Large	79 (4.1%)	99 (4.9%)	0.84 (0.62-1.14)	0.95 (0.66-1.35)
Body size at 30's *				
Medium	1,497 (77.7%)	1,573 (77.0%)	-Ref-	-Ref-
Thin	255 (13.2%)	273 (13.4%)	0.97 (0.80-1.17)	0.97 (0.77-1.22)
Large	176 (9.1%)	197 (9.6%)	0.96 (0.77-1.19)	1.00 (0.77-1.30)
Body size at 40's *				
Medium	1,291 (67.1%)	1310 (64.2%)	-Ref-	-Ref-
Thin	70 (3.6%)	91 (4.5%)	0.77 (0.56-1.06)	0.85 (0.58-1.23)
Large	563 (29.3%)	640 (31.4%)	0.91 (0.80-1.05)	1.00 (0.85-1.75)

[a] Age-adjusted regression model
[b] Multivariate adjusted regression model for age, education, ethnicity, study phase and family history of prostate cancer
*Body size at 30's and 40's adjusted further to body size at 20's in the multivariate model

Table 3.3 summarises odds ratios of self-reported body size changes and prostate cancer risk. Both cases and controls have similar percentages of self-reported body size change from medium to large in their 40's (~30%). The result suggests that there is no association with cancer risk for subjects whose self-reported body size increased from medium to large as compared to subjects with medium self-reported body size throughout their adulthood.

Table 3.3: Estimated risk of self-reported body size changes and prostate cancer risk.

Group	Cases	Controls	OR of prostate cancer[a] (95%CI)	OR of prostate cancer[b] (95%CI)
Body size remains thin or medium throughout adulthood	738	758	-Ref-	-Ref-
Body size increase to large in their 40s	319	341	0.97 (0.81-1.17)	1.07 (0.87- 1.33)
Total	1,057	1,099		

[a] Age-adjusted regression model
[b] Multivariate adjusted regression model for age, education, ethnicity, study phase and family history of prostate cancer

Table 3.4 presents estimated risks of different self-reported body shape and prostate cancer risk. Compared to a symmetrical shape, subjects with an apple shape were at 27% risk reduction (OR in the fully adjusted model = 0.73 with 95% CI 0.57-0.92). Both pear and oval shapes did not show any association with prostate cancer risk in the fully adjusted model of 1.44 (95% CI 0.77-2.69) and 0.82 (95% CI 0.59-1.13), respectively. Although the association is not significant, the direction of effect suggested that adipose tissue distributed around the hip and thigh (pear) is at higher risk, while abdominal fat distribution (apple and oval) is at lower risk.

Table 3.4: Odds ratio of self-reported body shape on Prostate Cancer Risk.

Self-reported body shape	Case	Control	OR of prostate cancer[a] (95%CI)	OR of prostate cancer[b] (95%CI)
Symmetric	349	173	-Ref-	-Ref-
Apple	735	504	0.67 (0.53-0.83)	0.73 (0.57-0.93)
Pear	51	17	1.57 (0.87-2.85)	1.47 (0.78-2.76)
Oval	194	118	0.76 (0.56-1.02)	0.82 (0.59-1.14)

[a] Age-adjusted regression model
[b] Multivariate adjusted regression model for age, education, ethnicity, and family history of prostate cancer

Results from sensitivity analysis (only in the control group) using the ANOVA test are presented in Table 3.5. The significant p-value suggested that the mean BMI in each group is a statistically significant difference. BMI increases with increased self-reported body size indicative of a good proxy between BMI and body size.

Table 3.5: BMI and self-reported body size in the control group.

Body size	Number	Mean*	Std. Dev.
2	6	20.23	1.69
3	17	21.78	2.07
4	48	22.97	1.67
5	103	24.02	2.39
6	168	25.44	2.07
7	254	27.46	3.13
8	135	30.18	3.56
9	35	34.14	4.49

***ANOVA F-test P-value <0.05**

3.4 Discussion

Three key areas potentially relating to increased risk for prostate cancer were explored in this study: self-reported body size at early and mid-adulthood, self-reported body size changes over decades in life, and self-identified body shape.

Self-reported body size (thin, medium, and large) ranging across three decades (20's, 30's and 40's) was explored and analysis suggested no associations between the self-reported body size at each stage of life among cases and control groups and the risk of prostate cancer. However, our analysis could be underpowered given the relatively small numbers in the 20's/large and 40's/thin category. Furthermore, the analysis suggested that 55% of both case and control subjects had a history of changes in self-reported body size. Our *ad hoc* analysis also showed that the magnitude of changes in self-reported body size from age 20's to 40's varies between individuals (result not shown here). Approximately 53% of those self-reported body size changes were of increase in size either for both periods-20s to 30s and 30s to 40s or at 20s to 30s and no change in 30s to 40s. The possible explanation for the increase in body size is because of decreased metabolic rate with ageing and accumulation over the years of unburned calorie intake. Environmental factors such as eating high-fat foods or lack of exercise, as well as Sedentary Lifestyle Syndrome (SeDS), could also be accountable for increases in body size [570]. These possible explanations are compatible with the considerable social and lifestyle changes that have occurred across the UK over the last 30 years.

The findings of previous studies regarding obesity in early and mid-adulthood are inconclusive. Our results are consistent with the majority of epidemiologic studies that found no associations between self-reported body size in early as well as middle to late adulthood and prostate cancer

risk [541, 548, 554, 566, 571, 572]. More recently, a research group (the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortium) investigated the potential causal relationship between BMI and prostate cancer using genetic approaches to analyse 20848 cases and 20214 controls. This also failed to identify any significant associations between BMI and prostate cancer [573]. Our study also did not find any association between changes in self-reported body size over the decades (increase in self-reported body size from medium to large in the 40's compared to remains medium throughout all decades) and prostate cancer risk. This finding is inconsistent with several other studies where some relationships with prostate cancer were observed [541, 546, 551, 565, 574, 575]. This inconsistency could be due to the different measurements used by these studies which used actual weight, BMI or waist circumference to indicate the change in body size. In contrast, in our study we used pictograms as a surrogate for body size. We also performed analyses of BMI and perceived body size within different social classes and education in the control group and the results suggested a very similar correlation to that seen in the main sensitivity analysis. Furthermore, the other studies used multiple parameters to measure body size when investigating the relationship in change of body size with prostate cancer. As such there was a higher possibility of obtaining statistically significant findings in at least one of the measurement parameters. The other limitation is that our data is restricted to middle age (40s) hence this may not be the period in life that obesity is associated with prostate cancer. Our results which failed to show association are in keeping with the majority of other studies that investigated the association between weight change and prostate cancer risk [539, 558, 559, 566, 571, 572, 576-580].

A limitation of using pictorial illustration is its inability to make an actual measurement of changes in body size in comparison with using other parameters such as weight, waist circumference/waist-hip ratio, BMI or body fat mass. As such, pictorial assessment of self-reported body size is relative, but it may be better for showing body size change over a long time window. Pictograms are considered to be a valid and useful method to assess self-reported body size and differentiate between thin and obese individuals [581]. The Stunkard Figure Rating (SFR) scale of body size [567] tool has been validated for historic recall of body size and was used in a large European population to explore the correlation between self-reported body silhouettes and the previously measured (9–23 years) BMI [582]. The authors reported an area under the curve of 0.92 (95% CI 0.87, 0.97) in women and 0.85 (95% CI 0.75, 0.95) in men for identifying obesity at age 30 using body silhouettes VS previously measured BMI at age 30 (±2y). The findings were also similar for previously self-reported BMI, 0.92 (95% CI 0.88, 0.95) and 0.90 (95% CI 0.85, 0.96) in women and men respectively. Another study assessing adolescent body size found that Stunkard's method was a useful indicator in absence of measured BMI [583]. It is also has been reported that recalled body size using pictograms showed a strong correlation with measured weight at age 20-40 years with a correlation ranging from 0.51 to 0.95 [584-586]. Our result from sensitivity analysis in controls suggested that pictogram can potentially be used for recall of body size. Nevertheless, personal perception of the body size of each individual could introduce biases such as classification bias.

Cohort studies often obtain more valuable data by longitudinally measuring and recording body weight, waist/hip circumference and body fat mass, however, implementing this approach was not possible in our study. Some medical conditions, such as hypo or hyperthyroidism, can affect body

size. However the prevalence of both these conditions in the UK is low (1-2% for both conditions) [587] and therefore unlikely to affect our results. As our study is subject to classification bias, we opted to broadly group body size into three groups to minimise any bias, i.e. thin, medium and large.

We are not aware of any published research on the prevalence of different types of body fat distribution in the population. However, waist and chest circumference measurements in males are the closest for describing whether a person's shape can be described as 'apple' or be a proxy of central adiposity [588]. The male shape seems to remain highly stable throughout adult life; therefore, it is reasonable to assume that characteristics of body fat distribution also remain the same.

Our results suggest that subjects with an 'apple' shape, indicative of body fat distributed mainly around the abdomen, were at reduced risk with both adjusted and unadjusted when compared to those with a 'symmetrical' shape. However, the 'pear' and 'oval' body shapes did not show any statistically significant associations. A recent cohort study reported by Barberio involving 26607 subjects found central body adiposity to be more associated with cancer risk than overall body size [589]. Although the cohort examined the association with cancer in general, our results of self-identify body shape indicative of the distribution of fat tissue around the body suggested similar findings.

In contrast to 'apple' or 'pear' body shape, hip circumference indicates increased amounts of subcutaneous fat. Thus 'apple' body shape in actual measurement would predict a wider waist circumference (WC) or higher waist-to-hip ratio (WHR). Studies using actual measurements have

shown increased risk of advanced or high-grade prostate cancer in such individuals [530, 543, 557, 590, 591].

Several possible explanations have been proposed regarding the association between central adiposity and prostate cancer. Adiposity can potentially impact through multiple hormonal pathways. Adiposity has been associated with higher levels of insulin, insulin like growth factor-I, leptin, and inflammatory cytokines. It has also been linked with lower levels of adiponectin and free testosterone. All of these may impact on prostate cancer development and progression [547, 592-599]. Moreover, some studies showed that adiposity lowered the risk of non-aggressive prostate cancer while at the same time increased the risk for aggressive and high-grade prostate cancer [530, 532, 535, 541, 543, 547, 548, 551, 552, 557, 560, 600]. However other studies have observed weak or no association with prostate cancer and disease subtypes [539, 601-603].

As yet no other study reported in the literature has used body shape as a proxy measure of body fat distribution to investigate possible associations with prostate cancer. Our findings suggest that abdominal fat deposition (apple body shape) maybe protective of prostate cancer.

Diabetes is known to be linked with obesity and also shows an inverse association with the risk of prostate cancer [604-606]. One of the limitations was that we collected data on diabetes only in period 2 with no details of diabetes type. However, we carried out a logistic regression analysis incorporating diabetes in our model, our results remained the same. Likewise, we also investigated the association of both smoking and physical activity with prostate cancer and there were no associations. Therefore, we did not include these variables in our final model.

In this study, we used self-reported descriptions within the questionnaire to capture the types of body fat distribution. This approach is likely to be less accurate than using 3-dimensional body

shape scanning as used in the UK National Sizing survey [588] conducted from 2001 to 2002. This cross-sectional study of 9617 adults found that male body shape remained highly stable throughout adulthood. Such quantitative approaches may reveal further insights into the role and influence of lipidosity and its site of deposition on prostate cancer risk and development.

In conclusion, the study findings suggest that body size and body shape as determinants of obesity and fat accumulation and as modifiable risk factors cannot be recommended, at this time, to be incorporated into a risk prediction model for prostate cancer in the community.

Therefore, in the following chapter, I conducted a systematic review of the literature to identify potential risk prediction models for prostate cancer that has the potential to be used in primary care and community settings and investigated whether there are other variables included in the reviewed study.

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Chapter 4 Prediction models for prostate cancer to be used in the primary care settings: systematic review

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Key words: Prostate cancer, prostate specific antigen, PSA, prostate cancer screening, risk Prediction, risk tools, prediction models, primary care, community.

Abstract

Objective

To identify risk prediction models for prostate cancer that can be used in the primary care and community health settings.

Design

Systematic review.

Data sources

MEDLINE and EMBASE databases combined from inception and up to the end of January 2019.

Eligibility

Studies were included based on satisfying all the following criteria; (i) presenting an evaluation of prostate cancer risk at initial biopsy in patients with no history of prostate cancer, (ii) studies not incorporating an invasive clinical assessment or expensive biomarker/ genetic tests, (iii) inclusion of at least two variables with PSA being one of them, and (iv) studies reporting a measure of predictive performance. The quality of the studies and risk of bias was assessed by using the PROBAST tool.

Data extraction and synthesis

Relevant information extracted for each model included; the year of publication, source of data, type of model, number of subjects, country, age, PSA range, mean/median PSA, other variables included in the model, number of biopsy cores to assess outcomes, study endpoint (s), cancer detection, model validation, and model performance.

Results

An initial search yielded 109 potential studies, of which five met the set criteria. Four studies were cohort-based and one was a case-control study. The prostate cancer detection rate was between 20.6% and 55.8%. AUC was reported in four studies and ranged from 0.65 to 0.75. All models showed significant improvement in predicting prostate cancer compared with being based on PSA alone. The difference in AUC between extended models and PSA alone was between 0.06 and 0.21.

Conclusions

Only a few prostate cancer risk prediction models have the potential to be readily used in the primary health care or community health setting. Further studies are needed to investigate other potential variables that could be integrated into models to improve their clinical utility for prostate cancer testing in a community setting.

Strengths and limitations

- The review focused on risk prediction models for prostate cancer for use in primary care.
- The PRISMA approach was followed in identifying relevant articles and reporting this study.
- We used the PROBAST tool to assess the quality and risk of bias in the included models.
- The search strategy was restricted to two databases and a manual search to retrieve original studies.

4.1 Introduction

Prostate cancer is the second most common cancer and the fifth leading cause of cancer-attributed death in men worldwide with an estimated incidence of 1,276,106 and 358,989 deaths in 2018 [607]. In the United Kingdom (UK), around 47,200 new cases of prostate cancer were reported in 2015, accounting for 26% of all new cancer cases in males. Prostate cancer deaths in the UK were around 11,600 in 2016 [608]. The global projections of prostate cancer incidence and mortality for 2030 are 1.7 and 0.5 million, respectively [609]. The highest incidence of prostate cancer is seen in western societies [610]. The significant increase of prostate cancer incidence and diagnosis over the last three decades can be attributed mainly to the widespread implementation of the prostate-specific antigen (PSA) serum test after it had been introduced in the late 1980s [611, 612].

The strong association of PSA with prostate cancer [613, 614], along with it being a relatively inexpensive test [615], has made PSA a key biomarker in the diagnostic process of prostate cancer and for the recommendation of a confirmatory prostate biopsy [613, 615]. PSA is, however, not a cancer-specific marker [611, 616]. Conditions such as benign prostate hypertrophy (BPH),

prostatitis, and other non-malignant prostatic conditions can also elevate the PSA level, thus introducing uncertainty to the application of the test [617-620]. This highlights the limitations of the PSA test regarding its specificity and sensitivity and it being largely dependent on setting a 'diagnostic' cut-off point, which often leads to an unacceptable number of false-positive and false-negative results [611, 616, 621, 622]. Such issues are likely to be the part of the explanation for the significant number of unnecessary biopsies currently performed each year. Such procedures are associated with adverse side effects for patients and also increases health care costs [623, 624]. To address such PSA test limitations, researchers have incorporated other measurable factors into approaches for the early detection of prostate cancer; these "risk assessment tools" are based on statistical models designed to improve the accuracy and performance of the PSA test [625-628]. Logistic regression and artificial neural network (ANNs) models are now considered to be the most common and effective statistical techniques in aiding development of new models to enhance early prostate cancer diagnosis [629]. These prostate cancer risk prediction models can be used to aid the testing of men for further investigations.

Currently in the UK, there is no population-based screening programme for prostate cancer. The ultimate goal of prostate cancer screening is to find intermediate and high risk of prostate cancer rather than low risk prostate cancer that would not require treatment but will give emotional burden to the patient once detected and unnecessary treatment in some patients. An important potential advantage of the extended risk models is their ability to provide a more accurate estimation of prostate cancer risk. This may ultimately lead to their use in patient counselling and decision-making [630-633]. Such models have already achieved better results in predicting probabilities of

outcome compared to clinical judgment [634, 635]. Furthermore, it has been reported that using such predictive models may minimize the rate of unnecessary biopsies [636].

Recently there has been a substantial increase in the development of predictive models to help clinicians assess risk and decide which man to send to clinical settings to further investigate for a possible diagnosis of prostate cancer [628, 632, 636-641]. The majority of these models are designed for use in clinical settings, where costs are less of an issue and most include the need for a clinical examination such as digital rectal examination (DRE) or transrectal ultrasonography (TRUS). One of the main limitations of DRE is that it has poor performance, especially at low PSA levels, and it is highly subjective to inter-observer variability [107, 642, 643]. A meta-analysis study revealed that DRE has a positive predictive value of only 18% [644]. Similarly, TRUS has been reported for having poor accuracy at low PSA levels [645, 646] and small prostate cancer might not be palpable on DRE or visualisation on TRUS [645]. Furthermore, less than 40% of prostate cancer detected by DRE are potentially curable, making it less beneficial for early diagnosis [647]. Several studies showed that there are fear, anxiety and embarrassment among some men, in particular black men, regarding the DRE test [648-651]. Another disadvantage of the DRE is the fact it is a potentially uncomfortable test [652-657]. This may explain why the DRE is a barrier for some men to participate in prostate cancer screening if it is including DRE test. Lee et al. reported that 74%-84% of black men may not maintain annual DRE screening [658], while another study found that it may prevent 22% of men to participate [659]. Since TRUS need to be performed by a skilled urologist, this means men have to make an appointment with a clinic in a different location, which makes the screening less convenient. As a result, men may feel reluctant to have such tests performed.

This systematic review of the literature was undertaken to identify risk prediction models that do not incorporate invasive or more costly clinical procedures or extensive biomarkers but have potential application for use in primary care and community settings. As low cost is a primary concern for community use, for this review we set an indicative threshold of approximately 3-5 times the cost of a PSA test for inclusion. This excluded a number of models that contain new and emerging biomarker or Single nucleotide polymorphism (SNP) panels. As the numbers of persons referred to clinical settings costs are less of an issue. The performance of the models reviewed for detecting prostate cancer at initial biopsy have been compared using 'reported area under the curve' (AUC) and/or sensitivity-specificity testing.

4.2 Methods

The approach used to identify and select relevant articles was based on the application of the '*Preferred Reporting Items for Systematic Reviews and Meta-Analysis'* (PRISMA) [660].

4.2.1 Data sources and search strategy

A literature search was performed using Medline (via OVID) and Embase databases. The 'medical subject heading' (MeSH) terms, combined with Boolean logic operators 'AND' and 'OR', were applied to retrieve relevant articles. The terms used for the search were "Prostatic Neoplasms" AND ("Initial biopsy" OR "first biopsy" OR "early detection of cancer") AND ("nomograms" OR "artificial neural networks" OR "risk assessment" OR "statistical model"). The full search strategy is provided in a supplementary file (Appendix A). All articles defined (published since the inception of the databases and up to the end of January 2019) were subsequently further filtered as being those only published in English language and with an abstract. Further to using the above

search databases, the research articles were selected manually from the reference lists of any relevant review articles. Google Scholar and Medline searches were also carried out to identify independent studies for external validation for each model included in this review. The results are presented in the supplementary table (Appendix B).

4.2.2 Eligibility criteria

As this review focuses on prostate cancer risk prediction based in community healthcare settings, all studies were selected based on the following inclusion criteria (i) evaluating the risk for prostate cancer at initial biopsy in subjects who had no prior history of prostate cancer (ii) studies that reported "low cost" risk assessment tools (i.e. those not including more expensive genetic, biomarker test) or "invasive" clinical tests/examinations (such as DRE or TRUS) (iii) studies that included a minimum of two variables of which PSA had to be one of them [on the basis that an elevated PSA test in UK primary care is usually the first sign and rationale for suggesting a need for further investigation of prostate cancer within National Institute for Health and Excellence (NICE) guidelines] and (iv) studies that reported AUC and/or sensitivity and specificity of the diagnostic/predictive tool. The exclusion criteria used were (i) articles with models that were built and based on repeat or mixed biopsies, (ii) studies that only validate an existing model (iii) articles that were not published in English. There were no time boundaries regarding the publication year. Screening of the titles, abstracts, and full texts was carried out by two reviewers (MA, AL). Any concerns about the eligibility of a study were resolved by discussion with a third reviewer (KM).

4.2.3 Data extraction

A data extraction form was developed to collect all relevant information. For each study used in this review, the items extracted included; the year of publication, source of data, type of model,

number of subjects, the country where it was performed, age, PSA range, mean/median PSA, number of biopsy cores, variables included in the model, study endpoint(s), cancer detection, model performance, and model validation.

4.2.4 Evaluating the performance of the risk models

Prediction models can be evaluated against various criteria. The most critical measurements of model performance are discrimination and calibration [633]. Discrimination refers to how well the prediction model can differentiate subjects in different outcome classes according to their predicted risks. It is often assessed by measuring the area under the receiver operating characteristic curve [661]. It also requires setting a series of thresholds to separate low and high ranges of predicted outcomes. A value of 0.5 indicates no discrimination, while a value of 1 indicates perfect discrimination. However, even with perfect discrimination, observed risk can differ from expected risk. Therefore, calibration has an important role in model evaluation [662]. Calibration represents the agreement between expected and observed outcomes [663]. A well-calibrated model is achieved when the calibration slope is close to 1. When the calibration slope is less than 1, it indicates that the model underestimates low risks and overestimates high risks [664].

Due to the heterogeneity of the studies included, conducting a meta-analysis was not applicable.

4.2.5 Study quality assessment

The quality of the studies included in this review was assessed using the PROBAST tool [665]. This tool has been developed specifically to assess the risk of bias and applicability for prediction model studies. The tool consists of four domains and has 20 signalling questions that facilitate to reach overall judgment of risk of bias, as well as issues relating to applicability.

4.2.6 Patient and public involvement

No patients were involved in setting the research question or the outcome measures, nor were they involved in the design and implementation of the study. There are no plans to involve patients in dissemination.

4.3 Results

A total of 102 publications were identified using the search strategy as shown in Figure 4.1. An additional nine articles were identified through manual searches from a bibliography of reviewed articles. At the first filter step, a total of 109 titles and abstracts were screened for eligibility after removing two duplicates. In the second filter step, 60 papers failed to meet the inclusion criteria and were excluded, resulting in 49 articles. The final step of filtering yielded only five studies that were considered to be eligible (i.e. passed all set criteria) and were thus included in this systematic review. There is no independent study identified for external validation for included models.

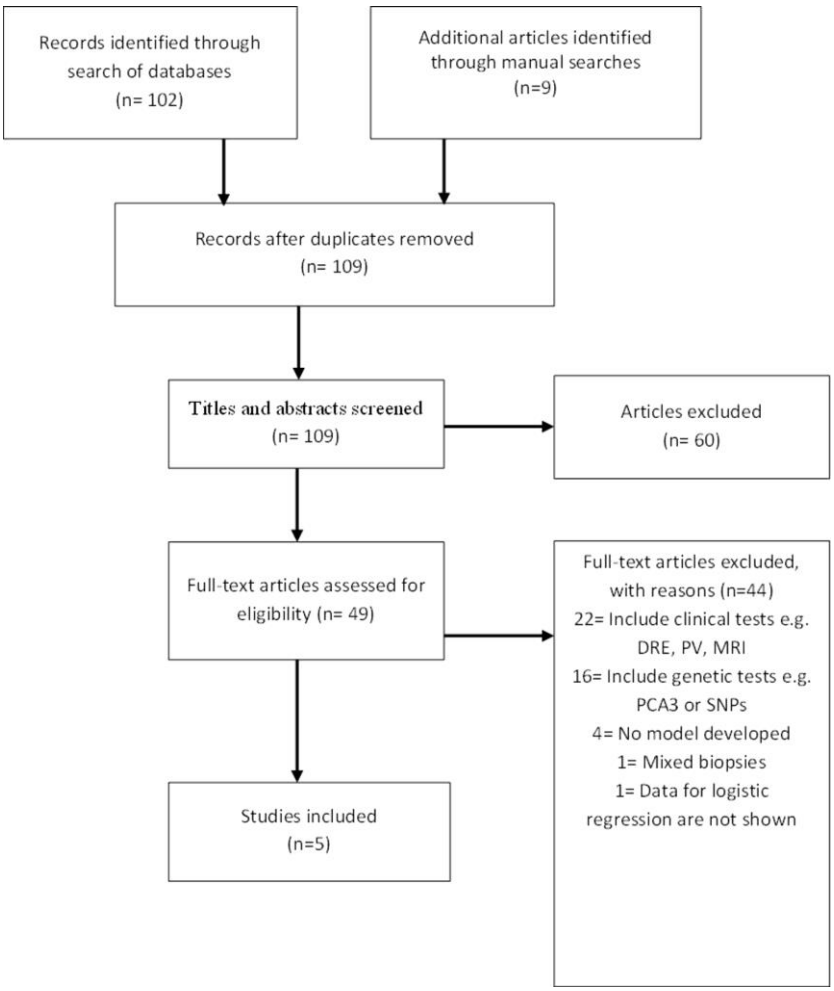

Figure 4.1: Flow diagram of studies included using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses method. DRE, digital rectal examination; PCA3, prostate cancer antigen 3; PV, prostate volume; SNP, single nucleotide polymorphism.

4.3.1 Study characteristics

Four of the five studies included were based on cohort studies and one was a case-control study. The characteristics of each of these studies and populations are summarised in Table 4.1. Details of PSA assays used in the models are presented in the supplementary table (Appendix C).

Table 4.1: Characteristics of the included studies.

Author & year	Type of model	Type of study	Sample No.	Location	Population type	Age	Median Age	PSA range	Median PSA	No. of biopsy cores	Cancer detection
Carlson, 1998 [666]	Logistic regression	Cohort	Model dev = 3773 Validation = 525	Baltimore, USA	Referral	≥ 45	—	4-20 ng/ml	—	Sextant biopsy	32%
Babaian, 2000 [667]	Neural network = 3 ANNs	Cohort	151	Texas, USA	Screening program	40-75	62	2.5-4 ng/ml	—	11 cores	24.5%
Jansen, 2010 [379]	Logistic regression	Cohort	Site 1 = 405 Site 2 = 351	Site 1 from the Rotterdam arm of the European Study of screening for Prostate cancer Site 2 Innsbruck, Austria	Screening program	≥ 50	Site 1 (66) Site 2 (60)	2-10 ng/ml	~ 4.4	≥6 cores	Site 1 = 55.8% Site 2 = 49.6%
Hill, 2013 [668]	Logistic regression	Case-control	1378	Florida, USA	Hospital referral	40-90	—	≥ 4 ng/ml	—	N/A	20.6%
Lazzeri, 2013 [669]	Logistic regression	Cohort	646	European multi-centre; Italy, Germany, France, Spain, and the UK	Referral	>45	—	2-10 ng/ml	~ 5.8	≥ 12 cores	40.1%

Subjects used to build the risk models varied across these studies. Of the five studies, three studies included men from referral populations [666, 668, 669] and two studies from screening programs [379, 667]. The sample sizes ranged from 151 to 3,773 with three studies derived from US cohorts [666-668] and two from Europe [379, 669]. Four studies used logistic regression methodology to build their model, whereas one study used an artificial neural network-based approach (ANN) [667]. The minimum age of participants was 40 years old [667, 668] and the minimum PSA level was 2 ng/ml [379, 669].

4.3.2 Variables in the model

Table 4.2 presents details of the variables used in each model. PSA level was used in all models, followed by free PSA (fPSA), age, and free-to-total PSA ratio (%fPSA). Other variables also reported in the models included; precursor of prostate-specific antigen (p2PSA), percentage of p2PSA to free PSA (%p2PSA), prostate health index (PHI), levels of haemoglobin, albumin, creatinine and red blood cell count (RBC), haematuria, mean corpuscular volume (MCV), and prostatic acid phosphatase.

Table 4.2: Variables used in the prostate cancer risk prediction models.

Author & year	Variables used in the model				
	Total PSA	**Free PSA**	**Percent free PSA**	**Age**	**Other variables***
Carlson, 1998 [666]	✓		✓	✓	
Babaian, 2000 [667]	✓	✓		✓	Creatinine Kinase, Prostatic acid phosphatase
Jansen, 2010 [379] *(Site 1)*	✓	✓			p2PSA
Jansen, 2010 [379] *(Site 2)*	✓	✓			p2PSA
Hill, 2013 [668] (Method 1)	✓			✓	HGB, RBC, Haematuria, Creatinine, MCV, and ethnicity "Black"
Hill, 2013 [668] *(Method 2)*	✓			✓	HGB, RBC, Creatinine, and MCV
Lazzeri, 2013 [669] *(Model 1)*	✓	✓	✓		
Lazzeri, 2013 [669] *(Model 2)*	✓	✓	✓		p2PSA
Lazzeri, 2013 [669] *(Model 3)*	✓	✓	✓		%p2PSA
Lazzeri, 2013 [669] *(Model 4)*	✓	✓	✓		PHI
*HGB= Haemoglobin. RBC= Red blood cells. MCV= Mean corpuscular volume. PHI= Prostate Health Index. p2PSA= Precursor of prostate specific antigen, %p2PSA= p2PSA/fPSA					

4.3.3 Outcome

The study endpoint also varied among the studies selected. Two studies evaluated the accuracy of detecting any prostate cancer [666, 667] and three studies examined the pathologic Gleason score [379, 668, 669]. Although Jansen et al., did not build a model to predict the aggressiveness of prostate cancer, they assessed the relationship of each variable individually with a Gleason score ≥7. Prostate cancer was determined by taking a needle biopsy. All subjects in the five studies underwent prostate biopsy. The least number of biopsy cores used was 6 [379, 666], and the highest was ≥ 12 [669]. One study did not report the number of biopsy cores taken [668]. Prostate cancer rates ranged from 20.6% to 55.8%.

4.3.4 Evaluating the performance of the risk models

For predicting any prostate cancer, the Jansen et al. model used data from the Rotterdam arm of the European Study of Screening for prostate cancer (ESPRC). Their model achieved the highest discrimination value when compared to PSA alone (AUC of 0.755 VS 0.585, respectively) [379]. The AUC values in other studies ranged from 0.648 to 0.74.

One study did not provide the AUC but instead reported an increase of 11% in specificity over percent free PSA alone with 95% sensitivity [666]. Lazzeri et al. [669] presented results from four separated models discriminating prostate cancer with a Gleason score of ≥7. Lazzeri's model 2 (which includes the base model PSA, fPSA and %fPSA in addition to p2PSA) and model 3 (which includes the base model plus PHI) showed the highest levels of discrimination out of the 4 models with an AUC of 0.67. In the study of Hill [668], the authors classified prostate cancer stages differently and built their two models accordingly. In Hill's first model, the difference in the discrimination was analysed and based on all prostate cancer versus non-cancerous prostate

conditions where the AUC for this model was 0.68 compared to 0.59 for PSA alone. In Hill's second model, the discrimination analysis was based on prostate cancer stages II, III, and IV versus prostate cancer stage I, prostatic interstitial neoplasm (PIN), BPH and prostatitis where stages I, II, III and IV are parallel to T1, T2, T3/T4, and metastatic prostate cancer respectively. The AUC for the second model was 0.72 compared to 0.63 for PSA alone. In general, four studies examined the AUC with PSA alone and all reported a benefit from the use of logistic regression or the trained ANN. Model performance and the differences between the AUC's for PSA alone and for the extended models are presented in Table 4.3.

Table 4.3: The difference of area under the curve (AUC) for PSA alone and extended model.

Study	**AUC for PSA**	**AUC for model**	**ΔAUC (Model – PSA)**
Carlson [666]	NA	NA	NA
Babaian [667]	0.64 %fPSA	0.74	0.1
Jansen [379] (*site 1)*	0.58	0.75	0.17
Jansen [379] *(site 2)*	0.53	0.7	0.16
Hill [668] *(method 1)*	0.59	0.68	0.09
Hill [668] *(method 2)*	0.63	0.72	0.09
Lazzeri [669]	0.50 for any prostate cancer 0.54 for Gleason score ≥7	Model 1 = 0.65 Model 1 (Gleason score≥7) = 0.60	0.15 0.06
		Model 2= 0.71 Model 2 (Gleason score≥7) = 0.67	0.21 0.13
		Model 3= 0.704 Model 3 (Gleason score≥7) = 0.67	0.2 0.13
		Model 4= 0.71 Model 4 (Gleason score≥7) = 0.672	0.21 0.13

Sensitivity and specificity data are presented in Table 4.4. At 95% sensitivity, the Babaian et al. model shows the highest specificity (51%), whereas the Jansen model for both sites had the lowest specificity (~23.5%). In the Hill study, with a sensitivity of ~90%, the specificity was lower than in other studies (~18% and 28%) for methods 1 and 2, respectively. In the study reported by Lazzeri, the sensitivity and specificity were not reported for the overall model; instead their study reports sensitivity and specificity for predictive variables individually. The highest sensitivity (90.5%) of %p2PSA and %fPSA achieved the highest specificity in predicting prostate cancer at 21.5% and 22.8%, respectively. Percentage p2PSA and PHI were more associated with Gleason scores.

Table 4.4: Sensitivity and specificity profile at different levels for each model.

Study	Sensitivity	Specificity	Probability cut-off	Positive predictive value	Negative predictive value
Carlson **[666]**	99	18	>15	≤47	N/A
	95	34	18	51	NA
	89	43	20	42	NA
Babaian **[667]**	95	51	NA	39	97
	92	62	NA	44	96
	89	62	NA	43	95
Jansen **[379]** (Site 1)	95	23.9	NA	NA	NA
Jansen **[379]** (Site 1)	90	30.1	NA	NA	NA
Jansen **[379]** (Site 2)	95	23.2	NA	NA	NA
Jansen **[379]** (Site 2)	90	36.2	NA	NA	NA
Hill **[668]** (Method 1)	90.9	17.6	33	47.1	70.5
Hill **[668]** (Method 2)	89.8	28	13	20.6	91.3
Hill **[668]** (Method 1)	80.5	37.1	37	50.9	70.2
Hill **[668]** (Method 2)	78.2	45	15	28.7	88.8
Hill **[668]** (Method 1)	39.9	81.4	48	63.4	62.6
Hill **[668]** (Method 2)	45.8	79.5	23	36.7	85
* Lazzeri **[669]** model reported only sensitivity and specificity for predictive variables individually and at sensitivity of 90, %p2PSA and %fPSA achieved the highest specificity					

Table 4.5 summarises the validation and calibration results for the studies included. Model calibration was reported in two studies [666, 669]. Carlson plotted the observed and expected risks using calibration plots, whereas Lazzeri used the Hosmer-Lemeshow goodness-of-fit test. In terms of validation, two studies did not report model validation [379, 668]. Only one study reported an external validation using an additional data set consisting of 525 patients [666]. Cross-validation using multiple re-sampling schemes was used in the Babaian Study, however, they did not report the number of times this was performed [667]. Lazzeri used 200 bootstrap re-samples to minimise overfitting bias [669].

Table 4.5: Validation and calibration for included models.

Author & Year	Validation	Calibration
Carlson,1998 [666]	External validation on additional data set consisting of 525 patients	Calibration plot
Babaian,2000 [667]	Cross-validation and separate data set of 151	N/A
Jansen, 2010 [379]	N/A	N/A
Hill, 2013 [668]	N/A	N/A
Lazzeri, 2013 [669]	Internal validation using 200 bootstraps resamples	Internal calibration using the Hosmer-Lemeshow goodness-of-fit test

4.3.5 Study quality assessment

Quality assessment was carried out by two reviewers (MA and AL) with any discordance resolved by a third reviewer (KM). The assessment of results suggested some issues of study quality

according to the criteria as set in the PROBAST tool, particularly in the analysis domain. For instance, one study applied univariable analysis to select predictors [666]. Three studies did not measure calibration [379, 667, 668]. Furthermore, two studies did not account for optimism and overfitting by using internal validation methods [379, 668]. Whereas one study did not use appropriate measures for model performance, i.e. AUC, this study reported the calibration [666]. The event per variable (EPV) was lower than recommended (< 10) [664, 670] in the Babaian [667] study indicating inadequate power. Four studies did not report missing data or how they handled it [379, 666, 667, 669]. The remaining study used complete-case analysis and excluded subjects with missing data on laboratory biomarkers (n=75) [668]. The PROBAST guidelines state that in a prediction model study where any risk of bias and applicability is low in all four domains, a regrading to high risk of bias should be considered when the study did not validate the model externally [665]. Thus, although the quality assessment for the Lazzeri study [669] was graded low risk in all the four domains, since the study did not report any external validation of the model, the assessment of the study has been regraded to high risk of bias according to the PROBAST criteria. A full quality assessment for all studies is presented in Table 4.6.

Table 4.6: Quality assessment for risk of bias and applicability concern for included studies.

Study	ROB*				Applicability			Overall	
	Participants	Predictors	Outcome	Analysis	Participants	Predictors	Outcome	ROB	Applicability
Carlson [666]	+	+	-	-	+	+	-	-	-
Babaian [667]	+	+	+	-	-	+	+	-	-
Jansen [379]	-	-	-	-	+	-	-	-	-
Hill [668]	-	+	+	-	-	+	+	-	-
Lazzeri [669]	+	+	+	+	+	+	+	-	+

* ROB- Risk of bias

+ indicates a low risk of bias or applicability; - indicates a high risk of bias or applicability.

4.4 Discussion

Despite the large number of prostate cancer risk prediction models, the majority still includes clinical inputs and/or more costly biomarker or genetic panels; few low-cost models exist that do not include specialist clinical input or more expensive further testing that limits their use for population-wide assessments. To our knowledge, this is the first study to examine risk prediction models for prostate cancer that are low cost and do not include clinical and genetic variables and are based on a single time point assessment.

Our study identified five unique models that met the set criteria. The Carlson model [666] has the largest population (3,773 subjects) when compared to the other four studies. Although they reported an 11% increase in specificity, they did not report AUC predictive estimates. It has been acknowledged that sensitivity and specificity results are dependent on the prevalence of the disease. Hence the comparison between populations where the prostate cancer prevalence may vary (especially in early detection) will be difficult [639]. More importantly, by not reporting the AUC estimate, the model raises some doubts regarding the reliability of the model and its implementation [639]. It will also make the comparison to other models not applicable [671].

Babaian [667] developed an algorithm and compared the performance of the ANN to PSA density (total PSA divided by prostate volume) (PSAD), %fPSA, and transition zone density (PSAD-TZ). Their ANN demonstrated a significant increase of model specificity that reached 51% when sensitivity was held at 95%. This was better than the specificity value of each individual variable such as %fPSA (10%), PSAD (39%), and PSAD-TZ (22%). In terms of AUC, the ANN achieved a moderate accuracy (0.74) being the second highest among all studies included. However, the ANN model did not

show significant improvement when compared with a model fitted with only individual variables (AUC for %fPSA= 0.64, PSAD= 0.74, PSAD-TZ= 0.75). They included a number of uncommon pre-biopsy inputs into their algorithm such as prostatic acid phosphatase and creatinine kinase [672]. Furthermore, they used a tight PSA range (2.5-4.0ng/ml) which meant that their model may be less suitable for patients with PSA levels below or above that range, thus limiting its generalisability.

The study by Jansen and colleagues [379] demonstrated that adding p2PSA to the base model of PSA and fPSA significantly enhanced the prostate cancer predictive value and specificity. The association and added value of p2PSA in the prediction and detection of prostate cancer have been reported by several other studies [622, 673-676]. Jansen [379] showed that p2PSA has no clear association with aggressive prostate cancer. However, the base model that includes p2PSA had the highest clinical significance in correlation to pathologic Gleason score with a p-value of 0.008 compared to %fPSA and PHI (p-value 0.01 and 0.02), respectively. Although they used archived blood samples and retrospective analysis, the results were similar to a prospective study of 268 patients [622].

Hill [668] used a case-control study to evaluate several laboratory biomarkers. They found that HGB, RBC, haematuria, creatinine, PSA, Age, MCV, and ethnicity ("being black") were statistically significantly associated in the first method ($p < 0.05$). In the second method, HGB, RBC, creatinine, PSA, age, and MCV were found to be statistically significantly correlated ($p < 0.001$) with prostate cancer. However, since this study was designed as a case-control study, it would have been more prone to uncontrolled confounding and selection bias. Moreover, the type of screening protocols used in Veterans' Administrations may vary from those conducted in other healthcare systems; therefore, the results may not be applicable to other populations. Furthermore,

subjects with a PSA level < 4.0 ng/ml have not been investigated, and thus, the performance of the models are unknown for individuals in this group.

Lazzeri et al. [669], in a European multi-centre study, have evaluated similar biomarkers as in the Jansen study with the same PSA range of 2-10 ng/ml prospectively. They found no difference in both %p2PSA and PHI as individual prostate cancer predictors with an AUC of 0.67 (95% confidence interval (CI) 0.64-0.71). However, the base model (consisting of PSA, fPSA, and %fPSA) that also included either p2PSA or PHI outperformed the base model alone, and the base model included %p2PSA. In the analysis, the additive value of both p2PSA and PHI is 0.064 and 0.056 for %p2PSA for predicting the risk of prostate cancer. These additive values increased to 0.076 for both p2PSA and PHI and 0.073 for %p2PSA in predicting Gleason scores ≥7 for the disease. The usefulness of PHI in improving the predictive accuracy of prostate cancer over total and free PSA has been confirmed and reported by several studies [622, 676-679].

In general, only one study has validated its model externally [666], whereas the remaining studies were either validated internally [667, 669] or did not report any validation methods [379, 668]. Prediction models may not be equally applicable to all data sets as patients' characteristics may vary [626, 680]. As a result, the generalisability of a model might be poor when it is used in populations other than that used in building the model. Therefore, external validation should be conducted before applying any new model to general practice [681, 682].

Another key performance measure of any model that needs careful evaluation is calibration. A calibration plot with a calibration slope is more preferable than the Hosmer-Lemeshow test; it has been acknowledged that evaluating a good and well calibrated model based on a large dataset can still fail the Hosmer-Lemeshow test. In

contrast, when evaluating a poorly calibrated model with a small dataset it can still pass the Hosmer-Lemeshow test [683]. In our analysis, three studies fail to report the calibration of the model [379, 667, 668], whilst the Carlson study [666] used a calibration plot, and Lazzeri [669] used the Hosmer-Lemeshow test. Excluding calibration from the majority of models may explain why some models are not currently used in practice [683].

With regard to biopsy cores, only two studies used extended biopsy cores. Babaian [667] used an 11-core multi-site biopsy, whereas Lazzeri [669] used at least 12 biopsy cores. Moreover, two studies used six cores biopsy in their model [379, 666]. The use of six-core biopsy has been criticized as not being adequate in detecting prostate cancer [684] and that models developed using sextant biopsy are less accurate than when a 10-core biopsy is used [685]. As a result, the European guideline for clinical prostate cancer recommended an extended biopsy as standard practice for prostate cancer detection [686].

It is worth noting that all five reviewed models performed better than just PSA alone. However, none of them has both high specificity and sensitivity. The level of sensitivity has been increased, and despite enhancement in the specificity, it is still considered low. Specificity is crucial when it comes to being used in a population setting as men without prostate cancer should be ruled out as much as possible from further invasive engagement with the health system.

Our review, therefore, suggests that none of the reviewed models provides an ideal performance in predicting prostate cancer with high sensitivity and high specificity. It is particularly important when considering the application of prostate cancer risk prediction at the population level that the tool used should be able to both detect the outcome and filter out people with no disease. As there is robust evidence suggesting

that the clinical relevance of PSA range to the detection of prostate cancer differs across age groups [90, 687, 688], any future model should consider the PSA threshold in relation to a specific age range. Risk prediction models for prostate cancer should therefore take account of age.

4.4.1 Comparison with other studies

To our knowledge, three systematic reviews of prostate cancer prediction tools have been published [626, 632, 633]. In the Louie et al. review, risk models were included that were externally validated in at least five study populations for the purpose of meta-analysis and only six studies were included in their analysis. Furthermore, all the studies included incorporated clinical tests such as DRE and/or TRUS prostate volume [632]. Schroder and Kattan [626] reviewed models that were built to predict the likelihood of having a positive prostate biopsy for cancer. However, it appears that they also included models where subjects had a previous negative biopsy. As such, some of the models included variables related to biopsy results and cores. The review by Shariat and colleagues examined different types of predictive tools [633]. They explored tools that predict prostate cancer on initial and repeat biopsy, pathologic stages, biochemical recurrence after radical prostatectomy, metastasis, survival, and life expectancy. Similarly, virtually all of the prediction tools that were based on initial biopsy included variables based on invasive procedures.

4.4.2 Strengths and limitations of this study

This report is the first to review risk prediction tools for prostate cancer that can be used in primary care and community settings. Any prediction model should therefore be simple to use, based on non-invasive tests, feasible at a population level and at low cost. We carried out an extensive data extraction relating to important features and characteristics for each study included, such as modelling method, source of data,

sample size, variables, discrimination, validation, and cancer detection rate. We have also followed PRISMA guidelines for identifying eligible articles as well as for reporting this study. In addition, the PROBAST tool was adopted to assess the quality and risk of bias for each prediction model.

Our study has some limitations. Our aim was to identify prediction models that have the potential to be implemented in primary care or community settings, and consequently our search strategy was to retrieve relevant studies for this specific purpose. Furthermore, we excluded articles that were not published in English or did not have an abstract. Moreover, only two databases were searched, besides manual search, to retrieve original studies.

A previous systematic review suggested that the majority of relevant studies could be identified through a manual search of articles reference lists instead of a database alone [626]. We identified four eligible studies using this approach. Given the small number of models identified by the approach we followed that can be applied in primary care settings compared to the large number relating to wider existing models, it is unlikely that we have not included any study that would affect the results of our review.

4.4.3 Implications and future research

It is now accepted that the PSA test and its derivatives have some limitations for detecting prostate cancer as defined by subsequent biopsy [689]. As a consequence, a considerable number of prostate cancer prediction models have been built to improve prediction accuracy. This has resulted in a plethora of prostate cancer risk prediction tools, with to date more than 100 models described [690, 691]. There is evidence that some of these models show benefit and have better performance over just PSA measurement alone [626]. It also has been demonstrated that some of these models outperformed clinical experts in predicting prostate cancer [634, 635]. Although such

models are not designed to replace specialist clinical judgment or patient preferences [680, 689, 692], they can help in patient counselling and aid clinicians to decide whether a prostate biopsy should be taken or not [681, 692, 693].

Given the small number of risk prediction models for prostate cancer that do not incorporate clinical or genetic tests, the discrimination of these reviewed models ranged from poor to moderate (AUC range ~0.65 to ~0.75); in addition there were some issues relating to their study design and analysis raises the risk of bias. Consequently, none of these models could be currently recommended for use in a primary care and community health care setting. Several guidelines are against using PSA test based screening for prostate cancer; the US Preventive Services task force, the Canadian task force on preventive health and the American College of Preventive Medicine do not currently recommend PSA-based testing due to insufficient evidence [694-696]. This has made it difficult, so far, to convince policymakers to adopt prostate cancer screening programme.

The first guideline of PROSTATE CANCER UK states “In the future, health professionals should look at a man’s PSA level alongside other known risk factors as part of a risk assessment tool, when one becomes available.” [697] However, the vast majority of the current prostate cancer risk prediction models are not suitable for routine use as they include clinical and genetic tests and are not validated externally in other cohorts. Therefore, the main challenge in the UK remains to develop a risk prediction tool that is reliable, cheap, applicable for as wide an ethnicity as possible, and most importantly, is easy to use and can be implemented at a primary care level [698].

The value of such risk tools is that they will help to stratify men at high risk of developing prostate cancer earlier so that they have appropriate management and/or surveillance programme as early as possible and therefore, may fit into the clinical

pathway. Such tools should help physicians have a better understanding of the risk for this disease and simplify the procedures and discussions with patients when recommending further specialist-led investigations such as DRE and/or MRI where a decision on whether a biopsy should or not be performed is concluded. Furthermore, using the appropriate risk prediction tool will avoid men from undergoing inappropriate further and frequent testing [698]. This will reduce any associated costs of inappropriate tests and decrease the burden on health care delivery systems.

It is crucial to address these issues by identifying all possible risk factors for prostate cancer that are non-clinical, non-genetic, and easy to use and interpret. There remains a pressing need to develop a risk prediction tool in the future using all appropriate factors (potentially also including genetics once there is infrastructure in place for genetic testing in the primary care and the cost comes down) into a robust multivariable analysis and validate the model externally to eliminate applicability and generalisability concerns. Only when this is achieved will it be possible to introduce a prostate cancer screening programme fit for purpose.

4.5 Conclusion

There is a paucity of suitable low cost risk models that incorporate non-clinical, non-genetic inputs and which can be used at a primary care level and in other community health services. Existing models have limitations reflecting both study design and reporting performance measures. Future research should take into account these key issues and explore other risk factors for incorporation into further models.

The findings of this chapter suggested a limitation of prostate cancer risk prediction that fits my set criteria. In the subsequent chapter, therefore I developed a new model – RISKMAN - that incorporates easy-to-measure and low cost variables suitable to be implemented in primary care and community settings.

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AUTHOR CONTRIBUTIONS

MA, AL and KM were involved in study conception, idea and design. Data acquisition and extraction was obtained by MA. Data synthesis and interpretation was carried out by MA, AL and WO. MA and AL drafted the manuscript. All authors approved the final version of manuscript. KM is the study guarantor.

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All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

ETHICAL APPROVAL

Not applicable as this study did not involve any personal data.

DATA SHARING

Data extraction sheet and the PROBAST assessment form for risk of bias is available upon request.

Figure legend
Figure 1: Flow diagram of studies included using PRISMA method

Chapter 5 Development of risk prediction model for prostate cancer for primary care settings

The work within this Chapter will be modified for publication.

5.1 Introduction

The previous chapter highlighted the scarcity of available risk prediction models for prostate cancer that can be used in primary care settings. However, as illustrated earlier, those prediction models have several drawbacks, and more importantly, most of them are not validated either internally or externally. Hence, their applicability in primary care settings and their clinical value remain unclear.

The rationale for building a risk prediction model for prostate cancer to be used in primary care settings is to facilitate the stratification of men according to their cancer risk, especially those who have a higher risk of developing aggressive or high-grade prostate cancer. It is critical for any prostate cancer prediction models to exclude cancer-free and indolent cases with low risk. In doing so, only men with a high risk of developing significant prostate cancer can then be advised to continue further investigations, such as with their general practitioner or at the secondary care level in specialist clinics. Individuals with low risk could be maintained under active surveillance. Such an approach would reduce the number of tests performed and referrals to clinics. Ultimately, many men would be saved from over and miss diagnosis, the cost of testing would be minimal and the burden of overload on secondary and specialist physicians could be reduced substantially. In recent years, magnetic resonance imaging (MRI) guided biopsy has been introduced in some large hospitals. Hopefully in the near future, MRI-guided biopsy for prostate cancer detection will become routinely available in the UK NHS. The introduction of such technology has emphasised even more the need for methods to accurately stratify men on the basis of their risk for prostate cancer so the appropriate patients can go forward with such procedures.

This component of the project aimed to develop a risk prediction model for prostate cancer for primary care settings (hereafter called RISKMAN) using two different data sources. The first data source was from the UK, where prostate cancer outcome was determined by MRI-guided biopsy. The second data source was from Poland and prostate cancer outcome was determined by conventional standard needle biopsy followed by histopathology. The RISKMAN model incorporates easily measured, low-cost and routinely collected data and variables. This study assessed the performance of the RISKMAN algorithm for these two distinctive clinical procedures to derive outcomes.

5.2 Methods

5.2.1 Study design and source of data

5.2.1.1 The UK cohort:

Data were provided by Mr V Gnanapragasam, a Consultant Urologist at Cambridge University Hospitals NHS Trust. The total cohort comprised 554 men from five centres located in the UK. Subjects were recruited from primary care referral settings as a result of elevated prostate-specific antigen (PSA) levels >4 ng/ml between January 2018 and June 2019. All patients had no history of a previous biopsy and had received multi-parametric magnetic resonance imaging (mpMRI). PSA and its derivative assays were assessed before a biopsy. Patients with a positive result on mpMRI underwent a targeted biopsy with a median number of cores of 2. Patients with a negative mpMRI result underwent a systematic biopsy with median cores of 16. In the original cohort, 9 men were excluded due to missing data and out of 545, at least 420 men had a positive mpMRI result. In this study, no observation was excluded as all observations have

complete records for age, PSA, and %fPSA (the ratio of free PSA to total PSA). Therefore, the results for the remaining 9 subjects are unknown.

Men were excluded if they had (i) a previous biopsy, (ii) pelvic metalwork interfering with mpMRI quality or no mpMRI and (iii) if no biopsy was done after mpMRI.

Details about this cohort were published elsewhere [699].

5.2.1.2 Polish cohort

Data were provided by Professor Cezary Cybulski from the Department of Genetics and Patho-morphology of the Pomeranian Medical University, Poland. The total cohort initially included 2907 men aged between 40 and 90, with no history of prostate cancer or other cancers. The enrolment period was between 2009 and 2012. Subjects were derived from two sources; the first was from an outpatient clinic in Szczecin. The second source was patients who were part of a population-based survey for family cancer in west Pomerania. The inclusion criteria were based on either having a positive family history of prostate cancer or carrying a specific gene mutation. The indication for prostate biopsy is an elevated PSA level ≥ 4.0ng/ml or an abnormal digital rectal examination (DRE) test. Based on that, only 323 men underwent a trans-rectal 24 core ultrasound-guided biopsy. More details of the cohort are described elsewhere [700].

5.2.2 Study measures

Outcomes of interest were the risk of developing any prostate cancer as determined by initial biopsy and confirmed by pathologists and high-grade prostate cancer defined as a Gleason score of ≥7. Predictors were pre-selected based on information from the literature review and results of meta-analyses, as well as their availability in the two cohorts and ease of use in primary care settings. The predictors included age, total prostate-specific antigen (PSA, ng/ml), and % fPSA (measured as free PSA/ total PSA × 100). All predictors' values were obtained prior to undertaking biopsies. Although

family history and ethnicity are established risk factors for prostate cancer, they were excluded from the final analysis of the model. Family history was excluded because the population from the Polish dataset was already selected based on positive family history, while ethnicity was excluded because populations in the UK and Polish data sets were both European Caucasian origin. Data on family history were not available for the UK cohort.

The UK cohort did not have any missing values for the selected variables. However, of 323 men in the Polish cohort, eight subjects had a missing value in free PSA and were removed, leaving the final cohort with 315 for final analyses.

5.2.3 Statistical analyses

For each variable, an estimated risk was evaluated using both univariate and multivariate logistic analyses. A Student's t-test was performed to assess the mean value difference between cases and controls. Model fit was assessed by checking specification errors following logistic analysis through regression coefficients. Model fit assessed if the model was properly specified. Consequently, it was not expected to find any additional predictors that were statistically significant except by chance. After running the model specification error test, results suggested that the model was well specified and it was indicative that no additional variables were required. Continuous data were kept on the original scale to maximise predictive ability, except for %fPSA in all analyses, where it was multiplied by 100 for better interpretation (the original scale caused problem with model convergence). All variables in the model were also checked for co-linearity; no co-linearity was detected. Odds ratio (OR) was used to assess the associations of prostate cancer.

Cross-validation with 10-fold repetition was used to internally validate each cohort. The predictive performance measures included the mean area under the curve (AUC),

and bootstrap bias-corrected 95% confidence interval (CI). The accuracy of the models was assessed by the receiver operating characteristic (ROC) and AUC calculated discrimination between those who have the disease from those without the disease. An AUC of 0.50 indicates a random chance and suggests no discrimination, and an AUC of 1.0 represents perfect discrimination. Calibration curves that assessed the agreement between the predicted probabilities and the actual results were also visually evaluated, with a slope of 1 indicating a perfect calibration. The added value of predictors was also evaluated by comparing the AUC in the multi-variate analyses in both cohorts.

The Polish cohort was subjected to similar investigations as the UK cohort. In addition, the number and percentage of biopsies saved, number and percentage of missing cases, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) according to multiple cut-point probabilities were assessed and compared between the cohorts. Cut-off points that yielded a sensitivity equal to or above 95% are highlighted in the results, and comparisons between the lowest and the highest cut-off points are also presented where appropriate. Continuous variables were presented as a mean with ± standard deviation (SD).

After applying the analyses to all participants in both cohorts, further analyses were applied to participants with PSA levels between 3-10 ng/ml to investigate any changes in discrimination and model performance in this “problematic” range. Age alone was considered model 1 as it has been established that prostate cancer risk increases with age, and also such information can be obtained at a minimal cost. Subsequently, PSA was added to age (model 2). Model 3 consisted of model 2 plus % fPSA (Full-model).

All statistical analyses were performed by using Stata version 16.1 (http://www.stata.com). A statistically significant value was set to a P-value of <0.05.

5.3 Results

The numbers and percentages of each sub-groups in both cohorts are presented in Table 5.1. The UK cohort consisted of 554 men with 359 men (64.8%) diagnosed with any prostate cancer, of whom 48% were high-grade cancers. The Polish cohort consisted of 315 men with 131 men (41.6%) diagnosed with any prostate cancer, of whom 17.8% were high-grade cancers. There was no significant difference in mean age between the UK and Polish cohorts; 65.3, and 65 years, respectively. The mean PSA level was much higher in the UK cohort (12.6 ng/ml) than in the Polish cohort (7.4 ng/ml), whereas the %fPSA mean was higher in the Polish cohort (20.5) compared to the UK cohort (14.7). All results are presented first for the UK cohort followed by the Polish cohort

5.3.1 The UK cohort:

Table 5.2 shows the mean value for each variable in each sub-group for cases and controls in both cohorts.

In the UK cohort, men with high-grade cancer are slightly older than men with any prostate cancer, as the mean age was 67.3 yrs and 66.8 yrs, respectively. Also, the mean age for all types of controls including a) controls without any form of cancer and b) controls with low-grade prostate cancer and no cancer, are slightly less than cases. Similarly, PSA mean value was higher in high-grade cases (17.4 ng/ml) compared to any prostate cancer (15.2 ng/ml). Apart from the group with PSA of 3-10 ng/ml, the PSA mean value of case groups was almost double as compared to control groups. For %fPSA, the mean value was slightly lower in high-grade cases than in any prostate cancer cases, 12.2 and 13.2, respectively. In addition, the mean %fPSA was higher in all controls compared to cases in all groups.

Table 5.3 to Table 5.5 show results from univariate and multivariate analysis with different comparison groups of controls including any prostate cancer case vs controls without prostate cancer for the UK cohort (Table 5.3), high-grade prostate cancer cases vs controls without prostate cancer (Table 5.4), and high-grade prostate cancer cases vs low-grade prostate cancer cases plus controls without prostate cancer (Table 5.5). Table 5.6 to Table 5.8 show the same comparison order as in Table 5.3 to Table 5.5 but only in PSA specific range of 3-10 ng/ml. Results from all comparisons suggested that all variables were independent and statistically significant predictors for both any and high-grade prostate cancers. Similar results were also applied to the PSA range between 3-10 ng/ml in the univariate logistic regression analysis. Multivariate analyses also confirmed that each of these variables was significantly associated with both types of prostate cancer cases (Tables 5.6-5.8).

5.3.1.1 Model performance

Discriminatory power

AUC plots are shown as follows; for any prostate cancer cases vs controls without prostate cancer (Figure 5.1), for high-grade prostate cancer cases vs controls without prostate cancer (Figure 5.2), and for high-grade prostate cancer cases vs low-grade prostate cancer cases plus controls without prostate cancer (Figure 5.3). In each figure, there are three separate lines representing AUC for age, AUC for age and PSA and AUC for the full model (age, PSA and %fPSA). In all figures, there is a difference in AUC between age alone, age and PSA, and full model. Each variable has an added value to the model performance in discriminating between cases and controls, with the full model being the highest performance in each comparison. The AUC of the full model is; 0.76 for any prostate cancer, 0.89 for high-grade vs controls without prostate cancer, and 0.78 for high-grade vs low-grade plus controls without prostate cancer.

Figure 5.4 to Figure 5.6 demonstrate a similar sequence of AUC plots as in Figure 5.1 to 5.3. These figures show AUC plots only in the PSA range of 3-10 ng/ml. The results are consistent with previous analyses, although the performance is slightly less than when compared to the entire cohort.

Internal validation

A 10-fold cross-validation of the UK cohort yielded a mean AUC of 0.75 with bootstrap bias-corrected 95% CI of 0.68-0.77 in any prostate cancer analyses. For high-grade prostate cancer vs control without prostate cancer, the mean AUC was 0.86 with bootstrap bias-corrected 95% CI of 0.82-0.89, whereas in high-grade vs low-grade plus controls without prostate cancer, it was 0.74 with bootstrap bias-corrected 95% CI of 0.70-0.78.

Model calibration

Figure 5.7, 5.8 and 5.9 illustrate calibration plot for the prediction model for any prostate cancer cases vs controls without prostate cancer (Figure 5.7), high-grade prostate cancer cases vs controls without prostate cancer (Figure 5.8), and high-grade prostate cancer vs low-grade plus controls without prostate cancer (Figure 5.9). Figure 5.10 to Figure 5.12 illustrate calibration plots for the same comparison order but with a PSA range of 3-10 ng/ml. The plots suggest models were not well calibrated in the UK cohort for all sub-groups analyses except in high-grade cases vs low-grade plus controls without prostate cancer. It is to be noted that a non-significant p-value is indicative of good model calibration. The p-value of high-grade vs low-grade plus controls without prostate cancer was 0.064 and <0.05 for the rest (Figures 5.7-5.12).

Table 5.1: Number of cases and controls in each group within cohorts.

Cohort	Group	Number (% of total cohort)	Definition
UK Total Subject =554	Low-grade	93 (16.79%)	Gleason score<=6
	High-grade cancer	266 (48.01%)	Gleason score>=7
	Any cancer	359 (64.80%)	Low-grade and high-grade
	Control with no cancer	195 (35.20%)	No cancer detected at biopsy
Polish Total Subjects = 315	Low-grade	75 (23.81%)	Gleason score<=6
	High-grade cancer	56 (17.78%)	Gleason score>=7
	Any cancer	131 (41.59%)	Low-grade and high-grade
	Control with no cancer	184 (58.41%)	No cancer detected at biopsy

Illustration of probability cut-off threshold

Table 5.9, Table 5.10 and Table 5.11 show the number and percentage of controls that would not be referred to biopsy, the number and percentage of cases that would miss biopsy, sensitivity, specificity, PPV, and NPV with their 95% CI set at threshold probabilities of 5, 9, 10, 12.5, 15, and 20%. For any prostate cancer, the results are shown in Table 5.9. For high-grade vs controls without prostate cancer, results are shown in Table 5.10. For high-grade vs low-grade plus controls without prostate cancer, results are shown in Table 5.11.

For any prostate cancer (Table 5.9), from a cut-off of 5% to 12.5%, there are no missing significant cases and as a result, the sensitivity was 100% in each of these cut-offs. At the highest cut-off of 20%, the model yielded 99.7% sensitivity and 7.18% specificity, whereas with the minimum cut-off of 5%, the sensitivity was 100% and the specificity was 2.05%. In the high-grade vs controls without prostate cancer, the model yielded a sensitivity of 93.6% and specificity of 34.9% at a cut-off of 20%, whereas at a cut-off of 5%, the sensitivity and specificity were 98.9% and 13.3%, respectively. For high-

grade vs low-grade plus controls without prostate cancer, the model yielded 94.4% sensitivity and 21.2% specificity at a cut-off of 20%, while with a cut-off of 5%, the sensitivity increased to 99.6% but the specificity decreased to 3.82%.

Table 5.12, Table 5.13 and Table 5.14 show similar comparison group order as previously described in Tables 5.9-5.11 but within the PSA range of 3-10 ng/ml. In any prostate cancer, there are not enough cases and controls in that range for the determined cut-offs (Table 5.12). However, in high-grade vs controls without prostate cancer, the model yielded a sensitivity of 91.3% and specificity of 30.3% at a cut-off of 20%. Lowering the cut-off to 5% yielded a 98.4% sensitivity and 5.63% specificity (Table 5.13). For high-grade vs low-grade plus controls without prostate cancer, when using a cut-off of 15%, the sensitivity was 96% while the specificity was 7.89%. Nevertheless, when using a 20% cut-off, the sensitivity was slightly less at 92.1%, but the specificity doubled at 16.3% (Table 5.14).

The summary of AUC values derived from univariate and multivariate modelling and their differences between model 1 (Age) and model 2 (PSA and Age) are shown in Table 5.27-Table 5.32. The AUC differences were statistically significant in all sub-groups except when restricting subjects with a PSA range of 3-10 ng/ml. The AUC difference between model 2 and model 3 (PSA and Age and %fPSA) was also statistically significant in all sub-groups. Similar results were also applied to the PSA range of 3-10 ng/ml (Table 5.27-Table 5.32).

5.3.2 The Polish cohort:

In the Polish cohort, men with high-grade cancer were slightly older than men with any prostate cancer as the mean age was 66.6 yrs and 66.2 yrs, respectively. Also, the mean age for all types of controls was slightly less than in cases in each group of analyses, except for any prostate cancer within the PSA range of 3-10 ng/ml. In contrast, PSA

mean was higher in high-grade cases (16.7 ng/ml) compared to any prostate cancer (11.0 ng/ml). Similar to the UK cohort (apart from restricting the analyses to PSA of 3-10 ng/ml), the PSA mean value more than doubled in case groups. For %fPSA, the mean was slightly lower in high-grade cases than in any prostate cancer cases (13.5 and 15.1, respectively). In addition, the mean %fPSA value was higher in all controls compared to cases in all groups, as shown in Table 5.2.

Table 5.15 to Table 5.17 summarise results from univariate and multivariate analyses with different comparison groups of controls including any prostate cancer cases vs controls without prostate cancer (Table 5.15), high-grade prostate cancer cases vs controls without prostate cancer (Table 5.16), and high-grade prostate cancer cases vs low-grade prostate cancer cases plus controls without prostate cancer (Table 5.17). Table 5.18 to Table 5.20 show the same comparison order as in Tables 5.15 to 5.17 but only with a PSA specific range of 3-10 ng/ml. Univariate analyses suggested that, except for 'age' where it has a borderline association, PSA and %fPSA were independent and statistically significant predictors for both any and high-grade prostate cancers. A borderline association was also seen with multivariate analyses in PSA in any prostate cancer and age in high-grade vs low-grade plus controls without prostate cancer. Similar results were also applied to the PSA range between 3-10 ng/ml in the univariate logistic regression analysis in the Polish cohort. Multivariate analyses confirmed that only %fPSA variable was significantly associated with both types of prostate cancer risk (Table 5.18-Table 5.20).

5.3.2.1 Model performance

Discriminatory power

Figure 5.13, Figure 5.14 and Figure 5.15 show AUC plots for any prostate cancer cases vs controls without prostate cancer (Figure 5.13), high-grade prostate cancer cases vs

controls without prostate cancer (Figure 5.14), and high-grade prostate cancer cases vs low-grade prostate cancer cases plus controls without prostate cancer (Figure 5.15). Similar to the UK cohort, there is a difference in AUC between age alone, age and PSA, and the full model, with the full model being the highest performance in each comparison. The AUC of the full model is; 0.76 for any prostate cancer, 0.83 for high-grade vs controls without prostate cancer, and 0.77 for high-grade vs low-grade plus controls without prostate cancer. Figure 5.16 to Figure 5.18 depict a similar order of model assessment as in Figure 5.13 to 5.15 but was restricted to a PSA range of 3-10 ng/ml. Likewise, each variable had an added value in model performance, although the performance was slightly less compared to the entire cohort.

Internal validation

The mean AUC in the Polish cohort after 10-fold cross-validation was 0.76 for any prostate cancer with bootstrap bias-corrected 95% CI of 0.67-0.79, 0.80 for high-grade prostate cancer versus controls without prostate cancer with 95% CI of 0.73-0.87, and 0.79 with 95% CI of 0.68-0.83 for high-grade versus low-grade plus controls without prostate cancer.

Model calibration

Figure 5.19, Figure 5.20 and Figure 5.21 illustrate calibration plots in the Polish cohort for the prediction model for any prostate cancer cases vs controls without prostate cancer (Figure 5.19), high-grade prostate cancer cases vs controls without prostate cancer (Figure 5.20), and high-grade prostate cancer vs low-grade plus controls without prostate cancer (Figure 5.21). Figure 5.22 to Figure 5.24 illustrate calibration plots for the same comparison order but with a PSA range of 3-10 ng/ml. Overall, the model was well calibrated in the Polish cohort for all sub-groups as all the p-values were above 0.05 in all analyses (Figure 5.19-Figure 5.24).

Illustration of probability cut-off threshold

Table 5.21, Table 5.22 and Table 5.23 show the number and percentage of controls that would not have been referred for biopsy, the number and percentage of cases that would miss biopsy, sensitivity, specificity, PPV, and NPV with their 95% CI at threshold probabilities of 5, 9, 10, 12.5, 15, and 20%, in the Polish cohort. For any prostate cancer, the results are shown in Table 5.21. For high-grade VS controls without prostate cancer, the results are shown in Table 5.22. For high-grade VS low-grade plus controls without prostate cancer, the results are shown in Table 5.23

For any prostate cancer, all cut-offs yielded a sensitivity of 90% and above with the highest specificity achieved at a cut-off of 20% where it was 28.3% (Table 5.21). In the high-grade vs controls without prostate cancer, only cut-offs of 12.5% and below yielded a sensitivity of 90% and above, whereas specificity ranges between 26.6% and 48.9% (Table 5.22). For high-grade vs low-grade plus controls without prostate cancer, only cut-offs of 10% and below yielded a sensitivity of 90% and above, where the specificity ranges between 18.9% and 41.3% (Table 5.23).

Table 5.24 to Table 5.26 show similar comparison order as Table 5.21 to 5.23 but within a PSA range of 3-10 ng/ml. In any prostate cancer, there are not enough cases and controls in that range for the cut-off of 5%. Similar to the analysis of the entire cohort, all cut-offs yielded a sensitivity above 90% with the highest specificity (16.7%) achieved at a cut-off of 20% (Table 5.24). In high-grade vs controls without prostate cancer, the model yielded a sensitivity of 90% and above in all cut-offs, except at 20%, and specificity ranges between 24.6% to 55.3% (Table 5.25). For high-grade vs low-grade plus controls without prostate cancer, only cut-offs of 5, 9, and 10 yielded a sensitivity of 90% and above where the specificity ranged between 25.2% and 45.4% (Table 5.26).

The summary of AUC values derived from univariate and multivariate modelling and their differences between model 1 (Age) and model 2 (PSA and Age) are shown in Table 5.27-Table 5.32. The difference in the Polish cohort between model 1 (Age) and model 2 (PSA and Age) was significant in all sub-groups except when restricting subjects with a PSA range of 3-10 ng/ml. However, the difference between model 2 and model 3 (PSA and Age and %fPSA) was significant in all sub-groups as well as when restricting subjects to a PSA range of 3-10 ng/ml (Table 5.27-Table 5.32).

Table 5.2: Variables means for each group in the UK and Polish cohorts.

Sub-groups / Variables	The UK cohort		Polish Cohort	
	Cases	Controls	Cases	Controls
Age, years mean (±SD)				
Any PCa VS Controls without PCa*†	66.8 (±7.1)	62.6 (±6.8)	66.2 (±7.7)	64.2 (±8.3)
Any PCa VS Controls without PCa within PSA 3-10ng/ml*	65.5 (±7.1)	62.5 (±6.7)	64.9 (±7.5)	65.0 (±7.8)
High-grade VS Controls without PCa*	67.3 (±7.1)	61.5 (±6.8)	66.6 (±8.0)	64.2 (±8.3)
High-grade VS Controls without PCa within PSA 3-10ng/ml*	65.9 (±7.0)	61.4 (±6.6)	65.1 (±7.7)	65.0 (±7.8)
High-grade VS Low-grade + Controls without PCa*	67.3 (±7.1)	63.4 (±7.0)	66.6 (±8.0)	64.7 (±8.1)
High-grade VS Low-grade + Controls without PCa within PSA 3-10ng/ml*	65.9 (±7.0)	63.2 (±7.0)	65.1 (±7.7)	64.9 (±7.7)
PSA, mean (±SD)				
Any PCa VS Controls without PCa*†	15.2 (±21.9)	7.9 (±5.6)	11.0 (±27.0)	4.8 (±4.4)
Any PCa VS Controls without PCa within PSA 3-10ng/ml*	6.7 (±1.8)	6.1 (±1.7)	5.6 (±1.7)	5.2 (±1.6)
High-grade VS Controls without PCa*†	17.4 (±24.6)	6.4 (±3.6)	16.7 (±40.3)	4.8 (±4.4)
High-grade VS Controls without PCa within PSA 3-10ng/ml*†	6.8 (±1.9)	5.9 (±1.6)	6.1 (±1.9)	5.2 (±1.6)
High-grade VS Low-grade + Controls without PCa*†	17.4 (±24.6)	8.1 (±6.5)	16.7 (±40.3)	5.4 (±4.7)
High-grade VS Low-grade + Controls without PCa within PSA 3-10ng/ml*†	6.8 (±1.9)	6.2 (±1.7)	6.1 (±1.9)	5.3 (±1.6)
%fPSA, mean (±SD)				
Any PCa VS Controls without PCa*†	13.2 (±6.5)	17.5 (±6.9)	15.1 (±8.8)	24.4 (±15.5)
Any PCa VS Controls without PCa within PSA 3-10ng/ml*†	14.7 (±5.9)	16.7 (±5.7)	15.9 (±8.0)	22.0 (±10.4)
High-grade VS Controls without PCa*†	12.2 (±5.9)	19.0 (±7.1)	13.5 (±7.7)	24.4 (±15.5)
High-grade VS Controls without PCa within PSA 3-10ng/ml*†	13.8 (±5.4)	17.8 (±6.1)	13.6 (±7.1)	22.0 (±10.4)
High-grade VS Low-grade + Controls without PCa*†	12.2 (±5.9)	17.0 (±7.0)	13.5 (±7.7)	22.0 (±14.4)
High-grade VS Low-grade + Controls without PCa within PSA 3-10ng/ml*†	13.8 (±5.4)	16.6 (±6.0)	13.6 (±7.1)	20.6 (±10.0)

PCa = Prostate cancer.

*The difference in mean between cases and controls are statistically significance in the UK cohort p-value <0.05.

†The difference in mean between cases and controls are statistically significance in the Polish cohort p-value <0.05.

Table 5.3: Univariate and multivariate logistic regression analyses in the UK cohort for any prostate cancer VS controls without prostate cancer.

Variables	Univariate logistic regression analysis			Multivariate logistic regression analysis		
	OR	95% CI	P Value	OR	95% CI	P value
Age	1.09	1.06 - 1.12	<0.001	1.10	1.07 - 1.13	<0.001
PSA	1.08	1.05 - 1.12	<0.001	1.03	1.01 - 1.06	<0.05
%fPSA	0.91	0.88 - 0.94	<0.001	0.90	0.87 - 0.93	<0.001

Table 5.4: Univariate and multivariate logistic regression analyses in the UK cohort for high-grade prostate cancer VS controls without prostate cancer.

Variables	Univariate logistic regression analysis			Multivariate logistic regression analysis		
	OR	95% CI	P Value	OR	95% CI	P value
Age	1.12	1.09 – 1.16	<0.001	1.20	1.15 – 1.26	<0.001
PSA	1.23	1.16 – 1.30	<0.001	1.12	1.06 – 1.19	<0.001
%fPSA	0.85	0.82 – 0.88	<0.011	0.80	0.76 – 0.84	<0.001

Table 5.5: Univariate and multivariate logistic regression analyses in the UK cohort for high-grade prostate cancer VS low-grade plus controls without prostate cancer.

Variables	Univariate logistic regression analysis			Multivariate logistic regression analysis		
	OR	95% CI	P Value	OR	95% CI	P value
Age	1.08	1.05 – 1.11	<0.001	1.09	1.06 – 1.13	<0.001
PSA	1.09	1.06 – 1.12	<0.001	1.05	1.02 – 1.07	0.001
%fPSA	0.89	0.86 – 0.91	<0.001	0.88	0.85 – 0.92	<0.001

Table 5.6: Univariate and multivariate logistic regression analyses in the UK cohort for any prostate cancer vs controls without prostate cancer within PSA range of 3-10 ng/ml.

Variables	Univariate logistic regression analysis			Multivariate logistic regression analysis		
	OR	95% CI	P Value	OR	95% CI	P value
Age	1.06	1.03 – 1.10	<0.001	1.08	1.03 – 1.12	<0.001
PSA	1.20	1.05 – 1.36	<0.05	1.05	0.91 – 1.21	0.487
%fPSA	0.94	0.91 – 0.98	<0.05	0.92	0.88 – 0.96	<0.001

Table 5.7: Univariate and multivariate logistic regression analyses in the UK cohort for high-grade prostate cancer vs controls without prostate cancer within PSA range of 3-10 ng/ml.

Variables	Univariate logistic regression analysis			Multivariate logistic regression analysis		
	OR	95% CI	P Value	OR	95% CI	P value
Age	1.10	1.06 – 1.15	<0.001	1.17	1.11 – 1.24	<0.001
PSA	1.33	1.16 – 1.53	<0.001	1.04	0.88 – 1.24	0.635
%fPSA	0.88	0.84 – 0.92	<0.001	0.82	0.76 – 0.87	<0.001

Table 5.8: Univariate and multivariate logistic regression analyses in the UK cohort for high-grade prostate cancer vs low-grade plus Controls without prostate cancer within PSA range of 3-10 ng/ml.

Variables	Univariate logistic regression analysis			Multivariate logistic regression analysis		
	OR	95% CI	P Value	OR	95% CI	P value
Age	1.06	1.02 – 1.09	<0.05	1.08	1.03 – 1.12	<0.001
PSA	1.22	1.07 – 1.39	<0.05	1.07	0.92 – 1.12	0.364
%fPSA	0.91	0.87 – 0.95	<0.001	0.89	0.85 – 0.94	<0.001

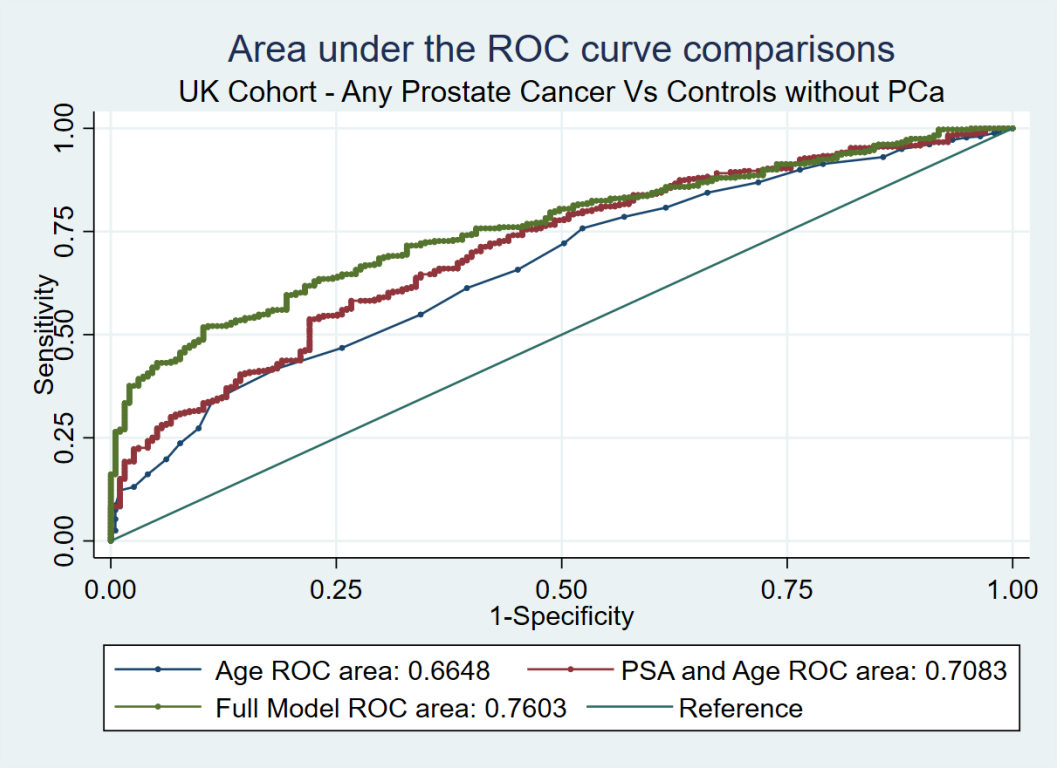

Figure 5.1: The receiver operating characteristic curve of the prediction model for any prostate cancer VS controls without prostate cancer in the UK cohort.

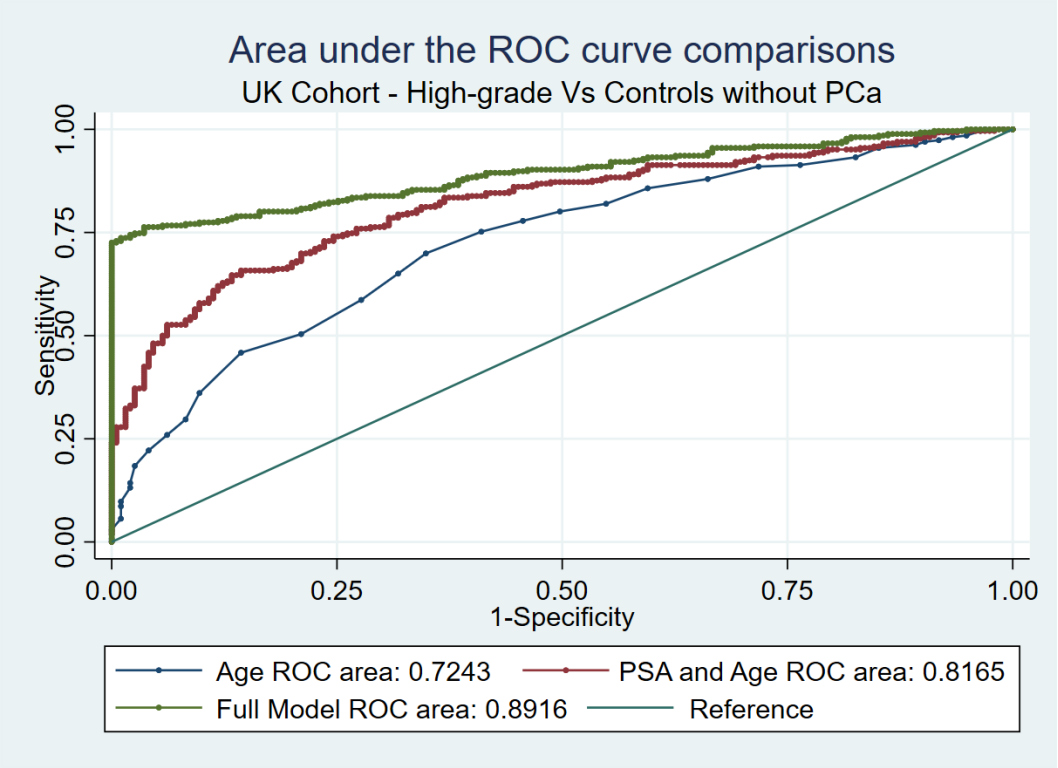

Figure 5.2: The receiver operating characteristic curve of the prediction model for high-grade prostate cancer VS controls without prostate cancer in the UK cohort.

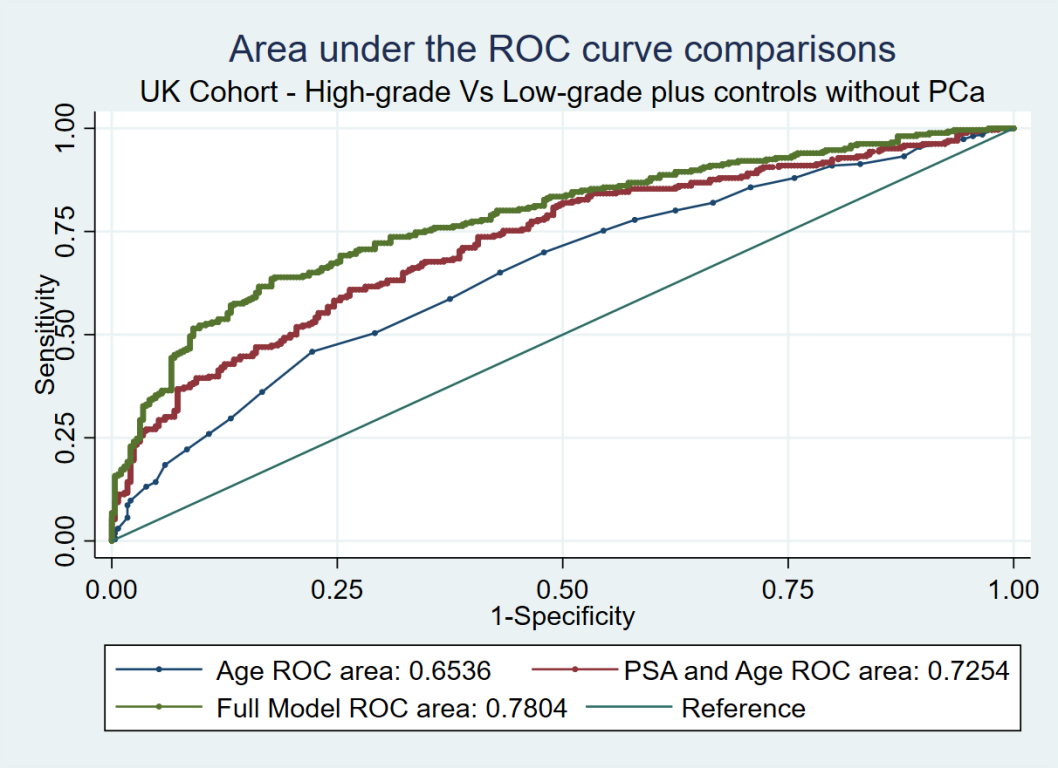

Figure 5.3: The receiver operating characteristic curve of the prediction model for high-grade prostate cancer VS low-grade plus controls without prostate cancer in the UK cohort.

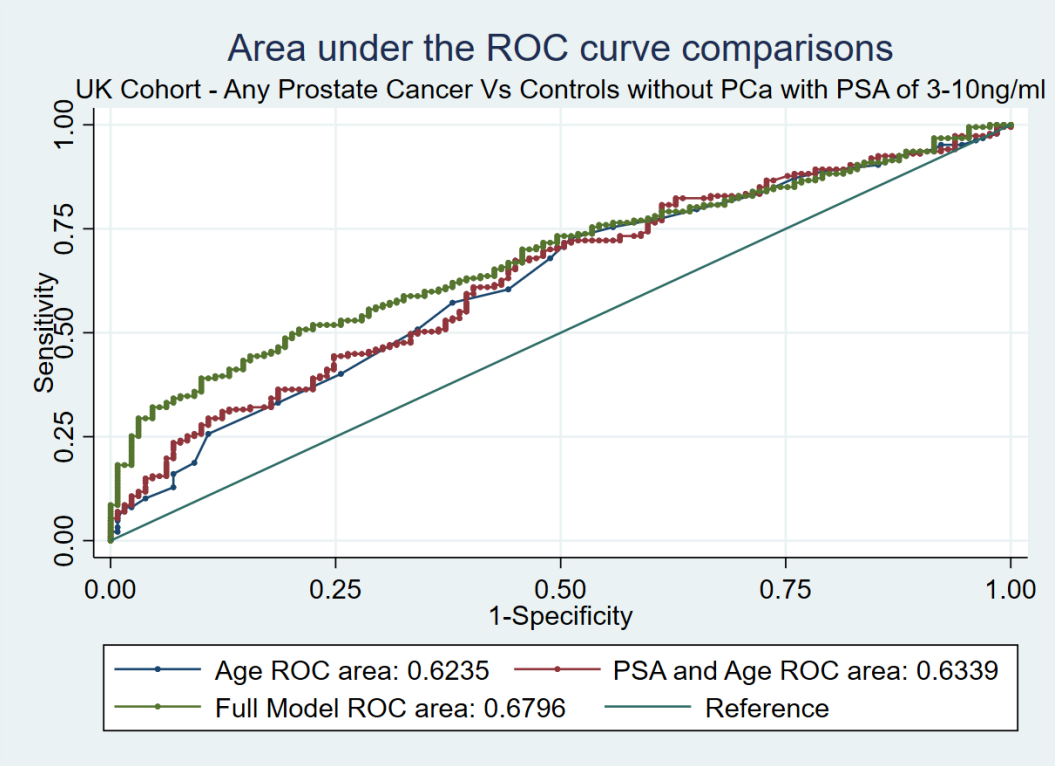

Figure 5.4: The receiver operating characteristic curve of the prediction model for any prostate cancer VS controls without prostate cancer with a PSA range of 3-10 ng/ml in the UK cohort.

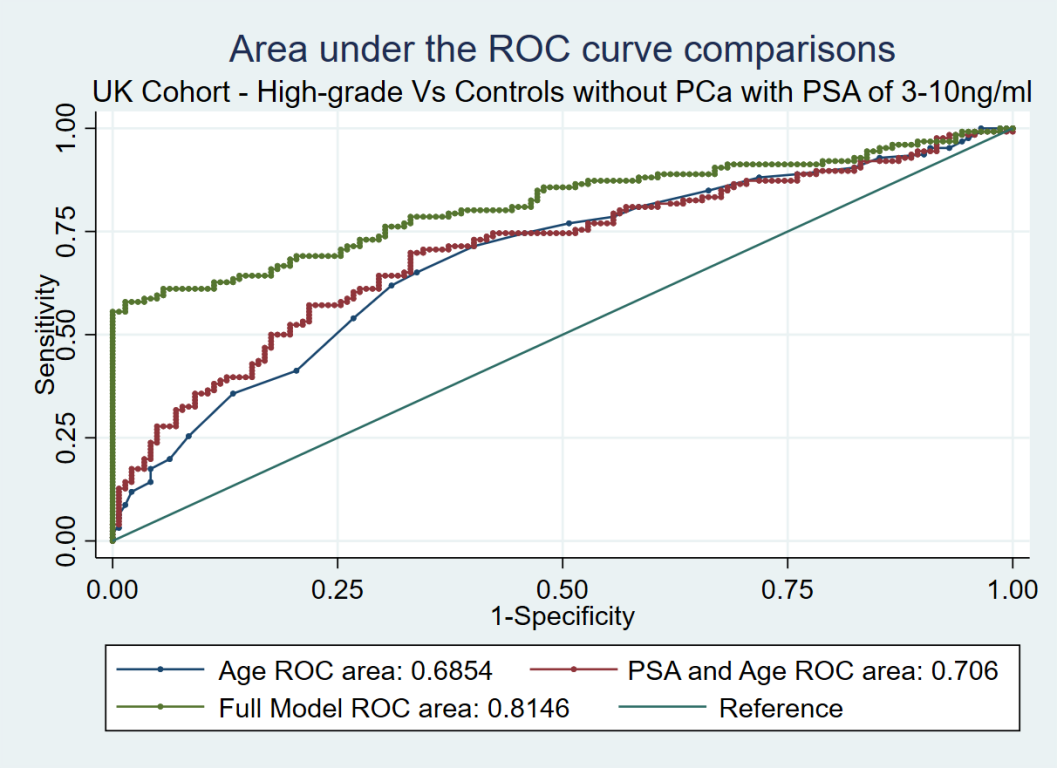

Figure 5.5: The receiver operating characteristic curve of the prediction model for high-grade prostate cancer VS controls without prostate cancer with a PSA range of 3-10 ng/ml in the UK cohort.

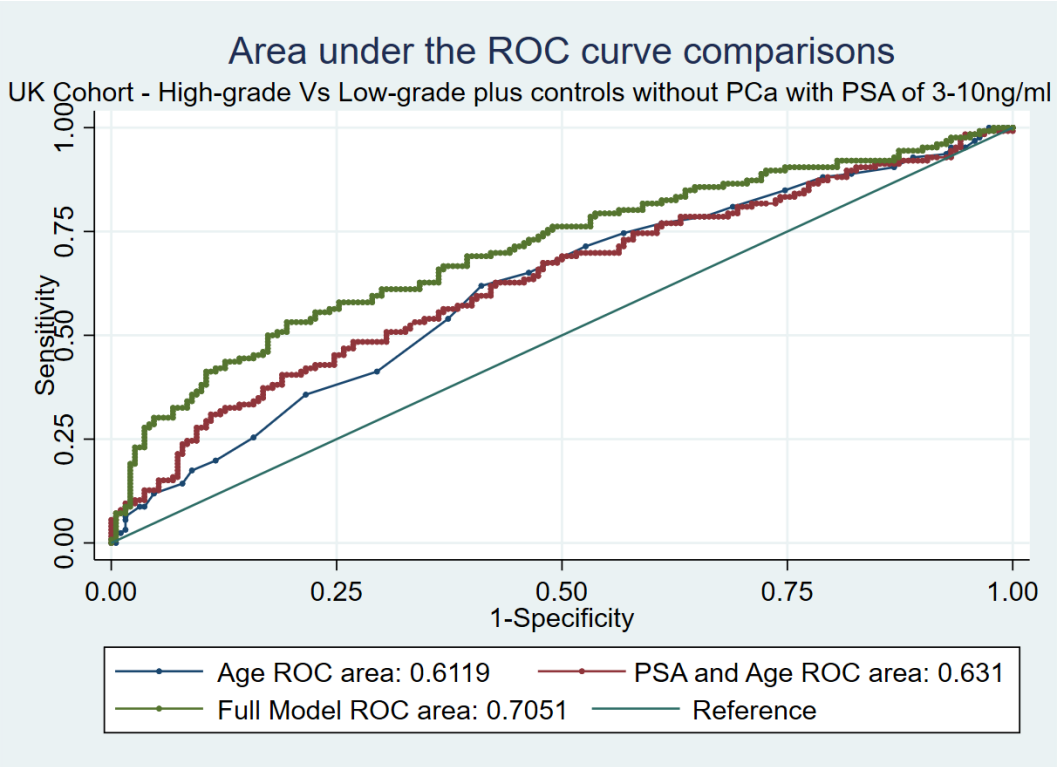

Figure 5.6: The receiver operating characteristic curve of the prediction model for high-grade prostate cancer VS low-grade plus controls without prostate cancer with a PSA range of 3-10 ng/ml in the UK cohort.

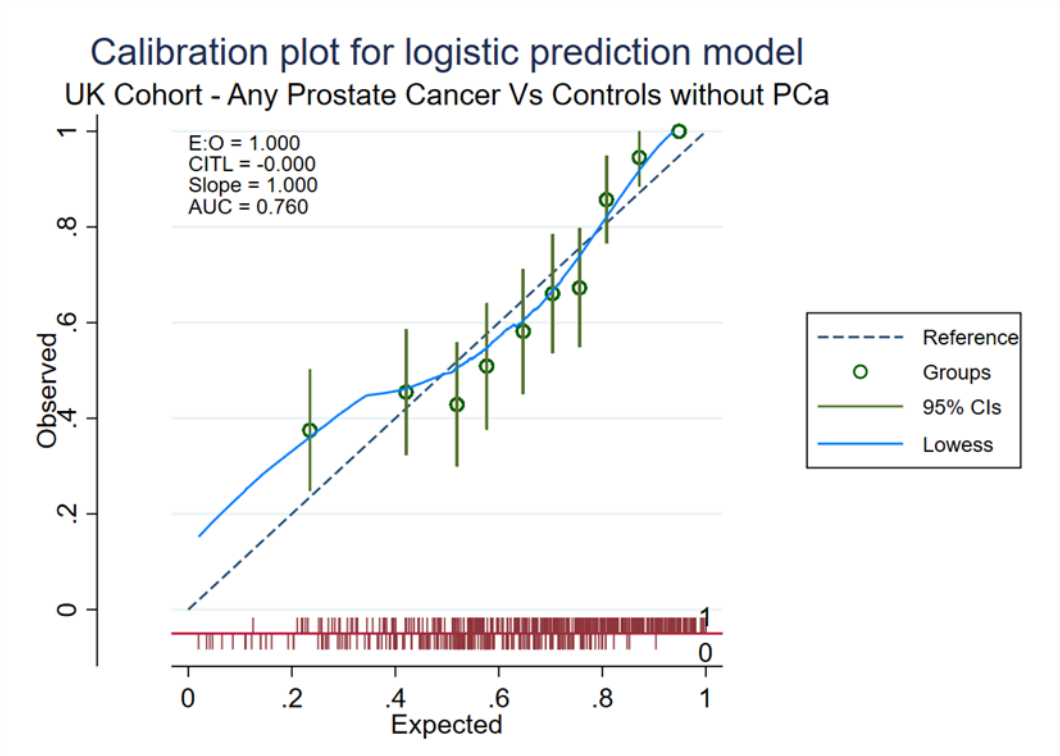

Figure 5.7: Calibration plot for logistic prediction model in the UK cohort for any prostate cancer VS controls without prostate cancer.

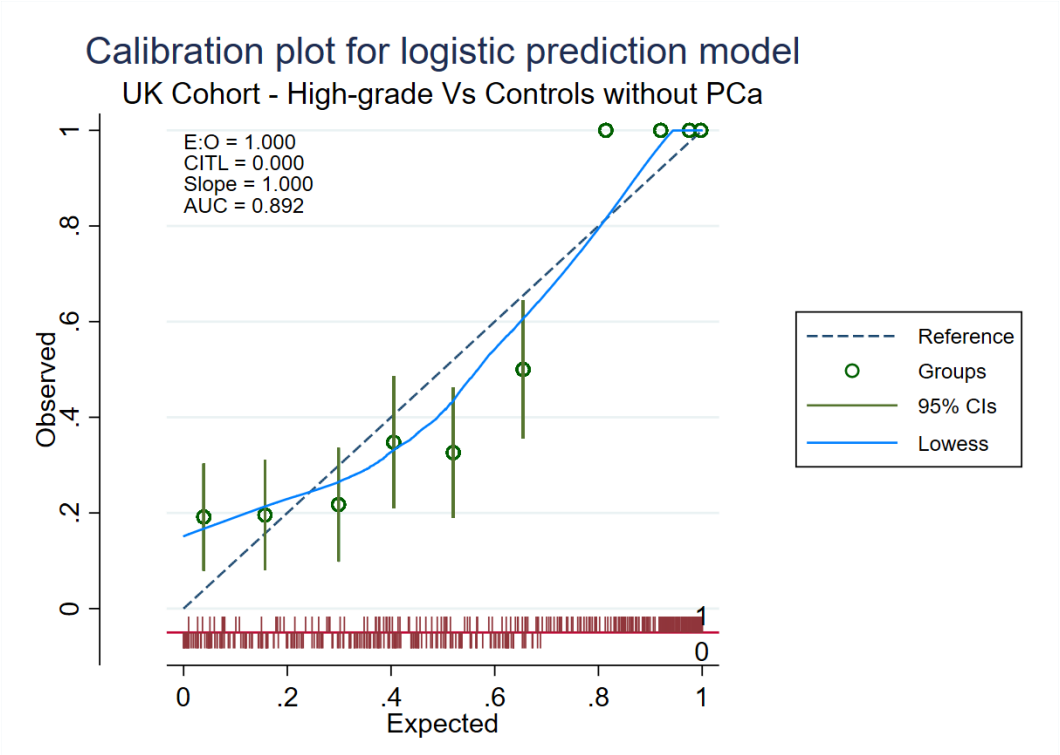

Figure 5.8: Calibration plot for logistic prediction model in the UK cohort for high-grade prostate cancer VS controls without prostate cancer.

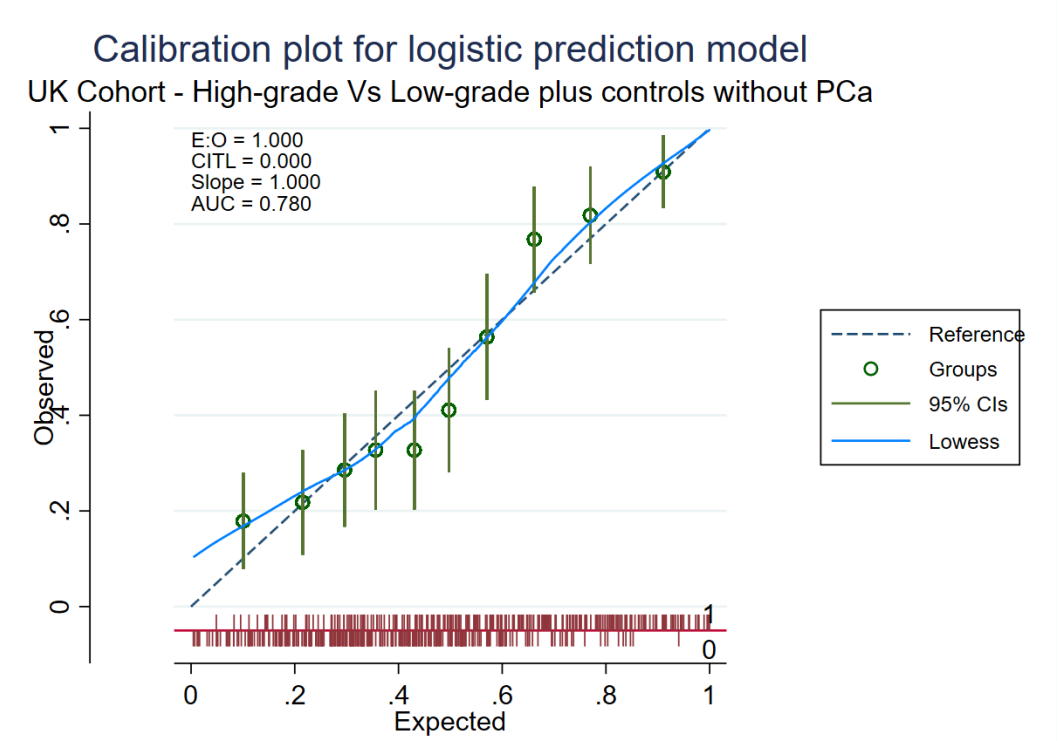

Figure 5.9: Calibration plot for logistic prediction model in the UK cohort for high-grade prostate cancer VS low-grade plus controls without prostate cancer.

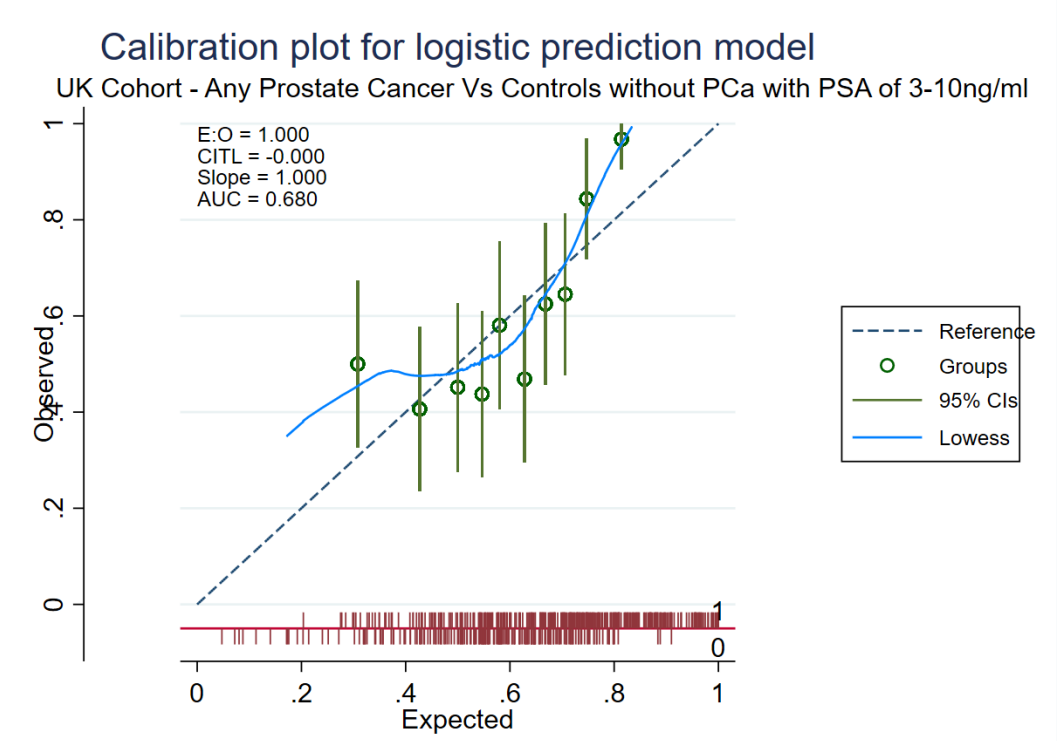

Figure 5.10: Calibration plot for logistic prediction model in the UK cohort for any prostate cancer VS controls without prostate cancer within PSA range of 3-10 ng/ml.

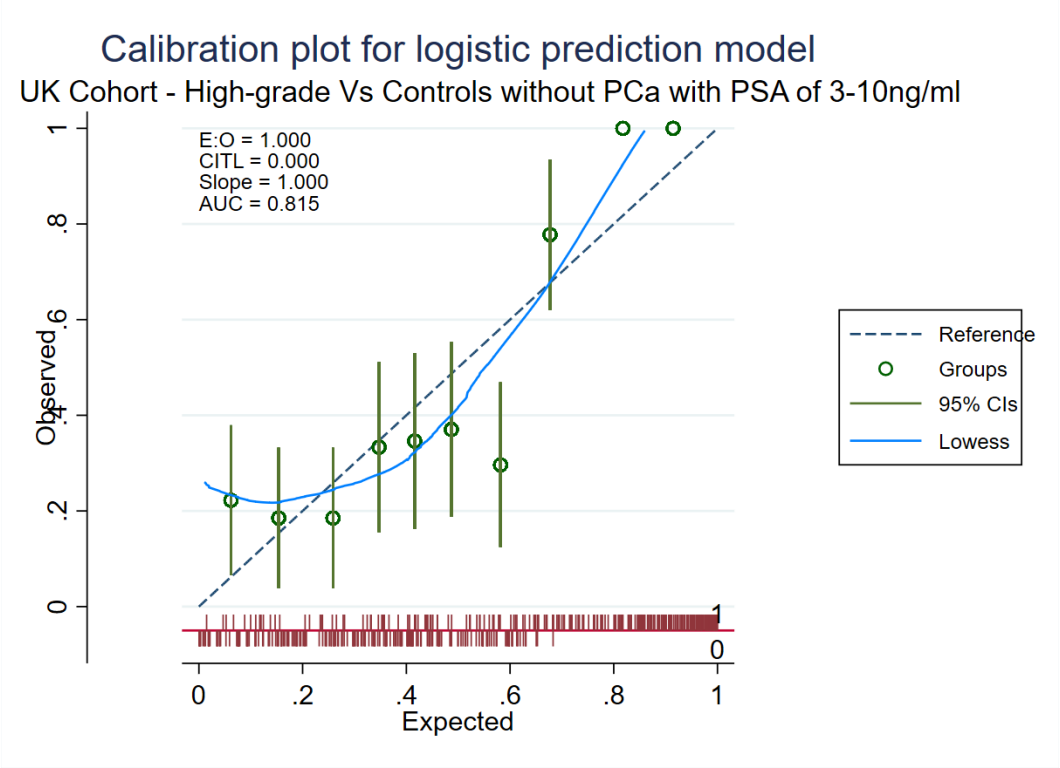

Figure 5.11: Calibration plot for logistic prediction model in the UK cohort for high-grade prostate cancer VS controls without prostate cancer within PSA range of 3-10 ng/ml.

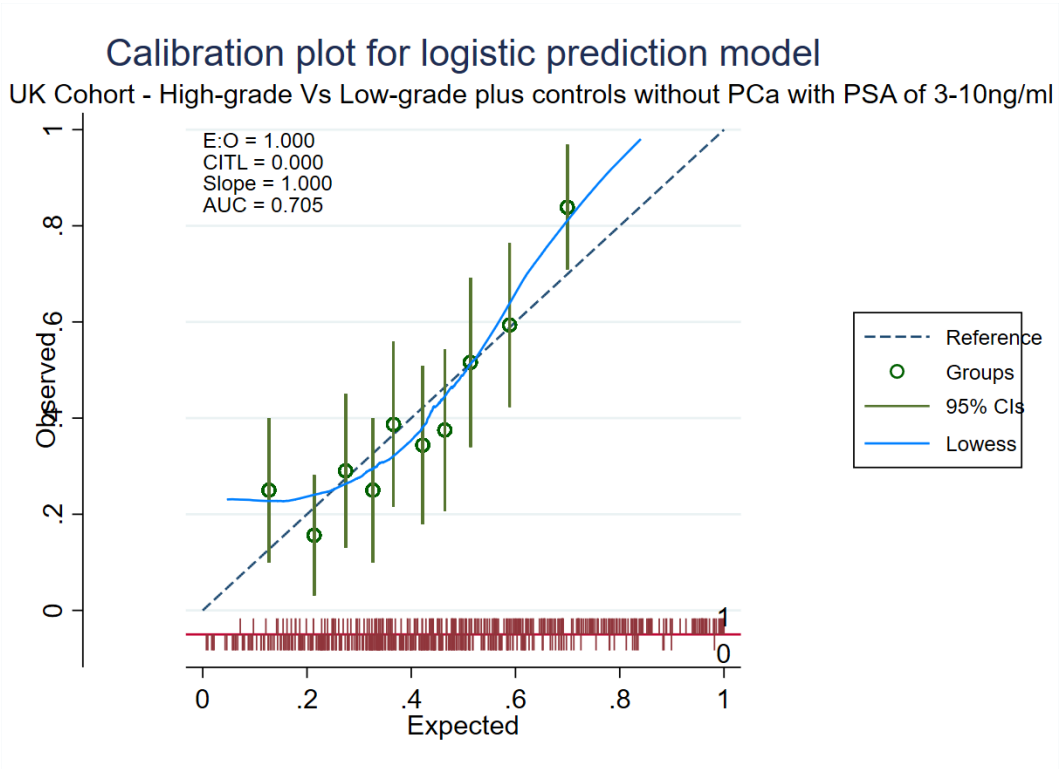

Figure 5.12: Calibration plot for logistic prediction model in the UK cohort for high-grade prostate cancer VS low-grade plus controls without prostate cancer within PSA range of 3-10 ng/ml.

Table 5.9: Cut-points probabilities in the UK cohort for any prostate cancer VS controls without prostate cancer.

Probability cut point	No. of controls that would not refer to biopsy (%)	No. of cases that would miss biopsy (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
5%	4 (2.05%)	0 (0.00%)	100% 99.00–100.00	2.05% 0.56-5.17	65.3% 61.10-69.30	100% 39.80–100.00
9%	6 (3.08%)	0 (0.00%)	100% 99.00–100.00	3.08% 1.14-6.58	65.5% 61.40-69.50	100% 54.10–100.00
10%	6 (3.08%)	0 (0.00%)	100% 99.00–100.00	3.08% 1.14-6.58	65.5% 61.40-69.50	100% 54.10–100.00
12.5%	9 (4.62%)	0 (0.00%)	100% 99.00–100.00	4.62% 2.13-8.58	65.9% 61.70-69.80	100% 66.40–100.00
15%	11 (5.64%)	1 (0.28%)	99.7% 98.50–100.00	5.64% 2.85-9.87	66.1% 61.90–70.00	91.7% 61.50-99.80
20%	14 (7.18%)	1 (0.28%)	99.7% 98.50–100.00	7.18% 3.98-11.8	66.4% 62.30-70.40	93.3% 68.10-99.80

Table 5.10: Cut-points probabilities in the UK cohort for high-grade VS controls without prostate cancer.

Probability cut point	No. of controls that would not refer to biopsy (%)	No. of cases that would miss biopsy (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
5%	26 (13.33%)	3 (1.13%)	98.9% 96.70-99.80	13.3% 8.90-18.90	60.9% 56.10-65.50	89.7% 72.60-97.80
9%	39 (20.00%)	9 (3.38%)	96.6% 93.70-98.40	20% 14.60-26.30	62.2% 57.40-66.90	81.3% 67.40-91.10
10%	41 (21.03%)	9 (3.38%)	96.6% 93.70-98.40	21% 15.50-27.40	62.5% 57.70-67.20	82% 68.60-91.40
12.5%	48 (24.62%)	11 (4.14%)	95.9% 92.70-97.90	24.6% 18.70-31.30	63.4% 58.50-68.20	81.4% 69.10-90.30
15%	55 (28.21%)	11 (4.14%)	95.9% 92.70-97.90	28.2% 22.00-35.10	64.6% 59.60-69.30	83.3% 72.10-91.40
20%	68 (34.87%)	17 (6.39%)	93.6% 90.00-96.20	34.9% 28.20-42.00	66.2% 61.20-71.00	80% 69.90-87.90

Table 5.11: Cut-points probabilities in the UK cohort for high-grade prostate cancer cases VS low-grade plus controls without prostate cancer.

Probability cut point	No. of controls that would not refer to biopsy (%)	No. of cases that would miss biopsy (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
5%	11 (3.82%)	1 (0.38%)	99.6% 97.90 – 100.00	3.82% 1.92 - 6.73	48.9% 44.60 - 53.20	91.7% 61.50-99.80
9%	20 (6.94%)	2 (0.75%)	99.2% 97.30 - 99.90	6.94% 4.29 - 10.50	49.6% 45.30 – 54.00	90.9% 70.80-98.90
10%	22 (7.64%)	3 (1.13%)	98.9% 96.70 - 99.80	7.64% 4.85 - 11.30	49.7% 45.40 - 54.10	88% 68.80-97.50
12.5%	31 (10.76%)	4 (1.50%)	98.5% 96.20- 99.60	10.8% 7.43 - 14.90	50.5% 46.10 - 54.90	88.6% 73.30-96.80
15%	38 (13.19%)	9 (3.38%)	96.6% 93.70 - 98.40	13.2% 9.51 - 17.70	50.7% 46.20 - 55.10	80.9% 66.70-90.90
20%	61 (21.18%)	15 (5.64%)	94.4% 90.90 - 96.80	21.2% 16.60 - 26.40	52.5% 47.90 - 57.10	80.3% 69.50-88.50

Table 5.12: Cut-points probabilities in the UK cohort for any prostate cancer VS controls without prostate cancer in patients with PSA range 3-10 ng/ml.

Probability cut point	No. of controls that would not refer to biopsy (%)	No. of cases that would miss biopsy (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
20%	2 1.55%	0 0.00%	100% 98.00–100.00	1.55% 0.19–5.49	59.6% 53.90–65.00	100% 15.80–100.00

Table 5.13: Cut-points probabilities in the UK cohort for high-grade prostate cancer patients VS controls without prostate cancer with PSA range 3-10 ng/ml.

Probability cut point	No. of controls that would not refer to biopsy (%)	No. of cases that would miss biopsy (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
5%	8 (5.63%)	2 (1.59%)	98.4% 94.40-99.80	5.63% 2.46-10.80	48.1% 41.80-54.30	80% 44.40-97.50
9%	15 (10.56%)	5 (3.97%)	96% 91.00-98.70	10.6% 6.03-16.80	48.8% 42.40-55.20	75% 50.90-91.30
10%	19 (13.38%)	6 (4.76%)	95.2% 89.90-98.20	13.4% 8.25-20.10	49.4% 42.90-55.80	76% 54.90-90.60
12.5%	23 (16.20%)	9 (7.14%)	92.9% 86.90-96.70	16.2% 10.60-23.30	49.6% 43.00-56.10	71.9% 53.30-86.30
15%	27 (19.01%)	10 (7.94%)	92.1% 85.90-96.10	19% 12.90-26.40	50.2% 43.60-56.80	73% 55.90-86.20
20%	43 (30.28%)	11 (8.73%)	91.3% 84.90-95.60	30.3% 22.90-38.50	53.7% 46.80-60.60	79.6% 66.50-89.40

Table 5.14: Cut-points probabilities in the UK cohort for high-grade prostate cancer patients VS low-grade plus Controls without prostate cancer with PSA range 3-10 ng/ml.

Probability cut point	No. of controls that would not refer to biopsy (%)	No. of cases that would miss biopsy (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
5%	1 0.53%	0 0.00%	100% 97.10–100.00	0.53% 0.01–2.90	40% 34.50–45.60	100% 2.50–100.00
9%	6 3.16%	1 0.79%	99.2% 95.70–100.00	3.16% 1.17–6.75	40.5% 34.90–46.20	85.7% 42.10–99.60
10%	7 3.68%	2 1.59%	98.4% 94.40–99.80	3.68% 1.49–7.44	40.4% 34.90–46.10	77.8% 40.00–97.20
12.5%	9 4.74%	3 2.38%	97.6% 93.20–99.50	4.74% 2.19–8.80	40.5% 34.90–46.20	75% 42.80–94.50
15%	15 7.89%	5 3.97%	96% 91.00–98.70	7.89% 4.49–12.70	40.9% 35.20–46.70	75% 50.90–91.30
20%	31 16.32%	10 7.94%	92.1% 85.90–96.10	16.3% 11.40–22.40	42.2% 36.30–48.30	75.6% 59.70–87.60

Table 5.15: Univariate and multivariate logistic regression analyses in Polish cohort for any prostate cancer VS controls without prostate cancer.

Variables	Univariate logistic regression analysis			Multivariate logistic regression analysis		
	OR	95% CI	P Value	OR	95% CI	P value
Age	1.03	1.00 – 1.06	<0.05	1.05	1.01 – 1.08	<0.05
PSA	1.12	1.06 – 1.18	<0.001	1.06	1.00 – 1.12	<0.05
%fPSA	0.91	0.88 – 0.94	<0.001	0.92	0.89 – 0.95	<0.001

Table 5.16: Univariate and multivariate logistic regression analyses in Polish cohort for high-grade prostate cancer VS controls without prostate cancer.

Variables	Univariate logistic regression analysis			Multivariate logistic regression analysis		
	OR	95% CI	P Value	OR	95% CI	P value
Age	1.04	1.00 – 1.07	0.054	1.06	1.02 – 1.11	<0.05
PSA	1.13	1.06 – 1.20	<0.001	1.08	1.01 – 1.15	<0.05
%fPSA	0.88	0.84 – 0.92	<0.001	0.89	0.84 – 0.94	<0.001

Table 5.17: Univariate and multivariate logistic regression analyses in Polish cohort for high-grade prostate cancer VS low-grade plus controls without prostate cancer.

Variables	Univariate logistic regression analysis			Multivariate logistic regression analysis		
	OR	95% CI	P Value	OR	95% CI	P value
Age	1.03	0.99 – 1.07	0.102	1.04	1.00 – 1.09	<0.05
PSA	1.10	1.05 – 1.15	<0.001	1.06	1.01 – 1.12	<0.05
%fPSA	0.90	0.87 – 0.94	<0.001	0.92	0.88 – 0.96	<0.001

Table 5.18: Univariate and multivariate logistic regression analyses in Polish cohort for any prostate cancer VS controls without prostate cancer within PSA range of 3-10 ng/ml.

Variables	Univariate logistic regression analysis			Multivariate logistic regression analysis		
	OR	95% CI	P Value	OR	95% CI	P value
Age	1.00	0.96 – 1.04	0.913	1.02	0.98 – 1.07	0.272
PSA	1.13	0.95 – 1.35	0.149	1.05	0.87 – 1.27	0.602
%fPSA	0.92	0.89 – 0.96	<0.001	0.92	0.88 – 0.96	<0.001

Table 5.19: Univariate and multivariate logistic regression analyses in Polish cohort for high-grade prostate cancer VS controls without prostate cancer within PSA range of 3-10 ng/ml.

Variables	Univariate logistic regression analysis			Multivariate logistic regression analysis		
	OR	95% CI	P Value	OR	95% CI	P value
Age	1.00	0.95 – 1.05	0.954	1.03	0.97 – 1.10	0.331
PSA	1.33	1.06 – 1.68	<0.05	1.20	0.93 – 1.56	0.158
%fPSA	0.87	0.81 – 0.94	<0.001	0.88	0.81 – 0.94	<0.001

Table 5.20: Univariate and multivariate logistic regression analyses in Polish cohort for high-grade prostate cancer VS low-grade plus Controls without prostate cancer within PSA range of 3-10 ng/ml.

Variables	Univariate logistic regression analysis			Multivariate logistic regression analysis		
	OR	95% CI	P Value	OR	95% CI	P value
Age	1.00	0.95 – 1.05	0.912	1.02	0.96 – 1.08	0.471
PSA	1.34	1.07 – 1.67	<0.05	1.22	0.96 – 1.56	0.106
%fPSA	0.89	0.83 – 0.95	<0.05	0.90	0.84 – 0.96	<0.05

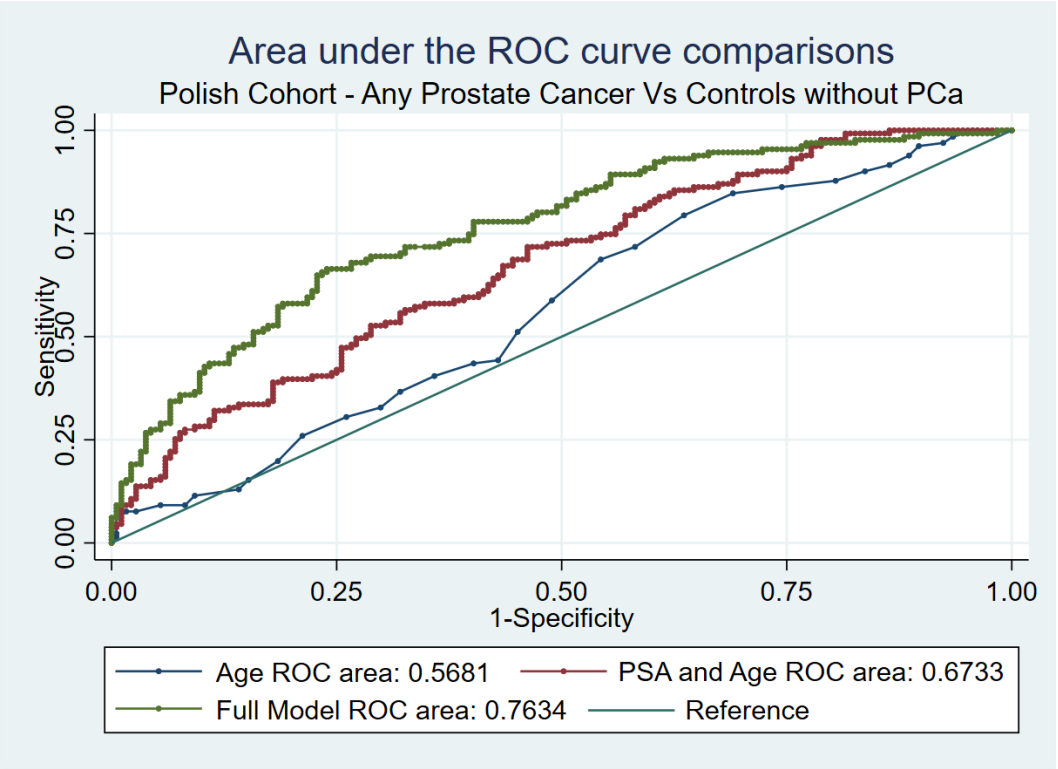

Figure 5.13: The receiver operating characteristic curve of the prediction model for any prostate cancer VS controls without prostate cancer in the Polish cohort.

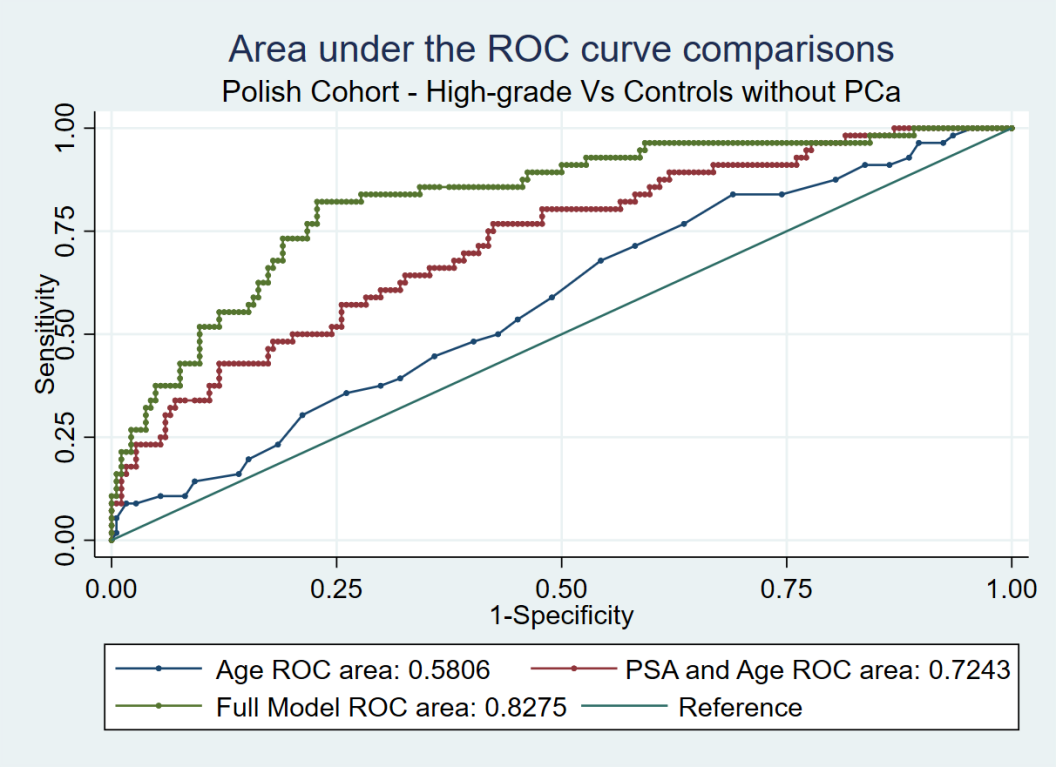

Figure 5.14: The receiver operating characteristic curve of the prediction model for high-grade prostate cancer VS controls without prostate cancer in the Polish cohort.

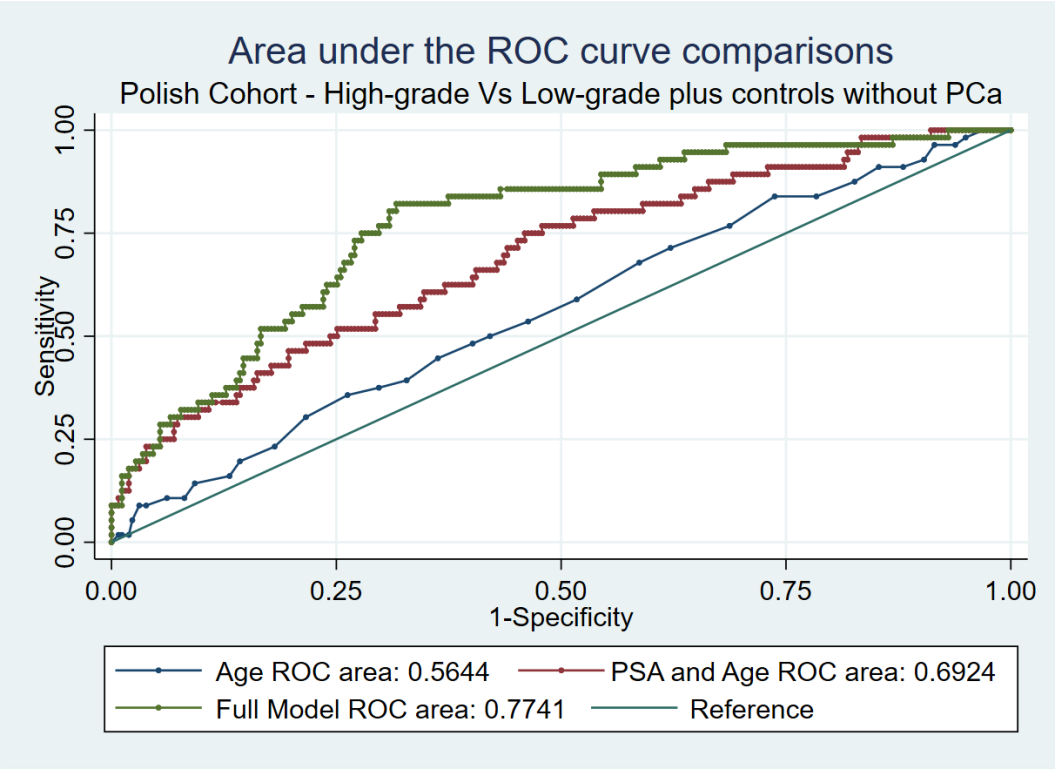

Figure 5.15: The receiver operating characteristic curve of the prediction model for high-grade prostate cancer VS low-grade plus controls without prostate cancer in the Polish cohort.

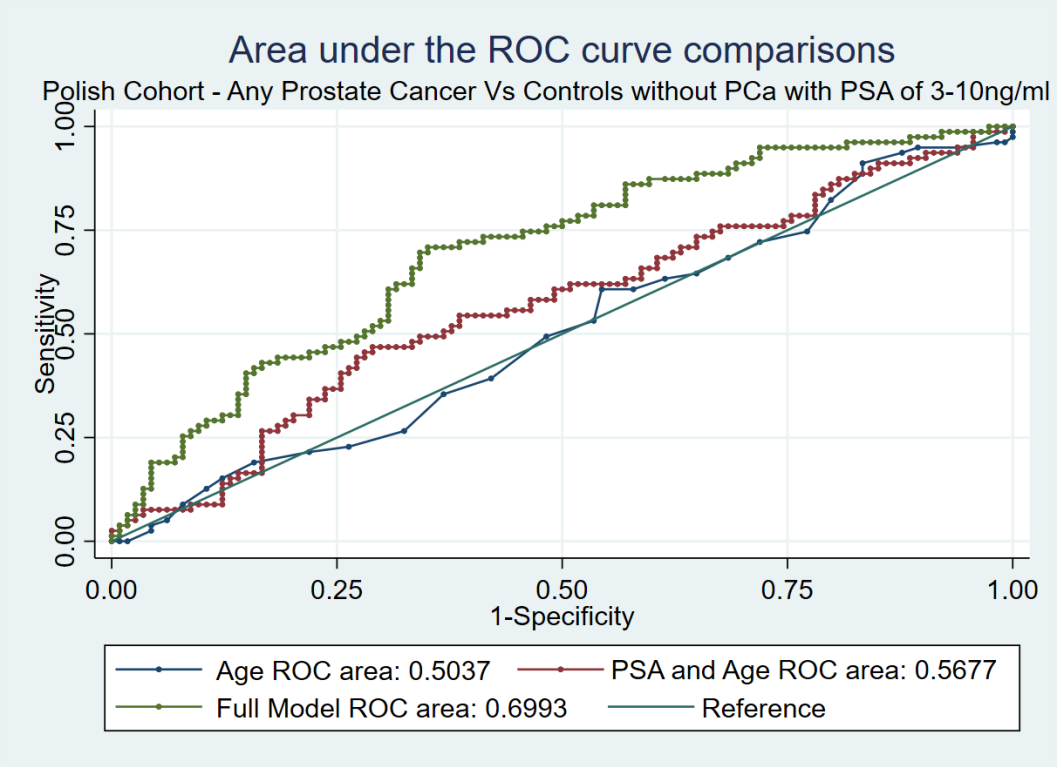

Figure 5.16: The receiver operating characteristic curve of the prediction model for any prostate cancer VS controls without prostate cancer with PSA range of 3-10 ng/ml in the Polish cohort.

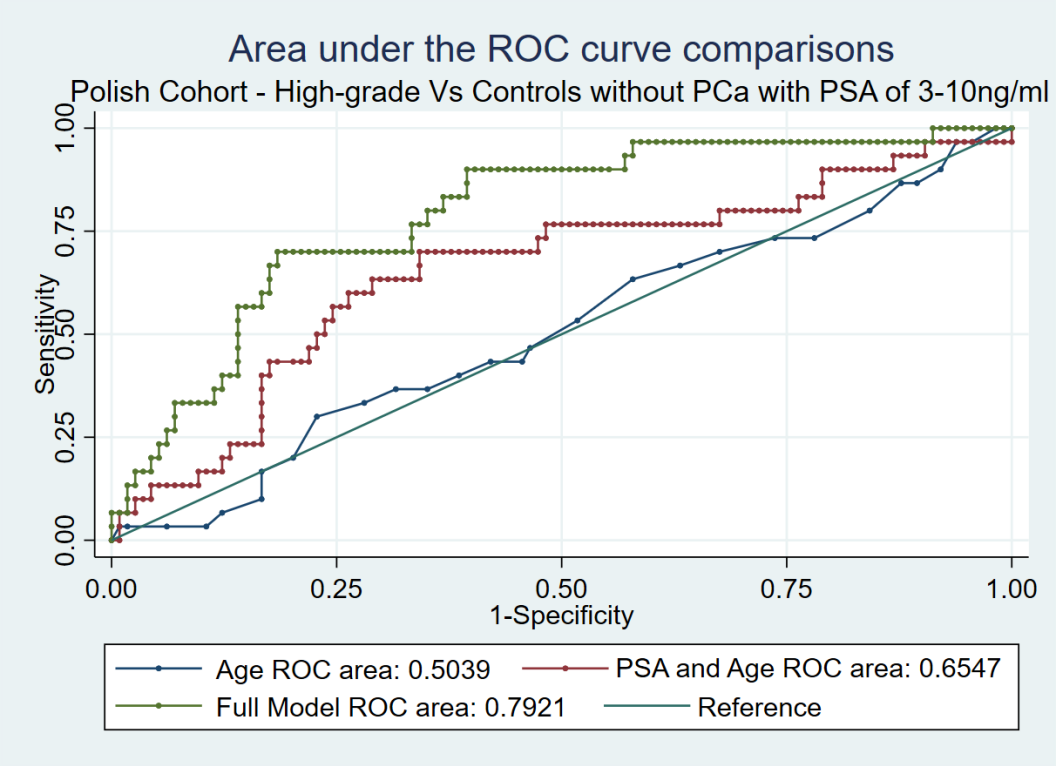

Figure 5.17: The receiver operating characteristic curve of the prediction model for high-grade prostate cancer VS controls without prostate cancer with PSA range of 3-10 ng/ml in the Polish cohort.

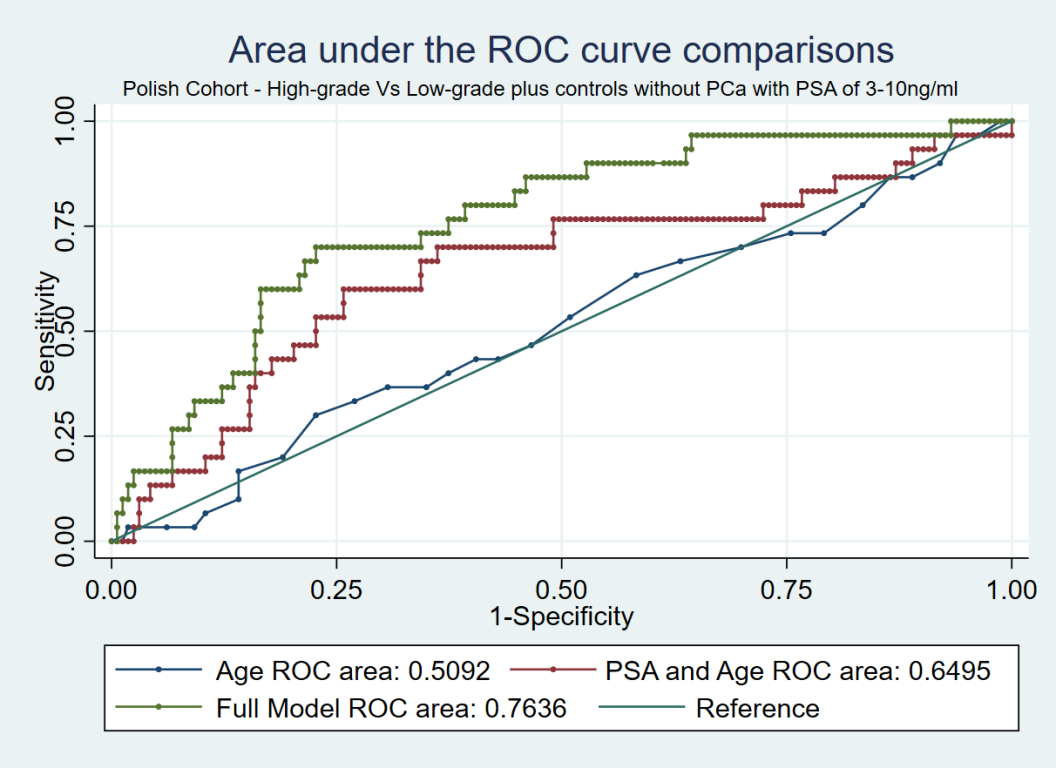

Figure 5.18: The receiver operating characteristic curve of the prediction model for high-grade prostate cancer VS low-grade plus controls without prostate cancer with a PSA range of 3-10 ng/ml in the Polish cohort.

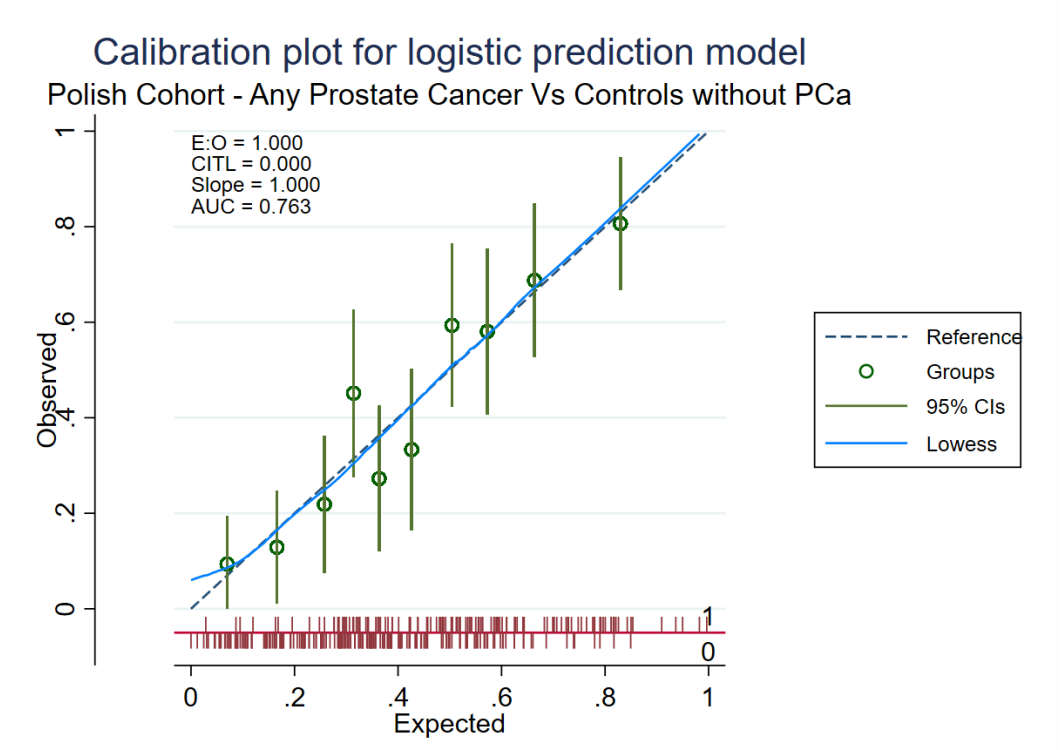

Figure 5.19: Calibration plot for logistic prediction model in Polish cohort for any prostate cancer VS controls without prostate cancer.

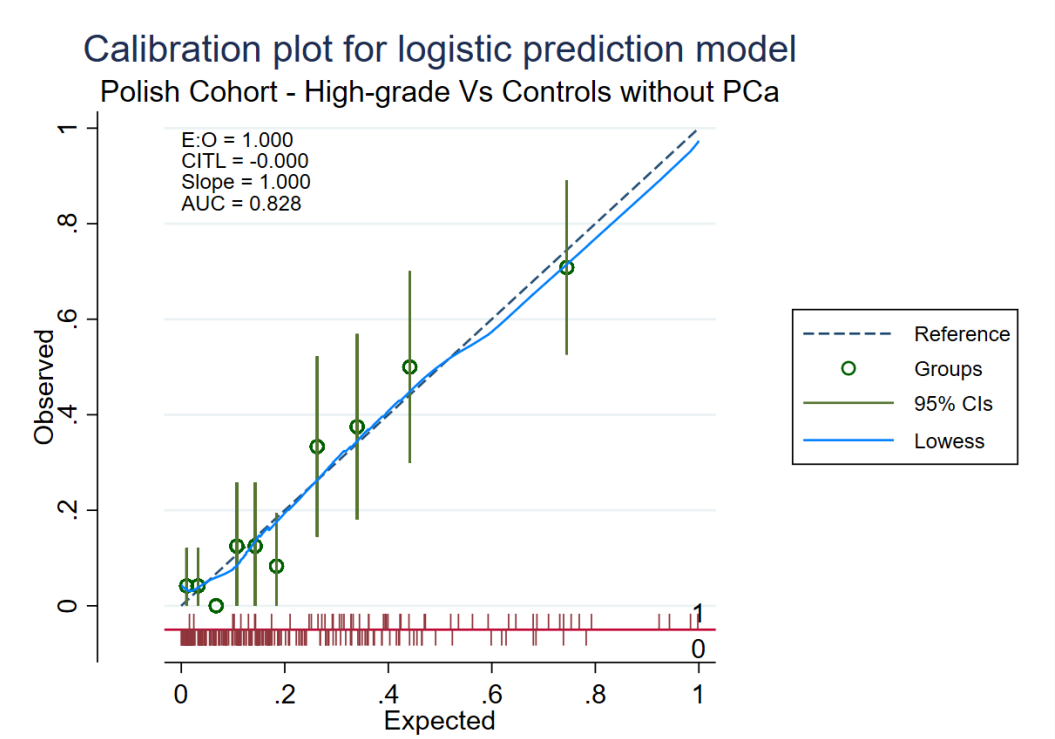

Figure 5.20: Calibration plot for logistic prediction model in Polish cohort for high-grade prostate cancer VS controls without prostate cancer.

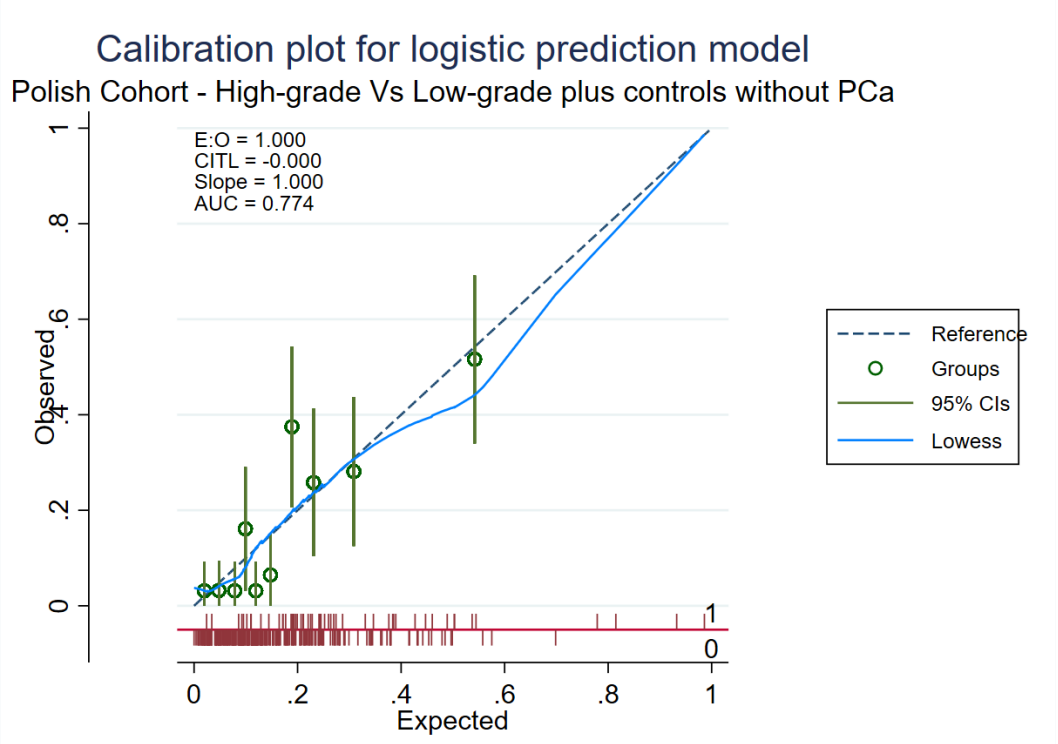

Figure 5.21: Calibration plot for logistic prediction model in the UK cohort for high-grade prostate cancer VS low-grade plus controls without prostate cancer.

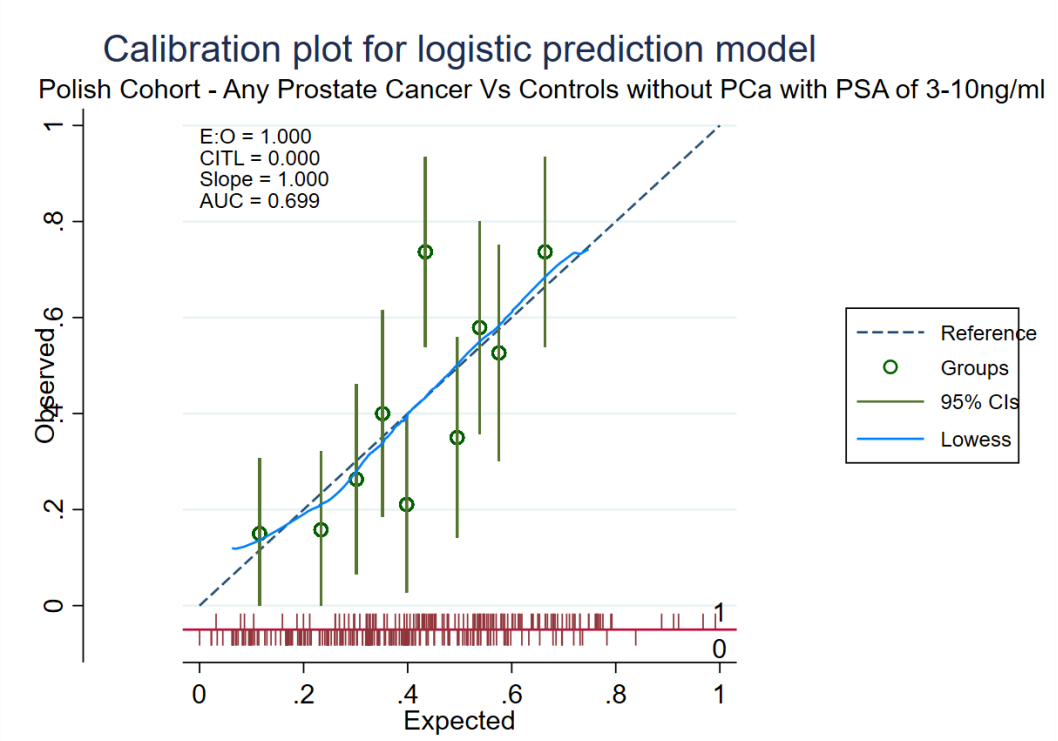

Figure 5.22: Calibration plot for logistic prediction model in Polish cohort for any prostate cancer VS controls without prostate cancer within PSA range of 3-10 ng/ml.

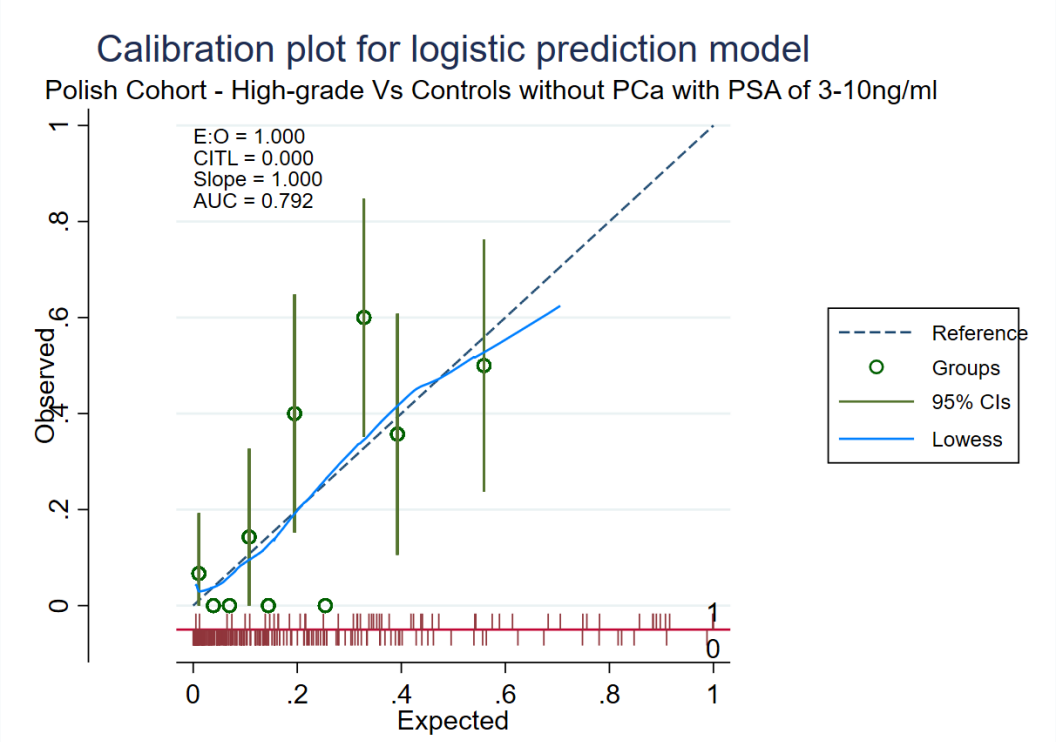

Figure 5.23: Calibration plot for logistic prediction model in Polish cohort for high-grade prostate cancer VS controls without prostate cancer within PSA range of 3-10 ng/ml.

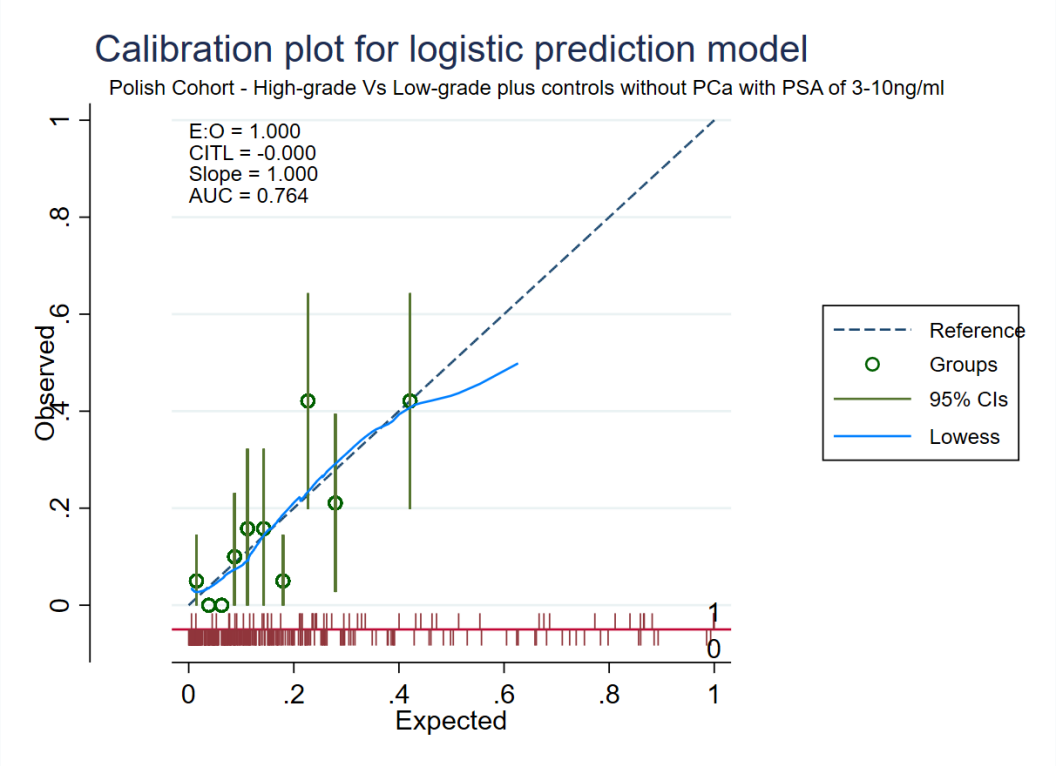

Figure 5.24: Calibration plot for logistic prediction model in Polish cohort for high-grade prostate cancer VS low-grade plus controls without prostate cancer within PSA range of 3-10 ng/ml.

Table 5.21: Cut-points probabilities in Polish cohort for any prostate cancer VS controls without prostate cancer.

Probability cut point	No. of controls that would not refer to biopsy (%)	No. of cases that would miss biopsy (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
5%	7 3.80%	1 0.76%	99.2% 95.80 – 100.00	3.8% 1.54 – 7.68	42.3% 36.80 – 48.10	87.5% 47.30 – 99.70
9%	21 11.41%	2 1.53%	98.5% 94.60 – 99.80	11.4% 7.21 – 16.90	44.2% 38.40 – 50.10	91.3% 72.00 – 98.90
10%	24 13.04%	3 2.29%	97.7% 93.50 – 99.50	13% 8.54 – 18.80	44.4% 38.60 – 50.40	88.9% 70.80 – 97.60
12.5%	32 17.39%	4 3.05%	96.9% 92.40 – 99.20	17.4% 12.20 – 23.70	45.5% 39.60– 51.60	88.9% 73.90 – 96.90
15%	37 20.11%	4 3.05%	96.9% 92.40 – 99.20	20.1% 14.60 – 26.60	46.4% 40.30 – 52.40	90.2% 76.90 – 97.30
20%	52 28.26%	7 5.34%	94.7% 89.30 – 97.80	28.3% 21.90 – 35.40	48.4% 42.20 – 54.70	88.1% 77.10 – 95.10

Table 5.22: Cut-points probabilities in Polish cohort for high-grade VS controls without prostate cancer.

Probability cut point	No. of controls that would not refer to biopsy (%)	No. of cases that would miss biopsy (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
5%	49 (26.63%)	2 3.57%	96.4% 87.70 – 99.60	26.6% 20.40 – 33.60	28.6% 22.20 – 35.60	96.1% 86.50 – 99.50
9%	72 39.13%	2 3.57%	96.4% 87.70 – 99.60	39.1% 32.00 – 42.60	32.5% 25.50 – 40.20	97.3% 90.60 – 99.70
10%	75 40.76%	3 5.36%	94.6% 85.10 – 98.90	40.8% 33.60 – 48.20	32.7% 25.60 – 40.50	96.2% 89.20 – 99.20
12.5%	90 48.91%	5 8.93%	91.1% 80.40 – 970	48.9% 41.50 – 56.40	35.2% 27.40 – 43.50	94.7% 88.10 – 98.30
15%	107 58.15%	8 14.29%	85.7% 73.80 – 93.60	58.2% 50.70 – 65.40	38.4% 29.80 – 47.50	93% 86.80 – 96.90
20%	129 70.11%	9 16.07%	83.9% 71.70 – 92.40	70.1% 62.90 – 76.60	46.1% 36.20 – 56.20	93.5% 88.00 – 97.00

Table 5.23: Cut-points probabilities in Polish cohort for high-grade prostate cancer cases VS low-grade plus controls without prostate cancer.

Probability cut point	No. of controls that would not refer to biopsy (%)	No. of cases that would miss biopsy (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
5%	49 (18.92%)	2 (3.57%)	96.4% 87.70 - 99.60	18.9% 14.30 - 24.20	20.5% 15.80 - 25.80	96.1% 86.50 - 99.50
9%	90 (34.75%)	3 (5.36%)	94.6% 85.10 - 98.90	34.7% 29.00 - 40.90	23.9% 18.40 – 30.00	96.8% 90.90 - 99.30
10%	107 (41.31%)	5 (8.93%)	91.1% 80.40 - 970	41.3% 35.30 - 47.60	25.1% 19.30 - 31.70	95.5% 89.90 - 98.50
12.5%	143 (55.21%)	8 (14.29%)	85.7% 73.80- 93.60	55.2% 48.90 - 61.40	29.3% 22.40 - 36.90	94.7% 89.80 - 97.70
15%	164 (63.32%)	10 (17.86%)	82.1% 69.60 - 91.10	63.3% 57.10 - 69.20	32.6% 25.00 – 41.00	94.3% 89.70 - 97.20
20%	194 (74.90%)	21 (37.50%)	62.5% 48.50 - 75.10	74.9% 69.20 - 80.10	35% 25.70 - 45.20	90.2% 85.50 - 93.90

Table 5.24: Cut-points probabilities in Polish cohort for any prostate cancer VS controls without prostate cancer in patients with PSA range 3-10 ng/ml.

Probability cut point	No. of controls that would not refer to biopsy (%)	No. of cases that would miss biopsy (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
9%	5 4.39%	1 1.27%	98.7% 93.10 – 100.00	4.39% 1.44 – 9.94	41.7% 34.60– 49.10	83.3% 35.90 – 99.60
10%	8 7.02%	1 1.27%	98.7% 93.10 – 100.00	7.02% 3.08 – 13.40	42.4% 35.20 – 49.90	88.9% 51.80 – 99.70
12.5%	11 9.65%	2 2.53%	97.5% 91.20 – 99.70	9.65% 4.92 – 16.60	42.8% 35.40 – 50.40	84.6% 54.60 – 98.10
15%	13 11.40%	2 2.53%	97.5% 91.20 – 99.70	11.4% 6.21 – 18.70	43.3% 35.90 – 50.90	86.7% 59.50 – 98.30
20%	19 16.67%	3 3.80%	96.2% 89.30 – 99.20	16.7% 10.30 – 24.80	44.4% 36.90 – 52.20	86.4% 65.10 – 97.10

Table 5.25: Cut-points probabilities in Polish cohort for high-grade prostate cancer patients VS controls without prostate cancer with PSA range 3-10 ng/ml.

Probability cut point	No. of controls that would not refer to biopsy (%)	No. of cases that would miss biopsy (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
5%	28 24.56%	1 3.33%	96.7% 82.80–99.90	24.6% 17.00–33.50	25.2% 17.60–34.20	96.6% 82.20–99.90
9%	41 35.96%	1 3.33%	96.7% 82.80–99.90	36% 27.20–45.50	28.4% 19.90–38.20	97.6% 87.40–99.90
10%	48 42.11%	2 6.67%	93.3% 77.90–99.20	42.1% 32.90–51.70	29.8% 20.80–40.10	96% 86.30–99.50
12.5%	53 46.49%	3 10.00%	90% 73.50–97.90	46.5% 37.10–56.10	30.7% 21.30–41.40	94.6% 85.10–98.90
15%	63 55.26%	3 10.00%	90% 73.50–97.90	55.3% 45.70–64.60	34.6% 24.20–46.20	95.5% 87.30–99.10
20%	73 64.04%	6 20.00%	80% 61.40-92.30	64% 54.50–72.80	36.9% 25.30–49.80	92.4% 84.20–97.20

Table 5.26: Cut-points probabilities in Polish cohort for high-grade prostate cancer patients VS low-grade plus Controls without prostate cancer with PSA range 3-10 ng/ml.

Probability cut point	No. of controls that would not refer to biopsy (%)	No. of cases that would miss biopsy (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
5%	41 (25.15%)	1 (3.33%)	96.7% 82.80 - 99.90	25.2% 18.70 - 32.50	19.2% 13.30 - 26.40	97.6% 87.40 - 99.90
9%	67 (41.10%)	3 (10.00%)	90% 73.50 - 97.90	41.1% 33.50 - 49.10	22% 15.00 - 30.30	95.7% 88.00 - 99.10
10%	74 (45.40%)	3 (10.00%)	90% 73.50 - 97.90	45.4% 37.60- 53.40	23.3% 15.90 – 32.00	96.1% 89.00 - 99.20
12.5%	91 (55.83%)	6 (20.00%)	80% 61.40 - 92.30	55.8% 47.90 - 63.60	25% 16.70 - 34.90	93.8% 87.00 - 97.70
15%	102 (62.58%)	7 (23.33%)	76.7% 57.70 - 90.10	62.6% 54.70 – 70.00	27.4% 18.20 - 38.20	93.6% 87.20 - 97.40
20%	125 (76.69%)	9 (30.00%)	70% 50.60 - 85.30	76.7% 69.40 - 82.90	35.6% 23.60 - 49.10	93.3% 87.6 0- 96.90

Table 5.27: Univariate and multivariate analyses to assess the discriminative value in any prostate cancer VS Controls without prostate cancer.

Univariate	**The UK Cohort**			**Polish Cohort**		
	AUC	95% CI	Δ AUC	AUC	95% CI	Δ AUC
Age (Model 1)	0.66	0.62 – 0.71	—	0.57	0.50 – 0.63	—
PSA, ng/ml	0.66	0.61 – 0.70	—	0.67	0.61 – 0.73	—
%fPSA	0.70	0.66 – 0.74	—	0.74	0.69 – 0.80	—
Multivariate						
PSA + Age (Model 2)	0.71	0.66 – 0.75	0.05[(1)]	0.67	0.61 – 0.73	0.10[(3)]
PSA + %fPSA + Age (Model 3)	0.76	0.72 – 0.80	0.05[(2)]	0.76	0.71 – 0.82	0.09 [(4)]

[1]The difference between model 1 and model 2, P-value <0.05. [2]The difference between model 2 and model 3, P-value <0.001.
[3]The difference between model 1 and model 2, P-value <0.001. [4]The difference between model 2 and model 3, P-value <0.001.

Table 5.28: Univariate and multivariate analyses to assess the discriminative value in high-grade prostate cancer VS Controls without prostate cancer.

Univariate	**The UK Cohort**			**Polish Cohort**		
	AUC	95% CI	Δ AUC	AUC	95% CI	Δ AUC
Age (Model 1)	0.72	0.68 – 0.77	—	0.58	0.50 – 0.66	—
PSA, ng/ml	0.77	0.73 – 0.81	—	0.73	0.65 – 0.81	—
%fPSA	0.79	0.75 – 0.83	—	0.79	0.73 – 0.86	—
Multivariate						
PSA + Age (Model 2)	0.82	0.78 – 0.85	0.10[(1)]	0.72	0.65 – 0.80	0.14[(3)]
PSA + %fPSA + Age (Model 3)	0.89	0.86 – 0.92	0.07[(2)]	0.83	0.76 – 0.89	0.11[(4)]

[1]The difference between model 1 and model 2, P-value <0.001. [2]The difference between model 2 and model 3, P-value <0.001.
[3] The difference between model 1 and model 2, P-value <0.001. [4] The difference between model 2 and model 3, P-value <0.05

Table 5.29: Univariate and multivariate analyses to assess the discriminative value in high-grade prostate cancer VS low-grade plus Controls without prostate cancer.

Univariate	The UK Cohort			Polish Cohort		
	AUC	95% CI	Δ AUC	AUC	95% CI	Δ AUC
Age (Model 1)	0.65	0.61 – 0.70	—	0.56	0.48 – 0.65	—
PSA, ng/ml	0.70	0.65 – 0.74	—	0.70	0.62 – 0.78	—
%fPSA	0.72	0.67 – 0.76	—	0.74	0.67 – 0.81	—
Multivariate						
PSA + Age (Model 2)	0.72	0.68 – 0.77	0.07[(1)]	0.69	0.61 – 0.77	13[(3)]
PSA + %fPSA + Age (Model 3)	0.78	0.74 – 0.82	0.06[(2)]	0.77	0.71 – 0.84	8[(4)]

[1]The difference between model 1 and model 2, P-value <0.001. [2]The difference between model 2 and model 3, P-value <0.001.
[3] The difference between model 1 and model 2, P-value <0.05. [4] The difference between model 2 and model 3, P-value <0.05.

Table 5.30: Univariate and multivariate analyses to assess the discriminative value in any prostate cancer VS Controls without prostate cancer in patients with PSA range 3-10 ng/ml.

Univariate	The UK Cohort			Polish Cohort		
	AUC	95% CI	Δ AUC	AUC	95% CI	Δ AUC
Age (Model 1)	0.62	0.56 – 0.68	—	0.50	0.42 – 0.59	—
PSA, ng/ml	0.59	0.52 – 0.65	—	0.56	0.48 – 0.64	—
%fPSA	0.62	0.55 – 0.68	—	0.70	0.62 – 0.77	—
Multivariate						
PSA + Age (Model 2)	0.63	0.57 – 0.69	0.01[(1)]	0.57	0.48 – 0.65	0.07[(3)]
PSA + %fPSA + Age (Model 3)	0.68	0.62 – 0.74	0.05[(2)]	0.70	0.62 – 0.77	0.13[(4)]

[1]The difference between model 1 and model 2, P-value =0.493. [2]The difference between model 2 and model 3, P-value <0.05.
[3]The difference between model 1 and model 2, P-value = 0.265.[4]The difference between model 2 and model 3, P-value <0.05.

Table 5.31: Univariate and multivariate analyses to assess the discriminative value in high-grade prostate cancer VS Controls without prostate cancer in patients with PSA range 3-10 ng/ml.

Univariate	**The UK Cohort**			**Polish Cohort**		
	AUC	95% CI	Δ AUC	AUC	95% CI	Δ AUC
Age (Model 1)	0.68	0.62 – 0.75	—	0.50	0.38 – 0.62	—
PSA, ng/ml	0.64	0.58 – 0.71	—	0.64	0.52 – 0.76	—
%fPSA	0.70	0.64 – 0.77	—	0.79	0.70 – 0.88	—
Multivariate						
PSA + Age (Model 2)	0.71	0.64 – 0.77	0.03(1)	0.65	0.54 – 0.77	0.15(3)
PSA + %fPSA + Age (Model 3)	0.81	0.76 – 0.87	0.10(2)	0.79	0.70 – 0.88	0.14(4)

[1]The difference between model 1 and model 2, P-value =0.194. [2]The difference between model 2 and model 3, P-value <0.001.
[3]The difference between model 1 and model 2, P-value =0.052. [4] The difference between model 2 and model 3, P-value <0.05.

Table 5.32: Univariate and multivariate analyses to assess the discriminative value in high-grade prostate cancer VS low-grade plus Controls without prostate cancer with PSA range 3-10 ng/ml.

Univariate	**The UK Cohort**			**Polish Cohort**		
	AUC	95% CI	Δ AUC	AUC	95% CI	Δ AUC
Age (Model 1)	0.61	0.55 – 0.67	—	0.51	0.39 – 0.62	—
PSA, ng/ml	0.60	0.54 – 0.67	—	0.64	0.52 – 0.76	—
%fPSA	0.65	0.59 – 0.71	—	0.75	0.66 – 0.84	—
Multivariate						
PSA + Age (Model 2)	0.63	0.57 – 0.69	0.02(1)	0.65	0.53 – 0.77	0.14(3)
PSA + %fPSA + Age (Model 3)	0.70	0.64 – 0.76	0.07(2)	0.76	0.67 – 0.85	0.11(4)

[1]The difference between model 1 and model 2, P-value =0.331. [2]The difference between model 2 and model 3, P-value <0.05.
[3]The difference between model 1 and model 2, P-value =0.0597. [4] The difference between model 2 and model 3, P-value <0.05.

5.4 Discussion

In the previous chapter, the scarcity was highlighted of appropriate risk prediction models for prostate cancer that can be used in primary care and community settings and the limitations inherent within them. In this chapter, the focus has been on developing a low-cost, feasible risk prediction model for prostate cancer that can be implemented in general practice, particularly in the UK.

The current practice from National Institute for Health and Care Excellence (NICE) guidelines recommends that men with suspected prostate cancer should receive a mpMRI as a first-line examination before the biopsy. Also, it recommends using nomograms with patients to assist them with decision making and predict biopsy results [57]. For primary care, the guideline instructs GPs to offer PSA testing to men above the age of 50 upon their request. Moreover, asymptomatic men should not be subjected to PSA testing, and the PSA test should be considered in men with symptoms such as lower urinary tract symptoms (LUTS), erectile dysfunction, visible haematuria, lower back pain, and weight loss [323].

PSA level alone as a screening test for prediction risk of prostate cancer or high-grade disease has been investigated extensively. However, the AUC for PSA level ranges from 0.62 to 0.69 for any prostate cancer and 0.70 to mid 0.70's for high-grade cancer [701-703]. Our findings are in line with these studies as the AUC of PSA alone was 0.66 and 0.67 for any prostate cancer in the UK and the Polish cohort, respectively. In contrast, it ranges between 0.70 and 0.77 for high-grade groups.

With regards to the benefit of PSA in mortality reduction, the results of the two most comprehensive prostate cancer screening trials, both reported in 2009, were

inconclusive. One study from the United States revealed no benefit [704], while another from Europe indicated that it reduced prostate cancer deaths by 20% after a follow-up of nine years [95]. More recently, the Cluster Randomised Trial of PSA Testing for Prostate Cancer (CAP) which included over 400,000 men, found that there was no significant difference in prostate cancer mortality between those who had a one-off PSA testing and those who had not done the test, although the detection of low-risk cancer increased [705]. This demonstrates the limitations of a single PSA test as a screening tool for prostate cancer, as well as the necessity for more precise methods of diagnosing tumours that require treatment.

To achieve this aim, two important datasets were acquired; one from the UK and the other from Poland. As MRI-guided biopsy is now increasingly being recommended to replace conventional biopsy needle cores in the UK, for the first time, it was possible to look at prediction using the same biomarkers but with different methods to yield the outcome; one by the traditional needle biopsy method, and the other by MRI-guided biopsy.

The key concept for prostate cancer risk prediction is to identify significant or high-grade prostate cancer cases. Therefore, the study compared and contrasted the performance of any prostate cancer and high-grade prostate cancer with different sets of control groups. The different sets of control groups included no prostate cancer and controls with low-grade prostate cancer plus controls without prostate cancer.

There are no comparative studies that employed the same variables and methodology in risk prediction models as our study, therefore, I chose to compare and contrast our results by each input variable into the model. Moreover, the results of risk prediction

with the MRI-guided outcome are novel, therefore, there are no similar studies available in the literature to make the comparisons.

Few studies have reported results from univariate analyses for each predictive variable that were incorporated in our model to assess its association with the risk of prostate cancer. In the univariate analysis within these studies, the odds ratio for age as a risk factor for prostate cancer ranged between 1.05 and 1.07 [706, 707]. One study reported the odds ratio for PSA and %fPSA in differentiating aggressive prostate cancer as 1.30 and 0.88 for all PSA ranges, and 1.37 and 0.91 for the PSA range of 4-10 ng/ml [708]. Our findings showed a similar odds ratio for age. The odds ratio ranged between 1.06 to 1.12 in the UK data and 1.00 to 1.04 in the Polish data. Moreover, a similar pattern was seen with PSA and %fPSA. The odds ratio of PSA in differentiating high-grade cancers was 1.23 in the UK and 1.13 in the Polish data for all PSA ranges, and 1.33 for both datasets in PSA specific range of 3-10ng/ml. The odds ratio of %fPSA in all PSA ranges was 0.85 and 0.88 and for PSA specific range of 3-10ng/ml was 0.88 and 0.87 in the UK and Polish data, respectively.

Overall, the analyses suggest that each variable (age, PSA, %fPSA) is associated with the risk of prostate cancer in both the existing conventional approach and the modern MRI-guided to detect prostate cancer outcomes. The performance of the full model that incorporates age, PSA, and %fPSA is better than PSA or age alone as well as both model 1 (Age) and model 2 (PSA and Age) in each sub-group in both datasets.

There is no difference in the discriminative ability of the full model in any prostate cancer between the UK and Polish cohorts, the AUC of the UK cohort was 0.76 (95% CI: 0.721-0.799) (Figure 5.1), whereas it was 0.76 (95% CI: 0.710-0.816) in the Polish cohort (Figure 5.13). The full model was slightly better in predicting high-grade

prostate cancer versus low-grade plus controls without prostate cancer, where the AUC for the UK and Polish cohorts are 0.78 and 0.77, respectively (Figure 5.3 and Figure 5.15). However, the model performance increased significantly in predicting high-grade patients against controls without prostate cancer as the AUC with the UK cohort was 0.89 (Figure 5.2) and 0.83 with the Polish cohort (Figure 5.14). Similarly, the model performance was better in the sub-group of high-grade versus controls without prostate cancer within the PSA range of 3-10 ng/ml, where the AUC in the UK and Polish were 0.81 and 0.79, respectively (Figure 5.5 and Figure 5.17).

In the Polish dataset, the univariate and multivariate analyses showed that, apart from %fPSA, both age and PSA have either borderline association or not significant association. This may be due to the insignificant differences in the mean between cases and controls, as shown in Table 5.2. The added value for each variable is higher in the Polish cohort than in the UK cohort. The model performance ranges between AUC of 0.70 to 0.83. It is worth noting that the model performed better in discriminating high-grade prostate cancer compared to any prostate cancer, even when restricting subjects to PSA ranges between 3 to 10 ng/ml. The best performance was achieved in predicting high-grade patients versus controls without prostate cancer as the AUC was 0.83. Also, the calibration assessment showed that the discriminative ability of the model was good as there was an agreement between observed and predicted outcomes with a p-value above 0.05.

Once models are developed, it is important to identify the optimal threshold to minimise missing numbers of prostate cancer cases, in particular, high-grade cases (false negatives) and to also minimise false positives for men that do not have prostate cancer; however, the model suggests otherwise.

At all cut-off point probabilities, the percentage of saved biopsies was better in the Polish cohort. However, the percentage of missing cases, sensitivity, and PPV were better in the UK cohort compared to the Polish cohort.

The most common way to diagnose biopsy-driven prostate cancer is to use PSA tests. According to the European Association of Urology Guidelines, higher PSA values imply an increased risk of prostate cancer [709]. Nonetheless, PSA levels between 4 and 10 ng/ml are known as the "Gray area". Some studies consider its relation to a range of 3-10 ng /ml [710, 711], where a prostate cancer diagnosis is debatable [712]. As shown in a survey, the positive rate of prostate biopsy was only ~16% to 20% when PSA levels were within this range. Thus it is essential to combine it with other parameters to enhance detection performance [713].

For any prostate cancer within the PSA range of 3-10 ng/ml, there were not enough observations of cases and controls below cut-point probabilities of 20% in the UK cohort, whilst in the Polish cohort, only a cut-point of 5% is not available. At a 20% probability, the percentage of missing cases and sensitivity was better in the UK than in the Polish cohort (Table 5.12 and Table 5.24).

In contrast, for high-grade prostate cancer versus controls without prostate cancer, the percentage of saved biopsies, specificity, and NPV at all cut-off probabilities were better in the Polish cohort than the UK cohort. Nevertheless, the percentage of missing cases, sensitivity, and PPV was slightly higher in the UK cohort (Table 5.10 and Table 5.22). When restricting the analyses to men with PSA levels between 3-10 ng/ml, the percentage of saved biopsies, specificity, and NPV were better in the Polish cohort. However, the percentage of missing cases and sensitivity was better in the UK cohort, except at a cut-off of 9% (Table 5.13 and Table 5.25).

For high-grade versus low-grade plus controls without prostate cancer, the percentage of saved biopsy, specificity, and NPV were better in the Polish cohort at all cut-off probabilities, whereas the percentage of missing cases, sensitivity, and PPV were better in the UK cohort (Table 5.11 and Table 5.23). For the same sub-group with a PSA range of 3-10 ng/ml, similar results were achieved except at a cut-off of 5% with NPV as it was better in the UK cohort (Table 5.14 and Table 5.26).

Moreover, for any prostate cancer in the Polish dataset, using a cut-off threshold of 15% would be justifiable as it saved more unnecessary biopsies while missing the same percentage of cases as a cut-off of 12.5%. Whereas a cut-off of 9% is more appropriate for high-grade versus controls without prostate cancer as well as with high-grade versus low-grade plus controls without prostate cancer. Also, a cut-off of 9% seems reasonable as being used in these sub-groups within the PSA range of 3-10ng/ml.

With regards to the UK cohort (MRI guided biopsy), all variables independently and accompanied by others were statistically significant and associated with prostate cancer. In particular, they were more associated with increased risks in high-grade prostate cancer versus controls with no prostate cancer. Similar to the Polish dataset, the model works better in discriminating high-grade prostate cancer from controls without any tumour as the AUC is 0.89 where it is 0.76 and 0.78 for any prostate cancer and high-grade versus controls with low-grade plus without prostate cancer, respectively. This is also the case when restricting subjects with a PSA range between 3-10 ng/ml. Although the model has high predictive values in the UK cohort, the calibration is still not stable. One explanation for that could be the difference in patient characteristics and disease prevalence among health centres or regions [528]. When a model is developed in an environment with a high incidence rate, it may produce

overestimated risk values when applied to an environment with a lower incidence rate [714]. Teaching hospitals, for example, may treat more patients with the outcome of interest than regional hospitals; this variability in settings can alter risk estimates and calibration [715].

Using a cut-point of 12.5% for any prostate cancer in the UK dataset would save about 5% of unnecessary biopsies while not missing any significant cancers. Whereas for any cancer with patients with a PSA range of 3-10 ng/ml, it is difficult to determine the appropriate cut-point as there were not enough subjects in both cases and control groups. For both high-grade cases against controls without prostate cancer and the same group when restricting PSA to 3-10 ng/ml, a cut-point of 10% could be used. Higher cut-off points of 12.5% and 15% may be used for high-grade versus low-grade and control without prostate cancer and the same group with PSA levels between 3 to 10 ng/ml, respectively.

Trans-rectal ultrasound-guided (TRUS) biopsy is the standard and commonly used procedure for detecting prostate cancer in men with an increased PSA level or abnormal digital rectal examination. On the other hand, this traditional approach is linked to the under-detection of clinically significant prostate cancer and the over-detection of indolent and low-grade prostate cancer [716]. Moreover, compared to MRI-guided biopsy, the TRUS-guided biopsy has a higher probability of cancer grade misclassification, which can lead to under or overtreatment [717]. In addition, TRUS-guided biopsy has also been linked to an increased risk of adverse effects such as bleeding and pain [718], which can result in higher healthcare costs and possibly life-threatening consequences [719]. Therefore, as a result of its high diagnostic accuracy for identifying high-grade prostate cancer, upfront mpMRI has been suggested as a

triage test to determine whether a biopsy is required for men with no previous biopsy and elevated PSA levels [720, 721].

In this study, the results presented show that using our model in the MRI-guided biopsy cohort misses less significant prostate cancer than the traditional pathway, which is in line with several reports. However, unlike the previous studies [722-724], it also saved less unnecessary biopsy percentage than the Polish cohort. Approximately 30% reduction in unnecessary biopsy has been reported in recent studies following the use of mpMRI in risk assessment [725-727]. The inconsistency of the results of this study concerning reducing the percentage of unnecessary biopsies with previous studies could be due to the difference in patients' characteristics and the variability of model inputs that have been used. Furthermore, a recent systematic review indicated that several factors could influence the performance of mpMRI and MRI-guided biopsy, the most important of which are radiologists' experience in reading the results and urologists' experience in interpreting a biopsy [728].

This is the first study that has examined the performance of a risk prediction model for prostate cancer that incorporates simple, low-cost, feasible inputs, which can be used in primary care settings with two different methods of obtaining the outcome of interest; the traditional approach, and MRI- guided pathway.

The findings suggest singular variable such as age, PSA and %fPSA do not have adequate predictability power to differentiate significant prostate cancer. Combining all these markers has improved the area under the curve significantly. This study has also shown that with the modern technique of MRI to identify prostate cancer outcomes, these markers can still be used to stratify men at risk of significance prostate cancer for further investigations. This approach will benefit not only men who acquire clinical

care but also the NHS service in that it will save money by finding significant cancers with less number of tests needing to be performed at a population level.

However, this study also has several limitations. First, the population of both cohorts were pre-screened subjects, which may lead to overestimates of the model's performance. Secondly, the low number of high-grade cases in the traditional cohort compared to the MRI-guided cohort. Thirdly, this study only investigated patients who were biopsy-naïve, therefore, the findings could not be applicable to recurrent biopsy cases. Fourthly, since the two cohorts are different in terms of method of outcome yield, only an internal validation was performed, and no external validation was conducted. To collect MRI-guided data to perform an external model validation would have taken multiple years. Family history is one of the important risk factors for prostate cancer along with ethnicity, but here we did not incorporate them because family history was already highly enriched in the Polish data set, as both cases and controls were selected based on family history and it was not available in the UK data. Similarly, ethnicity was excluded because the population was white in both cohorts as well as due to availability issues, so it was impossible to investigate these factors. Lastly, several research projects have shown that adding mpMRI after a positive PSA test and MRI-guided biopsy to a standard prostate cancer screening pathway is cost-effective [729-731]. In this study, the cost factor of this was not considered.

In the future, further improvement is needed by conducting large prospective and multi-centric studies that include the ability to externally validate the model on various patient populations to ensure that the model performance is fully assessed before being used in general practice. Furthermore, MRI imaging and biopsy procedures and protocols must be standardised to improve prostate cancer detection accuracy and targeted biopsies

[732]. This will help in comparing results of different regions and reduce the discrepancy in finding the outcome

In conclusion, these analyses and results indicated that the use of a multivariate model consisting of age, PSA, and %fPSA performed better than PSA alone in both the traditional and MRI-guided cohorts. Also, by using MRI-guided biopsy, less significant prostate cancer was missed compared to the traditional pathway.

Although our RISKMAN model cannot yet be generalised and applied as a screening program for the community yet, it shows promising results and could be enhanced further. Since there is no screening program for prostate cancer in the UK, with most of the guidelines being against it, in the following chapter, I presented results from a survey of a large group of men in the community to explore whether there is a demand for prostate cancer screening and whether men are interested in participating if one could be introduced in the future.

Chapter 6 End-user evaluation of a PSA home testing kit: survey results from UK men in the community

The work within this Chapter was prepared for publication and submitted to Cancer Reports and Reviews Journal.

The PSA home testing kit survey

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Abstract

Background

Prostate specific antigen (PSA) home testing provides a further opportunity for men to have a PSA test. GFCT commenced this service during the time of COVID-19 when face-to-face appointments in the health service were limited. This paper summarises men's views on this service and their view on incorporating a genetic test for prostate health.

Method

An email and link to an online questionnaire providing 10 questions were sent to men who had registered with the Charity and used a PSA home testing kit.

Results

The average overall service rating score is 4.6 out of 5, with 5 representing excellent service. Around 80% of men are willing to take a genetic risk assessment.

Conclusion

The PSA home kit test was a successful intervention during COVID-19. This is a continuing service and has opened up the opportunity for large scale testing in the future.

6.1 Introduction

Prostate cancer is the most common cancer among men and the second leading cause of cancer mortality worldwide [1]. In the United Kingdom (UK), about 129 men are diagnosed with prostate cancer every day and an estimated 12.5% of men will be diagnosed with the cancer in their lifetime. The incidence rates of prostate cancer are expected to rise by 12% in the UK by 2035, whereas prostate cancer is responsible for 13% of cancer deaths among males in the UK [2]. In 2007 data collected from 87 random general practices shows that prostate-specific antigen (PSA) testing remains low in the UK at a rate of 6% [733].

The Home Testing Kit service has made it possible to conduct large-scale screening for cancers in the comfort of their own homes without the cost or the need of having patients come to a clinic or general practitioner's surgery. In addition, with the current ongoing COVID-19 pandemic, the home testing service is an appropriate alternative way for testing men.

In this paper, we explore the eligibility of a PSA home testing kit for potential future screening for all men in the UK. Furthermore, as genetic testing to identify the high-risk group is on the horizon, we also asked in the questionnaire if men were willing to undertake a genetic test.

6.2 Methods

Men who used the PSA home testing service were asked to complete the 10-Qs survey. The survey was hosted online on the GFCT Ltd website (https:\\www.mypsatests.org.uk). The survey commenced on the 12th and ended on the 31st of May 2021. Data was exported from the source in the XML format and processed

further for data quality control check. For quantitative data, the analyses were performed using STATA statistical program version 15. Distribution and percentages are presented. For open-ended questions, the analysis was carried out using IBM SPSS Text Analytics for Survey4.

6.3 Results

1,902 men participated in the survey, of whom almost 89% (n=1691) completed the PSA home test successfully, whereas only 11% (211) could not complete the test. Out of the 211 men who did not complete the test, 200 men provided a reason. The main reason was being unable to provide sufficient blood for a sample (81.5%) (Table 6.1). The average service rating score is 4.6 out of 5, with 5 representing excellent service. 67% of respondents rated the service as “Excellent”, and almost 22% rated it as above average. Only 6% assessed the service rating as low/below average, as shown in Table 6.2.

Around 93% of the men were happy with the result being returned to them in an acceptable time and reported the results were easy to understand. Also, 90% of them will use the home kit PSA test again, and almost 92% will recommend this service to others. Responses from participants from open-ended questions can be found in (Appendix F).

The vast majority of men (80%) are willing to take a genetic risk assessment. Of the men who reported yes to the test, 47% will take the test if there is no cost implication. Moreover, about 72% of respondents agreed that the test could be broadened to other prostate health issues, and 87% reported that they would consider taking a test for other medical conditions via a postal system.

Overall, men were very happy with the service, and three quarters of men were willing to do a genetic test, either if it was free or at an extra cost. The comments provided were positive. Some men provided feedback for improvement. Almost 90% of respondents rated the service as excellent or good, resulting in an average score of 4.6 out of 5.

In summary, the PSA home testing service works very well and could potentially be expanded further.

Table 6.1: The main reasons for not completing the test.

Main reasons for not completing the test	**Number**	**Percentage**
Insufficient Blood	163	81.5
Instruction (not clear)	1	0.5
Sight Lancet	4	2
Other reasons	32	16
Total	200	100

Table 6.2: Overall rating of the service.

Rate Home Testing Kit service	**Number**	**Percentage**
Excellent	1278	67.19
Above average	413	21.71
Average	92	4.84
Below average	45	2.37
Low	74	3.89
Total	1902	100.00

6.4 Discussion

This study aimed to assess the feasibility of using a PSA home testing kit and men's views on the genetic test. There is no evidence at the current time on this specific topic for prostate cancer. Although the PSA test is not a cancer-specific marker [734], it is the only tool for screening for prostate cancer [735]. Scientists put great efforts into enhancing the performance of the test by adding more inputs and variables such as free to total PSA (%fPSA), age, and family history. Despite the alarming numbers of incidence and mortality, there is currently no screening programme for prostate cancer in the UK [736]. A 2007 data collected from 87 random general practices shows that PSA testing remains low in the UK at a rate of 6.%. Also, it shows a significant difference by age and geographical location among individuals who are tested, indicating the clinical need or current policy has not been reflected and represented [733]. A previous study explored the attitudes of men who had been diagnosed or suspected of prostate cancer towards PSA testing for prostate cancer in the UK; men believed that a nationwide screening programme should be offered as screening would motivate more males to be examined, while others believed that access to PSA testing is limited due to a lack of government support and resources [737]. Another study examined men's responses to the US Preventive Services Task Force recommendation in 2012, and despite the recommendation against PSA testing, men still favour PSA testing [738].

The At-Home self-sampling test has made it possible to conduct large-scale screening for cancers at their comfort without cost or the need to have patients come to a clinic. In addition, with the current ongoing pandemic, it is less likely men will have PSA tests at clinics.

The self-sampling method by sending the test kit through the mail has been used with patients at average or high risk of different types of cancer, such as colorectal cancer [62], prostate cancer [739], cervical cancer [740], and bladder cancer [741]. This method has increased the screening rate significantly in each type of cancer as well as enabled screening for other diseases.

The study has several strengths and limitations. This is the first study that investigated the viability of PSA home testing kits. Also, the survey was conducted by the GFCT – a non-profit organization. As a result, the results are less likely to be biased. Nonetheless, only men who received the kit had their opinions taken. The kit's reliability in terms of test accuracy and consistency has not been assessed in this study. Thus, we are unable to comment on the kit's use in this study. Moreover, questions about incorporating these tests into the National Health Service (NHS) were not included in the survey, as it would have an impact on future screening in the NHS.

In conclusion, men were very happy with the service, and three quarters of men were willing to do a genetic test either if it was free or at an extra cost. The comments provided were positive. Some men provided feedback for improvement. Almost 90% of respondents rated the service as excellent or good, resulting in an average score of 4.6 out of 5. The PSA home testing service works very well and could potentially be expanded further.

In the next chapter, I discussed all the findings derived from the thesis and highlighted strengths and weaknesses inherited within each chapter. Also, I described potential future work that could be done to improve prostate cancer risk assessment in primary care and community settings.

Chapter 7 Conclusion and future work

The work presented in this thesis focuses on providing a better prediction of prostate cancer risk. Specifically providing better risk prediction that can be used in the primary care and community settings.

In the preceding chapters, I discussed the limitations of existing risk factors and biomarkers (Chapters 2, 3), demonstrated the scarcity of appropriate risk models that do not include genetics or clinical tests for prostate cancer (Chapter 4), and developed a risk prediction model using two different cohorts and incorporating simple and readily available tests (Chapters 5). Following that, I present men's responses and views regarding home PSA testing for prostate cancer (Chapters 6).

Finally, I summarise contributions, reiterate results, and highlight future work in this chapter.

7.1 Overview of the main findings

The overview of the main findings of this thesis are summarised based on the objectives listed in Chapter 1.

Risk factors and biomarkers associated with prostate cancer (Chapter 2):

I started my thesis by conducting an extensive literature review to give a detailed overview of prostate cancer and its epidemiology and, more importantly, to identify and describe risk factors along with biomarkers that are associated with prostate cancer. The key findings of this chapter are:

- Prostate cancer is common and the incidence rate is increasing.
- Unlike breast cancer in women, there is no national screening program for prostate cancer due to the recognised problems of so-called "overdiagnosis" and "overtreatment".

- PSA, family history, ethnicity and age are established risk factors for prostate cancer. Other risk factors and biomarkers are still lacking evidence.
- For the genetic markers, to date there are over 200 Single nucleotide polymorphisms (SNPs) associated with the risk of prostate cancer.
- PSA is not prostate cancer-specific and can be elevated or lowered due to other prostatic conditions or medicines.

Assessing the relationship of body size and body shape to the risk of prostate cancer (Chapter 3):

Next, I investigated the association between body size and shape and prostate cancer risk. I used the data collected by our research group over the past 15 years. The UK Genetic Prostate Cancer Study (UKGPCS) was first established in 1993 and is the largest prostate cancer study of its kind in the UK. Other factors have been studied and published in reputable journals [742-745].

There is a link between male self-reported body size and the risk of prostate cancer, according to previous research. I explored the possibility of a link between prostate cancer risk and male self-reported body size in this UK-nationwide case-control study. I also looked into body shape as a proxy for fat deposition around the body. Because obesity and excessive adiposity have been associated with an increased risk of developing a variety of cancers, scientists attempted to look into self-reported body size and shape and their possible relationship with prostate cancer.

The goal of this study was to see if there were any underlying links between prostate cancer risk and male self-reported body size and shape. The key findings of this chapter are:

- Changes in self-reported body size in males from early to mid-adulthood are not a significant risk factor for prostate cancer development.
- When compared to a 'symmetrical' shape, body shape indicative of body fat distribution revealed that an 'apple' body shape was negatively related to prostate cancer risk.
- Further research into the risk of prostate cancer and possible links to genetic factors that influence body shape may throw further light on any underlying correlations.

Risk prediction models for prostate cancer for primary care settings (Chapter 4):

It has been documented that several attempts have been made to overcome key issues with prostate cancer screening by PSA test and its derivatives. As a result, several risk prediction models have been developed using mathematical approaches and included various biomarkers and risk factors to enhance the predictability accuracy and model performance to assess men in determining their potential risk of having or developing the disease. In the UK, currently although there is a risk assessment for prostate cancer available in some Primary Care Practices, it is not widely used as it is predominantly based on symptoms. The Q risk (https://www.qcancer.org/male/) consists of symptoms for urinary tract, family history and ethnicity.

There is, therefore, a need to identify better risk models that have wider potential to be used in primary care and community settings as this will help to identify high-risk men with no symptoms. The key findings of this chapter are:

- The vast majority of existing risk prediction models included either genetic and/ or invasive clinical tests, therefore, there are few risk models that do not include such variables (only five models identified).
- Most of the existing models have been developed to purposely be used in clinical practice, hence it includes either genetic biomarkers or clinical testing.
- Several limitations have been demonstrated within the potential risk models identified, such as study design, not being validated, and not using and reporting appropriate performance measurements.

Development of a Prostate Cancer Risk Prediction Model for Use in Primary Care (Chapter 5):

The findings of the systematic review in chapter 4 led me to carry out the work on developing a prostate cancer risk prediction model for use in primary care settings to help men stratify their risks, particularly those who are at a higher risk of developing aggressive or high-grade prostate cancer. It is also equally important that any prostate cancer prediction algorithm must also rule out cancer-free and indolent cases with low risk. Only men with a high risk of developing severe prostate cancer will be urged to pursue further investigations, such as with their primary care physician or at secondary care or specialist clinics, while those with a low risk will be kept under active monitoring. Magnetic resonance imaging (MRI) guided biopsy has been adopted in several large hospitals in recent years, and in the near future, MRI-guided biopsy for prostate cancer diagnosis will be routinely offered in the National Health Service (NHS). This has highlighted the need for risk-adapted risk stratification to determine which men should proceed with the surgery.

The goal of this study was to use two separate data sources to construct a risk prediction model for prostate cancer in primary care settings (RISKMAN). The first data source came from the United Kingdom, where the outcome of prostate cancer was assessed by MRI-guided biopsy. The second data source was Polish data, and the result of prostate cancer was evaluated by a traditional needle biopsy. The RISKMAN contains characteristics that are easily assessed, low-cost, and consistently gathered and available. The key findings of this chapter are:

- The results of this investigation showed that the RISKMAN algorithm performed well in two different clinical procedures.
- The developed model could be utilised to improve the quality and timing of health treatment by risk stratifying men and assisting in the early detection of those who are at high risk of developing severe prostate cancer while eliminating unnecessary biopsies.
- According to the findings, each variable (age, PSA, percent fPSA) is linked to the risk of prostate cancer in both traditional and new MRI-guided prostate cancer detection methods.
- In both datasets, the full model, which includes age, PSA, and percent fPSA, performs better than PSA or age alone, as well as model 1 (Age) and model 2 (PSA plus Age) in each sub-group.
- In any prostate cancer, there is no difference in the discriminative capability of the full model between the UK and Polish cohorts.
- In comparison to low-grade plus controls without prostate cancer, the full model was marginally better at predicting high-grade prostate cancer.

- In comparison to controls without prostate cancer, the model's performance in predicting high-grade patients improved significantly.
- Within the PSA range of 3-10 ng/ml, model performance was greater in the high-grade subgroup compared to controls without prostate cancer.
- The findings imply that single variables like age, PSA, and percent fPSA do not have enough predictive potential to distinguish between significant prostate cancer and non-significant prostate cancer.
- This study also revealed that these markers can still be used to stratify men at risk of significant prostate cancer for further inquiry using current techniques (MRI) to identify prostate cancer outcome. This method will help not only the men who receive clinical care but also the NHS service in the long run since it will save money by reducing the number of tests required to detect major cancers in the community.

PSA home kit survey (Chapter 6):

As PSA remains a key biomarker for prostate cancer. Our research group, together with the Graham Fulford Charitable trust, conducted a survey on home testing for prostate-specific antigen (PSA) as an option for getting a PSA test. GFCT (the charity) began providing this service during COVID-19 when face-to-face appointments in the health system were limited. Men who had registered with the charity and used a PSA home testing kit received an email with a link to an online questionnaire with ten questions. The key findings of the survey are:

- The average overall service rating is 4.6 out of five points, with 5 being exceptional service.

- 90% of them said they would use the home kit PSA test again, and nearly 92% said they would recommend it to others.
- The vast majority of men (80%) are willing to take a genetic risk assessment.
- During COVID-19, the PSA home kit test was a successful intervention. This service has made large-scale testing possible in the future.
- Future implication also includes potential wider reach to men with some ethnic minority background.

7.2 Implications of the RISKMAN algorithm

The RISKMAN algorithm aimed to address both the previous and current challenges of diagnosing prostate cancer at an early stage while also making use of recent breakthroughs in the clinical follow-up of men designated as high risk. Image-guided prostate cancer biopsy, in particular, has substantially improved the assessment of men at risk. However, the existing costs and NHS capability for administering large-scale MRI, as well as the MRI's unknown usefulness as a main diagnostic, limit its widespread usage as a screening test. Key advancements in genetics, which have enabled the stratification of prostate cancer predisposition, may be used to help identify higher-risk individuals, especially when combined with traditional approaches. It needs to be seen how such risk classification might be employed most effectively.

As a result, we have developed a simple incremental risk assessment approach with men's support groups that combines the best aspects of both PSA and other forms of PSA, as well as age, to better identify those men in the community who are at higher risk and should be referred for clinical assessment. The RISKMAN assessment process can be easily utilised in primary care or by post to promote testing uptake and improve

early detection of men at the highest risk and appropriate risk mitigation and monitoring for those groups of men who do not normally participate in early detection programs.

Up to a third of men could be spared from unnecessary biopsies and treatment if the RISKMAN algorithm is used. Men in the higher risk strata can also be assisted in lowering their risk profile through lifestyle changes that can significantly reduce their chances of developing fatal prostate cancer and other chronic diseases. Therefore, I propose that RISKMAN be implemented more broadly and evaluated as an effective method for identifying men who should be further clinically tested and/or enrolled in risk reduction programs.

An assessment of the efficacy of two different approaches to the onward clinical pathway in order to increase the number of men diagnosed with prostate cancer at an earlier stage when treatment is more successful should be done. The first step will be to screen men utilising the new MRI-guided biopsy method. This will be compared to a second method, in which those men who are most at risk are actively monitored using a risk-adapted tiered array of more expensive biomarkers. Also, an evaluation should be conducted on the two approaches for their ability to detect a higher proportion of clinically curable malignancies, as determined by a shift in stage distribution for those men who develop cancers over the follow-up period.

The RISKMAN strategy will improve the testing and management of prostate cancer at the regional level and eventually the entire UK by complementing other initiatives aimed at better detecting, mitigating, and monitoring men at risk of curable prostate cancer.

Originality and authenticity of RISKMAN

Since the RISKMAN results are derived from pre-screened populations, the findings need to be further tested and validated before it they can be applied to the general population in the community and primary care settings. Further studies using a non-screened population are therefore warranted. RISKMAN, however, is novel in that the prostate cancer risk prediction review from chapter 4 concluded that there was no algorithm with risk factors and markers as used in RISKMAN. Our study is also the first study to use MRI-guided biopsy outcome, which is assumed to be the future standard for detecting and diagnosing prostate cancer, to test the algorithm. Thus, there was no study in the literature that has data with such an outcome to compare and contrast results with this current study. Hence, the ideal approach to assess the RISKMAN model appropriately is by collecting data at the population and community level from multiple and different geographical areas within the UK to ensure the best representation of the broader population as possible. This will help to calibrate the model more accurately and enable validation of the model externally.

Furthermore, it is recommended to gather information on other potential factors that have been linked to prostate cancer risk, specifically factors that are easy to measure and obtain at a low cost. One such factor I would like to include in such a data collection going forwards is body shape (and possibly other anthropometric measures) as I described this factor associated with prostate cancer in Chapter 3 and it would be easy to collect. This will allow an assessment of its association with the disease on large multi-centric data and subsequently can be evaluated for the feasibility of incorporating it into the extended model in the future.

7.3 Strengths and limitations

A summary of strengths and limitations within this thesis will be presented for each chapter that has either been published, submitted or will be modified for publication separately.

Chapter 3 - Relationship of self-reported body size and shape with risk for prostate cancer: A UK case-control study:

Strengths:

- A large UK case-control data has been used to examine the potential association of three areas with the risk of prostate cancer; self-reported body size at early and mid-adulthood, self-reported body size changes over decades in life, and self-identified body shape.
- We are unaware of any published studies on the prevalence of various types of body fat distribution in the general population therefore this work adds further evidence to the literature.
- No other study has employed body shape as a proxy measure of body fat distribution to evaluate possible connections with prostate cancer in the literature.

Limitations:

- Our data is limited to middle age (40s), which means that this may not be the time in life when obesity is linked to prostate cancer.
- Pictograms were utilised as a surrogate for body size in our research. When compared to other metrics like weight, waist circumference or waist-hip ratio, body mass index (BMI) or body fat mass, pictorial illustration has the

disadvantage of not being able to measure actual changes in body size. As a result, pictorial assessment of self-reported body size is subjective, but it may be more useful for demonstrating changes in body size over lengthy periods of time. Pictograms are thought to be a reliable and useful tool for determining self-reported body size and distinguishing between thin and obese people.

- Individuals' perceptions of their own body size may induce bias, such as categorization bias.
- Body weight, waist/hip circumference, and body fat mass are often measured and recorded longitudinally in cohort studies to acquire more meaningful data. In our research, we were unable to put this strategy into practice.

Chapter 4 - Prediction models for prostate cancer to be used in the primary care settings: systematic review:

Strengths:

- To our knowledge, this is the first study to review risk prediction models for prostate cancer that are low cost and do not incorporate clinical and genetic tests and are based on single time point assessment, and therefore, have a potential to be used in primary care and community settings.

Limitations:

- The aim of this study was to find prediction models that might be used in primary care or community settings, and so the search strategy was focused on retrieving research that fit this purpose.
- Articles that were not published in English or did not contain an abstract were excluded.

Chapter 5 - Development of Risk Prediction Model for Prostate Cancer for Primary Care Settings:

Strengths:

- A low-cost, feasible prostate cancer risk prediction algorithm is developed that can be used in general practice, especially in the UK.
- Prediction performance was investigated, for the first time according to our knowledge, using the same biomarkers and inputs but with two different methods to obtain the outcome (prostate cancer); the traditional needle biopsy and MRI-guided biopsy.

Limitations:

- The subjects in both cohorts were pre-screened, which could lead to an overestimation of model performance.
- This study only examined patients with biopsy-naïve, therefore, the findings could not be applicable to repeat biopsy cases
- The two cohorts are different in terms of method of outcome yield, therefore, only an internal validation was carried out and no external validation was conducted.
- Family history is an important risk factor for prostate cancer; however, we did not include it because family history was enriched in the Polish data, as both cases and controls were chosen based on family history, and it was not available in the UK data. Ethnicity was also eliminated because the population in both cohorts was white, as well as due to a lack of data, thus I was unable to study these characteristics.

Chapter 6 – PSA home kit testing survey

Strengths:

- This is the first survey that looked at the feasibility of PSA home kit testing.
- The survey was carried out by the GFCT, which is a non-profitable organization. Therefore, the findings are less likely to be biased.

Limitations:

- The view is only reflected men who are being sent the kit.
- In this study, we did not evaluate the reliability of the kit in terms of test accuracy. Therefore, we cannot comment on the kit used in this study.
- We did not ask the question regarding incorporating these tests in the NHS as this will have an impact on future screening within the NHS.

7.4 Other areas for future work

- Future work could extend the RISKMAN to incorporate polygenic risk scores and be used to improve the identification of men at higher risk or prostate cancer.
- The extended RISKMAN algorithm can be used in MRI-guided biopsy outcome to investigate if it is effective as well as in conventional biopsy
- Decision analysis tools such as cost analysis, cost-effectiveness analysis, and cost-utility analysis will help to rationalise the best overall approach to expanding and implementing the overall program.

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Appendices

Appendix A Search Strategy

Search Strategy

1) * Prostatic Neoplasms/
2) Initial biopsy.mp
3) First biopsy.mp
4) * "Early Detection of Cancer"/mt [Methods]
5) 2 or 3 or 4
6) 1 and 5
7) Nomograms/
8) "Neural Networks (Computer)"/
9) Risk Assessment/
10) Models, Statistical/
11) 7 or 8 or 9 or 10
12) 6 and 11
13) Limit 12 to English language
14) Limit 13 to abstracts

Appendix B Search results for external validation for the included models

Study	No. of citations in Google Scholar	No. of citations in Medline	No. of external validation found in Google Scholar	No. of external validation found in Medline
Carlson, 1998	117	20	Null	Null
Babaian, 2000	128	13	Null	Null
Jansen, 2010	231	44	Null	Null
Hill, 2010	5	0	Null	Null
Lazzeri, 2013	195	0	Null	Null

Appendix C Type of assays used to measure PSA and free PSA

Study	PSA Assay	Free PSA Assay
Carlson, 1998	PA immunoassay (Tosoh)	Investigational double-antibody radioimmunometric assay (PSA II, DIANON Systems)
Babaian, 2000	PSA immunometric assay (Tosoh)	Tandem R assay (Beckman Coulter, San Diego, Calif)
Jansen, 2010	Access 2 Immunoassay System (Beckman Coulter, Brea, CA, USA)	Access 2 Immunoassay System (Beckman Coulter, Brea, CA, USA)
Hill, 2013	N/A	N/A
Lazzeri, 2013	Access 2 Immunoassay System (Beckman Coulter, Brea, CA, USA)	Access 2 Immunoassay System (Beckman Coulter, Brea, CA, USA)

Appendix D Publication 1: Relationship of self-reported body size and shape with risk for prostate cancer: A UK case-control study

PLOS ONE

RESEARCH ARTICLE

Relationship of self-reported body size and shape with risk for prostate cancer: A UK case-control study

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Data Availability Statement: The PRACTICAL consortium has defined policies and legal restrictions on sharing the data publicly, due to the consent process under which the data was collected. However, data are available upon request from The Institute of Cancer Research and The University of Manchester for researchers who meet the criteria for access to confidential data. Access requests may be sent to PRACTICAL@icr.ac.uk.

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Abstract

Introduction

Previous evidence has suggested a relationship between male self-reported body size and the risk of developing prostate cancer. In this UK-wide case-control study, we have explored the possible association of prostate cancer risk with male self-reported body size. We also investigated body shape as a surrogate marker for fat deposition around the body. As obesity and excessive adiposity have been linked with increased risk for developing a number of different cancers, further investigation of self-reported body size and shape and their potential relationship with prostate cancer was considered to be appropriate.

Objective

The study objective was to investigate whether underlying associations exist between prostate cancer risk and male self-reported body size and shape.

Methods

Data were collected from a large case-control study of men (1928 cases and 2043 controls) using self-administered questionnaires. Data from self-reported pictograms of perceived body size relating to three decades of life (20's, 30's and 40's) were recorded and analysed, including the pattern of change. The associations of self-identified body shape with prostate cancer risk were also explored.

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Competing interests: We can confirm that The British Association of Urological Surgeons' Section of Oncology as commercial affiliation does not alter adherence to PLOS ONE policies on sharing data and materials.

Results

Self-reported body size for men in their 20's, 30's and 40's did not appear to be associated with prostate cancer risk. More than half of the subjects reported an increase in self-reported body size throughout these three decades of life. Furthermore, no association was observed between self-reported body size changes and prostate cancer risk. Using 'symmetrical' body shape as a reference group, subjects with an 'apple' shape showed a significant 27% reduction in risk (Odds ratio = 0.73, 95% C.I. 0.57–0.92).

Conclusions

Change in self-reported body size throughout early to mid-adulthood in males is not a significant risk factor for the development of prostate cancer. Body shape indicative of body fat distribution suggested that an 'apple' body shape was protective and inversely associated with prostate cancer risk when compared with 'symmetrical' shape. Further studies which investigate prostate cancer risk and possible relationships with genetic factors known to influence body shape may shed further light on any underlying associations.

Introduction

Prostate cancer is the most prevalent cancer in men [1]. It is also the third most common cancer-specific cause of death for men living in Europe [2, 3]. In 2016, it accounted for approximately one quarter of all cancers diagnosed in men within the UK [4]. Apart from the established cancer risk factors, such as age, ethnicity and family history of prostate cancer in first degree-relatives, other potential risk factors include height, obesity/high body mass index (BMI) and levels of insulin-like growth factor-I [5–7].

Over the last few decades, obesity has increased by approximately 30% in European men [8, 9]. This has been linked to increased risk for developing several chronic diseases and cancers [10]. Extensive studies have investigated the association of both obesity and body size with prostate cancer risk. However, this relationship remains inconclusive [11–15]. Anthropometrics that have been used to measure obesity and body adiposity include waist circumference, waist-hip ratio and BMI [16]. The majority of epidemiologic studies investigating prostate cancer risk have used BMI to evaluate obesity rather than body fat distribution [3]. Previous studies have suggested that high BMI is associated with increased risks for advanced, aggressive and fatal prostate cancer [13, 15, 17–22]. In contrast, other studies have observed a decreased risk of localised/indolent cancer [13, 15, 23–25]. A large meta-analysis consisting of 27 prospective studies of prostate cancer observed no or weak association between BMI and total prostate cancer [26]. Similar findings have come from another systematic review examining the exposure in early adult life [27]. These conflicting results may in part be due to the fact that BMI has been criticised for its inaccuracy in measuring obesity and its ability to differentiate adipose and non-adipose tissues [28, 29]. This suggests that any association could be dependent on particular disease subtypes and the age of exposure [6, 12, 13, 30].

Both body shape and body size have often been used to describe the characteristics of the human body in health-related research. Defining obesity or adiposity through the use of clinical judgement including a consideration of body size appearance provides an alternative approach for determining the wider distribution of fat tissue over time.

The issue of whether weight change during adulthood is more strongly associated with prostate cancer than cross-sectional 'current' adiposity has not as yet been fully explored [31, 32]. Prostate cancer is characterised as being a slow developing disease. Thus the age that obesity develops in early adult life may be an important factor within the aetiology of this cancer [27, 33–35]. Moreover, early changes in prostate tissue have been seen in men during their early adulthood, suggesting that body size over lifetime is important [33, 36]. Adult weight change is a dynamic measure that could reflect imbalances in weight over time and it is thought to be more accurate than a static measure of adiposity such as BMI [19, 37]. However, these studies have reported inconsistent results [19, 31, 32]. Some studies found positive associations between weight gain and prostate cancer [38] whereas others have found an inverse association [39] or no association at all [14, 21]. In this study we specifically address the issues of whether male self-reported body size and overall body shape and self-reported body size and its change across three decades of life are associated with prostate cancer risk.

Methods

The 'Prostate Cancer Study on Gene-Environment Interactions' is a large scale case-control study identifying and investigating potential risk factors for the development of prostate cancer in the UK. The study used a self-administered questionnaire and written informed consent was obtained from each participant. Cases comprised adult men >36 years at diagnosis with histologically confirmed prostate cancer. Male adult controls were selected from the same general practices as cases. Eligible controls were men without history of prostate cancer and were within an age range of ±5 years of cases. This study received ethical committee approval MREC/99/4/013 (Trent Research Ethics Committee), 07/MRE04/29 (Nottinghamshire County Teaching and Primary Care Trust).

Epidemiological data were collected for two time periods; the first between 1997–2004 and the second between 2007–2009. In the second period, some additional questions were added and other questions expanded within the questionnaire to provide more in-depth information, including information on body shape. This was done following a preliminary analysis of data collected from the first period. Data collection from the two time periods involved different subjects and no repeated measurements were performed. Individuals did not contribute their data more than once.

Data on education was based on the UK educational system and social class was based on the UK occupational social class classification. Data on self-reported body size were available from both periods, but data on body shape were only available from the second period of data collection. Self-reported body size at different ages was assessed using a pictogram (Fig 1) with drawings of body silhouettes of nine different sizes ranging from 1 (very thin) to 9 (severely obese) [40]. Subjects were asked to recall information relating to their self-reported body size during their 20's, 30's and 40's. Cases and controls were asked to rate their perceived body size for the last 5 year period prior to diagnosis in case group and for the last 5 years prior to receiving the questionnaire in control group. Participants were excluded from the analysis if there were incomplete data (i.e. missing data for any decade). This was done to ensure each participant has data to investigate self-reported body size changes throughout decades. 1928 cases and 2043 controls were available for the analysis of self-reported body size in the 20's and 30's. Six subjects were younger than 40 years of age at the time data were collected; hence the number of cases and controls eligible for self-reported body size analysis in the 40's were 1924 and 2041 respectively. Ordinal scale data (scale of 1 to 9) for self-reported body size at age 20's, 30's, 40's were grouped into 'thin' (scale 1–3), 'medium' (scale 4–6) and 'large' (scale 7–9).

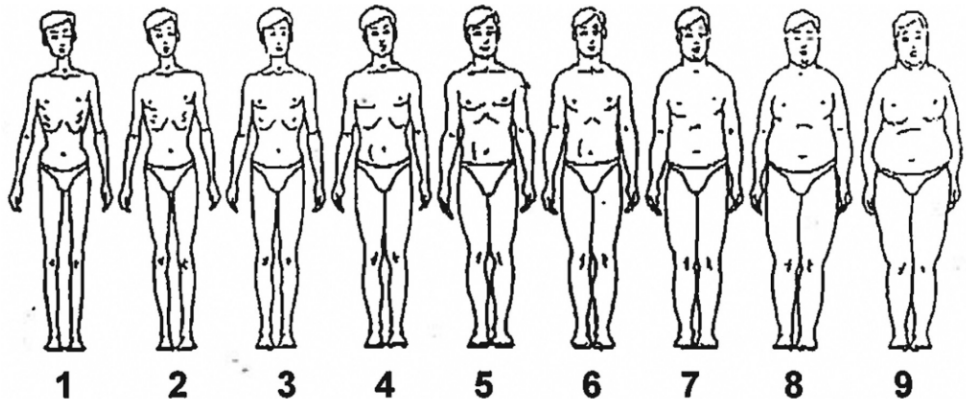

Fig 1. Pictogram with silhouette drawings used for recalling self-reported body size at each decade 20s, 30s, and 40s (taken from Stunkard et al, 1983).

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To explore the effect of self-reported body size increase during adulthood on prostate cancer risk, we restricted our analysis to include only subjects whose self-reported body size remained as medium size from 20's to 40's as our reference group and subjects whose self-reported body size was medium both in their 20s and 30s but increased to large in their 40's as our exposed group (Fig 1). There are 1057 cases and 1099 controls.

For body shape, participants were asked to select their body shape in four different forms (apple, pear, oval and symmetrical) that best represented their body shape throughout their life. Description of each body shape type was provided to aid subject's understanding on its meaning ('Apple' shape is where body fat is distributed mainly around the central abdominal area; 'Pear' shape is where body fat is distributed mainly around the hip and thigh; 'Oval' shape is where body fat is distributed around the neck, chest, abdominal area and thigh; 'Symmetrical' shape is where the person has lean body with no fat). The numbers of subjects included in this particular analysis were 1329 cases and 812 controls.

Statistical analysis

Logistic regression analysis was performed on the data using Stata version 15.0 [41]. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for total prostate cancer risk. Forward stepwise logistic regression was performed on demographic factors to identify potential confounders. The final multivariate logistic regression model included education, ethnicity, study phase (I and II) and family history of prostate cancer in first-degree relatives. Multivariable logistic regression was fitted with all confounders. Age was also included as an *a-priori* variable in all regression models. For self-reported body size, medium size was used as reference category and for pattern of change, no change from 20s to 40s was used as reference group. In the multivariate model, self-reported body size at age 30's and 40's were adjusted further to self-reported body size at age 20's to minimise the effect of correlation between self-reported body size at age 20's to age 30's and 40's. For body shape, symmetrical shape was used as reference category. Estimated risks were obtained from multivariate logistic regression models. A significant odds ratio is considered when 95% C.I does not include 1.

Sensitivity analysis

We collected data on current BMI from both periods. A sensitivity analysis was performed to explore if self-reported body size can be used as a proxy marker for BMI. We used the self-reported body size and BMI reported during the last 5 years prior to completing the questionnaire only in the controls due to the fact that prostate cancer may have affected current BMI in cases. Data were available in 766 controls. BMI as a continuous variable was normally distributed hence we applied Analysis of Variance (ANOVA) to explore the differences among group means. A finding was deemed to be statistically significant when the P-value was less than 0.05.

Study power

As they are no previous studies on body shape and prostate cancer, we computed our study power based on exposure in our study. Our study of 1329 case and 812 controls with a probability of exposure (apple body shape) among controls of 0.62, had a 95% study power to detect odds ratios for disease of 0.72 or 1.41 [42].

Results

The overall study response rates after initial consent to complete the questionnaire were 85.0% for cases and 74.4% for controls. Table 1 shows the study population characteristics. The median age for both case and control subjects was 60 and 59 years respectively. The vast majority of study subjects described themselves as white (98%).

Table 2 summarises the number of subjects and their self-reported body size at each of the three decades of their life. The majority of participants was medium across all three decades in both case and control groups.

Table 3 summarises odds ratios of self-reported body size changes and prostate cancer risk. Both cases and controls have similar percentage of self-reported body size change from medium to large in their 40's (~30%). The result suggests that there is no association with cancer risk for subjects whose self-reported body size increased from medium to large as compared to subjects with medium self-reported body size throughout their adulthood.

Table 4 presents estimated risks of different self-reported body shape and prostate cancer risk. Compared to symmetrical shape, subjects with an apple shape were at 27% risk reduction (OR in the fully adjusted model = 0.73 with 95% CI 0.57–0.92). Both pear and oval shape did not show any association with prostate cancer risk in the fully adjusted model of 1.44 (95% CI 0.77–2.69) and 0.82 (95% CI 0.59–1.13) respectively. Although, the association is not significant, but the direction of effect suggested that adipose tissue distributed around the hip and thigh (pear) is at higher risk, while abdominal fat distribution (apple, and oval) is at lower risk.

Results from sensitivity analysis (only in the control group) using ANOVA test is presented in Table 5. The significant p-value suggested that mean BMI in each group is a statistically significant difference. BMI increases with increased self-reported body size indicative of a good proxy between BMI and body size.

Discussion

Three key areas potentially relating to increased risk for prostate cancer were explored in this study; self-reported body size at early and mid-adulthood, self-reported body size changes over decades in life, and self-identified body shape.

Self-reported body size (thin, medium, and large) ranging across three decades (20's, 30's and 40's) was explored and analysis suggested no associations between the self-reported body

Table 1. Demographic and social characteristics of participants in the prostate cancer study on gene-environment interactions.

Characteristics	Cases (n = 1,928)	Controls (n = 2,043)	OR of prostate cancer	(95% CI)
	Median	Median		
Age (years)	60 (range 36–84)	59 (range 36–76)		
	n (%)	n (%)		
Marital Status				
Married or partnership	1,585 (82.2%)	1,691 (82.8%)	-Ref-	
Divorced, separated or widowed	227 (11.8%)	260 (12.7%)	0.93	0.77–1.13
Single	89 (4.6%)	68 (3.3%)	1.39	1.01–1.93
Missing	27 (1.4%)	24 (1.2%)		
Education				
No qualifications	433 (22.5%)	558 (27.31%)	-Ref-	
GCSE, O levels or equivalent	357(18.5%)	342 (16.74%)	1.35	1.11–1.64
A levels, higher or equivalent	132 (7.0%)	148 (7.24%)	1.16	0.89–1.51
Higher or professional qualification e.g. degree, HND	716 (37.0%)	742 (36.32%)	1.25	1.06–1.47
Others	252 (13.0%)	229 (11.21%)	1.42	1.14–1.76
Missing	38 (2.0%)	24 (1.17%)		
Ethnicity				
White	1,832 (95.0%)	2,000 (97.9%)	-Ref-	
Black	29 (1.5%)	4 (0.2%)	8.1	2.84–23.12
Asian	13 (0.7%)	7 (0.34%)	1.99	0.79–5.02
Other	26 (1.4%)	13 (0.64%)	2.19	1.12–4.29
Missing	28 (1.4%)	19 (0.93%)		
Social class				
I	236 (12.2%)	224 (11%)	-Ref-	
II	797 (41.3%)	851 (41.7%)	0.89	0.72–1.10
IIIN	193 (10.0%)	208 (10.2%)	0.88	0.67–1.15
IIIM	499 (26.0%)	528 (25.8%)	0.90	0.73–1.13
IV	108 (5.6%)	111 (5.4%)	0.93	0.67–1.28
V	18 (0.9%)	31 (1.5%)	0.56	0.30–1.02
Missing	77 (4.0%)	90 (4.4%)		
Family history of prostate cancer				
No	1,312 (68.0%)	1,880 (92.0%)	-Ref-	
Yes	533 (27.7%)	100 (4.9%)	7.61	6.08–9.54
Missing	83 (4.3%)	63 (3.1%)		

*Unadjusted OR. The rest of ORs were adjusted for age.

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size at each stage of life among cases and control group and risk of prostate cancer however our analysis could be underpowered given the relatively small numbers in the 20's/large and 40's/thin category. Furthermore, the analysis suggested that 55% of both case and control subjects had a history of changes in self-reported body size. Our *ad hoc* analysis also showed that the magnitude of changes of self-reported body size from age 20's to 40's varies between individuals (result not shown here). Approximately 53% of those self-reported body size changes were of increase in size (either for both periods-20s to 30s and 30s to 40s or at 20s to 30s and no change in 30s to 40s). The possible explanation for increase in body size is because of decreased metabolic rate with ageing and accumulation over the years of unburned calorie intakes. Environmental factors such as eating high-fat foods or lack of exercise, as well as Sedentary Lifestyle Syndrome (SeDS) could also be accountable for increasing in body size [43].

Table 2. Self-reported body sizes at each decade among cases and controls.

Body size at 20's	Cases	Controls	OR of prostate cancer[a]	OR of prostate cancer[b]
Medium	1,159 (60.1%)	1,208 (59.1%)	-Ref-	-Ref-
Thin	690 (35.8%)	736 (36.0%)	0.97 (0.85–1.11)	1.10 (0.95–1.28)
Large	79 (4.1%)	99 (4.9%)	0.84 (0.62–1.14)	0.95 (0.66–1.35)
Body size at 30's *				
Medium	1,497 (77.7%)	1,573 (77.0%)	-Ref-	-Ref-
Thin	255 (13.2%)	273 (13.4%)	0.97 (0.80–1.17)	0.97 (0.77–1.22)
Large	176 (9.1%)	197 (9.6%)	0.96 (0.77–1.19)	1.00 (0.77–1.30)
Body size at 40's *				
Medium	1,291 (67.1%)	1310 (64.2%)	-Ref-	-Ref-
Thin	70 (3.6%)	91 (4.5%)	0.77 (0.56–1.06)	0.85 (0.58–1.23)
Large	563 (29.3%)	640 (31.4%)	0.91 (0.80–1.05)	1.00 (0.85–1.75)

[a] Age-adjusted regression model

[b] Multivariate adjusted regression model for age, education, ethnicity, study phase and family history of prostate cancer

*Body size at 30's and 40's adjusted further to body size at 20's in the multivariate model

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These possible explanations are compatible with the considerable social and life style changes that have occurred across the UK over the last 30 years.

The findings of previous studies regarding obesity at early and mid-adulthood are inconclusive. Our results are consistent with the majority of epidemiologic studies that found no associations between self-reported body size in early as well as middle to late adulthood and prostate cancer risk [14, 21, 27, 39, 44, 45]. More recently, a research group (the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortium) investigated potential causal relationship between BMI and prostate cancer using genetic approaches to analyse 20848 cases and 20214 controls. This also failed to identify any significant associations between BMI and prostate cancer [46]. Our study also did not find any association between changes in self-reported body size over decades (increase in self-reported body size to large in the 40's compared to remains medium throughout) and prostate cancer risk. This finding is inconsistent with several other studies where some relationships with prostate cancer were observed [14, 19, 24, 38, 47, 48]. This inconsistency could be due to the different measurements used by these studies which used actual weight, BMI or waist circumference to indicate the change in body size. In contrast, in our study we used pictograms as a surrogate for body size. We also performed analyses of BMI and perceived body size within different social class and education in the control group and the results suggested a very similar correlation to that seen in the main sensitivity analysis. Furthermore, the other studies used multiple parameters to measure body size when investigating the relationship of change of body size with prostate cancer. As such there was therefore a higher possibility of obtaining statistical

Table 3. Estimated risk of self-reported body size changes and prostate cancer risk.

Group	Cases	Controls	OR of prostate cancer[a] (95%CI)	OR of prostate cancer[b] (95%CI)
Body size remains thin or medium throughout adulthood	738	758	-Ref-	-Ref-
Body size increase to large in their 40s	319	341	0.97 (0.81–1.17)	1.07 (0.87–1.33)
Total	1,057	1,099		

[a] Age-adjusted regression model

[b] Multivariate adjusted regression model for age, education, ethnicity, study phase and family history of prostate cancer

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Table 4. Odd ratios of self-reported body shape on prostate cancer risk.

Self-reported body shape	Case	Control	OR of prostate cancer[a] (95%CI)	OR of prostate cancer[b] (95%CI)
Symmetric	349	173	-Ref-	-Ref-
Apple	735	504	0.67 (0.53–0.83)	0.73 (0.57–0.93)
Pear	51	17	1.57 (0.87–2.85)	1.47 (0.78–2.76)
Oval	194	118	0.76 (0.56–1.02)	0.82 (0.59–1.14)

[a] Age-adjusted regression model

[b] Multivariate adjusted regression model for age, education, ethnicity, and family history of prostate cancer

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significant findings in at least one of the measurement parameters. The other limitation is that our data is only limited to middle age (40s) hence this may not be the period in life that obesity associates with prostate cancer. Our results which failed to show association are in keeping with the majority of other studies that investigated the association between weight change and prostate cancer risk [12, 31, 32, 39, 44, 45, 49–53].

A limitation of using pictorial illustration is its inability to make an actual measurement of changes in body size in comparison with using other parameters such as weight, waist circumference or waist-hip ratio, BMI or body fat mass. As such, pictorial assessment of self-reported body size is relative, but it may be better for showing body size change over long time windows. Pictograms are considered to be a valid and useful method to assess self-reported body size and differentiate thin and obese individuals [54]. The Stunkard Figure Rating (SFR) scale of body size [40] tool has been validated for historic recall of body size and was used in a large European population to explore correlation between self-reported body silhouettes and the previously measured (9–23 years) BMI [55]. The authors reported an area under the curve of 0.92 (95% CI 0.87, 0.97) in women and 0.85 (95% CI 0.75, 0.95) in men for identifying obesity at age 30 using body silhouettes vs previously measured BMI at age 30 (±2y). The findings were also similar for previously self-reported BMI, 0.92 (95% CI 0.88, 0.95) and 0.90 (95% CI 0.85, 0.96) in women and men respectively. Another study assessing adolescent body size found that Stunkard's method was a useful indicator in absence of measured BMI [56]. It is also has been reported that recalled body size using pictograms showed a strong correlation with measured weight at age 20–40 years with a correlation ranging from 0.51 to 0.95 [57–59]. Our result from sensitivity analysis in controls suggested that pictogram can potentially be used for recall of body size. Nevertheless, personal perception of body size of each individual could introduce bias such as classification bias.

Table 5. BMI and self-reported body size in control group*.

Body size	Number	Mean	Std. Dev.
2	6	20.23	1.69
3	17	21.78	2.07
4	48	22.97	1.67
5	103	24.02	2.39
6	168	25.44	2.07
7	254	27.46	3.13
8	135	30.18	3.56
9	35	34.14	4.49

*ANOVA F-test P-value <0.05

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Cohort studies often obtain more valuable data by longitudinally measuring and recording body weight, waist/hip circumference and body fat mass. Implementing this approach was not possible in our study. Some medical conditions, such as hypo or hyperthyroidism, can affect body size. However the prevalence of both these conditions in the UK is low (1–2% for both conditions) [60] and therefore unlikely to affect our results. As our study is subject to classification bias, we opted to broadly group body size into three groups to minimise any bias; i.e. thin, medium and large.

We are not aware of any published research on the prevalence of different types of body fat distribution in the population. However waist and chest circumference measurement in males are the closest for describing whether a person shape can be described as 'apple' or be a proxy of central adiposity [61]. Male shape seems to remain highly stable throughout adult life, therefore it is reasonable to assume that characteristic of body fat distribution also remains the same.

Our results suggest that subjects with an 'apple' shape indicative of body fat distributed mainly around the abdomen, were at reduced risk with both adjusted and unadjusted when compared to those with a 'symmetrical' shape. However, the 'pear' and 'oval' body shapes did not show any statistically significant associations. A recent cohort study reported by Barberio involving 26607 subjects, found central body adiposity to be more associated with cancer risk than overall body size [62]. Although the cohort examined the association with cancer in general, our results of self-identify body shape indicative of the distribution of fat tissue around the body suggested similar findings.

In contrast to 'apple' or 'pear' body shape, hip circumference indicates increased amounts of subcutaneous fat. Thus 'apple' body shape in actual measurement would predict wider waist circumference (WC) or higher waist to hip ratio (WHR). Studies using actual measurement have shown increased risk in advanced or high-grade prostate cancer in such individuals [3, 16, 30, 63, 64].

Several possible explanations have been proposed regarding association between central adiposity and prostate cancer. Adiposity can potentially impact through multiple hormonal pathways. Adiposity has been associated with higher levels of insulin, insulin like growth factor I, leptin, and inflammatory cytokines. It has also been linked with lower levels of adiponectin and free testosterone. All of these may impact on prostate cancer development and progression [20, 65–72]. Moreover, some studies showed that adiposity lowered the risk of non-aggressive prostate cancer while at the same time increased the risk for aggressive and high-grade prostate cancer [3, 5, 8, 14, 16, 20, 21, 24, 25, 30, 33, 73]. However other studies have observed weak or no association with prostate cancer and disease subtypes [12, 74–76].

As yet no other study reported in the literature has used body shape as proxy measure of body fat distribution to investigate possible associations with prostate cancer. Our findings suggest that abdominal fat deposition (apple body shape) maybe protective of prostate cancer.

Diabetes is known to be linked with obesity and also shows an inverse association with the risk of prostate cancer [77–79]. One of the limitations is that we collected data on diabetes only in period 2 with no details of diabetes type. However, we carried out logistic regression analysis incorporating diabetes in our model, our results remain the same. Likewise, we also investigated the association of both smoking and physical activity with prostate cancer and there were no associations. Therefore, we did not include these variables in our final model.

In this study, we used self-reported descriptions within the questionnaire to capture the types of body fat distribution. This approach is likely to be less accurate than using 3-dimensional body shape scanning as used in UK National Sizing survey [61] conducted in 2001 to 2002. This cross-sectional study of 9617 adults found that male body shape remained highly stable throughout adulthood. Such quantitative approaches may reveal further insights into

the role and influence of lipidosity and its site of deposition on prostate cancer risk and development.

Supporting information

S1 File. Collaborators.
(PDF)

S2 File. Q_section 10.
(DOCX)

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Appendix E Publication 2: Prediction models for prostate cancer to be used in the primary care settings: systematic review

BMJ Open

Prediction models for prostate cancer to be used in the primary care setting: a systematic review

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ABSTRACT

Objective To identify risk prediction models for prostate cancer (PCa) that can be used in the primary care and community health settings.

Design Systematic review.

Data sources MEDLINE and Embase databases combined from inception and up to the end of January 2019.

Eligibility Studies were included based on satisfying all the following criteria: (i) presenting an evaluation of PCa risk at initial biopsy in patients with no history of PCa, (ii) studies not incorporating an invasive clinical assessment or expensive biomarker/genetic tests, (iii) inclusion of at least two variables with prostate-specific antigen (PSA) being one of them, and (iv) studies reporting a measure of predictive performance. The quality of the studies and risk of bias was assessed by using the Prediction model Risk Of Bias ASsessment Tool (PROBAST).

Data extraction and synthesis Relevant information extracted for each model included: the year of publication, source of data, type of model, number of patients, country, age, PSA range, mean/median PSA, other variables included in the model, number of biopsy cores to assess outcomes, study endpoint(s), cancer detection, model validation and model performance.

Results An initial search yielded 109 potential studies, of which five met the set criteria. Four studies were cohort-based and one was a case-control study. PCa detection rate was between 20.6% and 55.8%. Area under the curve (AUC) was reported in four studies and ranged from 0.65 to 0.75. All models showed significant improvement in predicting PCa compared with being based on PSA alone. The difference in AUC between extended models and PSA alone was between 0.06 and 0.21.

Conclusion Only a few PCa risk prediction models have the potential to be readily used in the primary healthcare or community health setting. Further studies are needed to investigate other potential variables that could be integrated into models to improve their clinical utility for PCa testing in a community setting.

Strengths and limitations of this study

- ► The review focussed on risk prediction models for PCa for use in primary care.
- ► The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) approach was followed in identifying relevant articles and reporting this study.
- ► We used the Prediction model Risk Of Bias ASsessment Tool (PROBAST) to assess the quality and risk of bias in the included models.
- ► The search strategy was restricted to two databases and a manual search, to retrieve original studies.

INTRODUCTION

Prostate cancer (PCa) is the second most common cancer and the fifth leading cause of cancer-attributed death in men worldwide with an estimated incidence of 1 276 106 and 358 989 deaths in 2018.[1] In the UK, around 47 200 new cases of PCa were reported in 2015, accounting for 26% of all new cancer cases in males. PCa deaths in the UK of were around 11 600 in 2016.[2] The global prcjections of PCa incidence and mortality for 2030 are 1.7 and 0.5 million, respectively.[3] The highest incidence of PCa is seen in western societies.[4] The significant increase of PCa incidence and diagnosis over the last three decades can be attributed mainly to the widespread implementation of the prostate-specific antigen (PSA serum test after it had been introduced in the late 1980s.[5 6]

The strong association of PSA with PCa,[7 8] along with it being a relatively inexpensive test,[9] has made PSA a key biomarker in the diagnostic process of PCa and for the recommendation of a confirmatory prostate biopsy.[7 9] PSA is, however, not a cancer-specific marker.[5 10] Conditions such as benign prostate hypertrophy (BPH), prostatitis and other non-malignant prostatic conditions can also elevate PSA level, thus introducing uncertainty to the application of the test.[11–14] This highlights limitations of the PSA test regarding its specificity and sensitivity, and it being largely dependent on setting a 'diagnostic' cut-off point, which often leads to an unacceptable number of false-positive and false-negative results.[5 10 15 16] Such issues are likely to be the part of the explanation for

the significant number of unnecessary biopsies currently being performed each year. Such procedures are associated with adverse side effects for patients and also increases healthcare costs.[17 18]

To address such PSA test limitations, researchers have incorporated other measurable factors into approaches for the early detection of PCa; these 'risk assessment tools' are based on statistical models designed to improve the accuracy and performance of the PSA test.[19–22] Logistic regression and artificial neural network (ANN) models are now considered to be the most common and effective statistical techniques in aiding the development of new models to enhance early PCa diagnosis.[23] These PCa risk prediction models can be used to aid the testing of men for further investigations.

Currently in the UK, there is no population-based screening programme for PCa. The ultimate goal of PCa screening is to find intermediate and high risk of PCa rather than low-risk PCa that would not require treatment but will give emotional burden to the patient once detected and unnecessary treatment in some patients. An important potential advantage of the extended risk models is their ability to provide a more accurate estimation of PCa risk. This may ultimately lead to their use in patient counselling and decision-making.[24–27] Such models have already achieved better results in predicting probabilities of outcome compared with clinical judgement.[28 29] Furthermore, it has been reported that using such predictive models may minimise the rate of unnecessary biopsies.[30]

Recently, there has been a substantial increase in the development of predictive models to help clinicians assess risk and decide which man to send to clinical setting to further investigate for a possible diagnosis of PCa.[22 26 30–35] The majority of these models are designed for use in clinical settings, where costs are less of an issue and most include the need for a clinical examination such as digital rectal examination (DRE) or trans-rectal ultrasonography (TRUS). One of the main limitations of DRE is its poor performance, especially at low PSA levels, and it is highly subjective to inter-observer variability.[36–38] A meta-analysis study revealed that DRE has positive predictive value of only 18%.[39] Similarly, TRUS has been reported for having poor accuracy at low PSA levels[40 41] and small PCa might not be palpable on DRE or visualisation on TRUS.[40] Furthermore, less than 40% of PCa detected by DRE are potentially curable, making it less beneficial for early diagnosis.[42] Several studies showed that there is fear, anxiety and embarrassment among some men, in particular Black men, regarding the DRE test.[43–46] Another disadvantage of the DRE is the fact that it is a potentially uncomfortable test.[47–52] This may explain why the DRE is a barrier for some men to participate in PCa screening if it includes DRE test. Lee *et al* reported that 74% to 84% of Black men may not maintain annual DRE screening,[53] while another study found that it may prevent 22% of men from participating.[54] Since TRUS needs to be performed by a skilled urologist, this means men have to make an appointment with a clinic in a different location, which makes the screening less convenient. As a result, men may feel reluctant to have such tests performed.

This systematic review of the literature was undertaken to identify risk prediction models that do not incorporate invasive or more costly clinical procedures or extensive biomarkers but have potential application for use in primary care and community settings. As low cost is a primary concern for community use, for this review, we set an indicative threshold of approximately three to five times the cost of a PSA test for inclusion. This excluded a number of models that contain new and emerging biomarker or single nucleotide polymorphism panels. As a number of persons are referred to the clinical setting, costs are less of an issue. The performance of the models reviewed for detecting PCa at initial biopsy have been compared using 'reported area under the curve' (AUC) and/or sensitivity-specificity testing.

METHODS

The approach used to identify and select relevant articles was based on the application of the 'Preferred Reporting Items for Systematic Reviews and Meta-Analysis' (PRISMA).[55]

Data sources and search strategy

A literature search was performed using MEDLINE (via Ovid) and Embase databases. The 'medical subject heading' (MeSH) terms, combined with Boolean logic operators 'AND' and 'OR', were applied to retrieve relevant articles. The terms used for the search were 'Prostatic Neoplasms' AND ('Initial biopsy' OR 'first biopsy' OR 'early detection of cancer') AND ('nomograms' OR 'artificial neural networks' OR 'risk assessment' OR 'statistical model'). The full search strategy is provided in a online supplementary file 1. All articles defined (published since the inception of the databases and up to the end of January 2019) were subsequently further filtered as being those only published in English language and with an abstract. Further to using the above search databases, the research articles were selected manually from the reference lists of any relevant review articles. Google Scholar and MEDLINE searches were also carried out to identify independent study for external validation for each model included in this review. The results are presented in online supplementary file 2.

Eligibility criteria

As this review focusses on PCa risk prediction based in community healthcare settings, all studies were selected on the following inclusion criteria: (i) evaluating the risk for PCa at initial biopsy in patients who had no prior history of PCa, (ii) studies that reported 'low cost' risk assessment tools (ie, those not including more expensive genetic or biomarker test) or 'invasive' clinical tests/examinations (such as DRE or TRUS), (iii) studies that included a minimum of two variables of which PSA had

to be one of them (on the basis that an elevated PSA test in UK primary care is usually the first sign and rationale for suggesting a need for further investigation of PCa within NICE guidelines), and (iv) studies that reported AUC and/or sensitivity and specificity of the diagnostic/predictive tool. The exclusion criteria used were: (i) articles with models that were built and based on repeat or mixed biopsies, (ii) studies that only validate an existing model, and (iii) articles that were not published in English. There were no time boundaries regarding the publication year.

Screening of the titles, abstracts and full-texts was carried out by two reviewers (MA, AL). Any concerns about the eligibility of a study were resolved by discussion with a third reviewer (KM).

Data extraction

A data extraction form was developed to collect all relevant information. For each study used in this review, the items extracted included: year of publication, source of data, type of model, number of patients, country where it was performed, age, PSA range, mean/median PSA, number of biopsy cores, variables included in the model, study endpoint(s), cancer detection, model performance and model validation.

Evaluating the performance of the risk models

Prediction models can be evaluated against various criteria. The most critical measurements of model performance are discrimination and calibration.[27] Discrimination refers to how well the prediction model can differentiate patients in different outcome classes according to their predicted risks. It is often assessed by measuring the area under the receiver operating characteristic curve.[56] It also requires setting a series of thresholds to separate low and high ranges of predicted outcomes. A value of 0.5 indicates no discrimination, while a value of 1 indicates perfect discrimination. However, even with perfect discrimination, observed risk can differ from expected risk. Therefore, calibration has an important role in model evaluation.[57] Calibration represents the agreement between expected and observed outcomes.[58] A well-calibrated model is achieved when the calibration slope is close to 1. When the calibration slope is less than 1, it indicates that the model underestimates low risks and overestimates high risks.[59]

Due to the heterogeneity of the studies included, conducting a meta-analysis was not applicable.

Study quality assessment

The quality of the studies included in this review was assessed using the Prediction model Risk Of Bias ASsessment Tool (PROBAST).[60] This tool has been developed specifically to assess the risk of bias and applicability for prediction model studies. The tool consists of four domains and has 20 signalling questions that facilitate reaching overall judgement of risk of bias, as well as issues relating to applicability.

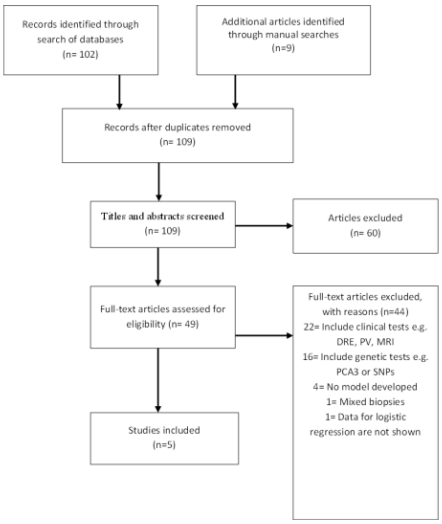

Figure 1 Flow diagram of studies included using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses method. DRE, digital rectal examination; PCA3, prostate cancer antigen 3; PV, prostate volume; SNP, single nucleotide polymorphism.

Patient and public involvement

No patients were involved in setting the research question or the outcome measures, nor were they involved in the design and implementation of the study. There are no plans to involve patients in dissemination.

RESULTS

A total of 102 publications were identified using the search strategy as shown in figure 1. An additional nine articles were identified through manual searches from a bibliography of reviewed articles. At the first filter step, a total of 109 titles and abstracts were screened for eligibility after removing two duplicates. In the second filter step, 60 papers failed to meet the inclusion criteria and were excluded, resulting in 49 articles. The final step of filtering yielded only five studies that were considered to be eligible (ie, passed all set criteria) and were thus included in this systematic review. There was no independent study identified for external validation for included models.

Study characteristics

Four of the five studies included were based on cohort studies and one was a case-control study. The characteristics of each of these studies and populations are summarised in table 1. Details of PSA assays used in the models are presented in online supplementary file 3.

Patients used to build the risk models varied across these studies. Of the five studies, three studies included men from referral populations[61–63] and two studies from screening programmes.[64 65] The sample sizes ranged from 151 to 3773, with three studies derived from US

Table 1 Characteristics of the included studies

Author and year	Type of model	Type of study	Sample no.	Location	Population type	Age	Median age	PSA range	Median PSA	No. of biopsy cores	Cancer detection
Carlson *et al*, 1998[61]	Logistic regression	Cohort	Model dev=3773 Validation=525	Baltimore, USA	Referral	≥45	—	4 to 20 ng/mL	—	Sextant biopsy	32%
Babaian *et al*, 2000[64]	Neural network=3 ANNs	Cohort	151	Texas, USA	Screening programme	40 to 75	62	2.5 to 4 ng/mL	—	11 cores	24.50%
Jansen *et al*, 2010[65]	Logistic regression	Cohort	Site 1=405 Site 2=351	Site 1 from the Rotterdam arm of the European Study of screening for Prostate cancer Site 2 Innsbruck, Austria	Screening programme	≥50	Site 1 (66) Site 2 (60)	2 to 10 ng/mL	~4.4	≥6 cores	Site 1=55.8% Site 2=49.6%
Hill *et al*, 2013[62]	Logistic regression	Case-control	1378	Florida, USA	Hospital referral	40 to 90	—	≥4 ng/mL	—	N/A	20.60%
Lazzeri *et al*, 2013[63]	Logistic regression	Cohort	646	European multicentre; Italy, Germany, France, Spain, and the UK	Referral	>45	—	2 to 10 ng/mL	~5.8	≥12 cores	40.10%

ANN, artificial neural network ; PSA, prostate-specific antigen.

Table 2 Variables used in the prostate cancer risk prediction models

Author and year	Variables used in the model				
	Total PSA	Free PSA	Per cent free PSA	Age	Other variables
Carlson *et al*, 1998[61]	✓		✓	✓	
Babaian *et al*, 2000[64]	✓	✓		✓	Creatine kinase, prostatic acid phosphatase
Jansen *et al*, 2010 (Site 1)[65]	✓	✓			p2PSA
Jansen *et al*, 2010 (Site 2)[65]	✓	✓			p2PSA
Hill *et al*, 2013 (Method 1)[62]	✓			✓	HGB, RBC, haematuria, creatinine, MCV and ethnicity 'Black'
Hill *et al*, 2013 (Method 2)[62]	✓			✓	HGB, RBC, creatinine and MCV
Lazzeri *et al*, 2013 (Model 1)[63]	✓	✓	✓		
Lazzeri *et al*, 2013 (Model 2)[66]	✓	✓	✓		p2PSA
Lazzeri *et al*, 2013 (Model 3)[63]	✓	✓	✓		%p2PSA
Lazzeri *et al*, 2013 (Model 4)[63]	✓	✓	✓		PHI

HGB, haemoglobin; MCV, mean corpuscular volume; PHI, prostate health index; p2PSA, precursor of PSA; %p2PSA, percentage of p2PSA to free PSA ; PSA, prostate-specific antigen; RBC, red blood cells.

cohorts[61 62 64] and two from Europe.[63 65] Four studies used logistic regression methodology to build their model, whereas one study used an ANN-based approach.[64] The minimum age of participants was 40 years[62 64] and the minimum PSA level was 2 ng/mL.[63 65]

Variables in the model

Table 2 presents details of the variables used in each model. PSA level was used in all models, followed by free PSA (fPSA), age and free-to-total PSA ratio (%fPSA). Other variables also reported in the models included: precursor of PSA (p2PSA), percentage of p2PSA to fPSA (%p2PSA), prostate health index (PHI), levels of haemoglobin (HGB), albumin, creatinine and red blood cell count (RBC), haematuria, mean corpuscular volume (MCV) and prostatic acid phosphatase.

Outcome

The study endpoint also varied among the studies selected. Two studies evaluated the accuracy of detecting any PCa[61 64] and three studies examined the pathologic Gleason score.[62 63 65] Although Jansen *et al*, did not build a model to predict the aggressiveness of PCa, they assessed the relationship of each variable individually with a Gleason score ≥7. PCa was determined by taking a needle biopsy. All patients in the five studies underwent prostate biopsy. The least number of biopsy cores used were six[61 65] and the highest were ≥12.[63] One study did not report the number of biopsy cores taken.[62] PCa rates ranged from 20.6% to 55.8%.

Evaluating the performance of the risk models

For predicting any PCa, the Jansen *et al*, model used data from the Rotterdam arm of the European Study of Screening for PCa (ESPRC). Their model achieved the highest discrimination value when compared with PSA alone (AUC of 0.755 vs 0.585, respectively).[65] The AUC values in other studies ranged from 0.648 to 0.74.

One study did not provide the AUC but instead reported an increase of 11% in specificity over per cent fPSA alone with 95% sensitivity.[61] Lazzeri *et al*,[63] presented results from four separated models discriminating PCa with a Gleason score of ≥7. Lazzeri's model 2 (which includes the base model total PSA, fPSA and %fPSA in addition to p2PSA) and model 3 (which includes base model plus PHI) showed the highest levels of discrimination out of the four models with an AUC of 0.67. In the study of Hill,[62] the authors classified PCa stages differently and built their two models accordingly. In Hill's first model, the difference in the discrimination was analysed and based on all PCa versus non-cancerous prostate conditions where the AUC for this model was 0.68 compared with 0.59 for PSA alone. In Hill's second model, the discrimination analysis was based on PCa stages II, III, IV versus PCa stage I, prostatic interstitial neoplasm, BPH and prostatitis where stages I, II, III and IV are parallel to T1, T2, T3/T4 and metastatic PCa, respectively. The AUC for the second model was 0.72 compared with 0.63 for PSA alone. In general, four studies examined the AUC with PSA alone and all reported a benefit from the use of logistic regression or the trained ANN. Model performance and the differences between the AUC's for PSA alone and for the extended models are presented in table 3.

Sensitivity and specificity data are presented in table 4. At 95% sensitivity, the Babaian *et al* model shows the highest specificity (51%) whereas the Jansen model for both sites had the lowest specificity (~23.5%). In the Hill study, with a sensitivity of ~90%, the specificity was lower than in other studies (~18% and 28%) for method 1 and 2, respectively. In the study reported by Lazzeri, the sensitivity and specificity were not reported for the overall

Table 3 The difference of AUC for PSA alone and extended model

Study	AUC for PSA	AUC for model	ΔAUC (Model – PSA)
Carlson *et al*[61]	NA	NA	NA
Babaian *et al*[64]	0.64 %fPSA	0.74	0.1
Jansen *et al* (Site 1)[65]	0.58	0.75	0.17
Jansen *et al* (Site 2)[65]	0.53	0.7	0.16
Hill *et al* (Method 1)[62]	0.59	0.68	0.09
Hill *et al* (Method 2)[62]	0.63	0.72	0.09
Lazzeri *et al*[63]	0.50 for any PC 0.54 for Gleason score ≥7	Model 1=0.65 Model 1 (Gleason score ≥7)=0.60	0.15 0.06
		Model 2=0.71 Model 2 (Gleason score ≥7)=0.67	0.21 0.13
		Model 3=0.704 Model 3 (Gleason score ≥7)=0.67	0.2 0.13
		Model 4=0.71 Model 4 (Gleason score ≥7)=0.672	0.21 0.13

AUC, area under the curve; %fPSA, free-to-total PSA ratio ; PC, prostate cancer; PSA, prostate-specific antigen.

model; instead their study reports sensitivity and specificity for predictive variables individually. The highest sensitivity (90.5%) of %p2PSA and %fPSA achieved the highest specificity in predicting PCa at 21.5% and 22.8%, respectively. Percentage p2PSA and PHI were more associated with Gleason scores.

Table 5 summarises the validation and calibration results for the studies included. Model calibration was reported in two studies.[61 63] Carlson plotted the observed and expected risks using calibration plots, whereas Lazzeri used the Hosmer-Lemeshow goodness-of-fit test. In terms of validation, two studies did not report model validation.[62 65] Only one study reported an external validation using an additional data set consisting of 525 patients.[61] Cross-validation using multiple re-sampling schemes was used in the Babaian study; however, they did not report the number of time this was performed.[64] Lazzeri used 200 bootstrap re-samples to minimise overfitting bias.[63]

Study quality assessment

Quality assessment was carried out by two reviewers (MA and AL) with any discordance resolved by a third reviewer (KM). The assessment of results suggested some issue of study quality according to the criteria as set in the PROBAST, particularly in the analysis domain. For instance, one study applied univariable analysis to select predictors.[61] Three studies did not measure calibration.[62 64 65] Furthermore, two studies did not account for optimism and overfitting by using internal validation methods.[62 65] Whereas one study did not use appropriate measures for model performance that is, AUC, this study reported the calibration.[61]

The event per variable was lower than recommended (<10)[59 66] in the Babaian[64] study, indicating inadequate

Table 4 Sensitivity and specificity profile at different levels for each model*

Study	Sensitivity	Specificity	Probability cut-off	Positive predictive value	Negative predictive value
Carlson *et al*[61]	99	18	>15	≤47	NA
	95	34	18	51	NA
	89	43	20	42	NA
Babaian *et al*[64]	95	51	NA	39	97
	92	62	NA	44	96
	89	62	NA	43	95
Jansen *et al* (Site 1)[65]	95	23.9	NA	NA	NA
Jansen *et al* (Site 1)[65]	90	30.1	NA	NA	NA
Jansen *et al* (Site 2)[65]	95	23.2	NA	NA	NA
Jansen *et al* (Site 2)[65]	90	36.2	NA	NA	NA
Hill *et al* (Method 1)[62]	90.9	17.6	33	47.1	70.5
Hill *et al* (Method 2)[62]	89.8	28	13	20.6	91.3
Hil *et all* (Method 1)[62]	80.5	37.1	37	50.9	70.2
Hill *et al* (Method 2)[62]	78.2	45	15	28.7	88.8
Hill *et al* (Method 1)[62]	39.9	81.4	48	63.4	62.6
Hill *et al* (Method 2)[62]	45.8	79.5	23	36.7	85

*Lazzeri[63] model reported only sensitivity and specificity for predictive variables individually and at sensitivity of 90, %p2PSA and %fPSA achieved the highest specificity
%fPSA, free-to-total PSA ratio ; NA, not applicable; %p2PSA, percentage of p2PSA to free PSA .

Table 5 Validation and calibration for included models

Author and year	Validation	Calibration
Carlson *et al*,1998[61]	External validation on additional data set consisting of 525 patients	Calibration plot
Babaian *et al*, 2000[64]	Cross-validation and separate data set of 151	NA
Jansen *et al*, 2010[65]	NA	NA
Hill *et al*, 2013[62]	NA	NA
Lazzeri *et al*, 2013[63]	Internal validation using 200 bootstrap resamples	Internal calibration using the Hosmer-Lemeshow goodness-of-fit test

power. Four studies did not report missing data or how they handled it.[61 63–65] The remaining study used complete case analysis and excluded patients with missing data on laboratory biomarkers (n=75).[62] The PROBAST guidelines state that in a prediction model study where any risk of bias and applicability is low in all four domains, a regrading to high risk of bias should be considered when the study did not validate the model externally.[60] Thus, although the quality assessment for the Lazzeri study[63] was graded low risk in all the four domains, since the study did not report any external validation of the model, the assessment of the study has been regraded to high risk of bias according to the PROBAST criteria. A full quality assessment for all studies is presented in table 6.

DISCUSSION

Despite the large number of PCa risk prediction models, the majority still include clinical inputs and/or more costly biomarker or genetic panels; few low cost models exist that do not include specialist clinical input or more expensive further testing that limits there use for population wide assessments. To our knowledge, this is the first study to examine risk prediction models for PCa that are low cost and do not include clinical and genetic variables, and are based on single time-point assessment.

Our study identified five unique models that met the set criteria. The Carlson model[61] has the largest population (3773 patients) when compared with the other four studies. Although they reported an 11% increase in specificity, they did not report AUC predictive estimates. It has been acknowledged that sensitivity and specificity results are dependent on the prevalence of the disease. Hence, the comparison between populations where the PCa prevalence may vary (especially in early detection) will be difficult.[33] More importantly, by not reporting the AUC estimate, the model raises some doubts regarding the reliability of the model and its implementation.[33] It will also make comparison to other models not applicable.[67]

Babaian[64] developed an algorithm and compared the performance of the ANN to PSA density (total PSA divided by prostate volume) (PSAD), %fPSA and transition zone density (PSAD-TZ). Their ANN demonstrated a significant increase of model specificity that reached 51% when sensitivity was held at 95%. This was better than the specificity value of each individual variable such as %fPSA (10%), PSAD (39%), and PSAD-TZ (22%). In terms of AUC, the ANN achieved a moderate accuracy (0.74), being the second highest among all studies included. However, the ANN model did not show significant improvement when compared with a model fitted with only individual variables (AUC for %fPSA=0.64, PSAD=0.74 and PSAD-TZ=0.75). They included a number of uncommon pre-biopsy inputs into their algorithm such as prostatic acid phosphatase and creatine kinase.[68] Furthermore, they used a tight PSA range (2.5 to 4.0 ng/mL) which meant that their model may be less suitable for patients with PSA level below or above that range, thus limiting its generalisability.

The study by Jansen and colleagues[65] demonstrated that adding p2PSA to the base model of PSA and fPSA significantly enhanced the PCa predictive value and specificity. The association and added value of p2PSA in the

Table 6 Quality assessment for ROB and applicability concern for included studies

	ROB*				Applicability			Overall	
Study	Participants	Predictors	Outcome	Analysis	Participants	Predictors	Outcome	ROB	Applicability
Carlson *et al*[61]	+	+	–	–	+	+	–	–	–
Babaian *et al*[64]	+	+	+	–	–	+	+	–	–
Jansen *et al*[65]	–	–	–	–	+	–	–	–	–
Hill *et al*[62]	–	+	+	–	–	+	+	–	–
Lazzeri *et al*[63]	+	+	+	+	+	+	+	–	+

+ indicates a ROB or applicability; – indicates a high ROB or applicability.
*ROB, risk of bias.

prediction and detection of PCa have been reported by several other studies.[16 69–72] Jansen[65] showed that p2PSA has no clear association with aggressive PCa. However, the base model that includes p2PSA had the highest clinical significance in correlation to pathologic Gleason score with a p value of 0.008 compared with %fPSA and PHI (p value 0.01 and 0.02, respectively). Although they used archived blood samples and retrospective analysis, the results were similar to a prospective study of 268 patients.[16]

Hill[62] used a case-control study to evaluate several laboratory biomarkers. They found HGB, RBC, haematuria, creatinine, PSA, age, MCV and ethnicity ('being Black') were statistically significantly associated in the first method ($p<0.05$). In the second method, HGB, RBC, creatinine, PSA, age and MCV were found to be statistically significantly correlated ($p<0.001$) with PCa. However, since this study was designed as a case-control study, it would have been more prone to uncontrolled confounding and selection bias. Moreover, the type of screening protocols used in Veterans' Administrations may vary to those conducted in other healthcare systems; therefore, the results may not be applicable to other populations. Furthermore, patients with a PSA level <4.0 ng/mL have not been investigated, and thus, the performance of the models are unknown for individuals in this group.

Lazzeri *et al*,[63] in a European multicentre study have evaluated similar biomarkers as in Jansen study with the same PSA range 2 to 10 ng/mL prospectively. They found no difference in both %p2PSA and PHI as individual PCa predictors with AUC of 0.67 (95% CI 0.64 to 0.71). However, the base model (consisting of PSA, fPSA and %fPSA) that also included either p2PSA or PHI outperformed the base model alone and the base model including %p2PSA. In the analysis, the additive value of both p2PSA and PHI is 0.064 and 0.056 for %p2PSA for predicting the risk of PCa. These additive values increased to 0.076 for both p2PSA and PHI, and 0.073 for %p2PSA in predicting Gleason scores ≥7 for the disease. The usefulness of PHI in improving the predictive accuracy of PCa over total and free PSA has been confirmed and reported by several studies.[16 72–75]

In general, only one study has validated their model externally,[61] whereas the remaining studies were either validated internally[63 64] or did not report any validation methods.[62 65] Prediction models may not be equally applicable to all data sets as patients' characteristics may vary.[20 76] As a result, the generalisability of a model might be poor when it used in populations other than that used in building the model. Therefore, external validation should be conducted before applying any new model into general practice.[77 78]

Another key performance measure of any model that needs careful evaluation is calibration. A calibration plot with a calibration slope is more preferable than the Hosmer-Lemeshow test; it has been acknowledged that evaluating a good and well calibrated model based on a large data set can still fail the Hosmer-Lemeshow test. In contrast, when evaluating a poorly calibrated model with a small data set it can still pass the Hosmer-Lemeshow test.[79] In our analysis, three studies fail to report the calibration of the model[62 64 65] while the Carlson study[61] used a calibration plot and Lazzeri[63] used the Hosmer-Lemeshow test. Excluding calibration from the majority of models may explain why some models are not currently used in practice.[79]

With regard to biopsy cores, only two studies used extended biopsy cores. Babaian[64] used an 11-core multisite biopsy, whereas Lazzeri[63] used at least 12 biopsy cores. Moreover, two studies used six cores biopsy in their model.[61 65] The use of six-core biopsy has been criticised as not being adequate in detecting PCa[80] and that models developed using sextant biopsy are less accurate than when a 10-core biopsy is used.[76] As a result, the European guideline for clinical PCa recommended an extended biopsy as standard practice for PCa detection.[81]

It is worth noting that all five reviewed models performed better than just PSA alone. However, none of them has both high specificity and sensitivity. The level of sensitivity has been increased, and despite enhancement in the specificity, it is still considered low. Specificity is crucial when it comes to being used in a population setting as men without PCa should be ruled out as much as possible from further invasive engagement with the health system.

Our review therefore suggests that none of the reviewed models provide an ideal performance in predicting PCa with high sensitivity and high specificity. It is particularly important when considering the application of PCa risk prediction at the population level that the tool used should be able to both detect the outcome and filter out people with no disease. As there is robust evidence suggesting the clinical relevance of PSA range to the detection of PCa differs across age groups,[82–84] any future model should consider PSA threshold in relation to a specific age range. Risk prediction models for PCa should therefore take account of age.

Out of the five reviewed models, the Lazzeri model 2, has the greatest potential to be implemented in primary care. It achieved the least risk of bias and had fair discrimination for both any and aggressive PCa. It also had the largest improvement in discrimination performance compared with PSA alone. Moreover, except for the p2PSA that requires a specific assay, the included variables are common and easy to measure. However, before it could be used, the model requires to be validated externally.

Comparison with other studies

To our knowledge, three systematic reviews of PCa prediction tools have been published.[20 26 27] In the Louie *et al* review, risk models were included that were externally validated in at least five study populations for the purpose of meta-analysis and only six studies were included in their analysis. Furthermore, all the studies included incorporated clinical tests such as DRE and/or TRUS-PV.[26] Schroder and Kattan[20] reviewed models that were

built to predict the likelihood of having a positive prostate biopsy for cancer. However, it appears that they also included models where patients had a previous negative biopsy. As such, some of the models included variables related to biopsy results and cores. The review by Shariat and colleagues examined different types of predictive tools.[27] They explored tools that predict PCa on initial and repeat biopsy, pathologic stages, biochemical recurrence after radical proctectomy, metastasis, survival and life expectancy. Similarly, virtually all the prediction tools that were based on initial biopsy included variables based on invasive procedures.

Strengths and limitations of this study

This report is the first to review risk prediction tools for PCa that can be used in primary care and community settings. Any prediction model should therefore be simple to use, based on non-invasive tests, be feasible at a population level and at low cost. We carried out an extensive data extraction relating to important features and characteristics for each study included, such as modelling method, source of data, sample size, variables, discrimination, validation and cancer detection rate. We have also followed PRISMA guidelines for identifying eligible articles as well as for reporting this study. In addition, the PROBAST was adopted to assess the quality and risk of bias for each prediction model.

Our study has some limitations. Our aim was to identify prediction models that have the potential to be implemented in a primary care or community setting, and consequently our search strategy was to retrieve relevant studies for this specific purpose. Furthermore, we excluded articles that were not published in English or did not have an abstract. Moreover, only two databases were searched, besides manual search, to retrieve original studies.

A previous systematic review suggested that the majority of relevant studies could be identified through a manual search of articles reference lists instead of a database alone.[20] We identified four eligible studies using this approach. Given the small number of models identified by the approach we followed, that can be applied in primary care settings compared with the large number relating to wider existing models, it is unlikely that we have not included any study that would affect the results of our review.

Implications and future research

It is now accepted that the PSA test and its derivatives have some limitations for detecting PCa as defined by subsequent biopsy.[85] As a consequence, a considerable number of PCa prediction models have been built to improve prediction accuracy. This has resulted in a plethora of PCa risk prediction tools, with to date more than 100 models described.[86 87] There is evidence that some of these models show benefit and have better performance over just PSA measurement alone.[20] It also has been demonstrated that some of these models out-performed clinical experts in predicting PCa.[28 29] Although such models are not designed to replace specialist clinical judgement or patient preferences,[76 85 88] they can help in patient counselling and aid clinicians to decide whether a prostate biopsy should be taken or not.[77 88 89]

Given the small number of risk prediction models for PCa that do not incorporate clinical or genetic tests, the discrimination of these reviewed models ranged between poor to moderate (AUC range ~0.65 to ~0.75); in addition there were some issues relating to their study design and analysis raises the risk of bias. Consequently, none of these models could be currently recommended for use in a primary care and community healthcare setting. Several guidelines are against using PSA test based screening for PCa; the US Preventive Services task force, the Canadian task force on preventive health and the American College of Preventive Medicine do not currently recommend PSA-based testing due to insufficient evidence.[90–92] This has made it difficult, so far, to convince policymakers to adopt PCa screening programme.

The first guideline of PROSTATE CANCER UK states, "In the future, health professionals should look at a man's PSA level alongside other known risk factors as part of a risk assessment tool, when one becomes available."[93] However, the vast majority of the current PCa risk prediction models are not suitable for routine use as they include clinical and genetic tests and are not validated externally in other cohorts. Therefore, the main challenge in the UK, remains to develop a risk prediction tool that is reliable, cheap, is applicable for as wide an ethnicity as possible, and, most importantly, is easy to use and can be implemented at a primary care level.[94]

The value of such risk tools is that they will help to stratify men at high risk of developing PCa earlier so that they have appropriate management and/or surveillance programme as early as possible and, therefore, may fit into the clinical pathway. Such tools should help physicians have a better understanding of the risk for this disease and simplify the procedures and discussions with patients when recommending further specialist-led investigations such as DRE and/or MRI where a decision on whether a biopsy should or not perform is concluded. Furthermore, using the appropriate risk prediction tool will avoid men from undergoing inappropriate further and frequent testing.[94] This will reduce any associated costs of inappropriate tests and decrease the burden on healthcare delivery systems.

It is crucial to address these issues by identifying all possible risk factors for PCa that are non-clinical, non-genetic, and easy to use and interpret. There remains a pressing need to develop a risk prediction tool in the future using all appropriate factors (potentially also including genetics once there is infrastructure in place for genetic testing in the primary care and the cost comes down) into a robust multivariable analysis and validate the model externally to eliminate applicability and generalisability concerns. Only when this is achieved will it be

possible to introduce a PCa screening programme fit for purpose.

CONCLUSION

There is a paucity of suitable low-cost risk models that incorporate non-clinical, non-genetic inputs and which can be used at a primary care level and in other community health services. Existing models have limitations reflecting both study design and reporting performance measures. Future research should take into account these key issues and explore other risk factors for incorporation into further models.

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Appendix F Responses from open-ended questionnaire

Open-ended comment-Text analysis

Out of 1902, 626 respondents provided comment with majority of positive feedback. Below comments are extracted to highlight positive comments.

"This was the first time that I had used this test. I was very impressed by how easy it was to carry out and how quick I received the results. Absolutely first class service."

"Excellent service apart from the fact that they sent a covid 19 test first, which they wouldn't check. After I complained the PSA test went perfectly."

"Excellent, easy to use and peace of mind without the faff of going to a doctor."

"Excellent service thank you very much especially as the doctors surgeries don't appear to want to help."

"It was just an excellent way of getting the test done. Much better than travelling to a centre, having to queue etc. The test kit was quite easy to use and the instructions were very clear."

"Excellent and rapid service"

"I thought the service was very good, clear ,concise and easy to understand. I shall be using this service again. I have recommended it to friends. May not have done a retest, had i got to pay again. Fortunately test was correctly done first time."

"Great to have a free test, thank you."

"I found it easy to do, well organised and quickly received, very good."

"Used this service as Lockdown prevented our normal local testing sessions and found it easy to use and that it provided speedy results at a reasonable cost. If local testing does return I will use that as the cost is covered by Burgess Hill Lions."

"Very well organized, and simple to use, certainly recommend your service."

"Very good"

"I found the procedure non invasive and results were returned very quickly."

"Good service recieved answer quickly."

"The time between my home test kit being delivered and results returned to me were very quick. This in turn led me to have a RARP in December 2020. I have since had two follow up PSA blood tests with PSA being undetectable. An excellent outcome"

"Easy to use and postage time to results was very quick."

"Very quick service"

"Quick and efficient."

"Think was a fast and efficient service. I recommendes into a big group and friends and colleagues. Sparked a debate about the accuracy of the test and the dilema of what to do next if psa number raises. All useful conversations in my book."

DUPLICATED!"Very straight forward and results very quick. A very reassuring service."

"Easy fast service"

"I was very pleased with kit and instructions provided. The kit was easy to use and the results came via e-mail the day after posting."

"This service is great no queuing at a venue for the test and fast results too. Thank you all"

"Great idea ,the way forward saves all the hassle of going to the doctors"

"Well done - excellent team service"

"Brilliant service and home testing kit makes this accessible for all."

"From posting the sample to getting the result by email was less than 24hrs, very impressive! Earlier Lions Club group test was cancelled due to Covid. Your test result was positive and I have since had a prostatectomy. Probably saved my life!"

There are comments which are also helpful for consideration to improve certain aspects of the home testing service.

"The test results were returned very promptly which was most appreciated. My one recommendation is to emphasise EXACTLY where the lance should be stuck in the finger. I had 3 tries before success to get the blood sample causing a bit of stress."

"Yes found the suggestion to prick finger on side difficult to load the sample vial, would be better if at end of finger as could then smear blood into it."

"A very good service. If the blood sample size could be reduced, I'd welcome that. Being on blood thinners, creating such a volume did cause me some concern, but the puncture did heal relatively quickly".

"This may be a personal issue with the blood flow in my fingers, but it took a lot of manipulation (and all the supplied lancets) to extract enough blood from my fingers to fill the tiny vial."

"In the instructions make it clearer that the "side of the finger" for the prick sample, means the "middle of the finger print area" and not the literal side of the finger (i.e.: not adjacent to the side of the finger nail)."