AN INVESTIGATION OF THE INFLAMMATORY RESPONSE IN PATIENTS WITH PROSTATE CANCER

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AN INVESTIGATION OF THE INFLAMMATORY RESPONSE IN PATIENTS WITH PROSTATE CANCER

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Contents **Page**

Dedication

I would like to dedicate this thesis to my parents, who between them have guided me over each and every one of life's hurdles. In particular, I would like to mention my dad, whose encouragement and unending support kept me going until the work in this project was completed.

Aknowledgements

I owe the utmost gratitude to the following people who helped me through my research project.

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Mr Mark Underwood	Department of Urology, Glasgow Royal Infirmary.
Dr Anne-Marie McNicol	University Department of Pathology, Glasgow Royal Infirmary.
Dr A Michael Wallace	University Department of Biochemistry, Glasgow Royal Infirmary.
Dr Wilson Angerson	University Department of Surgery, Glasgow Royal Infirmary.
Dr Roderick Campbell	University Department of Pathology, Glasgow Royal Infirmary.
Mr David Murray	University Department of Pathology, Glasgow Royal Infirmary.

Declaration

The work presented in this thesis was carried out in the University Departments of Surgery, Clinical Biochemistry, Pathology and the Department of Urology, Royal Infirmary, Glasgow.

In order to address the hypotheses posed in this thesis, three different cohorts of patients were identified, recruited and studied; ethics approval obtained for each cohort. In all, blood samples and/or pathological specimens were collected from 313 patients from seven hospitals in the West of Scotland.

I declare that the work carried out in this thesis was carried out by myself except where indicated below.

Appropriate tumour tissue specimens were selected with the assistance of Dr A.M McNicol (Consultant Pathologist) and Dr R. Campbell (Consultant Pathologist), Department of Pathology, Royal Infirmary, Glasgow.

Sections were cut and mounted by Mr D Murray (Senior Laboratory Technician). Immunohistochemical tissue staining was carried out by Mr D Murray. The resultant staining was checked by Dr A.M McNicol.

C-reactive protein and total, free and complex PSA concentrations were measured by the routine hospital laboratory service, using an automated analyser.

Sensitive C-reactive protein and sensitive interleukin-6 concentrations were measured under the supervision of Dr A.M Wallace (Principal Biochemist), Department of Biochemistry, Royal Infirmary, Glasgow.

The statistical analysis was performed under the supervision of Dr DC McMillan (Senior Lecturer) and Dr WJ Angerson (Reader: Biostatistician), Department of Surgery, Royal Infirmary, Glasgow.

The work presented in this thesis has resulted in the following publications:

McArdle PA, Pollock MA, Wallace AM, McMillan DC, Crooks JE, Underwood MA
Comparison of total, complexed and free prostate specific antigens and their ratios in the detection of prostate cancer in a non-screened population
Annals of Clinical Biochemistry 2004; 41: 201-206

McArdle PA, Canna K, McMillan DC, McNicol AM, Campbell R, Underwood MA
The relationship between T-lymphocyte subset infiltration and survival in patients with prostate cancer.
British Journal of Cancer 2004; 91: 541-3

McArdle PA, McMillan DC, Sattar N, Wallace AM, Underwood MA
The relationship between interleukin-6 and C-reactive protein in patients with benign and malignant prostate disease.
British Journal of Cancer 2004; 91: 1755-7

McArdle PA, Mir K, Almushatat ASK, Wallace AM, Underwood MA, McMillan DC
The inflammatory response, PSA and survival in patients with metastatic prostate cancer. Urologia Internationalis 2006;77 :127-9.

List of tables **Page**

List of figures **Page**

Summary of thesis

Prostate cancer remains a major global health problem. Each year, in the UK, more than 27,000 patients are diagnosed with prostate cancer and approximately 9,500 die of their disease. The therapeutic options range from treatments primarily aimed at cure, such as radical surgery or radical radiotherapy, in patients with organ-confined disease to palliative treatments such as hormone therapy or chemotherapy used in patients with advanced disease. In chapter 1, the epidemiology, pathology, clinical features and treatment of prostate cancer are discussed.

In chapter 2, the specific cellular and non-specific systemic inflammatory responses are discussed. It is now recognised that disease progression in cancer patients is not solely determined by the characteristics of the tumour, but also by the host response. Indeed, there is increasing evidence that both local and systemic inflammatory responses play an important role in disease progression in a variety of common solid tumours. Much of the initial work evaluating the role of the inflammatory response in cancer patients was carried out in patients with colorectal cancer. In these patients, it has been shown that both the local cellular inflammatory response and the systemic inflammatory response are important determinants of survival. The effects, however, of the local cellular and systemic inflammatory responses in patients with prostate cancer have not been established

The relationship between tumour bed lymphocyte infiltration and survival in patients with prostate cancer is as yet poorly understood. To date there have been two studies conducted in this area of research; however, the results appear contradictory. Moreover, the relationship between infiltration by specific lymphocyte subsets and survival has not been assessed. In chapter three, the influence of tumour-bed lymphocyte infiltration on survival in patients with prostate cancer was evaluated. It

was found that the presence of a prominent CD4+, but not CD8+, lymphocyte infiltrate in the tumour beds of patients with prostate cancer predicted poor survival, independent of stage. It has previously been suggested that this phenomenon may be explained by ineffective anti-tumoral immunity resulting from inactivation of lymphocytes in the tumour beds.

At presentation, there are a number of tumour-based factors which can predict survival in patients with prostate cancer. Predicting survival in the follow-up period is more difficult since PSA which is currently used for this purpose is normally suppressed by treatment. It is therefore of interest that the presence of a systemic inflammatory response has been shown to be an independent predictor of survival in patients with advanced gastrointestinal, lung and renal cancer. In chapter four, the effect of the systemic inflammatory response on outcome in patients with metastatic prostate cancer was investigated. It was found that the presence of a systemic inflammatory response, as evidenced by the presence of an elevated circulating C-reactive protein concentration predicted poor survival, independent of PSA. If these preliminary results were to be confirmed in a larger study, it might provide a rational basis for therapeutic intervention with anti-inflammatory drugs. This is potentially of particular importance in patients with hormone refractory disease where there are currently few effective treatment options.

The basis of the systemic inflammatory response in patients with prostate cancer is not well understood. However, it has been shown that many factors, including IL-6, TNF-α, IL-1, leukaemia inhibitory factor and ciliary neurotrophic factor can potentially induce the systemic inflammatory response and the production of C-reactive protein. In chapter five, the relationship between IL-6 and C-reactive protein in patients with untreated prostate cancer and benign prostatic disease was

evaluated. There was a close correlation between IL-6 and C-reactive protein both in patients with benign prostatic disease and those with prostate cancer. This suggests that IL-6 is the main factor mediating C-reactive protein production in patients with both benign prostatic disease and prostate cancer. Furthermore, in patients with prostate cancer there was no significant correlation between PSA and IL-6 concentrations. This observation suggests that in prostate cancer patients, the host may be responsible for production of IL-6 rather than the tumour itself.

Given that the inflammatory response appears to be important in predicting outcome in patients with prostate cancer, the question of whether C-reactive protein, could be used in the diagnostic setting to detect prostate cancer was explored in chapter 6. Although measurement of some PSA isoforms and their ratios could improve the specificity of cancer detection when compared to the use of the conventional total PSA test, C-reactive protein was unable to reliably distinguish between patients with prostate cancer and those with benign disease.

In conclusion, these studies suggest that in patients with prostate cancer, both the presence of a systemic inflammatory response and a profuse CD4+ lymphocyte infiltration of the tumour bed, predict poor survival. The negative impact of the systemic inflammatory response has been well documented in a variety of other common solid tumours. In contrast, the effect on survival of tumour bed lymphocyte infiltration appears to differ between tumour types. Whereas the presence of tumour-bed lymphocyte infiltration appears to be protective in patients with colorectal cancer, it appears to be detrimental in patients with prostate cancer. The reasons for these differences are as yet not clear. However, given that tumour lymphocyte infiltration parallels that of other inflammatory cells and that an elevated C-reactive protein is

associated with poor survival in both tumour types, it may be that the source of interleukin-6 differs in different tumours.

In colorectal cancer patients it has been reported that interleukin-6 concentrations increase with tumour stage and correlate with CEA concentrations. This would suggest that in colorectal cancer patients, interleukin-6 is produced by the tumour cells. In contrast, in patients with prostate cancer there is no significant correlation between serum interleukin-6 and PSA concentrations. It may therefore be that in patients with prostate cancer, interleukin-6 is produced by the host inflammatory cells rather than the tumour. If this were so, it might have implications for the treatment of the systemic inflammatory response in patients with different tumour types.

1.0 Introduction

Prostate cancer is the second commonest cause of cancer death in men in North America, Western Europe and the United Kingdom. In the United States, in 1998, more than 180,000 men developed prostate cancer and over 39,000 died of the disease (www.seer.cancer.gov). In the United Kingdom, in 2000, over 27,000 men developed prostate cancer and approximately 9,500 men died of their disease (www.crc.org.uk). In Scotland, over the same period, there were more than 1800 new cases detected and almost 800 deaths attributable to the disease. Incidence rates have been rising for many years and as a result the global health burden is increasing.

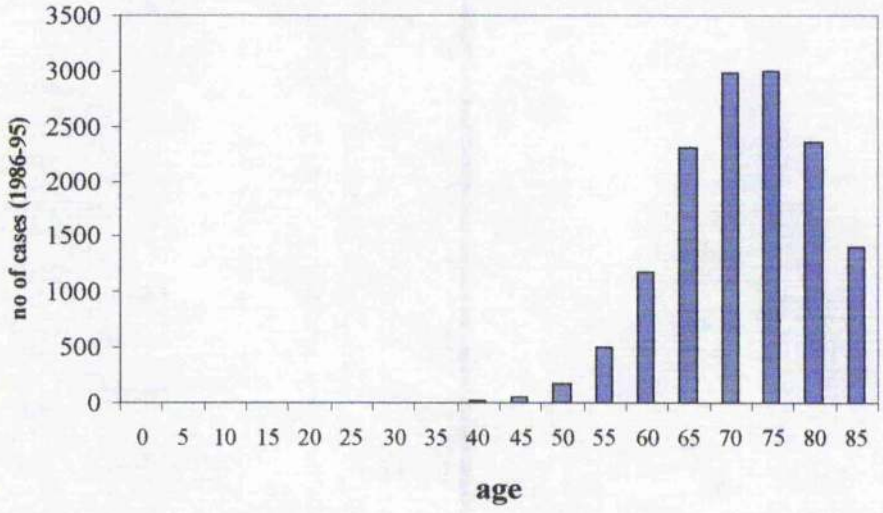
1.1 Incidence

The incidence of prostate cancer rises with age and varies significantly between different countries and ethnic groups.

Age

Prostate cancer is predominantly a disease of the elderly. For example, In Scotland, less than 0.5% of cases occur below the age of 50. The incidence rises rapidly with age, with 40% of cases occurring in patients over the age of seventy-five (ISD, 1998; Figure 1.1).

Figure 1.1: Prostate cancer and age at diagnosis in Scotland



These age related trends have been confirmed by a series of autopsy studies, which showed that approximately 30% of men in their fifties and up to 70% of those over the age of eighty showed evidence of latent prostate cancer (Breslow et al, 1977;Sheldon et al, 1980). Despite these findings, the life-time risk of developing clinically detectable prostate cancer up to the age of eighty is only 10%.

Geography

The incidence of prostate cancer varies seventy-fold between countries (SEER; Figure 1.2). The United States has by far the highest incidence. The incidence rates are higher in Northern Europe than Southern Europe. Incidence rates in the Far East are low.

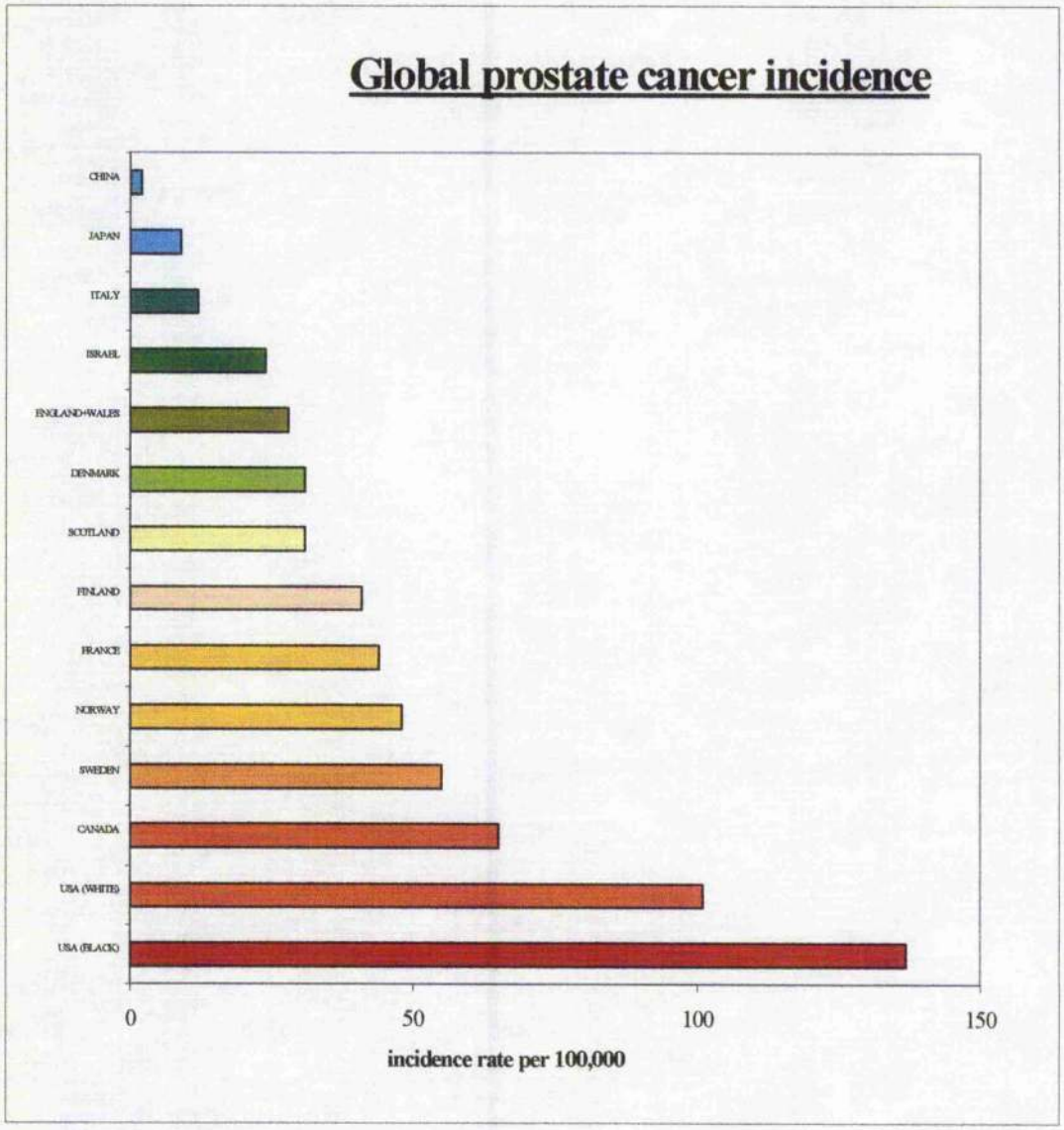


Figure 1.2 (Data obtained from SEER)

Ethnic groups

There are also marked ethnic variations in incidence. For example, in the US, the incidence rates vary almost five-fold among different ethnic groups, African Americans having the highest rates and native Americans the lowest.

Trends

Analysis of incidence rates has shown interesting trends. For example, in the United States, there was a three-fold increase in the incidence rate between 1973 and 1992. Thereafter, incidence rates declined (Figure 1.3). By comparison, the Scottish incidence rates have been consistently lower.

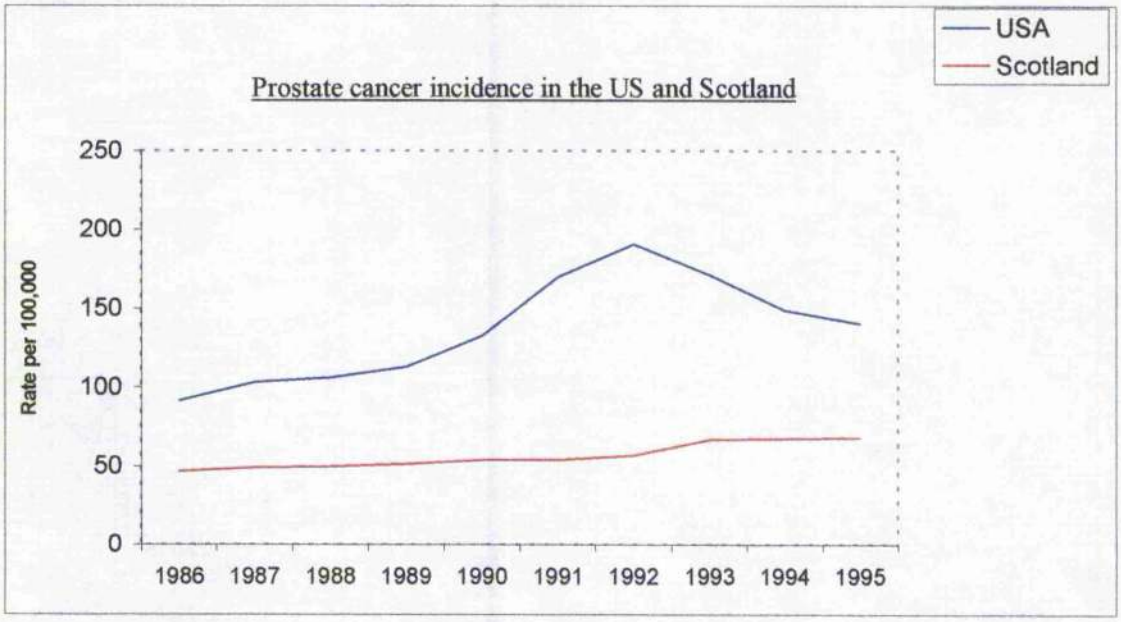


Figure 1.3 (Data obtained from SEER and ISD)

It has been suggested that the apparent increases in the incidence rates, particularly in the US, may have been, in part, due to increased detection of small, non-lethal cancers, as a direct result of the introduction of PSA as a diagnostic tool, rather than a true increase in the incidence of the disease (ISD, 1998; Brewster et al, 2000). Furthermore, in the US where screening is common, the high incidence reported may simply reflect the fact that more than 50% of white men over the age of 50 years will have had PSA measured (Gann, 1997).

1.2 Mortality

Cancer of the prostate is now the second commonest cause of cancer related death in men in the US (SEER), much of Europe and England and Wales (CRC). It is the third commonest cause of cancer related death in Scotland (CRC), (Figure 1.4).

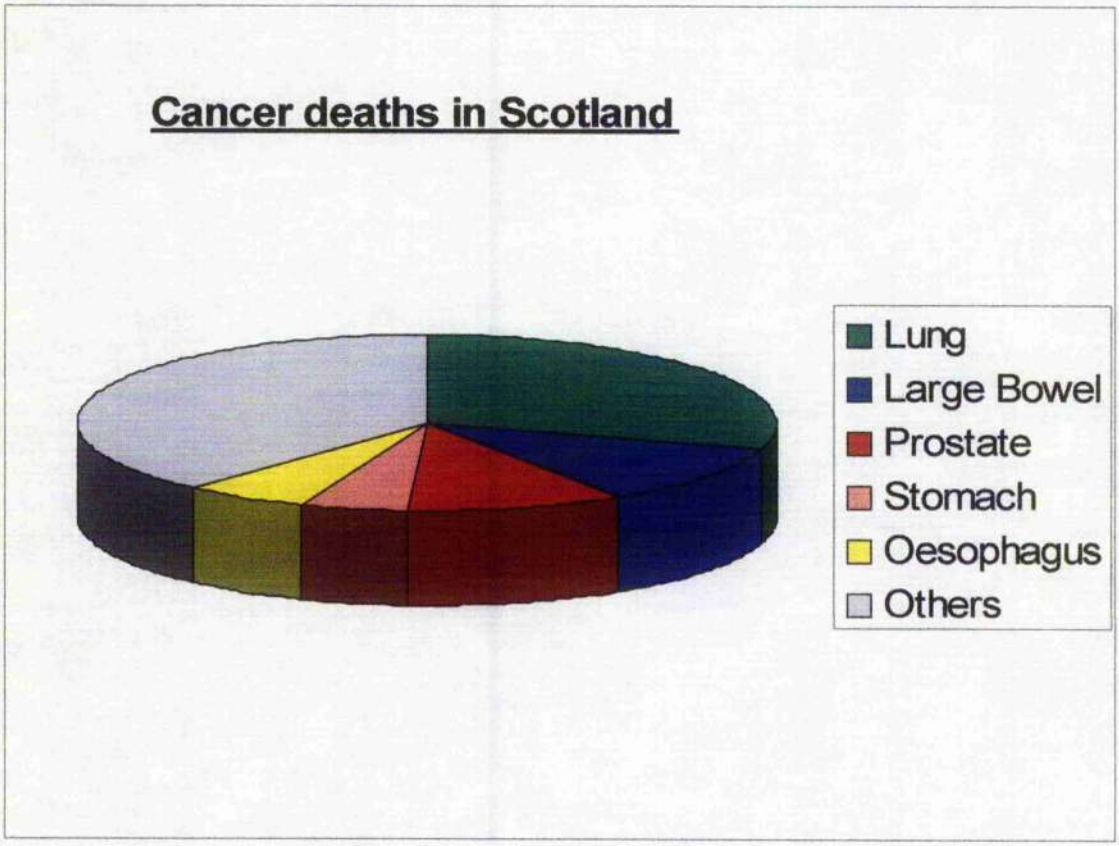


Figure 1.4 (Data obtained from CRC cancer stats 2002)

Prostate cancer related mortality varies between countries. In general, increases in the mortality rates over the past two decades have not been as marked as the increases in incidence rates over the same time period (ISD, 1998). For example, in the US, despite a three-fold rise in incidence rates between 1973 and 1991, the mortality rates remained relatively stable. This provides further evidence to suggest that the high

incidence rates reported in some countries may be due to the detection of latent disease.

1.3 Survival

There is evidence to suggest that improvements in the treatment of patients with prostate cancer have led to a genuine prolongation of survival. For example, in the US, five-year survival rose from 67% in the mid 1970's to 96% in the mid 1990's (SEER). In Scotland, the five year survival rate increased from 37% to 50% over the same period of time (ISD, 1998; Figure 1.5).

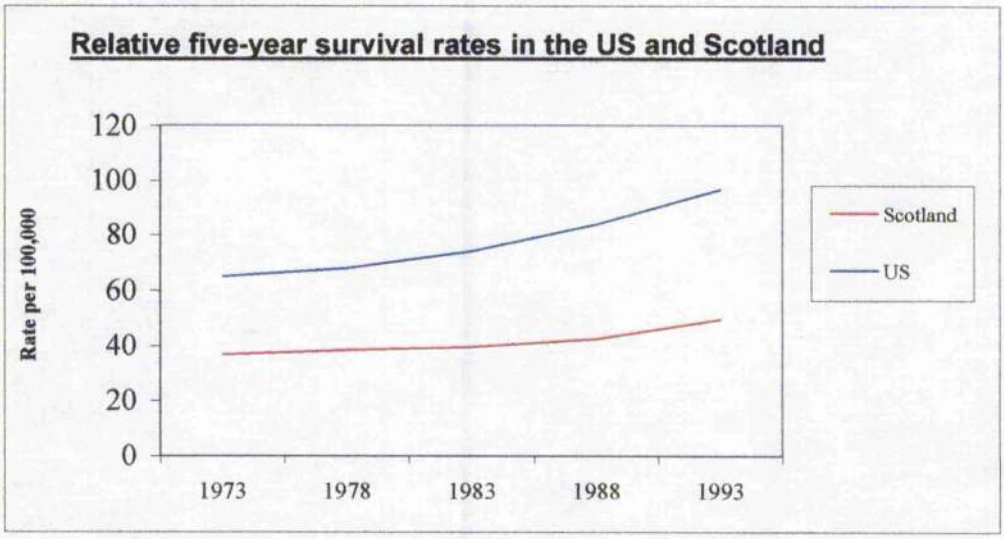


Figure 1.5 (Data obtained from SEER and ISD)

Clearly, survival rates in the UK appear to be lower than those in the United States. However, these apparent differences in survival must be interpreted with caution. These differences may in part be due to differences in case-mix, in particular the stage of disease at the time of diagnosis, variations in treatment or differences in the quality of registry-based data between countries.

A further possible source of bias is the variations in the use of prostate specific antigen (PSA) in different countries. The widespread use of PSA, either as a diagnostic test or as a screening tool, has led to an increase in the detection of small, localised tumours, most of which are unlikely to progress during the patients' lifetime. The inclusion of this additional cohort of patients may have contributed to the apparent increase in survival.

1.4 Aetiology of prostate cancer

In the West, prostate cancer is now one of the commonest malignancies to affect men. Despite the magnitude of the disease, little is known about the causes of prostate cancer. However, there is information to suggest that both genetic and environmental factors have a role to play in the development of the disease.

1.4.1 Environmental factors in prostate cancer

There is good evidence that migrants who move from areas of low incidence to areas with a higher incidence of prostate cancer acquire a higher risk of prostate cancer, often within one generation (Haenszel et al, 1968). For example, migrants from Japan and China to the United States have higher rates of prostate cancer than their original ethnic population (King et al, 1963) (Shimizu et al, 1991). There is also some evidence to show that special population groups, such as the mormons, whose lifestyle habits distinguish them from the general population, have higher incidence rates for prostate cancer (Lyon et al, 1980). These two different observations provide strong evidence that environmental factors contribute to the risk of developing prostate cancer.

Age and sex-hormones

It has long been known that male hormones (or androgens) such as testosterone, have an important role to play in the development and growth of the prostate gland. It is also thought that these androgens may also influence the development of prostate cancer (Kirby et al, 2000)

The precise role which androgens play in the development of prostate cancer is not fully understood. However, it appears that once established, the growth of prostate cancer may be partially dependant on androgen stimulation. It has been shown that prolonged administration of male sex hormones can also initiate prostate cancer growth in experimental models (Kirby et al, 2000). Furthermore, it has been noted that dihydrotestosterone (DHT) and testosterone levels are higher in neoplastic than benign prostate tissue (Habib et al, 1976). Despite this, serum levels of DHT and testosterone have not been correlated with the risk of developing prostate cancer (Ghanadian et al, 1979).

Dietary fat intake

High fat intake has been linked to an increased risk of developing prostate cancer in several studies (Rotkin, 1977; Schuman et al, 1977; Graham et al, 1983). Many mechanisms have been postulated to explain why high fat intake may encourage prostate tumour growth. These include modulaton of the inflammatory response, sex hormone metabolism, control of cell growth, apoptosis, lipid peroxidation and free radical formation (Pandian et al, 1999).

Vitamin A

It has been suggested by some studies that Vitamin A and its precursor beta-carotene may have a protective effect against prostate cancer (Rotkin 1977; Schuman et al, 1977). The possible protective effect of Vitamin A is supported by the observations that Vitamin A and synthetic retinoids inhibit and reverse the carcinogen-induced hyperplastic and anaplastic lesions in mouse prostate gland cultures (Chopra and Wilkoff, 1979).

Zinc, cadmium and selenium

A positive correlation has been established between estimated zinc and cadmium intake and mortality from prostate cancer. It has been suggested that zinc and cadmium may have this affect by antagonism with selenium (Zaridze and Boyle, 1987), which appears to have a protective effect to many cancers including prostate cancer (Schrauzer et al, 1977; Nomura et al, 2000; Brooks et al, 2001).

Vitamin D and calcium

It has been noted that prostate cancer seems to become more common in populations who live at increasing distances from the equator. Many have suggested that exposure to UV light and the production of vitamin D may, therefore, have a protective effect against the development of prostate cancer (Luscombe et al, 2001). This theory has been supported by the finding that a high calcium intake, normally associated with a high dairy food intake, is associated with a higher risk of developing prostate cancer (Chan et al, 1998). This observation may in part be explained by the theory that the high calcium intake, associated with a dairy diet, suppresses the production of vitamin D (Giovannucci, 1998).

Sexual activity

It has been suggested that sexual activity initiated early in life, multiple sexual partners and a history of sexually transmitted diseases (such as gonococcal infection) may increase the risk of subsequently developing prostate cancer. Others have, however, suggested that sexual activity may have little effect on the risk of developing the disease. One such study noted that 1400 reputedly celibate catholic priests had a similar incidence rate for prostate cancer as the rest of the male population (Ross et al, 1981).

Vasectomy

The relationship between vasectomy and risk of developing prostate cancer remains unproven. Some studies have suggested that a vasectomy almost doubled the risk of developing prostate cancer (Giovannucci et al, 1993). Other larger studies have failed to demonstrate a link between vasectomy and prostate cancer (Sidney,1987).

Occupation

Prostate cancer is more likely to develop in men who live and work in the city than those who live in a rural environment (Blair and Fraumeni, 1978). Moreover, workers in the rubber, textile, chemical, pharmacological, fertiliser and nuclear energy industries appear to be at particular risk. There is little evidence to conclusively identify any specific chemical agent. However, cadmium, tritium, and some isotopes of iron, cobalt, zinc and chromium have been suggested as candidates (Rooney et al, 1993).

Tobacco smoking and alcohol

Some studies have suggested a weak association between some types of smoking, such as cigar and pipe-smoking, and prostate cancer (Sharpe and Siemiatycki, 2001a). Overall however, a link between prostate cancer and smoking remains unproven (Lumey et al, 1997).

Most studies which have analysed the effect of alcohol consumption on the risk of developing prostate cancer have failed to demonstrate any convincing link (Breslow and Weed, 1998). However, it has been suggested that alcohol consumption at an early age (Sharpe and Siemiatycki, 2001b) or the consumption of spirits may be associated with increased risk (Sesso et al, 2001).

Non-steroidal anti-inflammatory drugs. (NSAIDs)

Non-steroidal anti-inflammatory drugs reduce the production of prostaglandins by inhibiting the cyclooxygenase enzymes. In recent years there has been interest in whether NSAIDs have the ability to decrease the risk of, or prevent the progression of human cancers. Indeed, there is evidence to suggest that NSAIDs can directly inhibit tumour growth in prostate cancer cell lines (Pollard and Luckert, 1986). Furthermore, there is growing evidence that regular consumption of NSAIDs reduces the risk of developing prostate cancer (Norrish et al, 1998; Roberts et al, 2002).

The use of anti-inflammatory drugs is not, however, without its hazards. Non-selective NSAIDs such as aspirin, indomethacin or ibuprofen which inhibit both the COX-1 and COX-2 enzymes are well known to cause platelet dysfunction, gastric ulceration and renal impairment (Henry DA, 1998). These side-effects are thought to be largely due to the COX-1 inhibitory effects. The development of selective COX-2 inhibitors or "coxibs" promised to provide effective anti-inflammatory action with a reduced side-effect profile. Unfortunately, however, it is now recognised that

although the coxibs do provide a lower risk of gastric ulceration, their use causes an increase in the risk of cardiovascular events such as mycocardial infarction. This has consequently led to a reduction in the use of coxibs.

1.4.2 Hereditary aspects of prostate cancer

Clinical studies have provided evidence of familial clustering in around 20% of prostate cancer cases (Aprikian et al, 1995; Keetch et al, 1995). However, only around 9% of cases are considered to be true hereditary cancers (Carter et al, 1992; Bastacky et al, 1995).

The earliest evidence for a genetic predisposition in prostate cancer came from studies which examined Mormons in Salt Lake City (Woolf et al, 1960). It was noted that prostate cancer appeared in clusters around specific families. Furthermore, Steinberg and his colleagues (1990) reported that first-degree relatives of patients with prostate cancer were almost twice as likely to develop the disease as the normal population. Furthermore, a subsequent study showed that men with two or three affected first- degree relatives had a 5 and 11-fold increase respectively, in the lifetime risk of developing the disease (Gronberg et al, 1996).

Moreover, data from a Swedish twin study has provided more convincing evidence to support the theory of genetic predisposition to prostate cancer (Gronberg et al, 1994). This study compared heterozygotic twins with their monozygotic counterparts. It was found that a monozygotic twin whose brother had developed prostate cancer was five times more likely to develop the disease than a brother of an affected heterozygotic twin (Gronberg et al, 1994).

The genetic basis for these observations is not clear. However, it is thought that many types of tumour may be initiated by specific genetic changes, which may be

inherited or occur spontaneously. In particular, it is known that alterations may occur in a group of quiescent genes called proto-oncogenes which convert them to active oncogenes. These oncogenes, once activated, are thought to initiate malignant change and promote tumour growth. Alternatively, tumour-suppressor genes, which normally act to inhibit tumour growth, may be "switched off".

The concept that there may be genetic factors which predispose to cancer in some patients has been reinforced by the discovery of specific gene mutations which have been associated with increased susceptibility to certain cancers, for example the BRCA1 gene in breast cancer (Lee and Boyer, 2001), and the APC gene in colorectal cancer (Su et al, 2000). It is therefore of interest that two highly penetrant genes which predispose to breast cancer (BRCA1 and BRCA2) also confer an increased risk of developing prostate cancer of 3 and 7 fold respectively (Gayther et al, 2000).

Furthermore, a major susceptibility locus for prostate cancer, the "Human Prostate Cancer Gene 1" has recently been identified on chromosome 1,24. This gene locus has been implicated in around 30% of the hereditary prostate cancers in one study (Smith et al, 1996).

1.5 Anatomy of the prostate

The name "prostate" is thought to have been derived from the Greek word "prohistani", which means to stand in front of, and was used to describe the anatomical relation of the prostate gland to the urinary bladder (Kirby et al, 2000).

The anatomy of the prostate gland is of importance when considering the distribution of benign and malignant disease to affect the gland. Furthermore, a clear understanding of the anatomy of the pelvis is essential when undertaking prostate surgery.

Gross Anatomy of the prostate gland

The prostate gland consists of right and left lateral lobes, which merge in front of the urethra. The middle lobe lies above the lateral lobes; enlargement of this lobe rapidly leads to urethral obstruction. The sphincter urethrae lies immediately beyond the prostate gland. Therefore, damage to the sphincter muscle fibres during prostate surgery can result in post operative urinary incontinence.

Important neurovascular bundles lying dorsolaterally to the prostate carry nerve fibres and blood to the corpora cavernosa of the penis, and are critical to the development of the normal erectile responses. Their division in the original radical prostatectomy procedure inevitably resulted in impotence. The observation that these neurovascular bundles lay outside the Dennovilliers fascial layer led to the development of the nerve sparing radical retropubic prostatectomy. This technique appears to be associated with a reduced incidence of post-operative impotence.

Zonal anatomy of the prostate gland

In 1968, McNeal described three distinct anatomical regions, namely the peripheral, transitional and central zones. The peripheral zone comprises almost 65% of the glandular volume. The central zone, comprises around 25% of the prostate volume. The smallest of these areas is the transitional zone. The distinctions between these areas are usually difficult to perceive, because, in the absence of disease, their anatomical boundaries are relatively subtle.

The clinical significance of the zonal anatomy of the prostate is in understanding which areas of the gland are susceptible to benign and malignant disease. Benign prostatic hypertrophy affects the transitional zone and frequently distorts and compresses the adjacent peripheral zone. In contrast, prostate cancer can

affect any zone of the gland, but is most commonly found to affect the peripheral zone.

1.6 Pathology of the prostate gland

Prostate cancer usually arises from tissue on the posterior aspect and the periphery of the gland (Quinn et al, 1990). Since both prostate cancer and benign prostatic hypertrophy are common histological findings, they are often found to co-exist.

The vast majority of prostate cancers are adenocarcinomas which arise from the epithelial lining of the secretory acini. Prostate cancer spreads within the gland, sometimes causing urethral obstruction. However, because it most frequently arises from the periphery of the gland, it has often spread locally to the adjacent structures or metastasised before urinary symptoms develop. Metastatic spread may be via the lymphatic system to the pre-sacral, iliac and para-aortic lymph nodes or blood borne to the skeleton, leading to the formation of characteristic osteosclerotic bone lesions (Muirs Textbook of Pathology, 1992).

Prostatic glandular tissues, including primary prostate adenocarcinomas and metastatic lesions, produce acid-phosphatase and prostate specific antigen (PSA). Specific antibodies to these markers can be used to confirm that metastatic deposits of adenocarcinoma originate from the prostate gland.

Wherever possible, cytological or histological confirmation of a diagnosis should be sought in any patient where there is a clinical suspicion of cancer. In the United Kingdom and the United States, most suspected cancers are confirmed by the use of spring-loaded devices, which can be used to take multiple core biopsies, often under ultrasound guidance.

Latent prostate cancer

Prostate cancer is unique among potentially lethal human cancers in that a wide discrepancy exists between the incidence of histologically-confirmed cancer and clinical disease.

In 1956, Franks reported that a high proportion of men examined at autopsy had microscopic foci of prostatic adenocarcinoma. These small tumours were found to affect around 30% of men over fifty years of age. Subsequent post-mortem studies have demonstrated a surprisingly high incidence of histologically proven prostate cancer in younger men. For example, in one study, Sakr and co-workers (1993) found small foci of prostate adenocarcinoma in 25-30% of men aged between 30 and 39 years of age.

Breslow and co-workers (1977) examined the geographical distribution of autopsy-detected prostate cancer. They reported that the incidence of small prostate tumours was similar in all seven countries studied, and that the incidence of these small tumour foci did not increase with age. However, the incidence of larger tumours detected at autopsy reflected the incidence of clinical disease in the local populations. Furthermore, the incidence of these larger lesions was found to increase with age and varied between ethnic population groups.

Scardino (1989) attempted to estimate the extent of the discrepancy between the high prevalence of the disease at autopsy and the low incidence of clinical disease. In 1985, in the United States, 86,000 men were diagnosed with prostate cancer and 25,500 men died of the disease. However, information from previous autopsy studies suggests that in the same year, over 8 million men would have harboured undiagnosed prostate cancer. Thus Scardino estimated that only 1 in 95 men with cancer were diagnosed with the disease, and only 1 in 323 men with prostate cancer died of the

disease. Clearly this suggests that the majority of small prostatic tumours, which can be detected at autopsy, do not progress to clinical prostate disease.

The term "latent prostate cancer" has been used to describe this phenomenon. It is thought that many of these small, well differentiated prostate cancers lack the ability to grow, de-differentiate and metastasise within a patients lifetime. Furthermore, it is known that prostate cancer cells grow slowly. Indeed, prostate cancers have been shown to have cell-doubling times of around two years (Stamey and Kabalin, 1989).

The biological significance of "latent carcinoma" remains unclear but it is known that many small prostate cancers are of little clinical importance. However, there is evidence that the increasing use of diagnostic tests, such as PSA, to detect prostate cancer is increasing the number of men diagnosed with "latent" disease. Unfortunately, there is no definitive method of identifying which of those lesions will progress to clinical disease and which are likely to remain quiescent over the patient's lifetime. Potentially, treatment of these latent cancers could result in inappropriate and unnecessary patient morbidity.

Premalignant change in the prostate gland

In 1986, McNeal and Bostwick identified the existence of dysplastic prostatic lesions which they called "intraductal dysplasia". It was noted that these lesions exhibited many features, including cytological atypia and nuclear polymorphism, which are associated with other premalignant conditions. In 1989, following a consensus meeting, intraductal dysplasia was renamed prostatic intraepithelial neoplasia or PIN. This brought the nomenclature into line with other pre malignant conditions such as CIN (cervical intraepithelial neoplasia).

The clinical implications of prostatic intraepithelial neoplasia are controversial. The presence of PIN has been shown to occur approximately a decade before the onset of prostate cancer (Sakr et al, 1996). Areas of PIN have been shown to be closely associated with foci of prostate cancer. Furthermore, the distribution of clinically relevant prostate cancer and PIN is similar (Quinn et al, 1990). It has also been shown that patients who possess widespread dysplastic change within their prostates are more likely to develop multi-focal malignancy (Quinn et al, 1990). This close correlation between PIN and malignancy therefore suggests that the two conditions are linked.

Pathological prognostic factors.

Many prognostic factors have been evaluated in prostate cancer; few have been shown to be of proven clinical value. A recent consensus conference organised by the College of American Pathologists (Bostwick et al, 2000) divided factors into three categories reflecting the strength of published evidence. Factors were ranked as: Category I, factors of proven prognostic importance, useful in clinical practice; Category II, factors that have been extensively studied but whose importance remains to be validated in statistically robust studies; and Category III, all other factors not sufficiently studied to demonstrate their prognostic value.

Category I prognostic factors include pre-operative serum PSA antigen level, TNM stage, histological grade and surgical margin status. The most commonly used grading system is that devised by Gleason (1977). Areas within the tumour were scored 1 to 5 according to the degree of dysplasia, the more dysplastic lesions being given higher scores. A "Gleason score" is calculated using combined scores of the two most prominent areas. This score was shown to provide important prognostic information and also to be readily reproducible (Gleason, 1992).

Category II factors include tumour volume, histological type and DNA ploidy. For example, tumour volume has been shown to correlate with tumour grade, larger volume tumours tending to be of higher grade than smaller prostate cancers (McNeal and Bostwick, 1986). Tumour volume has also been shown to relate to the frequency of capsular penetration and seminal vesicle invasion (McNeal et al, 1986).

Category III factors include perineural invasion, neuroendocrine differentiation, microvessel density, proliferation markers and oncogenes such as c-myc, c-erb-B2 and bcl-2. For example, angiogenesis, as evidenced by micro-vessel density, is thought by some to play an important role in tumour progression and metastasis (Halvorsen et al, 2000).

1.7 Clinical presentation

Over the past decade the pattern of presentation of patients with prostate cancer has changed dramatically. Historically, a relatively high proportion of men presented with locally advanced or metastatic disease. However, the introduction of more sensitive diagnostic tools such as trans-rectal ultrasound guided biopsy of the prostate has led to an increase in the numbers of men being diagnosed with early stage disease. For example, in the US, the number of patients presenting with localized prostate cancer more than doubled from 50 cases per 100,000 in 1985, to 110 cases per 100,000 of population in 1995 (SEER). During the same period, the number of newly diagnosed patients with metastases fell by 56%, from 14.9 to 6.6 cases per 100,000 head of population. As a result, there has been a marked shift in the pattern of clinical presentation at diagnosis.

1.7.1 Symptoms associated with prostate cancer

At diagnosis, most men with localised prostate cancer are asymptomatic. Although symptoms do occur in association with prostate cancer, they normally result from bladder outflow obstruction, local invasion by the tumour or metastatic disease.

Symptoms caused by bladder outflow obstruction are generally divided into irritative and obstructive symptoms. Irritative symptoms result from instability of the detrusor muscle of the bladder, secondary to obstruction, and include urgency and frequency. Obstructive symptoms result as a consequence of the mechanical obstruction of the urethra and include hesitancy, reduced urinary flow and incomplete bladder emptying. In extreme outflow obstruction, patients can develop a urinary retention, which requires urgent decompression.

In the US, 6% of men with prostate cancer have evidence of distant metastases at the time of their diagnosis (SEER). In the UK, the proportion is traditionally much higher. The spine is the commonest site of bone metastases (Gonzales et al, 1991). It is thought that blood draining from the prostate gland through the valve-less vertebral venous plexus may allow prostate cancer cells to metastasise directly to the lumbar vertebrae (Batson, 1940). In addition to the lumbar spine, prostate cancer metastases commonly affect other bones including the ribs, pelvis, sternum, humerus, neck of femur and skull. Bone metastases can produce a variety of complications including pain, pathological fracture, spinal cord compression and marrow failure.

1.7.2 Clinical examination

Examination of the prostate gland is facilitated by a digital rectal examination (DRE). This examination allows the clinician to assess the posterior surface of the prostate gland. Particular attention is paid to the size and symmetry of the gland, the

preservation of the median sulcus and the presence of any palpable prostatic nodules. Destruction of the median sulcus of the prostate or the presence of palpable nodules is suggestive of prostate cancer. DRE is also used to define the extent of local disease.

1.7.3 Prostate Specific Antigen

Prostate Specific Antigen (PSA) is a 28,400 Dalton glycoprotein comprising 237 amino acid residues and a oligosaccharide side chain. PSA is synthesised by the ductal and acinar epithelium of the prostate gland. It is secreted in the seminal fluid and acts to liquefy the seminal coagulum, thus releasing the entrapped sperm. The basement membrane of the prostatic epithelium normally creates a barrier preventing the escape of PSA into the circulation. Disruption of the basement membranes by disease allows PSA to enter the systemic circulation. The predominant molecular form present in the plasma is a complex of PSA and alpha 1-antichymotrypsin. Free PSA represents a small but variable proportion of the total PSA. Many disease processes within the prostate gland including benign prostatic hypertrophy, infection and inflammation are associated with elevated circulating PSA concentrations.

PSA and benign prostatic hypertrophy

Benign prostatic hypertrophy (BPH) is one of the commonest ailments affecting aging men, with more than 60% of men in their sixties having histological evidence of the disease. BPH leads to an increase in prostatic size and subsequently an increase in the circulating PSA concentrations. It has been estimated that for every gram of prostate tissue present there is an increase in circulating PSA concentrations of 0.3micrograms per litre (Stamey et al, 1987).

PSA and inflammation of the prostate

Inflammation of the prostate gland can be acute or chronic, bacterial or sterile. Acute infection within the prostate commonly produces a short-lived elevation in the concentrations of circulating PSA. In contrast, chronic prostatitis only produces an elevation in circulating PSA concentrations in around ten percent of patients (Pansadoro et al, 1996). Other non-infective causes of prostatic inflammation including urethral catheterisation, prostatic biopsy, digital rectal examination and acute urinary retention have also been shown to produce an increase in circulating PSA concentrations (Crawford et al, 1992).

PSA and Prostatic Intraepithelial Neoplasia

Prostatic Intraepithelial Neoplasia (PIN) is characterised by cytologically atypical cells lining architecturally normal ducts. As PIN does not lead to basement membrane disruption, serum PSA concentrations are unaffected.

PSA and prostate cancer

The rapid rise in the use of prostate specific antigen is a result of its close association with prostate cancer. The growth of prostate cancer within a gland leads to the disruption of the basement membranes and subsequently to leakage of PSA into the circulation. However, as discussed earlier, prostate cancer is not the sole cause of increased circulating concentrations of PSA.

Since the introduction of PSA, the mode of presentation of prostate cancer has changed markedly (Murphy et al, 1999). Prior to the introduction of PSA, nearly all prostate cancers presented at an advanced stage with either bladder outflow obstruction or skeletal metastases. In contrast, around one third of men currently

diagnosed with prostate cancer in the UK present with only a raised PSA (Milford Ward et al, 2001).

One of the problems with the use of PSA currently is the lack of an international standard. At present different assays may produce widely varying results from the same serum sample. The scale of this problem is put in proportion when it is considered that in Europe alone there are currently around eighty different PSA assays available. This causes difficulties in compiling reference ranges for benign and malignant disease. PSA values obtained using different assays are therefore not directly comparable. Furthermore, many manufacturers have not established normal control values for their assay.

Reference ranges of PSA

The most commonly quoted upper limit of "normal" was established using the original Hybritech PSA assay. Almost 500 men with no history of prostate disease were involved in the study. When circulating PSA concentrations were measured, 4 micro-grams per litre represented the 99th centile in the study population (Myrtle et al, 1986). Many clinicians therefore commonly use this value as an upper reference limit. However, in more recent studies approximately 18-25% of men found to have prostate cancer had serum PSA concentrations below this value (Kirby et al, 2000). Furthermore, around two thirds of men who have serum PSA values greater than 4 micro-grams per litre will have benign histology on initial prostate biopsy (Kirby et al, 2000).

This lack of specificity has led to the development of several methods of interpreting PSA with the aim of improving specificity without a significant reducing sensitivity. These include age specific reference ranges, PSA density and PSA velocity.

Age specific PSA reference ranges

It is known that circulating PSA concentrations rise with age. Age specific ranges were introduced as a stratagem to improve sensitivity in the older age groups, whilst preserving sensitivity in younger men. In practice, this means lowering the PSA threshold for investigation in younger patients. In older patients, the PSA threshold is often raised above the standard cut-off level of 4ng/mL to reduce unnecessary biopsies.

PSA Density

This technique relates circulating PSA concentration to glandular volume. It is thought that in some cases this may help separate large benign glands from malignant prostates.

PSA velocity

Another approach to improving assay specificity is PSA velocity. This approach measures the rate of change in circulating PSA concentrations over a given period of time. The measurement of PSA doubling times is similar. It is thought that the rate of change in PSA concentrations in patients with malignancy is greater than in those with benign disease. At present there is no clear evidence that PSA velocity or doubling times are superior to conventional PSA measurements in the diagnosis of prostate cancer (Perrin, 2006). However, recent studies have indicated that a PSA velocity of >2ng/mL/year is an important predictor of poor outcome in patients being treated with radical surgery (D'Amico et al, 2004) or radiotherapy (D'Amico et al, 2005).

PSA and stage of disease in prostate cancer

Serum PSA concentrations are also useful in distinguishing patients with localised disease from those who have metastatic disease at presentation. Oesteling et al (1993) showed that of approximately 300 men with a circulating PSA concentration of less than 20 micro-grams per litre, only one patient had evidence of skeletal metastases. Other groups have reported similar findings (Lee et al, 2000). The reliability demonstrated by PSA combined with the high cost of nuclear imaging has led some authorities to advise bone scanning only in those patients with PSA concentrations above 10 micrograms per litre.

1.7.4 Biopsy of the prostate gland.

Once abnormal biochemistry or suspicious findings on digital rectal examination have raised the suspicion of prostate cancer, the clinician will normally seek pathological confirmation of malignancy. Historically, prostatic biopsy to obtain a tissue diagnosis of cancer was performed blind. However, the advent of ultrasound technology offered a new way to evaluate the prostate, and biopsy techniques were adapted to incorporate ultrasound guidance.

Transrectal ultrasound and biopsy.

Transrectal ultrasound (TRUS) was first used to evaluate the prostate in the 1960's (Takahashi and Ouchi, 1963). However, the images obtained at that time were of poor quality. As ultrasound technology has become more sophisticated, the use of TRUS in the evaluation of prostate disease increased. By the mid 1980's, use of the 7 MHz ultrasound probe, which more clearly delineated the architecture of the prostate, had become an established diagnostic approach.

TRUS has three primary roles in the assessment of prostate disease. Firstly an estimate of the prostate volume can be made, secondly, abnormal areas of the gland can be targeted for biopsy and thirdly, the anatomy of prostate can be visualised allowing systematic biopsies of ultrasonically "normal" tissue to be taken.

Estimation of the prostate volume.

As circulating PSA concentrations are partially determined by the volume of prostate tissue within the gland, it is useful for the clinician to have an estimate of the size of the gland (Brawer and Chetner, 1997). The importance of prostate volume is apparent when considering the concept of PSA density. This method of interpreting PSA should offer a way of differentiating between those patients who have a raised PSA as a consequence of large glandular size, and those patients whose glands are producing an excess of PSA in proportion to their size. In theory, patients in the second group should be at higher risk of harbouring prostate cancer.

The use of TRUS to target suspicious lesions within the prostate gland.

Areas of the gland are said to be hyperechoic, isoechoic or hypoechoic depending on whether the ultrasound image produced is lighter, similar to, or darker than the image generated by normal peripheral zone tissue. Cancer of the prostate was initially thought to have a hyperechoic appearance on ultrasound; however, more recent studies have shown that the most common ultrasound finding in prostate cancer is a hypoechoic peripheral zone lesion (Lee et al, 1986).

However, TRUS is not without its weaknesses. For example, hypoechogenic images within the prostate gland may also occur in other diseases such as prostatitis, prostatic infarction, scarring of the gland and prostatic intraepithelial neoplasia (Langer, 1999). Furthermore, up to 40% of prostate cancers are isoechoic and

therefore "invisible" to TRUS. This number may be even higher today as more patients present with early stage disease.

The limitations in the sensitivity and specificity of TRUS as a method of identifying malignant lesions has led to the exploration of novel ultrasound techniques, such as the use of intravenous microbubble ultrasound contrast agents (Frauscher et al, 2001).

Systematic TRUS-guided biopsy of the prostate

In the PSA era patients are presenting with earlier disease, when tumours are more commonly non-palpable and isoechoic. An important function of TRUS has become the ability to demonstrate the anatomy of the prostate and therefore facilitate systematic tissue sampling from all relevant areas of the gland, even when the ultrasound image generated by that tissue is normal. In 1989, Hodge and co-workers demonstrated that cancer detection rates could be greatly improved if systematic sextant biopsies of sonographically "normal" prostate tissue were taken.

1.8 Staging of Prostate cancer

The aim of staging is to define the extent of the disease process. The first clinical classification system widely used in the assessment of prostate cancer stage was introduced by Whitmore in 1956, and later updated by Jewett in 1975. These classification systems have now largely been replaced by the TNM system of staging (see Table 1.1). The TNM classification breaks tumour stage into three components; the T component which reflects the extent of disease at the primary site, the N component which reflects the presence or absence of nodal metastases and the M component which indicates the presence or absence of distant tumour metastases. This method of classification became established internationally in the early 1990's

and was updated in 1997. The 1997 revision resulted in changes to the original system, including the amalgamation of the T2a and T2b stage groups and reassignment of T2c tumours as T2b cancers. In addition, the distinction between T3a and T3b tumours has been abandoned and T3c tumours are now assigned to the T3b category (Hoedemaeker et al, 2000).

Table 1.1- A comparison of the TNM and Whitmore-Jewett staging classifications

TNM (1992)	Whitmore-Jewett	Description of stage
Tx		Tumour can not be assessed
T0		No evidence of tumour
T1a	A1	Tumour an incidental finding at TURP (<5% of tissue involved)
T1b	A2	Tumour an incidental finding at TURP (>5% of tissue involved)
T1c	B0	Non palpable tumour, identified by raised PSA
T2a	B1	Tumour involves half a lobe or less
T2b	B1	Tumour involves more than half a lobe but not both lobes
T2c	B2	Tumour involves both lobes
T3a	C1	Unilateral extra capsular tumour extension
T3b	C1	Bilateral extra capsular tumour extension
T3c	C2	Tumour invading seminal vesicles
T4a		Tumour invades bladder, external sphincter or rectum
T4b		Tumour invades levator muscles or is fixed to pelvic side wall

1.8.1 The clinical assessment of local tumour extent.

The Digital Rectal Examination.

Accurate assessment of local tumour extent is particularly important in patients who are being considered for radical treatment. The traditional method of evaluating the local extent of prostate cancer is based on digital rectal examination (DRE). If an area

of abnormality, suggestive of prostate cancer, is found the extent of disease (T-stage) can be defined on the basis of the criteria outlined in Table 1.1.

Whilst there is some correlation between DRE and final pathological stage, this method of assessing local tumour extent has its limitations. In addition to being subject to clinical experience and inter-observer error, the DRE frequently underestimates the extent of disease (Ohori et al, 1994). For example, it has been shown in patients undergoing radical prostatectomy that only 41% of patients predicted to have T2b cancers and only 35% of patients predicted to have T2c prostate cancers had organ confined disease (Partin et al, 1993).

Transrectal ultrasound.

Transrectal ultrasound (TRUS) is the commonest modality used to image the prostate gland. Some cancers have variable echo characteristics, on ultrasound, which can be used to predict tumour extension through the capsule or into the seminal vesicles. The criteria for extracapsular extension include bulging or discontinuity of the boundary echo. Seminal vesicle invasion is suggested by fullness and loss of the normal tapering of the gland at the prostate base (Wilkinson and Hamdy, 2001). Unfortunately, recognising these subtle findings appears to be largely operator-dependant and this has led to variations in the accuracy of TRUS as a staging modality (Hardeman et al, 1989; Rifkin et al, 1991).

More recently two large trials have suggested that TRUS does not perform better than the digital rectal examination as a staging modality for predicting organ confined prostate cancer (Smith et al, 1997; Liebross et al, 1999). The authors concluded that DRE provided prognostic information at least equivalent to TRUS and was preferable because of its low cost.

Magnetic Resonance Imaging (MRI)

On MRI, extra-capsular prostate cancer extension is suggested by asymmetry of the neurovascular bundle, obliteration of the retroprostatic angle, asymmetry of the seminal vesicles and loss of the normal fat plane between the base of the prostate and the inferior aspect of the seminal vesicles (Wilkinson and Hamdy, 2001). Rifkin and co-workers (1991) assessed the ability of MRI to predict final pathological stage in patients with prostate cancer and found that MRI correctly staged 77% of cases with advanced disease and 57% of patients with localised prostate cancer

1.8.2 The assessment of lymph node status.

Historically, around 20% of patients undergoing radical prostatectomy had lymph node metastases at the time of surgery. More recently, improvements in nodal staging and better patient selection has meant that only 2-3% of patients undergoing radical prostate surgery have lymph node metastases (Kirby et al, 2000).

Since the techniques for screening potential surgical candidates for lymph node metastases are time-consuming, expensive and not always reliable, it is generally accepted that only those patients thought to be at high risk of having nodal disease should be investigated. For example, it has been reported that patients with high-grade tumours (Gleasons score 8+) and with circulating PSA concentrations greater than 20 are at high risk of having lymph node metastases (Partin et al, 1993) and therefore warrant investigation.

Computed tomography (CT).

The diagnosis of lymph node metastases by CT is based solely on the detection of lymphadenopathy. Any lymph node found to be larger than 1cm is considered to be abnormal (Wilkinson and Hamdy, 2001). However, although the reported specificity

of CT in detecting lymph node metastases is high, the sensitivity is low (Rorvik et al, 1998). The low sensitivity levels reported probably reflect the fact that most lymph node metastases are microscopic and do not therefore result in detectable lymph node enlargement.

Magnetic Resonance Imaging (MRI)

MRI has an advantage over CT in that it can be used to assess lymph node status at the same time as assessing the prostate gland itself. However, the sensitivity and specificity reported for the detection of lymph node metastases using MRI varies widely between studies (Wolf et al, 1995; Jager et al, 1996). The low detection rates limit the usefulness of CT and MRI in assessing lymph node status prior to radical therapy.

Staging lymphadenectomy

Lymph nodes infiltrated with prostate cancer are often not enlarged, and as a result non-invasive radiological imaging has often proven to be unreliable as a method of assessing lymph node status. Pelvic lymphadenectomy remains the "gold standard" method of obtaining a histopathological diagnosis of lymph node metastases.

Lymphadenectomy is not usually directed at all patients in whom radical treatment is being considered, but instead is targeted at groups of patients thought to be at high risk of having lymph node metastases. Lymphadenectomy can be carried out laparoscopically or as an open procedure before radical therapy or at the time of prostatectomy. High specificities, accuracies and predictive values are reported in patients undergoing staging lymphadenectomy (Alagiri et al, 1997).

1.8.3 The assessment of distant metastatic spread.

Prostate cancer most commonly spreads to the well vascularised areas of the skeleton, including the spine, ribs, skull and the proximal ends of the long bones. These metastases may cause bone pain, hypercalcaemia and pathological fractures. Infiltration of the bone marrow can cause marrow suppression and leukopenia. Less commonly, metastatic sites in the vertebral column can result in the impingement of nerve roots or the spinal cord giving rise to nerve root compression syndromes or spinal cord compression.

Historically, 30-35% of patients newly diagnosed with prostate cancer in the USA had evidence of bone metastases at presentation (Lee and Oesterling, 1997). Today, as a result of improved and earlier detection, the proportion of men presenting with advanced prostate cancer is much smaller. For example, it has been estimated that approximately 8% of white Americans now have metastatic disease at the time of presentation (Landis et al, 1999). However, a large proportion of patients will develop bone metastases despite attempts at curative therapy. Indeed, 85-100% of patients who die of prostate cancer have bone involvement (Carlin and Andriole, 2000).

The detection and monitoring of bone metastases is therefore important for a number of reasons. Firstly, the detection of bone metastases makes any use of radical therapy inappropriate. Secondly, it alerts the clinician to the possible complications of metastatic disease such as pathological fracture, pain or cord compression and allows the clinician to initiate appropriate therapy quickly. Finally, the detection of bone metastases has important prognostic implications for the patient.

Radionucleotide bone scanning

The most sensitive and widely used method of detecting osseous metastases in patients with prostate cancer is technetium 99m bone scanning. Although false negative results occur in less than 1% of cases, false positive results are more common. For example, hot spots may reflect a spectrum of benign disease including Pagets disease, trauma and degenerative bone disease as well as metastatic bone deposits (Kirby et al, 2000).

Traditionally, a high proportion of patients newly diagnosed with prostate cancer underwent bone scanning at diagnosis. More recently, several authors have been successful in identifying groups of patients at low risk of having bone metastases in whom bone scanning may not be indicated. Several groups have examined the relationship between PSA at diagnosis and the risk of having a positive bone scan Gleave et al, 1996). For example, Kosuda and co-workers (2002) reported that only 1% of patients with PSA concentrations less than 10ng/ml had a positive bone scan. Both these authors conclude that it was possible to omit base-line bone-scans in patients with serum PSA concentrations less than 10ng/ml.

Guidelines from the National Comprehensive Cancer network recommend a bone scan in those patients who have T1 or T2 disease only if there serum PSA concentrations are greater than 10ng/ml or whose Gleasons score is eight or above. It is also recommended that all patients who are symptomatic or who have T3 or T4 disease undergo bone scanning (Baker et al, 1996).

Plain film X-ray

The majority of bone metastases from prostate cancer are osteosclerotic. Plain film X-rays have low sensitivity levels for the detection of bone metastases, since a 50% change in bone density must occur before metastatic lesions can be detected on plain

film. In contrast, the high specificity for the detection of bone metastases associated with plain film X-ray makes them of use in differentiating between benign and malignant disease in patients with equivocal bone scans (Carlin and Andiole, 2000).

Magnetic Resonance Scanning (MRI).

It has been suggested that MRI might offer an alternative to bone scanning as a screening tool for bone metastases in prostate cancer patients. For example, Venz and co-workers (1994) demonstrated increased sensitivity and specificity for the detection of bone metastases using MRI when compared to routine bone scans. The main advantage of MRI was an increased ability to discriminate between benign and malignant bone disease.

Markers of bone turnover.

Alkaline phosphatase, one of the older biochemical tools used for the investigation of metastatic bone disease, remains a reliable and widely used marker of osteoblastic activity. In one recent study examining the relationship between bone scan results and serum PSA and alkaline phosphatase concentrations, the authors concluded that alkaline phosphatase concentrations correlated better with the outcome of the bone scans than did PSA (Wymenga et al, 2001).

Prostate specific antigen (PSA) and stage

Previous studied have shown that there is a good correlation between serum PSA concentrations and clinical stage in patients with prostate cancer. For example, one large study, which examined 703 men undergoing radical prostatectomy for prostate cancer, reported that 75% of patients whose serum PSA concentration were less than 4ng/ml, 53% of patients with PSA concentrations between 4 and 10ng/ml, 26% of

patients with PSA concentrations between 20 and 30ng/ml and only 8% of patients with serum PSA concentrations between 30 and 40ng/ml had organ confined disease (Partin et al, 1993). Furthermore, the authors reported that the incidence of capsular penetration, seminal vesicle involvement and lymph node metastases rose as PSA concentrations increased (Figure 1.6).

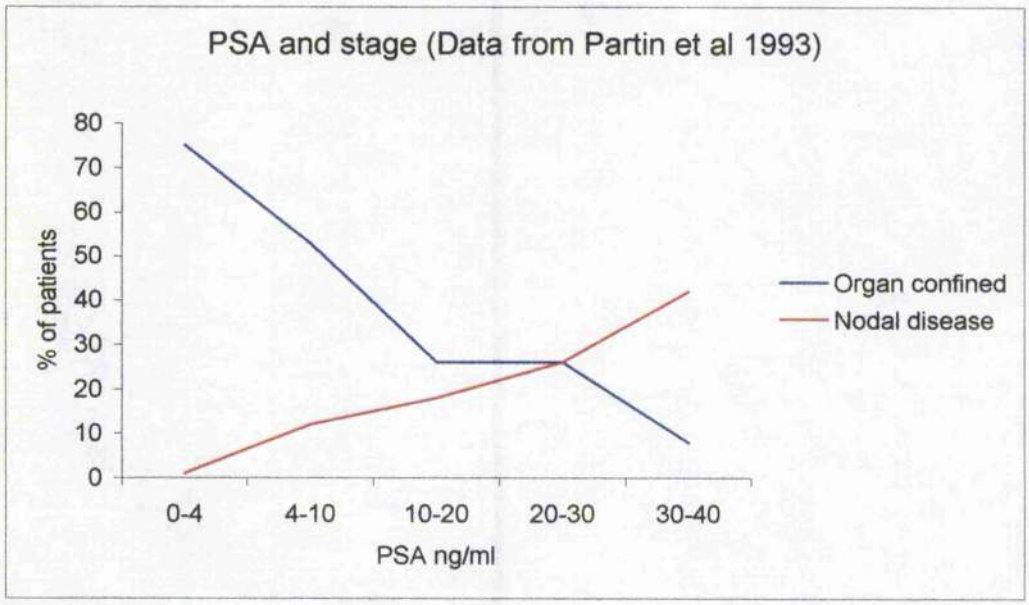
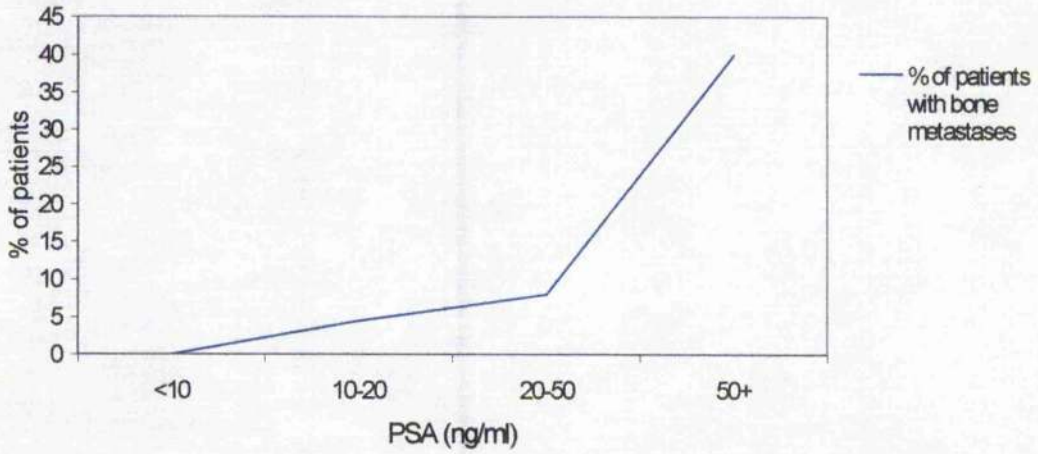


Figure 1.6 The relationship between PSA and extent of disease

Similarly, Gleave and co-workers reported that no patients with PSA concentrations below 10ng/ml had evidence of bone metastases on bone scan, whereas 5% of patients with PSA concentrations between 10 and 20 ng/ml, 8% of patients with PSA concentrations between 20 and 50ng/ml and 40% of patients with serum PSA concentrations above 50ng/ml had evidence of bone metastases (Gleave et al, 1996) (see Figure 1.7 on next page).

PSA and the incidence of bone metastases (Data from Gleave et al 1996)



Biopsy characteristics and staging

The vast majority of patients who are diagnosed with prostate cancer will have their diagnosis confirmed by biopsy. These tissue samples provide valuable information about the tumour, which may be used to help predict stage.

Gleason score.

Partin and co-workers (1993) examined 703 men undergoing radical prostatectomy for clinically localised prostate cancer. They reported that preoperative Gleasons scoring of biopsy material correlated strongly with final pathological stage in patients with low or high-grade disease. However, the majority of prostate cancer patients were reported to have intermediate grade tumours (Gleasons scores between 5 and seven). For example, of the 64 patients with low grade tumours (Gleasons score 2-4), 49 had organ confined disease, 12 had extra capsular extension, 2 had seminal vesicle involvement and one had lymph node metastases. In contrast, of the 38 patients with

high-grade disease (Gleasons score 8-10), only 5 had organ-confined disease, 9 had extracapsular extension, 8 had seminal vesicle involvement and 16 had nodal disease (Figure 1.8).

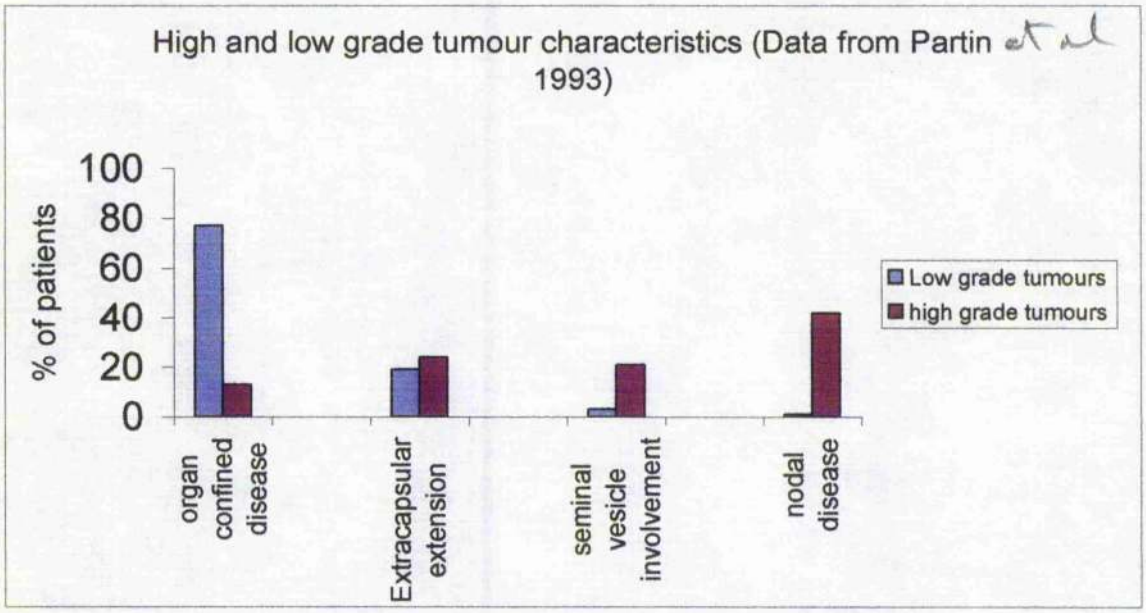


Figure 1.8 The relationship between tumour grade and stage

Tumour volume

It has long been recognised that low volume prostate cancers are far less likely to extend beyond the prostate capsule than high volume tumours (Voges et al, 1992). At present, imaging studies allow the clinician to accurately measure the total volume of the prostate gland, but not the tumour volume itself. As a result, some authors have advocated using the number or proportion of tumour positive core biopsy specimens as a surrogate indicator of tumour volume. For example, Ackerman and co-workers (1993) examined candidates for radical prostatectomy. They found that only 12% of patients with a single positive core biopsy had positive margins following surgery, compared with 50% of patients who had between 2 and 6 positive core biopsies.

Combining clinical predictors of stage.

There are a number of clinical indicators, which can be used to predict stage. However, all of these parameters have limitations when used in isolation. Several authors have attempted to combine some of these factors to improve their predictive value. For example, Partin and co-workers (1993; 1997) showed that the combination of clinical stage, preoperative PSA concentrations and Gleason scores improved the ability to predict pathological stage. They also constructed probability plots and nomograms to assist the preoperative prediction of the final pathological stage for patients with clinically localised prostate cancer.

Clearly this information is important to enable clinicians and patients to make more informed decisions about potential therapeutic options and to better understand the likely outcome of treatment.

1.9 Management of prostate cancer.

1.9.1 The management of localised disease.

The optimal management of prostate cancer is determined primarily by tumour stage and grade, age, general health and individual patient preference. The main strategies for managing localised prostate cancer include watchful waiting, external beam radiotherapy, brachytherapy and radical prostatectomy. However, there is ongoing debate amongst surgeons and oncologists about the relative merits of surgery and radiotherapy. The increasing numbers of localised prostate cancers, which are being detected by PSA testing, fuels this debate.

Watchful waiting

The watchful waiting approach to the treatment of localised prostate cancer is based on the knowledge that a large proportion of patients with prostate cancer will not die from the disease. Furthermore, aggressive treatment, such as radiotherapy or surgery, is often associated with considerable morbidity. Watchful waiting allows clinicians to monitor a patient's progress and intervene if the patient develops symptoms or evidence of disease progression.

Much of the information about the expectant management of prostate cancer comes from studies carried out in Sweden. For example, Johansson (1994) evaluated survival in more than 220 Swedish men with early stage prostate cancer. He reported that only 10% of the patients, managed conservatively, died of their cancer over a ten-year period. In contrast, Aus (1994) suggested that prostate cancer was a far more aggressive disease and that there was considerable morbidity and mortality associated with expectant management. In his study, almost two thirds of patients treated conservatively eventually died from their cancer.

The apparent differences between these studies probably reflect in part the tumour and patient characteristics of the two cohorts and the length of follow-up. It is therefore of interest that Zietman and colleagues (2001) recently reported that over half of men initially managed conservatively were commenced on active treatment within five years. Therapy was most commonly initiated following small but inevitable rises in circulating PSA concentrations. This observation reflects the unease that many patients and clinicians have in monitoring disease progress whilst withholding potentially curative treatment.

Overall, tumour grade appears to be the most important determinant of survival in patients with clinically localised prostate cancer treated conservatively.

For example, Denis (2000) compared survival in cohorts of patients with localised prostate cancer treated either conservatively, or with surgery or radiotherapy and found that grade was a strong predictor of 10 year survival. Furthermore, in patients with low-grade tumours (Gleasons score 2-4), ten-year cancer specific survival was comparable in all three patient groups. In contrast, patients with high-grade tumours (Gleasons score 8-10) treated conservatively had a poorer ten-year survival than those treated with either surgery or radiotherapy.

In conclusion, watchful waiting is probably an appropriate treatment option for some patients with low volume, low grade localised prostate cancers in whom life-expectancy does not exceed 10 years.

External beam radiotherapy

Radiotherapy is a potentially curative treatment in patients with localised prostate cancer. It is known that both increasing T-stage and a high gleason score compromises the chance of achieving cure with radiotherapy (Bagshaw et al, 1988; Roach et al, 1999). However, circulating PSA concentration at diagnosis is perhaps the most significant predictor of clinical outcome in patients being treated with radiotherapy. For example, Lee and colleagues (1995) reported that 86% of patients with pre-treatment PSA concentrations less than 15ng/ml had no evidence of biochemical relapse at 3 years, compared to only 38% of patients with PSA concentrations greater than 15ng/ml.

These studies suggest that the ideal candidate for radiotherapy would have an early stage, low-grade tumour and a pre-treatment PSA concentration less than 15ng/ml. Radiotherapy is therefore often the preferred treatment option in elderly patients or patients with significant co-morbidity which might make them unsuitable for radical surgery.

Radiotherapy is, however, not without risks and side effects. Shipley and colleagues (1994) reported a 0.2% mortality rate associated with treatment, a 0.9% incontinence rate and a potency rate of 33-60% following radiotherapy. Radiotherapy can also induce inflammatory changes in the rectum and bladder leading to proctitis, cystitis and urethral stricture formation.

Prostate specific antigen is used to assess the response to radiotherapy in patients with prostate cancer. For example, it has been reported that if a PSA nadir of <0.5ng/mL was achieved, the biochemical relapse rate, defined as three successive PSA rises after reaching a nadir, was <10%. In contrast, patients who failed to reach this nadir value had a biochemical relapse rate of around 54% (Zietman et al, 1996).

To date there have not been any randomised controlled trials comparing the results of external-beam radiotherapy and radical prostatectomy. Historically, comparisons between these treatment options have often reported results which favour surgery. However, patients undergoing surgery are more likely to undergo lymph-node dissection than those undergoing radiotherapy and are therefore more likely to be staged correctly. Since lymph-node metastases are unlikely to be detected in patients undergoing radiotherapy, there is an intrinsic survival bias in favour of surgery. In many recent studies, where staging is thought to be more accurate, similar results are reported for surgery and radiotherapy. For example, Kupelian et al (2002) reported that the eight-year biochemical relapse free survival rates for surgery and radiotherapy were 72% and 70% respectively.

Brachytherapy

Brachytherapy is an alternative method of delivering high radiation doses directly to the prostate whilst minimising exposure to the surrounding non-target tissues. Brachytherapy for the treatment of prostate cancer was popularised by Whitmore and

colleagues (1972). However, despite initial reports of encouraging local control and survival, there were a high proportion of late local failures. Interest was regenerated in the technique when Holm and colleagues (1983) described the use of transrectal ultrasound (TRUS) to guide the implantation of brachytherapy seeds. With a half-life of around 60 days and low-energy photon emissions, Iodine^{125} is the most commonly used implant in brachytherapy (Plowman, 2001).

Ragde and co-workers (1997) reported that for patients with low-grade localised disease the results of brachytherapy were comparable to those of radical prostatectomy. The ability to target dose delivery represents the greatest strength of brachytherapy, but is also its potential weakness, since any disease that extends beyond the range of the implant will not receive effective irradiation.

The American Brachytherapy Society recently published guidelines for the use of brachytherapy (Nag et al, 1999). They concluded that patients with T1-T2a disease, a Gleason score of 2-6 and a pre-treatment PSA concentration of less than 10ng/ml are good candidates for brachytherapy monotherapy. However, patients with T2b/c disease, a Gleason score of greater than 6 or a pre-treatment PSA concentration greater than 20ng/ml, are better treated with a combined treatment regime.

Radical prostatectomy.

Radical prostate surgery offers the advantage of a single potentially curative intervention in patients with localised prostate cancer. The first radical prostatectomy was performed by Hugh Hampton Young in 1904. The procedure involved the surgical removal of the prostate gland and seminal vesicles followed by the formation of an anastomosis between the bladder neck and the urethral stump. However, prior to the late 1970's the surgical anatomy of the prostate was poorly understood. Complications including haemorrhage, stricture formation, incontinence and

impotence were therefore common and this deterred many urologists from offering surgery as treatment.

More recently, a series of advances in our understanding of the relevant anatomy has reduced complication rates and most urologists now regard radical prostatectomy as the "gold standard" in the treatment of early stage disease. As a result, in the US, the proportion of newly diagnosed prostate cancer patients treated with surgery doubled between 1989 and 1991 (Lu-yao et al, 1993).

Nevertheless, complications associated with radical prostatectomy remain. One study, which evaluated 10,604 patients who underwent radical prostate surgery between 1991 and 1994, reported a post-operative mortality rate of 1% and a complication rate of 25% (Lu-yao et al, 1999).

Incontinence and erectile dysfunction are the two major long-term complications of radical prostatectomy. Many patients report some degree of urinary incontinence in the initial postoperative period. However, Walsh and colleagues recently reported that urinary continence gradually improves over the first 12 months and that at one year 93% of patients were dry (Walsh et al, 2000).

Impotence rates following surgery vary widely depending on the patient's age, the experience of the surgeon and whether a nerve sparing prostatectomy has been performed (Pirtskhalaishvili et al, 2001). For example, Stanford and colleagues (2000) recently reported that almost 60% of patients undergoing radical prostatectomy were impotent at 18 months. Recently, however, the use of sildenafil citrate (Viagra) has been shown to be effective in treating erectile dysfunction in a large proportion of patients undergoing nerve-sparing surgery (Zippe et al, 1998).

There are many studies examining the outcome of patients following radical prostatectomy. For example, Lu-Yao and Yao (1997) reported that the 10-year

disease specific survival rate following surgery was 94%, 87% and 67% for well, moderately and poorly differentiated tumours. The corresponding results for patients treated conservatively or with radiotherapy were 93%, 77%, 45% and 90%, 76% and 53% respectively.

Patients who are considered good candidates for radical prostatectomy are likely to be men who have localised but clinically significant disease in whom life expectancy is estimated to be at least 10 years. In such patients the expected benefits of surgery out-weigh the risk of morbidity. In summary, radical prostatectomy is still considered the "gold standard" treatment for clinically localised disease in patients who are younger than 70 years, are fit for surgery and have reasonable life expectancy.

Neoadjuvant hormone therapy.

Treatment with neoadjuvant hormone therapy is known to result in shrinkage of the prostate gland and a rapid reduction in the concentration of circulating PSA. When used prior to prostatectomy, neoadjuvant hormone therapy reduces the incidence of positive surgical margins, but has not been shown to improve survival or delay disease progression.

In contrast, there are a number of studies, which suggest that the use of neoadjuvant therapy prior to radiotherapy improves clinical outcome. For example, Zietman and colleagues (1997a) compared men treated with radiotherapy or with a combination of radiotherapy and neoadjuvant hormone therapy. They reported that at five years the local control and disease-free rates for the radiotherapy and the combination group were 54% vs. 29% and 36% vs. 15% respectively. The optimal sequence and length of treatment with neoadjuvant hormone therapy however has not been established.

1.9.2 Treatment of locally advanced prostate cancer

The optimal management of locally advanced prostate cancer also remains controversial. In general, patients described as having locally advanced disease have T3 or T4 disease with no evidence of lymph node or distant metastases. At this stage, the tumour extends through the prostatic capsule and may involve surrounding structures. A definitive treatment approach for patients with locally advanced disease remains unclear for several reasons. Firstly, the natural history of such cancer is not well understood. The longest study of untreated locally advanced prostate cancers was recently reported by Johansson and colleagues (1997). This study included 183 patients with T3 or T4 disease who were treated initially with observation. At 15 years, progression-free survival and disease-specific survival were reported as being 46% and 56%, respectively. Secondly, no randomised clinical trials have compared equivalent patients treated with different therapeutic modalities.

Single modality therapy for locally advanced disease

Radiotherapy.

External beam radiotherapy has traditionally been considered the treatment of choice in patients with locally advanced prostate cancer. Schmidt and co-workers (1986) reported a marked increase in the use of radiotherapy for the treatment of locally advanced disease from 9.1% in 1974, to 33.6% of patients in 1984. In general, good local control, low morbidity and overall survival comparable to other treatment modalities have been reported. For example, in one study, which evaluated 216 patients with locally advanced disease treated with radiotherapy, it was reported that 70% of patients remained free of local recurrence at 5 years, 65% at 10 years and 69%

at 15 years (Hanks et al, 1994). Overall survival rates were 56%, 32% and 23% at 5, 10 and 15 years respectively.

However, more recent studies carried out in the PSA era suggest higher levels of persistent disease. One study using circulating PSA concentrations >1ng/ml as evidence of treatment failure reported that of 589 patients treated with radiotherapy, only 21% were biochemically free of disease at 10 years (Zietman and Shipley, 1997). Furthermore, post-irradiation prostatic biopsies have been shown to contain residual tumour in up to half of patients (Oh and Kantoff, 1999). These findings probably represent the inability of external beam radiotherapy to eradicate most locally advanced cancers.

Radical prostatectomy

The use of radical surgery to treat locally advanced prostate cancer is controversial since the likelihood of achieving cure is low. Indeed, up to half of patients with T3 disease undergoing staging lymphadenectomy prior to surgery have evidence of lymph node metastases (Oh and Kantoff, 1999). Furthermore, Bosch and colleagues estimated that less than 30% of patients undergoing radical surgery for T3 disease had both clear resection margins and no evidence of lymph node metastases (Bosch et al, 1987). These reports suggest that radical prostatectomy is not an effective treatment in around 70% of patients with T3 disease.

Androgen deprivation therapy.

Androgens are known to stimulate growth in both normal and malignant prostate tissue. When androgens are withdrawn, cellular proliferation is inhibited and apoptosis may be promoted.

Neoadjuvant hormone therapy is given prior to definitive local treatment. GnRH receptor agonists, such as goserelin, have been used in the treatment of prostate cancer. These drugs mimic GnRH, a hormone produced by the hypothalamus. This hormone stimulates the release of FSH and LH from the anterior pituitary gland. LH in turn stimulates the production of androgens in the testes. Administration of these GnRH agonists initially causes a surge in circulating concentrations of testosterone and dihydrotestosterone which can lead to tumour flare. Eventually, however these agonists downregulate the hypothalamic-pituitary axis and consequently inhibit the production of LH and therefore androgen production. Anti-androgens are often given during the initial treatment period to limit the clinical sequelae related to tumour flare.

Combined treatment regimes for the treatment of locally advanced prostate cancer.

Androgen deprivation therapy and radiotherapy

There are a number of theoretical advantages in combining external-beam radiotherapy and androgen deprivation therapy. Elimination of androgen dependent prostate cancer cells increases the likelihood that a given dose of radiation will successfully treat the entire local tumour burden. Indeed, it is thought that androgen deprivation therapy and radiotherapy may have a synergistic effect in promoting cancer cell apoptosis. Furthermore, hormone therapy would potentially treat any undetected micrometastatic disease.

Several interesting studies have evaluated the combined use of radiotherapy and hormone treatment in the adjuvant and neoadjuvant setting. For example, Pilepich and co-workers (1997) randomised patients with T3 and N1 disease to receive adjuvant post-irradiation goserelin. Disease-free survival was significantly higher in those patients receiving goserelin. There was no difference in overall

survival between the two patient cohorts. However, when a sub group of patients with high grade tumours were examined survival was greater in the patients receiving hormone therapy. Bolla and co-workers (1997) conducted a randomised trial comparing radiotherapy alone with radiotherapy plus goserelin for three years. Five year survival was significantly higher in those patients receiving combined treatment.

Androgen deprivation therapy and radical prostatectomy.

One of the main problems associated with the use of radical surgery in the treatment of prostate cancer is the prevalence of non-organ confined disease. Several studies have now reported that the use of neo-adjuvant hormone therapy prior to radical prostatectomy significantly reduces the incidence of positive surgical resection margins and prostatic volume. Unfortunately, none of these studies has shown any improvement in survival, seminal vesicle or lymph node involvement or biochemical disease free survival (Chodak et al, 2002). Furthermore, despite reducing prostatic volume, neo-adjuvant hormone therapy has not been shown to improve the ease of surgery (Soloway et al, 1995).

1.9.3 The management of metastatic prostate cancer

Adenocarcinoma of the prostate gland most frequently metastasises to the well vascularised areas of the axial skeleton such as the spine, pelvis, ribs, skull and the proximal ends of the long bones. Bone metastases may cause intermittent or constant bone pain, bone marrow failure, hypercalcaemia, pathological fractures or spinal cord compression. Symptoms associated with these features of advanced disease can lead to a considerable reduction in the quality of life of affected patients.

Androgen deprivation therapy

There is now extensive evidence to support the observation first reported by Huggins and Hodges (1941) that androgen ablation is an effective palliative treatment in men with symptomatic metastatic prostate cancer. The clinical benefits of hormonal manipulation include a reduction in bone pain, an improvement in obstructive urinary symptoms, a reduction in prostatic bleeding, and a decrease in circulating PSA concentrations. As a result, hormone treatment is the mainstay of treatment in men with metastatic disease. However, the effects of androgen ablation on patients with prostate cancer are temporary. Such patients ultimately become unresponsive to androgen ablation and are then classified as having hormone-refractory disease. The median time from commencing hormonal therapy to developing hormone refractory disease is around 18 months. The median survival of patients with hormone refractory disease is only one year (Fournier, 1996).

Androgen deprivation therapy itself can be responsible for significant side-effects including loss of libido and potency, hot flushes, anaemia, alopecia, fatigue and psychological illness. Furthermore, Daniell (1997) recently reported that men treated with long term hormone therapy had reduced mineral bone density compared to age-matched controls. Indeed such patients were said to be at higher risk of developing osteoperotic fractures than pathological fractures resulting from metastatic bone disease. As a result of these factors, there is ongoing debate about the impact of hormone treatment on survival and the optimal timing for initiation of androgen deprivation therapy.

An important study carried out by the Medical Research Council (1997) aimed to answer some of these questions. The effect of immediate or delayed hormone therapy was evaluated in 261 men with asymptomatic metastases, 503 men with locally advanced disease and 174 men whose metastatic disease status was unknown.

Although the study initially reported improved survival among the cohort of patients receiving early hormone therapy, compared to those patients in whom treatment was deferred, this survival advantage was lost with additional follow-up. There was however, a statistically significant reduction in complications such as pathological fractures, spinal cord compression and ureteric obstruction in patients receiving early hormone treatment.

Chemotherapy in patients with hormone refractory prostate cancer

Traditionally hormone-refractory prostate cancer was thought to be a chemotherapy-resistant disease. However, recently chemotherapy has been shown to provide effective palliation of symptoms in some patients with hormone refractory disease.

Mitoxantrone is an amino anthracenedione and was originally used in the treatment of myelogenous leukaemias. Tannock and co-workers (1996) randomised patients with hormone refractory prostate cancer to receive either prednisone alone or prednisone in combination with mitoxantrone. They reported a statistically significant improvement in the palliation of symptoms, including bone pain, in those patients receiving combined steroid and mitoxantrone therapy. The length of effective palliation was also reported to be longer in the patients receiving combination therapy. Mitoxantrone combined with prednisone is therefore a therapeutic option in selected patients with symptoms related to hormone-refractory prostate cancer.

Taxanes have been shown to induce cell death in prostate cancer cell lines. This observation has generated interest in the use of taxane based chemotherapy regimes in vivo. For example Beer and colleagues (2001) sought to investigate the activity and toxicity of weekly docetaxel in patients with hormone independent prostate cancer and bone pain. They reported that approximately 50% of patients

receiving treatment experienced a reduction in bone pain. In a similar proportion of patients there was a >50% reduction in circulating PSA concentrations following treatment. The authors concluded that docetaxel was well tolerated in patients with hormone refractory disease and had significant therapeutic activity as measured by pain relief, reduction in PSA and reduction in measurable disease.

Bisphosphonates.

Bisphosphonates bind to hydroxyapatite crystals in bone and resist bone resorbtion. There is evidence to suggest that bone reabsorbtion may be necessary to allow the foundation of bone metastases in patients with prostate cancer. Treatment with bisphosphonates has been shown to be associated with a reduction in the number and activity of osteoclasts, probably through a direct effect on osteoclast activity as well as indirect effects on osteoblasts and macrophages. The net effect of bisphosphonate treatment is therefore a reduction in excessive bone turn-over and preservation of bone structure and mineralization.

Bisphosphonates have been shown to have a number of potential roles in patients with prostate cancer. Some studies have suggested that the use of bisphosphonates can inhibit the demineralisation of bone which is often associated with androgen deprivation therapy (Smith, 2003). Furthermore, in advanced disease, bisphosphonate treatment has been shown to be effective in controlling hypercalcaemia and bone pain associated with the presence of bone metastases (Saad et al, 2002). There is also some experimental evidence to suggest that bisphosphonates may directly inhibit tumour activity and delay the time to the first skeletal event (Goodin et al, 2002).

Radiotherapy

Radiotherapy can provide effective palliation for pain related to bone metastases. There are two main radiotherapy modalities which are of use in the treatment of metastatic prostate cancer. These include external beam radiotherapy and radionucleotide therapy.

External beam radiotherapy has long been known to have a favourable effect on pain caused by bone metastases. Local field radiation may be given as a single fraction of 8Gy, as five fractions of 4Gy or as ten fractions 3 Gy. The palliative effect begins within a few days and lasts around 4 months. However reports suggest that only approximately 25% of patients experience a complete cessation of pain (Di Lorenzo et al, 2003). Furthermore, in patients who develop spinal cord compression as a result of bone metastases, urgent external beam radiotherapy may be used to prevent permanent neurological injury.

Radionucleotide therapy is of use in patients with multiple painful bone metastases. It involves the intravenous administration of a bone seeking radioactive pharmaceutical. These compounds, such as Strontium 89, are then taken up by areas of bone undergoing rapid turnover, such as skeletal metastases. Such treatment therefore delivers targeted radiation directly to bone metastases.

2.0 The inflammatory response to cancer

2.0.1 The cell-mediated response

Host immune cells are able to eliminate foreign cells and altered self-cells by mounting a cell-mediated response which results in lysis of the target cells. There are two main mechanisms by which the host immune cells are able to initiate cell death. Firstly, cell destruction can be mediated by cytotoxic T-lymphocytes (CTL's) in response to a specific antigenic stimulus. Secondly, a non-specific cytotoxic response can be generated by cells such as natural killer cells (NK) and macrophages. Both these types of immune response are mounted in response to the presence of cancer cells and virus-infected cells.

This cell-mediated response is largely carried out by thymus-derived (T)-lymphocytes. In the circulation T-lymphocytes constitute 60-70% of peripheral lymphocytes. T-lymphocytes are also found in the paracortical areas of lymph-nodes and peri-arteriolar sheaths of the spleen. Each T-lymphocyte is genetically programmed to recognise a specific cell-bound antigen by means of an antigen-specific T-cell receptor. What distinguishes the T-cell receptor from membrane-bound antigen on B-lymphocytes is that it recognises antigen only when the antigen is associated with a self molecule encoded by genes within the major histocompatibility complex (MHC). Whereas the B-cell is capable of binding soluble antigen, the T-cell system is restricted to binding antigen on self-cells. This antigen may be displayed on the surface of antigen presenting cells or on virus-infected cells, cancer cells or grafts.

T-cell receptors are non-covalently linked to a cluster of five polypeptide chains, referred to as the CD3 molecular complex. The CD3 proteins are non-variable

and do not bind antigen but are involved in T-lymphocyte signalling after antigen binding.

In addition to CD3 proteins, T-lymphocytes express a variety of other antigens, including CD4, CD8 and many so-called accessory molecules, such as the CD2, CD28 and CD40 ligand. Of these CD4 and CD8 are particularly important. These antigens are expressed by two mutually exclusive T-lymphocyte subsets. Lymphocytes expressing the CD4 antigen account for approximately 60% of CD3+ T-lymphocytes. The majority of the remaining CD3+ lymphocytes express the CD8 antigen.

During the antigen presentation process, CD4 antigen molecules bind to portions of class II major histocompatibility complexes which are expressed on antigen presenting cells including macrophages, dendritic cells and B-lymphocytes. In comparison, CD8 molecules bind to portions of the class I MHC. Class I MHC molecules are found on the majority of nucleated cells. For this reason CD4+ helper cells can recognise antigen only if associated with a class II MHC, whereas CD8+ lymphocytes can only recognise cell-bound antigens associated with class I MHC antigens.

Cytotoxic T-Lymphocyte-mediated immunity

Activation of cytotoxic T-lymphocytes generates a population of cells with the ability to produce target-cell lysis. Most cytotoxic T-lymphocytes express the CD8 antigen complex and are therefore class I MHC restricted. As the vast majority of nucleated cells express the class I MHC, these lymphocytes have the potential to recognise and eliminate abnormal cells from most body tissues.

Non-activated cytotoxic T-lymphocytes are incapable of killing target cells and are therefore referred to as cytotoxic T-lymphocytes precursors to denote their

functionally immature state. Only after a cytotoxic T-lymphocyte has been immunologically activated will it develop the cell lytic capability of a mature cell.

Activation of a cytotoxic T-lymphocyte precursors requires two signals. The first signal occurs when the T-cell receptor and the CD8 antigen on the immature lymphocytes interact with an antigenic peptide on the class I MHC of a target cell. A second signal is provided by the release of cytokines from activated CD4+ T-helper cells. The most important of these cytokines appears to be interleukin-2 (IL-2) although others such as interleukin-6 and interleukin-4 may also have a role. Activation of IL-2 receptors on the precursor cell membranes stimulates proliferation and differentiation to produce mature cytotoxic T-lymphocytes. Precursor cells which have not encountered an antigenic stimulus do not express IL-2 receptors and therefore cytotoxic T-lymphocyte precursors require antigenic priming before they can respond to the IL-2 signal. This safe-guard ensures that only precursor cells responding to a specific antigenic stimulus are activated and undergo clonal expansion. Following antigenic stimulation cytotoxic T-lymphocytes are dependent on IL-2 to induce proliferation. Circulating IL-2 concentrations fall in response to antigen clearance and this triggers apoptosis of the antigen-specific cytotoxic T-lymphocytes clones. This mechanism ensures that the immune response is terminated promptly, following elimination of the biological stimulus, and this in turn limits the likelihood of collateral damage to healthy tissue and cells.

The destruction of a target-cell by activated cytotoxic T-lymphocytes requires a carefully orchestrated sequence of events culminating in target-cell lysis. The main phases of this sequence include conjugate formation, membrane attack, cytotoxic T-lymphocytes dissociation and target-cell destruction. When a cytotoxic T-lymphocyte comes into contact with an appropriate target cell the cells interact to form a

conjugate. The integrin receptors LFA-1 on the surface of the cytotoxic T-lymphocytes bind to the intercellular cell-adhesion molecules (ICAMs) on the target-cell membrane. Antigen activation of a cytotoxic T-lymphocytes by the target cell transforms LFA-1 from a low avidity state to a high avidity state resulting in cytotoxic T-lymphocyte-target cell binding. This mechanism ensures that cytotoxic T-lymphocytes bind only to cells which provide an appropriate antigenic stimulus. LFA-1 remains in a high avidity state for around five-10 minutes before reverting back to its original low-avidity state. This reduction in LFA-1 avidity is thought to promote cytotoxic T-lymphocyte-target-cell dissociation

Following cell binding, vesicles containing perforin are released into the junctional space between the two cells. As the perforin molecules make contact with the target-cell membrane they polymerise to form pores within the cell membrane. These pores are thought to facilitate the entry of further lytic substances which destroy the target cell. Cell death occurs approximately 15minutes to three hours after cellular dissociation.

Activation of CD4+ T-helper cells

Both the cell-mediated and humoral immune responses are dependent on activation of T-helper cells. This process starts when antigen receptors on the T-helper cells come into contact with antigenic peptide in combination with a MHC II complex. These complexes occur on the surfaces of antigen presenting cells such as macrophages, B-lymphocytes and dendritic cells. Such cells internalise antigen by phagocytosis or endocytosis before re-expressing part of that antigen on the cell surface in conjunction with a MHC II complex. The T-helper cell is then able to recognise the antigen associated with the MHC on the surface of the antigen-presenting cell. This recognition generates a signal which results in the production of interleukin-2 which

in turn results in clonal expansion of the T-helper cell population and activation of cytotoxic T-cells.

Natural killer cell-mediated cytotoxicity

Natural killer (NK) cells comprise approximately 5-10% of the circulating lymphocyte population. These cells have been implicated in anti-viral immunity and are involved in the host defence against tumour cells. The origin of NK cells is not clear as they express some membrane markers of T-lymphocytes and also some membrane markers of monocytes and granulocytes. NK cells appear to kill tumour cells and virus-infected cells by a process similar to CTL mediated lysis. After a NK cell adheres to the target cell, release of perforin-containing granules occurs. This release of perforin causes target cell membrane damage which in turn triggers target cell death.

Since NK cells do not express antigen specific receptors, a mechanism exists to prevent them from attacking normal cells. It is known that tumour and virus-infected cells generally express lower levels of class 1 MHC molecules than normal. It has been suggested that NK cells may have membrane receptors for class 1 MHC molecules, and that activation of these receptors inhibits target cell destruction. This mechanism means that when NK cells encounter normal cells, cell lysis is inhibited by activation of the class 1 MHC receptors. In contrast when a tumour cell or virus infected cell with reduced class 1 MHC expression is encountered cell lysis is initiated leading to the death of the target cell.

The cell-mediated immune response in cancer.

The immune system is a complex defence system which has evolved primarily to protect the body against infection by micro-organisms and not necessarily against

malignancy. Despite this, the immune system is thought to provide protection against many cancer types. The immune surveillance hypothesis proposes that the immune system continually surveys the body for malignancy, eliminating many or most tumours, and possibly slowing the growth of others (Burnet, 1967). Indeed as early as 1909, Paul Ehrlich had proposed the concept of cellular immunity directed against tumours cells (Ehrlich, 1909). The tumour surveillance hypothesis fell into disrepute after reports that non-virus related cancers were not increased in immunodeficient animals and humans. Furthermore, some studies reported that the incidence of cancer was not increased in (nu/nu) mutant mice which lack a thymus gland and are consequently deficient in most T-lymphocytes (Stutman, 1974). In retrospect, however, the conclusions of these studies are flawed as the animals studied did not completely lack T-lymphocytes.

It is now generally believed that the cell-mediated immune response does have an important role to play in anti-tumoral immunity and there is increasing evidence to support this theory. For example, it has been shown that patients suffering from some types of cancer produce T-cell lymphocyte clones specific for tumour antigens (Boon et al, 1994). Moreover, some authors report tumour regression in patients from whom tumour infiltrating lymphocytes are harvested, expanded in vitro and then re-infused. Furthermore, a substantially increased spontaneous tumour rate has been reported in aged mutant mice specifically lacking T and B-lymphocytes, as a result of a targeted mutation on the Rag-2 gene (Shankaaran et al, 2001). Other studies have demonstrated an increased incidence of certain tumour types in mice lacking perforin, the pore forming protein which is an essential component of the cytotoxic T-lymphocyte and natural killer cell-mediated response (Van der Broek et al, 1996).

It is also reported that, in many human cancers the degree to which the tumour is infiltrated with inflammatory cells predicts recurrence-free and cancer-specific survival. For example, the presence of macrophages around lung and gastric cancers is associated with reduced tumour progression (Ropponen et al, 1997). In many other solid tumours including colorectal (Ropponen et al, 1997), breast (Aaltomaa et al, 1992) and melanoma (Clark et al, 1989), tumour lymphocyte infiltration has been shown to have prognostic implications. Much of the research investigating the prognostic importance of tumour infiltrating lymphocytes (TILs) in predicting survival has been undertaken in patients with colorectal cancers.

The colorectal model

The survival advantage conferred by a pronounced tumour lymphocytic infiltration in large bowel cancer has been known for many years. Indeed, MacCarty described this relationship as early as 1931. Similar observations have been made in many subsequent studies. For example, in 1987, Jass and colleagues devised a prognostic scoring system which identified four independent pathological variables including lymphocytic infiltration which predicted outcome. It was suggested that this new system was superior to the well established Dukes classification because it placed twice as many patients into groups which provided a confident prediction of clinical outcome. In addition to providing useful prognostic information the Jass classification allowed clinicians to identify groups of patients who might receive the most benefit from adjuvant chemotherapy.

For such a system to be clinically useful it must be possible to reliably reproduce the results. Jass and co-workers reported interobserver agreement to be fair to excellent. However these assessments were made by comparing specialist

colorectal pathologists. When the system was re-assessed using general pathologists, the intra-observer and inter-observer agreement was reported as being little better than chance (Dundas et al, 1988). The technical difficulties in using the Jass classification system and problems with its reproducibility have meant that it has not become widely established in hospital laboratories. It is of interest, however, that the Jass classification is the only pathological system which makes an assessment of the host response to the presence of the tumour. Similar findings were subsequently reported by Nielsen and co-workers who demonstrated that poor inflammatory tumour infiltrate was associated with poor outcome in patients undergoing surgery for primary operable colorectal cancer (Neilsen et al 1999).

More recently, the ability to identify lymphocyte subsets, by the use of specific immunohistochemical staining, has led to renewed interest in this area. For example, Naito and his colleagues (1998) reported that, in a cohort of 131 patients with colorectal cancer, the pattern of lymphocytic infiltration of the tumour had important prognostic implications. More specifically they reported that the degree to which CD8+ lymphocytes invaded the tumour, predicted survival. Patients in whom the CD8+ lymphocytes infiltrated the cancer cell nests did better than those patients in whom the lymphocytes only invaded the stroma or appeared at the tumour margin. In contrast, other studies have suggested that CD4+ lymphocytes are more important than CD8+ cells in determining outcome in patients with colorectal cancer. For example, Ali and co-workers (2004) reported that, in patients with colorectal cancer, a marked CD4+ lymphocyte infiltration of the tumour was associated with good prognosis.

The cell mediated response in renal carcinoma.

Renal cell carcinoma is one of the few cancer types in which immunotherapy is routinely used in patients with disseminated disease. As a result, the role of the cell-mediated response in patients with renal carcinoma is an area of great interest. However, the two main studies which have investigated the role of tumour infiltrating lymphocytes in renal carcinoma have reported that a marked T-cell response was associated with poor survival (Nakano et al, 2001; Bromwich et al, 2003). It was suggested that in renal cell carcinoma at least, tumour-lymphocyte infiltration may be determined by tumour grade or by tumour cytokine production rather than a reflection of anti-tumoral immunity.

The cell mediated response in prostate cancer

The relationship between the presence of an inflammatory cell infiltrate and prognosis has only recently been investigated in patients with prostate cancer. To date, few studies have addressed this area of research, and their results are somewhat contradictory.

The largest study, undertaken by Vesalainen and colleagues (1994), examined 325 patients with prostate cancer; of the patients studied, 101 were found to have metastatic disease at the time of diagnosis. Histological specimens were assessed and divided into three groups according to the magnitude of the lymphocytic infiltrate. Patients who were judged as having a dense lymphocytic infiltration were found to have improved survival compared to those groups who had only moderate or scanty infiltration. However, patients within the cohort received a variety of different treatments including radiotherapy, chemotherapy and androgen deprivation therapy during the course of follow-up. Irani and co-workers (1999) examined a cohort of

161 men undergoing radical prostatectomy for localised prostate cancer. Prostatic tissue from each patient was examined and the degree of tumour infiltration by inflammatory cells assessed. The inflammatory cell infiltrate was not separated by cell type (i.e. lymphocyte, monocyte, plasma cell or leukocyte). The specimen was classified as having high grade inflammation if there was evidence of inflammatory nodules, glandular epithelium disruption and interstitial inflammatory infiltrate. The specimen was considered to have low grade inflammation if none of these features were present. In contrast to the results of Vesalainen and co-workers, it was reported that patients who were assessed as having high grade inflammation had significantly higher rates of biochemical recurrence than those with low grade inflammation. Other studies have also reported a relationship between tumour infiltration by inflammatory cells other than lymphocytes and survival. For example, it has been reported that scanty infiltration of the tumour by macrophages predicts poor prognosis (Shimura et al, 2000).

The role of a local cellular immune response to prostate cancers remains unclear. It seems logical that such an infiltrate would help control and even destroy tumour cells. However, it has been suggested that many of the lymphocytes found in tumour beds are inactive and therefore do not contribute to effective anti-tumoral immunity. Furthermore, the down-regulation of MHC II expression of some cancers, including prostate cancer, may help the tumour cells escape immune surveillance (Naoe et al, 2002). Elevations in PSA concentrations, normally associated with aggressive disease, have also been shown to have a direct immunosuppressive effect on T-lymphocyte function (Kennedy-Smith et al, 2002). Recently, it has also been reported that androgen deprivation induces a marked cellular response in prostate cancer tissues. The reasons for this are not known but the authors suggest a number

of possibilities. Firstly it is suggested that a mechanism may exist whereby the presence of androgens down-regulate the auto-immune response directed at prostatic tissue, thereby protecting prostate cancer cells from the immune system. Secondly, such a T-cell mediated response may be generated in response to androgen deprivation induced apoptosis rather than as direct anti-tumoral immunity (Mercader et al, 2001). Other studies have suggested that tumour-infiltrating lymphocytes may encourage disease progression directly. For example, Freeman et al (1995) suggested that in some circumstances lymphocytes infiltrating prostate cancers produce angiogenic factors, such as vascular endothelial growth factor, and thereby directly promote tumour growth and dissemination. In conclusion, the role of the cell-mediated immune response in patients with prostate cancer has not yet been established. Furthermore, the function of specific T-lymphocyte subsets in prostate cancer has not been evaluated.

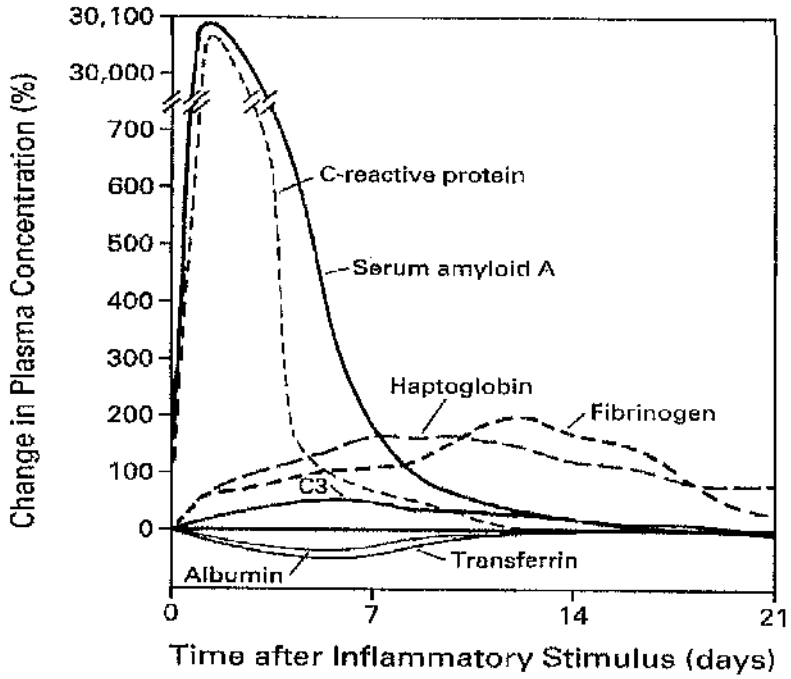
2.0.2 The non-specific inflammatory response

Any process, such as trauma, infection or malignancy, which results in cellular damage, can evoke a non-specific inflammatory response. This is a primitive yet essential innate host immune response to injury aimed at the restoration of tissue integrity. When activated, the non-specific inflammatory response triggers a cascade of physiological changes throughout the body which can involve many organ systems distant to the source of the inflammatory stimulus. This response is characterised by a complex series of non-specific responses, including fever, leucocytosis and breakdown of muscle protein. It also results in the dramatically increased synthesis and secretion of a variety of plasma proteins (Bengmark, 2001). These proteins are known as the acute phase proteins. Indeed, this whole phenomenon was initially

called the acute phase response. However, it was later discovered that this response often persisted in patients with chronic infective or inflammatory disorders and in those with malignancy. As a result, it has been suggested that the term "systemic inflammatory response" is more accurate and should be used to avoid confusion (Gabay and Kushner, 1999).

The acute phase proteins are defined as proteins whose plasma concentrations increase, or decrease by a minimum of twenty-five percent as part of the systemic inflammatory response (Morley and Kushner, 1982). For example, the circulating concentrations of the complement proteins and clotting factors can increase by 50 to 100 percent, whereas some of the proteinase inhibitors may increase several fold. Indeed, the most dynamic acute phase proteins, C-reactive protein and serum amyloid A, can change by more than three orders of magnitude (Figure 2.1). The changes in the circulating concentrations of the acute phase proteins result largely from changes in their production by hepatocytes. Regulation of the hepatic synthesis of the acute-phase proteins is controlled by a number of cytokines including tumour necrosis factor, interleukin-1 and interleukin-6. Although the concentrations of multiple components of the systemic inflammatory response commonly increase together, not all of them increase uniformly in all patients with the same illness. This observation suggests that components of the systemic inflammatory response are individually regulated by specific cytokines.

Figure 2.1: Characteristic patterns of change in plasma concentrations of some acute phase proteins after a moderate inflammatory stimulus (Gabay and Kushner,1999).



The acute phase proteins

The systemic inflammatory response leads to the greatly enhanced synthesis of a number of plasma proteins along with a decrease in the plasma concentrations of some others. The major acute phase proteins are listed in Table 2.1. The role of the systemic inflammatory response is not fully understood but certain aspects appear to be of benefit to the individual. For example, increases in C-reactive protein and the complement proteins help eliminate infection, increased fibrinogen help prevent blood loss and protease inhibitors prevent excessive tissue necrosis when lysosomal enzymes are released by damaged cells.

Table 2.1: The major acute-phase proteins

Protein Types	Increased	Decreased
Proteinase inhibitors	α-antitrypsin	
Coagulation proteins	Fibrinogen Prothrombin Plasminogen Factor VIII	
Complement Proteins	C1-5 C56	Properdin
Miscellaneous	C-reactive protein Serum Amyloid A Haptoglobulin Caeruloplasmin	Albumin HDL LDL

Fibrinogen

The erythrocyte sedimentation rate (ESR) is defined as the rate at which red blood cells settle out of unclotted blood in one hour and is an indirect measurement of the circulating concentrations of fibrinogen. Fibrinogen is a soluble coagulation protein which can be converted by the enzyme thrombin to form solid fibrin clot. Fibrinogen concentrations are usually elevated as part of the systemic inflammatory response.

The ESR is raised in many different conditions including infection, renal insufficiency, collagen diseases and malignancy. The ESR is also affected by physiological states such as age and pregnancy. This fact often makes interpretation of ESR results difficult. Furthermore, the ESR responds slowly to a stimulus and is slow to normalise after the stimulus has resolved. This means that ESR often does not accurately reflect disease activity at the time of sampling.

The complement proteins.

Consisting of a group of 20 plasma proteins, the complement system plays an important role in host defence against bacterial infections. The complement system can be activated by two independent pathways, called the classical and the alternative

pathways. Activation of either of the complement pathways gives rise to the production of a membrane attack complex. This complex is extremely hydrophobic and has a high affinity for cell membranes. Binding between the membrane attack complex and a cellular membrane results in membrane disruption and cell lysis.

C-reactive protein.

A member of the pentraxin family of proteins, C-reactive protein (CRP) was first described by Tillet and Francis in the 1930's, who noted its ability to bind to the C-polysaccharide component of the pneumococcal cell wall. Complexed or activated CRP was found to activate the complement pathway resulting in the opsonization and phagocytosis of bacteria or damaged cells. Since its discovery CRP has become established as a highly sensitive but non-specific marker of infection. Its synthesis in hepatocytes is induced by pro-inflammatory cytokines including tumour necrosis factor alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) (Du Clos, 2000). The production of CRP increases rapidly within the first four hours of exposure to the inflammatory stimulus and doubles every eight hours thereafter. The peak circulating concentrations of CRP occur approximately thirty-six to fifty hours after the onset of inflammation. The relatively short half-life of CRP (approximately 19 hours) means that once the inflammatory stimulus has resolved, the circulating concentrations quickly return to normal. The rapid kinetics of CRP metabolism, which closely follow the inflammatory course make it the most useful clinical tool in measuring the course of inflammation-inducing disease processes. Although CRP has both pro and anti-inflammatory functions, its overall effect is anti-inflammatory because it prevents adhesion of neutrophils to the endothelial cells of blood vessels, inhibits the production of oxygen free radicals and stimulates the production of IL-1 receptors by mononuclear cells.

Serum amyloid A

The serum amyloid A family consists of a number of differentially expressed apolipoproteins which are synthesised primarily by the liver. The serum amyloid A proteins are found throughout most vertebral species, implying an important generic role during inflammation. However, as yet the precise function of serum amyloid A is not fully understood. During the systemic inflammatory response the circulating concentrations of serum amyloid A may rise from resting levels by 1000 fold (Uhlar and Whitehead, 1999). As with CRP, circulating levels of serum amyloid A fall rapidly with resolution of the inflammatory stimulus.

Albumin

Synthesised in the liver, albumin is the major plasma protein. It normally accounts for around half of the hepatic protein production. Albumin has a long plasma half-life of around twenty days. During the systemic inflammatory response, albumin concentrations may fall. This phenomenon may be due to in part to nutritional depletion secondary to cytokine-induced anorexia. However, it is now recognised that albumin may also be catabolised along with skeletal muscle proteins to provide the amino acids required to support the increased production of other acute phase proteins.

The Cyclooxygenase enzymes.

The cyclooxygenases are enzymes involved in the conversion of arachidonic acid to prostaglandins and other eicosanoids. The cyclooxygenase (COX) enzymes mediate the conversion of arachidonic acid to prostaglandins by a two-step process. The first step involves the incorporation of oxygen to arachidonic acid to form prostaglandin G2. The second step results in peroxidation of prostaglandin G2 to form

prostaglandin H2, which in turn can be converted to a number of different prostaglandins. Oxygen free radicals are released as a by-product of these reactions (Kirschenbaum et al, 2001)

Two different isoforms of the enzyme have been identified. The COX-1 isoform is commonly expressed by a majority of mammalian cells and is involved in regulating water reabsorbtion, gastric acid production , blood flow and platelet aggregation. (Pairet and Engelhardt, 1996). The COX-2 isoform, however, is induced by a variety of factors including cytokines such as IL-6, growth factors and tumour promoters. COX-2 has been shown to be highly expressed in a number of human cancers and cancer cell-lines (Lee DW et al, 2002; Wu T, 2005; Liu J et al, 2005). Furthermore, over-expression of COX-2 has been linked to the promotion of carcinogenesis, resistance to apoptosis and abnormal cell cycle regulation.

Regulation of the systemic inflammatory response.

The systemic inflammatory response is regulated by cytokines. Cytokines are intracellular signalling polypeptides which are produced by cells activated in response to an inflammatory stimulus. The cytokines that are produced as part of the inflammatory response are the chief stimulators of the production of the acute phase proteins. These cytokines include tumour -necrosis factor, interleukin-6 (IL-6), Interleukin-1 (IL-1) and possibly interleukin-8 (IL-8). They are produced by a number of cell types, but the most important sources are macrophages and lymphocytes. In addition to stimulating the production of the acute-phase proteins, cytokines can also regulate the production of other cytokines. Furthermore, the effects of individual cytokines on target cells may also be affected by the presence of other cytokines. In other words cytokines may enhance or inhibit the effects of other cytokines during an inflammatory response. Cytokines exert their influence on the

expression of the acute phase proteins by regulating transcription of the genes encoding these proteins (Gabay and Kushner, 1999)

Interleukin-1

Interleukin-1 is produced by macrophages and other nucleated cells in response to activation by contact with endotoxin, antigen or lymphokines such as interferon-γ. IL-1 triggers T-helper cell differentiation and the production of cytokines.

Interleukin-6

IL-6 belongs to the "IL-6 type cytokine family" which includes leukaemia inhibitory factor, IL-11, ciliary neurotropic factor, cardiotrophin-1 and oncostatin-M. In the normal homeostatic state, IL-6 levels are typically very low. However, in response to the appropriate stimulus (e.g. inflammation) a wide variety of cells can produce IL-6. Indeed, some adenocarcinoma cells have also been shown to secrete large quantities of IL-6. Many physiologic functions are attributed to IL-6 including promotion of antibody production from B-lymphocytes, induction of thrombopoiesis, promotion of osteoclastic –mediated bone remodelling and modulation of acute-phase protein synthesis. Indeed, IL-6 is the chief regulator of production of most acute-phase proteins (Gauldie et al, 1987). High concentrations of IL-6 have been associated with fatigue, somnolence, depression, anorexia and day-time sleepiness. Il-6 mediates its activity, in part, through the IL-6 receptor complex (Corcoran and Costello, 2003).

Tumour-necrosis factor

TNF functions as a pyrogen, activates the clotting system, stimulates the hepatic production of the acute-phase proteins and over time can induce cachexia. TNF can be detected in malignant or human stromal cells in patients with ovarian, breast, prostate, bladder and colorectal cancers. In malignant disease, high levels of TNF

destroys tumour blood vessels. However, when chronically produced, TNF can act as an endogenous tumour promoter, contributing to the tissue remodelling necessary for tumour growth and spread (Balkwell and Mantovani, 2001).

Inflammation and cancer

In 1863, Rudolf Virchow first suggested an association between inflammation and cancer. He hypothesised that leucocytes found in some malignant tissues were the origin of cancer. The evidence to support a link between inflammation and malignant disease has expanded over the last decade.

It is now recognised that approximately 15% of cancers worldwide are caused by an infectious agent (Balkwell and Mantovani, 2001). For example, cervical and penile cancers are associated with the human papilloma virus, hepatocellular carcinoma is caused by hepatitis B and C and gastric cancer is linked to H-pylori infection. Furthermore, some cancers have also been associated with chronic inflammation caused by chemical and physical elements. For example, mesothelioma is related to asbestos exposure, bronchial carcinomas to smoking and skin cancers to ultra violet light. Recently, there have also been a number of studies which indirectly implicate inflammation in the development of cancer. It has been shown that people who take non steroidal anti-inflammatory drugs (NSAIDS) have a reduced risk of developing colorectal cancer (Thun et al, 1993). There is also evidence to suggest that the use of NSAID's reduces the risk of developing a number of other cancers including breast, bladder, stomach and prostate.

It is not clear how inflammation facilitates the development of malignancy, but several potential mechanisms have been proposed (Balkwell and Mantovani, 2001). It is thought that oxygen radicals generated by inflammation can oxidise DNA, resulting in mutagenic changes and damage to the DNA repair proteins.

Another link between inflammatory cytokines and DNA damage come from studies examining the regulation of the tumour suppressor gene p53 (Hudson et al, 1999). It has been suggested that some cytokines may be able to inactivate p53, thus increasing cellular proliferation and inhibiting apoptosis of potential tumour cells. In addition to inhibiting apoptosis, it has also been shown that many cytokines have the potential to directly stimulate tumour cell proliferation by acting as autocrine growth factors.

Once a malignancy is established it needs to secure an adequate blood supply to allow growth and metastasis. It is thought that some proinflammatory cytokines, such as TNF and IL-6, can promote tumour angiogenesis by stimulating the production of angiogenic factors such as vascular endothelial growth factor (VEGF). Cytokines may also encourage metastatic spread by acting as chemokines. Many tumour types express cellular adhesion molecules and it is thought that cytokines may promote migration and the use of these cellular adhesion molecules to seed cancer cells to distant anatomical sites.

Most malignant tumours, especially when extensive or metastatic, induce a systemic inflammatory response. This is particularly pronounced with those malignancies, such as renal cell carcinoma, which may produce systemic symptoms such as fever and weight loss. Indeed, markers of the systemic inflammatory response such as CRP have been shown to provide useful predictors of extent of disease, disease recurrence and survival in a variety of common solid tumours. Furthermore, CRP has been shown to be useful in monitoring progression and regression of some tumour types.

CRP and survival in patients with cancer.

The impact of the presence of a systemic inflammatory response, as evidenced by elevated circulating concentrations of CRP, on patient survival has been examined in

number of different malignancies. C-reactive protein has been shown to predict survival in a number of tumour types including renal (Ljungberg et al, 1995), melanoma (Tartour et al, 1994), lymphoma (Legouffe et al, 1998) and ovarian carcinoma (Kodama et al, 1999). Colorectal cancer is, however, the most widely studied model for assessing the impact of the systemic inflammatory response on survival. Indeed, there is increasing evidence to support the hypothesis that raised pre-operative or post-operative circulating C-reactive protein concentrations correlate with poor survival in patients with colorectal cancer, independent of stage (McMillan et al, 1995; Nozoe et al, 1998, Nielsen et al, 2000)

The systemic inflammatory response and cancer cachexia.

Cancer cachexia is one of the most debilitating conditions associated with advanced malignancy and has a significant impact on quality of life and survival. It has been estimated that 20% of patients with advanced cancers die directly as a result of cachexia rather than tumour burden (Argiles et al, 2001). Cachexia is a metabolic condition characterised by weight-loss and anorexia and the depletion of adipose tissue and skeletal muscle. Cachexia has been shown to be related to the presence of a systemic inflammatory response in a number of studies (Barber et al, 1999). There are a number of possible ways by which the systemic inflammatory response may produce the cancer cachexia syndrome. The experimental administration of cytokines such as TNF-α induces many of the metabolic features of cachexia (Warren et al, 1987). In particular the presence of pro-inflammatory cytokines is associated with skeletal muscle breakdown and loss of lean body cell mass. Moreover, the presence of the prolonged acute phase protein response often seen in patients with advanced cancer can contribute to cachexia. The ongoing production of acute phase proteins such as CRP is thought to result in reprioritising of amino-acid use away from

peripheral tissues, such as muscle, in favour of the liver where the acute phase proteins are produced. It is now generally accepted that cachexia is not reversed by nutritional supplementation alone. However, it is of great interest that recent studies have suggested that modulation of the systemic inflammatory response through the use of NSAIDs can reverse weight loss and improve quality of life in weight losing cancer patients (McMillan et al, 1999)

The cyclooxygenase enzymes and cancer.

It is thought that the COX enzymes may contribute directly to the carcinogenic process through the production of oxygen free radicals which results in DNA damage. Furthermore, some prostaglandin H2 is converted to malondialdehyde which is also known to stimulate mutagenic changes in DNA (Kirschenbaum et al, 2001). It is therefore of interest that patients taking non-steroidal anti-inflammatory drugs, which inhibit the actions of the COX enzymes, are at reduced risk of developing some cancers such as cancer of the colon (Samoha and Arber, 2005) and breast (Xu XC, 2002).

Besides initiating carcinogenesis, there is evidence to suggest that the cyclooxygenase enzymes may also be implicated in promoting tumour growth and dissemination. For example, there is growing evidence to suggest that in a number of tumour types, prostaglandins may have a role in protecting tumour cells from undergoing apoptosis and therefore encourage tumour growth. It is therefore of interest that several studies have demonstrated that non-steroidal anti-inflammatory drugs, which inhibit the production of prostaglandins, induce apoptosis in a number of tumour types, including prostate cancer cells (Liu XH et al, 1998). There is also evidence that COX-2 may promote disease progression and dissemination in cancer patients. For example, in many tumour types, such as breast cancer, COX-2

expression has been strongly associated with expression of vascular endothelial growth factor (VEGF) (Timoshenko et al, 2006). This pro-angiogenic factor is known to promote neovascularisation and thus facilitate tumour growth and spread of tumour cells. It has also been postulated that the production of prostaglandins may result in apoptosis of immune cells such as natural killer cells. This in turn may allow tumour cells to grow and disseminate whilst escaping from immune surveillance mechanisms (Myers et al, 2001).

The systemic inflammatory response in prostate cancer.

For many years clinicians have sought prognostic markers which would help distinguish between prostate cancers with good prognosis and those which were likely to progress. This is of importance in patients with prostate cancer as treatment often carried significant morbidity and there was concern that some patients may be unnecessarily treated. It was in this context that the first studies examining the relationship between the systemic inflammatory response and prostate cancer were performed. For example, Johansson and colleagues (1992) reported that raised ESR levels were associated with disease progression in patients with prostate cancer. The prognostic value of ESR was later shown to be independent of PSA (Borre et al, 1997). Furthermore, Trautner and colleagues (1980) reported that elevations in circulating CRP concentration were associated with disease progression in patients receiving oestrogen therapy. The presence of abnormal cytokine levels has also been associated with increased stage and disease progression. For example, Adler and co-workers (1999) reported significantly higher IL-6 concentrations in patients with metastatic prostate cancer. These results were later confirmed by Shariat and colleagues (2001). It has also been reported that patients with hormone-escaped prostate cancer had significantly higher levels of IL-6 than patients with hormone

sensitive disease (Wise et al, 2000). It has been suggested that IL-6 may participate directly in disease progression by acting as a growth factor (Okamoto et al, 1997) and by inhibiting apoptosis (Chung et al, 2000).

Cancer cachexia is of particular importance in patients with prostate cancer. It has been estimated that approximately 60-70% of patients with advanced prostate cancer suffer and die from cachexia (Argiles et al, 2001). As with other tumour types, the basis of cachexia in advanced prostate cancer patients appears to be multi-factorial and is poorly understood. However, several cytokines including IL-1, IL-6, TNF-α and IL-8 have been implicated as agents responsible for the features of cachexia. For example, Nakashima and colleagues (1998) reported that TNF-α contributed to the complex syndrome of cachexia in patients with prostate cancer. Furthermore, Pfitzenmaier and co-workers (2003) demonstrated that several cytokines including TNF-α, IL-6 and IL-8 were elevated in patients with advanced prostate cancer suffering from cachexia when compared with patients who were without cachexia.

The cyclooxygenase enzymes and prostate cancer

It is now widely accepted that the COX enzymes, and in particular COX-2, have an important role to play in carcinogenesis and disease progression in many tumour types.

Weak expression of both the COX isoforms has been reported in normal prostate tissue and benign prostatic hyperplasia (Yoshimura et al, 2000). Indeed, Madaan and colleagues (2000) reported that COX-1 appeared to be equally expressed in both cancer and benign tissue. There was, however, a significant over expression of COX-2 in prostate cancer tissue when compared with benign controls. The authors also found a significant correlation between increasing tumour grade and COX-2 expression. This suggests that COX-2 expression is related to a malignant phenotype.

Tjandrawinata and Huges-Fulford (1997) reported that increased prostaglandin synthesis has growth-promoting and positive feedback effects in prostate cancer. For example, they found that cellular proliferation rates increased in prostate cancer cells treated with exogenous prostaglandins. Furthermore, they found that prostaglandins increased COX-2 expression, and that this resulted in further prostaglandin production via a positive feedback loop. COX-2 is also reported to promote tumour growth by inhibiting apoptosis of prostate cancer cells (Liu et al, 2005).

The cyclooxygenases may also be involved in dissemination of tumour cells by encouraging angiogenesis. For example, Wang and colleagues (2005) reported that angiogenesis, as measured by microvessel density, is increased in prostate cancer tissue expressing COX-2. Interestingly, the authors also reported that COX-2 expression is increased in tissue infiltrated by inflammatory cells such as T-lymphocytes. It is thought that cytokines, such as IL-6, produced by lymphocytes and other inflammatory cells induce the expression of COX-2.

It is therefore of interest that several epidemiological studies have demonstrated a reduction in the risk of developing prostate cancer in men taking regular non-steroidal anti inflammatory drugs (NSAIDs). For example, Nelson and Harris (2000) reported a 66% reduction in the risk of developing prostate cancer in men taking daily doses of aspirin or ibruprofen. Another study found a lower incidence of prostate cancer in men over the age of sixty who regularly consumed NSAIDs compared to age-matched controls in the general population (Roberts et al, 2002). Norrish and colleagues (1998) have also reported a trend towards a reduction in risk of advanced prostate cancer following the regular administration of aspirin.

3.1.0 The relationship between T-lymphocyte subset infiltration and survival in patients with prostate cancer.

3.1 Introduction

It has long been recognised that disease progression in cancer patients is not solely determined by the characteristics of the tumour but also by the host response. Indeed there is increasing evidence that both local and systemic inflammatory responses play an important role in the progression of a variety of common solid tumours (Coussens and Werb, 2002).

For example, in patients with colorectal cancer, there is good evidence that the presence of a pronounced lymphocytic infiltration within the tumour is associated with improved survival (Jass et al., 1987; Ropponen et al., 1997, Nielsen et al., 2000). More recently, the ability to identify lymphocyte subsets has led to renewed interest in the relationship between the tumour inflammatory infiltrate and outcome. Indeed, increased infiltration of the tumour by CD8+ and CD4+ T-lymphocytes has been shown to be associated with increased survival in patients with colorectal cancer (Niato et al., 1998; Ali et al., 2004). In contrast, the presence of an increased infiltration by CD4+ or CD8+ T-lymphocytes has been associated with decreased survival in patients with renal cancer (Nakano et al 2001, Bromwich et al 2003).

The relationship between lymphocytic infiltration and survival in other urological cancers, for example prostate cancer, is less clear. Indeed, at the time that this work was carried out, only two papers had been published in a peer-reviewed journal. Vesalainen and coworkers (1994) reported that, in a cohort in which approximately 30% of patients had metastatic disease, tumours with a dense tumour

lymphocyte infiltration were associated with higher survival rates than tumours with absent or decreased infiltrates. In contrast, Irani and his co-workers (1999) reported that, in patients undergoing radical prostectomy, an increased inflammatory cell infiltrate within the tumour was associated with an increased risk of tumour recurrence.

To date, the relationship between lymphocyte subset infiltration and survival does not appear to have been assessed in patients with prostate cancer. The aim of the present study was to examine the relationship between CD4+ and CD8+ T-lymphocyte infiltration and survival in patients with prostate cancer.

3.2 Patients and Methods

Patients

Patients who underwent radical local treatment for histologically proven prostate cancer (n= 11) or who were diagnosed as having prostate cancer following transurethral resection of prostate for bladder outflow obstruction (n= 69) were included in the study. Clinical details recorded included age, stage, tumour grade, circulating concentrations of PSA and haemoglobin at diagnosis and subsequent treatment.

All patients were followed up in the Department of Urology. The date and cause of death was obtained from the cancer registry.

The study was approved by the local ethics committee.

Methods

Immunohistochemistry: Blocks from the primary tumour were fixed in 10% buffered formalin and embedded in paraffin wax. One representative block of tumour was selected from each patient. Sections (4 μm) were cut and mounted on slides coated with aminopropyltriethoxysilane.

Sections were then immunostained using the peroxidase-based Envision (Dako, Cambridgeshire, UK) technique as previously described (Bromwich et al., 2003). The primary antibody for CD4 was mouse monoclonal (Vector, Peterborough, UK) and that for CD8 was mouse monoclonal (Dako, Cambridgeshire, UK). Sections were dewaxed and rehydrated. Endogenous peroxidase was blocked by incubation in 0.3% hydrogen eroxide for 10 minutes. Antigen retrieval for CD8 was performed by micro-waving in 1mM EDTA buffer, pH 8 for 5 minutes at full pressure in a plastic pressure cooker in a microwave oven. Antigen retrieval for CD4 was achieved by immersing the sections in high pH buffer (9.9 Dako) in a coplin jar, maintained at 99˚C for 75 minutes in a water bath.

The sections were then incubated with the primary antibodies at dilutions of 1:50 (CD4) and 1:100 (CD8) for 30 minutes at room temperature. Sites of binding were detected using the Envision kit with 3`3` diaminobenzidine as chromogen according to the manufacturers instructions. Sections were counterstained with haematoxylin, dehydrated, cleared and mounted with Pertex.

Formalin fixed tonsil was included in each run as a positive control. The negative control for each case was the omission of the primary antibody. Examples of the immunohistochemical staining for CD4+ and CD8+ lymphocytes are provided in figures 3.1 and 3.2.

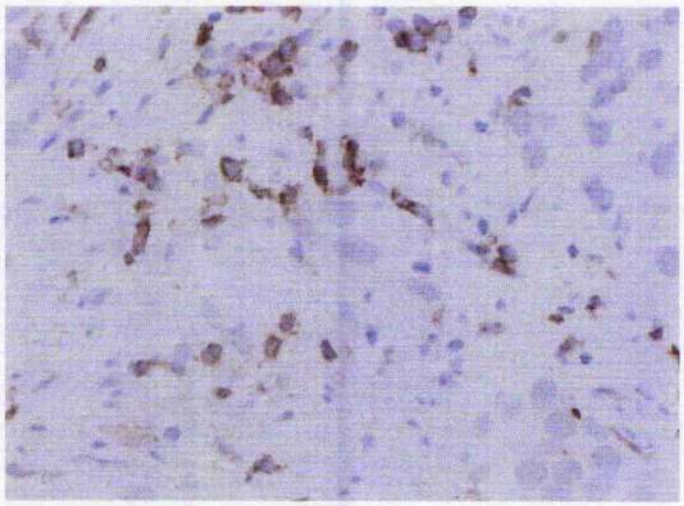


Figure 3.1 Example of the immunohistochemical staining for CD4+ lymphocytes in prostate cancer.

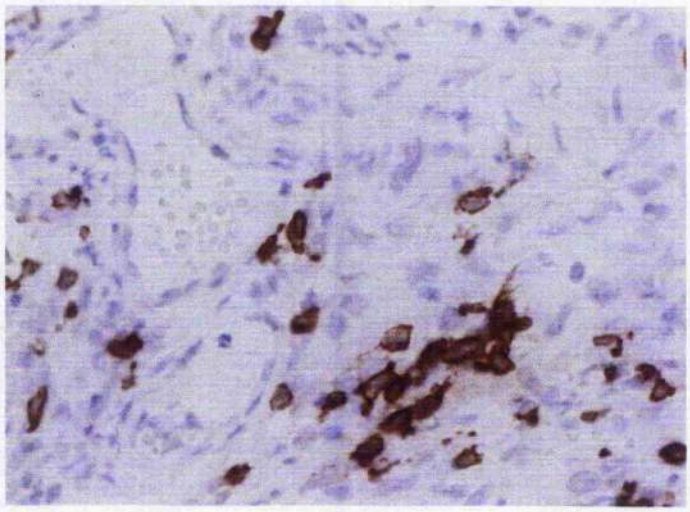


Figure 3.2 Example of the immunohistochemical staining for CD8+ lymphocytes in prostate cancer.

Morphometry: Quantitative analysis of the lymphoid infiltrate was performed using point counting (Anderson and Dunnill, 1965) with a random sampling technique. With this method, the volume occupied by any given component (volume density) is expressed as a percentage of the total volume of the tissue. A 100-point ocular grid was used at X400 magnification and 30 fields were counted per case for CD4+ and CD8+ immunopositive cells. Only fields within the tumour (including cancer cell nests and surrounding tissue stroma) were counted. Any normal tissue on the slide was excluded from the analysis.

This final method was designed on the basis of a pilot study, which demonstrated that the volume density of CD4+ and CD8+ of two observers reached a plateau after 25–30 fields. This pilot study also demonstrated that CD4+ and CD8+ counts were equivalent to the CD3+ counts (Ali et al, 2004). The observers (McArdle and Canna) were blinded to the clinical outcome of the patient.

Statistics

Data are presented as median and range. Where appropriate, comparison of patient groups was carried out using contingency table analysis (X^2) and the Kruskal-Wallis test for analysis of variance. For the purpose of survival analysis T-lymphocyte subset counts were grouped into tertiles as previously described (Nielsen et al., 1999). Survival analysis was performed using the Cox proportional hazard model. Deaths up to August 2003 have been included in the analysis. Analysis was performed using SPSS software (SPSS Inc., Chicago, Illinois, U.S.A.).

3.3 Results

Eighty patients were included in the study. The baseline characteristics, according to stage, are shown in Table 3.1 Patients with metastatic disease were older ($p<0.05$), had higher circulating concentrations of PSA ($p<0.001$) and had lower haemoglobin ($p<0.05$) concentrations. Twenty one patients underwent radical local therapy (prostatectomy or radical radiotherapy) as primary treatment. The remaining 59 patients received androgen deprivation therapy. The median (range) for percentage volume of CD4+ T-lymphocytes was 0.40 (0.03-1.90). The median (range) for percentage volume of CD8+ T-lymphocytes was 0.78 (0.27-3.00). More patients with metastatic disease died of prostate cancer

The baseline characteristics, according to PSA concentration, are shown in Table 3.2. Patients with higher PSA concentrations had more advanced disease ($p<0.001$), had higher grade tumours ($p<0.05$) and were more likely to die of their diease ($p<0.05$).

The baseline characteristics, according to tumour CD4+ T-lymphocytic infiltration (tertiles 1, 2, 3), are shown in Table 3.3. There were no significant associations with increasing CD4+ T-lymphocytic infiltration.

The minimum follow-up was 26 months; the median follow-up of survivors was 71 months. Forty-one patients died during follow-up, 22 of prostate cancer and 19 of intercurrent disease. The mean cancer specific-survival for those with localised, locally advanced and metastatic disease was 120, 98, and 66 months respectively ($p=0.001$).

On univariate analysis, advanced stage ($p<0.01$), elevated PSA concentrations ($p<0.01$), decreased haemoglobin ($p<0.05$) and increased CD4+ T-lymphocyte counts

($p<0.05$) were associated with reduced cancer specific survival (Table 3.4). On multivariate analysis PSA (HR 2.47, 95%CI 1.27-4.83, p=0.008), haemoglobin (HR 3.48, 95%CI 1.30-9.30, p=0.013) and CD4+ T-lymphocyte count (HR 2.29, 95%CI 1.25-4.22, p=0.008) retained independent significance. There was no significant correlation between PSA concentrations and either CD4+ (p=0.539) or CD8+ (p=0.202) T-lymphocytes.

When those patients with localised or locally advanced prostate cancer (n=57) were examined in univariate analysis, only CD4+ T-lymphocyte count achieved statistical significance (HR 2.88, 95%CI 1.15-7.22, p=0.024).

3.4 Discussion

The results of the present study show that the presence of an increase in CD4+ T-lymphocyte infiltrate was associated with poor cancer specific survival, independent of stage, in patients with prostate cancer. Furthermore, when the analysis was confined to those patients with localised or locally advanced disease, only CD4+ T-lymphocyte count predicted survival. These results are consistent with those of Irani and coworkers (1999) but not with those of Vesalainen and coworkers (1994).

The reasons for the difference in the results of the present study and those of Vesalainen and coworkers (1994) are not clear. Both cohorts included a wide spectrum of disease and both had a similar proportion of patients with metastatic disease. However, the apparent discrepancies may reflect differences in methodology including the way in which the inflammatory infiltrate was assessed.

Vesalainen and his colleagues studied a heterogeneous group of 325 patients with prostate cancer, of whom 101 had metastatic disease at the time of diagnosis.

The majority of patients received at least form of treatment, mainly in the form of oestrogens or an orchidectomy. However, it is not clear whether any of the patients in this cohort had received treatment prior to tissue sampling. Potentially this is a confounding factor since it is now known that androgen withdrawal is associated with an increase in tumour infiltrating lymphocytes (Mercader et al, 2001).

Tissue samples obtained from surgical or needle specimens were studied. Since only eight patients underwent a radical prostatectomy, it is likely that the majority of tissue samples were collected from a "needle biopsy". It is not clear whether the "needle biopsies" were obtained by a tru-cut biopsy or fine-needle aspiration. In the case of a tru-cut biopsy, only a small amount of tissue is removed and this may not be representative of the tumour as a whole. In the latter case, although it is possible to harvest tumour cells and lymphocytes, the normal tissue architecture is lost.

The density of tumour infiltrating lymphocytes was assessed on simple staining with haematoxylin and eosin. The type of lymphocyte was not assessed. A pronounced lymphocytic infiltration of the primary tumour was found to be associated with improved survival.

In contrast Irani and colleagues, evaluated a cohort of 161 men undergoing radical prostatectomy for localised prostate cancer. None of the patients had nodal involvement; none had received any treatment prior to surgery. Prostatic tissue from each patient was examined and the degree of tumour infiltration by inflammatory cells assessed. The inflammatory cell infiltrate was not separated by cell type (i.e. lymphocyte, monocyte, plasma cell or leukocyte). The specimen was classified as having high grade inflammation if there was evidence of inflammatory nodules, glandular epithelium disruption and interstitial inflammatory infiltrate. The specimen

was considered to have low grade inflammation if none of these features were present. The authors reported that patients who were assessed as having high grade inflammation had significantly higher rates of biochemical recurrence than those with low grade inflammation. Furthermore, this effect was found to be independent of Gleason score, pre-operative PSA and pathological stage.

In the present study, none of the patients had received treatment prior to surgery. T-lymphocyte subsets were identified by immunohistochemistry and the density was assessed using a point counting technique. This approach provided a more objective assessment and circumvented the problem of variation in distribution of lymphocytes within an individual tumour.

High CD4+ T-lymphocyte counts were associated with reduced cancer specific survival. This effect was independent of pre-operative circulating PSA concentrations.

In view of the apparent differences in the relationship between lymphocytic infiltration and outcome in the different studies, it is of particular interest that, since our work has been published, a further paper from the original institution has been published (Karja et al, 2005). One hundred and eighty-eight patients undergoing radical prostatectomy were evaluated. Immunohistochemistry was used to assess the degree of lymphocytic infiltration. Low levels of lymphocytic infiltration within the tumour were found to be associated with organ confined disease, whilst strong lymphocytic infiltration was associated with perineural invasion, capsular invasion and shortened biochemical recurrence free survival.

Why the presence of an increase in CD4+ T-lymphocyte infiltrate would be associated with poor cancer specific survival in patients with prostate cancer is unclear since it would seem logical that an increased T-lymphocyte infiltrate would

help control and even destroy tumour cells. However, it has been suggested that many of the T-lymphocytes found in tumour beds are inactive and therefore do not contribute to effective anti-tumoral immunity. Indeed, this concept is consistent with the observation that downregulation of MHC I expression may help tumours cells escape immune surveillance (Naoe et al., 2002).

In summary, the results of the present study have shown that the presence of increased CD4+ T-lymphocyte infiltration within the tumour was associated with poor outcome, independent of stage and PSA, in patients with prostate cancer.

Table 3.1 Baseline characteristics, according to stage, of patients with prostate cancer.

		Localised (n= 20)	Locally advanced (n= 37)	Metastatic (n= 23)	p-value
Age group (≤70/ >70)		11/ 9	22/ 15	5/ 18	0.013
Tumour grade (low/ intermediate/ high)		5/ 10/ 5	10/ 16/ 11	3/ 10/ 10	0.635
PSA (<4/ 4-10/ 11-100/ >100 ng/ml)		7/ 5/ 7/ 0	4/ 6/ 22/ 3	0/ 1/ 12/ 10	<0.001
Haemoglobin (≥12/ <12 g/l)		18/ 0	28/ 7	15/ 7	0.035
% tumour volume	CD4+*	0.42 (0.03-1.70)	0.40 (0.07-1.90)	0.53 (0.07-1.63)	0.475
	CD8+*	0.87 (0.27-1.70)	0.73 (0.27-3.00)	0.83 (0.27-1.90)	0.882
	CD4+ plus CD8+*	1.30 (0.30-3.13)	1.17 (0.33-4.90)	1.23 (0.50-3.20)	0.801
Treatment (radical local/ androgen deprivation)		11/ 9	9/ 28	1/ 22	0.001
Alive/ Dead		11/ 9	19/ 18	9/ 14	
(cancer specific/ intercurrent)		2/ 7	8/ 10	12/ 2	0.018

*Median (range)

Table 3.2 Baseline characteristics, according to PSA, of patients with prostate cancer.

	PSA <4ng/ml (n= 11)	PSA 4-10ng/ml (n= 12)	PSA 11-100ng/ml (n= 41)	PSA >100ng/ml (n= 13)	p-value
Age group (≤70/ >70)	4/ 7	6/ 6	22/ 19	4/ 9	0.449
Stage (localised/ locally advanced/ metastatic)	7/ 4/ 0	5/ 6/ 1	7/ 22/ 12	0/ 3/ 10	<0.001
Tumour grade (low/ intermediate/ high)	6/ 4/ 1	4/ 3/ 5	5/ 22/ 14	2/ 5/ 6	0.029
Haemoglobin (≥12/ <12 g/l)	9/ 1	10/ 1	32/ 6	9/ 4	0.448
% tumour volume CD4+*	0.37 (0.10-0.60)	0.35 (0.07-0.87)	0.40 (0.30-1.90)	0.43 (0.07-1.63)	0.643
CD8+*	0.73 (0.40-1.43)	0.85 (0.50-1.53)	0.87 (0.27-3.00)	0.73 (0.27-1.57)	0.333
CD4+ plus CD8+*	1.07 (0.57-2.00)	1.27 (0.70-2.27)	1.30 (0.30-4.90)	1.03 (0.60-3.20)	0.298
Treatment (radical local/ androgen deprivation)	3/ 8	7/ 5	11/ 30	0/ 13	0.013
Alive/ Dead	5/ 6	7/ 5	22/ 19	5/ 8	
(cancer specific/ intercurrent)	0/ 6	2/ 3	13/ 6	7/ 1	0.017

*Median (range)

Table 3.3 Baseline characteristics, according to CD4+ T-lymphocytes (tertiles), of patients with prostate cancer.

	Percentage volume of CD4+ T-lymphocytes			
	Tertile 1 (n= 26)	Tertile 2 (n= 28)	Tertile 3 (n= 26)	p-value
Age group (≤70/ >70)	11/ 15	14/ 14	13/ 13	0.812
Stage (localised/ locally advanced/ metastatic)	7/ 12/ 7	9/ 14/ 5	4/ 11/ 11	0.332
Tumour grade (low/ intermediate/ high)	7/ 10/ 9	9/ 9/ 9	2/ 16/ 8	0.144
PSA (<4/ 4-10/ 11-100/ >100 ng/ml)	4/ 4/ 13/ 4	6/ 5/ 13/ 4	1/ 3/ 15/ 5	0.677
Haemoglobin (≥12/ <12 g/l)	20/ 5	22/ 2	19/ 7	0.236
Treatment (radical local/ androgen deprivation)	8/ 18	7/ 21	6/ 20	0.806
Alive/ Dead	16/ 10	13/ 15	10/ 16	
(cancer specific/ intercurrent)	4/ 6	7/ 8	11/ 5	0.240

*Median (range)

Table 3.4. Clinicopathological characteristics in patients with prostate cancer: univariate survival analysis.

	Patients (n= 80)	Hazard ratio (95% CI)	p-value
Age group (≤70/ >70)	38/ 42	2.38 (0.97-5.85)	0.059
Stage			
(localised/ locally advanced/ metastatic)	20/ 37/ 23	2.99 (1.52-5.88)	0.002
Tumour grade (low/ intermediate/ high)	18/ 36/ 26	1.41 (0.80-2.51)	0.238
PSA (<4/ 4-10/ 11-100/ >100 ng/ml)	11/ 12/ 41/ 13	2.50 (1.38-4.52)	0.002
Haemoglobin (≥12/ <12 g/l)	61/ 14	3.38 (1.28-8.90)	0.014
Tumour counts			
CD4+ T-lymphocyte count (tertiles)	26/ 28/ 26	2.03 (1.15-3.59)	0.015
CD8+ T-lymphocyte count (tertiles)	26/ 28/ 26	1.46 (0.85-2.52)	0.170
CD4+ plus CD8+ count (tertiles)	26/ 28/ 26	1.69 (0.97-2.97)	0.065
Treatment			
(radical local/ androgen deprivation)	21/ 59	2.08 (0.70-6.17)	0.188

4.1.0 The systemic inflammatory response and survival in patients with metastatic prostate cancer

4.1 Introduction

Prostate cancer is the second commonest cause of death from cancer in men in the UK. Each year there are more than 10,000 deaths from the disease and prostate cancer accounts for 13% of all deaths from cancer in men. Overall survival is approximately 60% at 5 years (Cancerstats 2002; www.cancerresearchuk.org).

At presentation, there are a number of tumour-based factors, which can be used to predict the extent of disease and therefore survival in patients with prostate cancer. These include clinical stage, tumour grade and circulating concentration of prostate specific antigen (PSA). Furthermore, the value of PSA as a predictor of survival in patients with metastatic disease is well established.

However, it is increasingly recognised that, in cancer patients, disease progression is dependent on a complex interaction of the tumour and the host inflammatory response (Balkwill and Mantovani, 2001; Coussens and Werb, 2002). It is therefore of interest that the presence of a systemic inflammatory response, as evidenced by elevated circulating concentrations of C-reactive protein, has been shown to be an independent predictor of survival in patients with advanced gastrointestinal, lung and renal cancer (O'Gorman et al., 2000; Scott et al., 2002; Bromwich et al, 2003).

The aim of the present study was examine the relationship between the systemic inflammatory response, PSA and survival in patients with metastatic prostate cancer.

4.2 Patients and methods

Patients with metastatic prostate cancer attending hospitals in the North Glasgow NHS Trust between January 1997 and December 2002 were included in the study. All patients had histologically proven prostate cancer and had radiologically confirmed metastatic disease. All patients had been established on androgen deprivation therapy. Some patients had previously been treated with radiotherapy. A blood sample was taken for the measurement of total PSA and C-reactive protein. At this time no patient showed clinical evidence of infection or other inflammatory condition.

The study was approved by the Research Ethics Committee of the North Glasgow NHS Trust.

Total PSA was measured using the Bayer ADVIA Centaur Assay system (Bayer PLC, Bayer House, Newbury, UK). Inter-assay variability was <6% for total PSA.

C-reactive protein was measured by Fluorescence Polarisation Immunoassay using an Abbott TDX analyser and Abbott reagents Abbott Laboratories, Abbott Park, IL, USA). The limit of detection of the assay is a C-reactive protein concentration of less than 5mg/l. The coefficient of variation, over the range of measurement, was less than 5% as established by routine quality control procedures.

Statistics

Based on previous work (O'Gorman et al., 2000), a C-reactive protein concentration greater than 10mg/l was considered to indicate the presence of a

systemic inflammatory response. Univariate survival analysis was performed using the Kaplan–Meier method. Multivariate survival analysis and calculation of hazard ratios (HR) were performed using the Cox regression analysis with prognostic scores as covariates. Deaths up to 1st June 2005 were included in the analysis. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

4.3 Results

The baseline characteristics of the patients with metastatic prostate cancer (n= 62) are shown in Table 4.1. The majority of patients were aged over 70 years and had intermediate or high-grade tumours. Approximately half the patients had a PSA greater than 10ng/ml and a third had an elevated circulating C-reactive protein concentration. Fifty one patients had received androgen deprivation therapy alone and 11 had received a combination of androgen deprivation therapy and radiotherapy.

In all, 41 (66%) of patients died, 38 (61%) of their disease during the follow-up period. The median follow-up of the survivors was 62 months. On univariate survival analysis, PSA ($p<0.05$, Figure 4.1) and C-reactive protein ($p<0.05$, Figure 4.2) were significant predictors of cancer specific survival. On multivariate analysis, both PSA (HR 1.96, 95%CI 1.00-3.83, p=0.049) and C-reactive protein (HR 1.97, 95% CI 0.99-3.92, p=0.052) were predictors of cancer specific survival. PSA concentrations were significantly correlated with those of C-reactive protein ($r_s= 0.46$, $p<0.001$).

4.4 Discussion

The results of the present cross-sectional study show that, in patients with metastatic prostate cancer, both an elevated circulating PSA concentration and the presence of a systemic inflammatory response, as evidenced by an elevated C-reactive protein concentration, were associated with poorer cancer specific survival. Furthermore, on multivariate analysis, the prognostic value of both was similar and both were significantly correlated. These results would suggest that the systemic inflammatory response is associated with disease progression. However, it remains to be determined whether an elevated C-reactive protein will provide useful additional prognostic information.

Few studies have examined the role C-reactive protein as a prognostic factor in prostate cancer. Trautner and co-workers (1980) reported that, in a cohort of 102 patients including 31 patients with metastatic disease, the rise in C-reactive protein concentrations was associated with tumour progression even when the patient was receiving oestrogen therapy. Lewenhaupt and co-workers (1990) also reported that, in a cohort of patients including 31 (30%) patients with metastatic disease, an elevated C-reactive protein concentration was associated with survival on univariate analysis but not on multivariate analysis. However, the above studies included patients with localised disease who were unlikely to progress and patients with metastatic disease who had already progressed. This is likely to have confounded the assessment of the prognostic value of an elevated circulating C-reactive protein concentration.

The mechanism by which a systemic inflammatory response might impact on cancer-specific survival is not clear. There is evidence to suggest that interleukin-6, which is largely responsible for the production of C-reactive protein (Ljungberg et

al,1997; McArdle et al, 2004; McKcown et al., 2004) can be produced by prostatic tumours (Corcoran et al, 2003). Furthermore, increased circulating concentrations of interleukin-6 have been shown to be associated with reduced survival in patients with prostate cancer (Nakashima et al, 2000). Interleukin-6 has also been shown to act as an autocrine growth factor (Okamoto et al, 1997; Lou et al, 2000) and to inhibit apoptosis in prostate cancer (Chung et al, 2000). However, it is not clear whether interleukin-6 is superior to C-reactive protein in predicting survival in patients with metastatic prostate cancer.

In summary, the results of the present study suggest that, in patients with metastatic prostate cancer, the presence of a systemic inflammatory response during the follow-up period may predict poor outcome, independent of PSA.

Table 4.1. The characteristics of patients with metastatic prostate cancer. Univariate survival analysis performed using the Kaplan-Meier method with log-rank test.

		Patients	Survival (months)	p-value
		62 (%)	Mean (95%CI)	
Age	≤70yrs	27 (44)	52.6 (40.8-64.5)	
	>70 yrs	35 (56)	38.5 (28.5-48.5)	0.132
Gleeson score	2-6	17 (27)	51.9 (36.7-67.1)	
	7	19 (31)	39.8 (26.9-52.7)	
	8-10	17 (27)	37.4 (23.5-51.3)	0.301
PSA	≤10 ng/ml	33 (53)	53.4 (43.8-62.9)	
	>10 ng/ml	29 (47)	3.4.7 (23.1-46.3)	0.012
C-reactive protein	≤10mg/l	43 (69)	51.8 (42.8-60.8)	
	>10mg/l	19 (31)	30.2 (16.2-44.1)	0.010
Treatment				
Androgen deprivation		51 (82)	44.9 (36.5-53.3)	
Combination therapy		11 (18)	41.8 (22.4-61.2)	0.639

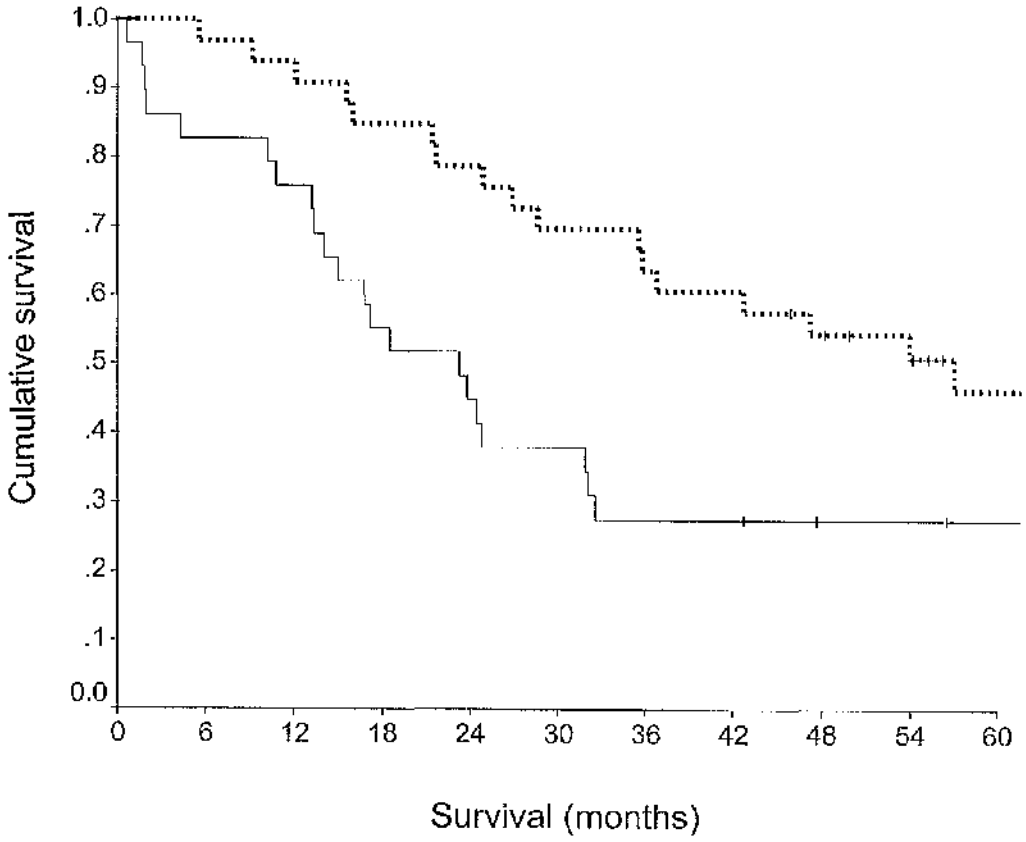


Figure 4.1. The relationship between PSA (≤10/ >10ng/ml from top to bottom) and cancer specific survival in patients with metastatic prostate cancer (n=62).

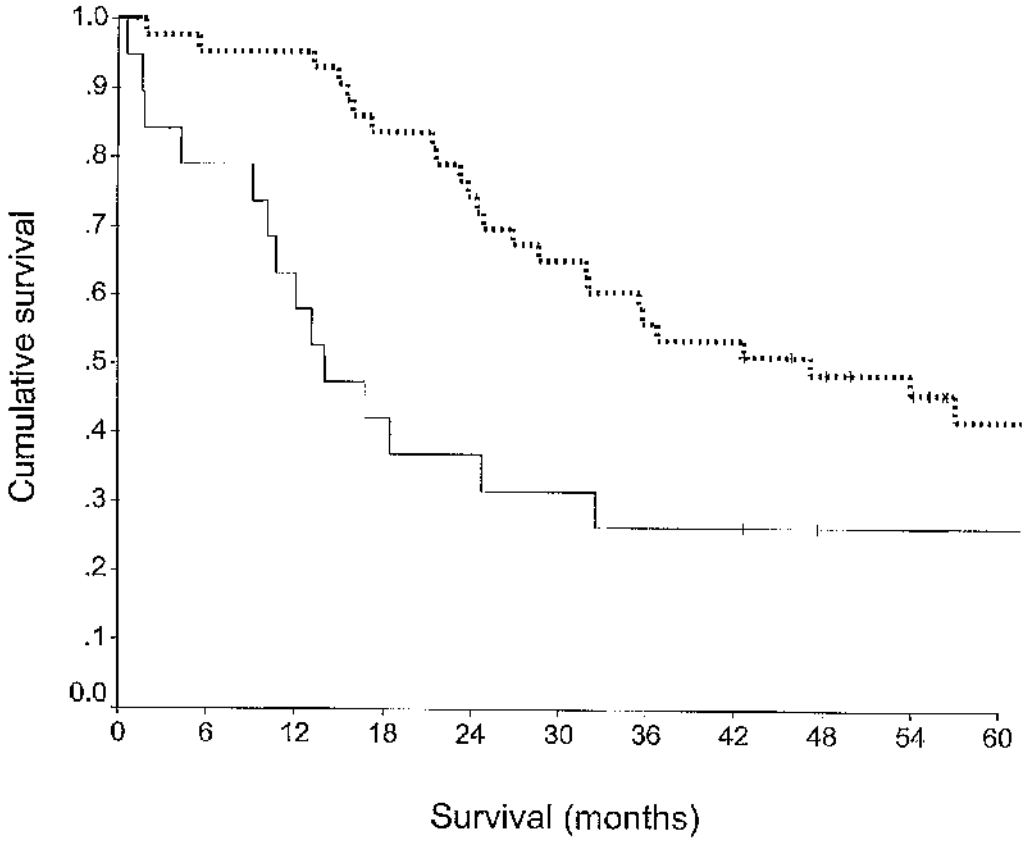


Figure 4.2. The relationship between C-reactive protein (≤10/ >10mg/l from top to bottom) and cancer specific survival in patients with metastatic prostate cancer (n=62).

5.0.1 The relationship between interleukin-6 and C-reactive protein in patients with benign and malignant prostate disease

5.1 Introduction

The systemic inflammatory response is an obligatory response of the body to infection, surgery or trauma. There is also increasing evidence that the presence of a systemic inflammatory response, as evidenced by increased circulating concentrations of C-reactive protein, is associated with early recurrence and poor survival in a variety of common hormone independent tumours (Nielsen et al., 2000; Scott et al., 2002; McMillan et al., 2003). There is also some evidence that a similar relationship exists in hormone dependent tumours (Lewenhaupt et al., 1990; Alberquerque et al., 1995).

A number of factors appear to mediate the increased production of C-reactive protein. In injury, the pro-inflammatory cytokines, interleukin-1, TNF-alpha and interleukin-6 in particular, have been shown to stimulate the production of C-reactive protein (Gabay and Kushner, 1999). In cancer, the factors which determine circulating concentrations of C-reactive protein are less clear since studies in cell lines and animal tumour models have demonstrated that a number of factors stimulate C-reactive protein production. For example, it has been shown that leukaemia inhibitory factor and ciliary neurotrophic factor are also capable of inducing an acute phase protein response from the liver (Baumann et al 1993, Henderson et al 1994, Akiyama et al 1997). There is also evidence that soluble receptor subunits involved in interleukin-6 signal transduction, the soluble interleukin-6 receptor and the soluble gp130 receptor, may be important in the regulation of interleukin-6 activity and the acute phase protein response (Narazaki et al., 1993; Jones et al., 2002). Therefore, if

in cancer patients, C-reactive protein was stimulated by factors other than interleukin-6 then it might be expected that the relationship between interleukin-6 and C-reactive protein would less strong than that in patients with benign disease.

The aim of the present study was to examine the relationship between interleukin-6 and C-reactive protein in patients with benign disease (BPH) and in prostate cancer.

5.2 Patients and methods

Consecutive patients undergoing diagnostic transrectal ultrasound guided (TRUS) biopsy of prostate were included in the study. The indication for TRUS biopsy was either a total PSA concentration greater than 4ng/mL and/or an abnormal digital rectal examination.

Prior to biopsy, a blood sample was obtained and the serum stored at –20°C prior to analysis. No patient had a digital rectal examination within a two-week period prior to sampling. No patients were taking non-steroidal anti-inflammatory drugs or were receiving treatment for prostate cancer at the time of blood sampling.

None of the patients had participated in a formal screening programme. All patients underwent a minimum of systematic sextant biopsy. A Gleason score was allocated for each tumour. The Gleason score reflects tumour heterogeneity by combining primary and secondary patterns into a composite score which has been shown has been shown to be an important predictor of disease recurrence and survival (Epstein et al., 1993). Gleason scores were compressed as previously recommended (Bostwick et al., 2000).

The study was approved by the local Research Ethics Committee. All subjects were informed of the purpose and procedure of the study and all gave written consent.

Total PSA was measured using the Bayer ADVIA Centaur Assay system (Bayer PLC, Bayer House, Newbury, UK). Inter-assay variability was <6% for total PSA.

Interleukin-6 concentrations were measured using a sensitive solid phase enzyme linked immunosorbent assay (R & D Systems Europe Ltd, Abingdon, UK). The lower level of detection was 0.2 pg/l and the intra - assay variability was less than 6% over the sample concentration range.

C-reactive protein concentration was measured using a sensitive double antibody sandwich ELISA with rabbit anti-human C-reactive protein and peroxidase conjugated rabbit anti-human C-reactive protein. The assay was linear from 0.1 mg/l to 5mg/l and logarithmic thereafter. Inter-assay variation was less than 10% over the sample concentrations range.

Statistics

Data are presented as the median (range), and where appropriate, differences between control and cancer groups were examined using the Mann-Whitney U test. Correlations between two variables were calculated using Spearman's rank correlation test. As the distribution of interleukin-6 and C-reactive protein concentrations were skewed, they were logarithmically transformed to illustrate their relationship in patients with benign prostatic disease and prostate cancer. Analysis was carried out using the statistics package SPSS (SPSS Inc., Chicago, Illinois USA).

5.3 Results

One hundred and forty five patients (59 with benign prostatic disease and 86 diagnosed with prostate cancer) were included in the study. The characteristics of the patients with benign prostatic disease and prostate cancer are shown in Table 5.1 and 5.2. The majority of patients with prostate cancer had localised or locally advanced disease and had a low Gleason score. Compared to patients with benign prostatic disease, the cancer patients had higher circulating concentrations of total PSA ($p<0.001$). There were no significant differences in circulating concentrations of interleukin-6 and C-reactive between patients with benign disease and prostate cancer.

The relationship between circulating concentrations of interleukin-6 and C-reactive protein in patients with benign prostatic disease and prostate cancer is shown in Figure 5.1. The correlation coefficients for patients with benign prostatic disease and prostate cancer were $r_s = 0.632$, $p<0.001$ and $r_s = 0.663$, $p<0.001$ respectively.

The relationship between Gleason score, PSA, interleukin-6 and C-reactive protein in the patients with prostate cancer is shown in Table 5.3. With increasing Gleason scores there were significant increases in total PSA ($p<0.05$), interleukin-6 ($p<0.01$) and C-reactive protein ($p<0.01$). There was a significant correlation between Gleason score and total PSA ($r_s = 0.364$, $p=0.001$), interleukin-6 ($r_s = 0.311$, $p=0.004$) and C-reactive protein ($r_s = 0.304$, $p=0.004$). There was no significant association between total PSA and either interleukin-6 or C-reactive protein.

5.4 Discussion

It is recognised that diagnosis of prostate cancer using TRUS guided biopsy carries a recognised false negative rate of approximately 20% (Romics, 2004). In the present

study, however, there were no significant differences in the circulating concentrations of either interleukin-6 or C-reactive protein between patients with benign prostatic disease and those with prostate cancer. Furthermore, the relationship between interleukin-6 and C-reactive protein was similar in both patient groups. It is therefore unlikely that misclassification of a small number of cancer patients would significantly alter the results of this study.

In the cancer patients, there was a significant increase in both interleukin-6 and C-reactive protein concentration with increasing tumour grade. These results are also consistent with previous observations that concentrations of interleukin-6 and its receptor are increased in patients with a Gleason score greater than 7 (Shariat et al., 2001). This might suggest that the tumour was responsible for the increased production of interleukin-6. However, the increases in interleukin-6 and C-reactive protein were independent of total PSA.

An alternative explanation would be that the source of interleukin-6 is the host inflammatory cells. It is therefore of interest that recent studies have shown that a pronounced inflammatory infiltrate in the tumour was associated with poor outcome in patients with prostate cancer (Irani et al., 1999; McArdle et al., 2004).

Taken together, these observations confirm that interleukin-6 is predominantly responsible for the elaboration of C-reactive protein. Furthermore, they suggest that interleukin-6 is produced primarily by the host rather than the tumour. Moreover, it may also be that interleukin-6, produced by tumour infiltrating leucocytes, stimulates tumour cell proliferation and promotes leucocyte recruitment as part of an autocrine growth factor loop (Corcoran and Costello, 2003).

In summary, the results of the present study indicate that the relationship between interleukin-6 and C-reactive protein is similar in patients with benign and malignant prostate disease.

Table 5.1 The characteristics of patients with benign prostatic disease and prostatc cancer.

	Benign prostatic disease (n= 59)	Prostate cancer (n= 86)	p-value
Age (≤70/ >70 yrs)	45/ 14	52/ 34	0.069
Stage (T1-T2/ T3-T4/ Met)		37/ 37/ 12	
Gleason score (2-6, 7, 8-10)		46/ 24/ 16	
Total PSA (ng/ml)*	7.0 (0.8-39)	14.6 (0.5-146)	<0.001
Interleukin-6 (pg/ml)*	2.3 (0.7-12.9)	2.3 (0.8-28.1)	0.526
C-reactive protein (mg/l)*	2.0 (0.1-21.1)	1.8 (0.3-50)	0.891

Median (range)

Table 5.2 The relationship between stage, PSA, interleukin-6 and C-reactive protein in patients with benign prostatic disease and prostate cancer.

	Benign prostatic disease (n= 59)	T1-T2 cancer (n= 37)	T3-T4 cancer (n= 37)	Metastatic (n= 12)	p-value
Age (≤70/ >70 yrs)	45/ 14	26/ 11	19/ 18	7/ 5	0.073
Gleason score (2-6, 7, 8-10)		30/ 4/ 3	14/ 17/ 6	2/ 3/ 7	<0.001
Total PSA (ng/ml)*	7.0 (0.8-39.0)	7.6 (0.5-36.1)	20.3 (3.7-116)	23.2 (9.7-146)	<0.001
Interleukin-6 (pg/ml)*	2.3 (0.7-12.9)	2.0 (0.8-7.7)	2.5 (1.0-28.1)	2.5 (1.3-4.5)	0.123
C-reactive protein (mg/l)*	2.0 (0.1-21.1)	1.9 (0.3-50)	2.2 (0.4-42.0)	1.2 (0.4-6.2)	0.556

* Median (range)

Table 5.3 The relationship between inflammatory parameters and Gleason score in patients with prostate cancer.

	Gleason score 2-6 (n= 46)	Gleason score 7 (n= 24)	Gleason score 8-10 (n= 16)	p-value
Age (≤70/ >70 yrs)	31/ 15	12/ 12	9/ 7	0.343
Stage (T1-T2/ T3-T4/ Met)	30/ 14/ 2	4/ 17/ 3	3/ 6/ 7	<0.001
Total PSA (ng/ml)*	11.0 (0.5-76)	19.8 (3.7-116)	18.7 (4.8-146)	0.025
Interleukin-6 (pg/ml)*	1.9 (0.8-11.4)	3.1 (0.8-28.1)	3.0 (1.1-9.3)	<0.005
C-reactive protein (mg/l)*	1.1 (0.3-18.7)	2.7 (0.3-50)	3.3 (0.8-12.5)	<0.005

* Median (range)

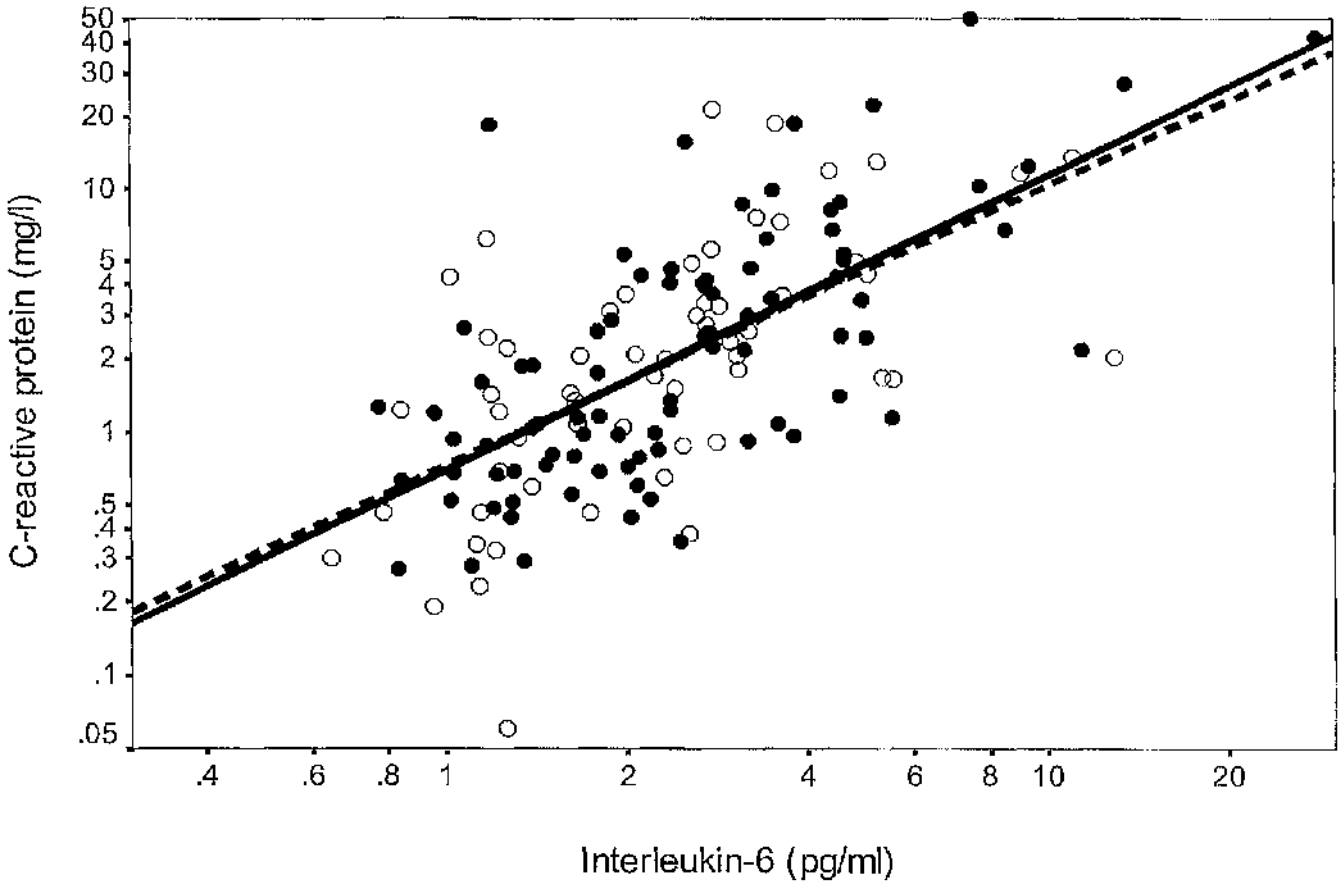


Figure 5.1. The relationship between circulating concentrations of interleukin-6 and C-reactive protein in patients with benign prostatic disease (O, n=59) and prostate cancer (●, n=86).

6.1.0 An evaluation of prostate specific antigen and C-reactive protein in the detection of prostate cancer in a non-screened population.

6.1 Introduction

Since its introduction in the late 1980's, prostate specific antigen (PSA) remains the most widely used marker for the detection of prostate cancer. However, although PSA testing is associated with a high level of sensitivity using conventional cut-off concentrations, the specificity of the test is low since patients with benign prostatic disease may also have elevated PSA levels.

A number of alternative approaches have been devised in an attempt to improve the specificity of the total PSA test, including the use of age adjusted reference ranges, PSA density, PSA transitional zone density and PSA velocity. However, none of these derived values has gained widespread acceptance in routine clinical practice.

Recently, the discovery of different isoforms of PSA has generated further interest. Circulating PSA can be divided into two main forms, namely complexed and free PSA. Most circulating PSA is bound to a variety of protease inhibitors, most commonly alpha-1-antichymotrypsin; the proportion of free or unbound PSA is relatively small. Initially, there was no reliable method of measuring complexed PSA directly and it was therefore calculated by subtracting the unbound or "free" PSA from the total PSA. More recently, a method of directly measuring the concentrations of complexed PSA has been developed (Allard et al, 1998). Several studies have suggested that the use of these PSA isoforms or their ratios provides greater specificity than total PSA in the detection of prostate cancer (Bangma et al, 1997;Catalona et al, 1998; Brawer et al, 1998; Brawer et al, 2000; Okihara et al,

2002). Nevertheless, despite these encouraging results, there is a continuing need to evaluate new potential markers of malignancy.

It is therefore of interest that, as described in chapter four, the presence of a systemic inflammatory response, as evidenced by elevated circulating concentrations of C-reactive protein, is associated with poor prognosis in patients with metastatic prostate cancer. Furthermore, there is evidence that the systemic inflammatory response may play an important role in promoting disease progression. However, the value of measuring C-reactive protein in the diagnostic setting does not appear to have been assessed.

The aim of the present study was therefore 1) to evaluate the role of PSA and its isoforms in the detection of prostate cancer and 2) to assess whether measurement of C-reactive protein concentrations was useful in discriminating between patients with benign disease and prostate cancer.

6.2 Patients and methods

Consecutive patients undergoing diagnostic transrectal ultrasound guided (TRUS) biopsy of prostate were studied prospectively. The indication for TRUS biopsy was either a total PSA concentration greater than 4 μg/L and/or an abnormal digital rectal examination. None of the patients had participated in a formal screening program. All patients underwent a minimum of systematic sextant biopsy.

Prior to biopsy, a blood sample was obtained and the serum stored at –20°C. No patient had a digital rectal examination within a two-week period prior to sampling. No patient was receiving treatment at the time of blood sampling.

Total and complexed PSA was measured using the Bayer ADVIA Centaur Assay system (Bayer PLC, Bayer House, Newbury, UK). Total and free PSA was measured using the Immulite 2000 Immunoassay system (Diagnostic Products Corporation UK, Glyn Rhonwy, Llanberis, UK). The inter-assay variability, as quoted by the manufacturer was <6% and <3% for total and complexed PSA (Bayer assay system) with an assay sensitivity of 0.01 and 0.03μg/L respectively. The inter-assay variability was <4% and <6% for total and free PSA (Immulite assay system) with an assay sensitivity of 0.01 and 0.03 μg/L respectively.

C-reactive protein concentrations were measured by a fluorescence polarization immunoassay and using an Abbott TDXTM analyser and Abbott reagents (Abbott Laboratories, Abbott Park, Illinois, USA). The limit of the detection of the assay is a C-reactive protein concentration lower than 5mg/L. The coefficient of variation, over the range of measurement, was less than 5 per cent, as established by routine quality control procedures. Based on previous work a C-reactive protein concentration of greater than 10mg/L was considered to indicate the presence of a systemic inflammatory response.

The study was approved by the local ethics committee.

Statistics

Data are presented as median and range. Where appropriate comparison between unpaired data was carried out using the Mann-Whitney U-test. Agreement between the two methods of measuring total PSA was assessed using a paired sample T-test. Correlations between two variables were calculated using Spearman's rank correlation test.

Receiver operating characteristics (ROC) curves were generated by plotting the sensitivity versus (1-specificity) as previously described (Babian et al, 1996). The area under the ROC curve was calculated for total PSA, complexed PSA, free PSA, complexed-to-total and free-to-total PSA. Analysis was performed using SPSS software (SPSS Inc, Chicago, Illinois, USA).

6.3 Results

One hundred and seventy one men were included in the study. Of these, 103 (60%) were found to have histologically proven prostate cancer, 8 (5%) had prostatic intraepithelial neoplasia and the remaining 60 (35%) had benign prostatic disease. For the purpose of this study, patients with prostatic intraepithelial neoplasia were included in the non-cancer group. The median ages in the benign and cancer groups were 66 (range 45-79) and 69 (range 46-88, $p<0.01$) respectively.

The results of the total, complexed and free PSA assays are shown in table 6.1. When the two methods of measuring total PSA were compared, the difference between the values obtained using the two assays was shown to vary in proportion to the PSA concentration. Using a paired sample T-test the values obtained using the Immulite 2000 assay were significantly higher by a mean of 12.5% (95% CI 10%-15%, $p<0.001$) than the values obtained with the Bayer assay.

There was a significant positive correlation between the values of total PSA and complexed ($r=0.978$, $p<0.001$) and free PSA ($r=0.807$, $p<0.001$). There was also a significant negative correlation between total PSA and the free/total ratio ($r= -0.346$, $p<0.001$).

Total PSA, measured using both techniques, was significantly higher in the patients with histologically confirmed prostate cancer compared to patients with benign disease ($p<0.001$; table 6.1). There were also significant differences in complexed PSA ($p<0.001$), complexed/total PSA ($p=0.033$) and free/total PSA ($p<0.001$) between patients with prostate cancer and those with benign disease.

There was no significant difference in C-reactive protein concentrations between patients with benign and malignant prostate disease.

ROC analysis showed that, when all patients were examined, the areas under the ROC curves for total and complexed PSA, and the free/total ratio were similar (Figure 6.1, table 6.1).

Using the conventional diagnostic cut-off of 4 μg/L, as measured by the Bayer assay, the sensitivity and specificity of total PSA was 90% and 24% respectively (table 6.2). Fixing the sensitivity of the other assays at 90%, the corresponding specificities for complexed PSA and the free/total PSA ratio were 24% and 21% respectively.

Seventy-seven patients had total PSA concentrations between 2 and 10 μg/L as measured using the Bayer assay (table 6.3). ROC curve analysis showed that total and complexed PSA failed to discriminate between benign and malignant disease. In contrast, the areas under the curve were greater for the complexed to total ($p=0.057$) and the free to total ($p=0.033$) ratios (Figure 6.2).

Using the conventional cut-off of 4μg/L, the specificities for the complexed to total and free to total ratios were 40% and 42% respectively compared with approximately 20% for total and complexed PSA (table 6.4).

6.4 Discussion

The use of prostate specific antigen to detect prostate cancer is well established. However, although PSA has been shown to be a highly sensitive method of detecting the presence of prostate cancer, concern has been expressed about the relative lack of specificity, particularly in screened populations where low specificity results in a high proportion of unnecessary biopsies (Bangma et al, 1997;Catalona et al, 1998; Brawer et al, 1998; Brawer et al, 2000; Okihara et al, 2002). As a result, there is increasing interest in the use of the complexed and free isoforms of PSA as a potential method of increasing the ability to discriminate between those patients with benign disease and those with prostate cancer.

Most of the information about free and complexed PSA has been derived from retrospective studies based on asymptomatic screened populations in North America. For example, Bangma and his co-workers (1997) found that the free to total PSA ratio was more specific than total PSA, but only in patients with a total PSA greater than 7 μg/L. Subsequently, Catalona and his colleagues (1998) reported that the use of the free-to-total PSA ratio increased specificity between 4-10 μg/L, while maintaining sensitivity. Brawer and his co-workers (1998) initially reported that complexed PSA performed better than either total PSA or the free to total PSA ratio. Brawer and his colleagues (2000) subsequently reported that the specificity of complexed PSA was equivalent to that of the free-to-total PSA. In contrast, Okihara and his co-workers (2002) reported that complexed PSA was equivalent to total PSA and that free-to-total PSA was superior to both. Some of these apparent contradictions might be explained by the retrospective nature of some of these studies, the use of non-consecutive

subjects and the use of different total PSA concentrations to define the patient population of interest.

The clinical value of complexed and free PSA, and their ratios, especially as it pertains to routine clinical practice in an unscreened population in the UK remains uncertain. Some studies have failed to show an advantage in the use of the free to total PSA ratio compared to total PSA (Masters et al, 1998; Klingler et al, 1998); however, the numbers studied were small. In contrast, Jung and his colleagues (2000) reported that, in patients with total PSA concentrations between 2 and 10 μg/L, complexed PSA was equivalent to total PSA, but that specificity increased substantially if either the complexed-to-total or free-to-total ratio was used.

The results of the present study, in an unscreened population show that, overall, there were significant differences in the total and complexed PSA concentrations, the complexed to total and free-to-total PSA ratios between patients with prostate cancer and those with benign disease. Indeed, ROC curve analysis showed that the areas under the curves for total and complexed PSA, and the free to total PSA ratio were similar. Using the conventional cut-off of 4 μg/L for total PSA, the corresponding specificities for total PSA, complexed PSA and the free-total PSA ratio were also similar.

Confining the analysis to the 77 patients with total PSA concentrations between 2 μg/L and 10 μg/L, ROC curve analysis demonstrated that the complexed to total and the free to total ratios were better than total PSA in discriminating between patients with benign and malignant disease. These results are therefore similar to those reported by Jung and his co-workers.

In contrast the results of the present study indicated that C-reactive protein concentrations were not of value in differentiating between patients with benign and malignant disease. The failure of C-reactive protein to differentiate between benign and malignant disease may be due to the fact the presence of a systemic inflammatory response in patients with symptomatic prostatic disease may reflect either the presence of cancer or other disease processes such as urinary tract infection. Furthermore, whereas PSA is produced directly by the tumour, C-reactive protein is a non-specific host response. It is therefore not surprising that PSA had greater discriminatory power than C-reactive protein.

In summary, the results of the present study, in a non-screened population, show that, overall, the use of the PSA isoforms and their ratios did not provide additional discriminatory power. However, in a subset of patients with total PSA concentrations between 2 and 10 μg/L, the free to total PSA ratio was superior to total PSA in discriminating between patients with benign and malignant disease.

Table 6.1. The relationship between total, free and complexed PSA in patients with benign disease or prostate cancer (entire cohort).

	Benign (n=68)	Cancer (n=103)	Area under ROC curve (95%CI)	P value
Total PSA- (Bayer) (μg/L)	6.9 (0.4-39.0)	12.7 (0.5-146.0)	0.698 (0.619-0.777)	<0.001
Complexed PSA (μg/L)	5.0 (0.3-26.7)	10.3 (0.3-80.0)	0.707 (0.629-0.784)	<0.001
Complexed/ total PSA (%)	74.5 (7.5-92.0)	78.6 (27.0-97.0)	0.597 (0.510-0.683)	0.033
Total PSA- (Immulite) (μg/L)	8.1 (0.4-39.6)	14.8 (0.3-264.0)	0.710 (0.632-0.788)	<0.001
Free PSA (μg/L)	1.1 (0.1-6.7)	1.3 (0.1-85.5)	0.581 (0.494-0.669)	0.072
Free/ total PSA (%)	14.3 (4.0-52.5)	9.3 (3.0-32.4)	0.718 (0.642-0.795)	<0.001

Median (range)

Table 6.2. Relative specificity of total, free and complexed PSA at 90% sensitivity

	Cut-off	Sensitivity	specificity
Total PSA (Bayer)	4μg/L	90%	24%
Complexed PSA	2.9μg/L	90%	24%
Complexed/total ratio	62.5%	90%	10%
Total PSA- (Immulite)	4.8μg/L	90%	27%
Free/total ratio	20%	90%	21%

Table 6.3. The relationship between total, free and complexed PSA in patients with benign disease or prostate cancer (within a total PSA range of 2-10 µg/L)

	Benign (n=40)	Cancer (n=37)	Area under ROC curve (95% CI)	P value
Total PSA- (Bayer) (µg/L)	6.1 (2.1-10.0)	6.6 (2.2-10.0)	0.522 (0.391-0.653)	0.740
Complexed PSA (µg/L)	4.6 (1.5-7.9)	4.9 (1.5-8.4)	0.548 (0.418-0.679)	0.466
Complexed/total PSA (%)	74.5 (7.5-91.0)	81.0 (50.9-96.0)	0.626 (0.501-0.752)	0.057
Total PSA- (Immulite) (µg/L)	6.8 (2.3-11.1)	7.2 (2.3-12.5)	0.544 (0.412-0.675)	0.511
Free/total PSA (%)	14.1 (5.4-40.6)	11.9 (3.0-27.8)	0.642 (0.517-0.766)	0.033

Median (range)

Table 6.4. Relative specificity of total, free and complexed PSA at 80% sensitivity.

	Cut-off	Sensitivity	specificity
Total PSA (Bayer)	4μg/L	80%	20%
Complexed PSA	3.1μg/L	80%	20%
Complexed/ total ratio	72.3%	80%	40%
Total PSA- (Immulite)	4.9μg/L	80%	25%
Free/total ratio	16.5%	80%	42%

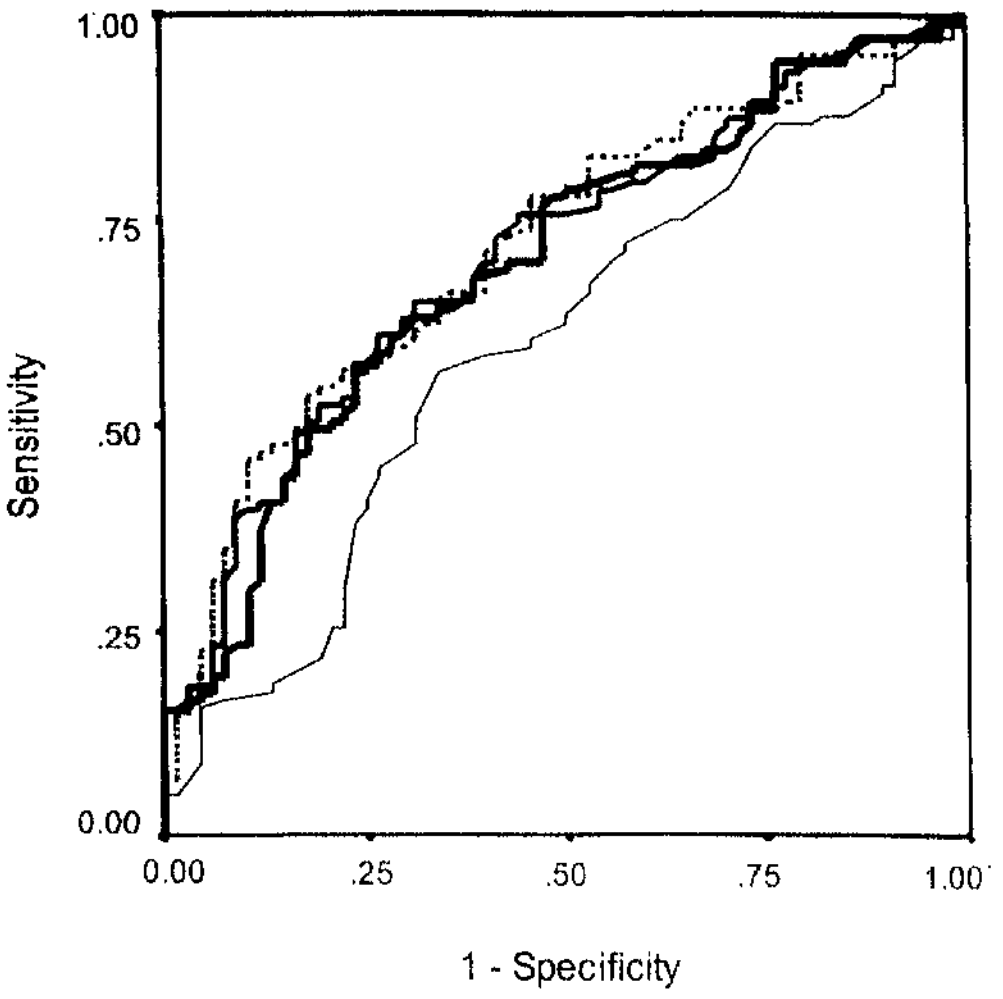


Figure 6.1. ROC curves for entire patient cohort (n= 171)

Total PSA ━, complexed PSA ── , free to total PSA ····· , complexed to total PSA ──

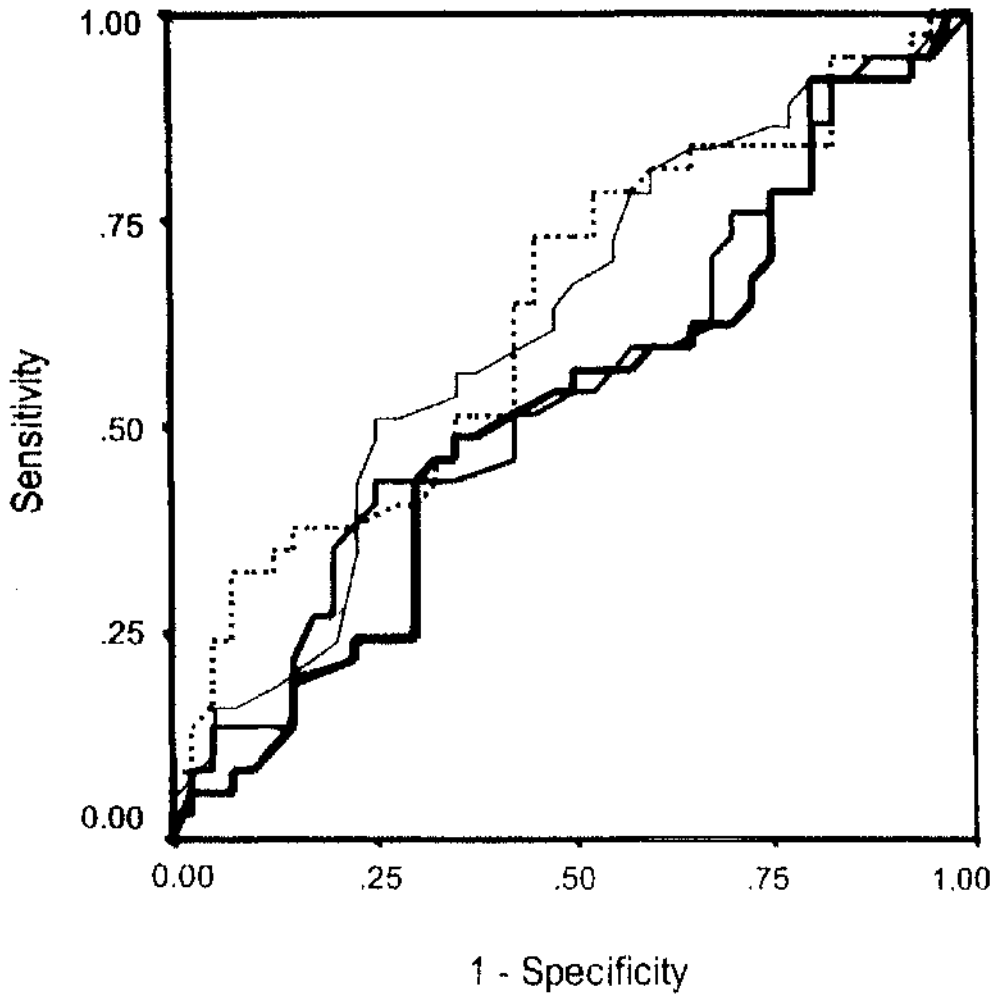


Figure 6.2. ROC curves for patient cohort with total PSA concentrations 2-10μg/L (n= 77).

Total PSA ━, complexed PSA —, free to total PSA ····, complexed to total PSA —

7.0.1 Discussion

It has long been recognised that disease progression in cancer patients is not solely determined by the characteristics of the tumour but also by the host response (Balkwill and Mantovani, 2001). Indeed there is increasing evidence that both local and systemic inflammatory responses play an important role in the progression of a variety of common solid tumours (Coussens and Werb, 2002).

For example, in patients with colorectal cancer, there is good evidence that the presence of a pronounced lymphocytic infiltration within the tumour is associated with improved survival (Jass et al, 1987; Ropponen et al, 1997; Nielsen et al, 1999). Conversely, the presence of a systemic inflammatory response, as evidenced by the presence of an elevated circulating C-reactive protein concentration, is associated with poor prognosis (Nielsen et al., 2000; McMillan et al., 2003).

The relationship between lymphocytic infiltration and survival in patients with urological cancers is unclear. At the time that this work was carried out, only two studies had evaluated the impact of tumour lymphocyte infiltration on outcome in patients with prostate cancer. However, the conclusions reached by these studies appeared to be contradictory. Furthermore, the relationship between specific lymphocyte subset infiltration and survival does not appear to have been assessed in these patients.

In chapter 3, the relationship between lymphocytic subset infiltration and survival was examined. In a cohort of 80 patients with untreated prostate cancer, it was shown that the presence of a profuse CD4+ but not CD8+ lymphocytic infiltration within the tumour bed was associated with a poor prognosis, independent of stage, grade and subsequent treatment. This study therefore provides evidence that

the presence of a local inflammatory response is an important factor in determining the prognosis in patients with prostate cancer.

At presentation, there are a number of tumour based factors, which can be used to predict the extent of disease and therefore survival in patients with prostate cancer. These include clinical stage, tumour grade and circulating concentrations of PSA. However, predicting prognosis during the follow-up period is more difficult, since treatment for prostate cancer may suppress circulating PSA concentrations to varying degrees.

Few studies have examined the value of an elevated circulating C - reactive protein concentration, as a prognostic factor in patients with prostate cancer. It is therefore of interest that Trautner and co-workers (1980) reported that, in a heterogeneous group of patients, a rise in circulating C-reactive protein concentrations was associated with disease progression even when patients were receiving hormone therapy.

In chapter 4, the impact of an elevated circulating C-reactive protein concentration on survival was assessed in a cohort of 62 patients receiving hormone treatment for metastatic prostate cancer. This study showed that in patients with metastatic prostate cancer, the presence of a systemic inflammatory response during the follow-up period may predict poor outcome, independent of PSA.

The mechanism by which a systemic inflammatory response might impact on cancer-specific survival is not clear. There is evidence to suggest that interleukin-6, which is largely responsible for the production of C-reactive protein can be produced by prostatic tumours. Furthermore, increased circulating concentrations of interleukin-6 have been shown to be associated with reduced survival in patients with prostate cancer. Interleukin-6 has also been shown to act as an autocrine growth

factor (Okamoto et al, 1997) and to inhibit apoptosis in prostate cancer (Chung et al, 2000).

A number of factors appear to mediate the increased production of C-reactive protein. In injury, the pro-inflammatory cytokines, interleukin-1, TNF-alpha and interleukin-6 in particular, have been shown to stimulate the production of C-reactive protein (Gabay and Kushner, 1999). In cancer, the factors which determine circulating concentrations of C-reactive protein are less clear since studies in cell lines and animal tumour models have demonstrated that a number of factors stimulate C-reactive protein production. It might therefore be anticipated that the relationship between C-reactive protein and IL-6 would be different in patients with prostate cancer from those with benign disease.

In chapter 5, the relationship between circulating concentrations of C-reactive protein and IL-6 was assessed in a cohort of 145 patients (59 with benign disease and 86 with prostate cancer). Compared to patients with benign prostatic disease, the cancer patients had higher circulating concentrations of total PSA. There were no significant differences in circulating concentrations of interleukin-6 and C-reactive between patients with benign disease and prostate cancer. Moreover, the relationship between C-reactive protein and its corresponding pro-inflammatory cytokine interleukin-6 was found to be similar in both benign and malignant disease. Taken together this might suggest that interleukin-6 is primarily produced by the host inflammatory cells, possibly by tumour infiltrating leucocytes.

Given that we have shown that the inflammatory response is important in disease progression in patients with prostate cancer, we postulated that measurement of circulating C-reactive protein concentrations might prove a useful tool in helping to diagnose patients with prostate cancer. The process of investigation and diagnosis of

patients suspected of having prostate cancer is currently problematical since total PSA which is currently used to identify patients at risk of having prostate cancer lacks specificity. This inherent weakness means that a large proportion of patients currently subjected to prostatic biopsy have benign disease.

In chapter 6, we examined the ability of C-reactive protein , total PSA and PSA isoforms to discriminate between benign disease and prostate cancer in a cohort of 171 patients attending a number of hospitals for a diagnostic TRUS biopsy. In a subset of patients with total PSA concentrations between 2 and 10 μg/L, the free to total PSA isoform ratio was found to be superior to total PSA in discriminating between patients with benign and malignant disease. There was no significant difference in circulating C-reactive protein concentrations between patients with benign and malignant prostate disease and therefore it is unlikely to be useful in the discrimination between benign and malignant disease.

The mechanisms by which the inflammatory response might influence disease progression and impact on cancer-specific survival are not clear. However, interleukin-6, which is largely responsible for the production of C-reactive protein, has also been shown to act as an autocrine growth factor and to inhibit apoptosis in prostate cancer. It is also not clear why the presence of an increase in CD4+ T-lymphocyte infiltrate would be associated with poor survival in patients with prostate cancer. It may be that many of the T-lymphocytes found in tumour beds are inactive and therefore do not contribute to effective anti-tumoral immunity (Naoe et al., 2002).

The relationship between the local and systemic inflammatory responses is not clear. However, it may be that both the local and systemic inflammatory responses impact on disease progression and survival through the cyclooxygenase pathway. For example, it is thought that the COX enzymes may contribute directly to the

carcinogenic process through the production of oxygen free radicals and malondialdehyde which are known to stimulate mutagenic changes in DNA (Kirschenbaum et al, 2001).

There is also evidence to suggest that the COX enzymes may also be implicated in promoting tumour growth and dissemination. For example, there is evidence to suggest that prostaglandins may have a role in protecting tumour cells from undergoing apoptosis and thereby encourage tumour growth (Liu XH et al, 1998). Furthermore, COX-2 expression has been strongly associated with expression of vascular endothelial growth factor which is known to promote neovascularisation and thus facilitate tumour growth and spread of tumour cells (Timoshenko et al, 2006). It has also been postulated that the production of prostaglandins may result in apoptosis of immune cells such as natural killer cells (Myers et al, 2001). This in turn may allow tumour cells to grow and disseminate whilst escaping from immune surveillance mechanisms. It is therefore of interest that recent studies have shown that COX-2 expression can be induced by both tissue infiltration by T-lymphocytes and pro-inflammatory cytokines such as IL-6 (Wang et al, 2005).

The results of the above studies suggest that tumour progression and the inflammatory response are intimately linked in patients with prostate cancer. Specifically, these studies have demonstrated that both the presence of a pronounced lymphocytic infiltration within the primary tumour bed and the presence of a systemic inflammatory response are associated with poor outcome in patients with prostate cancer. This raises the prospect that it might be possible to delay tumour progression and improve survival by modifying the inflammatory response through the use of NSAIDs. This might be of particular importance in some patients with advanced disease, for example those patients with hormone-escaped prostate cancer and

evidence of a systemic inflammatory response, where treatment options are currently limited.

Moreover, it is well recognised that a high proportion of patients with advanced prostate cancer develop the loss of weight, reduced appetite and reduced performance status characteristic of cancer cachexia syndrome. These symptoms lead to a significant reduction in quality of life and are currently resistant to conventional treatment. It is therefore of great interest that previous studies have shown that NSAIDs can reverse the above changes in gastro-intestinal cancer patients with weight loss (McMillan et al., 1999; Lundholm et al., 2004). To date, no studies have evaluated the role of NSAIDs in the management of patients with prostate cancer and cachexia.

It has long been recognised that inflammation contributes to carcinogensis in many common solid tumours. There is also evidence to suggest that modification of the inflammatory response may reduce the risk of developing some cancers. At present, it is not clear whether inflammation precedes the development of prostate cancer. However, a recent meta-analysis reported that the use of aspirin reduced the risk of developing prostate cancer (Mahmud et al, 2004). These studies suggest that there may be a further role for NSAIDs as chemopreventative agents.

In summary, the results of the present studies are consistent with the hypothesis that the host inflammatory response is associated with disease progression in patients with prostate cancer.

Areas of ongoing research

This work presented in thesis has led to a number of avenues of research;

How is T-lymphocytic infiltration regulated in prostate cancer? We are currently examining the relationship between COX-2 expression and lymphocytic infiltration in patients undergoing radical local treatment for histologically proven prostate cancer.

What is the basis of the association between an elevated C-reactive protein concentration and poor cancer specific survival in patients with metastatic prostate cancer? We are currently examining the relationship between the presence of a systemic inflammatory response, as evidenced by an elevated circulating C-reactive protein concentration, and lean body mass (a primary determinant of survival) as measured by total body potassium in patients with metastatic prostate cancer.

What is the cytokine profile associated with the systemic inflammatory response in patients metastatic prostate cancer? We are currently examining the effect of the administration of a NSAID (Ibuprofen) on the plasma cytokine and acute phase protein profile of patients with metastatic prostate cancer.

We have obtained ethics approval and are currently recruiting patients for the above studies.

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APPENDICES TO CHAPTER 3

Patient no	Age	Follow Up	Alive/ Dead	Survival (months)	CD4 count	CD4(%)	CD4 tertile	CD8 count	CD8(%)	CD8 tertile	CD4+CD8	CD4+CD8(%)	CD4+CD8 tertile	Specimen Type	Stage	Gleason score	Subsequent Treatment	PSA	Pretreatment
1	77.48	31-Aug-2003	alive	133.67	17.00	.5667	1.00	43.00	1.43	2.00	60.00	2.00	2.00	TURP chips	t1/2	3+3	Hormonal manipulation	.50	none
2	71.53	02-Oct-1998	dead	42.27	18.00	.6000	1.00	33.00	1.10	2.00	51.00	1.70	2.00	TURP chips	t1/2	1+1	Hormonal manipulation	.70	none
3	71.01	31-Aug-2003	alive	117.30	4.00	.1333	.00	21.00	.70	1.00	25.00	.83	.00	TURP chips	t1/2	1+2	Radical prostatectomy	1.90	none
4	87.55	20-Jan-1998	dead	38.67	2.00	.0667	.00	8.00	.27	.00	10.00	.33	.00	TURP chips	t3/4	1+2	Hormonal manipulation		none
5	66.00	11-Aug-1999	dead	34.27	2.00	.0667	.00	46.00	1.53	2.00	48.00	1.60	1.00	TURP chips	t1/2	1+2	Hormonal manipulation	5.00	none
6	71.05	24-Jul-1998	dead	24.73	10.00	.3333	1.00	8.00	.27	.00	18.00	.60	.00	TURP chips	METS	1+3	Hormonal manipulation	228.00	none
7	75.14	08-May-1995	dead	8.77	3.00	.1000	.00	15.00	.63	.00	22.00	.73	.00	TURP chips	t1	2	Radical prostatectomy	2.70	none
8	62.42	31-Aug-2003	alive	54.30	18.00	.6000	2.00	31.00	1.03	1.00	49.00	1.63	2.00	TURP chips	t3	2+3	Radical Radiotherapy	5.00	none
9	68.30	31-Aug-2003	alive	63.67	7.00	.2333	.00	26.00	.87	1.00	33.00	1.10	1.00	TURP chips	t3/4	2+3	Radical Radiotherapy	40.10	none
10	77.48	31-Aug-2003	alive	25.60	18.00	.6000	1.00	17.00	.57	.00	35.00	1.17	1.00	TURP chips	t1/2	2+3	Hormonal manipulation	13.20	none
11	61.96	16-Jun-2001	dead	16.93	19.00	.6333	2.00	57.00	1.90	2.00	76.00	2.53	2.00	TURP chips	METS	2+4	Hormonal manipulation	48.30	none
12	80.84	20-Jan-1999	dead	23.37	4.00	.1333	.00	15.00	.50	.00	19.00	.63	.00	TURP chips	mets	2+5	Hormonal manipulation	38.10	none
13	85.24	18-Oct-1994	dead	11.93	12.00	.4000	1.00	51.00	1.70	2.00	63.00	2.10	2.00	TURP chips	t3/4	3	Hormonal manipulation	17.00	none
14	72.30	20-Feb-2003	alive	96.63	2.00	.0667	.00	32.00	1.07	1.00	34.00	1.13	1.00	TURP chips	mets	3	Hormonal manipulation	6.80	none
15	66.76	05-Jun-2001	dead	93.70	16.00	.5333	1.00	20.00	.67	1.00	36.00	1.20	1.00	Radical Prostatectomy	t1/2	3+2	Radical prostatectomy	9.70	none
16	64.26	31-Aug-2003	alive	42.17	13.00	.4333	1.00	24.00	.80	1.00	37.00	1.23	1.00	TURP chips	t1/2	3+2	Hormonal manipulation	2.20	none
17	60.39	05-Oct-2001	dead	75.97	6.00	.2000	.00	23.00	.77	1.00	29.00	.97	.00	TURP chips	t3/4	3+3	Hormonal manipulation	2.30	none
18	82.75	31-Aug-2003	alive	48.73	6.00	.2000	.00	25.00	.83	1.00	31.00	1.03	1.00	TURP chips	mets	3+3	Hormonal manipulation	170.00	none
19	73.29	23-Mar-2003	dead	54.00	23.00	.7667	2.00	36.00	1.20	2.00	59.00	1.97	2.00	TURP chips	t3/4	3+3	Hormonal manipulation	91.70	none
20	63.93	25-Jan-2001	dead	8.37	51.00	1.7000	2.00	43.00	1.43	2.00	94.00	3.13	2.00	Radical Prostatectomy	t2a	3+3	Radical prostatectomy		none
21	61.89	31-Aug-2003	alive	76.70	2.00	.0667	.00	22.00	.73	1.00	24.00	.80	.00	Radical Prostatectomy	t3/4	3+4	Radical prostatectomy	30.40	none
22	59.01	31-Aug-2003	alive	75.33	3.00	.1000	.00	34.00	1.13	2.00	37.00	1.23	1.00	Radical Prostatectomy	t3/4	3+4	Radical prostatectomy	27.40	none
23	63.53	31-Aug-2003	alive	35.20	35.00	1.1667	2.00	51.00	1.70	2.00	86.00	2.87	2.00	Radical Prostatectomy	t1/2	3+4	Radical prostatectomy	16.80	none
24	74.61	04-Feb-2001	dead	67.50	30.00	1.0000	2.00	47.00	1.57	2.00	77.00	2.57	2.00	TURP chips	mets	3+4	Hormonal manipulation	68.00	none
25	70.23	05-Jun-1998	dead	16.17	2.00	.0667	.00	20.00	.67	1.00	22.00	.73	.00	TURP chips	t3/4	3+4	Hormonal manipulation	740.00	none
26	69.81	25-Jun-2000	dead	52.87	25.00	.8333	2.00	45.00	1.50	2.00	70.00	2.33	2.00	TURP chips	t3/4	3+4	Hormonal manipulation	92.70	none
27	80.98	19-Dec-1998	dead	44.97	18.00	.6000	2.00	13.00	.43	.00	31.00	1.03	1.00	TURP chips	METS	3+4	Hormonal manipulation	360.00	none
28	63.05	31-Aug-2003	alive	39.60	27.00	.9000	2.00	49.00	1.63	2.00	76.00	2.53	2.00	TURP chips	METS	3+4	Hormonal manipulation	67.20	none
29	75.45	18-May-1999	dead	5.33	16.00	.5333	1.00	41.00	1.37	2.00	57.00	1.90	2.00	TURP chips	t3/4	3+4	Hormonal manipulation	41.70	none
30	67.45	06-Oct-2001	dead	9.43	27.00	.9000	2.00	35.00	1.17	2.00	62.00	2.07	2.00	TURP chips	t3/4	3+4	Hormonal manipulation		none
31	72.20	31-Aug-2003	alive	25.57	21.00	.7000	2.00	16.00	.53	.00	37.00	1.23	1.00	TURP chips	mets	3+4	Hormonal manipulation	226.90	none
32	70.56	16-Jun-2002	dead	61.50	11.00	.3667	1.00	24.00	.80	1.00	35.00	1.17	1.00	TURP chips	t3/4	3+4	Hormonal manipulation	3.50	none
33	79.34	31-Aug-2003	alive	40.03	29.00	.9667	2.00	12.00	.40	.00	41.00	1.37	1.00	TURP chips	mets	3+4	Hormonal manipulation	68.00	none
34	72.08	16-Jul-2001	dead	22.50	26.00	.8667	2.00	23.00	.77	1.00	49.00	1.63	2.00	TURP chips	t1/2	3+5	Radical Radiotherapy	7.10	none
35	60.31	31-Aug-2003	alive	89.53	12.00	.4000	1.00	28.00	.93	1.00	40.00	1.33	1.00	Radical Prostatectomy	t1/2	3+5	Radical prostatectomy	8.40	none
36	81.39	02-Sep-1997	dead	17.53	11.00	.3667	1.00	13.00	.43	.00	24.00	.80	.00	TURP chips	mets	3+5	Hormonal manipulation	1158.00	none
37	80.48	31-Aug-2003	alive	70.77	6.00	.2000	.00	14.00	.47	.00	20.00	.67	.00	TURP chips	METS	3+5	Hormonal manipulation	3992.00	none
38	77.74	10-Sep-2000	dead	24.13	20.00	.6667	2.00	20.00	.67	1.00	40.00	1.33	1.00	TURP chips	t3/4	3+5	Hormonal manipulation	44.80	none
39	82.73	31-May-1999	dead	62.47	16.00	.5333	1.00	22.00	.73	1.00	38.00	1.27	1.00	TURP chips	t3/4	4	Hormonal manipulation	389.00	none
40	71.81	20-Jul-1996	dead	36.23	16.00	.5333	1.00	51.00	1.70	2.00	67.00	2.23	2.00	TURP chips	mets	4	Hormonal manipulation	37.00	none
41	69.22	31-Aug-2003	alive	88.13	12.00	.4000	1.00	32.00	1.07	2.00	44.00	1.47	1.00	TURP chips	t1/2	4+2	prostatectomy.zoladex	22.60	none
42	68.19	31-Aug-2003	alive	46.73	6.00	.2000	.00	35.00	1.17	2.00	41.00	1.37	1.00	Radical Prostatectomy	t2b	4+3	Radical prostatectomy	6.20	none
43	48.95	31-Aug-2003	alive	90.20	8.00	.2667	1.00	30.00	1.00	1.00	38.00	1.27	1.00	Radical Prostatectomy	t1/2	4+3	Radical prostatectomy	16.50	none
44	67.39	13-Jul-1996	dead	20.67	18.00	.6000	2.00	21.00	.70	1.00	39.00	1.30	1.00	TURP chips	mets	4+3	Hormonal manipulation	94.00	none
45	74.93	08-May-1997	dead	32.43	4.00	.1333	.00	26.00	.87	1.00	30.00	1.00	1.00	TURP chips	mets	4+3	Hormonal manipulation	974.00	none
46	63.98	31-Aug-2003	alive	51.90	27.00	.9000	2.00	22.00	.73	1.00	49.00	1.63	2.00	TURP chips	T3/4	4+3	Hormonal manipulation	42.70	none
47	75.62	31-Aug-2003	alive	23.97	41.00	1.3667	2.00	36.00	1.20	2.00	77.00	2.57	2.00	TURP chips	t3/4	4+3	Hormonal manipulation	50.50	none
48	74.27	31-Aug-2003	alive	41.23	3.00	.1000	.00	12.00	.40	.00	15.00	.50	.00	TURP chips	mets	4+4	Hormonal manipulation	81.00	none
49	51.59	31-Aug-2003	alive	46.27	39.00	1.3000	2.00	30.00	1.00	1.00	69.00	2.30	2.00	TURP chips	mets	4+4	Hormonal manipulation	560.00	none

50	70.72	30-Aug-1997	dead	11.83	28.00	.9333	2.00	37.00	1.23	2.00	65.00	2.17	2.00	TURP chips	METS	4+5	Hormonal manipulation	30.50	none
51	79.19	25-Jul-1996	dead	22.87	4.00	.1333	.00	13.00	.43	.00	17.00	.57	.00	TURP chips	T1/2	4+5	Hormonal manipulation	3.80	none
52	80.52	03-Apr-2003	alive	91.67	3.00	.1000	.00	19.00	.63	.00	22.00	.73	.00	TURP chips	t3/4	4+5	Hormonal manipulation	41.00	none
53	60.89	19-Nov-2000	dead	69.90	21.00	.7000	2.00	46.00	1.53	2.00	67.00	2.23	2.00	Radical Prostatectomy	t1/2	5	Radical prostatectomy	46.00	none
54	64.33	31-Aug-2003	alive	65.67	6.00	.2000	.00	28.00	.93	1.00	34.00	1.13	1.00	Radical Prostatectomy	t3/4	5+3	Radical prostatectomy	23.30	none
55	70.07	31-Aug-2003	alive	71.27	12.00	.4000	1.00	42.00	1.40	2.00	54.00	1.80	2.00	TURP chips	t3/4	5+3	Hormonal manipulation	40.90	none
56	60.72	31-Aug-2003	alive	64.97	57.00	1.9000	2.00	90.00	3.00	2.00	147.00	4.90	2.00	TURP chips	t3	5+4	Radical Radiotherapy	19.10	none
57	74.33	31-Aug-2003	alive	26.70	9.00	.3000	1.00	19.00	.63	.00	28.00	.93	.00	TURP chips	t3	5+4	Radical Radiotherapy	9.10	none
58	91.32	01-Jan-2001	dead	1.57	49.00	1.6333	2.00	47.00	1.57	2.00	96.00	3.20	2.00	TURP chips	mets	5+4	Hormonal manipulation	260.00	none
59	76.05	31-Aug-2003	alive	28.63	9.00	.3000	1.00	17.00	.57	.00	26.00	.87	.00	TURP chips	t3/4	5+4	Hormonal manipulation	89.30	none
60	79.64	14-Jul-2002	dead	13.37	7.00	.2333	.00	23.00	.77	1.00	30.00	1.00	.00	TURP chips	mets	5+4	Hormonal manipulation	67.90	none
61	70.67	31-Aug-2003	alive	78.00	6.00	.2000	.00	15.00	.50	.00	21.00	.70	.00	TURP chips	t3.	5+5	Radical Radiotherapy	7.90	none
62	63.16	31-Aug-2003	alive	112.17	4.00	.1333	.00	18.00	.60	.00	22.00	.73	.00	TURP chips	t3/4	6	Hormonal manipulation	11.00	none
63	67.74	24-Jul-1998	dead	39.87	12.00	.4000	1.00	65.00	2.17	2.00	77.00	2.57	2.00	TURP chips	t3/4	7	Hormonal manipulation	99.00	none
64	71.23	16-Nov-1999	dead	62.47	24.00	.8000	2.00	44.00	1.47	2.00	68.00	2.27	2.00	TURP chips	t3/4	8	Hormonal manipulation	6.30	none
65	70.48	16-Oct-1997	dead	32.50	12.00	.4000	1.00	18.00	.60	.00	30.00	1.00	.00	TURP chips	t3/4	moderate	Radical Radiotherapy	18.00	none
66	72.14	31-Aug-2003	alive	132.23	10.00	.3333	1.00	29.00	.97	1.00	39.00	1.30	1.00	TURP chips	mets	poor	Hormonal manipulation	23.00	none
67	73.81	05-May-1920	dead	95.50	15.00	.5000	1.00	21.00	.70	1.00	36.00	1.20	1.00	TURP chips	mets	poor	Hormonal manipulation	41.00	none
68	63.51	27-May-1995	dead	21.37	23.00	.7667	2.00	35.00	1.17	2.00	58.00	1.93	2.00	TURP chips	mets	poor	Hormonal manipulation	178.00	none
69	70.79	31-Aug-2003	alive	118.70	5.00	.1667	.00	9.00	.30	.00	14.00	.47	.00	TURP chips	t1/2	poor	Hormonal manipulation	22.00	none
70	59.95	27-Mar-1997	dead	15.40	14.00	.4667	1.00	25.00	.83	1.00	39.00	1.30	1.00	TURP chips	t3/4	POOR	Hormonal manipulation	36.10	none
71	80.76	14-Jan-1997	dead	16.87	1.00	.0333	.00	8.00	.27	.00	9.00	.30	.00	TURP chips	T1/2	POOR	Hormonal manipulation	13.00	none
72	64.55	31-Aug-2003	alive	87.40	13.00	.4333	1.00	27.00	.90	1.00	40.00	1.33	1.00	TURP chips	t3/4	POOR	Hormonal manipulation	240.00	none
73	62.54	31-Aug-2003	alive	133.53	15.00	.5000	1.00	22.00	.73	1.00	37.00	1.23	1.00	Radical Prostatectomy	t1/2	well	Radical prostatectomy	2.20	none
74	72.32	09-Jan-1998	dead	60.30	24.00	.8000	2.00	16.00	.53	.00	40.00	1.33	1.00	TURP chips	t3/4	well	Hormonal manipulation	19.00	none
75	74.58	04-Jul-1998	dead	68.03	7.00	.2333	1.00	12.00	.40	.00	19.00	.63	.00	TURP chips	t3/4	well	Hormonal manipulation	1.90	none
76	76.07	10-Nov-1998	dead	66.07	14.00	.4667	1.00	16.00	.53	.00	30.00	1.00	.00	TURP chips	t3/4	well	Hormonal manipulation	4.00	none
77	74.67	11-Mar-2003	alive	120.17	18.00	.6000	2.00	14.00	.47	.00	32.00	1.07	1.00	TURP chips	t3/4	well	Hormonal manipulation	1.30	none
78	70.41	31-Aug-2003	alive	96.23	3.00	.1000	.00	22.00	.73	1.00	25.00	.83	.00	TURP chips	t3/4	WELL	Hormonal manipulation	15.20	none
79	62.37	31-Aug-2003	alive	66.23	3.00	.1000	.00	10.00	.33	.00	13.00	.43	.00	TURP chips	t3/4	WELL	Hormonal manipulation	29.50	none
80	62.56	31-Aug-2003	alive	78.50	8.00	.2667	1.00	18.00	.60	.00	26.00	.87	.00	TURP chips	t3	WELL	Hormonal manipulation	4.90	none

Appendices to Chapter 4

Patient no.	Age	Date of Diagnosis	Date of blood sampling	Date of follow-up	Status at follow-up	Survival (Months)	Gleason Score	Treatment	PSA	CRP
1	84.63	06-Apr-1995	01-Dec-1998	19-Dec-1998	Cancer Death	.60	7.00	Radiotherapy+ hormones	106.00	119.00
2	78.33	26-Jan-2000	15-Feb-2002	06-Apr-2002	Cancer Death	1.67	3.00	Hormone treatment	55.10	27.00
3	71.54	06-Sep-1996	07-Jul-1997	30-Aug-1997	Cancer Death	1.80	9.00	Hormone treatment	274.00	219.00
4	75.77	01-Aug-1995	15-Apr-1997	12-Jun-1997	Cancer Death	1.93		Hormone treatment	500.00	6.00
5	59.72	19-Oct-1999	19-Oct-1999	25-Feb-2000	Cancer Death	4.30	8.00	Hormone treatment	488.00	101.60
6	89.00	30-Aug-1999	30-Aug-1999	13-Feb-2000	Cancer Death	5.57	5.00	Hormone treatment	9.50	.88
7	78.27	18-May-1996	25-Mar-1999	25-Dec-1999	Cancer Death	9.17	6.00	Hormone treatment	3.60	21.65
8	67.54	06-Jan-1995	25-May-1999	26-Mar-2000	Cancer Death	10.20	7.00	Radiotherapy+ hormones	213.80	12.25
9	75.15	18-Aug-1998	18-Aug-1998	07-Jul-1999	Cancer Death	10.77	10.00	Hormone treatment	111.50	54.71
10	73.53	11-Nov-1998	29-Nov-2002	27-Nov-2003	Cancer Death	12.10	7.00	Hormone treatment	10.40	24.00
11	71.66	30-Mar-1999	03-Apr-2000	05-May-2001	Cancer Death	13.23	7.00	Hormone treatment	250.00	58.00
12	74.21	05-Jun-1997	20-Nov-1998	25-Dec-1999	Cancer Death	13.33		Hormone treatment	30.00	6.00
13	55.44	26-Jun-2001	02-Jul-2001	27-Aug-2002	Cancer Death	14.03	9.00	Hormone treatment	358.50	11.00
14	67.00	30-Oct-2001	30-Oct-2001	24-Jan-2003	Cancer Death	15.03	8.00	Hormone treatment	10.60	1.23
15	73.00	03-Jul-1999	03-Jul-1999	12-Oct-2000	Cancer Death	15.57	3.00	Radiotherapy+ hormones	2.00	5.51
16	87.08	07-Aug-1997	19-May-2001	11-Sep-2002	Cancer Death	16.00	7.00	Hormone treatment	.90	6.00
17	67.00	18-Jun-1998	18-Jun-1998	04-Nov-1999	Cancer Death	16.80	9.00	Hormone treatment	540.00	100.00
18	86.79	01-Oct-2002	30-Oct-2002	29-Mar-2004	Cancer Death	17.20	5.00	Hormone treatment	20.00	1.48
19	55.64	01-Nov-1995	17-Nov-2000	26-May-2002	Cancer Death	18.50	7.00	Hormone treatment	120.00	11.00
20	79.69	16-Jul-1998	22-Sep-1998	26-Jun-2000	Cancer Death	21.43	8.00	Hormone treatment	3.80	2.08
21	54.73	01-Feb-1999	26-Oct-1999	07-Aug-2001	Cancer Death	21.70	7.00	Hormone treatment	.70	3.05
22	56.65	11-Nov-1997	01-Jan-1999	29-Nov-2000	Cancer Death	23.27	8.00	Radiotherapy+ hormones	269.00	.71
23	73.00	10-Jul-2001	23-May-2001	08-May-2003	Cancer Death	23.83		Radiotherapy+ hormones	23.70	4.59
24	61.76	26-Nov-1997	22-Mar-2003	24-Mar-2005	Cancer Death	24.43	7.00	Radiotherapy+ hormones	977.00	6.00
25	80.82	07-Jan-1997	07-Jan-1997	20-Jan-1999	Cancer Death	24.77	7.00	Hormone treatment	39.00	30.00
26	65.74	01-Jan-2000	25-Aug-2001	11-Sep-2003	Cancer Death	24.90	8.00	Radiotherapy+ hormones	3.30	6.00
27	80.15	20-Jun-1997	14-Jul-1998	29-Sep-2000	Cancer Death	26.93	8.00	Hormone treatment	3.00	4.23
28	84.29	20-Apr-1998	22-Jan-1999	31-May-2001	Cancer Death	28.67	7.00	Hormone treatment	3.10	.67
29	64.25	02-Jul-1998	07-Jun-1998	19-Jan-2001	Cancer Death	31.90	6.00	Hormone treatment	17.80	.24
30	77.52	12-Apr-1995	18-Jun-1998	04-Feb-2001	Cancer Death	32.07	7.00	Hormone treatment	35.00	6.00
31	73.41	30-May-2000	18-Jul-2000	25-Mar-2003	Cancer Death	32.60	6.00	Hormone treatment	54.00	61.00
32	75.99	01-Sep-2000	12-Oct-2000	14-Sep-2003	Cancer Death	35.57	8.00	Hormone treatment	1.40	3.06
33	79.82	16-May-2000	10-Nov-2000	20-Oct-2003	Cancer Death	35.80	7.00	Hormone treatment	8.60	6.00
34	63.57	08-Nov-1996	09-Jun-1997	17-Jun-2000	Cancer Death	36.80	7.00	Hormone treatment	1.50	6.00
35	53.64	01-Nov-1999	28-Nov-2001	01-Jun-2005	Alive	42.70		Radiotherapy+ hormones	29.60	12.00
36	69.95	22-Jan-2001	22-Jan-2001	27-Jul-2004	Cancer Death	42.73		Hormone treatment	1.70	2.38
37	79.47	26-Nov-2001	26-Nov-2001	01-Jun-2005	Alive	42.77	7.00	Hormone treatment	63.60	.44
38	84.71	30-Aug-1999	22-Aug-2001	01-Jun-2005	Alive	45.97	6.00	Hormone treatment	.10	6.00
39	63.98	26-Dec-1998	17-May-1999	02-Apr-2003	Cancer Death	47.20	9.00	Hormone treatment	.50	1.59
40	72.13	01-Jul-2001	01-Jul-2001	01-Jun-2005	Alive	47.70	7.00	Hormone treatment	226.90	20.00
41	74.76	10-Apr-2000	15-Jun-2001	01-Jun-2005	Alive	48.23	5.00	Radiotherapy+ hormones	4.80	7.56
42	76.29	13-Feb-1996	26-Apr-2001	01-Jun-2005	Alive	49.90	3.00	Hormone treatment	.60	7.00
43	90.00	31-Jan-1998	31-Jan-1998	09-Jul-2002	Cancer Death	54.00	6.00	Hormone treatment	1.10	2.76
44	65.06	18-Dec-2000	18-Dec-2000	01-Jun-2005	Alive	54.20		Hormone treatment	1.40	6.28

45	65.57	25-Oct-1999	17-Nov-2000	01-Jun-2005	Alive	55.23	5.00	Hormone treatment	.10	6.00
46	68.71	25-Aug-2000	18-Oct-2000	01-Jun-2005	Alive	56.23	4.00	Hormone treatment	.10	1.26
47	67.24	07-Jun-1999	10-Oct-2000	01-Jun-2005	Alive	56.50	7.00	Hormone treatment	33.90	6.00
48	79.64	11-May-1999	24-Sep-1999	01-Jun-2004	Cancer Death	57.07	8.00	Hormone treatment	.50	.39
49	68.21	07-Apr-1999	07-Apr-1999	23-Apr-2004	Non-Cancer Death	61.43	8.00	Hormone treatment	20.80	7.00
50	68.78	05-Mar-2000	17-Apr-2000	01-Jun-2005	Alive	62.37	4.00	Hormone treatment	2.40	2.12
51	63.86	28-Mar-2001	28-Mar-2000	01-Jun-2005	Alive	63.03		Radiotherapy+ hormones	.20	2.70
52	74.03	28-Mar-2000	27-Mar-2000	01-Jun-2005	Alive	63.07		Hormone treatment	.20	3.72
53	79.08	17-Mar-1997	09-Feb-1999	14-May-2004	Non-Cancer Death	64.03	8.00	Hormone treatment	.10	1.26
54	79.73	21-Jan-2000	21-Jun-2000	01-Jun-2005	Alive	65.27		Hormone treatment	.10	1.10
55	68.98	01-Sep-1999	09-Oct-1999	01-Jun-2005	Alive	68.73	2.00	Hormone treatment	.50	.60
56	57.90	30-Jun-1999	30-Jun-1999	01-Jun-2005	Alive	72.10	7.00	Hormone treatment	17.20	1.16
57	84.59	04-Dec-1996	06-May-1999	01-Jun-2005	Alive	73.93	9.00	Hormone treatment	.50	3.20
58	65.00	04-May-1999	04-May-1999	01-Jun-2005	Alive	74.00	3.00	Hormone treatment	14.70	1.17
59	84.00	10-Mar-1999	10-Mar-1999	01-Jun-2005	Alive	75.83	7.00	Hormone treatment	2.30	14.00
60	63.12	27-Aug-1998	30-Oct-1998	01-Jun-2005	Alive	80.20	7.00	Hormone treatment	6.80	12.00
61	80.27	19-Sep-1997	01-Nov-1997	14-Jun-2004	Non-Cancer Death	80.57	3.00	Hormone treatment	1.30	11.44
62	62.26	23-Jun-1998	01-Jul-1998	01-Jun-2005	Alive	84.23	9.00	Radiotherapy+ hormones	14.30	1.12

Appendices to Chapter 5

Patient no.	Age	Date of sampling	TRUS Result	Stage	Gleason score	IL-6	CRP	PSA
1	44.87	22-Oct-2001	Benign			2.85	3.27	36.70
2	47.60	22-Jul-2002	Benign			2.48	.88	2.41
3	50.16	25-Oct-1999	Benign			3.62	7.24	17.60
4	52.92	23-Jan-2002	Benign			1.21	.32	12.30
5	53.08	25-Oct-1999	Benign			4.35	11.90	1.10
6	54.70	16-May-2002	Benign			1.88	3.12	5.75
7	55.60	03-Nov-1999	Benign			3.55	18.52	1.80
8	55.62	22-Oct-2001	Benign			2.81	.90	1.40
9	56.59	21-Feb-2002	Prostatitis			1.97	1.05	6.30
10	57.07	08-Mar-2000	Benign			2.30	.65	7.00
11	57.66	11-Aug-1999	Benign			1.27	2.18	6.60
12	58.37	25-Jan-2000	Benign			1.32	.95	2.80
13	58.89	06-Dec-2001	Benign			1.73	.46	22.40
14	59.10	28-Feb-2000	Benign			.79	.46	7.80
15	59.12	08-Sep-1999	Benign			5.24	12.91	1.30
16	60.21	12-Jan-2000	Benign			1.38	.59	9.70
17	60.56	05-May-1999	Benign			12.94	2.01	6.50
18	61.21	20-Dec-2001	Benign			5.31	1.67	2.20
19	62.95	28-Feb-2000	Benign			5.03	4.43	4.90
20	63.35	14-Mar-2002	Benign			2.07	2.06	7.75
21	63.55	08-Mar-2000	Benign			1.26	.06	11.20
22	63.62	20-Dec-2001	Benign			2.32	1.98	8.10
23	63.83	15-Dec-1999	Benign			2.41	1.50	5.40
24	64.13	03-Nov-1999	Benign			2.57	4.87	12.80
25	64.35	24-Jan-2000	Benign			3.62	3.60	5.40
26	64.80	30-Oct-2001	Benign			1.67	2.05	5.20
27	65.29	28-Jan-2002	Benign			5.55	1.63	5.10
28	65.55	24-Dec-2001	Benign			1.18	2.44	3.30
29	65.68	14-Jun-2002	Benign			1.19	1.42	15.28
30	65.69	28-Jul-1999	Benign			3.30	7.59	.80
31	65.76	05-Apr-2000	Benign			11.01	13.51	8.80
32	65.99	22-Sep-1999	Benign			2.22	1.68	34.00
33	66.04	15-Dec-1999	Benign			1.17	6.19	19.30
34	66.25	10-Jan-2002	Benign			2.73	2.51	8.10
35	67.30	08-Sep-1999	Benign			9.04	11.57	10.90
36	68.57	11-Apr-2002	Benign			1.99	3.66	3.00
37	68.64	28-Nov-2001	Benign			1.13	.23	5.20
38	69.15	22-Nov-1999	Benign			1.12	.34	11.80
39	69.49	15-Dec-1999	Benign			1.14	.46	17.10
40	70.15	28-Jan-2002	Benign			4.82	4.93	8.10
41	70.92	24-Dec-2001	Prostatitis			2.98	2.32	2.10
42	71.35	23-May-2002	Benign			1.02	4.26	1.80
43	71.62	09-Feb-2000	Benign			2.55	.38	6.60
44	71.92	22-Nov-2001	Benign			1.22	.69	4.30

45	72.73	24-Jan-2000	Benign			1.64	1.34	10.00
46	73.24	13-Dec-1999	Benign			2.76	5.57	6.70
47	73.77	23-Jan-2002	Benign			3.07	1.80	5.70
48	73.80	22-Sep-1999	Benign			2.62	2.96	22.50
49	73.98	16-Jun-1999	Benign			1.23	1.20	8.60
50	74.94	17-Nov-1999	Benign			1.61	1.43	11.10
51	76.98	26-Nov-2001	Benign			1.65	1.07	24.50
52	77.02	19-May-1999	Benign			2.78	21.08	14.50
53	77.93	03-Nov-1999	Benign			.95	.19	9.40
54	62.09	26-Jan-2000	PIN			2.70	3.36	4.50
55	63.32	08-Mar-2000	PIN			.84	1.23	7.80
56	64.29	23-Feb-2000	PIN			2.71	2.73	3.50
57	66.04	30-Jun-1999	PIN			.65	.30	6.50
58	68.13	22-Jul-2002	PIN			3.05	2.04	13.57
59	74.17	21-Feb-2002	PIN			3.20	2.58	39.00
60	45.88	30-Jun-1999	Adenocarcinoma	T1/2	8.00	1.07	2.66	4.80
61	48.32	20-Sep-2001	Adenocarcinoma	T1/2	3.00	.84	.63	2.60
62	57.83	17-Dec-2001	Adenocarcinoma	T1/2	3.00	1.78	1.73	5.10
63	58.12	28-Jan-2002	Adenocarcinoma	T1/2	6.00	1.79	2.56	18.00
64	58.77	06-Oct-1999	Adenocarcinoma	T1/2	8.00	4.56	8.68	8.00
65	59.56	22-Feb-2002	Adenocarcinoma	T1/2	5.00	4.61	5.31	.46
66	59.65	22-Mar-2000	Adenocarcinoma	T1/2	3.00	.78	1.26	11.50
67	61.91	17-Jan-2002	Adenocarcinoma	T1/2	4.00	1.30	.69	4.80
68	61.98	12-Jan-2000	Adenocarcinoma	T1/2	5.00	1.63	.79	1.00
69	62.29	22-Jul-2002	Adenocarcinoma	T1/2	6.00	1.02	.52	24.08
70	62.76	22-Mar-2000	Adenocarcinoma	T1/2	7.00	.83	.27	5.50
71	62.91	24-Dec-2001	Adenocarcinoma	T1/2	6.00	2.08	.60	13.80
72	63.08	10-Jan-2002	Adenocarcinoma	T1/2	6.00	2.25	.84	14.90
73	63.10	26-Nov-2001	Adenocarcinoma	T1/2	5.00	1.33	1.85	2.20
74	63.42	28-Feb-2002	Adenocarcinoma	T1/2	5.00	4.50	4.30	8.30
75	63.83	21-Feb-2002	Adenocarcinoma	T1/2	6.00	2.09	.78	14.80
76	64.37	26-Jan-2000	Adenocarcinoma	T1/2	5.00	2.78	2.24	12.70
77	65.35	12-Jan-2000	Adenocarcinoma	T1/2	3.00	1.42	1.08	17.70
78	66.24	11-Aug-1999	Adenocarcinoma	T1/2	5.00	2.12	4.36	6.10
79	66.82	22-Feb-2002	Adenocarcinoma	T1/2	6.00	1.28	.44	10.60
80	67.05	06-Oct-1999	Adenocarcinoma	T1/2	6.00	3.82	18.65	7.50
81	67.32	22-Sep-1999	Adenocarcinoma	T1/2	6.00	1.18	18.29	11.80
82	67.81	19-May-1999	Adenocarcinoma	T1/2	6.00	2.70	3.91	1.30
83	67.93	28-Jul-1999	Adenocarcinoma	T1/2	6.00	1.34	.29	3.70
84	68.32	21-Mar-2002	Adenocarcinoma	T1/2	4.00	2.37	4.61	9.27
85	69.84	14-Mar-2002	Adenocarcinoma	T1/2	4.00	.96	1.19	7.56
86	70.53	14-Jun-2002	Adenocarcinoma	T1/2	2.00	1.10	.28	6.78
87	73.79	22-Oct-2001	Adenocarcinoma	T1/2	7.00	7.71	10.20	7.40
88	73.88	13-Dec-1999	Adenocarcinoma	T1/2	5.00	2.75	2.55	10.00
89	74.10	23-May-2002	Adenocarcinoma	T1/2	2.00	1.21	.67	3.70
90	75.00	06-Oct-1999	Adenocarcinoma	T1/2	5.00	4.41	6.69	6.50
91	75.19	21-Feb-2002	Adenocarcinoma	T1/2	4.00	3.57	1.07	6.60

92	75.43	26-Jan-2000	Adenocarcinoma	T1/2	6.00	1.17	.88	36.10
93	75.53	02-Jun-1999	Adenocarcinoma	T1/2	7.00	2.69	4.04	7.10
94	76.27	14-Jun-2002	Adenocarcinoma	T1/2	7.00	7.47	50.00	7.73
95	77.07	28-Nov-2001	Adenocarcinoma	T1/2	5.00	4.59	5.00	13.60
96	77.44	17-Jan-2002	Adenocarcinoma	T1/2	9.00	1.97	5.31	8.60
97	58.16	17-Nov-1999	Adenocarcinoma	T3/4	7.00	1.80	.69	20.60
98	60.08	03-Oct-2001	Adenocarcinoma	T3/4	6.00	2.45	.35	33.00
99	60.11	23-Jan-2002	Adenocarcinoma	T3/4	7.00	1.65	1.14	3.70
100	62.50	05-Apr-2000	Adenocarcinoma	T3/4	9.00	4.39	8.14	4.80
101	62.54	29-Dec-1999	Adenocarcinoma	T3/4	2.00	11.38	2.17	10.30
102	64.15	25-Aug-1999	Adenocarcinoma	T3/4	6.00	2.36	4.03	18.10
103	65.12	28-Jul-1999	Adenocarcinoma	T3/4	8.00	1.88	2.86	20.30
104	66.18	14-Mar-2002	Adenocarcinoma	T3/4	7.00	1.38	1.04	16.57
105	67.71	24-Dec-2001	Adenocarcinoma	T3/4	7.00	4.55	2.45	7.30
106	67.96	08-Mar-2000	Adenocarcinoma	T3/4	7.00	28.11	42.03	21.70
107	68.41	25-Oct-2001	Adenocarcinoma	T3/4	6.00	2.00	.72	10.00
108	68.56	22-Jul-2002	Adenocarcinoma	T3/4	7.00	13.48	27.00	25.11
109	68.65	28-Feb-2000	Adenocarcinoma	T3/4	7.00	5.17	22.25	17.60
110	68.82	21-Mar-2001	Adenocarcinoma	T3/4	7.00	3.49	3.47	18.44
111	69.21	25-Aug-1999	Adenocarcinoma	T3/4	7.00	1.39	1.86	116.00
112	69.61	17-Nov-1999	Adenocarcinoma	T3/4	5.00	1.03	.93	56.00
113	69.62	15-Dec-1999	Adenocarcinoma	T3/4	7.00	1.46	.73	17.60
114	69.77	22-Oct-2001	Adenocarcinoma	T3/4	5.00	1.14	1.39	5.00
115	70.18	22-Nov-1999	Adenocarcinoma	T3/4	5.00	2.72	4.13	68.00
116	71.06	28-Nov-2001	Adenocarcinoma	T3/4	5.00	5.02	2.44	14.30
117	71.58	24-Jan-2000	Adenocarcinoma	T3/4	7.00	8.52	6.75	44.80
118	71.85	01-Jan-2002	Adenocarcinoma	T3/4	5.00	1.19	.48	11.35
119	72.02	10-Jan-2002	Adenocarcinoma	T3/4	6.00	1.03	.68	76.00
120	72.31	09-Feb-2000	Adenocarcinoma	T3/4	6.00	1.70	.97	7.40
121	72.50	17-Dec-2001	Adenocarcinoma	T3/4	8.00	9.30	12.45	18.10
122	72.96	25-Aug-1999	Adenocarcinoma	T3/4	9.00	2.78	3.67	17.60
123	73.42	30-May-2002	Adenocarcinoma	T3/4	5.00	3.80	.96	42.82
124	73.42	21-Dec-2001	Adenocarcinoma	T3/4	7.00	1.93	.97	22.10
125	74.23	28-Feb-2002	Adenocarcinoma	T3/4	8.00	3.50	9.74	11.90
126	74.99	19-Sep-2001	Adenocarcinoma	T3/4	7.00	4.93	3.44	21.30
127	75.88	22-Jul-2002	Adenocarcinoma	T3/4	6.00	2.18	.53	25.26
128	77.44	23-Jan-2002	Adenocarcinoma	T3/4	8.00	2.22	.99	20.90
129	77.74	22-Jul-2002	Adenocarcinoma	T3/4	7.00	5.51	1.13	11.31
130	78.36	28-Nov-2001	Adenocarcinoma	T3/4	6.00	2.36	1.34	55.60
131	81.53	06-Oct-1999	Adenocarcinoma	T3/4	7.00	2.51	15.64	22.00
132	82.17	22-Nov-1999	Adenocarcinoma	T3/4	7.00	3.11	8.64	99.00
133	84.35	25-Oct-2001	Adenocarcinoma	T3/4	7.00	3.18	2.97	40.00
134	57.90	30-Jun-1999	Adenocarcinoma	Mets	7.00	1.80	1.16	19.00
135	57.95	21-Dec-2001	Adenocarcinoma	Mets	10.00	2.70	2.47	103.00
136	64.15	24-Jan-2000	Adenocarcinoma	Mets	8.00	1.50	.80	19.30
137	64.51	25-Oct-1999	Adenocarcinoma	Mets	5.00	1.61	.55	23.20
138	65.65	25-Oct-2001	Adenocarcinoma	Mets	10.00	2.36	1.23	9.70

139	69.07	22-Jul-2002	Adenocarcinoma	Mets	8.00	3.18	.92	23.27
140	69.28	17-Nov-1999	Adenocarcinoma	Mets	9.00	3.41	6.20	146.00
141	75.09	25-Oct-2001	Adenocarcinoma	Mets	7.00	3.15	2.16	22.50
142	75.48	30-Oct-2001	Adenocarcinoma	Mets	8.00	3.22	4.68	75.40
143	78.70	30-May-2002	Adenocarcinoma	Mets	9.00	4.53	1.39	89.52
144	79.47	26-Nov-2001	Adenocarcinoma	Mets	7.00	2.03	.44	68.00
145	82.79	17-Dec-2001	Adenocarcinoma	Mets	6.00	1.29	.51	15.60

Appendices to Chapter 6

Patient no.	Age	Date of sample	TRUS Result	Gleason score	CRP	Total PSA (immulite)	Free PSA	Free/ Total PSA ratio	Total PSA (Bayer)	Complex PSA	Complex/ Total PSA ratio
1	59.56	22-Feb-2002	Adenocarcinoma	5.00	5.31	.34	.11	32.40	.46	.29	63.00
2	61.98	12-Jan-2000	Adenocarcinoma	5.00	.79	1.30	.33	25.40	1.00	.59	59.00
3	67.81	19-May-1999	Adenocarcinoma	6.00	3.91	.82	.06	7.32	1.30	.70	54.00
4	63.10	26-Nov-2001	Adenocarcinoma	5.00	1.85	2.71	.54	19.90	2.20	1.50	68.00
5	72.44	26-Nov-2001	Adenocarcinoma	6.00	.25	2.27	.33	14.50	2.20	1.60	73.00
6	48.32	20-Sep-2001	Adenocarcinoma	3.00	.63	3.02	.09	2.98	2.60	2.50	96.00
7	64.72	21-Mar-2002	Adenocarcinoma	9.00		5.03	.64	12.70	3.65	2.87	78.60
8	60.11	23-Jan-2002	Adenocarcinoma	7.00	1.14	4.68	.27	5.78	3.70	3.10	84.00
9	67.93	28-Jul-1999	Adenocarcinoma	6.00	.29	5.03	1.29	25.60	3.70	2.20	59.00
10	74.10	23-May-2002	Adenocarcinoma	2.00		4.29	.53	12.40	3.70	2.72	73.50
11	77.74	11-Apr-2002	Adenocarcinoma	7.00		4.76	.98	20.50	4.23	3.49	82.50
12	45.88	30-Jun-1999	Adenocarcinoma	8.00	2.66	5.02	.57	11.40	4.80	3.80	79.00
13	61.91	17-Jan-2002	Adenocarcinoma	4.00	.69	5.20	.62	11.90	4.80	3.70	77.00
14	62.50	05-Apr-2000	Adenocarcinoma	9.00	8.14	4.01	.43	10.70	4.80	4.00	83.00
15	57.15	17-Jan-2002	Adenocarcinoma	8.00	5.68	5.66	.50	8.83	4.90	4.20	86.00
16	69.77	22-Oct-2001	Adenocarcinoma	5.00	1.59	6.23	.83	13.30	5.00	3.00	60.00
17	57.83	17-Dec-2001	Adenocarcinoma	3.00	1.73	5.45	.90	16.50	5.10	3.60	71.00
18	62.76	22-Mar-2000	Adenocarcinoma	7.00	.27	4.93	.59	11.90	5.30	4.30	81.00
19	75.03	14-Feb-2002	Adenocarcinoma	3.00	73.60	6.75	1.38	20.40	5.50	3.80	69.00
20	66.24	12-Aug-1999	Adenocarcinoma	5.00	4.36	5.87	1.01	17.20	6.10	5.00	82.00
21	75.00	06-Oct-1999	Adenocarcinoma	5.00	6.69	5.42	1.51	27.80	6.50	4.70	72.00
22	75.19	21-Feb-2002	Adenocarcinoma	4.00	1.07	7.60	.35	4.60	6.60	4.90	74.00
23	70.53	14-Jun-2002	Adenocarcinoma	2.00		8.78	1.76	20.00	6.78	4.90	72.30
24	75.53	02-Jun-1999	Adenocarcinoma	7.00	4.04	8.04	.62	7.71	7.10	6.00	85.00
25	67.71	24-Dec-2001	Adenocarcinoma	7.00	2.45	9.61	.45	4.68	7.30	6.50	89.00
26	72.31	09-Feb-2000	Adenocarcinoma	6.00	.97	7.86	.52	6.62	7.40	6.70	91.00
27	73.79	22-Oct-2001	Adenocarcinoma	7.00	10.20	7.15	.91	12.70	7.40	6.20	84.00
28	67.05	06-Oct-1999	Adenocarcinoma	6.00	18.65	8.52	1.12	13.10	7.50	5.60	75.00
29	69.84	14-Mar-2002	Adenocarcinoma	4.00		10.40	.68	6.50	7.56	6.99	92.50
30	73.48	16-May-2002	Adenocarcinoma	6.00		10.00	1.13	11.30	7.64	6.31	82.60
31	76.27	14-Jun-2002	Adenocarcinoma	7.00		11.20	.89	7.90	7.73	3.94	50.90
32	58.77	06-Oct-1999	Adenocarcinoma	8.00	8.68	7.48	.46	6.15	8.00	7.30	91.00
33	83.84	24-Dec-2001	Adenocarcinoma	6.00	6.59	11.20	.68	6.07	8.10	6.90	85.00
34	73.18	29-Nov-2001	Adenocarcinoma	6.00	7.14	9.91	1.24	12.50	8.20	6.70	82.00
35	63.42	28-Feb-2002	Adenocarcinoma	5.00	4.30	9.52	.88	9.24	8.30	6.10	73.00
36	77.44	17-Jan-2002	Adenocarcinoma	9.00	5.31	10.80	.72	6.67	8.60	7.60	88.00
37	66.88	14-Feb-2002	Adenocarcinoma	4.00	62.89	11.60	.90	7.76	8.70	7.30	84.00
38	68.32	21-Mar-2002	Adenocarcinoma	4.00		12.10	1.61	13.30	9.27	6.99	75.40
39	68.41	25-Oct-2001	Adenocarcinoma	6.00	.72	12.50	1.81	14.50	10.00	7.60	76.00
40	73.88	13-Dec-1999	Adenocarcinoma	5.00	2.55	10.20	1.26	12.40	10.00	8.40	84.00
41	62.54	29-Dec-1999	Adenocarcinoma	2.00	2.17	10.20	1.05	10.30	10.30	8.40	82.00
42	66.82	22-Feb-2002	Adenocarcinoma	6.00	.44	13.00	.92	7.08	10.60	8.60	81.00
43		28-Jun-2002	Adenocarcinoma	8.00	.75	13.60	1.16	8.53	10.90	10.50	96.00
44	69.85	08-Mar-2002	Adenocarcinoma	4.00		15.70	1.58	10.00	10.99	8.18	74.40

45		09-Feb-2000	Adenocarcinoma	3.00	7.69	11.80	.88	7.46	11.30	10.30	91.00
46	77.74	22-Jul-2002	Adenocarcinoma	7.00		14.00	1.49	10.60	11.31	8.82	78.00
47	71.85	01-Jan-2002	Adenocarcinoma	5.00		13.40	1.01	7.50	11.35	9.98	87.90
48	70.31	20-Dec-2001	Adenocarcinoma	6.00	.44	13.90	1.78	12.80	11.40	8.10	71.00
49	59.65	22-Mar-2000	Adenocarcinoma	3.00	1.26	14.90	.83	5.57	11.50	9.00	78.00
50	67.32	22-Sep-1999	Adenocarcinoma	6.00	18.29	12.80	.62	4.84	11.80	11.50	97.00
51	74.23	28-Feb-2002	Adenocarcinoma	8.00	9.74	13.60	1.66	12.20	11.90	9.30	78.00
52	64.37	26-Jan-2000	Adenocarcinoma	5.00	2.24	14.10	1.89	13.40	12.70	9.50	75.00
53	77.07	28-Nov-2001	Adenocarcinoma	5.00	5.00	24.80	1.44	5.81	13.60	10.80	79.00
54	62.91	24-Dec-2001	Adenocarcinoma	6.00	.60	14.30	.53	3.71	13.80	12.00	87.00
55	71.06	28-Nov-2001	Adenocarcinoma	5.00	2.44	14.90	1.91	12.80	14.30	11.70	82.00
56	88.24	06-Dec-2001	Adenocarcinoma	6.00	2.13	17.20	1.71	9.94	14.60	1.70	80.00
57	63.83	21-Feb-2002	Adenocarcinoma	6.00	.78	17.20	2.98	17.30	14.80	10.00	68.00
58	63.08	10-Jan-2002	Adenocarcinoma	6.00	.84	19.10	1.28	6.70	14.90	13.00	88.00
59	82.79	17-Dec-2001	Adenocarcinoma	6.00	.51	20.70	2.26	10.90	15.60	12.50	80.00
60	66.18	14-Mar-2002	Adenocarcinoma	7.00		22.20	2.03	9.10	16.57	14.19	85.70
61	69.36	17-Jan-2002	Adenocarcinoma	6.00	.75	17.10	1.29	7.54	17.00	13.90	82.00
62		22-Oct-2001	Adenocarcinoma	2.00	1.81	19.00	1.05	5.53	17.60	14.50	82.00
63	68.65	28-Feb-2000	Adenocarcinoma	7.00	22.25	14.80	2.98	20.10	17.60	12.50	71.00
64	69.62	15-Dec-1999	Adenocarcinoma	7.00	.73	18.80	3.20	17.00	17.60	12.40	70.00
65	72.96	25-Aug-1999	Adenocarcinoma	9.00	3.67	22.00	.89	4.05	17.60	15.10	83.00
66	65.35	12-Jan-2000	Adenocarcinoma	3.00	1.08	18.80	1.39	7.39	17.70	14.70	83.00
67	58.12	28-Jan-2002	Adenocarcinoma	6.00	2.56	19.80	1.19	6.01	18.00	14.80	82.00
68	64.15	25-Aug-1999	Adenocarcinoma	6.00	4.03	20.00	1.60	8.00	18.10	14.10	78.00
69	72.50	17-Dec-2001	Adenocarcinoma	8.00	12.45	21.80	3.33	15.30	18.10	11.10	61.00
70	68.82	21-Mar-2001	Adenocarcinoma	7.00		24.90	1.00	4.00	18.44	16.28	88.40
71	57.90	30-Jun-1999	Adenocarcinoma	7.00	1.16	17.20	3.45	20.10	19.00	14.10	74.00
72	64.15	24-Jan-2000	Adenocarcinoma	8.00	.80	17.30	1.25	7.23	19.30	14.80	74.00
73	65.12	28-Jul-1999	Adenocarcinoma	8.00	2.86	23.40	1.90	8.12	20.30	15.80	78.00
74	58.16	17-Nov-1999	Adenocarcinoma	7.00	.69	27.70	3.15	11.37	20.60	14.20	69.00
75	77.44	23-Jan-2002	Adenocarcinoma	8.00	.99	22.90	1.21	5.28	20.90	16.00	77.00
76		19-Sep-2001	Adenocarcinoma	7.00	3.44	24.10	1.91	7.93	21.30	17.10	80.00
77	67.96	08-Mar-2000	Adenocarcinoma	7.00	42.03	28.20	1.79	6.35	21.70	19.70	91.00
78	81.53	06-Oct-1999	Adenocarcinoma	7.00	15.64	22.80	3.38	14.80	22.00	15.30	70.00
79	73.42	21-Dec-2001	Adenocarcinoma	7.00	.97	24.30	3.71	15.30	22.10	15.10	68.00
80	75.09	25-Oct-2001	Adenocarcinoma	7.00	2.16	26.30	.96	3.65	22.50	19.10	85.00
81	64.51	25-Oct-1999	Adenocarcinoma	5.00	.55	25.60	2.43	9.49	23.20	18.00	78.00
82	69.07	22-Jul-2002	Adenocarcinoma	8.00		28.00	2.20	7.90	23.27	20.50	88.10
83	62.29	22-Jul-2002	Adenocarcinoma	6.00		28.30	1.37	4.80	24.08	19.40	80.60
84	68.56	22-Jul-2002	Adenocarcinoma	7.00		28.40	2.91	10.20	25.11	19.79	78.90
85	75.88	22-Jul-2002	Adenocarcinoma	6.00		32.70	1.76	5.40	25.26	22.65	89.90
86		03-Oct-2001	Adenocarcinoma	6.00	.35	36.50	1.24	3.39	33.00	30.80	94.00
87	75.43	26-Jan-2000	Adenocarcinoma	6.00	.88	32.60	5.63	17.30	36.10	26.00	72.00
88		25-Oct-2001	Adenocarcinoma	7.00	2.97	40.10	5.30	13.20	40.00	24.00	60.00
89	73.42	30-May-2002	Adenocarcinoma	5.00		54.70	5.28	9.60	42.82	34.20	79.90
90	66.91	04-Apr-2002	Adenocarcinoma	7.00		49.20	2.20	4.47	43.41	34.18	78.80
91		19-Sep-2001	Adenocarcinoma	6.00	30.47	41.60	1.41	3.39	43.60	35.80	82.00

92	71.58	24-Jan-2000	Adenocarcinoma	7.00	6.75	45.70	1.96	4.29	44.80	35.40	79.00
93	78.36	28-Nov-2001	Adenocarcinoma	6.00	1.34	53.10	3.66	6.89	55.60	41.00	73.00
94	69.61	17-Nov-1999	Adenocarcinoma	5.00	.93	46.90	9.14	19.50	56.00	36.00	64.00
95	70.18	22-Nov-1999	Adenocarcinoma	5.00	4.13	58.20	4.10	7.00	68.00	43.00	63.00
96	79.47	26-Nov-2001	Adenocarcinoma	7.00	.44	63.60	4.66	7.33	68.00	42.00	62.00
97	75.48	30-Oct-2001	Adenocarcinoma	8.00	4.68	81.20	13.30	16.40	75.40	32.90	44.00
98	72.02	10-Jan-2002	Adenocarcinoma	6.00	.68	77.20	2.76	3.56	76.00	54.00	71.00
99	78.70	30-May-2002	Adenocarcinoma	9.00		112.00	10.40	9.30	89.52	65.08	72.70
100	82.17	22-Nov-1999	Adenocarcinoma	7.00	8.64	107.00	13.00	12.10	99.00	64.00	65.00
101	57.95	21-Dec-2001	Adenocarcinoma	10.00	2.47	114.00	8.94	7.84	103.00	74.00	72.00
102	69.21	25-Aug-1999	Adenocarcinoma	7.00	1.86	111.00	6.41	5.78	116.00	80.00	69.00
103	69.28	17-Nov-1999	Adenocarcinoma	9.00	6.20	264.00	85.50	32.40	146.00	39.00	27.00
104	55.05	23-Feb-2000	Benign		.89	.38	.05	13.10	.43	.31	72.00
105	65.69	28-Jul-1999	Benign		7.59	.61	.32	52.50	.80	.50	63.00
106	68.88	22-Mar-2000	Benign		3.75	.90	.17	18.90	.95	.73	77.00
107	53.08	25-Oct-1999	Benign		11.90	1.18	.23	19.50	1.10	.80	73.00
108	59.12	08-Sep-1999	Benign		12.91	1.40	.11	7.86	1.30	1.20	92.00
109	55.62	22-Oct-2001	Benign		.90	1.07	.17	15.90	1.40	1.00	71.00
110	55.60	03-Nov-1999	Benign		18.52	1.36	.22	16.20	1.80	1.30	72.00
111	71.35	23-May-2002	Benign			2.47	.54	21.90	1.80	1.11	61.70
112	61.21	20-Dec-2001	Benign		1.67	2.42	.48	19.80	2.20	1.60	73.00
113	47.60	22-Jul-2002	Benign			2.84	.35	12.30	2.41	1.84	76.00
114	58.37	26-Jun-2000	Benign		.95	2.77	.38	13.70	2.80	2.20	79.00
115	75.12	14-Mar-2002	Benign			3.12	.86	27.60	2.82	2.03	71.90
116	68.57	11-Apr-2002	Benign			4.49	1.41	31.40	3.00	2.00	66.70
117	65.55	24-Dec-2001	Benign		2.44	3.63	.39	10.70	3.30	2.30	70.00
118	71.92	22-Nov-2001	Benign		.69	4.69	1.07	22.80	4.30	3.20	74.00
119	62.95	28-Feb-2000	Benign		4.43	6.20	1.05	16.90	4.90	3.50	71.00
120	65.29	28-Jan-2002	Benign		1.63	5.65	.64	11.30	5.10	4.20	82.00
121	64.80	30-Oct-2001	Benign		2.05	5.66	.57	10.10	5.20	4.00	77.00
122	68.64	28-Nov-2001	Benign		.23	6.68	1.18	17.70	5.20	3.50	67.00
123	63.83	15-Dec-1999	Benign		1.50	5.80	1.07	18.40	5.40	4.00	74.00
124	64.35	24-Jan-2000	Benign		3.60	5.71	.58	10.20	5.40	4.70	87.00
125		03-Oct-2001	Benign		1.64	6.70	.57	8.50	5.50	4.70	85.00
126	73.77	23-Jan-2002	Benign		1.80	6.72	1.04	15.50	5.70	4.30	7.50
127	54.70	16-May-2002	Benign			8.15	1.54	18.90	5.75	4.18	72.70
128	60.56	05-May-1999	Benign		2.01	6.61	.73	11.00	6.50	5.10	78.00
129	57.66	11-Aug-1999	Benign		2.18	6.83	1.18	17.30	6.60	5.00	76.00
130	71.62	09-Feb-2000	Benign		.38	7.29	2.96	40.60	6.60	3.40	52.00
131	73.24	13-Dec-1999	Benign		5.57	8.13	1.51	18.50	6.70	4.40	66.00
132	57.07	08-Mar-2000	Benign		.65	9.39	1.20	12.80	7.00	5.50	79.00
133	61.42	08-Sep-1999	Benign		.71	7.72	.90	11.70	7.10	6.00	85.00
134	63.35	14-Mar-2002	Benign			10.40	2.38	22.90	7.75	5.01	64.60
135	59.10	28-Feb-2000	Benign		.46	11.10	1.03	9.28	7.80	7.10	91.00
136	63.62	20-Dec-2001	Benign		1.98	9.17	1.13	12.30	8.10	5.50	68.00
137	66.25	10-Jan-2002	Benign		2.51	8.41	1.15	13.70	8.10	7.00	86.00
138	70.15	28-Jan-2002	Benign		4.93	10.60	2.18	20.60	8.10	4.80	59.00

139	73.98	16-Jun-1999	Benign	1.20	9.06	1.53	16.90	8.60	6.10	71.00
140		21-Dec-2001	Benign	82.02	9.26	1.63	17.60	8.70	7.40	85.00
141	65.76	05-Apr-2000	Benign	13.51	8.86	1.46	16.50	8.80	6.60	75.00
142	77.93	03-Nov-1999	Benign	.19	11.10	.80	7.20	9.40	7.90	84.00
143	60.21	12-Jan-2000	Benign	.59	9.51	.83	8.73	9.70	7.00	72.00
144	72.73	24-Jan-2000	Benign	1.34	9.37	1.14	12.20	10.00	7.20	72.00
145	67.73	22-Nov-1999	Benign	100.00	11.40	1.87	16.40	10.30	8.00	78.00
146	67.30	08-Sep-1999	Benign	11.57	11.00	2.31	21.00	10.90	7.00	64.00
147	74.94	17-Nov-1999	Benign	1.43	10.20	1.09	10.70	11.10	9.70	87.00
148	63.55	08-Mar-2000	Benign	.06	11.10	1.60	14.40	11.20	8.40	75.00
149	69.15	22-Nov-1999	Benign	.34	14.10	1.80	12.80	11.80	10.10	86.00
150	52.92	23-Jan-2002	Benign	.32	13.00	.52	4.00	12.30	10.50	85.00
151	64.13	03-Nov-1999	Benign	4.87	13.10	1.72	13.10	12.80	9.70	76.00
152	77.02	19-May-1999	Benign	21.08	17.00	3.63	21.40	14.50	9.40	65.00
153	65.68	14-Jun-2002	Benign		18.10	1.91	10.60	15.28	12.84	83.80
154	69.49	15-Dec-1999	Benign	.46	19.90	1.80	9.05	17.10	13.90	81.00
155	50.16	25-Oct-1999	Benign	7.24	17.30	2.13	12.30	17.60	13.20	75.00
156	66.04	15-Dec-1999	Benign	6.19	19.80	1.47	7.42	19.30	14.80	77.00
157	58.89	06-Dec-2001	Benign	.46	26.10	1.47	5.63	22.40	18.60	86.00
158	73.80	22-Sep-1999	Benign	2.96	21.30	6.66	31.30	22.50	12.00	53.00
159	76.98	26-Nov-2001	Benign	1.07	24.80	6.38	25.70	24.50	12.90	53.00
160	65.99	22-Sep-1999	Benign	1.68	35.80	3.57	9.97	34.00	26.00	76.00
161	44.87	22-Oct-2001	Benign	3.27	37.50	4.28	11.40	36.70	22.20	59.00
162	64.29	23-Feb-2000	PIN	2.73	3.25	.20	6.15	3.50	2.80	80.00
163	62.09	26-Jan-2000	PIN	3.36	4.46	.40	8.97	4.50	4.10	91.00
164	63.32	08-Mar-2000	PIN	1.23	8.18	.44	5.38	7.80	6.70	86.00
165	68.13	22-Jul-2002	PIN		20.60	4.82	23.40	13.57	8.79	64.80
166	74.17	21-Feb-2002	PIN	2.58	39.60	5.60	14.10	39.00	26.70	68.00
167	73.98	10-Jan-2002	PIN	2.24	6.13	1.36	22.10	5.90	3.70	63.00
168	66.04	30-Jun-1999	PIN	.30	6.79	.98	14.40	6.50	5.00	77.00
169	79.37	20-Dec-2001	PIN	1.72	31.90	5.93	18.60	32.00	22.00	69.00
170	70.92	24-Dec-2001	Prostatitis	2.32	2.27	.36	15.90	2.10	1.50	71.00
171	56.59	21-Feb-2002	Prostatitis	1.05	7.13	.76	10.70	6.30	5.00	79.00