An Evaluation of Modified Transperineal Template Guided Saturation Biopsy in the diagnosis of Prostate Cancer

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Abstract

The unique situation where there is persistent clinical suspicion of prostate cancer (PCa) despite repeated benign histology from conventional prostate biopsy is a diagnostic dilemma for clinicians and patients. Transperineal template guided saturation biopsy (TTSB) has a high cancer yield in this group, but is associated with unacceptably high morbidity particularly high rates of acute urinary retention (AUR). As localised PCa rarely involves periurethral area at the base, we hypothesised that urethral trauma from extensive sampling of this area is a major trigger factor for AUR. This thesis presents data from modified periurethral sparing TTSB technique to determine whether the risk of AUR is reduced compared with rates reported in the literature, without compromising cancer yield and investigates biochemical predictors of pathological outcome and role of pre-biopsy MRI in this group.

Three hundred and three men with persistent clinical suspicion of PCa despite a median of 2 (range 1-6) sets of negative biopsies were investigated. Patients prospectively completed a questionnaire evaluating bleeding, pain (visual analogue scale, range 0-10) and analgesic requirements which were assessed at 1 hour post biopsy and on days 1, 3 and 7. Serum PSA, PSAD and %fPSA were documented and evaluated for their ability to predict PCa diagnosis, Gleason score and cancer volume. Furthermore, a pre-biopsy magnetic resonance imaging (MRI) was performed and abnormalities in 4 anatomical quadrants were compared with TTSB to assess whether pre-biopsy MRI could defer need for TTSB or allow a more targeted biopsy regime.

Median age was 64 years (range 43-85). PCa was diagnosed in 167 of 303 men (55.1%) from median of 29 cores [range 16-43, Gleason 6 (29.9%), 7 (45.5%) and 8-10 (24.6%)] and 140 (83.8%) were clinically significant with 77.2% of cancers involving the anterior region. AUR occurred in 23 men (7.6%). On multivariate analysis, only prostate volume predicted AUR (P=0.004). Pain was minimal (peak on day 1, mean 0.8 out of 10), requiring simple analgesia in 27% on day 1, mostly for mild perineal discomfort. Haematuria (75%) and rectal bleeding (20%) significantly decreased over one week, with 33% still experiencing haematospermia at this stage. PSAD (AUC 0.76) was more predictive of cancer diagnosis compared to 0.71 for %fPSA and pre-TTSB PSA respectively. The overall sensitivity and specificity of MRI for cancer diagnosis were 65% and 77% respectively with multiparametric MRI showing more accuracy with sensitivity and specificity of 83% and 95% respectively compared to other MRI sequences. Of the 24 false negative MRI cases, 16 (67%) were clinically significant. So complete prostate mapping should continue at present.

In conclusion, this thesis demonstrates that clinical suspicion of PCa despite negative histology from conventional TRUS Biopsy should not be disregarded, as a significant proportion harbour aggressive disease. Modified TTSB is well tolerated with low risk of AUR. Presentations of aspects of this thesis at key regional, national and international urology meetings have generated interest and helped set up dedicated units in the region for PCa diagnosis in this group. Questions raised in this study provide backbone for future research in this field.

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List of Abbreviations

ADC	Apparent diffusion coefficient
ATLPC	Aggregate Tumour Length from all Positive Cores
AUR	Acute Urinary Retention
BPH	Benign Prostatic Hyperplasia
DHT	Dihydrotestosterone
DWI	Diffusion Weighted Imaging
MPC	Maximum Percentage Core involvement
MRI	Magnetic Resonance Imaging
mpMRI	Multiparametric MRI
MTL	Maximum Tumour Length
NPC	Number of Positive Cores
PCa	Prostate cancer
PPC	Percentage of Positive Cores
PSA	Prostate Specific Antigen
PSAD	PSA Density
%fPSA	Percent of Free PSA
PSAV	PSA Velocity
T2WI	T2 Weighted Imaging
TRUS	Transrectal Ultrasound
TRUSB	Transrectal Ultrasound Guided Biopsy
TRSB	Transrectal Saturation Biopsy
TTSB	Transperineal Template Guided Saturation Biopsy

Chapter 1 Introduction

1.1 Overview of Cancer

The origin of the term 'cancer' frequently used to describe all malignant tumours is unclear. However, it has been used in the ancient Egyptian medical book, Papyrus Ebers, dating back to 1500 BC. Nevertheless, investigators attribute the first use of the term 'cancer' and 'carcinoma' to Hippocrates (460 – 370 BC) who used these terms in his writings to describe benign and malignant growths respectively (Garrison, 1926). Although human beings are noted to be affected by cancer since record began, it still remains a disease with huge public health concern till date and a leading cause of death in both economically developed and developing countries (World Health Organization, 2008). In fact, there are very few diseases with such profound psychological, emotional and life changing effects on an individual when it is diagnosed.

Histologically, carcinoma is used to describe malignant tumours arising from epithelial cells hence can affect any organ or system in the body. Carcinomas are further classified into adenocarcinoma when they posses glandular growth pattern microscopically and squamous cell carcinoma where they produce recognisable squamous cells in any epithelium of the body. Nevertheless, unlike benign conditions, malignant tumours have the ability to transform affected cells, invade surrounding tissues and metastasise to a distant area of the body. When normal body cells are transformed, they show considerable difference compared to unaffected cells. The degree to which malignant cells differ from normal is referred

to as differentiation. Therefore, malignant tumours range from the well differentiated category with close resemblance to a normal cell hence making it difficult to distinguish microscopically, to the poorly differentiated or anaplastic types which exhibit considerable morphological and functional variation to the normal cell.

1.2 Epidemiology of Cancer

Interest in the study of cancer has never been greater, partly due to the huge public health concern that it presents and the need to plan and manage limited healthcare resources. Hence, the study of patterns of cancer occurrence in populations is vital as it provides useful knowledge of the extent of disease burden and possible associations.

Over the last 30 years, the number of new cases of cancer has increased. In 2002, there were 10.9 million new cancer cases, 6.7 million deaths, and 24.6 million persons living with cancer within 5 years of diagnosis worldwide, excluding non-melanoma skin cancer (Parkin *et al*, 2005). According to 2004 estimate, cancer is responsible for 25.2 percent of deaths making it the third leading cause of death globally (World Health Organization, 2008). Unfortunately, the number of new cancer cases continues to rise. According to recent report by the International Agency for Research on Cancer, there were more new cancer cases diagnosed in 2008, with an estimated 12.7 million new cases worldwide, resulting in 7.6 million deaths (Jemal *et al*, 2011). In Europe, the incidence of cancer rose by 300,000 in 2

years from 2004 to 2006 with an estimated 3.2 million new cases, resulting in 1.7 million deaths (Ferlay *et al*, 2007).

In the United Kingdom (UK) a similar trend is observed. The number of new cancer cases excluding non-melanoma skin cancer in the last 30 years (1978 – 2007) has increased by 14% and 32% in males and females respectively (Cancer Research UK, 2008). In 2008 alone, 156,723 cancer deaths were recorded in the UK (Cancer Research UK, 2009). It is suggested from Thames Cancer Registry and Macmillan cancer support that over 2 million people in the UK are living with or beyond cancer diagnosed at any time with annual increase of 3.2% (Maddams J *et al*, 2008).

The risk of developing cancer increases with age. Amongst women aged 40 to 79 years and among men aged 60 to 79 years in the Unites States, cancer is the leading cause of death (Jemal *et al*, 2009). Similar data is reported for the United Kingdom. Office of National Statistics' report showed that rates of cancer were higher in males than females from the 60–64 age group onwards, with an increasing difference in rates between the sexes with age up to 85 years in 2008 (Office for National Statistics, 2010).

The rise in incidence of cancer with age could be attributed to increasing longevity (Thun *et al*, 2010), use of cancer screening programmes (Nishizawa *et al*, 2009) or rising world population (World Health Organization, 2008). It is estimated that the world population will increase from 6.1 billion in 2000 to 8.9 billion by 2050, but the most important changes would occur in the elderly population; the number of those aged more than 65 years worldwide is expected to rise from 6.9% in year 2000 to 16.4% by 2050 (Bray & Moller, 2006). Therefore it is expected that the total

number of people living with cancer is likely to rise in parallel to this change in global demographics.

As expected, this is likely to further add pressure to existing healthcare resources.

This increasing economic burden of cancer has been observed in the past twenty years. Tangka and his colleagues (Tangka *et al*, 2010), in their medical expenditures survey, observed that the total medical cost of cancer in the United States of America has nearly doubled from $24.7 billion dollars in 1987 to $48.1 billion in 2005. Consequently, in order to combat this growing economic burden, strategies to improve early cancer detection and prevention have been advocated (Kim *et al*, 2009).

1.3 Prostate Cancer

The prostate is a gland that lies just beneath the bladder and encircles the urethra, a tube that carries urine from the bladder and also semen from the testes and prostate to the tip of the penis. In the normal adult male, the prostate is about the size of a walnut, with an average weight of 11 grams, ranging from 7 to 16 grams (Leissner & Tisell, 1979).

The term prostate originated from the Greek word ‘prostates’ which literally means ‘to stand before someone or something’; but the English anatomist, William Cheselden is credited with the first suggestion of prostate being a single organ rather that two glands in 1792 (Josef Marx & Karenberg, 2009). Prostate cancer is most commonly adenocarcinoma, however, other rare subtypes including sarcomas

in 0.1 – 0.2% and primary urothelial prostate cancers accounting for 1 to 4% of all prostate cancers (Epstein, 2012).

1.4 Epidemiology of Prostate cancer

Prostate adenocarcinoma (PCa) is the most common male cancer and a leading cause of death. In 2008, PCa was the second most frequently diagnosed male cancer worldwide, accounting for 14% (903,500) of the total new cancer cases and 6% (258,400) of the total male cancer deaths worldwide (Jemal *et al*, 2011). The incidence is 25-fold more in developed countries of Oceania, Europe, and North America largely due to wide use of routine Prostate Specific Antigen (PSA) testing. Thus, in Europe, PCa is the commonest cancer diagnosed in men, comprising 22.2% (382,000) of cases and resulting in 89,319 deaths in 2008 (Ferlay *et al*, 2010). In the UK, it is estimated that there is a 1 in 9 lifetime risk of being diagnosed with PCa and 37,051 new cases of PCa were diagnosed in 2008, making it the commonest male cancer, accounting for 10,168 deaths (Cancer Research UK, 2011). One study suggests that the increased mortality in PCa for successive birth cohorts is real and cannot be explained by increased detection from widespread use of PSA screening (Post *et al*, 1999).

The cause of PCa is unclear, but several studies have looked into possible risk factors for developing the disease. The three most established risk factors for PCa are age, ethnic origin and family history. Other less established risk factors include physical activity and diet.

1.4.1 Age and Prostate Cancer

The risk of developing PCa with advancing age is well reported in literature. Franks (Franks, 1973) observed that PCa is rare before 50 years, after which the incidence rises rapidly in a linear fashion until 80 years of age. However, despite the contemporary widespread use of PSA testing in many European countries, the number of deaths from PCa increased by 16% from 1995 to 2006 mainly due to the rapid increase in the number of men reaching older ages (Ferlay *et al*, 2007). In the UK, the risk of developing PCa is low in men less than 50 years but rises significantly from above 50 years of age (Cancer Research UK, 2011). It is therefore a bigger health concern in western countries with increased elderly populations. For example, the age standardized Incidence of PCa in North America is 120 per 100,000 compared to 4.4 per 100,000 in South Central Asia (Parkin *et al*, 2005). Regional variations exist amongst western countries and are thought to be influenced by their demographic differences. Thus, in Sweden with increasing life expectancy, PCa is the commonest male cancer accounting for 36.8% of all new cases in 2004 (The National Board of Health Welfare Centre for Epidemiology, 2006). In a recent study, it was reported that PSA concentration ≤1 ng/mL at 60 years predicts lifetime risk of metastasis and death from PCa (Vickers *et al*, 2010). Of the 1167 men aged 60 years who were followed up for 25 years, Vickers and colleagues observed only 0.5% and 0.2% risk of metastasis and death respectively if the initial PSA was ≤1 ng/mL prompting the authors to propose targeted screening at 60 years.

1.4.2 Ethnicity and Prostate Cancer

Several studies have demonstrated wide variations in the incidence and mortality of PCa amongst racial groups; in particular, African-Americans (AA) who are the 2nd largest ethnic minority group in America. The incidence of PCa in AA men is 35 to 58 percent higher than in white men (Ghafoor *et al*, 2002; Parker *et al*, 1998; Wingo *et al*, 1996). Furthermore, AA are twice more likely to die from PCa than their white counterparts (Parker *et al*, 1998; Wingo *et al*, 1996). In a study of 369 consecutive men (120 AA and 249 white) who had radical prostatectomies at a single institution, Powell and colleagues found that AA men had more locally advanced PCa than the white American men [69% among blacks compared with 57% among whites] with higher rate of positive surgical margin [P = 0.002] (Powell *et al*, 1997). However, the high mortality and incidence rate of PCa seen in African-Americans is not observed in blacks from nations in Africa where incidence remains low (Walker *et al*, 1993).

Several migrant studies suggest that the incidence of clinical PCa varies widely across geographical regions alluding that environmental factors may play a dominant role in development of PCa. For example, when Japanese men migrated to Los Angeles County, their incidence of PCa rose compared to their native homeland populations (Shimizu *et al*, 1991). Similar observation was found amongst the same group who migrated to Sao Paulo, Brazil (Iwasaki *et al*, 2008) and in Asian American migrants (Cook *et al*, 1999). In another study, it was observed that Korean immigrants to the US had a 3.5 times higher incidence of PCa compared to their native counterparts (Lee *et al*, 2007).

1.4.3 Hereditary Prostate Cancer

There have been recent interests from investigators looking at familial and hereditary associations for PCa. In a metanalysis of familial PCa risk, Johns and Houlston found a 2.5 fold increased risk in first degree relatives. When the relatives of cases were diagnosed before 60 years; the relative risk dramatically increased 4.3 fold and 3.5 fold where 2 first degree relatives are affected (Johns & Houlston, 2003). Hereditary prostate cancer is reported to occur in 9% of PCa cases. This is defined as nuclear families with 3 cases of prostate cancer, families with prostate cancer in each of 3 generations and families with 2 men diagnosed before age 55 years (Carter *et al*, 1992a). Using segregation analysis, autosomal dominant inheritance of rare high-risk alleles which predisposes 88 - 97% of all carriers to become affected by 85 years of age compared with 5 - 10% of non-carriers has been suggested (Carter *et al*, 1992a; Verhage *et al*, 2001). A recent report by Lange and co-workers (Lange *et al*, 2012) suggested that early onset PCa cases had a significantly greater average number and frequency of risk alleles than in the control group. An inheritance of one defective copy of either of the two breast-cancer susceptibility genes (BRCA1 and BRCA2) has been implicated in familial PCa amongst other cancers. BRCA1 and BRCA2 are tumour suppressor genes which encode large proteins that function in multiple cellular pathways (Venkitaraman, 2001). Carriers of BRCA1 and BRCA2 mutations have a 1.8 to 3.75 and 7.3 to 8.6 fold increased risk of PCa by the age of 65 years (Consortium, 1999; Kote-Jarai *et al*, 2011; Leongamornlert *et al*, 2012; Thompson *et al*, 2002). Increasingly, evidence from published reports suggests that carriers of these mutations are more likely to

present with aggressive form of PCa compared to non-carriers (Castro *et al*, 2013; Edwards *et al*, 2010). It has been suggested that targeted screening may be beneficial in this group as the age of onset is 6 -7 years earlier for hereditary PCa and as a consequence, a greater proportion would die of their disease compared to sporadic cases (Bancroft *et al*, 2014; Bratt, 2002). However, hereditary PCa does appear not differ in any other way from sporadic forms (Bratt, 2002; Siddiqui *et al*, 2006).

1.4.4 Lifestyle factors

It is believed that exogenous factors play a key role in the progression from latent to clinical PCa. Modifiable lifestyle factors like physical activity, diet and obesity have been suggested as possible risk factors. In a recent metanalysis of 88,294 cases from 19 studies, it was demonstrated that increasing total physical activity was significantly associated with a decreased risk of PCa with a relative risk reduction of 10% (Liu *et al*, 2011).

Dietary and nutritional factors that may influence disease development include total energy intake (as reflected by body mass index), dietary fat, cooked meat, micronutrients and vitamins (carotenoids, retinoids, vitamins C, D, and E), fruit and vegetable intake, minerals (calcium, selenium), and phyto-oestrogens (isoflavonoids, flavonoids, lignans) (Heidenreich *et al*, 2011). Therefore, Western diet, with its high content of processed animal products containing saturated fat may be of importance. For example, compared to Far East Asian countries like China and Japan where traditional food is rich in fresh vegetables, the incidence of

PCa is low. The influence of diet in the development of PCa and definition of an ideal prostate diet were the subject of a recent review (Hori *et al*, 2011). Other factors such as occupational exposure to cadmium common to farmers by inhalation (Elghany *et al*, 1990; Nakamura *et al*, 2002) and vasectomy (Dennis *et al*, 2002) have been discussed as being of aetiological importance.

1.5 Diagnosis of Prostate Cancer

A diagnosis of PCa is made based on a combination of different modalities including prostate specific antigen (PSA), digital rectal examination (DRE), transrectal ultrasound (TRUS) and magnetic resonance imaging (MRI). However, transrectal ultrasound and biopsy (TRUSB) is required to provide the ultimate tissue sample for definitive diagnosis and grading of the disease by histological analysis of the TRUSB specimen.

1.6 Prostate Specific Antigen (PSA)

PSA is a 34 kD glycoprotein made up of a single polypeptide chain of 240 amino acids, manufactured almost exclusively by the epithelium of the prostate gland (Watt *et al*, 1986). It is a serine protease belonging to the human kallikrein (hK) gene family located on chromosome 19q 13.3 to 13.4 (Lilja, 2003). PSA is produced as an inactive form which is later converted to the active serine proteinase (Lundwall & Lilja, 1987; Watt *et al*, 1986). The physiological function of PSA is unclear, but the hypothesis proposed by Lilja (Lilja, 1988) has been widely accepted.

According to Lilja, within the seminal fluid, the proteolytic action of PSA liquefies the gel-forming proteins from the seminal vesicles, initiating liquefaction of the ejaculate, thereby increasing the motility of sperm cells and aiding fertilization.

Antigens with structure similar to PSA had been reported by several workers in the 1970s, however, Wang and his co workers are credited with the purification of a human PSA in 1979 and considering its use as a useful marker for assessing treatment responses and follow-up among patients with PCa (Wang *et al*, 1979). However, Stamey and colleagues were first to show its clinical use as a marker for monitoring responses and recurrence after radical prostatectomy by demonstrating that PSA fell to undetectable level after prostatectomy (Stamey *et al*, 1987) with a serum half life of 2 to 3 days after prostatectomy (Oesterling *et al*, 1988; Stamey *et al*, 1987).

The use of PSA as a biomarker for early PCa detection is limited by its lack of cancer specificity. Consequently, elevated serum level has been reported in benign prostatic hypertrophy [BPH] and prostate massage (Clements *et al*, 1992; Kane *et al*, 1992; Stamey *et al*, 1987), prostatitis (Morote Robles *et al*, 1988), acute urinary retention (McNeill & Hargreave, 2000), after coronary stent implantation (Ozcan *et al*, 2009) and other non-cancerous conditions (Glenski *et al*, 1992).

Furthermore, there is no universally accepted optimal PSA cut off limit despite availability of numerous commercial test kits. In a study of 2950 men with normal PSA less than 4ng/ml, PCa was identified in 15% (Thompson *et al*, 2004). The difficulty in finding a PSA cut-off value that would result in a sufficiently high specificity concurrently with a reasonably high sensitivity (i.e. above 50%) was highlighted by Holmstrom and colleagues. In their study, they observed that PSA

failed to attain likelihood ratios (i.e. the likelihood that a given test result would be expected in a person with a disease compared with the likelihood that the same result would be expected in a person without the disease) required for a screening test irrespective of cut-off value even though PCa occurred in only 3.9% of the 439 men with initial PSA of 1ng/mL or less (Holmstrom *et al*, 2009).

In contrast, a PSA screening study including 5855 men with a mean age of 58 years followed up for a median of 7.6 years showed that no man with initial PSA level less than 0.5ng/mL was diagnosed with PCa. Of the 1,992 men with PSA ranging from 0.55 – 0.99ng/mL, PCa detection rate was only 0.9% (Aus *et al*, 2005). The authors proposed introduction of strategies aimed at individualised screening programmes based on initial PSA levels.

Several studies have reported the potential use of PSA as a screening tool to identify men who are likely to be harbouring PCa, resulting to its widespread use in clinical practice for the diagnosis and management of PCa but the benefit for PCa screening remains controversial. Results from two randomised studies to determine if PCa screening using PSA decreased PCa mortality have been published recently. In the European Randomized Study of Screening for Prostate Cancer [ERSPC] trial of 182,000 men, PSA based screening was associated with a 20% reduction in PCa mortality at a median follow-up of 9 years. However, a total of 1410 men needed to be screened and 48 men with PCa treated to save 1 death (Schroder *et al*, 2009). Compared with the US Prostate, Lung, Colorectal and Ovarian cancer [PLCO] screening trial of 76,693 men, screening was associated with a relative increase of 22% in the rate of PCa diagnosis compared with control group but did not provide

reduction in death rates at 7 years (Andriole *et al*, 2009). There was no indication of a benefit appearing with 67% of the subjects having completed 10 years of follow-up. It is suggested that the high rates of contamination (52%) in the control group compared to 20% in the ERSPC study could account for this disparity.

In another randomised population-based trial (Hugosson *et al*, 2010) of Twenty thousand men with 14 years of follow-up, the number who needed to be invited to screening (corresponding to NNS) to prevent one prostate cancer death was 293, and the number who needed to be diagnosed (corresponding to NNT) was 12, with a relative risk of 0.56. Importantly, the difference between the cumulative risks of death in the screening and control groups was not apparent before 10 years (Hugosson *et al*, 2010; Schroder *et al*, 2009).

One concern against the use of PSA screening is the fact that PCa has a long lead time and natural course resulting to an increase in the rate of over diagnosis (i.e. the diagnosis in men who would not have clinical symptoms during their lifetime) depending on the age at commencement of screening programme. This is estimated to range from 27 – 50% (Draisma *et al*, 2003; Etzioni *et al*, 2002). McGregor and co-workers (McGregor *et al*, 1998) estimate that only 16% of men with screen detected PCa could have their lives extended by invasive therapy, since PCa rarely causes death before the age of 85 years. Furthermore, a recent Cochran review concluded that screening did not significantly decrease all-cause or PCa-specific mortality and recommending that any benefits from PCa screening may take more than 10 years to accrue; therefore, men who have a life expectancy of less than 10 to 15 years should be warmed against undergoing screening for PCa (Ilic *et al*, 2011).

Despite these concerns, PSA remains widely used in clinical practise as a screening tool for early detection of PCa with the aim of reducing mortality from the disease. Currently, there is no long term data to support its use or an optimum PSA threshold to diagnose non-palpable but clinically significant PCa.

These inconsistencies prompted investigators to search for alternative PSA modifications in order to improve its specificity for early detection of PCa. The PSA modifications have been proposed include:- age specific PSA ranges, free to total PSA, PSA density, PSA velocity (PSAV) and PSA doubling time (PSADT).

1.6.1 Age-specific PSA reference range

In order to enhance PSA as a predictor of PCa and improve its ability to distinguish cancer from BPH, the concept of age specific reference range was proposed. In a population-based study of healthy 471 white Caucasian men aged 40 to 79 years divided into four 10 year age groups, Oesterling and colleagues (Oesterling *et al*, 1993) found that PSA correlated with patients' age, increasing at the rate of 0.04ng/ml per year. Using regression analysis, their proposed reference range for serum PSA (95th percentile) for men aged 40 to 49 years is 0.0 to 2.5 ng/ml; for 50 to 59 years, 0.0 to 3.5 ng/ml; 60 to 69 years, 0.0 to 4.5 ng/ml; and 70 to 79 years, 0.0 to 6.5 ng/ml. The expectation is that age-specific PSA range would increase PSA sensitivity in younger men who are more likely to benefit from invasive therapy whilst increasing specificity in the older age group. In one study, el-Galley and colleagues showed that the use of age-specific PSA reference range increased PSA

sensitivity in men younger than 60 years whilst avoiding 22% of TRUS biopsies in those 70 years and over (el-Galley *et al*, 1995).

Age-specific PSA shows considerable variation amongst racial groups. In Asians, the observed value is lower and may be accounted for by their smaller prostate size compared to whites (Ku *et al*, 2002; Lee *et al*, 2000; Oesterling *et al*, 1995). In contrast, PSA value is slightly higher in healthy AA men compared to whites (Cooney *et al*, 2001). A potential source of bias is the high participation by younger men with prostatic symptoms and positive family history of PCa in whom higher PSA values are usually observed (Heeringa *et al*, 2001). Nevertheless, the use of race-specific PSA reference range remains controversial (Morgan *et al*, 1996a; Whittemore *et al*, 1995) so further study is required to ascertain the clinical relevance of the minor variations seen in black men compared to Caucasians.

1.6.2 Free and Total PSA

The lack of PCa specificity is a drawback in the ability of PSA to reliably discriminate PCa from other non-cancerous conditions involving the prostate which can result in an elevated serum PSA level. The dilemma is in correctly identifying men with BPH with borderline raised PSA in the intermediate range of 4 to 10ng/ml [diagnostic 'gray zone'], who are unlikely to be harbouring cancer. If correctly identified, unnecessary biopsy which is invasive and costly could be avoided in this group.

Although benign conditions can cause a raised PSA, studies have also shown that 22 to 32% of men diagnosed with PCa have PSA within the normal range (Kellokumpu-Lehtinen *et al*, 1989; Van Cangh *et al*, 1996). This means that if PSA is used as the

sole diagnostic tool for early PCa detection, a significant number of PCa would have been missed. Furthermore, 78% men who underwent prostate biopsy purely for a raised PSA in the range of 4.0 to 9.9ng/ml had a negative biopsy (Catalona *et al*, 1991).

The discovery that PSA exists in various forms in the serum coupled with development of commercial assays that accurately measure these forms helped to increase knowledge of their possible utility as an adjunct to further increase the ability of PSA to discriminate benign prostatic disease from cancer. Using monoclonal antibodies produced against PSA from serum of 64 patients, Lilja and co-workers (Lilja *et al*, 1991) demonstrated the existence of PSA predominantly in an 80 to 90 kDa complex to α1-antichymotrypsin with an immunoreactive 25 to 40 kDa free (non-complexed) form. Subsequent report by Christensson and colleagues characterised PSA in 3 different forms and highlighted differences in their serum concentrations in men with BPH compared to those with PCa (Christensson *et al*, 1993).

In a prospective multicentre study of 773 men, it was demonstrated that percentage of free PSA [%fPSA] is more predictive of cancer than total PSA [tPSA] level with an area under the receiver operating characteristic curve [AUC] of 0.72 compared to 0.53 for total PSA. Furthermore, at a cut off of 25%, %fPSA yielded a sensitivity of 95% whilst avoiding unnecessary biopsy in 20% of those patients with BPH (Catalona *et al*, 1998). In another large, well controlled population based trial of 11,644 men with PSA level between 4 to 10ng/ml, %fPSA was significantly more predictive of PCa than tPSA with an AUC of 0.72 compared to 0.60. furthermore, at

a cut off of 20%, 95% of cancers were detected and whilst avoiding 15% of unnecessary biopsy (Luboldt *et al*, 2001).

To determine predictive value of %fPSA in the group of men whose PSA concentration remained in intermediate range after an initial negative TRUS biopsy, Djavan performed a repeat extended biopsy showing a sensitivity of 90% and at a cut-off of 30%, unnecessary biopsy was avoided in 50% of the patients (Djavan *et al*, 2000). In another study of men with negative initial histology who underwent repeat biopsy for a persistently elevated PSA, not only was %fPSA significantly lower in the cancer detected group compared to the no-cancer group (8% vs 14%, $p < 0.01$), it was the single most important predictor for the detection of PCa at repeat biopsies (Uemura *et al*, 2004).

However, limitations of %fPSA include the variability of assay characteristics, dilutional effect of enlarged prostate (Moon *et al*, 2000; Stephan *et al*, 1997), instability of free PSA at room temperature and its unreliability at higher PSA concentration (above 10ng/ml). Furthermore, %fPSA is not clinically useful for the follow-up of patients with prostate cancer.

1.6.3 PSA Density (PSAD)

PSAD is the quotient of serum PSA and prostate volume (PSA ng/ml/prostate volume cc). It was developed by Benson and colleagues aimed at increasing the specificity of PSA in the intermediate range of 4 to 10ng/ml without influencing sensitivity with biopsy based on a PSAD of 0.15 or more (Benson *et al*, 1992a; Benson *et al*, 1992b).

According to Benson and colleagues, the concept of PSAD is based on the logic that by accounting for PSA elevations resulting from BPH, it would be possible to differentiate BPH from those due to PCa. The normal prostatic stromal/epithelial relationship which is maintained in BPH is disrupted in PCa. Benign tumours are known to grow by expansion, unlike malignant tumours which grow by a combination of both expansion and infiltration; resulting to an increase in prostatic epithelial cell number (hence PSA) but only minimal effect on gland volume (Benson & Olsson, 1994). Several studies have investigated the value of PSAD in discriminating BPH from PCa in men with serum PSA concentration within this diagnostic gray zone (Benson *et al*, 1993; Schmid *et al*, 1996). Of 142 healthy men with intermediate range PSA and normal DRE and TRUS, a PSAD cut-off of 0.15 provided a sensitivity of 91% and reduced the number needed to biopsy to detect one cancer from six to three (Bazinet *et al*, 1994). In contrast, some conflicting reports suggest that PSAD cut-off of 0.15 is an unreliable predictor of PCa in this cohort (Brawer *et al*, 1993; Cookson *et al*, 1995). PSAD is highly dependent on accurate volume determination and this is believed to explain the mixed observations by various reports in literature (Benson & Olsson, 1994). Furthermore, the cost of having to perform a TRUS scan and the operator variability of TRUS prostate volume measurement are limitations to its routine use in clinical practice.

1.6.4 PSA and Kinetics

There are two methods of measuring changes in serum PSA value over time (PSA Kinetics), including PSA Velocity (PSAV) and PSA Doubling Time (PSADT).

PSAV is based on the observation by Carter and co-workers (Carter *et al*, 1992b) in a small cohort of men, that the most significant factor affecting PSA levels with age is the development of PCa and the use of PSA rate of change of 0.75 µg/L/yr not only maintained sensitivity, but was more significantly associated with PCa with specificity rising to 90% compared to PSA alone which had a specificity of 60%. One study found that PSAV of 0.35 ng/ml/yr determined ten to fifteen years before diagnosis can identify men with life-threatening PCa at a period when their serum PSA levels are associated with curable disease (Carter *et al*, 2006). Similar observation was reported more recently, using a PSAV threshold of 0.4ng/ml/yr and showing PSAV to be useful for PCa risk stratification several years prior to actual diagnosis of PCa when most men would usually have low PSA levels (Loeb *et al*, 2011b). In the time period prior to diagnosis, longitudinal PSA changes in men with and without PCa are so significantly different that annual testing of men with initial PSA of ≤ 1.0ng/ml/yr may not be necessary (Berger *et al*, 2005).

Furthermore, PSAV ≥ 2.0ng/ml/yr during the year prior to diagnosis has been associated with a ten to twelve fold increase in the rate of PCa specific mortality following radical prostatectomy or radiation therapy (D'Amico *et al*, 2004; D'Amico *et al*, 2005). The suggestion is that PSAV is more useful than PSA doubling time in the prediction of high risk PCa (Loeb *et al*, 2008).

The concept of PSADT was described by Schmid and co-workers (Schmid *et al*, 1993) who observed that serial PSA determinations in untreated PCa conform to an exponential model suggesting that PCa has a log linear growth pattern. In their study, 43 men aged 51 to 83 years with untreated PCa were observed for a mean of 30 months and found to have a significantly longer PSADT (>24 months) when PCa

is locally advanced (93% vs. 53%) than with higher histological grade. In another study, D'Amico and Hanks (D'Amico & Hanks, 1993) used regression analysis to show that PSADT of recurrent PCa after radiation therapy is a constant [$r > 0.98$], indicating that PSA rises exponentially and correlates linearly with the interval to clinical manifestation after PSA failure. The slope of the correlation curve is the number of PSADT determinations (4.5, 95% CI 3 – 6) needed before disease is clinically manifested after PSA failure and can be useful for grouping this group into those with aggressive [PSADT ≤ 3.8] and less aggressive tumour biology [PSADT ≥ 3.8]. Recent report suggest that PSADT is useful for predicting disease progression in men with non-metastatic biochemical relapse after radical prostatectomy or radiation therapy who are treated with intermittent androgen hormone therapy (Keizman *et al*, 2011). Nonetheless, the diagnostic use of both PSA kinetics is limited by the dilution effect of BPH, racial differences (Tang *et al*, 2011), circadian fluctuation of PSA values (Mermall *et al*, 1995), variations in interval of PSA determinations, acceleration/deceleration of PSAV and PSADT over time. Furthermore, a prospective study failed to show any clear evidence that PSAV or PSADT substantially enhances the predictive accuracy compared to a single pre-treatment PSA alone (O'Brien *et al*, 2009) and is not useful for monitoring of men on active surveillance (Ross *et al*, 2010).

1.7 Digital Rectal Examination (DRE)

DRE was the principal method for detection of PCa prior to introduction of PSA in the 1980s. Using DRE, PCa volume of 0.2ml or greater can be detected because

majority are located in the peripheral zone of the prostate. A suspect DRE is an absolute indication for prostate biopsy. The use of suspect DRE alone for screening will correctly detect PCa in up to 18% of patients (Crawford, 1996; Schroder *et al*, 1998). In a screening programme of healthy volunteers aged 50 years and over, the positive predictive value of suspect DRE at a serum PSA value 1.0 ngm/ml or greater ranged from 14 – 30% (Carvalhal *et al*, 1999). A metanalysis of published report showed that when a patient has abnormal PSA levels or DRE findings, the chance of having cancer is 20 – 25% (Mistry, 2003). The combined use of PSA and DRE for screening is more effective than either test alone for early detection of localised PCa (Carvalhal *et al*, 1999; Crawford, 1996). However, its low reproducibility and inter-examiner variability (Smith, 1995); and the need for an examination room and trained examiners are limitations. Furthermore, it is hypothesised that negative perception of DRE may deter some men from screening programmes (Underwood, 1991).

1.8 Transrectal Ultrasonography (TRUS)

Since its description by Watanabe and colleagues, TRUS has become widely accepted for clinical use (Watanabe *et al*, 1971). Prior to this, DRE was the primary modality for assessing the local extent of PCa. Technical advances have resulted in better transducers allowing clearer delineation of internal architecture of the prostate. Earlier investigators explored the possible role of TRUS as a tool for early detection of PCa. Lee and co-workers (Lee *et al*, 1985) described visualisation of hypoechoic lesion on ultrasound as the criterion for diagnosis of PCa on TRUS.

Using this criterion, Kenny and Hutchinson found 81.1% correlation between TRUS and biopsy findings with a false positive rate of 15.1% (Kenny & Hutchinson, 1988). In another prospective study of 784 men to compare the usefulness of TRUS for screening over DRE, it was demonstrated that TRUS correctly detected twice more PCa than DRE and interestingly, 59% of tumours measuring less than 1.5cm were not palpable (Lee *et al*, 1988). Overall, the probability that a hypoechoic area is malignant ranges from 36 to 37% (Andriole *et al*, 1988; Chodak *et al*, 1986). On the other hand, 31.8% of PCa are isoechoic whilst 7.6% are found to be hyperechoic (Spajic, 2007).

To improve differentiation of benign from malignant hypoechoic lesions, several investigators have modified TRUS technique. Sperandeo and colleagues described the use of compression of suspicious lesions with the ultrasound probe to determine if they are deformable or non-deformable. In their report, 92.6% of non-deformable hypoechoic lesions were shown to have PCa (Sperandeo *et al*, 2003). Using contrast enhanced ultrasound, Tang and co-workers reported a statistically significant difference between malignant and benign hypoechoic nodules by demonstrating that malignant nodules are more likely to enhance (Tang *et al*, 2008).

Despite these advances, the specificity of TRUS alone for predicting PCa remains disappointing. In one study that evaluated 256 hypoechoic lesions, the PPV of TRUS when used alone was 41% which fell to 24% when DRE is normal (Lee *et al*, 1989). In contrast, when PSA, DRE and TRUS are used together, the number of biopsy cores needed to diagnose one cancer decreased from 26.5 for TRUS alone to 1.3 (Bangma *et al*, 1995).

Furthermore, focal non-malignant lesions like benign prostatic hyperplasia, prostatitis, prostatic atrophy and infarction produce sonographic appearances diifficult to distinguish from PCa (Brawer & Lange, 1989).

Therefore, in clinical setting, the established role of TRUS in early detection of PCa is for accurate measurement of prostate volume and to facilitate biopsy by providing guidance for needles through the channel in the probe.

1.9 Prostate Biopsy

1.9.1 Anatomy of the Prostate, Biopsy and Cancer Location

Improvements in TRUS technology from the first description by Watanabe have expanded its clinical use for PCa detection. It is essential to understand prostate anatomy seen at TRUS because although current improvements in ultrasound technology have vastly improved prostate image resolution; its accuracy depends on the concise demonstration and interpretation of the visualised internal architecture of the prostate.

Lowsley was the first to publish a detailed description of lobar division of prostate. He divided the prostate into five lobes based on anatomical findings on human embryos at different stages of development. Lowsley's five lobes include an anterior, posterior, 2 lateral, and 1 middle lobes with the anterior lobe regressing after birth (Lowsley, 1912). This observation was supported by Robert Moore in his report of 678 prostates from consecutive autopsies (Moore, 1936). In contrast, Lowsley's lobar pattern could not be confirmed in the adult prostates investigated by Le Duc using ductile injection of india ink (Le Duc, 1939). Instead, he concluded

that the prostate has no posterior lobe but is rather comprised of two lateral and one middle lobe. In support of Le Duc's observations, Franks concluded that Lowsley's lobar anatomy did not exist in the adult prostate and proposed that the prostate is comprised of two functionally distinct outer and inner gland, with benign hyperplasia said to arise exclusively from the later (Franks, 1954a).

This controversy existed until 1968 when McNeal, in a sequence of related investigations of the adult prostate reported for the first time, the existence of distinct functional and histologically separate zones within the prostate (McNeal, 1968; McNeal, 1981; McNeal, 1988). These histological distinctions cause differences in the reflection of sound waves on ultrasound hence enabling identification of different zones within the prostate. McNeal proposed that although the prostate developed in relation to the urethra, its subsequent development and function are related to the Wolffian duct, so he examined the prostate by making his cuts in a plane along the course of the ejaculatory duct from the base of the seminal vesicles through to verumontanum, a plane at right angle to usual plane of section and described three zones and an anterior fibromuscular stroma.

The peripheral zone (PZ) comprises 70% of the glandular prostate is the most susceptible to inflammation and some 70 – 80% of PCa. It has a simplified, rounder sacculations with less prominent intraluminal partitions and pale simple columnar epithelium.

In contrast, the central zone (CZ) is a vertical wedge of glandular tissue lateral to each ejaculatory duct with its apex at the verumontanum. It comprises about 25% of the total mass of the glandular prostate with more elaborate acini and large

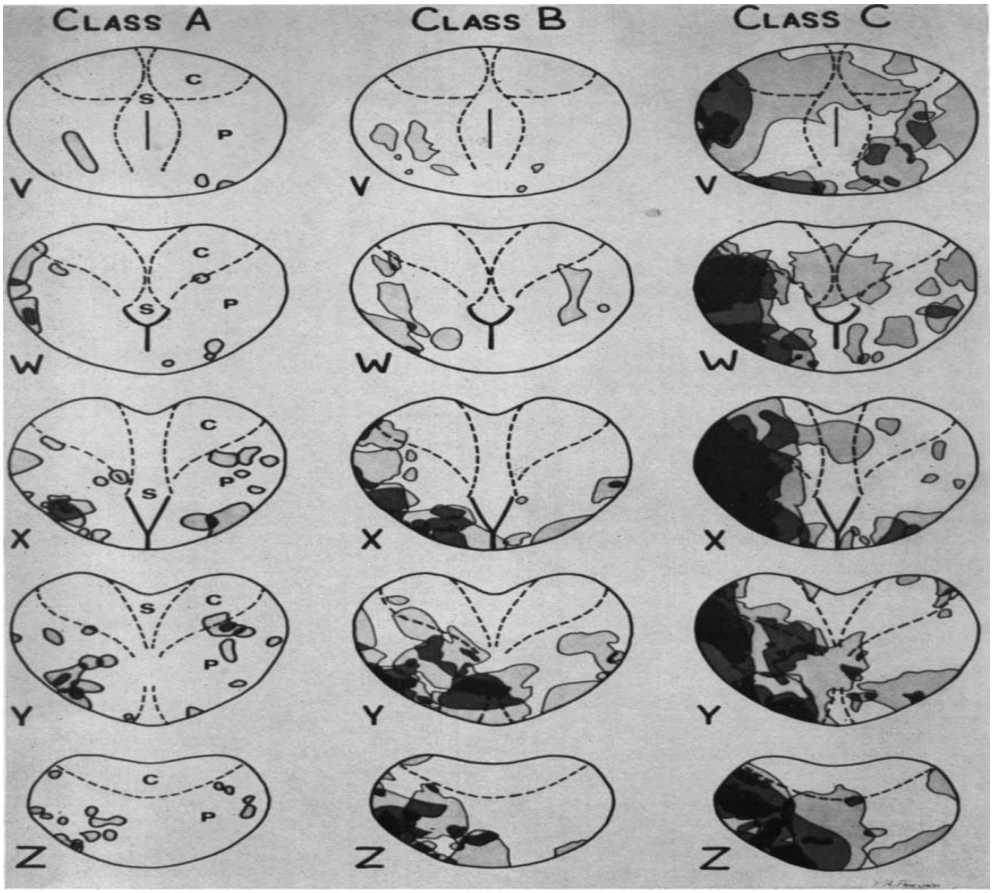
rectangular sacculations which are concentrated peripherally. It makes up almost the entire base of the prostate and is seldom involved with PCa. Lee and colleagues believe that the point of entry of the ejaculatory duct through the capsule of central zone at the base creates an invaginated extraprostatic space down to the apex of the prostate resulting in anatomical defect which creates a pathway for tumours of apical origin to invade the CZ (Lee, 1989).

The transition zone (TZ) consists of two independent small lobes comprising 5-10% of glandular prostate. This region is believed to be the main site of BPH origin, but 10 – 20% cancers are believed to originate from here. Because of their central location, this zone is not palpable. The final zone described by McNeal corresponds to the anterior fibromuscular stroma (AFMS). This is a non-glandular region at the anterior surface of the prostate. Because it is non-glandular, it is not thought to be a site of origin for prostate cancer

The precise locations of cancer within the prostate has been shown in autopsy and radical prostatectomy whole mount specimens with the suggestion that the periurethral area at the base of the prostate is rarely the only site of tumour and when involved, is usually due to invasion from adjacent zones (Chen *et al*, 2000; McNeal, 1969). McNeal (McNeal, 1969) reported on 134 autopsy prostate glands of which 45 had PCa and demonstrated that the central area at the base surrounding the urethra and ejaculatory duct was consistently spared of PCa involvement. Cancer involvement of the periurethral area is a late progression event which strongly correlated with increasing tumour size. Of the 44 small tumours [<0.1cc], none originated solely from the central zone and more interestingly, this area was

consistently spared even in larger tumours [0.1 to >1cc]. McNeal thus postulated that this restricted pattern of PCa spread was likely due to increased density of the central zone and the propensity of tumours to spread along planes of least resistance (figure 1-1).

Figure 1-1: Cancer distribution in the prostate at 5 serial planes [V-Z] and 3 ranges of tumour volumes [A-C]. Light gray = one tumour; dark gray = two tumours overlapping; black = three or more tumours overlapping. C = central zone; P = peripheral zone; S = central stromal core. Adapted from McNeal (McNeal, 1969).



In agreement with McNeal's observation, Chen et al (Chen *et al*, 2000) reported on a computer generated cancer distribution plot obtained from step sectioned 180 radical prostatectomy specimens and showed that the periurethral region at the central area of the base was rarely involved with small volume PCa [**figure 1-2**].

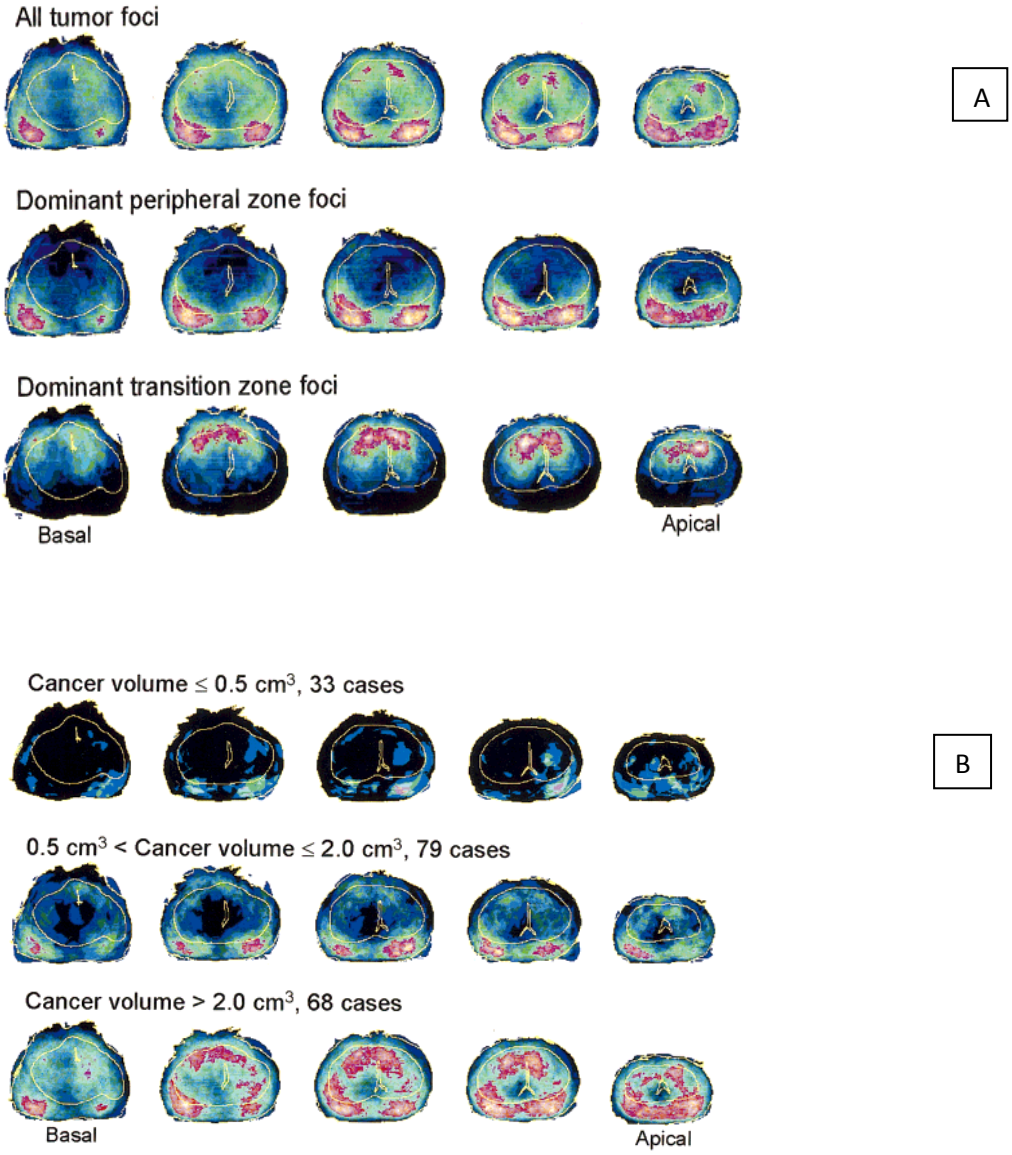


Figure 1-2: Five sections of the computer plots showing distribution of all cancer foci [A] and distribution stratified according to tumour volume [B]. The sections nearest the base are represented by the first section to the left whilst the apical sections are represented on the right. Adapted from Chen et al (Chen *et al*, 2000)

1.9.2 TRUS Biopsy (TRUSB)

In 1989, Hodge and colleagues described the random systematic sextant biopsy and demonstrated that systematic sextant biopsy technique was significantly better than lesion directed biopsy. Of 136 patients who underwent their six systematic, spatially separated paramedian biopsies, 83 were positive for PCa and 96% of these were correctly identified using the sextant technique (Hodge *et al*, 1989). In support of sextant biopsy technique, Stamey advocated its use in routine clinical practice, but suggested that rather than sampling the mid lobar plane, cores should be taken more laterally in order to adequately sample cancers located in the anterior horn of the peripheral zone (Stamey, 1995).

As technology evolved, investigators began to use computer simulations to determine the optimum biopsy sites in order to improve cancer detection. Kawata et al reconstructed 86 autopsy prostates and 40 radical prostatectomy specimens to demonstrate that a modified laterally directed sextant biopsy scheme detected significantly more life threatening PCa (tumour ≥0.25cc and or ≥Gleason 7) compared to routine sextant scheme (Kawata *et al*, 2003). In a similar study, Chen et al used a computer to simulate 10 biopsy schemes and demonstrated that an 11-core multisite-directed biopsy scheme comprising a sextant, one posterior midline, two TZ and anterior horn biopsies had the highest detection rate for cancers greater than 0.5 cc (Chen *et al*, 1999). Using Chen's 11-core scheme, Babaian et al detected 33% more cancers and demonstrated that the most commonly positive non sextant site is the anterior horn (Babaian *et al*, 2000). To minimise this rather

high false negative rate of sextant biopsy, investigators began to explore alternate site sampling or extended biopsy schemes.

1.9.3 Extended TRUS Biopsy

Sextant biopsy as described by Hodge was designed to sample the PZ which harbours most of the cancers and it quickly became the gold standard biopsy technique; until reports began to emerge suggesting that significant numbers of cancers ranging from 20 to 35% are missed by this regimen (Babaian *et al*, 2000; Eskew *et al*, 1997; Eskicorapci *et al*, 2004; Presti *et al*, 2000). These studies show that laterally directed biopsy scheme significantly improved the diagnostic yield compared to the sextant technique. Gore et al (Gore *et al*, 2001) performed laterally directed biopsies in addition to the standard sextant regimen in 396 patients and observed that although 8 core strategy which included mid lobar and laterally directed basal and apical cores optimally detected PCa in smaller prostates with volume <50cc; overall, a 10 core regimen that combined laterally directed biopsy cores obtained from the base, mid gland and apex of the prostate with cores obtained from the paramedian areas at the base and apex achieved an optimal detection rate in all patient subgroups independent of prostate volume or PSA level.

However, in a systematic review of 20,698 patients from 87 studies based on Eskew's 5 anatomical region biopsy model (Eskew *et al*, 1997), modified sextant scheme involving 12 laterally directed cores identified 31% more cancers without additional side effect compared to the sextant scheme (Eichler *et al*, 2006).

Furthermore, increasing the number of biopsy cores above 12 did not significantly alter the cancer yield.

Another advantage of extended biopsy is that it improves the accuracy of Gleason score after radical prostatectomy. In a prospective study comparing laterally directed sextant biopsy to extended 12 core biopsy scheme, the concordance between biopsy and prostatectomy specimen was significantly higher in the extended biopsy group compared to the group undergoing sextant biopsy [85.2% vs 50% p = 0.026] (Elabbady & Khedr, 2006). Similar observations was reported in another study (Divrik *et al*, 2007). Mian et al demonstrated that extended biopsy scheme comprising 10 or more samples was significantly less likely to be upgraded compared to sextant biopsy [17% vs 41% p = 0.001] (Mian *et al*, 2006). Consequently, it is now recommended that sextant biopsy should no longer be performed but instead, at least 8 cores should be sampled in a prostate 30 – 40mls in size (Heidenreich *et al*, 2011).

1.10 Repeat Biopsy: Indications

In the presence of persistent clinical suspicion of PCa despite a negative initial biopsy, majority of men would undergo a repeat and sometimes multiple repeat biopsies. The indications for repeat prostate biopsy include a persistently elevated or rising PSA, suspicious DRE, atypical small acinar proliferation (ASAP) of prostate and multifocal high grade prostatic intraepithelial neoplasia (HGPIN) (Heidenreich *et al*, 2011). The incidence of PCa at repeat biopsy in patients initially diagnosed with ASAP ranges from 39% - 40% and no usual preoperative parameters including

PSA, DRE results, TRUS findings, PSAD and prostate volume significantly predicted cancer diagnosis in this group (O'dowd *et al*, 2000; Scattoni *et al*, 2005). Because of this high risk of cancer diagnosis with ASAP and the lack of clinical or pathological factors to predict which men will have cancer on repeat biopsy, a systematic review by Epstein and Herawi recommended that all men diagnosed with ASAP should undergo repeat biopsy within 3 to 6 months (Epstein & Herawi, 2006).

The cancer detection rate for HGPIN has fallen over the years probably due to shift from sextant biopsy to extended biopsy schemes. Nevertheless, the role of HGPIN as a risk factor for subsequent PCa detection at repeat biopsy remains controversial. In one Canadian study of men diagnosed with HGPIN who underwent repeat biopsies, multifocal HGPIN was an independent risk factor for PCa diagnosis (Merrimen *et al*, 2009). Recent study of identical design by the same group limited to only a cohort who underwent 10 or more extended biopsy regimen at both initial and repeat biopsy performed at a mean of 0.98yr showed similar result (Merrimen, 2010). Therefore, an isolated HGPIN is no longer an indication for repeat biopsy and such patients should be followed up with PSA and DRE (Moore *et al*, 2005). The optimum time for repeat biopsy is unclear, but the longer the time interval between initial and repeat biopsy, the higher the PCa detected (Lefkowitz *et al*, 2002).

1.11 Transrectal Saturation Biopsy (TRSB)

Although several studies had shown that extended biopsy schemes significantly increased PCa detection rate with superior concordance of Gleason score between

needle biopsy and radical prostatectomy, there remain significant number of men who would require repeat prostate biopsies because of either persistently raised PSA, rising PSA, abnormal DRE or inconclusive initial histology (Djavan *et al*, 2000; Nam *et al*, 2004).

Unfortunately, the cancer detection rates from repeat biopsies show a diminishing return. In one study, 34% of 1136 men who had initial biopsies for raised PSA and or abnormal DRE were diagnosed with PCa. Of the 427 men who subsequently had serial prostatic biopsies for persistent clinical suspicion of PCa, 19% had PCa at 2nd biopsy and this number decreased to 8% and 7% for subsequent repeat biopsies 3 and 4 respectively (Keetch *et al*, 1994). In another study of 1051 men with total PSA between 4 and 10 ng/ml who had sextant plus two TZ biopsies, PCa detection rates of 22%, 10%, 5% and 4% for biopsies 1, 2, 3 and 4 respectively (Djavan *et al*, 2001). These studies were based largely on sextant biopsy schemes even though Djavan included two cores from the TZ. However, even with laterally directed extended biopsy schemes, significant false negative rates still persist (Kawakami *et al*, 2007). This prompted investigations to explore either increasing the number of samples or varying the distribution of biopsy sites in order to improve PCa detection rate.

Several transrectal saturation biopsy techniques have been reported with varying cancer detection rates (table 1-1 overleaf).

Table 1-1: Transrectal saturation biopsy series and cancer detection rates

Author	Sample Size (N)	Mean Age (yrs)	Number of Cores	PCa (%)
Borboroglu et al 2000	57	61.4	22.5	30
Stewart et al 2001	224	64.2	23	34
Jones et al 2002	15	61	24	33
Rabbets et al 2004	116	62	24	29
Walz et al 2006	161	63.7	24	41
Stav et al 2008	27	62.1	61.7	11.1
Zaytoun et al 2011	663	64.5	20-24	32.7

Borboroglu et al were the first to report an extensive TRUS guided prostate biopsies on men with persistent clinical suspicion of PCa after a mean of 2 previous negative sextant biopsies (Borboroglu *et al*, 2000). Of 57 men who underwent a mean of 22.5 biopsy cores (range 15 – 31) from six sagittal zones, PCa was detected in 30%. Subsequently, Stewart et al reported a 34% cancer yield in 224 men undergoing repeat biopsy (Stewart *et al*, 2001). They described a 'saturation' biopsy comprising a mean of 23 cores (range 14 – 45) performed under general anaesthesia. Two studies from the same group reported 24 core TRSB as an office based procedure under local anaesthesia (LA) with a 33 - 41% cancer detection rate (Jones *et al*, 2002; Rabets *et al*, 2004). They achieved periprostatic nerve block by injecting lignocaine lateral to a fat notch between the prostate and seminal vesicle which they named Mount Everest sign.

In another study, 41% of 161 patients who underwent an average of 24 cores TRSB were diagnosed with PCa. The authors proposed a model with 72% accuracy for prediction of PCa diagnosis (Walz *et al*, 2006). Similar to initial biopsy protocol, Patel et al demonstrated that more effort should be spent on laterally directed biopsies (Patel *et al*, 2004). Of the 25 cancers detected in their series, none were detected in the parasagittal biopsy cores alone. Only in one study (Stav *et al*, 2008) was the cancer detection rate of TRSB low (11.1%) after an average of 61.7 cores (range 41 – 76) using a technique aimed at extensive coverage of the peripheral zone. However, the mean prostate volume of the 27 patients in this series was 89.6cc (range 35 – 210).

The largest TRSB series reported in literature was by Zaytoun et al (Zaytoun *et al*, 2011). In their study comparing cancer office based TRSB (n = 663) and extended transrectal biopsies (n = 393) after initial negative biopsy using a sampling scheme of 20 cores focused on the lateral and apical regions of the prostate. PCa was identified in 32.7% of the TRSB cohort which was 31.3% higher than detection rate in the extended biopsy group.

The complication rates for TRSB is poorly reported in literature, but in two studies, this ranged from 2.5 to 12% (Borboroglu *et al*, 2000; Walz *et al*, 2006). However, haematuria described as self limiting was found in 100% of patients in one study (Stav *et al*, 2008), whilst routine use of urethral catheterisation for variable period of time was part of the procedure in other series (Fleshner & Klotz, 2002; Stewart *et al*, 2001). Nevertheless, the suggestion in literature is that complications of TRSB are self limiting and comparable to standard biopsy schemes (Rabets *et al*, 2004).

Despite this apparent improvement in PCa detection rate, there are physical and technical limitations with the use of transrectal approach resulting from the inability to adequately sample the anterior and apical regions of the prostate.

TRSB lacks precision for localisation of cancer within the prostate. This was demonstrated in the study of Falzarano et al (Falzarano *et al*, 2010) in their series including 72 men diagnosed with prostate cancer from saturation needle biopsy who underwent radical prostatectomy. Their data showed that 90% of 39 men who were diagnosed with unilateral cancer by saturation biopsy had bilateral disease after radical prostatectomy. Saturation biopsy missed 12 potentially clinically significant cancers. Furthermore, there is a potentially increased risk of sepsis and rectal bleeding arising from multiple needle sticks injuries to anterior rectal wall. Lastly, the transrectal approach relies heavily on operator dependent 3D visual recall for guidance when the procedure is performed. Coupled with the lack of fixation devise, it means that TRSB is devoid of precision which can lead to sampling inaccuracies.

1.12 Transperineal Template Guided Saturation Biopsy (TTSB)

In the current PSA era, prostate biopsy is a frequently performed procedure and remains an essential tool for prostate cancer diagnosis (Welch *et al*, 2007). It is estimated that more than 1 million prostate biopsies are performed annually in the United States of America and Europe (Loeb *et al*, 2011a; Wagenlehner *et al*, 2013). In the UK, Cross and McPhail from the National collaborating centre for cancer

analysed data from the hospital episode statistics (HES) showing a rising trend for prostate biopsies and an estimated 89,000 biopsies per annum in England and Wales (Cross & McPhail, 2008). With a recent update of a European randomised controlled trial after 11 years follow-up showing that PSA based screening significantly reduced mortality from PCa by 21%, it is likely that the demand for prostate biopsies would increase (Schröder *et al*, 2012). Furthermore, a significant proportion of men with negative first biopsy would proceed to have repeat biopsies. In one study, it was shown that the risk of subsequent repeat biopsy when initial procedure is negative was 11.6% after 1 year rising to 38% after 5 years (Welch *et al*, 2007). Further compounding this huge workload demand is the diminished accuracy of conventional TRUSB technique originally designed to sample the peripheral zone of the gland (Hodge *et al*, 1989). This led to increasing use of extended biopsy schemes including saturation biopsy in order to improve cancer detection rate.

In 2001, by adapting the brachytherapy template grid into their technique to enable systematic sampling of all regions of the prostate, Igel and colleagues described a systematic transperineal ultrasound guided template biopsy of the prostate (TTSB) in patients at high risk of harbouring potentially life threatening PCa (PSA >10ng/ml, PSAV >0.75, previous ASAP or PIN on biopsy) despite at least one previous negative set of TRUS biopsies (Igel *et al*, 2001). Depending on the size of the prostate, 4 cores were obtained anterior to posterior from 4 coronal planes similar to the technique used for interstitial radioactive seed insertion at brachytherapy. In order to ensure complete sampling of the prostate in patients with prostate volume

greater than 45cc, additional 2 cores were obtained from each of 2 distal coronal planes. Of 88 patients who were biopsied, PCa was detected in 38 (43%) from a mean of 17 cores. Subsequent report from the same group on 210 high risk patients who underwent TTSB showed similar result with 37% PCa detection rate from a mean of 21.2 cores (Pinkstaff *et al*, 2005).

Several groups have since described various techniques for TTSB with high cancer detection rates (22.7 – 68%) which is comparable to that reported for transrectal approach in repeat biopsy population (Bittner *et al*, 2009; Bittner *et al*, 2013; Demura *et al*, 2005; Dimmen *et al*, 2012; Furuno *et al*, 2004; Gershman *et al*, 2012; Mabjeesh *et al*, 2012; Merrick *et al*, 2007; Pal *et al*, 2011; Satoh *et al*, 2005).

Merrick et al divided the prostate into 24 predetermined regions corresponding to areas covering sextant, lateral PZ, TZ and apex (Merrick *et al*, 2007); and depending on prostate size; one to three cores were obtained from 24 regional locations. Other series have described an equally distributed and systematic sampling of the entire prostate such that on transverse image, the biopsy spots had a diamond shape appearance in most cases (Demura *et al*, 2005; Furuno *et al*, 2004). In contrast, Barzell and Whitmore described a TTSB technique in which the prostate was divided into eight regions using transverse, sagittal and coronal planes which were chosen arbitrarily (Barzell & Whitmore, 2003). Rather than a predetermined number of cores, 4 to 8 cores are obtained from each of the 8 prostate regions at 5mm intervals depending on the size of the prostate to allow complete mapping of the whole gland. In another study, a technique aimed at ensuring maximum sampling and shortening of the procedure time was described (Bott *et al*, 2006). In this technique, the prostate was divided equally into right and left halves and

anterior, middle and posterior areas transversely in addition to inferior and superior longitudinal subdivisions using an indelible marker made on the ultrasound monitor. In order to sample the prostate, between 6 to 12 needles were inserted into each area divisions at the same time and biopsies obtained from each needle respectively. More recently, a standardised 36 core technique has been described (Pal *et al*, 2011).

There are potential advantages of TTSB over transrectal saturation approach. Firstly, the use of fixation devise should allow precise sampling of all regions of the prostate, especially tumours located anteriorly within TZ, resulting to increased diagnostic accuracy. In a study of 210 high risk patients after at least one previous negative TRUS biopsies, TZ cancer was identified in 60 of the 78 patients [77%] who were diagnosed with PCa at TTSB (Pinkstaff *et al*, 2005). Furthermore, in 36 patients [46%], PCa was exclusively identified in the TZ. In another series, it was demonstrated that the cancer core rate [ratio of the number of cancer cores to the number of biopsy cores] was significantly higher in the anterior region compared to the posterior region in patients who underwent repeat biopsy using TTSB (Furuno *et al*, 2004).

Secondly, prostate mapping with TTSB should result to an improved accuracy of staging in men with low volume disease who are considering active surveillance [AS]. In a recent study, 34% of 101 men who underwent TTSB re-biopsy as part of an AS programme for low risk disease diagnosed from extended TRUS biopsy were found to harbour higher risk disease (Ayres *et al*, 2012); furthermore, PCa was located predominantly in the anterior part of the gland in 44% of those men with worse disease on TTSB. More interestingly, PCa upgrading was identified in 38% of

the 34 men whose re-biopsy was done within 6 months of commencing AS. This suggests that these were most likely missed tumours from their initial TRUS biopsies rather than true progression of an indolent tumour in the time interval between biopsies.

Lastly, mapping of the prostate with TTSB should provide detailed information to enable better assessment of patient's suitability for focal therapeutic options. In a study of 110 patients initially diagnosed with unilateral disease on TRUS biopsy who underwent a TTSB; bilateral disease was demonstrated in 55%; furthermore, Gleason score was increased in 23% of patients over the TRUS biopsy (Onik & Barzell, 2008). In another study to evaluate the usefulness of transperineal mapping biopsy [3-DPM] as a staging procedure in the appropriate selection of patients for treatment with focal cryoablation, 54% of the 80 patients who were re-biopsied were found to be unsuitable; furthermore, repeat TRUS biopsies had a false-negative rate of 47% when compared with 3-DPM in assessing patient's suitability for focal cryoablation (Barzell & Melamed, 2007). These findings are supported by reports from radical prostatectomy series which suggest that patients have far more advanced disease at their final pathological result than suggested from their seemingly low volume disease diagnosed at TRUS biopsies (Boccon-Gibod *et al*, 2005; D'Amico, 2000; Wang *et al*, 1997). In two studies, significant disease was identified at radical prostatectomy in 57 to 70% of patients with insignificant PCa diagnosed at their preoperative TRUS biopsy (Boccon-Gibod *et al*, 2005; Wang *et al*, 1997).

Prostate cancer is unique because of the discrepancy between reported frequencies of cancer occurrence at autopsy compared to lifetime clinically manifested cancer cases. Whilst autopsy series show a 30 to 38% rate of incidental cancer in men older than 50 years (Franks, 1954b), the prevalence of clinically significant cancer in a man's life is 9.5% which is far less (Seidman *et al*, 1985). Consequently, there are concerns that increasing the number of cores as in TTSB would lead to a rise in detection of clinically insignificant cancer. According to Ploussard et al (Ploussard *et al*, 2011), insignificant cancers are PCa diagnosed in the absence of cancer related symptoms that would not have caused disease-specific morbidity or mortality during the patient's life if left untreated. The first concept of insignificant cancer was proposed by Stamey et al (Stamey *et al*, 1993). The authors examined 139 consecutive unselected cystoprostatectomies from patients with bladder cancer and identified PCa in 55 (40%). By applying previously determined 8% prevalence of prostate cancer within a man's life to isolate clinically significant disease from the 139 cystoprostatectomies; they concluded that tumours greater than 0.5cm^3 which represented 8% of the total cohort is clinically significant cancer.

By using Stamey's classification of insignificant tumours and combining preoperative pathological and clinical criteria along with PSAD, Epstein et al (Epstein, 1994) demonstrated that fewer than 3 positive cores, no core with more than 50% involvement, no Gleason pattern 4 or 5 and PSAD less than 0.15 identified most significant and potential insignificant PCa. In another study by the same group to predict insignificant cancer rate at TTSB; an average of 44 cores were obtained on 103 radical prostatectomy specimens whose preoperative TRUS biopsies had suggested insignificant cancer. Their study showed that 29% of tumours were

misclassified as insignificant by conventional biopsy schemes; but when TTSB was used as the predictive tool, the false positive rate of insignificant cancer diagnosis was only 8 to 11.5% with specificity of 95.8 to 97.1% in the two TTSB schemes tested (Epstein *et al*, 2005). In fact, the suggestion in literature is that increasing the number of cores at needle biopsy identifies more significant PCa at an earlier stage when it is potentially curable and may make the diagnosis of insignificant biopsy more accurate (Chan *et al*, 2001; Miyake *et al*, 2004).

However, by far the greatest concern regarding the use of TTSB is the high rate of morbidity, particularly acute urinary retention [AUR] ranging from 11 to 39.4% reported in literature (Merrick *et al*, 2007; Merrick *et al*, 2008; Pinkstaff *et al*, 2005). In one study that evaluated the morbidity of TTSB, 39.4%, 7.1% and 1.6% of the 120 patients who underwent TTSB for persistent clinical suspicion of PCa after a mean of 2 previous negative conventional TRUS biopsies were catheter dependent on the day of the procedure and at 3 and 6 days afterwards respectively (Merrick *et al*, 2008). The overall catheter dependency rate in this study was 1 day even though that in virtually all patients, their international prostate symptom scores [IPSS] returned back to baseline within 30 days. The authors postulated that the AUR may have been instigated by TTSB. In a recently published large TTSB series including 485 men, 27% developed AUR after a mean of 56 cores (Bittner *et al*, 2013).

To prevent AUR, investigators have tried various manoeuvres including the use of tamsulosin - an α-adrenergic blocker known to cause relaxation of smooth muscles of the prostate and bladder neck resulting in an improvement in urinary symptoms

(Milicevic *et al*, 2012). The utility of tamsulosin administered prior to prostate biopsy in order to minimise biopsy induced urinary symptoms is not clear. In a randomised controlled trial of 66 patients to study voiding impairment after prostate biopsy, Bozlu and colleagues (Bozlu *et al*, 2003) treated 33 patients with tamsulosin commenced the day prior to a 12 core biopsy procedure for 30 days. They reported a significantly lower rate of voiding difficulty in the tamsulosin group compared to the control group (9 vs. 42%) with only one patient developing AUR in the tamsulosin group compared to three for the control group. Consequently, investigators have initiated tamsulosin from between 2 hours to 2 days prior to TTSB and continued this postoperatively for up to 2 weeks in some series (Bittner *et al*, 2013; Bott *et al*, 2006; Merrick *et al*, 2007). Nonetheless, despite prophylactic tamsulosin, 27 – 38% of patients developed AUR in two studies (Bittner *et al*, 2013; Merrick *et al*, 2007).

Others have incorporated the use of prophylactic urethral catheterisation which was left insitu for several days in their TTSB technique (Ayres *et al*, 2012; Bott *et al*, 2006; Demura *et al*, 2005; Furuno *et al*, 2004; Igel *et al*, 2001; Onik & Barzell, 2008; Pal *et al*, 2011; Pinkstaff *et al*, 2005). However, despite the use of prophylactic catheterisation, 9 of 110 patients who underwent mapping biopsies in Onik and Barzell series developed AUR and more noteworthy is that all AUR incidence occurred in 9 of the 58 (15%) patients who had bilateral mapping TTSB as in our study (Onik & Barzell, 2008). Thus when patients were not routinely catheterised, the rates of retention in vast majority of the studies were much higher as summarised in table 1-2 overleaf.

Table 1-2: Key TTSB publications indicating the incidence of prostate cancer (PCa) diagnosis and reported acute urinary retention (AUR) rates.

‡ Denotes studies where prophylactic urethral catheter was left insitu as part of TTSB techniques for several days. X: Not reported

Author	Sample Size (N)	Mean Age (yrs)	Number of Cores	PCa (%)	AUR (%)
Igel et al 2001	88	X	17	43	2‡
Buskirk et al 2004	157	69	22	X	11
Furuno et al 2004	113	65	19	43	1‡
Demura et al 2004	371	67	20	36	2‡
Pinkstaff et al 2004	210	66	17	37	11
Satoh et al 2005	128	67	22	23	2‡
Bott et al 2006	60	64	24	38	3‡
Merrick et al 2007	102	65	51	42	38
Merrick et al 2008	129	65	54	46.5	39
Bittner et al 2008	217	64	24 - 72	45.9	X
Pal et al 2011	40	63	36	68	2.5‡
Mabjeesh et al 2012	92	64	30	26	X
Gershman et al 2012	34	66	25	50	X
Bittner et al 2013	485	65	56	46.6	27

1.13 Processing of Biopsy Specimen

The quality of prostate biopsy specimen and its processing can influence the outcome of the histopathological analysis. It is recommended that biopsies taken from different regions of the prostate be sent to the pathology laboratory in separate pots with full clinical information (Heidenreich *et al*, 2014). In the laboratory, the cores are transferred into cassettes and prepared by fixation, dehydration and paraffin impregnation.

The optimum embedding technique including number of cores embedded in one cassette remains subject of debate. It is believed that embedding multiple cores in a single block would result in less tissue being analysed because of the difficulty with alignment of all cores in one plane to allow optimal tissue representation. In a retrospective study, Gupta and colleagues (Gupta *et al*, 2004) observed that individual submission and processing of biopsy specimens significantly reduced the rates of equivocal diagnosis. The UK Prostate Cancer Risk Management Programme recommends that one core is embedded per cassette (PCRMP Guide, 2006). In one series using computer simulation of biopsy core, it was demonstrated that sectioning a biopsy core at a 0-degree angle provided optimal sectioning with maximum surface area for analysis and this was more likely when each core is embedded individually (Kao *et al*, 2002).

Perhaps, the main drawback to widespread use of single embedding technique is that it is time consuming and costly especially with increasing number of cores with saturation biopsies. In order to allow embedding of multiple biopsy cores in one cassette, optimized techniques including flattening of cores between two nylon

meshes or enveloping them in a piece of paper is recommended. Furthermore, the cores should be pushed down with a tamper to keep the cores in the same plane during embedding in paraffin and allow sectioning at multiple levels through the entire length of the core (PCRMP Guide, 2006). It is recommended that blocks should be cut at three different levels each three to five sections (10 – 20 µm) apart in order to optimize detection of small lesions (Van der Kwast *et al*, 2013).

1.14 Magnetic Resonance Imaging (MRI) and Prostate Biopsy

The use of MRI as a non invasive modality for defining anatomical and pathological lesions in the prostate has evolved over the last 30 years. Conventional MRI (cMRI) reveals the morphological information of the prostate using a combination of T1 (T1W-MRI) and T2 (T2W-MRI) weighted images with or without endorectal coil (Aigner *et al*, 2007). Initially used for staging of men diagnosed with PCa prior to radical therapy (Steyn & Smith, 1982), recent technological advancement has expanded its role to included screening prior to biopsy, risk stratification in patients with persistent clinical suspicion of PCa despite negative TRUSB, monitoring of patients on active surveillance and treatment follow-up (Türkbey *et al*, 2012). Furthermore, pre-biopsy MRI offer additional advantage with reduction of post-biopsy artefact caused by haemorrhage which manifests as low signal on T2W-MRI similar to cancer and can lead to overestimation of cancer burden in 20% of cases (Ahmed *et al*, 2009). cMRI has a sensitivity and specificity of 70% and 76% respectively after previous negative TRUSB when additional targeted biopsies of suspicious areas on MRI are added to a sextant biopsy protocol in men with PSA

level ranging from 4 to 20ng/ml (Vilanova *et al*, 2001). Conventional MRI is limited in its diagnostic ability because not all low signal intensity in the peripheral zone is due to PCa. Benign conditions such as chronic prostatitis, hormonal treatment effects, atrophy and post-biopsy haemorrhagic artefact resulting in low signal on T2WI are difficult to distinguish from cancer (Ahmed *et al*, 2009). Furthermore, cancer in the central and transition zones are more difficult to discern on T2WI due to BPH (Hoeks *et al*, 2011).

Recently, functional MRI techniques including diffusion weighted imaging (DWI), dynamic contrast enhanced MRI (DCE-MRI) and MR spectroscopy imaging (MRSI) have been introduced. Using differences in apparent diffusion coefficients, vascularity values and metabolic ratios between prostate tumour and non tumour tissues, these functional MRI sequences show improved tumour differentiation (Yoshizako *et al*, 2008). When combined with cMRI to provide both functional and anatomic information, it is called Multiparametric MRI (mp-MRI).

Compared to cMRI, functional techniques may improve accuracy for PCa diagnosis. A review by Kirkham et al (Kirkham *et al*, 2006) to assess the ability of MRI to localise PCa within the prostate using whole mount histology as gold standard found that cancer detection rates are highly variable. The sensitivity of conventional T2W-MRI ranged from 37 to 96% whilst DCE-MRI had a narrower sensitivity range of 57 to 89%. When compared with whole mount prostatectomy, DCE-MRI has a sensitivity, specificity and negative predictive value of 86%, 94% and 95% respectively for detection of tumours greater that 0.5cc (Puech *et al*, 2009; Villers *et al*, 2006). Literature suggests that when both sequences are combined, the validity of MRI for cancer detection is likely to be improved (Amsellem-Ouazana *et al*, 2005;

Tanimoto *et al*, 2007). Amsellem-Ouazana et al (Amsellem-Ouazana *et al*, 2005) combined MRSI and cMRI prior to TRUSB after 2 negative biopsies and reported a specificity of 96.3% and sensitivity of 73.3% when supplementary cores from suspicious areas on MRI were added to standard 10 core scheme in 42 men. Similarly, Tanimoto et al (Tanimoto *et al*, 2007) found a statistically significant difference in sensitivity, specificity and accuracy in favour of combined MRI sequence compared with either modality alone.

In patients with persistent clinical suspicion of PCa, MRI may guide the area of biopsy and reduce the false negative rates of repeat conventional TRUSB. However, the critical question is whether the use of MRI would increase detection of clinically significant PCa to justify the operating time and high running cost of this technology. To achieve this, the use of MRI should allow targeted biopsy of suspicious areas to decrease number of cores and improve detection rate. Using MRI guided TRUS biopsies in 68 men with PSA greater than 4ng/ml and at least 2 previous negative TRUSB, Hambrock et al (Hambrock *et al*, 2010) reported that a median of 4 MR image guided biopsy detected significantly more cancers compared to a matched standard biopsy protocol. A recent systematic review reported that MRI-targeted biopsy using 4 cores has equivalent cancer detection rates compared with standard 12 cores TRUSB (Moore *et al*, 2013). Furthermore, targeted biopsy resulted in a third fewer men being biopsied and avoided diagnosis of clinically insignificant cancer in 10% of patients.

In the current PSA era, with more men increasingly requiring extended repeat prostate biopsy especially with saturation scheme due to persistent clinical suspicion of PCa, it is surprising that despite the literature suggesting improved

diagnostic yield from pre-biopsy MRI information in transrectal biopsy series in this cohort of men, few studies have correlated pre-biopsy MRI and TTSB outcomes. Hadaschik et al (Hadaschik *et al*, 2011) developed software interfaces to superimpose suspicious areas of pre-biopsy MRI over peri-intervention ultrasound image at transperineal biopsy. Of the 106 patients, PCa was identified in 59.4% and highly suspicious image on MRI correlated with cancer diagnosis in 95.8%. Furthermore, lesion targeted cores had a significantly higher positivity rate compared to non-targeted cores. In another study using similar software interface (Miyagawa *et al*, 2010) , cancer was identified in 61% of 85 men undergoing combination of transrectal and transperineal biopsies after one negative sextant TRUSB. Targeted biopsy detected PCa uniquely in 35% of cancer positive cases with higher number of positive cores compared to non targeted scheme (32% vs 9%). In a recent study, Kasivisvanathan and colleagues (Kasivisvanathan *et al*, 2013) described a cognitive registration technique to determine clinically significant cancer diagnosis rate. Of the 182 men with suspicious lesions on mp-MRI who underwent transperineal MRI-targeted biopsies, no significant difference was observed with respect to clinically significant cancer (maximum core length 4 or greater and Gleason score 3+4 or greater) diagnosis (57% vs. 62%). However, targeted biopsy diagnosed fewer clinically insignificant cancers compared to non targeted biopsy and this was statistically significant (9.3% vs. 17%).

Although emerging studies described thus far suggest a role for MRI-targeted lesion biopsy in the cohort of men studied in this field of science, there are inherent limitations to its use as a screening tool to determine whether or not to offer prostate biopsy in clinical practice. In one study, it was demonstrated that 52 of 92

patients on active surveillance had inapparent tumour on MRI. On multivariate analysis, there was no association between imaging findings and outcome prompting the authors to conclude that tumour apparency or inapparency in PCa patients on active surveillance is of no prognostic value (Cabrera *et al*, 2008).

Herein, we compare pre-TTSB MRI and subsequent TTSB and assesses whether pre-biopsy MRI would decrease the need for TTSB or allow a more targeted regime of repeat biopsy.

1.15 Hypothesis

The composite studies comprising this thesis are based on a novel modified TTSB technique which avoids sampling of the periurethral area at the base of the prostate rarely involved with localised PCa based on the following hypothesis:-

1. That urethral trauma from extensive sampling of the basal periurethral area is a major instigating factor for AUR in TTSB.
2. That modifying TTSB technique so as to avoid sampling of this periurethral area at the base would reduce risk of AUR compared to the literature, without influencing cancer yield.

1.16 Objectives

1. To determine the detection rate of PCa using a modified TTSB.
2. To determine the location, distribution and characteristics of cancer detected in men undergoing modified TTSB.
3. To determine short term complications of modified TTSB and whether the risk of AUR would be reduced compared to rates reported in the literature.
4. To evaluate the value of pre-biopsy PSA and its derivatives for their ability to predict pathological outcomes of modified TTSB.
5. To evaluate role of pre-biopsy MRI in predicting outcome of modified TTSB

Chapter 2 Patients and Methods

2.1 Patients

303 patients were recruited for this study from July 2007 to January 2013. Prior to the introduction of modified TTSB technique to the Wirral University Hospital trust, there were discussions between urologists, management and purchasers. As new technology committee had not been developed at the time, a thorough audit of this technique was mandated. Our initial information leaflet included details of the procedure as discussed in section 2.2.2 of this report, but with results from published reports of TTSB. Patients were given an information leaflet in advance and full informed consent obtained prior to procedure in the day unit. Regular audit was undertaken and presented to the audit department on the Wirral in fulfilment of the Trust's clinical governance policy.

All patients underwent modified TTSB which was performed at the Wirral University Teaching Hospital, Merseyside. Patients were largely recruited from the Wirral but a significant proportion was referred by urologists from hospitals within and outside of the Mersey region (table 2-1 overleaf).

Table 2-1: Sources of patients referred for TTSB

Hospital	Number of Patients (%)
Wirral University Hospital	132 (43.5)
Warrington Hospital	71 (23.4)
Countess of Chester Hospital	32 (10.6)
Southport and Ormskirk Hospital	19 (6.3)
University Hospital Aintree	15 (5)
Royal Liverpool University Hospital	21 (6.9)
Whiston Hospital	6 (2)
Nobles Hospital	2 (0.7)
Leighton Hospital	2 (0.7)
Bangor Hospital Gwynedd	1 (0.3)
Wrexham Maelor Hospital	1 (0.3)
Coventry University Hospital	1 (0.3)

Patients were referred for TTSB when there was a persistent clinical suspicion of PCa despite at least one negative conventional TRUSB; including PSA progression whilst on surveillance following negative TRUSB, raised PSA with family history of PCa, extensive high grade prostatic intraepithelial neoplasia (HGPIN) and atypical small acinar proliferation (ASAP, table 2-2 overleaf).

Table 2-2: Clinical Indications for repeat prostate biopsy in men referred for modified TTSB

Indication	Number of Patients (%)
Rising PSA	242 (79.9)
Family History of Prostate Cancer	20 (6.6)
Extensive HGPIN	23 (7.6)
ASAP	18 (5.9)

Within the study population are 66 men whose initial prostate volume was greater than 60cc. During the early phase of the study it became apparent that technical difficulty is encountered as a result of pubic arch interference, which precludes adequate sampling of the peripheral zone of the gland, in those with very large prostates. Therefore, a protocol was adopted allowing for the use of dutasteride 0.5mg daily for 3 to 6 months in order to downsize the prostate so as to minimise pubic arch interference in such patients. Subsequently, the prostate was scanned with TRUS for confirmation of size reduction to less than or equal to 60cc prior to TTSB. Dutasteride is a potent inhibitor of 5α-reductase types 1 and 2 isoenzymes which convert testosterone to dihydrotestosterone (DHT) leading to the reduction in androgenic drive of BPH development and hence a reduction in prostate volume (Andriole *et al*, 2004). Both 5α-reductase types 1 and 2 are normally expressed in prostate tissue but the type 2 is the predominant isoenzyme (Anderson *et al*, 2001). The level of expression of both 5α-reductase isoenzymes are significantly higher in BPH compared to normal prostate (Iehle *et al*, 1999) and dutasteride 0.5mg daily for 3 months in men awaiting TURP for BPH reduced intraprostatic DHT by

approximately 94% (Wurzel *et al*, 2007). The use of 5α-reductase inhibitors consistently decreased prostate volume by 24% to 25.7% when compared with placebo in randomised trials (Roehrborn *et al*, 2002; Tsukamoto *et al*, 2009).

As potent antiandrogens, 5α-reductase inhibitors have been explored as potential chemopreventive agents for PCa. The prostate cancer prevention trial (PCPT) randomised 18,882 aged 55 years or older to 5mg daily of finasteride or placebo. They reported a 25% risk reduction of PCa over a 7 year period but with 6.4% increased risk of high grade PCa (Thompson *et al*, 2003). A similar study with identical design found that dutasteride 0.5mg daily resulted in a relative risk reduction of 22.8% but with 12 more high grade tumours diagnosed (Andriole *et al*, 2010). Studies examining the effects of 5α-reductase inhibitor therapy on prostate pathology have reported conflicting results (Bostwick *et al*, 2004; Yang *et al*, 1999). Bostwick et al reported that finasteride therapy results in significantly higher Gleason grade which can result in grading bias. Dutasteride induces an involution and atrophy of epithelium relative to the stroma in benign prostatic tissue (Iczkowski *et al*, 2005). On the contrary, Yang et al (Yang *et al*, 1999) did not find any significant histologic differences between benign and cancerous prostates in finasteride treated men compared to placebo. Others have questioned the validity of the increased high grade tumour reported in the PCPT trial in the 5α-reductase inhibitor treated arm suggesting that this may be a result of an increased biopsy sensitivity with finasteride therapy (Redman *et al*, 2008). Nevertheless, the pathologist should be made aware when a patient is on 5α-reductase inhibitor therapy in view of above controversies.

2.2 Modified TTSB procedure

2.2.1 Equipment

All modified TTSB procedures were carried out using biplanar transrectal ultrasound transducer (BK Medical, Herlev, Denmark) mounted on a brachytherapy stepping unit (DK Technologies®, Barum, Germany) as shown in figure 2-1 below.

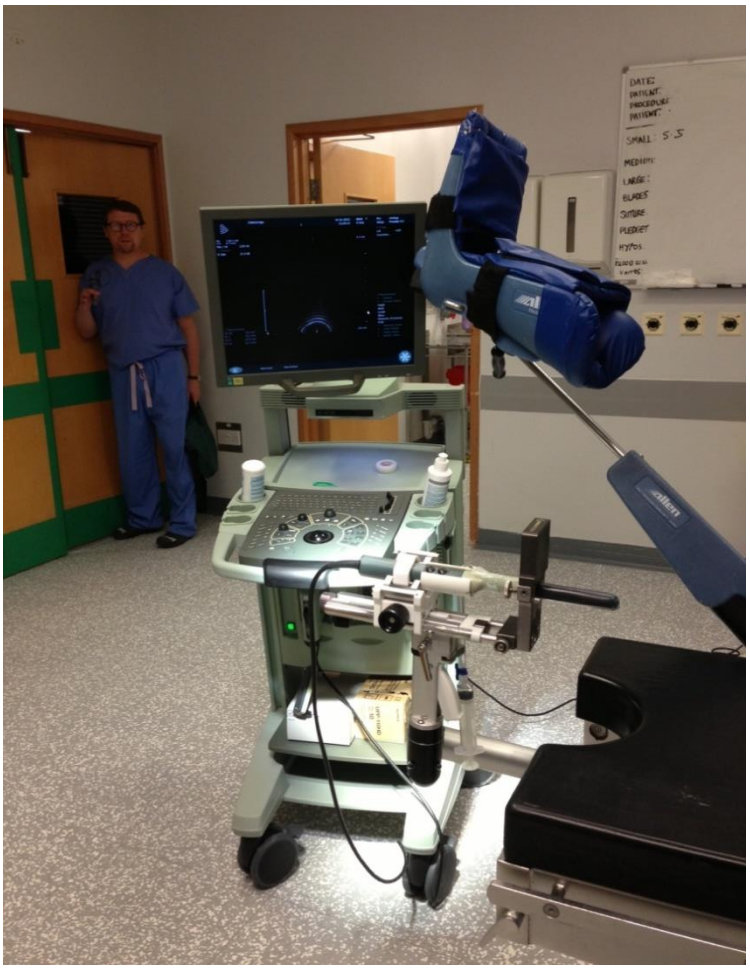


Figure 2-1: Flex focus 800 ultrasound machine with biplanar implant probe mounted on a brachytherapy stepping unit

The image of the prostate is obtained with the flex focus 800 ultrasound scanner (BK Medical, Denmark). A Magnum spring loaded biopsy gun (BARD, Covington, USA) was used to obtain the transperineal prostate biopsy samples.

2.2.2 Modified TTSB Technique

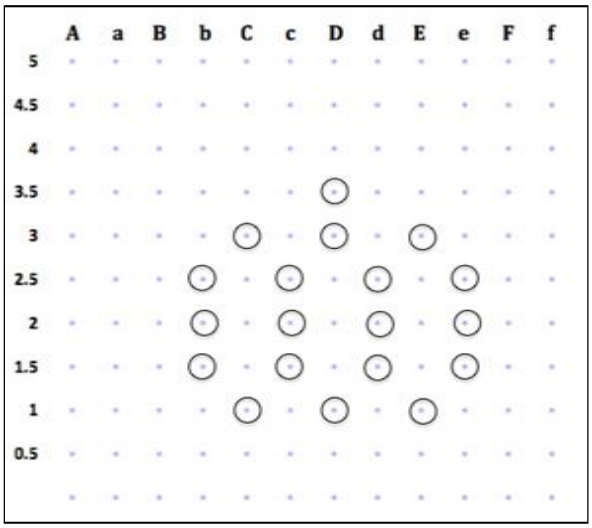
Modified TTSB was performed under general anaesthesia and as a day case procedure. Patients were admitted to a day ward one hour preoperatively and given a phosphate enema, which decreases the amount of faeces in the rectum, thus enhancing the acoustic image of prostate on TRUS (Trabulsi *et al*, 2012). Each received a single dose of intravenous gentamicin 0.24g on induction of anaesthesia, plus metronidazole 1g suppository at the end of the procedure according to local hospital protocol. No patient was catheterised prophylactically.

Once anaesthetised and placed in extended lithotomy position, the scrotum is secured anteriorly with adhesive op-tape (Barrier®, Göteborg, Sweden); the perineal skin is shaved and prepared. An endocavity balloon (CIVCO®, Iowa, USA) is placed over the implant probe. Water is injected and withdrawn from the balloon until all bubbles are removed, so as to avoid acoustic interference. The side viewing, biplanar implant probe attached to a brachytherapy stepping unit is then inserted into the rectum. Water is injected into the endocavity balloon so as to improve contact between the probe and the anterior rectal wall. An image of the prostate is obtained in the broadest transverse section and centred on the D line of the template grid. The stepper is set for 5mm slices. Prostate volume was measured

and calculated as an ellipsoid using the formula, height x width x length x 0.5236 as previously described (Terris, 1991).

A Magnum biopsy gun set on 22mm pass is used to take biopsy cores in rows systematically from right to left using an 18G needle. The interval between biopsy cores on a row is 10mm, but with 5mm between rows (figure 2-2).

Figure 2-2: Transverse view of prostate superimposed on the template grid showing sampled sites



The number of biopsies within a row is dictated by the width of the prostate, but we always ensured that the most lateral cores are near the capsule to cover the peripheral zone.

After inserting the biopsy needle through the aperture in the template and into the prostate, the image is switched to the longitudinal view. The needle is then withdrawn so that a 22mm core is taken from the apex inwards. Each biopsy site is recorded on a copy of the image of broadest transverse section and each biopsy individually potted in formalin.

Previous studies have estimated the average length of the prostate (Kälkner *et al*, 2006; Terris, 1991). Kälkner et al compared prostate lengths assessed on computerised tomography (CT), step-sectioned TRUS and conventional TRUS on 31 men diagnosed with localised PCa prior to combined external beam radiotherapy and high dose rate brachytherapy (Kälkner *et al*, 2006). The mean lengths of the prostate were 4.5 cm on CT compared to 3.6 cm for both step section and conventional TRUS respectively. In another study, Terris and Stamey (Terris, 1991) reported average sagittal cephalocaudal diameter of 3.4cm on TRUS volume estimation in 150 men prior to radical prostatectomy. Therefore, a 22mm peripheral biopsy cores taken anteriorly, posteriorly and laterally where prostate length is shortest should sample the full length of the prostate. However, as the cephalocaudal length of the gland increases when moving more centrally, the 22mm length biopsies fall progressively short of the base, sparing this area (figure 2-3).

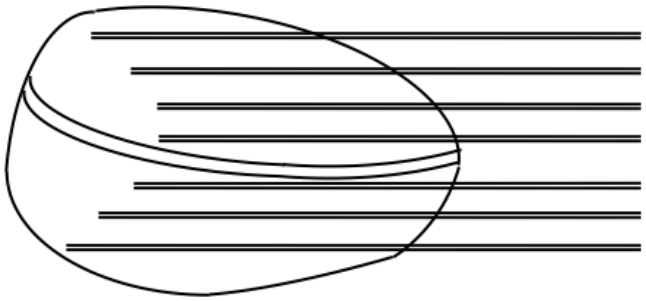
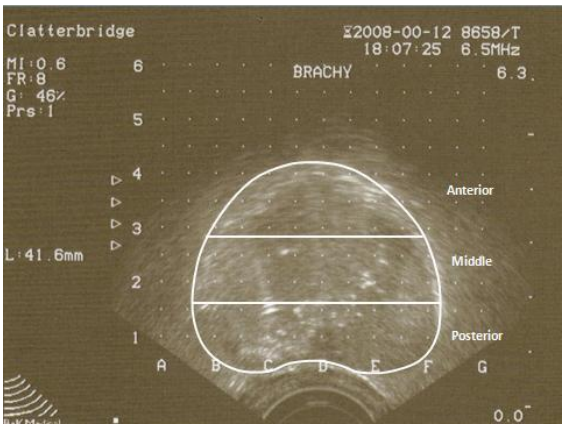


Figure 2-3: Schematic diagram showing sparing of the periurethral basal area with modified TTSB

Postoperatively, bladder emptying was not performed. Patients are discharged once they have successfully voided urine, with a 5 day course of ciprofloxacin 0.5g twice daily.

All specimens were reported by a single pathologist. Tumour location at TTSB was marked as anterior, middle, posterior or either combination by dividing the prostate equally into anterior, middle and posterior regions on transverse image similar to the description by Bott et al (Bott *et al*, 2006) but without further division into right and left halves. This results in 7 possible tumour locations namely: - anterior, anterior + middle, middle, middle + posterior, posterior, anterior + posterior and anterior + middle + posterior areas (figure 2-4).

Figure 2-4: Subdivisions of the prostate into anterior, middle and posterior regions



2.3 Processing of Biopsy Specimen

Following TTSB, each individually potted specimen is sent to the histopathology laboratory where they are accessioned (ordered) with date, time and a unique lab number is assigned. The specimens are then manually placed and processed in a workstation prior to embedding. The total routine overnight tissue processing time is 15 hours. Three cores are embedded in a cassette (figure 2-5) and cut in three levels using a microtome as recommended by the pathology committee of the European Randomised Study of Screening for Prostate Cancer (ERSPC) guidelines on processing and reporting of prostate biopsies (Van der Kwast *et al*, 2013). The slides batch are stained with Haematoxylin and Eosin stain using an automated staining machine followed by automated cover-slipping.

Figure 2-5: Three biopsy cores embedded in single cassette

2.4 Morbidity of Modified TTSB

To further evaluate morbidity of modified TTSB, a questionnaire was designed and administered to all patients undergoing modified TTSB on the day of their procedure once they were admitted to the day ward. The survey questionnaire consisted of multiple questions in 3 domains seeking to explore patient's experience of bleeding, pain and analgesic requirements after TTSB which were assessed from 1hour post biopsy and on days 1, 3 and 7 (see accompanying material). More specifically, the questions asked about severity of bleed using colour such as fresh, pale or dark and source or location including rectal and urine (haematuria) or semen (haematospermia, which was assessed from day 1 post biopsy). Pain was assessed and scored using visual analogue scale (range 0-10, with an increasing score indicating worsening pain). Furthermore, the location of pain was explored including perineal, rectal or pain elsewhere. Finally, the need for analgesic was documented and patients were required to document what analgesic agent they required for pain relief.

Once completed, patients returned the questionnaire by post using a prepaid envelope. All questionnaires were independently collated by a urology nurse practitioner and blinded to patient's name or hospital identification detail.

2.5 Biomarkers of prostate cancer prior to modified TTSB

Prior to modified TTSB, a venous blood sample was obtained for serum PSA and %fPSA determination. The obtained blood specimen was immediately sent to the

biochemistry laboratory and refrigerated on receipt at 4°C. The total PSA and %fPSA were subsequently analysed from the refrigerated serum using Roche Elecsys free PSA assay (Roche Diagnostics Corporation, Basel, Switzerland). PSA Density (PSAD) was calculated by dividing the preoperative PSA (ng/mL) by the calculated prostate volume (mL) at TTSB as previously described by Benson et al (Benson *et al*, 1992b) and discussed in detail in chapter 1, subsection 1.6.3 of this report.

The pre-biopsy PSA and its derivatives (PSAD and %fPSA) were then assessed for their ability to predict prostate cancer diagnosis and biopsy cancer volume including total number of cores positive with cancer (NPC), maximum tumour length (MTL), aggregate tumour length from all positive cores (ATLPC), maximum percent core involved (MPC) and percentage of positive cores (PPC). These prostate biopsy histological parameters have been shown to correlate with pathological tumour volume and recurrence after radical prostatectomy by several studies (Nelson *et al*, 2002; Ochiai *et al*, 2005; San Francisco *et al*, 2004).

In a study of 207 men who underwent radical prostatectomy for cancer detected after extended biopsy, the total number of positive cores correlated with total tumour volume ($p<0.001$) and the incidence of insignificant cancer was significantly higher amongst those with only 1 positive core (42.5%) compared to (16.4%) with 2 positive cores and (5.5%) for 3 or more positive cores (Ochiai *et al*, 2005). Nelson et al (Nelson *et al*, 2002) determined predictors of PSA-free survival from 588 radical prostatectomy specimens after 4 years follow-up. Of all the preoperative variables, greatest percentage of biopsy core involved with cancer, PSA and Gleason score significantly predicted PSA-free survival on multivariate analysis. Another study demonstrated that percentage of positive cores and biopsy Gleason score were the

only preoperative predictors of recurrence after radical prostatectomy (San Francisco *et al*, 2004).

To describe significant cancer at TTSB, the full saturation biopsy classification scheme described by Epstein (Epstein *et al*, 2005) was utilised. After performing a 44 core saturation biopsy on 103 radical prostatectomy specimens which were predicted to have insignificant PCa at their initial needle biopsy, Epstein and colleagues showed that using a classification scheme based on Gleason score ≥7, NPC ≥4, MTL ≥4.5mm and ATLPC ≥5.5mm predicted significant cancer with a sensitivity of 71.9%, specificity 95.8% and false negative rate of only 11.5% (Epstein *et al*, 2005). Consequently, using this classification, this study's cohort were stratified into six groupings as follows:- NPC less than 4 and 4 or greater, MTL less than 4.5mm and 4.5mm or greater, ATLPC less than 5.5mm and 5.5mm or greater and maximum Gleason score (MGS) less than 7 and 7 or greater. Additionally, we also compared the group with MPC less than 50% and 50% or greater and PPC less than 15% and 15% or greater according to the median value.

2.6 MRI prior to Modified TTSB

Prior to TTSB, patients were imaged using a 1.5 Tesla (1.5 T Achieva Philips Medical Systems, Best, Netherlands) MRI scanner with a Synergy body coil.

Firstly, a multiplanar reference images were acquired. This was followed by acquisition of T2 weighted images (T2WI) in the axial and coronal planes through the prostate, time to repetition (TR) 3300 ms, time to echo (TE) 125 ms, using a field of view (FOV) of 220 mm; image matrix 256 X 256; section thickness 3 mm

with 0.3 mm interval covering the prostate gland and seminal vesicles with 24 sections and image acquisition time of 3 min 30 s.

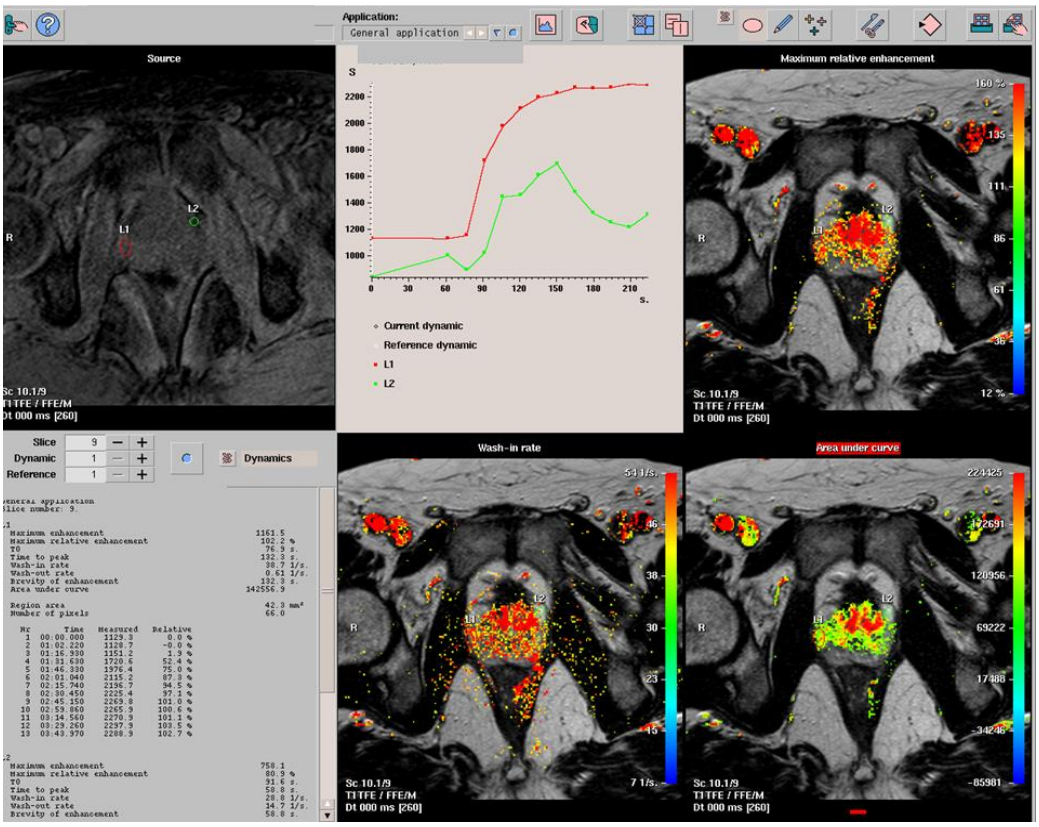
After acquiring the T2WI, diffusion image was then performed. Diffusion-weighted images (DWI-MRI) were obtained in a transaxial plane using a single-shot echo-planar sequence; TReff/TEeff, 3,580/62; parallel imaging factor, 2; b factors, 0 and 600 s/mm2; 80 x 71 matrix; 3-mm section thickness with a 1-mm intersection gap; 18 sections obtained in 2 minutes 33 seconds. Spectral inversion recovery fat suppression was used to eliminate chemical shift artefacts. The isotropic motion-probing gradient pulses were placed along three orthogonal oblique directions to achieve a shorter TE and an improved signal-to-noise ratio (Gradient Overplus, Philips Healthcare). The parameters derived from DWI include the *b* value (the amount of diffusion weighting) and Apparent Diffusion Coefficient (ADC, movement of water molecules within the interpulse time representing capillary and diffusion characteristics). As prostate cancer damages normal glandular structure resulting in higher cellular density, the ADC is restricted compared to healthy prostate tissue (Hoeks *et al*, 2011).

Dynamic contrast-enhanced MR imaging (DCE-MRI) was performed with application of a fast three-dimensional T1-weighted fat saturated gradient-echo sequence (THRIVE) with a section thickness 7 mm with 3.5 mm overlap covering the prostate gland and seminal vesicles with 20 sections using a field of view (FOV) of 180 mm. Three-dimensional data sets were acquired once before contrast agent administration and then after contrast agent administration at the following time intervals; 25 seconds, 50 seconds, 1 minute 15 seconds, 1 minute 35 seconds, 2 minutes, 2 minutes 45 seconds, 3 minutes 45 seconds, 4 minutes 45 seconds, 5

minutes 45 seconds, 6 minutes 45 seconds and 7 minutes 45 seconds. The MR contrast agent, Gadobutrol (Gadovist; Bayer Schering Pharma, Berlin, Germany), was injected as a bolus at 1mL per kilogram of body weight. For this purpose, a dual headed automated injection system (Spectris Solaris EP; Medrad, Pittsburgh, Pa) was used at a flow rate of 2.5mL/sec. Immediately afterwards, a 100mL saline flush was administered at a rate of 2.5 mL/sec.

On DCE-MRI, prostate cancer would classically show an earlier or faster enhancement with mainly earlier washout of contrast agent compared to normal prostate tissue as shown in figure 2-5.

Figure 2-6: DCE-MRI showing rapid increased enhancement of the right peripheral zone tumour compared to normal left peripheral zone with range of interest graph.



2.6.1 Prostate Anatomical Division

In the axial plane, the largest sections of the prostate and central gland were selected and the maximum anterior-posterior dimension (APD) and maximum transverse dimension (TD) was taken. In the coronal plane the maximum superior-inferior dimension (SID) was taken. The elliptical method was used to calculate the prostate and central gland volumes using the formula APD X TD X SID X π/6 as previously described (Al-Rimawi *et al*, 1994).

The prostate was subdivided into 4 anatomical quadrants as shown in figure 2-6 (right anterior (RA), left anterior (LA), right and left posterior (RP and LP) in the axial plane at the point of largest anterior-posterior dimension (APD).

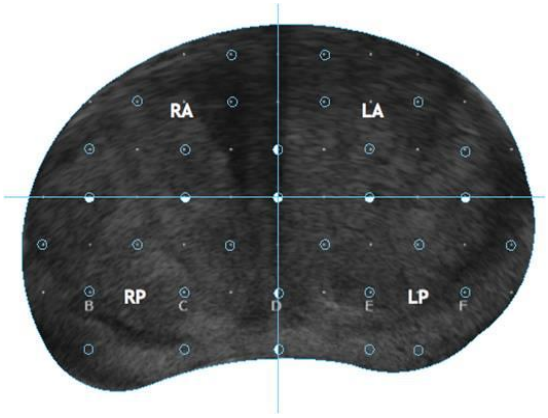


Figure 2-7: The four anatomical quadrants of the prostate

Abnormality on MRI was reported in relation to the quadrants by an expert Uro-radiologist who was blinded to the result of TTSB. Any area suspicious of tumour was localised to one or more quadrants and staged using the extent of primary tumour (T), regional lymph nodes (N) and presence of distant metastasis (M; TNM) classification (Sobin *et al*, 2009) as shown on table 2.3.

Table 2-3: The new Union for International Cancer Control (UICC) recommended TNM Classification of Prostate Cancer (Sobin *et al*, 2009).

Primary Tumour (T)
TX Primary tumour cannot be assessed
T0 No evidence of primary tumour
T1 Clinically unapparent tumour not palpable or visible by imaging
T1a Tumour incidental histologic finding in 5% or less of tissue resected
T1b Tumour incidental histologic finding in more than 5% of tissue resected
T1c Tumour identified by needle biopsy (e.g. because of elevated PSA)
T2 Tumour confined within the prostate
T2a Tumour involves one half of one lobe or less
T2b Tumour involves more than half of one lobe, but not both lobes
T2c Tumour involves both lobes
T3 Tumour extends through the prostatic capsule
T3a Extracapsular extension (unilateral or bilateral) including microscopic bladder neck involvement
T3b Tumour invades seminal vesicle(s)
T4 Tumour is fixed or invades adjacent structures other than seminal vesicles: external sphincter, rectum, levator muscles, and/or pelvic wall
Regional Lymph Nodes (N)
NX Regional lymph nodes cannot be assessed
N0 No regional lymph node metastasis
N1 Regional lymph node metastasis
Distant Metastasis (M)
MX Distant metastasis cannot be assessed
M0 No distant metastasis
M1 Distant metastasis
M1a Non-regional lymph node(s)
M1b Bone(s)
M1c Other site(s)

2.7 Grading of Prostate Cancer

Donald Gleason described the grading system for PCa as a sum of the two most common architectural pattern of tumour growth identified, ranging from 1 to 5. In their report, Gleason and Melinger demonstrated that combination of the histological grade and clinical stage was a far better prognostic marker than clinical staging alone (Gleason & Melinger, 1974; Mellinger *et al*, 1967). The Gleason score as its now called ranges from 2 to 10, with 2 being the least score and 10 representing the most aggressive cancer.

More recently, in recognition of changing trends in PCa due to increasing use of PSA screening, TRUSB with thinner cores; increased number of radical prostatectomies and widespread use of immunohistochemical staining, the International Society of Urological Pathology (ISUP) consensus conference recommended some modifications to the original Gleason grading (Epstein, 2010). The worst grade is now assigned because any amount of high-grade tumour sampled on needle biopsy most likely indicates a more significant amount of high-grade tumour within the prostate because of the correlation of grade and volume coupled with the problems inherent with needle biopsy (Epstein, 2005). Summary of the 2005 modified Gleason grading system for prostate cancer is outlined table 2-4 overleaf.

Table 2-4: Summary of the 2005 ISUP Modified Gleason System

Pattern 1: Circumscribed nodule of closely packed but separate, uniform, rounded to oval, medium-sized acini (larger glands than pattern 3)
Pattern 2: Like pattern 1, fairly circumscribed, yet at the edge of the tumour nodule there may be minimal infiltration Glands are more loosely arranged and not quite as uniform as Gleason pattern 1
Pattern 3: Discrete glandular units Typically smaller glands than seen in Gleason pattern 1 or 2 Infiltrates in and amongst non-neoplastic prostate acini Marked variation in size and shape Smoothly circumscribed small cribriform nodules of tumour
Pattern 4: Fused microacinar glands Ill-defined glands with poorly formed glandular lumina Large cribriform glands with an irregular border Hypernephromatoid
Pattern 5: Essentially no glandular differentiation, composed of solid sheets, cords, or single cells Comedocarcinoma with central necrosis surrounded by papillary, cribriform, or solid masses

2.8 Statistical Methods

Continuous numeric data in this thesis are presented with mean, median and range. Statistical analysis was performed in four parts:-

1. Majority of the pre and post-biopsy data are continuous variables. In this part of the analysis, Shapiro-Wilk test was first performed to determine normality of the sample data. This revealed that data is not normally distributed ($W = 0.629$, $P = 0.0001$). Consequently, Mann-Whitney U test was used to compare the difference between continuous and categorical variables with dichotomous outcomes (for example cancer and no cancer, retention and no retention) and Wilcoxon signed-rank test to compare two related samples for statistical difference. Furthermore, Bivariate Spearman's correlation coefficient (ρ) was used to determine correlation between two continuous variables and Chi square to assess relationship between two categorical variables, but where sample size is less than 5, Fisher's exact test was utilised instead.

2. To evaluate morbidity of TTSB including bleeding, pain and analgesic requirement, the questionnaire data was entered into an electronic spreadsheet and analysed using generalised linear model (GLM) for repeated measures since these variables were measured multiple times over a 7 day period following biopsy.

3. Clinical and TTSB variables predictive of prostate cancer diagnosis, Gleason score and cancer volume were determined by binary logistic regression analysis. Firstly, univariate analysis was applied to identify variables with a significance of $p \leq 0.05$. Variables predictive of prostate cancer diagnosis, Gleason score and cancer volume on univariate analysis were then included into multivariate analysis by

forward stepwise method. Prior to multivariate analysis, bivariate Spearman's correlation was performed to determine whether related variables are suitable for inclusion into the same multivariate model. When two related variables are highly correlated, they are not included into the same model as previously described (Mabjeesh *et al*, 2012). 'Goodness of fit' for the different models was assessed using -2 log likelihood difference and Nagelkerke's R^2. Odds ratio and 95% confidence interval were derived from regression analysis using Wald's method. A receiver operating characteristics (ROC) curve was determined by plotting sensitivity (true positive) against 1-specificity (false positive). The area under the ROC curve (AUC) was used to assess PSA, PSAD and %fPSA for their accuracy to predict for cancer diagnosis. From the coordinates points of the ROC curve, cut-offs that would detect at least 90% of cancers including their corresponding specificity (i.e. number of unnecessary biopsies that would be avoided by using the cut-offs) was derived as described by Catalona et al (Catalona *et al*, 1998).

4. Cross-tabulation was used to examine the relationship between outcome of MRI and TTSB. Using crosstab, sensitivity, specificity, positive predicted and negative predictive values were calculated.

All statistics were 2-tailed with significant difference assumed where $p < 0.05$. Analysis was performed using the IBM SPSS statistics version 20 (IBM Corporation, Armonk, New York).

Chapter 3 Results

3.1 Patient characteristics

The preoperative clinical characteristics of the 303 men who underwent modified TTSB is summarised in table 3-1.

Table 3-1: Clinical parameters of men undergoing modified TTSB

	Mean	Median	Range
Age (yrs)	63.2	64	43 – 85
Baseline PSA (ng/mL)	9.7	8.0	1.0 – 57
Pre-TTSB PSA (ng/mL)	12.3	10	2.0 – 114
%fPSA	11.9	10	1.0 – 35
PSAD (ng/mL/cm^3)	0.29	0.21	0.01 – 2.99
Prostate Volume (mL)	46.7	46	17 – 106
Prostate Length (mm)	48.5	48	30 – 90

On average, patients had undergone median of 2 (range 1 – 6) sets of negative conventional TRUSB prior to referral for TTSB. There was a statistical significant difference between patients' median baseline PSA level at their initial presentation compared to their pre-TTSB PSA levels (Wilcoxon signed-rank test, P = 0.0001). Of

the 217 men in whom detail of number of conventional biopsy cores taken prior to referral for TTSB was available, analysis revealed that they had undergone a mean of 19 (range 6 – 58) transrectal biopsy cores prior to undergoing modified TTSB.

3.2 TTSB parameters

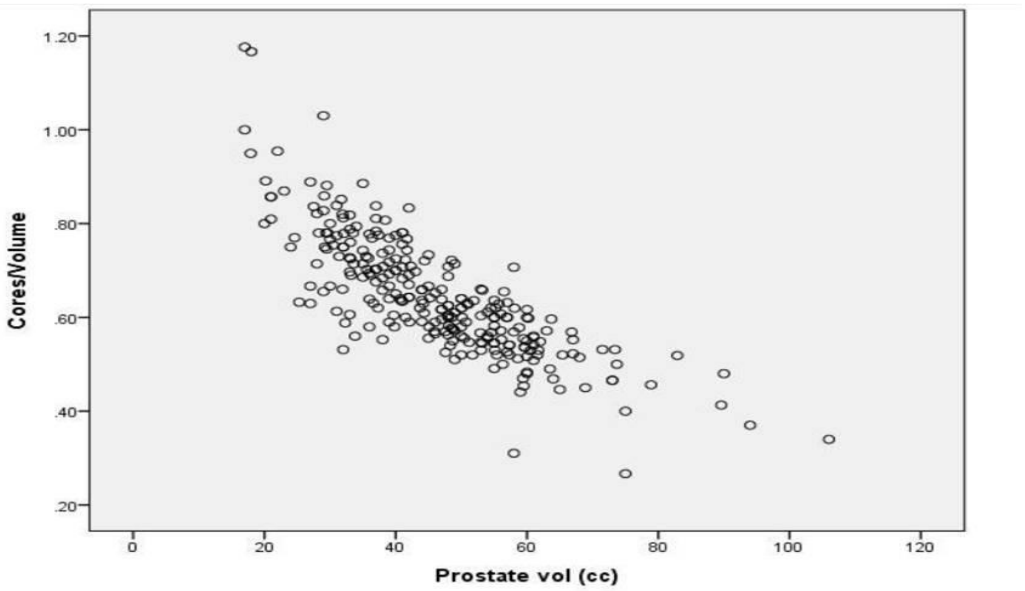
Parameters of the study population at saturation biopsy are summarised in table 3-2.

Table 3-2: TTSB Parameters of the study population

	Mean	Median	Range
Number of cores at TTSB	28	29	16 – 43
Number of rows	7	7	3 – 9
Core/volume (core/cm^3)	0.6	0.6	0.3 – 1.3
Peripheral cores	13.7	14	6 – 18
Central cores	14.4	14	6 – 25

The mean total number of central cores obtained at TTSB is comparable to the number of peripheral cores (14.4 vs. 13.7 cores). However, the sampling density (number of cores obtained at TTSB per unit prostate volume) is significantly higher in smaller prostates (Spearman coefficient $\rho = -0.84$, $P = 0.0001$, figure 3-1)

Figure 3-1: The relationship between sampling density of modified TTSB and prostate volume



3.3 Modified TTSB Outcome

3.3.1 Incidence of prostate Cancer

Prostate cancer was detected in 167 of 303 men who underwent TTSB (55.1%). Of the 66 patients who were on dutasteride, cancer was identified in 26 (39.4%) compared to 141 of 237 (59.5%) not on dutasteride. When compared with Mann-Whitney U test, men on dutasteride had significantly older (*P* = 0.001) with higher baseline PSA (*P* = 0.014), pre-TTSB PSA (*P* = 0.004). However, PSAD (0.22 vs 0.31 ng/ml/cc) and mean sampling density (0.6 vs. 0.7 core/cc) were significantly lower in patients having dutasteride than without (*P* = 0.0001 and *P* = 0.0001 respectively). However, the number of prior TRUSB and number of cores at TRUSB were not statistically different between the 2 groups (*P* = 0.529 and 0.580 respectively).

The TTSB variables of the study population stratified according to cancer positive and cancer negative cohorts are compared in table 3-3 overleaf.

Table 3-3: Clinical parameters of cancer positive and cancer negative population. *Denotes statistically significant P value of Mann-Whitney U test.

	Cancer Positive (N = 167)			Cancer Negative (N = 136)			
	Mean	Median	Range	Mean	Median	Range	*P**
Age (yrs)	64.2	64.4	43 – 85	61.9	61.7	47 - 78	0.003*
No. of TRUSB	1.8	2.0	1 – 5	1.9	2.0	1 - 6	0.511
No. Cores TRUSB	19.2	16	6 – 58	18.5	14	6 – 55	0.586
Prostate volume (cm^3)	43.1	41	17 – 94	51.2	50.4	21 – 106	0.0001*
No. of Cores at TTSB	27.3	27	17 – 41	29.6	30	16 – 43	0.0001*
Core/Volume (core/cm^3)	0.7	0.6	0.4-1.3	0.6	0.6	0.3 – 0.9	0.0001*

The number of prior negative TRUSB and total number of cores taken at TRUSB prior to undergoing TTSB were comparable between men with and without PCa. However, men diagnosed with cancer were on average older (p = 0.003), with smaller prostate (*P* = 0.0001) requiring less number of cores but with higher sampling density at modified TTSB compared to the cancer negative cohort.

3.3.2 Cancer Grade and Volume

The grade of tumour (maximum Gleason score) and volume of cancers identified is summarised in table 3-4. Cancer grade ranged from 6 to 10 with the vast majority having Gleason 7 cancer (44.3%).

Table 3-4: Outline of Gleason score and cancer volume after modified TTSB

	Count	Percent (%)
Maximum Gleason Score (MGS)		
6	50	29.9
7	76	45.5
8-10	41	24.6
Total	167	100.0
No. Positive Cores (NPC)		
1	35	21
2-5	70	41.9
>5	62	37.1
Total	167	100.0
Percentage Positive Cores (PPC, %)		
<10	56	33.5
10-49	105	62.9
≥50	6	3.6
Total	167	100.0
Maximum Percent Core (MPC, %)		
<10	21	12.6
10-30	46	27.5
>30	100	59.9
Total	167	100.0
Maximum Tumour Length (MTL, mm)		
<3	48	28.7
3-6	63	37.7
>7	56	33.6
Total	167	100.0
Aggregate Tumour Length (ATLPC, mm)		
<3	35	30
3-10	43	25.7
>10	89	44.3
Total	167	100.0

The vast majority of patients were diagnosed with Gleason 7 to 10 cancers (70.1%). The distribution of positive cores identified was most heavily weighted towards patients with greater than 2 positive cores (79%), PPC greater than 10 (66.5%) and MPC greater than 10 (87.4%). Both the maximum tumour length in any single positive core (MTL) and the aggregate tumour length from all positive cores (ATLPC) were 3mm or greater in the majority of cases (71.3% & 70% respectively).

3.3.3 Clinical significance of cancers identified

To determine the rate of insignificant cancer diagnosis at TTSB, the full saturation biopsy scheme proposed by Epstein et al (Epstein *et al*, 2005) was utilised. According to Epstein's criteria, all cancer grades 7 or greater are clinically significant. A clinically insignificant cancer was defined as having Gleason score 6 or less cancer, with 3 or less positive cores, maximum tumour length less than 4.5mm and total tumour length less than 5.5mm.

Applying Epstein's criteria to the 167 tumours detected, 117 were Gleason 7 or greater which is clinically significant. In addition, there were 50 Gleason 6 tumours of which 23 had adverse features making then clinically significant giving a total of 140 (83.8%) clinically significant tumours overall. The remaining 27 Gleason 6 tumours (16.2%) were clinically insignificant by Epstein's criteria.

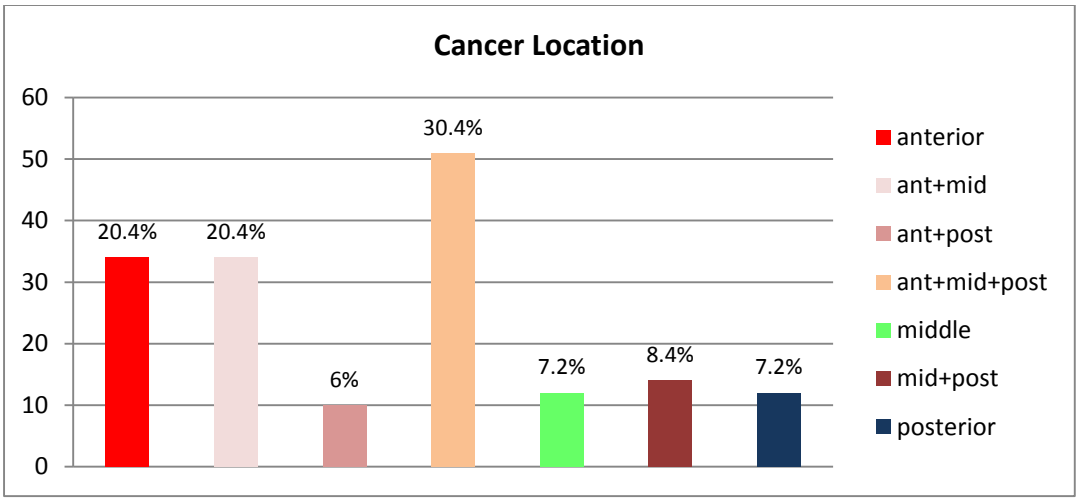
When stratified according to the number of previous TRUSB, the proportion with clinically significant cancer was 56 of 73 (76.7%) patients with 1 negative TRUSB, 57 of 65 (87.7%) after 2 negative TRUSB and 27 of 29 (93.1%) in patients after 3 or more prior negative biopsies.

3.3.4 Cancer Location

Figure 3-2 shows the anatomical distribution of tumours within the prostate which were identified at TTSB. Tumours were confined solely to the anterior, middle and posterior regions in 34 (20.4%), 12 (7.2%) and 12 (7.2%) patients respectively. However, some involvement of the anterior region was observed in 129 cases (77.2%). Nevertheless, 52% of tumour involved the posterior third of the gland.

Figure 3-2: Location of cancers detected by TTSB

Ant+mid = anterior and middle, ant+post = anterior and posterior, ant+mid+post = anterior, middle and posterior, mid+post = middle and posterior



Of the 12 posterior tumours, 10 (83.3%) were in patients who underwent TTSB after only 1 negative TRUSB. No patient with more than 2 previous negative TRUSB was diagnosed with cancer involving the posterior third. In addition, of the 34 anteriorly

located tumours, 23 (67.6%) were in patients who have had 2 or more negative TRUSB.

Table 3-5 summarises clinical variables of the patients whose tumours were confined solely to the anterior, middle and posterior thirds of the prostate. Statistical analysis by Kruskal-Wallis test showed that baseline PSA (*P* = 0.021), number of prior TRUSB (*P* = 0.009), pre-TTSB PSA (*P* = 0.023) and PSAD (*P* = 0.032) significantly differed between the three anatomical regions.

Table 3-5: Clinical characteristics of men with tumours confined to the anterior, middle and posterior regions.

Data presented as median (range). ‡Denotes statistically significant *P* value.

Unique cancer location (N = 58)

	Anterior (N=34)	**Middle (N=12)**	**Posterior (N=12)**	***P***
Age (yrs)	65 (43-85)	60 (52-66)	59 (54-71)	0.107
Baseline PSA	8 (1-57)	6.5 (5-22)	6 (4-12)	0.021‡
No. of TRUSB	2 (1-4)	1 (1-3)	1 (1-2)	0.009‡
Pre-TTSB PSA	10 (3-57)	8 (3-14)	7 (3-14)	0.023‡
Prostate volume	44 (18-94)	55 (33-65)	45 (28-56)	0.052
PSAD	0.26 (0.07-1.5)	0.16 (0.04-0.33)	0.14 (0.1-0.46)	0.032‡
%fPSA	9 (2-24)	14 (6-21)	12 (4-27)	0.428

There is a trend to smaller prostate volume in anterior tumours but this did not reach statistical significance (*P* = 0.052). However, the distribution of age (*P* = 0.107), number of cores at TRUSB (*P* = 0.144), %fPSA (*P* = 0.428) and number of

cores at TTSB (P = 0.273) were not significantly different. Similarly, pathological findings including distribution of number of positive cores (P = 0.216), percent positive cores (P = 0.115), maximum percent core involvement (P = 0.487), aggregate of tumour lengths (P = 0.206), maximum tumour length (P = 0.313) and maximum Gleason score (P = 0.346) were not significantly different between the unique tumour locations.

3.4 Morbidity of TTSB

3.4.1 Acute Urinary Retention (AUR)

Following TTSB, 23 of 303 men developed AUR (7.6%). Table 3-6 compares the clinical parameters of patients with or without retention.

Table 3-6: Comparison of TTSB variables for retention and no retention groups

*Denotes significant difference by Mann-Whitney U test.

	Retention (n = 23)			**No Retention (n = 280)**			
	Mean	Median	Range	Mean	Median	Range	P*
Age (yrs)	64.6	66	43 –78	63	63.9	43 – 85	0.89
Baseline PSA (ng/ml)	12.5	8.5	5 – 57	9.5	7	1 - 47	0.129
No. of TRUSB	1.5	1	1–3	1.9	2	1 – 6	0.093
No of Core TRUSB	15.7	12	12–28	19	16	6 – 58	0.444
Pre-TTSB PSA (ng/ml)	11.3	8.5	3 – 57	12.4	10	2 – 114	0.201
PSAD ($ng/mL/cm^3$)	0.24	0.15	0.04–1.5	0.3	0.21	0.01 – 2.99	0.059
Prostate volume (cc)	56.3	57.9	31 – 90	45.9	45	17 – 106	0.002*
Sampling density ($core/cm^3$)	0.6	0.5	0.4–0.8	0.7	0.6	0.3 – 1.25	0.001*

See page 85 for definition of sampling density

There was no statistical significant difference between retention and no retention group for age, baseline PSA prior to their initial TRUSB, number of prior negative TRUSB, number of cores taken at previous TRUSB, pre-TTSB PSA or PSAD. However, prostate volume and sampling density significantly differed between the 2 groups. Of the 66 patients on dutasteride, 9 (13.6%) developed AUR compared to 14 of 237 (5.9%) in patients not on dutasteride and this was statistically significant (Chi-Square, *P* = 0.036). Univariate and multivariate logistic regression analysis was performed to determine which clinical and TTSB variable predicted occurrence of AUR following TTSB (table 3-7).

Table 3-7: Predictors of AUR by binary logistic regression analysis

Parameters	**Univariate**	**Multivariate**
Age	0.263	
Baseline PSA	0.070	
No. of TRUSB	0.076	
No. of cores at TRUSB	0.155	
Pre-TTSB PSA	0.684	
PSAD	0.456	
Prostate volume	0.001	**0.004**
No. of cores at TTSB	0.079	
No. of Rows at TTSB	0.042	0.862
Core/volume	0.005	0.459
Operation time	0.905	
Dutasteride therapy	0.041	0.457

On multivariate analysis and controlling for the effect of dutasteride, larger prostate volume was the only predictor of AUR (P = 0.004).

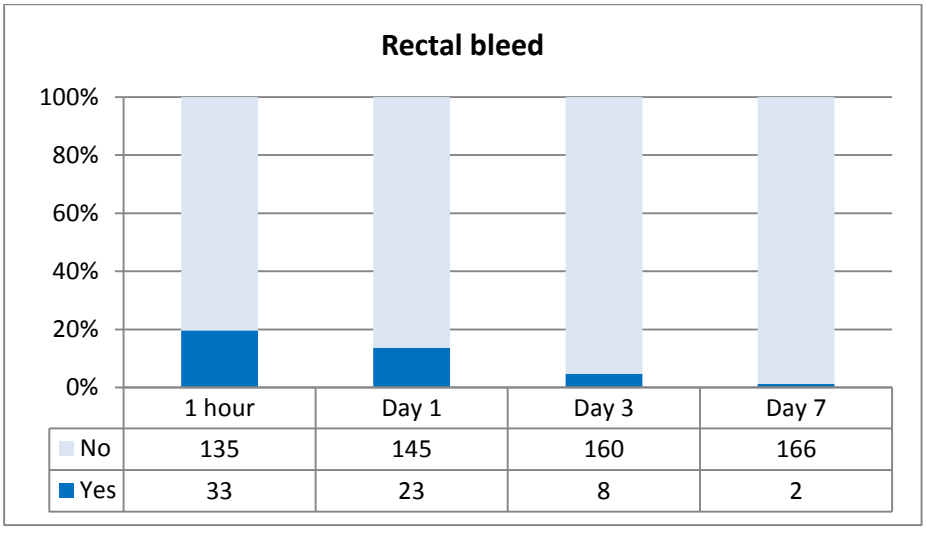
3.4.2 Bleeding, Pain and Analgesic requirement after modified TTSB

168 of 303 patients responded to the questionnaire, a response rate of 55.4%.

3.4.2.1 Bleeding

The incidence of the three categories of post TTSB bleeding (rectal bleed, haematuria and haematospermia) evaluated are summarised in figures 3-3, 3-4 and 3-5. Assessment of haematospermia was undertaken from day 1 post TTSB as obviously, no patient would have experienced this event in the hour following biopsy.

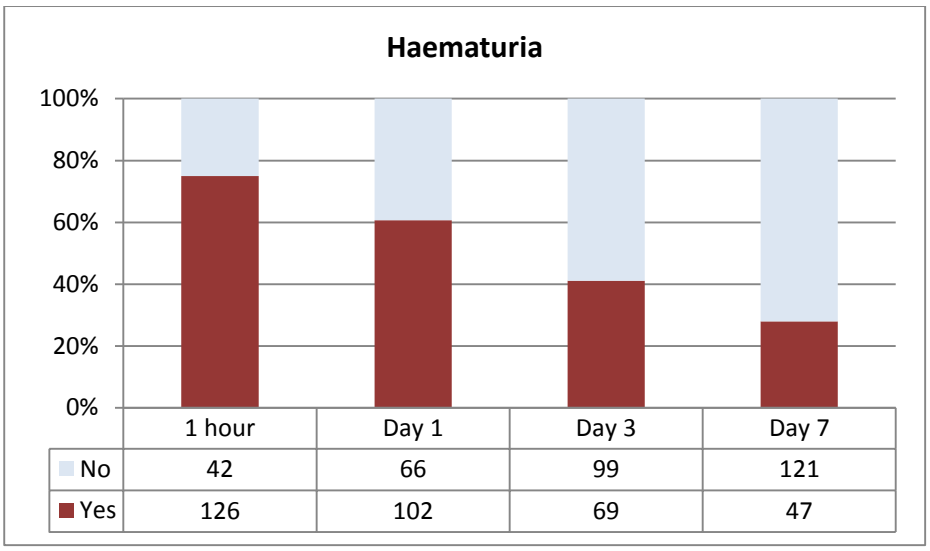
Figure 3-3: Incidence of rectal bleed after TTSB



Following TTSB, 19.6% experienced rectal bleed within the 1st hour whilst 13.7%, 4.8% and 1.2% reported minor, self limiting rectal bleed on days 1, 3 and 7 after

TTSB. Analysis of the within-subject contrasts by generalised linear model for repeated measure showed a statistically significant decrease in rectal bleed with time (*P* = 0.0001).

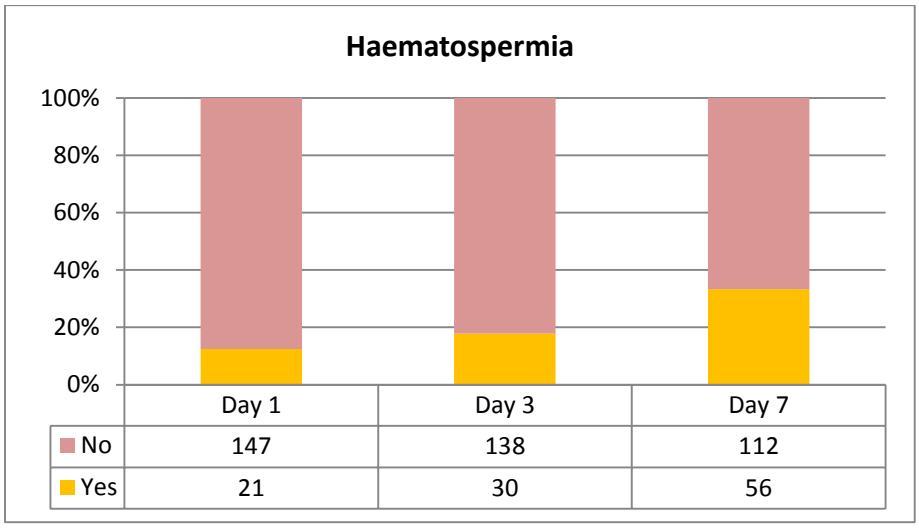
Figure 3-4: Incidence of haematuria after TTSB



Similarly and as expected, the vast majority of the patients (75%) experienced minor and self limiting haematuria following TTSB which significantly decreased to 60.7%, 41.1% and 28% on days 1, 3 and 7 respectively (*P* = 0.0001).

On the contrary, the incidence of haematospermia shown in figure 3-5 increased over time. Following TTSB, 12.5%, 17.9% and 33.3% reported haematospermia on days 1, 3 and 7 respectively which was statistically significant (*P* = 0.0001).

Figure 3-5: Incidence of haematospermia after TTSB



3.4.2.2 Pain

The incidences of the two domains of pain examined (perineal and rectal) are shown in figures 3-6 and 3-7 whilst figure 3-9 shows the overall mean pain score of the study respondents.

Figure 3-6: Incidence of perineal pain after modified TTSB

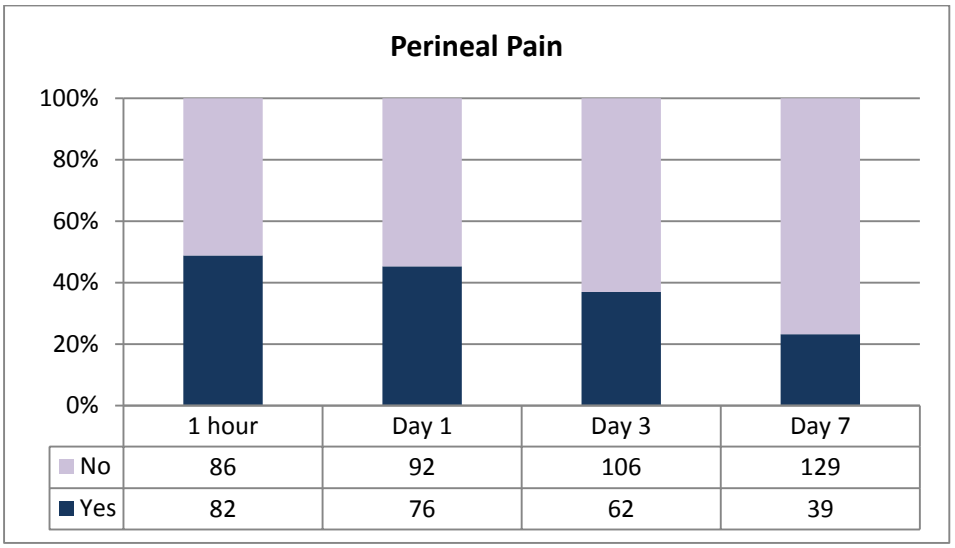
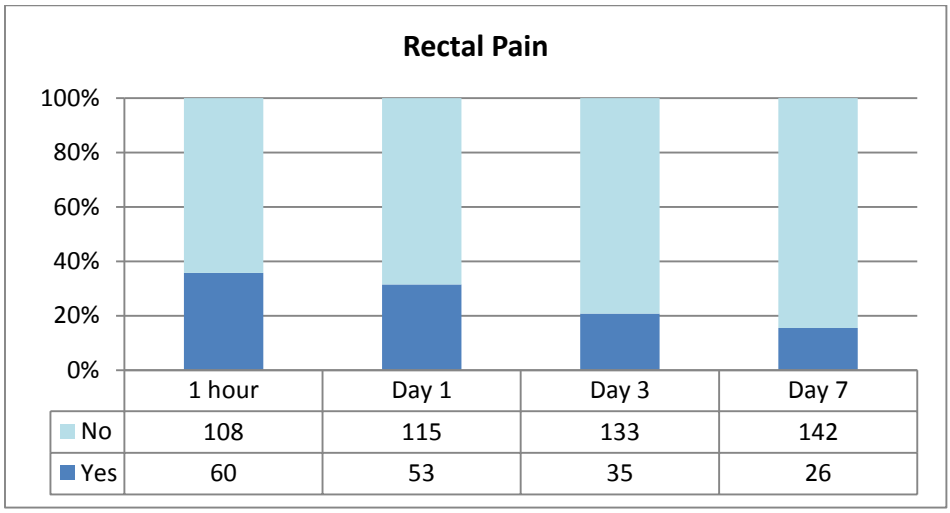


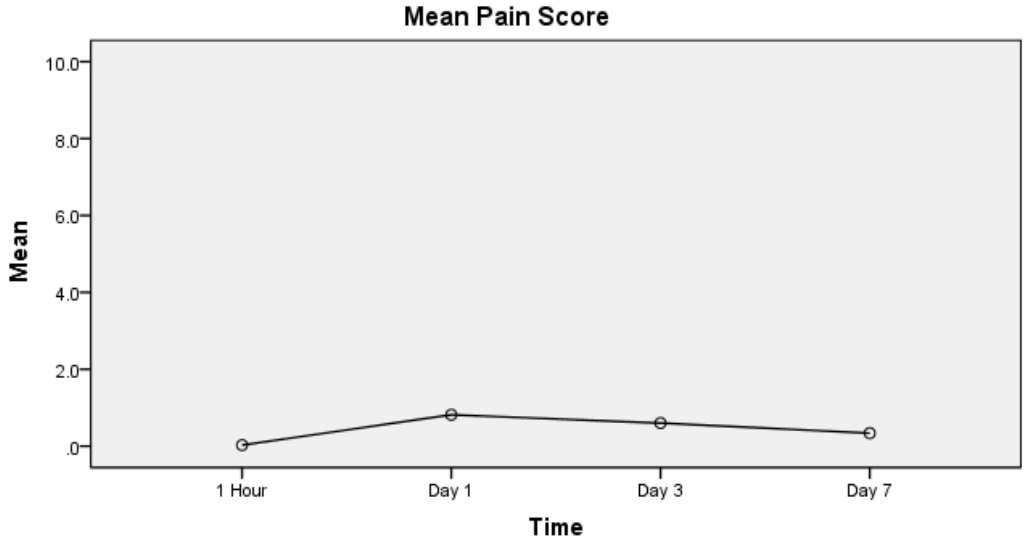
Figure 3-7: Incidence of rectal pain after modified TTSB



The commonest location of patient reported pain immediately following TTSB is perineal (48.8% at 1 hour) compared to 35.7% reporting rectal pain at the same period. On days 1, 3 and 7 following TTSB, 45.2%, 36.9% and 23.2% reported perineal pain compared to 31.5%, 20.8% and 15.5% for rectal pain over the same period. Analysis using General Linear Model (GLM) for repeated measures showed a statistically significant reduction in perineal and rectal pain over time (P = 0.0001 and P = 0.0001 respectively).

Figure 3-8 shows the overall pain scores reported by patients following TTSB. Pain following TTSB rose from 0.03 1hr post-biopsy and peaked on day 1 with a mean score of 0.8 out of 10 on the visual analogue scale. Subsequently, there was a significant reduction in pain experience to 0.6 and 0.3 on days 3 and 7 respectively (P=0.0001).

Figure 3-8: Overall mean pain score after modified TTSB



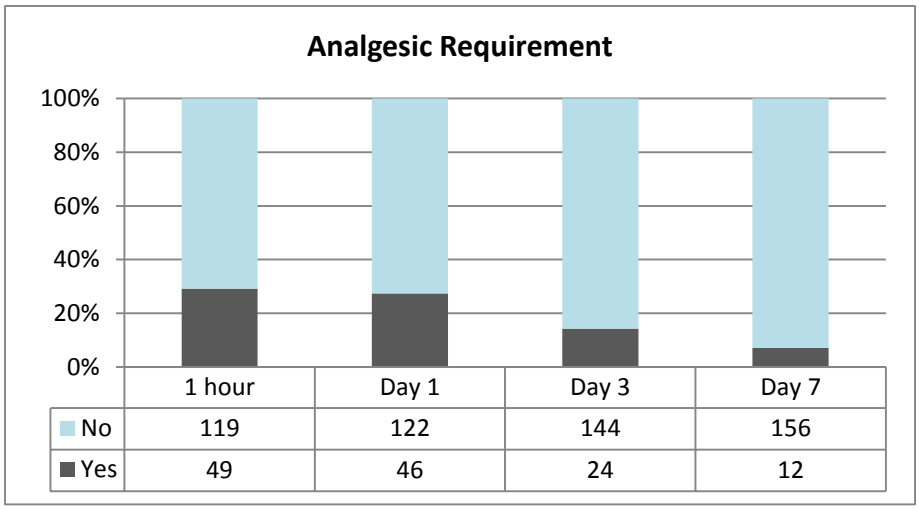
Analysis of the within-subject contrast using General Linear Model (GLM) for repeated measures showed a statistically significant increase in pain score between 1 hour and day 1 post TTSB (*P* = 0001). However, after the peak of pain on day 1, there was statistically significant reduction in pain between days 1 and 3 (*P* = 0.003) and between days 3 and 7 (*P* = 0.002).

3.4.2.3 Analgesic requirement

The numbers of patients requiring analgesics are shown in figure 3-9. When required, pain relief was satisfactorily achieved with simple analgesics including paracetamol in 25 patients (14.9%), codeine and paracetamol in 11 (6.5%) and ibuprofen in 5 (3%). The vast majority did not require any analgesia after TTSB. One

patient required morphine for pain control 1 hour after TTSB but was discharged successfully on simple analgesia afterwards (figure 3-9).

Figure 3-9: Analgesic requirements after modified TTSB



Overall, analysis using General Linear Model (GLM) for repeated measures showed that analgesic requirement reduced significantly over time following TTSB (*P* = 0.0001).

3.5 Evaluation of Biochemical Predictors of PCa Diagnosis and Adverse Pathological Outcome in patients Undergoing Modified TTSB

3.5.1 Description of study population

Of the 303 patients who underwent modified TTSB, 245 had complete information regarding %fPSA. Nevertheless, there was no statistical significant difference between the 58 patients without %fPSA and the 245 patients with complete data for age, baseline PSA, number of TRUSB, total number of TRUSB, prostate volume, number of cores at TTSB and sampling density between (table 3-8).

Table 3-8: Comparison of clinical parameters of patients with and without %fPSA information. *Denotes Mann-Whitney U test

	%fPSA Available			**%fPSA Not available**			
	Mean	Median	Range	Mean	Median	Range	P*
Age	62.9	63.1	43 – 85	64.3	65	43 – 78	0.055
Baseline PSA	9.6	8.0	1 – 47	9.9	7.0	1 – 57	0.075
No. of TRUSB	1.8	2	1 – 6	2	2	1 – 5	0.125
No. of core TRUSB	18.7	12.5	6 – 58	20	18	8 – 55	0.365
Prostate Volume	46.9	46	17 – 106	45.9	46	18 – 76	0.532
No. of core TTSB	28.6	29	16 – 43	27.2	27	17 – 38	0.086
Core/Volume	0.6	0.6	0.3 – 1.25	0.6	0.6	0.3 – 1.0	0.934

Of the 245 men with complete %fPSA data, cancer was diagnosed in 133 (54.3%). Table 3-9 shows the pre-TTSB total PSA and its derivatives stratified according to cancer positive and cancer negative cohorts.

Table 3-9: Total PSA, Percent free PSA (%fPSA) and PSA density (PSAD) of cancer positive and cancer negative groups. *Denotes Mann-Whitney U test

Total Patients (N = 245)

	Cancer Positive (N = 133)			**Cancer Negative (N = 112)**			
	Mean	Median	Range	Mean	Median	Range	*P**
Baseline PSA (ng/ml)	10.4	8	1 - 39	8.7	7	2 - 47	0.011
Pre-TTSB PSA (ng/ml)	15	11	2 - 114	8.2	8	2 - 22	0.0001
%fPSA	10.2	9	2 - 35	14	13	1 - 32	0.0001
PSAD (ng/ml/cc)	0.38	0.27	0.04 – 2.99	0.16	0.15	0.01 – 0.38	0.0001

Both total serum PSA values (Baseline and pre-TTSB PSA) and their derivatives including %fPSA and PSAD showed statistically significant difference between cancer positive and cancer negative patients. Similar to findings from analysis of the overall cohort (table 3-3), there was statistical significant difference for age (*P* = 0.002), prostate volume (*P* = 0.0001), number of cores at TTSB (*P* = 0.0001) and sampling density (*P* = 0.001) between cancer positive and cancer negative groups for the cohort of 245 men with complete %fPSA and PSAD information. The

distribution of number of TRUSB and number of cores taken at TRUSB was similar between the two groups (*P* = 0.338 and 0.656 respectively).

3.5.2 Histopathological outcome of the cancer positive cases

Detail of the grade and cancer volume parameters of the 133 positive cases are outlined in table 3-10. The vast majority of patients were diagnosed with Gleason 7 to 10 (70.7%) with a mean NPC of 5, PPC 19, MPC 49.6, MTL 5.5mm and ATLPC 21.2mm.

Table 3-10: Summary of cancer grade and volume

	Cancer Positive (N = 133)		
	Mean	Median	Range
Maximum Gleason Score	7	7	6 - 10
NPC	5	4	1 – 17
PPC (%)	19	15.6	2 – 67
MPC (%)	49.6	50	1 – 100
MTL (mm)	5.5	4.2	1 – 16
ATLPC (mm)	21.2	12.3	1 - 145

3.5.3 Predictors of cancer diagnosis, grade and volume

3.5.3.1 Predictors of cancer diagnosis

3.5.3.1.1 Univariate Analysis

Univariate analysis was first performed in order to identify variables which are predictive for cancer diagnosis at $P < 0.05$ (table 3-11).

Table 3-11: Univariate predictors of cancer diagnosis

	Univariate *P*
Age	0.001
Baseline PSA	0.046
No. of TRUSB	0.279
No. Of cores TRUSB	0.418
Pre-TTSB PSA	0.0001
%fPSA	0.0001
PSAD	0.0001
Prostate volume	0.0001
No. of cores at TTSB	0.0001
Core/volume	0.0001

The vast majority of the pre-TTSB clinical parameters of the study population including age, baseline PSA, pre-TTSB PSA, %fPSA, PSAD, prostate volume, number

of cores at TTSB and sampling density were statistically significant predictors of cancer diagnosis on univariate analysis except for number of previous TRUSB and number of prior TRUSB cores (*P* = 0.279 and 0.418 respectively)

3.5.3.1.2 Multivariate analysis for prediction of cancer diagnosis

Variables which were significant on univariate analysis were entered included in a multivariate binary logistic regression analysis by forward stepwise method. Bivariate Spearman's correlation was performed to determine the suitability of related variables for inclusion into the same multivariate model. This identified that pre-TTSB PSA and PSAD were highly correlated (Spearman's correlation coefficient $\rho = 0.9$, $P = 0.0001$). Consequently, two separate models were constructed in order to avoid inclusion of PSAD and pre-TTSB PSA into the same model (tables 3-12). The fitness of the two models thus constructed was assessed using -2 log likelihood difference and Nagelkerke's R^2. More importantly, the models were controlled for the effect of dutasteride.

Table 3-12: Two models predicting cancer diagnosis by multiple binary logistic regressions.

Exp B: odd ratio, CI: confidence interval

‡Denotes significant *P* value

Model 1 (Including Pre-TTSB PSA)

-2 Log likelihood - 240.7
Nagelkerke's R^2 - 0.353

	Exp B	95%CI	*P*‡
Age	1.06	1.01 – 1.12	0.032‡
Baseline PSA	0.99	0.92 – 1.05	0.638
%fPSA	0.93	0.88 – 0.99	0.021‡
Prostate volume	0.96	0.94 – 0.98	0.001‡
No. of cores TTSB	0.91	0.85 – 0.98	0.107
Core/volume	1.30	0.97 – 1.74	0.081
Dutasteride	1.66	0.74 – 3.71	0.218
Pre-TTSB PSA	1.12	1.04 – 1.20	0.002‡

Model 2 (Including PSAD)

-2 Log likelihood - 239.8
Nagelkerke's R^2 - 0.362

	Exp B	95%CI	*P*
Age	1.05	1.00 – 1.11	0.046‡
Baseline PSA	0.98	0.92 – 1.04	0.514
%fPSA	0.94	0.88 – 0.99	0.026‡
Prostate volume	1.06	0.97 – 1.17	0.198
No. of cores	0.87	0.73 – 1.03	0.111
TTSB			
Core/volume	1.60	0.73 – 3.50	0.243
Dutasteride	1.47	0.64 – 3.38	0.365
PSAD	3.69	1.79 – 7.61	0.0001‡

Multivariate regression analysis revealed that age, %fPSA, smaller prostate volume and pre-TTSB PSA were significant predictors of cancer diagnosis at TTSB in model 1 whilst age, %fPSA and PSAD were significantly predicted cancer diagnosis in model 2. PSAD was more predictive of cancer diagnosis (OR, 3.7, 95% confidence interval [CI], 1.79-7.61) and contributed significantly more than %fPSA (OR, 0.9, 95% CI 0.88-0.99), age (OR 1.1, 95% CI 1.00-1.11) and pre-TTSB PSA (OR 1.1, 95% CI 1.04-1.20).

3.5.3.2 Predictors of tumour grade and volume

3.5.3.2.1 Univariate analysis

Based on Epstein's definition of significant cancer at saturation biopsy, biopsy cancer grade and volume parameters were divided into two categorical groups of variables including NPC less than 4 and 4 or greater, MTL less than 4.5mm and 4.5mm or greater, ATLPC less than 5.5mm and 5.5mm or greater and maximum Gleason score (MGS) less than 7 and 7 or greater, MPC less than 50% and 50% or greater and PPC less than 15 and 15 or greater according to the median value.

Using the categorical groups thus created as the dependent variables, univariate analysis was performed on clinical and biopsy parameters including age, baseline PSA, number of TRUSB, number of cores taken at TRUSB, pre-TTSB PSA, %fPSA, PSAD, prostate volume, number of TTSB cores and sampling density in order to determine predictors of significant cancer (MGS ≥7, NPC ≥4, MTL ≥4.5mm, ATLPC ≥5.5mm, MPC ≥50% and PPC ≥15%) at TTSB (table 3-13 overleaf).

Variables which were statistically significant at $P<0.05$ on univariate analysis for each of the categorical groups were then entered into the multivariate analysis.

Table 3-13: Univariate predictors of cancer grade and volume

P <0.05 is statistically significant

	Univariate Analysis																	
Median	**MGS**			**NPC**			**MTL (mm)**			**ATLPC (mm)**			**MPC (%)**			**PPC (%)**		
	<7	≥7	*P*	<4	≥4	*P*	<4.5	≥4.5	*P*	<5.5	≥5.5	*P*	<50	≥50	*P*	<15	≥15	*P*
Age	63.2	65.0	0.012	63.8	65.1	0.007	63.0	66.4	0.0001	62.7	65.0	0.003	63.5	65.0	0.009	63.0	65.6	0.001
Baseline PSA	7	9	0.001	7	9	0.002	7	9	0.0001	6	9	0.001	7.5	9.0	0.015	7	9	0.002
No. of TRUSB	1	2	**0.214**	2	2	**0.084**	2	2	**0.223**	1	2	0.042	1	2	0.016	2	2	**0.336**
No. of cores TRUSB	12	18	**0.175**	12	19	0.023	14	18	0.545	12	18	**0.123**	12	18	**0.136**	13	18	**0.192**
Pre-TTSB PSA	9.0	13.0	0.001	9	14	0.0001	9	14	0.0001	9	12	0.0001	9.0	13.5	0.0001	9	13	0.0001
%fPSA	12	8	0.010	11.5	7.5	0.001	11	7.5	0.005	13	8	0.0001	11	7	0.0001	11.0	7.5	0.004
PSAD	0.19	0.33	0.0001	0.19	0.40	0.0001	0.19	0.37	0.0001	0.18	0.35	0.0001	0.19	0.39	0.0001	0.19	0.40	0.0001
Prostate volume	46.7	39.8	0.041	45.8	38.1	0.007	44	39	**0.076**	49	38	0.0001	47.6	38.0	0.001	45.8	38.1	0.003
No. of cores at TTSB	29	26	0.046	28	26	0.024	28	26	**0.056**	29	26	0.0001	29	26	0.003	28	26	0.002
Core/volume	0.6	0.7	**0.101**	0.6	0.7	0.022	0.7	0.6	**0.281**	0.6	0.7	0.008	0.6	0.7	0.021	0.6	0.7	0.048

3.5.3.2.2 Multivariate Analysis for prediction of tumour volume and grade

Tables 3-14 to 3-19 summarises results of the two logistic regression models created. Multivariate binary logistic regression analysis by forward stepwise method was performed to determine predictors of Gleason score and biopsy tumour volume. As previously described, pre-TTSB PSA and PSAD were not included in the same model because they are highly correlated.

Table 3-14: Multivariate analysis for prediction of Gleason score.

‡Denotes significant *P* value

Model 1

-2 Log likelihood - 129.4
Nagelkerke's R^2 - 0.229

	Exp B	95%CI	*P*‡
Age	1.05	0.96 – 1.16	0.295
Baseline PSA	1.05	0.90 – 1.23	0.549
%fPSA	0.97	0.87 – 1.09	0.625
Prostate volume	0.97	0.94 – 1.01	0.106
No. of cores TTSB	0.96	0.77 – 1.20	0.734
Dutasteride	1.64	0.36 – 7.38	0.522
Pre-TTSB PSA	1.16	1.06 – 1.26	0.001‡

Model 2

-2 Log likelihood	**- 130.4**		
Nagelkerke's R^2	**- 0.219**		
	Exp B	95%CI	*P*‡
Age	1.06	0.97 – 1.16	0.221
Baseline PSA	1.07	0.92 – 1.25	0.395
%fPSA	0.98	0.87 – 1.09	0.662
Prostate volume	0.98	0.87 – 1.10	0.697
No. of cores TTSB	1.01	0.80 – 1.27	0.927
Dutasteride	1.94	0.45 – 8.31	0.370
PSAD	2.69	1.55 – 4.67	0.0001‡

Controlling for the effect of dutasteride, the two models constructed were equally fitted to predict Gleason score ≥7 as demonstrated by their comparable -2 log likelihood difference and Nagelkerke's R2 values. Multivariate analysis showed that pre-TTSB PSA and PSAD were the only significant predictors of Gleason score ≥7 in models 1 and 2 respectively (*P* = 0.001 and 0.0001).

Table 3-15: Multivariate analysis for prediction of number of positive cores (NPC)

‡Denotes significant *P* value

Model 1

-2 Log likelihood - 112.3
Nagelkerke's R^2 - 0.270

	Exp B	95%CI	*P*‡
Age	1.04	0.95 – 1.13	0.392
Baseline PSA	0.91	0.78 – 1.06	0.224
No. of cores TRUSB	1.07	0.96 – 1.19	0.200
%fPSA	0.92	0.83 – 1.02	0.124
Prostate volume	1.02	0.89 – 1.16	0.829
No. of cores TTSB	0.90	0.82 – 0.98	0.020‡
Core/volume	1.25	0.56 – 2.80	0.586
Dutasteride	0.67	0.16 – 2.80	0.584
Pre-TTSB PSA	1.10	1.04 – 1.17	0.001‡

Model 2

-2 Log likelihood - 112.3
Nagelkerke's R^2 - 0.270

	Exp B	95%CI	*P*‡
Age	1.06	0.96 – 1.14	0.304
Baseline PSA	1.07	0.80 – 1.05	0.228
No. of cores TRUSB	0.99	0.97 – 1.21	0.139
%fPSA	0.98	0.84 – 1.04	0.189
Prostate volume	0.98	0.87 – 1.14	0.974
No. of cores TTSB	1.01	0.76 – 1.26	0.879
Core/volume	0.97	0.42 – 2.23	0.945
Dutasteride	1.94	0.17 – 2.74	0.587
PSAD	2.44	1.54 – 3.85	0.0001‡

The two models constructed were equally fitted to predict NPC ≥4 positive cores as demonstrated by the equal values of their respective goodness of fit parameters (-2 log likelihood difference and Nagelkerke's R2). Multivariate analysis showed that pre-TTSB PSA and number of cores at TTSB significantly predicted NPC ≥4 in model 1 (*P* = 0.020 and 0.001 respectively), whilst PSAD was the only significant predictor in model 2 (*P* = 0.0001).

Table 3-16: Multivariate analysis for prediction of Maximum tumour length (MTL)

‡Denotes significant *P* value

Model 1

-2 Log likelihood - 106.4
Nagelkerke's R^2 - 0.345

	Exp B	95%CI	*P*‡
Age	1.09	1.01 – 1.18	0.028‡
Baseline PSA	1.01	0.87 – 1.16	0.930
No. of cores	0.99	0.89 – 1.09	0.769
TRUSB			
%fPSA	1.00	0.89 – 1.11	0.928
Dutasteride	1.20	0.28 – 5.08	0.806
Pre-TTSB PSA	1.11	1.04 – 1.19	0.001‡

Model 2

-2 Log likelihood - 103.2
Nagelkerke's R^2 - 0.376

	Exp B	95%CI	*P*‡
Age	1.10	1.02 – 1.19	0.012‡
Baseline PSA	1.01	0.89 – 1.15	0.892
No. of cores TRUSB	0.99	0.90 – 1.10	0.892
%fPSA	1.00	0.90 – 1.12	0.988
Dutasteride	1.25	0.31 – 5.09	0.760
PSAD	2.34	1.51 – 3.63	0.0001‡

In the two equally fitted models constructed, age and pre-TTSB PSA significantly predicted MTL ≥4.5mm in model 1 (*P* = 0.028 and 0.001 respectively) whilst age and PSAD were significant predictors in model 2 (*P* = 0.013 and 0.0001 respectively).

Table 3-17: Multivariate analysis for prediction of aggregate tumour lengths from positive cores (ATLPC)

‡Denotes significant *P* value

Model 1

-2 Log likelihood - 131.8
Nagelkerke's R^2 - 0.265

	Exp B	95%CI	*P*‡
Age	1.01	1.00 – 1.14	0.047‡
Baseline PSA	0.99	0.86 – 1.14	0.877
No. of TRUSB	0.92	0.22 – 3.90	0.906
%fPSA	0.93	0.86 – 1.02	0.104
Prostate volume	0.95	0.92 – 0.98	0.002‡
No. of cores TTSB	0.93	0.72 – 1.20	0.054
Core/volume	1.11	0.54 – 2.32	0.773
Dutasteride	0.65	0.14 – 3.02	0.583
Pre-TTSB PSA	1.01	1.02 – 1.16	0.015‡

Model 2

-2 Log likelihood - 135.3 **Nagelkerke's R^2 - 0.233**			
	Exp B	95%CI	*P*‡
Age	1.08	1.01 – 1.17	0.036‡
Baseline PSA	1.00	0.87 – 1.14	0.947
No. of TRUSB	0.87	0.20 – 3.83	0.854
%fPSA	0.94	0.86 – 1.02	0.142
Prostate volume	0.97	0.93 – 1.00	0.062
No. of cores TTSB	0.96	0.75 – 1.25	0.782
Core/volume	1.02	0.48 – 2.19	0.956
Dutasteride	0.69	0.15 – 3.08	0.626
PSAD	2.00	1.16 – 3.33	0.012‡

On multivariate analysis controlling for effect of dutasteride therapy, age, prostate volume and pre-TTSB PSA were significant predictors of aggregate tumour lengths from positive cores (ATLPC) ≥5.5mm in model 1 (*P* = 0. 047, 0.002 and 0.015) whilst patients age and PSAD significantly predicted ATLPC ≥5.5mm in model 2 (*P* = 0.036 and 0.012 respectively).

Table 3-18: Multivariate analysis for prediction of maximum percent core involvement (MPC)

‡Denotes significant *P* value

Model 1

-2 Log likelihood - 139.1
Nagelkerke's R^2 - 0.297

	Exp B	95%CI	*P*‡
Age	1.04	0.95 – 1.13	0.03
Baseline PSA	0.99	0.85 – 1.14	0.947
No. of TRUSB	1.22	0.32 – 4.65	0.854
%fPSA	0.96	0.86 – 1.07	0.023‡
Prostate volume	0.99	0.86 – 1.14	0.743
No. of cores TTSB	0.89	0.81 – 0.97	0.008‡
Core/volume	1.30	0.63 – 2.69	0.754
Dutasteride	1.36	0.31 – 5.91	0.626
Pre-TTSB PSA	1.13	1.06 – 1.20	0.0001‡

Model 2

-2 Log likelihood - 140.2
Nagelkerke's R^2 - 0.288

	Exp B	95%CI	*P*‡
Age	1.05	0.96 – 1.14	0.293
Baseline PSA	1.00	0.88 – 1.15	0.959
No. of TRUSB	1.20	0.31 – 4.65	0.789
%fPSA	0.97	0.87 – 1.08	0.557
Prostate volume	0.98	0.86 – 1.13	0.817
No. of cores TTSB	0.98	0.77 – 1.26	0.879
Core/volume	1.06	0.51 – 2.20	0.880
Dutasteride	1.43	0.34 – 6.02	0.624
PSAD	2.70	1.71 – 4.27	0.0001‡

In the two equally fitted models constructed, number of cores obtained at TTSB, %fPSA, number of cores at TTSB and pre-TTSB PSA significantly predicted maximum percent core involvement (MPC) ≥50% in model 1 (*P* = 0.023, 0.008 and 0.0001 respectively). In model 2 however, PSAD was the only significant predictor of MPC ≥50% (*P* = 0.0001).

Table 3-19: Multivariate analysis for prediction of percentage of positive cores (PPC).

‡Denotes significant *P* value

Model 1

-2 Log likelihood - 111.9
Nagelkerke's R^2 - 0.274

	Exp B	95%CI	*P*‡
Age	1.06	0.99 – 1.14	0.109
Baseline PSA	0.90	0.77 – 1.05	0.166
%fPSA	0.92	0.86 – 0.99	0.278
Prostate volume	1.02	0.89 – 1.17	0.771
No. of cores TTSB	0.90	0.82 – 0.98	0.012‡
Core/volume	1.15	0.83 – 1.59	0.731
Dutasteride	0.44	0.10 – 1.92	0.275
Pre-TTSB PSA	1.10	1.04 – 1.17	0.001‡

Model 2

-2 Log likelihood	**- 110.7**		
Nagelkerke's R^2	**- 0.288**		
	Exp B	95%CI	*P*‡
Age	1.07	1.00 – 1.15	0.055
Baseline PSA	0.92	0.80 – 1.05	0.203
%fPSA	0.92	0.86 – 0.99	0.260
Prostate volume	1.00	0.87 – 1.14	0.959
No. of cores TTSB	0.95	0.84 – 1.06	0.345
Core/volume	0.84	0.55 – 1.29	0.424
Dutasteride	0.48	0.12 – 1.97	0.307
PSAD	2.10	1.37 – 3.24	0.001‡

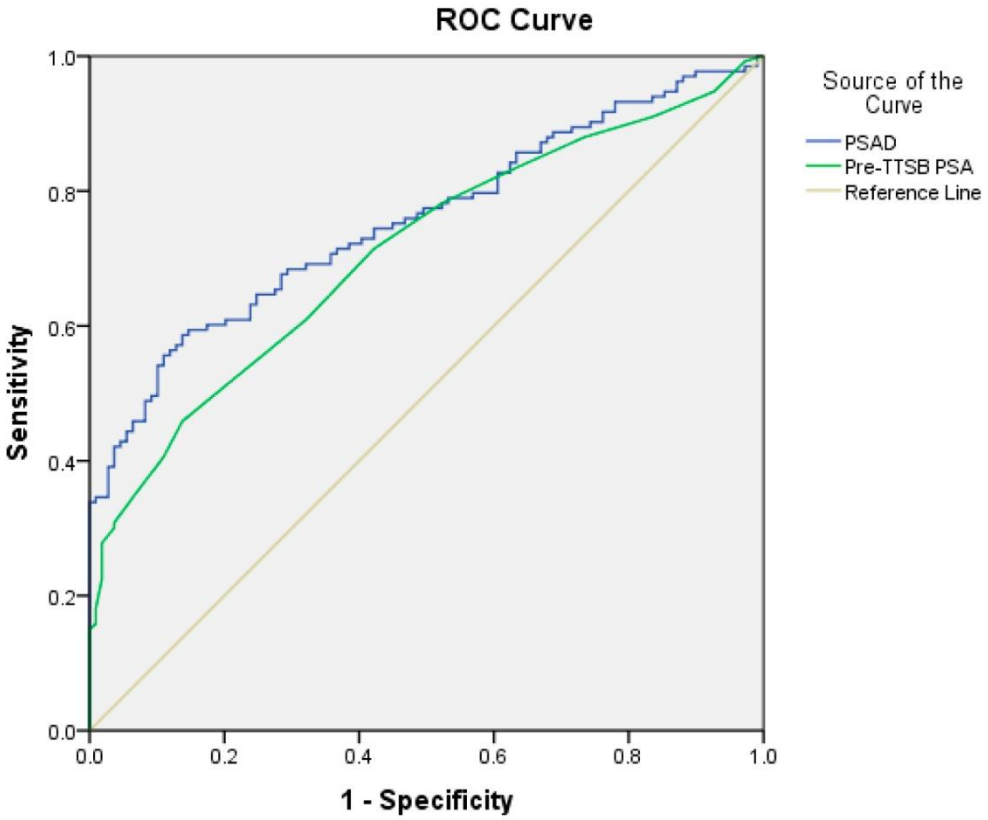
The two multivariate regression models constructed were equally fitted to predict percentage of positive cores (PPC) ≥15% as shown by the equal values of their respective goodness of fit parameters (-2 log likelihood difference and Nagelkerke's R2). Number of cores at TTSB and pre-TTSB PSA significantly predicted PPC ≥15% in models 1 whilst only PSAD was predictive in model 2.

Figure 3-10 shows the ROC curve of PSA Density (PSAD), pre-TTSB PSA and percentage of free PSA (%fPSA) for prostate cancer diagnosis. The PSAD (AUC 0.76; 95% CI, 0.70 – 0.82, P=0.0001) was more predictive of cancer than pre-TTSB PSA

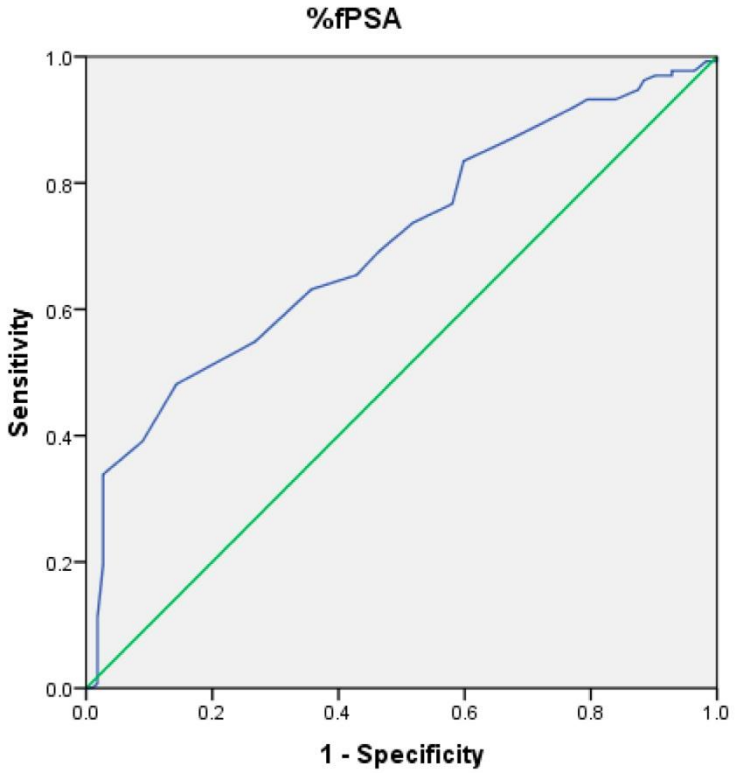
(AUC 0.71; 95% CI, 0.65 – 0.77, P=0.0001) and %fPSA (AUC 0.71; 95% CI, 0.64 – 0.77, P=0.0001).

Figure 3-10: Receive operating characteristics (ROC) curve for (A) PSA Density (PSAD) and pre-TTSB PSA; (B) percentage of free PSA (%fPSA)

A



B



The cut-off values at which at least 90% of cancers would be detected (sensitivity) by the PSA parameters include ≥0.10ng/mL/cm^3 for PSAD, ≥4.5ng/mL for pre-TTSB PSA and ≤17.5% for %fPSA. At these cut-offs, the number of unnecessary biopsies that would have been avoided (specificity) were 25% for PSAD, 16% for pre-TTSB PSA and 23% for %fPSA.

Using these cut-offs, the number of significant cancer that would be predicted (sensitivity) and corresponding specificity (proportion of clinically insignificant cancer) according to Epstein's criteria are shown in table 3-20

Table 3-20: Sensitivity and specificity of PSAD, %fPSA and pre-TTSB PSA for predicting Gleason score and tumour volume

		Sensitivity (%)	Specificity (%)
Gleason score ≥7	PSAD ≥0.10	95.7	17.9
	%fPSA ≤17.5	89.4	17.9
	Pre-TTSB PSA ≥4.5	93.6	25.6
NPC	PSAD ≥0.10	95.7	12.9
	%fPSA ≤17.5	91.4	16.1
	Pre-TTSB PSA ≥4.5	92.9	17.7
PPC (%)	PSAD ≥0.10	95.8	13.1
	%fPSA ≤17.5	88.9	14.8
	Pre-TTSB PSA ≥4.5	93.1	18.0
MPC (%)	PSAD ≥0.10	98.6	16.1
	%fPSA ≤17.5	85.9	11.3
	Pre-TTSB PSA ≥4.5	97.2	22.6
MTL (mm)	PSAD ≥0.10	97.0	13.4
	%fPSA ≤17.5	86.4	11.9
	Pre-TTSB PSA ≥4.5	97.0	20.9
ATLPC (mm)	PSAD ≥0.10	95.5	15.6
	%fPSA ≤17.5	88.6	15.6
	Pre-TTSB PSA ≥4.5	94.3	24.4

3.6 Utility of MRI before TTSB

Of the 303 men included in this study, 158 underwent an MRI scan prior to modified TTSB (52.1%). Table 3-21 shows a breakdown of the MRI sequences undertaken by the cohort.

Table 3-21: Pre-TTSB MRI technique

	N (%)
Conventional MRI (T1/T2WI)	76 (48.1)
Diffusion weighted imaging MRI + T2WI	49 (31.0)
Full Multiparametric MRI (T2WI + dwi=MRI + DCE-MRI)	33 (20.9)

3.6.1 MRI stage

Majority of cases were T1c. Table 3-22 presents the stage of cancers identified on MRI.

Table 3-22: Cancer stage on MRI

MRI Stage	**N (%)**
T1c	93 (58.9)
T2a	61 (38.6)
T3a	4 (2.5)

3.6.2 Comparison of pre-biopsy MRI to TTSB for cancer diagnosis

Analysis of MRI data was performed to determine how well pre-saturation biopsy MRI scan result correlated with TTSB as the gold standard in order to determine whether MRI scan could decrease the need for TTSB. Firstly, a 2x2 crosstabulation table outlining MRI and TTSB outcomes was constructed (table 3-23). This enabled sensitivity, specificity to be determined.

Table 3-23: MRI and TTSB results

	TTSB positive	**TTSB negative**	**Total**
MRI positive	44	21	65
MRI negative	24	69	93
Total	68	90	158

MRI correctly detected cancer in 44 of 68 patients (64.7%) and was correctly negative when TTSB outcome is benign in 69 of 90 patients (76.7%). This observation was statistically significant by Chi-Square test (P = 0.0001). However, MRI failed to identify 24 of 68 cancers (35.3%) detected by TTSB and of the 90 negative cases on TTSB, MRI incorrectly found abnormality in 21 (23.3%).

To further determine the contribution of the different MRI sequences to the observation above, we cross-tabulated the TTSB result with the three MRI sequences (table 3-24).

Table 3-24: MRI sequence and TTSB result

		TTSB positive	TTSB negative	Total
cMRI	positive	30	13	43
cMRI	negative	17	16	33
	Total	47	29	76
dwi-MRI + T2WI	positive	4	7	11
dwi-MRI +T2WI	negative	5	33	38
	Total	9	40	49
mp-MRI	positive	10	1	11
mp-MRI	negative	2	20	22
	Total	12	21	33

The cross tabulation above shows that there is a huge difference between the three MRI sequences in their ability to detect abnormality. Multiparametric MRI (mp-MRI) is significantly more accurate for identifying and excluding prostate abnormality compared to the other sequences (Fisher's Exact test $P = 0.0001$). Conventional MRI (cMRI, Chi-Square test $P = 0.104$) and diffusion weighted imaging

MRI (DWI-MRI, Fisher's Exact test *P* = 0.179) have no statistical significant relationship with TTSB for cancer detection.

The sensitivity, specificity, positive and negative predictive values of MRI overall and for each of the three MRI sequences are shown in table 3-25

Table 3-25: Diagnostic accuracy of MRI

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Overall MRI	64.7	76.7	67.7	74.2
cMRI (T1/T2WI)	63.8	55.2	69.8	48.5
DWI-MRI + T2WI	44.4	82.5	36.4	86.8
mp-MRI	83.3	95.2	90.9	91.0

Multiparametric MRI (mp-MRI) outperformed the other MRI sequences in all parameters for detection of abnormality in the prostate with a sensitivity of 83.3% compared to 44.4% and 63.8% for diffusion weighted imaging and conventional MRI respectively. When mp-MRI identifies an abnormality, it is more likely to be positive at TTSB compared to other MRI sequences (PPV 90.9%).

Importantly, analysis of the clinical characteristics of the patients in the three MRI sequences using Kruskal-Wallis test showed no difference for age (*P* = 0.524), pre-TTSB PSA (*P* = 0.074), prostate volume ((*P* = 0.321), number of cores at TTSB (*P* = 0.453) and sampling density (*P* = 0.282). Furthermore, the Gleason score (*P* = 0.089)

and tumour volume parameters including number of positive cores, percent of positive cores and maximum percentage core involvement (*P* = 0.296) were the same across the three groups of MRI sequence.

3.6.3 Characteristics of tumours missed by MRI

From the crosstabulation in table 3-22, it was shown that MRI missed 24 cancers which were identified by TTSB. Stratification of the tumours missed by MRI into the three sequences showed that majority of the missed tumours were by conventional MRI (17 of 24, 70.8%), DWI-MRI 5 (20.8%) and mp-MRI 2 (8.4%). Further analysis was performed on the 24 missed tumours (false negative) in order to determine their histological characteristics compared with the correctly identified (true positive) tumours (table 3-26).

Table 3-26: Histological parameters of cancers detected by TTSB which were missed compared with those correctly identified by MRI.
Data presented as median (range).

	Histology				
MRI	**Gleason score**	**NPC**	**MTL (mm)**	**ATLPC (mm)**	**MPC (%)**
False negative (n = 24)	7 (6-10)	2.5 (1-14)	2.5 (1-11)	5 (1-57)	25 (1-100)
True positive (n = 44)	7 (6-9)	5.0 (1-16)	5.3 (1-20)	17 (1-140)	60 (1-100)

There was no difference in tumour grade (Gleason score) between the missed cancers (false negative) and those that were accurately identified by MRI (Mann-Whitney U Test, *P* = 0.179). Furthermore, a trend towards less median number of positive cores (NPC) in men diagnosed with cancer on TTSB which were missed by MRI was observed, but this did not reach statistical significance (Mann-Whitney U Test, *P* = 0.107). However, the median tumour volume parameters (MTL, ATLPC and MPC) of the MRI missed cancers were significantly smaller than those accurately identified on MRI (*P* = 0.002, 0.013 and 0.005 respectively).

3.6.4 Characteristics of cancers missed by MRI according to Epstein's criteria

Analysis of the 24 missed tumours was performed to determine their clinical significance by Epstein's criteria (Epstein *et al*, 2005). This criteria for classification of insignificant cancer at saturation biopsy described by Epstein et al has been discussed in detail in chapter 2, section 4.0 of this report.

Using Epstein's criteria, 16 of the 24 (66.7%) missed tumours on MRI could be classed as clinically significant (Gleason score ≥7, number of positive cores ≥4, maximum tumour length ≥4.5mm and aggregate tumour lengths from positive cores ≥5.5mm).

3.6.5 Correlation of cancer location between MRI and TTSB

The location of abnormalities in any of the 4 anatomical quadrants on MRI scan was analysed and correlated with the ultimate location of tumour identified by TTSB in order to determine whether a limited TTSB might be feasible.
Abnormalities were located in multiple quadrants in vast majority of the patients. Consequently, MRI scan demonstrated an abnormality in 89 quadrants compared to TTSB which detected cancer in 144 quadrants. Of the 89 abnormal MRI quadrants, 82 (92%) were diagnosed with cancer by TTSB.

Abnormality reported on MRI was concordant with result of TTSB in 113 of 158 patients (71.5%) including 69 cases correctly reported as normal by MRI (true negative) and 44 cases with abnormality in at least one anatomical quadrant on MRI which was correctly diagnosed with cancer at TTSB (true positive).

The characteristics of the tumours inaccurately localised to a quadrant (false negative) compared to correctly localised tumours (true positive) on MRI quadrants are summarised in table 3-27.

Table 3-27: Grade and volume of tumours correctly localised to anatomical quadrant on MRI compared to the missed cases.

Data is presented as median (range)

	Histology				
MRI	**Gleason score**	**NPC**	**MTL (mm)**	**ATLPC (mm)**	**MPC (%)**
False negative quadrant (N = 62)	7 (6-10)	2.5 (1-14)	1 (1-11)	1.6 (1-83)	30.0 (1-100)
True positive quadrant (N = 82)	7 (6-9)	5.0 (1-16)	6.5 (1-20)	17.5 (1-140)	65.0 (1-100)

MRI identified quadrants with higher tumour load as shown by the statistically significant difference between the number of positive cores (NPC), maximum tumour length (MTL), aggregate of tumour lengths of positive cores (ATLPC) and mean maximum percent core involved with cancer (MPC) for the quadrant concordant MRI detected tumours (true positive) compared to the false negative quadrants (Mann-Whitney U test, P = 0.027, 0.001, 0.006 and 0.006 respectively). However, Gleason score between the missed quadrant (false negative) and the accurately identified tumour quadrant by MRI was not significantly different (Mann-Whitney U test, P = 0.123).

Multiparametric MRI was better for identifying small tumours because of the 62 quadrants from 24 patients which were falsely negative on MRI but diagnosed with cancer by TTSB, cMRI missed 40 (64.5%) quadrants, DWI-MRI 16 (25.8%) and only 6 (9.7%) quadrants were missed by mp-MRI.

Chapter 4 Discussion

4.1 Modified TTSB technique and prostate cancer diagnosis

Modified TTSB identified PCa in 55.1% of men undergoing repeat prostate biopsy after a median of 2 previous negative TRUSB, from a median of 29 cores. The cancer detection rate is superior to the 11.1 to 41% incidence reported for transrectal saturation biopsy series listed in table 1-1 and comparable to 23 to 68% rates reported for other TTSB approaches which are summarised in table 1-2. This suggests that the periurethral sparing modified TTSB technique has no significant impact on cancer detection, a finding in keeping with previous studies showing that the basal periurethral area is hardly ever the sole site to be involved by localised prostate cancer.

In a study of 88 men with persistent clinical suspicion of PCa despite having undergone a mean of 15.1 biopsy cores from their previous negative TRUSB, Igel et al (Igel *et al*, 2001) detected cancer in 43% at TTSB. In another study, Furuno et al (Furuno *et al*, 2004) performed TTSB on 113 men, 86 of which had no previous biopsies. Overall cancer detection rate was 43%. However, in the 27 patients with previous negative biopsy, cancer was detected in 7 (26%). The same group reported a cancer detection rate of 49% in 371 patients undergoing TTSB (first biopsy in 312 and repeat biopsy in 59 patients). The cancer detection rate in the repeat group was only 26% (Demura *et al*, 2005). Pinkstaff et al reported a 37% detection rate in 78 men from 21.2 cores after 2 or more negative TRUSB whilst Satoh and

colleagues performed TTSB on 128 high risk men after a median of one negative sextant tranrectal biopsy. They reported a PCa detection rate of 22.7%. Other TTSB series in patients with persistent clinical suspicion of PCa after negative TRUSB histology record cancer detection rates of 26 to 68% (Bittner *et al*, 2009; Bott *et al*, 2006; Gershman *et al*, 2012; Mabjeesh *et al*, 2012; Merrick *et al*, 2007; Merrick *et al*, 2008; Pal *et al*, 2011). Bott et al (Bott *et al*, 2006) modified their technique aimed at reducing operating time by sampling the prostate from 6 to 12 needles placed simultaneously into the anterior, middle or posterior regions of the gland. Of the 67 men who underwent TTSB using their modified approach, cancer was detected in 38%. Merrick and colleagues (Merrick *et al*, 2007) reported a 42.2% cancer detection rate after 2 negative TRUSB from a mean of 22.4 cores using anatomic based TTSB technique in 102 patients. The same group reported a 45.9 to 46.5% detection rates from two studies (Bittner *et al*, 2009; Merrick *et al*, 2008). Another study by Pal et al (Pal *et al*, 2011) in a relatively small cohort of 40 men undergoing TTSB due to high PSA of 22ng/ml and two negative sets of TRUSB detected PCa in 68% from a standardised 36 core approach. Mabjeesh et al studied 92 patients and reported PCa incidence of 26% after TTSB whilst Gershman and colleagues (Gershman *et al*, 2012) reported 50% cancer detection in 34 patients who had repeat TTSB. More recently, a large North American series involving 485 patients reported PCa detection rate of 46.6% from 56 cores at TTSB (Bittner *et al*, 2013).

In addition to the advantages generic to TTSB which have been discussed in chapter 1, section 9.0 of this report, this modified technique provided some unique

advantages. Firstly, the sampling density which is inversely proportional to the prostate volume (figure 3-1) means that contrary to previous reports where the number of TTSB cores rose with increasing prostate size resulting up to 50 to 76 cores in some series (Merrick *et al*, 2007; Stav *et al*, 2008), modified TTSB results in far less number of cores (mean 28 cores) which in the current climate of tight hospital resources, provides reasonable workload for the pathologist.

Secondly, modified TTSB approach ensures an evenly spaced prostate sampling 10mm apart horizontally and 5mm vertically between rows. This is similar to the description by Demura et al (Demura *et al*, 2005). In their study, they postulated based on their experience that if tumour focus is a sphere, then tumour foci >10mm in dimension would be equivalent to a tumour volume of 0.5cc and should be detected. Hence, biopsy spot with diagonal length measuring 10mm as in modified TTSB should detect tumour focus that is 0.5cc or greater which has been shown to be of clinically significant size by Epstein et al (Epstein *et al*, 2005). Using mathematical modelling to analyze evenly spaced transperineal biopsy pattern in order to determine the volume of a small spherical tumour that might be missed at TTSB, Kepner et al (Kepner & Kepner, 2010) demonstrated that a 1cm core spacing at TTSB should detect 1cc tumour with 99% accuracy, although some tumours in the volume range 0.5-1cc may be cinically significant. This should not occur in our modified technique with 1cm horizontal, but 0.5cm vertical spacing.

This study's protocol allowed for the downsizing of prostate volume to less than $60cm^3$ using dutasteride prior to biopsy which was aimed at ensuring adequate

sampling of the lateral peripheral zone of the gland. We found that the group of patients who received dutasteride were older, with higher PSA parameters compared to the patients not on dutasteride. This observation is not surprising as data from placebo arm of randomised controlled trials have demonstrated correlation between serum PSA, prostate volume and BPH progression. Crawford et al (Crawford *et al*, 2006) analysed data from placebo arm of a randomised controlled trial including 737 men to determine clinical predictors of BPH progression. In their study, the risk of BPH progression was significantly greater in those with baseline prostate volume 31cm^3 or greater and PSA 1.6ng/m or greater.

In another randomised controlled trial of 3040 men with BPH comparing finasteride versus placebo to determine whether baseline PSA and prostate volume were associated with long-term changes in symptoms, Roehrborn et al (Roehrborn *et al*, 1999) determined that baseline PSA 1.4ng/mL or greater, and enlarged prostate were best long-term response to finasteride predictors compared to placebo. In another placebo controlled randomised trial of 3047 men to compare the effects of placebo, doxazosin, finasteride and combination therapy on measures of the clinical progression of benign prostatic hyperplasia (BPH), it was shown that in men with PSA levels greater than 4ng/mL or a baseline prostate volume more than 40 cm^3, the number needed to treat to prevent BPH progression was 4.7 and 4.9 respectively compared to 8.4 for the entire cohort (McConnell *et al*, 2003).

4.2 Location and characteristics of tumours identified by modified TTSB

We observed that tumours were confined to the anterior region of the gland in 20.4% with some involvement of the anterior third in 77.2% of all identified cancers. This observation is consistent with published reports (Bott *et al*, 2006; Demura *et al*, 2005; Mabjeesh *et al*, 2012; Merrick *et al*, 2007). In a prospective study of 92 men who underwent TTSB to evaluate the cancer detection rate and location, Mabjeesh et al (Mabjeesh *et al*, 2012) demonstrated a high cancer detection rate of 83.3% in the anterior region of the prostate. These anteriorly located tumours had significantly higher number of positive cores compared to posterior tumours. In another study, Bott et al (Bott *et al*, 2006) reported anterior tumour location in 60% of the 23 cancers identified in their series. Dumura et al (Demura *et al*, 2005) obtained a total of 1224 biopsy cores at TTSB from 59 patients undergoing repeat biopsy using TTSB. Of the 57 cancer positive cores identified, 34 (60%) were distributed in the anterior compared to posterior region of the gland. Similarly, Merrick and colleagues (Merrick *et al*, 2007) studied the distribution of PCa identified by TTSB. In their study, they found that although none of their 24 arbitrary regions of the prostate was spared from cancer, 32 of 43 cancers they detected involved the anterior apex.

Investigators now postulate that this anterior location make these tumours difficult to reach using conventional transrectal approach despite numerous repeat biopsies. This is supported by the observations of Demura et al (Demura *et al*, 2005). In their series including 371 men who underwent TTSB of which 312 had

never had any previous biopsy whilst 59 were repeat biopsy. The cancer core rate (ratio of the number of cancer cores to the number of biopsy cores) in the anterior region was found not to be statistically different from that of the posterior region in the primary TTSB group. However, in the repeat biopsy group, the cancer core rate progressively increased anteriorly resulting in a statistically significant higher cancer core rate in the anterior region compared to posterior region. The difficulties associated with detecting anterior tumours were identified by Chen et al (Chen *et al*, 1997) using a stochastic computer simulation model of cancer foci. In their study of 607 tumour foci from 180 serially sectioned radical prostatectomy specimens, sextant biopsy identified cancer in 73% with tumour volume greater than 0.5cc compared to 18% less than 0.5cm^3. The tumour distribution of the 40 cases with tumour volume greater than 0.5cm^3 which were missed by sextant biopsy was mainly in the anterior region above and lateral to the urethra. When simulated biopsies were performed in this area, an additional 17% of cancers previously missed were identified. Bott el al (Bott *et al*, 2002) identified anterior prostate tumours in 21% of 547 radical prostatectomy specimens. Patients with anterior tumours required significantly more biopsy sessions to diagnose PCa with fewer numbers of positive cores and summated tumour lengths than those with posterior tumours.

Our findings that men with anteriorly located tumours were older, with significantly higher baseline PSA, PSAD and number of previous TRUSB attempts is consistent with published reports and not surprising given that anterior location of these tumours make them less likely to be palpable and more likely to be under-sampled

with transrectal biopsy approach (Bott *et al*, 2002). Furthermore, anteriorly located tumours are difficult to detect by imaging, either with transrectal ultrasound (Terris *et al*, 1991) or MRI (Zakian *et al*, 2003). Differentiation of BPH from transition zone tumours using functional MRI scan is difficult because of the broad range of metabolic profile exhibited by transition zone cancer (Zakian *et al*, 2003). Hence majority of these patients as in ours are subjected to multiple repeat biopsies due to persistent clinical suspicion of PCa. Consequently, when diagnosed, they are older as our data suggests. However, contrary to the observation of Mabjeesh et al (Mabjeesh *et al*, 2012) who reported significantly higher number of positive cores in the anterior zone, we did not find a statistically significant difference in the distribution of all biopsy tumour volume surrogates including number of positive cores.

Owing to the difficulty associated with detecting these awkwardly located anterior tumours, some authors have recommended the use of MRI prior to biopsy to aid their identification (Lawrentschuk *et al*, 2010). In a review of 31 patients diagnosed with anterior predominant tumours (14 on active surveillance and 17 with previous negative TRUSB) in order to determine the role of MRI in this cohort, Lawrentschuk et al found that MRI scan had a positive predictive value of 87%. Furthermore, 57% of cores from the anterior prostate had cancer and 10 of 13 patients who eventually underwent surgery were locally advanced (pT2 and above). Of the 8 active surveillance patients with positive surgical margin, 5 were positive in the anterior region only and a third of the patients had biochemical recurrence at 1 year follow-up. To highlight this potential aggressive nature, the authors coined the

name ‘prostate evasive anterior tumours’. Similarly, in a study comparing pathological characteristics of 259 patients with pure anterior tumours to 594 with posterior tumours, Koppie et al (Koppie *et al*, 2006) reported that anteriorly located tumour cohort had more negative biopsy sessions before cancer diagnosis, but fewer number of positive biopsy cores and proportion of cancer positive cores which were significantly different. Patients with anterior tumours had higher tumour volume and positive margin rates.

However, whether anterior tumours possess different biologic potential remains a subject of debate amongst investigators. Most of the earlier studies suggesting lower degree of biologic aggressiveness for anterior tumours are limited by the fact that they were based on inferences drawn from comparing transition and peripheral zone tumours (Greene *et al*, 1991; Grignon & Sakr, 1994; King *et al*, 2009). However, McNeal’s anatomical descriptions utilising the prostatic urethra as the reference point showed that the anterior region of the prostate above the urethra is composed not only of transition zone, but also by anterior peripheral zone and the non-glandular anterior fibromuscular stroma (AFMS) (McNeal, 1981; McNeal, 1988). In another study, Fine et al (Fine *et al*, 2007) reported topographic anatomy of 197 whole-mount radical prostatectomy specimens noting that the anterior prostatic anatomy exhibit considerable diversity at different levels of the gland. At the apex, all of the glandular tissue in the anterior region was composed of peripheral zones bilaterally prompting the authors to conclude that anterior anatomy of the prostate differed from apex, mid and base of the gland depending on the relationship of AFMS to the glandular zones suggesting that these diversities

be taken into account in order to accurately characterise the true nature of these tumours. Another study demonstrated that histological features of transition zone-like tumours are non specific as they can be found in anteriorly located peripheral zone tumours (Garcia *et al*, 2008). The authors cautioned against assigning zone of origin based on histological appearance especially in needle biopsy specimen.

Contemporary studies are now beginning to emerge showing that zonal origin of tumours did not affect outcome after radical prostatectomy (Al-Ahmadie *et al*, 2008; Chun *et al*, 2007). Al-Ahmadie et al (Al-Ahmadie *et al*, 2008) validated observations of Fine et al (described above) using detailed histopathological analysis of 197 prostatectomy specimens with predominant anterior tumours with emphasis on the variability in anterior prostate anatomy from apex to base in order to determine zonal origin and pathological staging. By utilising Fine's approach, they observed that 97 of 197 anterior tumours (49.2%) were from anterior peripheral zone, 70 (35.5%) transition zone, 14 (7.1%) from both zones and 16 (8.1%) were indeterminate. Anterior peripheral zone tumours were mostly localised within the apical third of the gland. Importantly, there was no statistical significant difference for Gleason score, incidence of extracapsular extension, overall surgical positivity rate or laterality. The authors concluded that anterior tumours of peripheral zone origin are more prevalent than those from transition zone. Chun et al (Chun *et al*, 2007) assessed zonal origin of 1262 radical prostatectomy specimens using a modified computer assisted planimetric method. On multivariate cox model analysis, the zone of origin does not affect the rate of biochemical recurrence and

addition of zone of origin to the multivariate model did not improve its predictive accuracy.

Another issue is whether extensive prostate sampling using TTSB will lead to over diagnosis of insignificant tumours. Modified TTSB identified insignificant cancer in 16.2% of the 167 tumours detected in this series. This rate of insignificant cancer diagnosis is higher than the 11.5% incidence rate reported by Epstein et al (Epstein *et al*, 2005). Merrick et al (Merrick *et al*, 2007) reported an insignificant cancer detection rate of 7.1% from their TTSB series. However, they based their classification on criteria derived from conventional tranrectal biopsy series which is not an adequate assessment tool for saturation biopsies. This is the first study to utilise full saturation biopsy classification proposed by Epstein to analyse true incidence of potential over diagnosis of indolent cancer at TTSB.

More interestingly and contrary to reports from tranrectal biopsy series suggesting a high risk of clinically insignificant cancer diagnosis after more than 2 negative TRUS biopsies (Djavan *et al*, 2001; Zaytoun *et al*, 2012), the rate of significant cancer diagnosis in this study correlated with increasing number of prior negative biopsy and was highest amongst those with more than 3 negative TRUSB (93.1%). Zaytoun and colleagues (Zaytoun *et al*, 2012) reported overall clinically insignificant cancer rate of 63% from 749 serial transrectal biopsies in men with persistent clinical suspicion of PCa after 2 negative biopsies. In their series, 74.6% of cancers detected by transrectal saturation biopsy were clinically insignificant prompting the authors to advise a high threshold for recommending repeat biopsy. In a

prospective European Prostate Cancer Detection study of 1051 men with raised PSA who underwent serial prostate biopsies; Djavan et al (Djavan *et al*, 2001) reported a significantly lower Gleason score, stage and tumour volume for cancers detected on biopsies 3 and 4. On the contrary, Tan et al (Tan *et al*, 2008) showed that although cancers diagnosed on repeat biopsy were smaller volume, there were significant number of higher grade tumours detected after second prostate biopsy. Of the 905 cancers they investigated, the insignificant cancer diagnosis rate were 7.7%, 7% and 8.2% on initial, first and two or greater numbers of prostate biopsy. Similar to findings in this study, Bittner et al recently reported a clinically significant cancer diagnosis rate of 86.7% after TTSB using Epstein's criteria as in this study. There has been a consistent trend towards an upward Gleason score migration after prostate biopsy reported in the last 20 years. Two recent UK studies have reported an upward shift in Gleason score in men who underwent prostate biopsies over time (Oxley *et al*, 2014) and after radical prostatectomy (Laird *et al*, 2014). From a randomised controlled study data in which 3282 cancer containing biopsies were analysed, Oxley and colleagues (Oxley *et al*, 2014) showed a shift to a higher Gleason score category for each of the 10 year duration of the study (Oxley *et al*, 2014). The odds of been diagnosed with a higher Gleason score increased by 4.9% for each year of the study. Similarly, analysis of data from the UK radical prostatectomy database reported that intermediate or high risk disease increased from 82.5% preoperatively to 97.2% following radical prostatectomy (Laird *et al*, 2014). This could be a direct result of the recent update to the original Gleason score system that has made lower Gleason grades to be less likely reported (Epstein, 2010).

Furthermore, in the subset of patients who have had more than 2 negative biopsies prior to undergoing TTSB, 83% were diagnosed with clinically significant cancer which is comparable to the 93% in this report. This apparent discrepancy is likely due to the anterior location of tumours making them evade detection by conventional biopsy strategies. Our observation that 67.6% of the tumours confined to the anterior region were in patients who have had 2 or more negative TRUSB is definitely suggestive. Secondly, data from TTSB series show that conventional TRUSB underestimates extent of disease and Gleason score. Recently, Taira and colleagues (Taira *et al*, 2013) performed TTSB on 64 men who were initially diagnosed with insignificant cancer by TRUSB suitable for active surveillance. They identified clinically significant cancer in 71.9% and 44.6% had Gleason 7 or more. In another study, Ayres et al (Ayres *et al*, 2012) re-staged 101 men on active surveillance for PCa using TTSB. They found that 34% of patients had more significant cancer after TTSB compared to their previous TRUSB and 44% of these had disease predominantly in the anterior region of the gland.

4.3 Morbidity of modified TTSB

The incidence of acute urinary retention (AUR) in this study is 7.6%. Routine catheterisation was not part of modified TTSB technique. Our rate of AUR is lower than the 11 to 39% rates reported by other series where prophylactic catheterisation was also not utilised (table 1-2).

The mechanism for development of AUR in patients undergoing TTSB remains enigmatic. One theory suggests that needle trauma and subsequent prostate oedema are the likely cause of AUR after TTSB (Buskirk *et al*, 2004). In their study performed to determine the influence of needle trauma on AUR, the authors reviewed 157 men who underwent TTSB after at least one negative TRUSB. AUR was reported in 11.5%; and age, median prostate volume and number of cores being significantly higher in men with retention. On multivariate analysis, only number of cores predicted for AUR prompting the authors to suggest that needle trauma and subsequent prostate oedema are likely causes of urinary retention.

On the contrary, our data showed that men with greater sampling density were less likely to develop AUR. In fact, on multivariate analysis, only prostate volume predicted AUR in this series. This observation suggests that rather than number of needle incursion, prostate volume appears to play a confounding role in the development of AUR after TTSB. This is supported by prospective data investigating morbidity of TTSB reported by Merrick et al (Merrick *et al*, 2008) showing that median catheter dependency and urinary symptoms after TTSB worsened with increasing prostate volume. In their study, the median catheter dependency for prostate volumes <60, 60–90, 90–120 and >120mL were 0, 1, 2 and 3 days

respectively. Overall, there was a transient deterioration in the mean International Prostate Symptom Score (I-PSS) at 7 days post TTSB but 94% of patients had normal IPSS by 30 days with smallest (<40mL) and the largest (>120mL) prostate groups having the lowest rates of symptom resolution. Using the same symptom questionnaire, Zisman et al (Zisman *et al*, 2001) reported on post transrectal biopsy urinary symptom in 204 men. 52% reported new onset urinary symptoms at 7 days and 8% were severe. Of the patients reporting severe urinary symptoms, 5 developed AUR and larger prostate transition zone volume was the only independent predictor of impaired voiding on logistic regression analysis.

Another hypothesis is that temporary prostatic oedema from extensive prostate biopsy results in bladder outlet obstruction leading to AUR (Borboroglu *et al*, 2000). However, this proposal is contradictory to published reports from transrectal biopsy data which suggests no difference in morbidity with increasing number of biopsy cores (Berger *et al*, 2004; Ghani *et al*, 2004; Naughton *et al*, 2000; Paul *et al*, 2004). In a prospective randomised trial to study effect of increasing core number at TRUSB on morbidity, Paul and colleagues (Paul *et al*, 2004) investigated three different biopsy regimens with different core numbers and areas of sampling. There was no statistical difference between patient's experience of pain and other biopsy side effects with increasing number of sapling cores. Berger et al performed TRUSB in 5957 men to assess complication rates of 6, 10 and 15 core strategies. Apart for haematospermia, there was no difference in haematuria, rectal bleeding, AUR and infective complications between the groups. In another questionnaire based study with 760 respondents, there was no difference in severity and duration of post biopsy bleeding between groups after 6, 8 and 12 core biopsy regimens. A similar

trend was reported in a randomised trial comparing 6 and 12 core biopsy schemes (Naughton *et al*, 2000).

The incidence of bleeding, pain and analgesic need after TTSB has not been reported previously. This is surprising for an invasive procedure which has been established in the last 12 years. Analysis of our data showed comparable morbidity result to transrectal series with lower incidence of rectal bleeding. For example, immediately after TTSB, 75% and 13.7% of the patients experienced haematuria and rectal bleeding respectively which by day 7 had significantly reduced to only 28% and 1.2% respectively by day 7. This is comparable to report from a randomised controlled trial investigating the morbidity of different transrectal biopsy regimens (Paul *et al*, 2004). Similar to our study, Paul and colleagues reported a 70% and 25.3% incidence of gross haematuria and rectal bleeding amongst cohort undergoing repeat biopsy. The higher incidence of rectal bleeding reported for transrectal approach is not surprising given that the technique involves repeated rectal wall punctures compared to transperineal technique's needle trajectory which is parallel to the rectal wall. Furthermore we observed that the rate of haematospermia increased over time. This is similar to previous report suggesting high incidence of persistent haematospermia after prostate biopsy (Emiliozzi *et al*, 2001; Paul *et al*, 2004; Peyromaure *et al*, 2002). Emiliozzi and colleagues (Emiliozzi *et al*, 2001) reported haematospermia lasting up to 2 months in 66% of men after 12 core transperineal biopsy. Paul et al (Paul *et al*, 2004) reported a 71% incidence of haematospermia lasting between 10 to 12.8 days in men who underwent repeat transrectal biopsy. In another study, 78.3% of

respondents reported haematospermia after 1 month of biopsy (Peyromaure *et al*, 2002).

Perhaps the unexpected finding of this study is the low degree of pain experienced by patients after TTSB. Contrary to expectation for an extensive biopsy strategy, on the 1st day after TTSB when pain peaked, the mean pain score was only 0.8 out of 10 on the visual analogue scale and 73% of patients did not require analgesics. This is significantly lower when compared to one previous transrectal series in which 18.7% of patients reported moderate to severe pain one month after biopsy (Paul *et al*, 2004). Peyromaure and colleagues (Peyromaure *et al*, 2002) also evaluated pain after 10-core TRUSB from a visual analogue scale in 275 men and reported that 36% of respondents still experienced perineal pain one month after biopsy.

Our data show that overall rate of morbidity after modified TTSB is low. Although the 7.6% incidence of AUR is higher than reported for TRUSB series (Berger *et al*, 2004; Zisman *et al*, 2001); it is nonetheless lower than reported in other TTSB series where routine catheterisation was not performed with the additional advantage of avoiding prophylactic urethral catheterisation which can add to patient morbidity (Hale *et al*, 2012). Although not studied in this series, transient erectile dysfunction has been reported after TTSB (Losa *et al*, 2013; Tsivian *et al*, 2013). However, using a validated questionnaire, Merrick et al (Merrick *et al*, 2008) reported that TTSB did not significantly influence erectile function in men who were potent prior to biopsy. The question is whether the additional gain of increased precision and cancer detection of TTSB outweighs the risk of increased morbidity or vice versa to the patient. Further study is required to answer this question. However, given the huge

psychological stress experienced by men following a negative biopsy especially in the setting of persistent clinical suspicion of cancer, it is probable that majority of men would accept to undergo repeat biopsy and a procedure with less morbidity would be more readily acceptable.

4.4 Predictors of histopathological outcomes for TTSB

Our data showed that PSAD, %fPSA and pre-TTSB PSA independently predicted cancer diagnosis after TTSB on multivariate analysis. When deciding to use at least 90% sensitivity to set the cancer detection thresholds for PSAD, %fPSA and total PSA, we reasoned that physicians and patients were likely to be more concerned with ensuring a high detection rate of clinically significant cancer. Consequently, we observed that a cut-off of 0.10ng/mL/cm^3 for PSAD and 4.5ng/mL for pre-TTSB PSA (i.e. perform TTSB at or above these cut-off levels) and 17.5% for %fPSA (i.e. perform TTSB at or below this cut-off) would identify at least 90% of cancer whilst sparing 16 to 25% from undergoing unnecessary biopsy. Given that 38% of men would undergo repeat biopsy within 5 years of their initial biopsy (Welch *et al*, 2007); this represents a substantial proportion of men who would potentially avoid unwarranted repeat biopsy. Furthermore, when these cut-offs were applied to detect cancers with aggressive features relating to cancer volume and Gleason score, we found that 86 to 99% of clinically significant tumours would have been identified correctly whilst avoiding 12 to 26% of insignificant disease.

This study to our knowledge is the first study to explore the potential correlation between PSA and its derivatives pathological outcomes of TTSB. The vast majority of studies that evaluated predictive accuracy of PSAD, %fPSA and serum PSA for pathological outcomes have been performed in the setting of transrectal biopsies (see chapter 1, section 5.1). These studies report mixed observations. For example, in a recent large study performed to analyse the performance of PSAD as a predictor of Gleason upgrade after radical prostatectomy in 1516 men, Corcoran et al (Corcoran *et al*, 2012) reported that PSAD was the strongest predictor of subsequent tumour upgrade. Magheli et al (Magheli *et al*, 2008) determined the utility of PSA and PSAD for predicting pathological stage and biochemical recurrence in 13,434 men who underwent radical prostatectomy for clinically localized prostate cancer over a 22 year period. When stratified by Gleason score (≤ 6, 7, and ≥ 8), PSAD was more predictive of extracapsular extension and biochemical recurrence than PSA in patients with biopsy Gleason ≤ 6 whilst PSA better predicted for seminal vesicle invasion, lymph node metastasis and biochemical recurrence in biopsy Gleason 7 group. For men with biopsy Gleason scores ≥ 8, there was no statistical difference between PSA and PSAD in prognostic value for pathological or clinical outcomes. Others have suggested that low %fPSA increased probability of cancer diagnosis and might be associated with more aggressive disease (Morgan *et al*, 1996b; Southwick *et al*, 1999; Uemura *et al*, 2004). Southwick et al (Southwick *et al*, 1999) reported that higher %fPSA levels were associated with more favourable histopathological findings after radical prostatectomy, suggesting that a cut-off of 15% or greater provided the greatest discrimination in predicting favourable disease. On Multivariate logistic regression

analysis, %fPSA was the strongest predictor of adverse pathological outcome (odds ratio, OR 2.25), followed by biopsy Gleason sum (OR 2.06) and patient age (OR 1.35). Morgan et al (Morgan *et al*, 1996b) reported that %fPSA can help avoid repeat biopsies as %fPSA was significantly lower in men diagnosed with cancer even after 2 previous negative conventional biopsies. They suggested that a %fPSA cut-off of 10% has 91% sensitivity. Another study of similar design showed higher probability of cancer diagnosis using 11% as %fPSA cut-off level (Uemura *et al*, 2004).

Although there is paucity of literature correlating PSAD or %fPSA with saturation biopsy findings, outcome prediction using PSA velocity (PSAV) has been described. Bittner et al (Bittner *et al*, 2009) reported on the effect of PSA velocity (PSAV) on PCa diagnosis, Gleason score, tumour location and cancer volume in 217 men undergoing repeat biopsy using TTSB. In their study, they found that a greater PSAV did not correlate with PCa diagnosis or histological findings. On the contrary, Mabjeesh and colleagues (Mabjeesh *et al*, 2012) reported that only PSAV and PSA doubling time (PSADT) independently predicted cancer diagnosis amongst the 92 men who underwent TTSB after 2 previous negative TRUSB. More recently, Ayres et al reported on 101 men on active surveillance for prostate cancer who underwent re-staging TTSB. Similar to the observation by Bittner et al, they found that PSA, PSAV and PSA doubling time (PSADT) did not correlate with TTSB outcomes (Ayres *et al*, 2012). Although not highlighted by the authors, a closer review of the data from both studies actually suggest a trend between increasing PSAD and TTSB

outcomes with one showing that PSAD was actually the strongest predictor of cancer diagnosis at multivariate logistic regression (Bittner *et al*, 2009).

The AUC was higher for PSAD (0.76) than for pre-TTSB PSA (0.71) and %fPSA (0.71) indicating that PSAD is slightly more predictive of cancer diagnosis in patients undergoing modified TTSB in this series. Our data showed that across all pathological outcomes including cancer diagnosis, tumour volume and Gleason score, PSAD was consistently more predictive than either PSA or %fPSA. This finding is consistent with previous reports (Busch *et al*, 2012; Elliott *et al*, 2008; Oh *et al*, 2012). Similar to current study, a retrospective review of data from 1708 prostate biopsies from a single institution reported that PSAD had a statistically higher AUC than PSA for detecting all prostate cancers (0.737 vs. 0.633, P<0.001) as well as high grade (0.766 vs. 0.673, P<0.001) and high volume (0.843 vs. 0.755, P<0.001) disease respectively. Busch et al (Busch *et al*, 2012) reported from 1,334 radical prostatectomy data and showed that total PSA and PSAD significantly increased with increasing tumour aggressiveness as indicated by a rising Gleason score. PSAD but not %fPSA predicted biochemical free survival on multivariate Cox regression analysis. Another study compared the accuracies of PSA and PSAD for predicting Gleason score upgrading at radical prostatectomy in 505 men diagnosed with low grade by extended transrectal biopsies. The multivariate model incorporating PSAD was found to have significantly higher predictive accuracy for Gleason score upgrading compared to PSA model (Oh *et al*, 2012). Horiguchi et al (Horiguchi *et al*, 2003) studied 114 men after radical prostatectomy to determine which preoperative variables including PSA based parameters and MRI predicted adverse

pathological stage. Similar to our observation, PSAD had the largest AUC (0.73) amongst the parameters tested and on multivariate analysis, PSAD, Gleason score and adverse MRI findings predicted extra-prostatic cancer.

On the contrary, a few studies correlating PSAD and Gleason score have suggested diminished utility for PSAD as a predictive parameter. Corcoran et al (Corcoran *et al*, 2012) reported from preoperative data of 1516 radical prostatectomies that the predictive ability of PSAD diminished with increasing tumour aggressiveness. They observed that for Gleason score 6 and 3 + 4, PSAD was the strongest predictor of subsequent tumour upgrade (OR 1.46 and 1.37 respectively. However, for tumours that were upgraded from Gleason 7 to greater than 7, PSAD was not predictive even on univariate analysis prompting the authors to postulate that this loss of predictive ability was due to less PSA production per unit volume in poorly differentiated tumours. Another study reported that PSAD provided only minimal and statistically insignificant improvement in predicting adverse pathological findings and biochemical recurrence compared to preoperative PSA (Freedland *et al*, 2003). However, the suggestion in literature is that preoperative PSA and Gleason score have a linear relationship (Oh *et al*, 2012; Pierorazio *et al*, 2009); which would be contrary to Corcoran's hypothesis (Corcoran *et al*, 2012).

There are two possible explanations for the superior predictive accuracy of PSAD observed in this study compared to TRUSB based series. Firstly, conventional TRUSB technique fails to detect significant amount of tumours in this cohort of men undergoing repeat biopsy (refer to chapter 1, section 7.0). Even with extended

biopsy approach, a high false negative rate has been reported (Eskew *et al*, 1997). Compared to saturation biopsy, Merrick et al (Merrick *et al*, 2007) demonstrated that only 53.5% and 76.7% of the cancers diagnosed with TTSB would have been diagnosed by a sextant or standard 12-core biopsy respectively. Secondly, the use of fixation devise at TTSB removes operator dependent free hand probe manipulation of TRUSB and ensures a more accurate prostate volume measurement. As PSAD is highly dependent on accurate determination of prostate volume (Benson & Olsson, 1994), a correct volume estimation at TTSB should improve sensitivity of this parameter. This could explain the mixed reports from transrectal ultrasound based series regarding the utility of PSAD.

In addition to the PSA based parameters, we found that other pre-saturation biopsy variables also independently predicted pathological outcome in the cohort of men investigated in this study including age in both logistic models 1 and 2 for cancer diagnosis and prostate volume in model 1 (incorporating pre-TTSB PSA) whilst a lesser number of TTSB cores independently predicted tumour volume parameters including number of positive cores, maximum percent core involvement and percentage of positive cores in the logistic model incorporating pre-TTSB PSA.

Our observation that age independently predicted cancer diagnosis is not surprising given that previous studies have demonstrated that prostate cancer is a disease of advancing age (chapter 1, section 4.1). In one study, only 2% of men below the age of 50 years had prostate cancer compared to 34% for 60 to 69 year olds (Jani *et al*, 2008). Data from the United State's Surveillance Epidemiology and End Result

(SEER) database show that the median age for prostate cancer diagnosis is 67 years with 61% of men diagnosed after 65 years of age (Altekruse *et al*, 2010). This is consistent with our data showing that older men were significantly more likely to be diagnosed with cancer on TTSB. Potter et al reported on data from 2054 men to determine the likelihood of finding prostate cancer on transrectal TRUSB. Similar to our finding, age, PSA and DRE result independently predicted probability of cancer diagnosis on prostate biopsy on multivariate analysis.

Prostate volume has been reported in other series to be associated with pathological outcome both at biopsy and after radical prostatectomy with most studies reporting an inverse relationship between prostate volume and adverse pathology. Amongst 1995 men who underwent a 21-core extended prostate biopsy, prostate volume <50mL results in a 2 fold increase in risk of cancer diagnosis at repeat biopsy (Ploussard *et al*, 2013). Another study showed that prostate volume significantly predicted Gleason score upgrading after radical prostatectomy in 451 men initially diagnosed with low grade disease (Kim *et al*, 2013). In one TTSB series, smaller prostate volume emerged as the only independent predictor of prostate cancer diagnosis on multivariate logistic regression analysis. Furthermore, patients with prostate volume ≤60mL had a substantially higher rate of prostate cancer diagnosis compared with patients with prostate volume ≥60mL (65.9% vs.29.8%) and no patient with prostate volume larger than 105mL was diagnosed with cancer (Merrick *et al*, 2007). Similar association between small prostate volume and prediction of cancer diagnosis on biopsy have also been reported (Al-Azab *et al*, 2007; Campos-Fernandes *et al*, 2009; Leibovici *et al*, 2011). The reason why smaller

prostates have more aggressive disease remains controversial. Some authors have proposed that cancer in smaller volume prostates are biologically different compared to larger glands (Freedland *et al*, 2005) whilst others postulate that lead time bias because of PSA-driven biopsies in larger glands (D'Amico *et al*, 1998; Kim *et al*, 2013).

Interestingly, we found that number of cores obtained at TTSB independently predicted biopsy tumour volume parameters. This is likely due to our modified TTSB technique which ensures an even sampling distribution across the prostate such that the number of cores obtained is dictated by the size of the prostate. Consequently, smaller glands required less number of cores to diagnose cancer. This is demonstrated by the univariate analysis which showed that number of cores at TTSB was significantly less in patients with larger tumour volume including NPC ≥4, MPC ≥50% and PPC ≥15 (table 3-13).

This study is timely as deciding when and who to subject to TTSB in this very challenging cohort remains enigmatic. Accumulating evidence suggests that significant proportion of men with low risk prostate cancer will not progress to clinically life threatening disease in their lifetime, it is now increasingly common to offer these patients all options including active surveillance whereby invasive curative options are deferred until the tumour shows signs of progression (NICE, 2008). Consequently, an ability to predict the likelihood of harbouring significant cancer prior to diagnosis has become even more important.

4.5 MRI and TTSB outcomes

Data from this study confirm that an abnormality on MRI correctly identifies cancer in 64.7% of cases which is similar to cancer detection rates previously reported in literature and outlined in chapter 1 section 10.0 of this report. However, a crucial question in this cohort of men with persistent clinical suspicion of prostate cancer is whether a normal MRI is precise enough to permit limited prostate sampling or allow deference of the need for TTSB. In this study, MRI missed 24 tumours of which 16 (66.7%) where clinically significant cancers. Our findings compare with that of other series from biopsy naive men with 'normal' MRI scan who underwent prostate biopsy and where cancer was diagnosed in 11.7 to 35% (Hadaschik *et al*, 2011; Haffner *et al*, 2011; Kuru *et al*, 2013; Labanaris *et al*, 2011). Haffner et al (Haffner *et al*, 2011) performed 10 to 12 core extended prostate biopsies on 555 consecutive biopsy naive men after pre-biopsy dynamic contrast enhanced MRI. Abnormality on MRI was reported using a 24 region scheme with additional 2 cores taken from each of the suspicious areas on MRI. Overall cancer detection rate was 54%. Of the 204 men with negative MRI report, cancer was detected in 50 (24.5%) on biopsy. MRI had a sensitivity and specificity of 83% and 61% respectively for cancer detection. In another study, Labanaris et al (Labanaris *et al*, 2011) determined whether a patient with clinical suspicion of PCa but inapparent tumour on multiparametric MRI could be spared of prostate biopsy. Of the 109 patients with normal MRI who underwent 18 core biopsies, cancer was identified in 19.2% with 47.6% classified as clinically significant and 38.1% having high grade tumours. In another series of 106 men who underwent transperineal biopsy of suspicious,

questionably suspicious or not suspicious lesions from 3 Tesla Multiparametric MRI; cancer was diagnosed in 13 of 37 (35%) men with non-suspicious MRI (Hadaschik *et al*, 2011). Similarly, Kuru and colleagues (Kuru *et al*, 2013) found that 14 of 94 (15%) men with non-suspicious MRI prior to targeted fusion biopsy had prostate cancer diagnosed by systematic biopsy and 11 of the cancers were intermediate risk disease. Hence, although the cancer detection rates of MRI is promising, our data and that of others described above suggest that cases with a negative MRI should continue to be investigated.

When we compared tumour burden between accurate MRI and those that missed cancers, we found that MRI identified cancers with significant tumour load as shown by the significantly larger biopsy tumour volume parameters in true positive cases compared to the false negatives. It is therefore not surprising that majority of tumours identified in studies incorporating targeted biopsy of suspicious areas on MRI are clinically significant as our data show that when MRI is positive, the tumour is mostly of significant size. For example, Sonn et al (Sonn *et al*, 2013) performed multiparametric MRI on 105 men with prior negative biopsy followed by a fusion biopsy of suspicious areas to determine clinically significant cancer (Gleason ≥3 + 4 or Gleason 6 with maximal cancer core length ≥4 mm) detection rate compared to systematic biopsy. Fusion biopsy detected cancer in 34%. Additionally, 21 of 23 men (91%) with PCa on targeted biopsy had significant cancer compared to 15 of 28 (54%) for systematic biopsy. Furthermore, the proportion of significant cancer positively correlated with worsening MRI suspicion grade with highly suspicious areas (grade 5) having 75% clinically significant cancer in at least one of the

targeted biopsy core. Similarly, a recent prospective series of 347 men who underwent 3T Multiparametric MRI followed firstly by MRI-targeted TRUS biopsies in case of MRI abnormalities and then systematic sector biopsies reported that 74% of PCa identified were clinically significant (Kuru *et al*, 2013). Of the 104 with highly suspicious MRI, cancer was identified in 83% with 72% having Gleason score 7 and above. Nevertheless, our observation that MRI missed 24 tumours of which 66.7% were clinically significant in this series is of concern. Given this finding, it should be considered inappropriate and potentially dangerous to base the decision on whether or not to offer repeat biopsy to a patient with persistent clinical suspicion of prostate cancer on MRI report.

In clinical practice, routine pre-biopsy MRI is not included as part of the diagnostic strategy for prostate cancer. The reasons for this are probably due to increased cost and potential staff burden. However, should MRI result in reduction of the number of biopsies needed to diagnose prostate cancer especially in this cohort who are prone to multiple repeat biopsies; its cost and staff burden could be overlooked. One recent study showed that mp-MRI performed prior to a 21 core systematic transperineal biopsy reduced the number of initial prostate biopsies when combined with prostate volume (Numao *et al*, 2013). Of the 151 men classed as low risk (PSA <10 and a normal DRE), the negative predictive value (NPV) of a combination of positive MRI and prostate volume <33mL for significant cancer diagnosis was up to 98% and at this NPV, 33% of biopsies could have been spared. However, in the same study, amongst men classed as high risk (PSA ≥10 and abnormal DRE) with normal MRI report, a high cancer detection rate of 47 - 51%

was identified depending on the definition of significant cancer utilised; prompting the authors to conclude that the role of pre-biopsy MRI in reducing biopsy in high risk men might be limited.

Another critical question is whether MRI would be accurate enough to allow TTSB with less number of cores. An accurate localisation of tumour and subsequent targeted biopsy could enhance feasibility of focal therapy for prostate cancer. To evaluate this potential for MRI, we divided abnormity location on MRI into 4 anatomical quadrants and correlated this with the eventual cancer location on TTSB. Our data showed that when MRI is abnormal in a quadrant, 92% would harbour cancer on TTSB. Furthermore, as previously noted, MRI also identified quadrants harbouring larger volume tumours more accurately that smaller volume tumours. However, MRI missed cancer from 62 quadrants with median Gleason score of 7 indicating that MRI is limited in its ability to spot abnormality within a quadrant. As it is important in the selection process for focal therapy that those patients with clinically significant, unsuspected cancer outside the target area for focal ablative therapy are not inadvertently excluded from treatment, an ideal MRI sequence should spot most if not all abnormality. A recent review of image-guided biopsies using MRI-derived target reported that MR-directed prostate biopsies using 4 cores show detection of clinically significant cancer equivalent to standard 12-core biopsy (Moore *et al*, 2013). The authors suggested that MR imaging could allow 1 in 3 men to avoid biopsy and 1 in 10 to avoid a diagnosis of clinically insignificant prostate cancer. However, this report was based on conclusions drawn from comparisons between MRI targeted biopsies and conventional transrectal

biopsy strategy which has been shown to lack accuracy with regards to cancer diagnosis. Transperineal template saturation biopsy strategy has a high precision (Crawford *et al*, 2005) and allows representative sampling of entire prostate making it a good reference test for validation of prostate MRI. Using TTSB as a reference test would remove the positive selection bias from studies comparing MRI to prostatectomy specimens as all men with suspicious MRI can be included as it allows for complete sampling of the entire prostate with good correlation to final prostatectomy outcome.

Unfortunately, there is paucity of studies comparing MRI-targeted biopsy and complete prostate mapping using TTSB. In a series of 64 men using TTSB as the reference standard to determine the potential utility of mp-MRI for identification of clinically significant PCa and evaluate its diagnostic performance in cancer detection; the negative predictive value of mp-MRI for clinically significant cancer was 89 to 95% depending on the definition used for clinical significance (Arumainayagam *et al*, 2013). Kasivisvanathan et al (Kasivisvanathan *et al*, 2013) reported on 182 men who had transperineal MR-targeted biopsy followed immediately by systematic TTSB. In their study, MRI-targeted biopsy detected 5% fewer clinically significant cancers compared to systematic TTSB (57% vs. 62%). However, MR-targeted biopsy required less number of cores per patient (median 5 vs. 30); but missed more clinically significant cancer compared to TTSB (21% vs. 13%).

Perhaps not surprisingly, we found that multiparametric MRI was superior to the other sequences for cancer detection. Our data shows a higher sensitivity and negative predictive value for mp-MRI (83.3% and 91%) compared to 44.4% and 86.8% for dwi-MRI and 63.8% and 48.5% for T1/T2 sequence. In a standard T2WI a focus of cancer is identified as low signal intensity relative to surrounding tissues. Consequently, T2WI has low specificity as benign conditions such BPH, prostatitis and post biopsy haemorrhage can mimic cancer. Furthermore, tumours of transition zone (TZ) origin are difficult to detect on T2WI due to overlap between signal intensity characteristics of TZ and cancer. On the contrary, mp-MRI which combines high resolution T2WI with at least two functional MRI sequences demonstrate increased accuracy in the detection of transition zone (TZ) and anterior tumours. In a small retrospective series of 23 patients to evaluate the value of dwi-MRI and DCE-MRI in combination with T2-MRI for the diagnosis of prostate TZ cancer, the addition of DCE-MRI and dwi-MRI to T2-MRI improved accuracy for TZ cancer detection from 64.3% to 78.6% (Yoshizako *et al*, 2008). In 28 patients with similar characteristics, Wang et al analysed 31 TZ cancers and demonstrated an improved accuracy from 63% to 73% from addition of dwi-MRI and diffusion-weighted magnetic resonance imaging to conventional MRI (Wang *et al*, 2011). The area under the ROC curve (AUC) of combined modality was increased from 0.659 to 0.712. Furthermore, in the cohort studied in this series with significant proportion having predominantly anterior tumours; mp-MRI has been shown to have increased ability to detect these awkwardly located tumours. In a limited population of 31 men who underwent prostate biopsy for PSA ≥10ng/mL, Lawrentschuk et al

(Lawrentschuk *et al*, 2010) reported that mp-MRI had a positive predictive value of 87% for detection of anterior prostate tumours.

Similar to our findings, data from other series confirm that mp-MRI provides better characterisation of prostate abnormality than either T2WI alone or in combination with either one of the functional sequences (Amsellem-Ouazana *et al*, 2005; Kirkham *et al*, 2006; Tanimoto *et al*, 2007). Franiel et al (Franiel *et al*, 2011) investigated the incremental value of mp-MRI compared to T2WI for detection of cancer areas in 55 men after 2 previous negative biopsies. Addition of dwi-MRI and DCE-MRI to standard T2WI identified 94% of abnormal areas and subsequent targeted biopsy detected prostate cancer in 100% of cases compared to 86% for T2WI/DCE-MRI or T2WI alone. In another study, analysis of functional MRI parameters of 20 men with histologically proven PCa prior to prostatectomy showed that combination of two functional parameters significantly improved cancer detection over use of any parameter alone (Riches *et al*, 2009). The area under the ROC curves for a combination of ADC and choline/citrate ratio was 0.94 compared to 0.71 and 0.73 for either parameter alone.

One of the main drawbacks of MRI is its reduced sensitivity and specificity for detecting small tumours. This study found that mp-MRI was more accurate than other MRI sequences at localising cancers with small tumour load. Multiparametric MRI missed 2 of 24 (8.4%) tumours compared to 70.8% and 20.8% for T1/T2-MRI and dwi-MRI in this series. Similar finding was reported in a prospective study of 347 patients in which mp-MRI prior to biopsy missed 11 of 94 small tumours (Kuru

et al, 2013). The authors hypothesised that poor image resolution and slice thickness or nearby adenoma with similar appearance are likely causes of MRI missing small tumours in their cohort.

4.6 Study Limitations

There are a few limitations inherent to this technique including the definition of insignificant cancer utilised in this study, use of a non-validated morbidity questionnaire and MRI abnormality reporting predating recently proposed guideline.

This was not a randomised study. Consequently there was no direct comparison of cancer detection and urinary retention rates with other biopsy approaches. Additionally, analysis of whole mount radical prostatectomy specimen will be needed in order to validate cancer locations, tumour volume and Gleason score from this technique. Furthermore, 16.2% clinically insignificant cancer rate reported in this study was based on a widely recognised definition proposed by Epstein for saturation biopsy. This is the first study to apply these criteria in such a setting. The optimum system for classification of clinically insignificant cancer remains a debate amongst investigators. In a recent series, one group from London analysed data from 500 simulated transperineal mapping biopsies from 107 whole mount prostatectomy specimens to define characteristics of clinically significant disease (Ahmed *et al*, 2011). The presence of Gleason ≥7, maximum cancer core length

(MCCL) ≥ 6mm and total cancer core length (TCCL) ≥10mm provided 95% or greater sensitivity for prediction of tumour ≥0.5mL whilst MCCL and TCCL values ≥ 4mm and 6mm predicted tumours 0.2mL or greater. However, this data is yet to be validated.

Additionally, the cohort of men studied in this series is a unique group hence our findings should be interpreted with caution and not be generalised to the entire PCa population. Nonetheless, for men persistently suspected of harbouring PCa despite multiple negative transrectal biopsies who represent applicable study population, it provides a useful adjunct to aid clinicians in decision making and patient counselling prior to offering TTSB. A further limitation is that we utilised a single (Roche Elecsys) free PSA assay to determine %fPSA values, as is standard clinical practice. However, despite calibrations against WHO standard, one study demonstrated highly significant differences amongst five different commonly used commercial assays especially when fixed threshold are utilised (Stephan *et al*, 2007).

Our patient reported outcome questionnaire used for morbidity data acquisition is yet to be validated. However, similar in-house designed questionnaires have been utilised by others (Kuru *et al*, 2013). Furthermore, an element of recall bias cannot be completely eliminated, but the possible confounding effect of receiving the disturbing news of prostate cancer and reporting complications after biopsy at the same time was eliminated by conducting the questionnaire before the biopsy

outcome was revealed. Further prospective series in large cohort would be required to validate it.

Additionally, at the time of initiation of this study, the recently proposed Magnetic Resonance Prostate Imaging Reporting and Data System (MR PI-RADS) by the European Society of Urogenital Radiology (ESUR) for prostate MRI was not in existence (Barentsz *et al*, 2012). The PI-RADS score informs the probability of cancer risk and its aggressiveness plotted on a scheme. The use of such guideline might lead to an improved cancer risk stratification and prognostication. However, it is noteworthy that data from a recent series of 351 men using 5-point MR reporting system prior to biopsy showed results comparable to ours (Numao *et al*, 2013).

4.7 Concluding Remarks

The optimum diagnostic pathway for men with persistent clinical suspicion of prostate cancer despite repeated negative biopsy remains enigmatic. This unfortunate group of men with a diagnostic conundrum suffer from severe psychological stress which has been under-reported in literature. Experience from clinical practice suggests that for this cohort, an improved diagnostic strategy with limited morbidity is desirable.

This thesis describes a modified TTSB technique with equivalent, if not superior cancer detection rate compared to rates reported in the literature. The procedure is well tolerated with lower retention rates when compared with similar series. In the

current climate of limited resources, modified TTSB provides an acceptable number of biopsy cores and workload for the pathologist.

An important observation from this study is that rising PSA in the context of failed histological confirmation of cancer should not be disregarded. Hence TTSB should be offered to all patients with reasonable life expectancy in the presence of persistent clinical suspicion of cancer. However, the challenge remains how to predict outcome of saturation biopsy in this cohort in order to identify those who are harbouring potentially life threatening disease whilst sparing unnecessary biopsy in low risk men. The thesis identified preoperative predictors of positive cancer and aggressive disease diagnosis, thus laying foundation for the development of a predictive model specific for this cohort in the future. The impact of an accurate predictive model in this group will be huge given the large number of biopsies performed worldwide.

The potential of MRI as a screening tool and subsequent targeted biopsy were explored in this thesis. As MRI technology continues to evolve, its impact is likely to progress geometrically. In the words of Dr Patrick Walsh at the lecture in honour of Willet Whitmore who is considered to be the father of modern urologic oncology, "by far the most important discovery that would have the greatest impact in our field would be the development of accurate imaging of tumour within the prostate" (Walsh, 2009). A more accurate MRI could potentially result in less number of biopsy cores and morbidity without compromising detection of significant cancer.

Currently, our data support a complete prostate mapping in this group regardless of MRI finding.

Although currently there are wide ranging data which have become available in this field of science since initiation and compilation of studies reported in this thesis. Nevertheless, the impact from presentation of various aspects of the studies in the thesis at key regional, national and international urology meetings have generated lots of interests which have impacted on clinical practice far beyond the Mersey region. As a consequence of this research, a dedicated prostate cancer diagnostic service for this cohort of men has been developed on the Wirral. Other questions raised herein are timely as we continue to explore the optimum diagnostic strategy for prostate cancer detection in this population.

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Appendix A Morbidity Questionnaire

Wirral University Teaching Hospital NHS
NHS Foundation Trust

0151 334 4000 ext. 4367

Dear

You are having a template biopsy of the prostate under the care of Mr Parr. This is a technique that we have only introduced over the past year. As a department we are seeking information from patients who undergo this to evaluate their experience. This ensures that we are able to give future patients accurate information regarding what to expect after the procedure.

Your thoughts and input would be much appreciated should you wish to take part and **your involvement in this service evaluation is entirely voluntary and confidential**.

If you choose not to take part, at any point, this will not affect your care.

Please return forms completed or otherwise using the stamped addressed envelope enclosed.

If you have any questions about this form, please do not hesitate to contact me on the above number.

Wishing you a speedy recovery,

Clare Hanson

Advanced Nurse Practitioner in Urology

Please tick relevant boxes for all questions.

Please ask for the nursing staff to help you if required.

- 1 **hour** after the procedure:

Any Bleeding?

1. Bleeding from your back passage yes ☐ no ☐

Please state colour of bleeding from back passage

1.1. Fresh bright red blood ☐
1.2. Pale (pink) ☐
1.3. Dark (pink-red) ☐
1.4. Dark red, old blood ☐

Other, please state_____

2. Bleeding in your urine yes ☐ no ☐

Please state colour of bleeding in urine

2.1. Fresh bright red blood ☐
2.2. Pale (pink) diluted in the urine ☐
2.3. Dark (pink-red) diluted in the urine ☐
2.4. Dark red, old blood ☐

Other, please state_____

3. Not passed urine ☐ Comments _____

Pain:

4. Is there any pain? Yes ☐ No ☐
   4.1. If yes, please state where it is;
   4.2. Pain in your perineum (the bit of skin between your scrotum and back passage) ☐
   4.3. Pain in or around your back passage/rectum ☐

Other, please state_____

5 Please rate your pain on a scale of 0-100, 0= no pain 100= the worst pain ever

Use the scale below to mark your response

Perineum

0_____100

Back passage/rectum

0_____100

Other, please state_____

0_____100

6. Have you needed any painkillers? **Yes** ☐ **No** ☐

If so, please state which you used or which you were given_____

(Please ask the nursing staff if you are not sure)

This completes this Questionnaire, Thank you

Please tick relevant boxes for all questions.

- 1 **day** after the procedure: date ___/___/___

Any Bleeding?

1. Bleeding from your back passage yes ☐ no ☐

Please state colour of bleeding from back passage

1.1. Fresh bright red blood ☐
1.2. Pale (pink) ☐
1.3. Dark (pink-red) ☐
1.4. Dark red, old blood ☐

Other, please state_____

2. Bleeding in your urine yes ☐ no ☐

Please state colour of bleeding in urine

2.1. Fresh bright red blood ☐
2.2. Pale (pink) diluted in the urine ☐
2.3. Dark (pink-red) diluted in the urine ☐
2.4. Dark red, old blood ☐

Other, please state_____

3. Not passed urine ☐ Comments _____

4. Bleeding in your semen yes ☐ no ☐

4.1. Fresh bright red blood ☐
4.2. Pale (pink) diluted in the urine ☐
4.3. Dark (pink-red) diluted in the urine ☐
4.4. Dark red, old blood ☐

Other, please
state_____

Pain:

5. Is there any pain? Yes ☐ No ☐
   5.1. If yes, please state where it is;
   5.2. Pain in your perineum (the bit of skin between your scrotum and back passage) ☐
   5.3. Pain in or around your back passage/rectum ☐

Other, please state_____

6. Please rate you pain on a scale of 0-100, 0= no pain 100= the worst pain ever

Use the scale below to mark your response

Perineum

0_____100

Back passage/rectum

0_____100

Other, please state_____

0_____100

7. Have you needed any painkillers? **Yes** ☐ **No** ☐
If so, please state which you
used_____

This completes this Questionnaire, Thank you

Please tick relevant boxes for all questions.

- **3 days** after the procedure: date ___/___/___

Any Bleeding?

1. Bleeding from your back passage yes ☐ no ☐

Please state colour of bleeding from back passage

1.1. Fresh bright red blood ☐
1.2. Pale (pink) ☐
1.3. Dark (pink-red) ☐
1.4. Dark red, old blood ☐

Other, please state_____

2. Bleeding in your urine yes ☐ no ☐

Please state colour of bleeding in urine

2.1. Fresh bright red blood ☐
2.2. Pale (pink) diluted in the urine ☐
2.3. Dark (pink-red) diluted in the urine ☐
2.4. Dark red, old blood ☐

Other, please state_____

3. Not passed urine ☐ Comments _____

4. Bleeding in your semen yes ☐ no ☐

4.1. Fresh bright red blood ☐
4.2. Pale (pink) diluted in the urine ☐
4.3. Dark (pink-red) diluted in the urine ☐
4.4. Dark red, old blood ☐

Other, please state_____

Pain:

5. Is there any pain? Yes ☐ No ☐
   5.4. If yes, please state where it is;
   5.5. Pain in your perineum (the bit of skin between your scrotum and back passage) ☐
   5.6. Pain in or around your back passage/rectum ☐

Other, please state_____

Please rate you pain on a scale of 0-100, 0= no pain 100= the worst pain ever

Use the scale below to mark your response

Perineum

0_____100

Back passage/rectum

0_____100

Other, please state_____

0_____100

7. Have you needed any painkillers? **Yes** ☐ **No** ☐
If so, please state which you used_____

This completes this Questionnaire, Thank you

Please circle your responses for all questions

- **7 days** after the procedure: date ___/___/___

Any Bleeding?

1. Bleeding from your back passage yes ☐ no ☐

Please state colour of bleeding from back passage

1.1. Fresh bright red blood ☐
1.2. Pale (pink) diluted ☐
1.3. Dark (pink-red) ☐
1.4. Dark red, old blood ☐

Other, please state_____

2. Bleeding in your urine yes ☐ no ☐

Please state colour of bleeding in urine

2.1. Fresh bright red blood ☐
2.2. Pale (pink) diluted in the urine ☐
2.3. Dark (pink-red) diluted in the urine ☐
2.4. Dark red, old blood ☐

Other, please state_____

3. Not passed urine ☐ Comments _____

4. Bleeding in your semen yes ☐ no ☐

4.1. Fresh bright red blood ☐
4.2. Pale (pink) diluted in the urine ☐
4.3. Dark (pink-red) diluted in the urine ☐
4.4. Dark red, old blood ☐

Other, please
state_____

Pain:

5. Is there any pain? Yes ☐ No ☐
   5.1. If yes, please state where it is;
   5.2. Pain in your perineum (the bit of skin between your scrotum and back passage) ☐
   5.3. Pain in or around your back passage/rectum ☐

Other, please state_____

6. Please rate you pain on a scale of 0-100, 0= no pain 100= the worst pain ever

Use the scale below to mark your response

Perineum

0_____100

Back passage/rectum

0_____100

Other, please state_____

0_____100

7. Have you needed any painkillers? **Yes** ☐ **No** ☐
If so, please state which you
used_____

Any further comments about any stage of your recovery or experience:

This completes this Questionnaire
Please return in the pre-paid envelope provided

Appendix B Peer reviewed Publication

This appendix contains a publication in a peer reviewed journal of aspects of this thesis (Ekwueme K, Simpson H, Zakhour H, Parr NJ. Transperineal template-guided saturation biopsy using a modified technique: outcome of 270 cases requiring repeat prostate biopsy. BJU Int. 2013 Jun;111(8):E365-73. doi: 10.1111/bju.12134).

It covers the technical aspects of modified TTSB and outcomes in 270 patients.

Please see overleaf.

Appendix C Crude data

This is an appendix of crude data that I generated over the course of this study

Please see attached CD