AN INVESTIGATION OF THE RELATIONSHIP BETWEEN THE INFLAMMATORY RESPONSE AND OUTCOME IN PATIENTS WITH BREAST CANCER

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AUTHOR'S DECLARATION

I declare that, the work presented in this dissertation, has been carried out solely by myself except where indicated below.

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DEDICATION

Dedicated to my family, friends and all who supported and encouraged me in my career and academic study.

SUMMARY OF THESIS

Breast caner is the commonest malignancy in women and a major cause of morbidity and mortality in the western World. Well established risk factors for breast cancer are mostly related to women's reproductive history such as early menarche, late first pregnancy and late menopause. In recent years, survival is improving due to a combination of better health education with efficient large-scale use of screening mammography and earlier detection of breast cancer, as well as, improved surgical techniques and, in particular, widespread use of adjuvant therapy.

Conventional clinico-pathologic features of the patient and primary tumour at initial presentation such as age, tumour size and grade, nodal status and hormonal receptor status, are considered to be the key prognostic factors for predicting recurrence and survival and are used as a guide for appropriate therapy. However, the clinical course of breast cancer may vary from an indolent slowly progressive one to that of rapid progression and metastatic spread, and with highly variable outcomes even when these factors are taken into account. Therefore, new prognostic and predictive indicators are required to enable accurate assessment of outcomes and direct therapy towards those most likely to benefit.

It is increasingly recognised that variations in outcome in cancer patients are not solely determined by the characteristics of the tumour but also by the host-response factors. Cancer development and progression is dependent on a complex interaction of the tumour and the host inflammatory response. The local immune response with cell-mediated immune response and, in particular, the local environment of cytokines, proteases,

angiogenic and growth factors, and its subsequent systemic inflammatory response, may, in turn, stimulate tumour growth and metastasis. Recently, the systemic inflammatory response, as evidenced by elevated circulating concentrations of C-reactive protein and/or lowered albumin concentrations, has been shown to be stage independent prognostic factors in patients with a variety of cancers, including advanced systemic diseases, as well as, following a potentially curative resection of primary operable tumours. However, this complex tumour-host interaction in patients with different staged breast cancer and its prognostic value remains unclear.

In patients with metastatic breast cancer, the prognosis is generally poor and prediction of outcome remains problematical. This prognostic uncertainty has driven the search for well standardised laboratory-based parameters which have prognostic value in systemic disease. Therefore, the value of systemic inflammation-based score (Glasgow Prognostic Score, GPS) was evaluated in patients with metastatic breast cancer (n=96). The GPS was constructed as follows; patients with both an elevated C-reactive protein (>10 mg/l) and hypoalbuminaemia (<35g/l) were allocated a score of 2. Patients in whom only one or none of these biochemical abnormalities was present were allocated a score of 1 or 0, respectively. The minimum follow-up was 7 months and the median follow-up of the survivors was 16 months. During this period 51 patients died of their cancer. On multivariate analysis, only the GPS (HR 2.26, 95% CI 1.45–3.52, $P<0.001$) remained significantly associated with cancer-specific survival. The median survival in these patients was 24 months, 13 months and 1 month for a GPS of 0, 1 and 2, respectively. Therefore, the presence of a systemic inflammatory response, the GPS that based on simple routinely available and well-standardised measurements, appears to be a useful independent indicator of poor outcome in patients with metastatic breast cancer.

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In patients with early-staged invasive disease, the prognostic value of the relationship between the systemic inflammatory response; as evidenced by elevated C-reactive protein and lowered albumin concentrations that were measured prior to surgery, standard clinico-pathologic factors and cancer outcome was examined in 300 patients with operable primary cancer. The median follow-up of the survivors was 46 months. During this period, 37 patients relapsed and 25 died of their cancer. On multivariate analysis only tumour size ($P<0.05$), albumin ($P<0.01$), and systemic treatment ($P<0.0001$) were significant independent predictors of relapse-free, cancer-specific and overall survival. Lower serum albumin concentrations ($\leq 43g/l$) were associated with deprivation ($P<0.05$), hormonal-receptor negative tumours ($P<0.01$) and significantly poorer 3-year relapse-free (85% vs 93%, $P=0.001$), cancer-specific (87% vs 97%, $P<0.0001$) and overall survival (84% vs 94%, $P=0.001$) rates. Therefore, these results suggest that lower pre-operative albumin concentrations, but not elevated C-reactive protein concentrations, predict relapse-free, cancer-specific and overall survival, independent of clinico-pathologic status and treatment in patients undergoing potentially curative surgery for primary operable breast cancer.

The host-tumour interaction was further extended to examine the cell-mediated immune response, as evidenced by T-lymphocytic and macrophage infiltration, within the tumour microenvironment in association with tumour proliferative activity and microvessel density, in a subset of 168 patients, to clarify their relationship with cancer outcome following potentially curative resection for primary operable breast cancer. Tumour CD4+ and CD8+ T-lymphocyte infiltration, CD68+ macrophage infiltration and CD34+ microvessel density were assessed using immunohistochemistry and slide counting techniques. The median follow-up of the survivors was 60 months. During this period, 20

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patients relapsed and 15 died of their cancer. On univariate analysis, increased lymph node involvement ($P<0.01$), negative hormonal-receptor status ($P<0.05$), lower albumin concentrations ($P<0.01$), increased tumour Ki-67 labelling index ($P<0.05$), increased tumour microvessel density ($P<0.05$), the extent of loco-regional control ($P<0.0001$) and limited systemic treatment ($P\leq0.01$) were associated with both relapse-free and cancer-specific survival. Increased tumour grade and Ki-67 proliferative activity were associated with greater tumour infiltration of CD4+ and CD8+ T-lymphocytes ($P<0.05$), CD68+ macrophages ($P<0.05$) and CD34+ microvessel density ($P<0.01$) but not lowered albumin concentrations. On multivariate analysis, only pre-operative albumin ($P<0.05$), loco-regional treatment ($P<0.01$) and systemic treatment ($P<0.05$) were significant independent predictors of relapse-free and cancer-specific survival.

In conclusion, the results of these studies suggest that the systemic inflammatory response, as measured by GPS, may be a useful tool in the assessment of survival in patients with systemic metastatic breast cancer. Moreover, the host inflammatory responses are closely related to poor tumour differentiation and hormone-negativity, and malignant disease progression in patients with early-staged invasive breast cancer. Furthermore, that the tumour-based cell mediated immune response and pathological factors are subordinate to the systemic factors, such as albumin, in determining survival in patients with primary loco-regional operable disease. Future prognostic studies should be large enough and over adequate follow-up time and should include all potential prognostic factors in the multivariate survival analysis.

CHAPTER 1: INTRODUCTION

1.1   Epidemiology of breast cancer

1.1.1   Incidence and mortality

Breast cancer is the most common malignancy in women and a major cause of mortality and morbidity. Worldwide, it accounts for more than a fifth of all cancers, with an estimated recent incidence of more than a million new patients per year, resulting in almost 373,000 deaths (Parkin *et al.*, 2001; Boyle *et al.*, 2003).

The incidence and mortality of breast cancer varies widely around the world, with North European countries, North American and Australia having the highest rates, and Asian and African countries the lowest (Key *et al.*, 2001; Sasco, 2001; Boyle *et al.*, 2003). In recent years, breast cancer incidence has been increasing in most countries, particularly in several Asian and African countries (Sasco, 2001). Recent mortality rates have levelled off or started to decline in many western countries, such as UK, United States, Canada and Australia. Analysis of age-specific mortality rates shows that the change in mortality has occurred primarily in middle-age women and, to a lesser extent, in older women. These trends are, at least in part, attributable to large-scale use of mammograghic screening, early detection of breast cancer and, in particular, to the widespread use of adjuvant therapy (Peto *et al.*, 2000; Sasco, 2001; Jatoi and Miller, 2003).

In the UK, breast cancer continues to be the commonest cancer to affect women, with a recent annual incidence of nearly 41,000 new patients per year, accounting for almost 30 per cent of all new cancer cases in women, and with estimated lifetime risk of one in nine.

However, despite the increase in incidence, mortality rates have being falling steadily since 1990, probably due to a combination of factors including earlier diagnosis and improved treatment. In the year 2001, there were 12,994 breast cancer deaths compared to around 15,186 breast cancer deaths in 1992. It has been overtaken by lung cancer as the most common cause of death from malignancy in women (Cancer Research UK, 2003).

In Scotland, breast cancer is by far the most frequent cancer among women, accounting for 27% of cancer in women and with approximately 3,533 cases in 2001. Over the last decade, a substantial improvement in breast cancer survival has been observed with an obvious increase from 60% for patients diagnosed in 1977-1981 to 77% in 1997-2001. This improvement is likely to be due to a combination of new treatments, particularly hormonal therapy, earlier diagnosis of cancers in women participating in Scottish Breast Screening Programme, and better organization and delivery of care for patients. Around 1.2% of women are living with breast cancer and the prevalence of breast cancer is increasing due to improvements in prognosis and increasing incidence (Scottish Cancer Registry, ISD, NHS National Services Scotland; www.isdscotland.org.).

1.1.2 Aetiology and risk factors

The search for specific breast cancer risk factors has been stimulated by the large difference in rates of breast cancer among countries worldwide, within countries over time, and by changes in rates among migrating population. In general, the causes of breast cancer are not fully understood, but epidemiological researches have clearly identified important factors that influence breast cancer risk (Table: 1.1). Known and suspected risk factors include the following:

1.1.2.1 Geographical variation

Marked geographical variation in the age-adjusted incidence and mortality of breast cancers is observed between countries. The difference between Far Eastern and Western countries is diminishing, but the relative-risk difference is still around five-fold. Studies of immigrants from countries with a low-risk of breast cancer (eastern Asia) to one of higher-risk (United States) show that immigrants experience an increase in incidence of breast cancer, with a doubling of incidence rates within 10 years of arrival, and assume levels just below that of their adopted country within one or two generations, indicating that lifestyle and environmental factors are of greater importance than genetic factors (Spicer and Pike, 1999; McPherson *et al.*, 2000; Key *et al.*, 2001; Sainsbury, 2001).

1.1.2.2 Age

The incidence of breast cancer increases rapidly with age during the reproductive years, being rare before the age of 25 and doubling about every 10 years until the menopause (about 50 years of age), when there is a slight levelling off before the incidence again rises, although at a reduced rate compared with the premenopausal years (McPherson *et*

al., 2000; Sainsbury, 2001). The cumulative incidence of breast cancer among women in Europe and North America is about 2.7% by age 55, about 5% by age 65, and about 7.7% by age 75 (Key *et al.*, 2001).

1.1.2.3 Age at menarche and menopause

Women who have more menstrual cycles with an early onset menarche (under 12 years of age) and late menopause (over 55 years of age) have an increased risk of developing breast cancer, resulting from increasing the time of exposure to the mitogenic effect of ovarian hormones, in particular oestrogens (Gendy and Rainsbury, 2001a; Sasco, 2001; Cuzick, 2003). For each one-year delay in menarche, the risk decreases by around 5%, and for one-each year older at menopause, the risk increases by about 3% (Key *et al.*, 2001; Cuzick, 2003). Women who have a natural menopause after the age of 55 are twice as likely to develop breast cancer compared with those whose menopause occurs before the age of 45. And those women who have had bilateral oophorectomy under the age of 35 years have only 40% of the risk of breast cancer of women who have a natural menopause (McPherson *et al.*, 2000; Sainsbury, 2001).

1.1.2.4 Childbearing

Childbearing appears to have a dual effect on the risk of breast cancer. Although, the risk is increased in the immediate period following childbirth; possibly reflecting the effects of oestrogen in pregnancy in causing extensive terminal ducts differentiation and their subsequent involution, this excess risk gradually diminishes and then, for the rest of women's life, the effect of birth is rather to protect against the disease (Key *et al.*, 2001; Cuzick, 2003). Late age at first full-term pregnancy and nulliparity increase the lifetime

risk of developing breast cancer. With an increase of around 3% for each one-year delay, the risk of breast cancer in women who have their first child after the age of 30 years is about twice that of women who have their first child before the age of 20 years. The highest risk is for those who have their first child after the age of 35; these women seem to have even a higher risk than that associated with nulliparous women. An increasing protection against breast cancer is also observed with increasing the numbers of full-term pregnancies; with a decrease of around 7% per birth. Furthermore, an early age at birth of a second child further reduces the risk of breast cancer (McPherson et al., 2000; Key et al., 2001; Cuzick, 2003).

1.1.2.5 Breast feeding

The effect of breastfeeding on the risk of breast cancer has been examined. Studies from Asian and less developed countries, where the duration of breastfeeding is substantially longer, have reported a protective association between breast-feeding and breast cancer. Also, some studies from more developed countries have found that women who had breastfed for a total of 25 months or more had a 33% lower risk compared to those who had never breastfed (Key et al., 2001).

1.1.2.6 Family history of breast cancer

Hereditary breast cancer accounts for approximately 5-10% of overall breast cancer in Western countries. It includes mutations in the genes *BRCA1, BRCA2, p53, PTEN* and *ATM* (Key et al., 2001). Breast cancer susceptibility is generally inherited as an autosomal dominant gene with limited penetrance, which may be transmitted through either maternal or paternal lines (McPherson et al., 2000; Sainsbury, 2001). Two breast

cancer genes, *BRCA1 and BRCA2*, have been responsible for a substantial proportion of very high-risk families, with a considerable life-time risk of developing breast cancer estimated as high as 50-80% among mutation carriers compared to the general population (Sasco, 2001; Cuzick, 2003). Inherited mutations in other rare genes, such as *p53* and *PTEN*, are associated with familial syndromes (Li-Fraumeni cancer syndrome and Cowden's disease, respectively) that include a high risk of breast cancer (McPherson *et al.*, 2000; Key *et al.*, 2001). Breast cancer risk increases with the number of relative affected (at least two different affected first-degree relatives), younger age at diagnosis, presence of bilateral breast cancer disease, male breast cancer or multiple primaries in the same women such as ovarian cancer and *BRCA1* and *BRCA2* gene mutations (Table 1.2; McPherson *et al.*, 2000; Gendy and Rainsbury, 2001a; Sainsbury, 2001; Cuzick, 2003).

1.1.2.7   Previous benign breast disease and mammographic parenchymal patterns

History of prior breast biopsy for benign breast disease of specific epithelial abnormality increases the risk of breast cancer. Non–proliferative lesions are generally associated with little or no increase in breast cancer risk. Proliferative lesions without atypia confer an increase in the risk of about two-fold. While atypical hyperplasia is associated with an approximately four to five-fold increase in breast cancer risk, women with this change and also a family history of breast cancer (first degree relative) have a nine-fold increase in the risk (McPherson *et al.*, 2000; Key *et al.*, 2001).

The extent of mammographic opaque areas have been shown to be an important measure of the risk of developing breast cancer. Women with a large proportion of the breast consisting of radiodense tissue are at a higher risk of breast cancer than women with more

radiolucent breasts. Although, breast density decreases after the menopause, the risk is apparent for both pre- and post- menopausal women (Key *et al.*, 2001; Cuzick, 2003).

1.1.2.8 Radiation

Ionising radiation is the most well established environmental risk factor for increasing breast cancer later in life, particularly when exposure is during rapid breast formation. A doubling of breast cancer risk was observed among teenage girls exposed to radiation during the Second World War (McPherson *et al.*, 2000). The relative risk for women exposed to ionising radiation before the age of 40 years ranges between 1.1 – 2.7 at 1 Gy (Key *et al.*, 2001).

1.1.2.9 Oral contraceptive

There is a small increase in the relative risk of developing breast cancer among women while taking oral contraceptive and for 10 years after stopping these agents. The use of oral contraceptives late in a women's reproductive life will result in an increase of the relative risk at a time when the background risk is becoming appreciable. However, at young ages and when the use of oral contraceptives is common, the absolute risk is low (Key *et al.*, 2001; Cuzick, 2003). Cancers diagnosed in women taking oral contraceptive are less likely to be clinically advanced than those diagnosed in women who have never used these agent, with a relative risk of 0.88 (0.81-0.95) (McPherson *et al.*, 2000).

1.1.2.10 Hormonal replacement therapy

The use of hormonal replacement therapy for the menopause occurs during the prime risk period for breast cancer. Among current and recent users of hormonal replacement

therapy, the relative risk of breast cancer increases with increasing the duration of use, but this excess diminishes after cessation of use. Long-term users ($\geq$ 5 years) of oestrogen replacement therapy were observed to have a 35% increase in the risk of breast cancer; this risk appears to be higher with combined oestrogen and progestin preparations (Brewster and Helzlsouer, 2001; Key et al., 2001). The increase in breast cancer risk is consistent with the effect of delay in the menopause (McPherson et al., 2000). Current evidence, however, suggests that women taking hormonal replacement therapy do not develop poorer prognosis tumours (Stallard et al., 2000).

1.1.2.11 Weight

Postmenopausal obesity is associated with an increase in the risk of breast cancer; about 50% higher among obese women (body-mass index > 30 kg/m^2) compared with lean women (body-mass index 20 kg/m^2). But during the premenopausal years, it actually reduces the risk (McPherson et al., 2000; Key et al., 2001). This inverse relationship can be explained in terms of the differential effects of premenopausal and postmenopausal obesity on endogenous hormone levels. Premenopausal obesity decreases sex hormone binding globulin and minimally increases exposure to oestrogen, but decreases breast exposure to progesterone. Postmenopausally, the decrease in risk is gradually eliminated, and eventually, the increased bioavailable oestrogen levels associated with postmenopausal obesity, and which are determined by the conversion of fat in adipose tissue to oestrogen via aromatisation, produce an increase in the risk of breast cancer (Spicer and Pike, 1999; Cuzick, 2003).

1.1.2.12 Life style and other environmental factors

Breast cancer is more common among women of higher socio-economic status than those of lower socio-economic status (Sainsbury, 2001).

Diet and nutrition are controversial factors in the risk of breast cancer. Although there is a close correlation between the incidence of breast cancer and dietary fat intake in populations, the true relation between fat intake and breast cancer does not appear to be particularly strong or consistent. Also, the evidences for the protective roles of fruits, vegetables, specific antioxident micronutrients, and dietary vitamins, such as vitamin C and E, have been inconclusive (McPherson *et al.*, 2000; Brewster and Helzlsouer, 2001; Key *et al.*, 2001).

Alcohol consumption is associated with a moderate increase in the risk of breast cancer. Within the range of light to moderate alcohol intake, and with an increase risk in the order of 10% for each 10g alcohol (1 unit) / day, breast cancer seems to increase linearly. So, an intake of around 30g (3 units) per day is associated with an increased risk of around 30%. The basis for the increased risk associated with alcohol consumption is unclear, but may be caused by the influence of alcohol on the liver and so on hormone profiles (Brewster and Helzlsouer, 2001; Key *et al.*, 2001; Boyle *et al.*, 2003; Cuzick, 2003).

The possible role of cigarette smoking as a risk factor for breast cancer has remained controversial (Key *et al.*, 2001; Sasco, 2001).

Moderate physical activity has been reported to be associated with a lower risk of breast cancer, which seems to be stronger for premenopausal women than for postmenopausal

women (Key *et al.*, 2001). The risk reduction may result from the differences in endogenous sex steroid levels. In premenarcheal girls, strenuous exercise can delay the onset of menarche. And moderate exercise increases the probability of anovulatory cycles (Spicer and Pike, 1999).

Table 1.1: Established and probable risk factors for breast cancer (from McPherson *et al.*, 2000).

Factor	Relative risk	High risk group
Age	>10	Women aged over 50 years
Geographical location	5	Developed country
Age at menarche	3	Menarche before age 11
Age at menopause	2	Menopause after age 54
Age at first full pregnancy	3	First child in early 40s
Family history	≥ 2	Breast cancer in first degree relative when young (before age 50)
	4-6	Breast cancer in two first degree relatives (one before age 50)
Previous benign breast disease	4-5	Atypical hyperplasia
Cancer in other breast	> 4	
Socio-economic group	2	Group I and II
Diet	1.5	High intake of saturated fat
Body weight:		
- Premenopausal	0.7	Body mass index > 35
- Post menopausal	2	Body mass index > 35
Alcohol consumption	1.3	Excessive intake
Exposure to ionising radiation	3	Abnormal exposure in young females after age 10
Taking exogenous hormones:		
- Oral contraceptives	1.24	Current use
- Hormone replacement therapy	1.35	Use for ≥ 10 years
- Diethylstilbestrol	2	Use during pregnancy

Table 1.2: Familial breast cancer (from McPherson *et al.*, 2000).

Criteria for identifying women at substantial increased risk:

The following categories identify women who have three or more times the population risk of developing breast cancer.

A women who has:
- One first degree relative with bilateral breast cancer or breast and ovarian cancer **or**
- One first degree relative with breast cancer diagnosed under the age of 40 years or one first degree male relative with breast cancer diagnosed at any age **or**
- Two first or second degree relatives with breast cancer diagnosed under the age of 60 years or ovarian cancer at any age on the same side of the family **or**
- Three first or second relatives with breast and ovarian cancer on the same side of the family.

First degree relative is mother, sister, or daughter.
Second degree female relative is grandmother, granddaughter, aunt, or niece.

Criteria for identifying women at very high risk in whom gene testing might be appropriate:

- Families with four or more relatives affected with either breast cancer or ovarian cancer in three generations and one alive affected relative.

1.2   Anatomy of the breast

The basic structural unit of the mammary gland is the duct-lobular unit, which vary enormously in their number and size, from about 10 to over 100 lobules that empty via ductules in to a lactiferous duct; of which there are around 15-20 ducts. The glandular tissue, with its various relative amounts of supporting connective tissue and fat, lies within a superficial fasica, in which fibrous processes (Cooper's ligaments) attach firmly to the deep and superficial layers of this fasica, and thereby to the overlying breast skin. The main bulk of the breast tissue is usually localized to its upper outer-quadrant (Figure: 1.1; Ellis, 2004; Sainsbury, 2004).
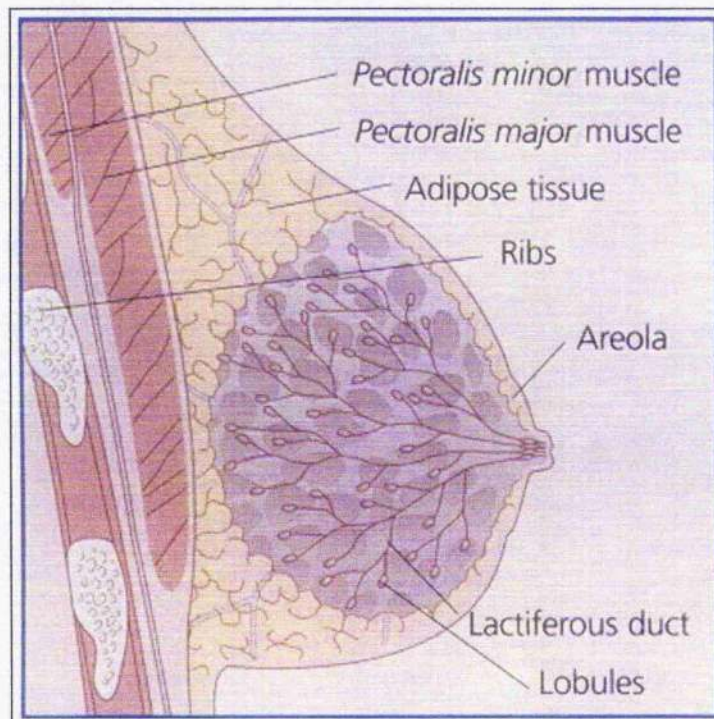
Figure 1.1: Cross-section of the breast (from McCready, 2003).

The adult female breast extends from the second rib above to the sixth rib below, and from the lateral border of the sternum medially to the mid-axillary line laterally; with its superiolateral extremity extending into the axilla as the axillary tail. On its deep aspect, about two-thirds of the breast lies on the pectoralis major. It overlaps the serratus anterior laterally and the upper part of rectus sheath inferiorly (Ellis, 2004; Sainsbury, 2004).

The nipple with its surrounding areola is usually situated at the level of the fourth intercostals space in nulliparous women, but this position tends to be variable with pendulous breasts. The nipple contains about 15-20 lactiferous ducts openings, and the areola contains numerous sweat and sebaceous glands (Ellis, 2004; Sainsbury, 2004).

The blood supply of the breast is a rich anastomotic network which is derived from internal thoracic artery (internal mammary), intercostals artery, and axillary artery that branches into superior thoracic, pectoral branch of the acromiothoracic, lateral thoracic, and subscapular. These arteries are accompanied by their corresponding veins (Ellis, 2004).

The lymphatics of the breast drain predominantly into the axillary lymph nodes, which receive nearly 75-85% of the breast drainage, and into the internal thoracic (internal mammary) lymph nodes (Figure: 1.2). The axillary lymph nodes vary in number from 20-30 nodes, and often defined by clinicians and pathologists into three levels; as level I (nodes inferior to pectoralis minor), level II (nodes behind pectoralis minor) and level III (nodes above pectoralis minor). The apical nodes are also in continuity with the supraclavicular nodes and drain into the subclavian lymph trunk, which enters the great veins directly, or via thoracic duct or jugular trunk. The internal thoracic (internal

mammary) lymph nodes are small, few (3-5 nodes) and lie along the internal thoracic

vessels, deep to the plane of the costal cartilage (Figure: 1.2; Ellis, 2004; Sainsbury,

2004).
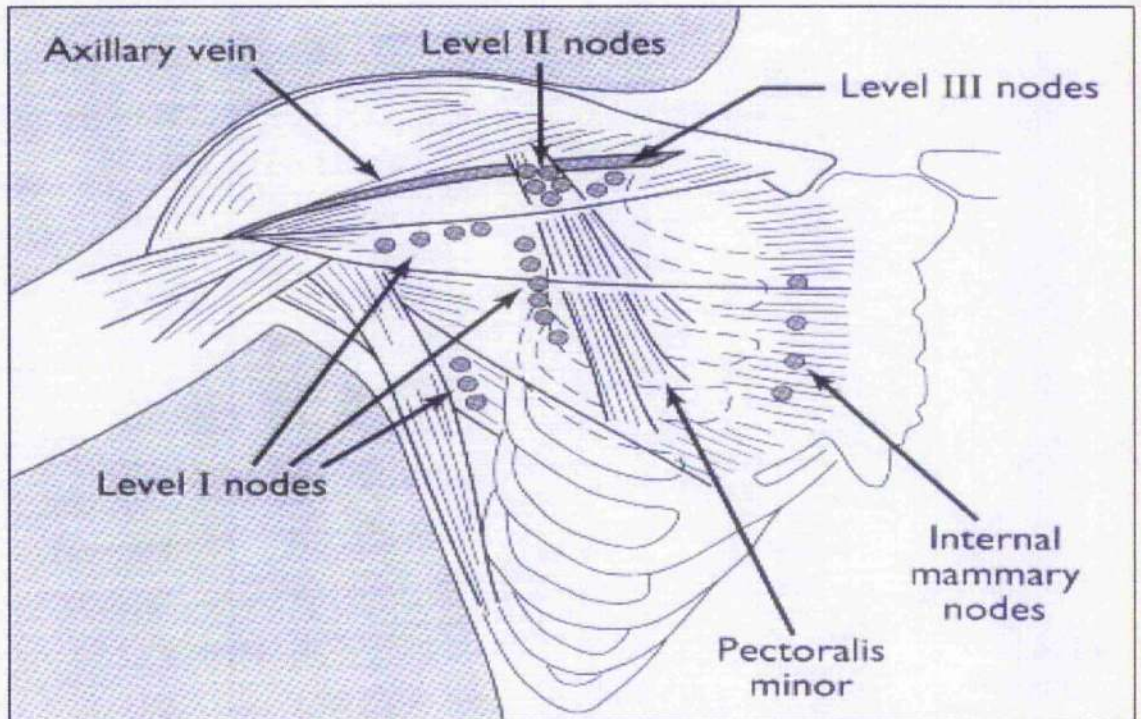
Figure 1.2: Levels of axillary lymph nodes (from Bundred *et al.*, 2000).

1.3   Pathology of breast cancer

Breast cancers arise from the epithelial cells that line the terminal duct-lobular unit (Sainsbury *et al.*, 2000). The transition from a normal breast-epithelial cell into cancer cell is assumed to proceed in a stepwise fashion in the multi-step phenomenon of breast carcinogenesis (Figure: 1.3; Sakorafas and Tsiotou, 2000; Burstein *et al.*, 2004).

Breast cancer can be classified into pre-invasive (*in situ* carcinoma) and invasive cancer as follows:

1.3.1   *In situ* carcinomas of the breast

In situ carcinoma is the proliferation of epithelial cells that have undergone malignant transformation but remain confined within the basement membrane at their site of origin within the terminal duct-lobular unit and draining duct. As there are no lymphatics or blood vessels in the epithelial layer, *in situ* carcinoma offers no risk of metastasis until malignant cells cross the basement membrane (Sainsbury *et al.*, 2000; Sakorafas and Tsiotou, 2000).

Two types of *in situ* carcinoma of the breast have been described and comprise ductal carcinoma *in situ* (DCIS) and lobular carcinoma *in situ* (LCIS). Both arise from the terminal duct-lobular unit. However, there are significant clinical, morphological, and biological behavioural differences between the two types (Table: 1.3; Lagios and Page, 1998; Pinder, 2001).

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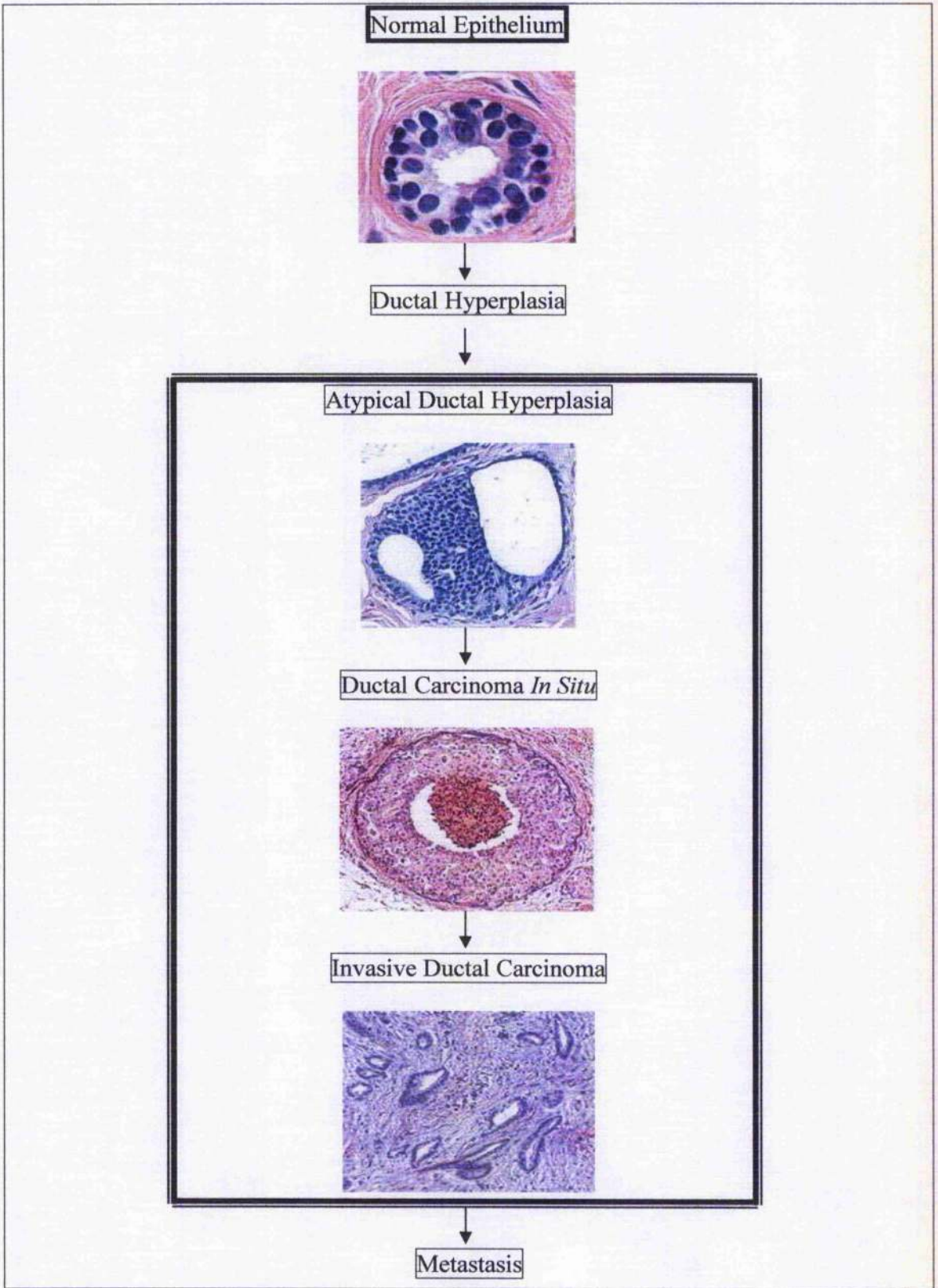
Figure 1.3: Multi-step breast carcinogenesis (from Sakorafas and Tsiotou, 2000; Burstein *et al.*, 2004).

1.3.1.1   Ductal carcinoma *in situ* (DCIS)

Ductal carcinoma *in situ* of the breast is a heterogeneous group of non-invasive neoplastic proliferations of the ductal epithelium that have several morphological variants, which differ markedly in gross and histological appearance, in molecular and cellular characteristics, and in their clinical behaviour. DCIS appears to behave as premalignant lesion in the process of multi-step breast carcinogenesis (Figure: 1.3). However, the biology of the process is relatively poorly understood. Because previous studies, which based on small series of patients with missed lesions or disease for which patients refused excision, indicate that about 50% of high-grade DCIS progresses to invasive malignancy relatively rapidly within 3-5 years. Whereas, low-grade DCIS may take 10-15 years to progress to invasive carcinoma. Also, previous autopsy studies suggest that latent DCIS is relatively common, however, not all DCIS progress to invasive breast cancer (Sakorafas and Tsiotou, 2000; Pinder, 2001).

The frequency of DCIS in symptomatic series of breast cancer is 2-5%, often presenting as a palpable mass. However, DCIS is increasingly identified in asymptomatic women at breast-screening mammography as microcalcification, and in such series the frequency accounts for around 20-25% of all breast cancers (Pinder, 2001; Poller, 2001).

DCIS may extend to the nipple along the major ducts and produce the characteristic eczematous appearance of Paget's disease of the nipple. Paget's disease is always accompanied by DCIS in the underlying subareolar ducts, although invasive carcinoma may also be present (Pinder, 2001).

Traditionally, DCIS has been subclassified on the basis of architectural and growth patterns into: comedo, cribriform, papillary, solid and micropapillary. However, this system has been superseded by a pathological classification that includes the nuclear grade of the tumour cells and the presence or absence of necrosis (Figure: 1.4), as these two features have been shown to be prognostically important, as well as, having high interobserver reproducibility among pathologists. Based on such criteria, tumour size and tumour- free margin, local treatment options include either: local excision alone, local excision plus radiotherapy, or mastectomy (Pinder, 2001; Poller, 2001; Silverstein and Buchanan, 2003; Burstein *et al.*, 2004).
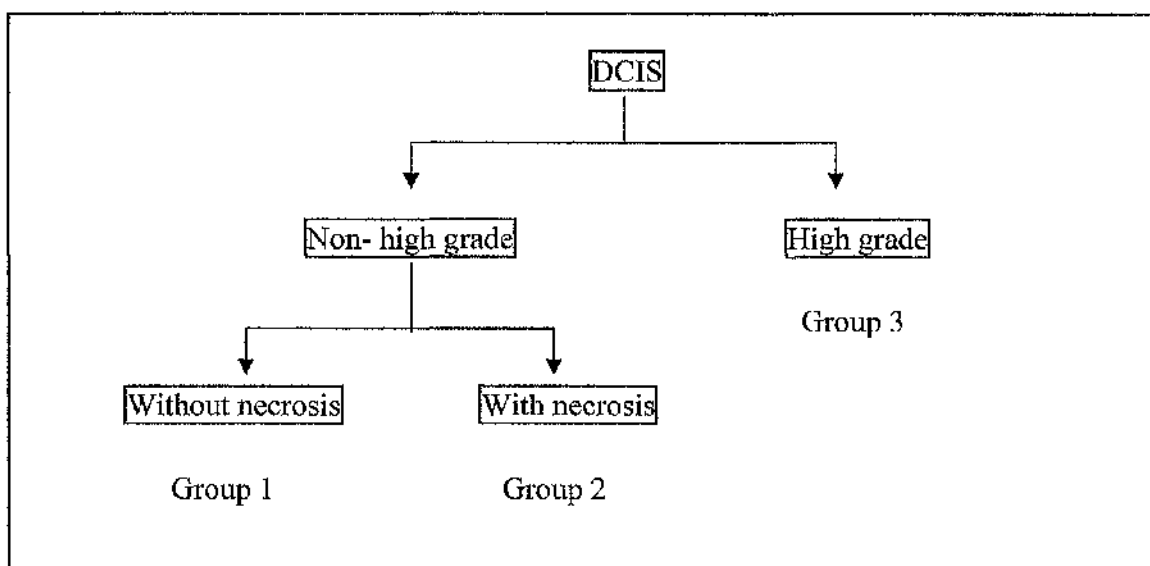
Figure 1.4: Illustration of Scheme for the Van Nuys ductal carcinoma *in situ* (DCIS) Classification (from Poller, 2001).

1.3.1.2   Lobular carcinoma *in situ* (LCIS)

Lobular carcinoma *in situ* is less common than DCIS. It is usually an incidental finding in breast tissue that has been removed for another disease process, such as fibrocystic change. LCIS is often multifocal and bilateral involvement occurs in up to 30% of cases. Follow-up studies have shown that LCIS is generally considered to be a marker of increased risk (about tenfold) of future invasive malignancy rather than a true anatomic precursor lesion of invasive disease; any subsequent malignancy does not necessarily occur at the site of LCIS (Sakorafas and Tsiotou, 2000; Pinder, 2001).

Table 1.3: Comparative features of ductal and lobular carcinoma *in situ* (from Lagios and Page, 1998).

	__DCIS__	__LCIS__
Average age	Late 50s	Late 40s
Menopausal status	70% Postmenopausal	70% Premenopausal
Clinical signs	- Breast mass - Paget's disease - Nipple discharge	None
Mammographic signs	Microcalcification	None
Risk of subsequent carcinoma	30% to 50% at 10 to 18 years	23% to 30% at 15 to 20 years
Site of subsequent invasive carcinoma		
- Same breast	99%	50% to 60%
- Other breast	1%	40% to 50%

1.3.2   Invasive carcinomas of the breast

Invasive breast carcinoma is defined as a malignant neoplasm of the mammary epithelial cells that have invaded beyond the native basement membrane at their site of origin of the terminal duct-lobular unit into the surrounding stroma. Invasive tumour cells may access lymphatic and blood vessels within the surrounding adjacent normal tissue through which it may metastasis to both regional lymph nodes and distant sites (Mizrachi, 1999).

Invasive breast cancers constitute a heterogeneous group of lesions that differ with regard to their morphological types, pathological features and biological potential. They are classified on the basis of the cytologic-morphology features and growth pattern of the invasive tumour cells rather than structure of origin within the mammary duct system, as ductal carcinoma does not necessarily arise from the duct (Schnitt and Guidi, 2000; Sainsbury et al., 2000; Pinder, 2001). The main types are as follow:

1.3.2.1   Invasive ductal carcinoma

Invasive ductal carcinoma is the most common histological type of invasive breast cancer, accounting for more than 50-70% of cases (Pinder, 2001; Sainsbury, 2001). It includes tumours with a range of histological appearance; from those with well-formed glands to those that have little or no evidence of specific differentiation, alternatively known as "carcinoma of no special type (NST)" and "carcinoma not otherwise specified (NOS)", in which they are further categorised according to histological grade that takes into account the variation in appearance (Mizrachi, 1999). Histologically, it is often composed of cords and sheets of large pleomorphic malignant epithelial cells that penetrate the stromal fibrous tissue haphazardly (Figure: 1.3; Pinder, 2001).

1.3.2.2 Invasive lobular carcinoma

Invasive lobular carcinoma is the second most frequent form, accounting for about 10-15% of all invasive breast cancer (Pinder, 2001). It is characterized by multifocality in the ipsilateral breast and more frequently bilateral (Schnitt and Guidi, 2000). Histologically, it is formed from moderately sized, regular malignant cells often arranged in linear cords that diffusely infiltrate within fine collagen bands, giving the "Indian file" appearance pattern (Pinder, 2001).

1.3.2.3 Invasive tubular carcinoma

Invasive tubular carcinoma is a special type cancer that is typically associated with limited metastatic potential and excellent prognosis (Schnitt and Guidi, 2000). It is uncommon in routine symptomatic practice, accounting for about 2% of invasive carcinomas. However, in screen-detected populations it comprises about 15% of invasive carcinoma. Histologically, there is central elastosis and elongated tubular structures radiated through a fibroblastic stroma. The tubules are lined by a single layer of malignant cells that form central lumina (Pinder, 2001).

1.3.2.4 Other invasive breast cancers

Other special histological types of invasive breast carcinomas such as mucinous, medullary, papillary and invasive cribriform are usually have a better prognosis (Gendy and Rainsbury, 2001a). Other rare cancers may include adenoid cystic carcinoma, spindle cell/ metaplastic and squamous cancers (Schnitt and Guidi, 2000; Pinder, 2001).

1.3.3 Spread of breast cancer

The spread of primary breast cancer occurs by:

1.3.3.1 Local invasion

Local spread of malignant cells through the breast parenchyma occurs along mammary ducts, and as the tumour increases in size, by direct infiltration into other portions of the breast, and by the breast lymphatics. In advanced and untreated cases, it tends to involve the overlying skin and to penetrate deep to the pectoral muscles, and even the chest wall (Hellman and Harris, 2000; Sainsbury, 2004).

1.3.3.2 Lymphatic spread

The most common regional lymphatic node involvements in breast cancer occur primarily to the axillary lymph nodes and to the internal mammary lymph nodes chain. The site of the primary tumour within the breast does not dictate which nodes will be involved, because medial tumours spread to the axillary nodes just as readily as lateral tumours. In advanced disease, the supraclavicular nodes and any contralateral lymph nodes may also be involved by cancer spread (Hellman and Harris, 2000; Sainsbury, 2004).

1.3.3.3 Haematogenous spread

Tumour cells may disseminate, by the lymphatic and blood vascular systems, to a variety of organs, including most commonly bone, lung, liver, and brain. Skeletal metastasis occurs in the lumbar vertebrae, femur, thoracic vertebrae, ribs and skull; they are generally osteolytic. Occasional sites of metastasis may also occur in the adrenal glands and ovaries (Hellman and Harris, 2000; Singletary *et al.*, 2002a,b; Sainsbury, 2004).

1.3.4   Histological grading of breast cancer

Because of the histological diversity of breast cancer, histological grading is considered
as an essential component of the pathological assessment of breast cancer. The grading
system measures the degree of differentiation of a carcinoma by microscopic
examination, and based on a combination of scores for glandular (tubule) formation,
nuclear grade; nuclear size/ degree of pleomorphism, and on mitotic rate (Table: 1.4;
Elston and Ellis, 1991; Schnitt and Guidi, 2000).

1.3.5   Staging of breast cancer

The prognosis of breast cancer is related to the stage of the disease at presentation
(Sainsbury *et al.*, 2000). The staging system for breast carcinoma applies to both *in situ*
and invasive (including microinvasive) carcinoma (Singletary *et al.*, 2002a,b). The
clinical staging that depends on the size of the primary tumour (T) along with regional
lymph nodes status (N) and the presence or absence of distant metastases (M) is a clinical
assessment and hence may be inaccurate. Therefore, a separate pathological classification
has been added which allows tumour size and lymph nodes status, as assessed by
pathologist, to be taken into account. Patients with certain additional features, such as
peau d'orange, skin involvement by tumour or inflammatory cancer (i.e., advanced local
breast cancer), have a worse prognosis (Tables: 1.5 & 1.6; Sainsbury *et al.*, 2000;
Bundred, 2001; Singletary *et al.*, 2002a,b).

Table 1.4: Histological grading system of invasive breast cancer; Elston and Ellis modification of Bloom and Richardson grading system (from Elston and Ellis, 1991; Schnitt and Guidi, 2000).

Components of grading	Score
- Tubules	
> 75% of tumour composed of tubules	1 point
10-75% of tumour composed of tubules	2 points
< 10% of tumour composed of tubules	3 points
- Nuclear grade	
Nuclei small and uniform	1 point
Moderate variation in nuclear size and shape	2 points
Marked nuclear pleomorphism	3 points
- Mitotic rate	
Dependent on microscopic field area	1-3 points
Histological grade	**Total points**
1 (Well differentiated)	3-5
2 (Moderately differentiated)	6-7
3 (Poorly differentiated)	8-9

Table 1.5: TNM Staging system for breast cancer (from Singletary _et al._, 2002a,b).

Primary tumor (T):

TX	Primary tumor cannot be assessed.
T0	No evidence of primary tumor.
Tis	Carcinoma in situ:
Tis (DCIS)	Ductal carcinoma in situ.
Tis (LCIS)	Lobular carcinoma in situ.
Tis (Paget)	Paget's disease of the nipple with no tumor. Note: Paget's disease associated with a tumor is classified according to the size of the tumor.
T1	Tumor ≤ 2 cm in greatest dimension:
T1mic	Microinvasion ≤ 0.1 cm in greatest dimension.
T1a	Tumor > 0.1 cm but not > 0.5 cm in greatest dimension.
T1b	Tumor > 0.5 cm but not > 1 cm in greatest dimension.
T1c	Tumor > 1 cm but not > 2 cm in greatest dimension.
T2	Tumor > 2 cm but not > 5 cm in greatest dimension.
T3	Tumor > 5 cm in greatest dimension
T4	Tumor of any size with direct extension to (a) chest wall or (b) skin, only as described below:
T4a	Extension to chest wall, not including pectoralis muscle.
T4b	Oedema (including peau d'orange) or ulceration of the skin of the breast, or satellite skin nodules confined to the same breast.
T4c	Both T4a and T4b.
T4d	Inflammatory carcinoma.

Regional lymph nodes (N):

NX	Regional lymph nodes cannot be assessed (eg, previously removed).
N0	No regional lymph node metastasis.
N1	Metastasis in movable ipsilateral axillary lymph node(s).
N2	Metastases in ipsilateral axillary lymph nodes fixed or matted, or in clinically apparent* ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node metastasis:
N2a	Metastasis in ipsilateral axillary lymph nodes fixed to one another (matted) or to other structures.
N2b	Metastasis only in clinically apparent* ipsilateral internal mammary nodes and in the absence of clinically evident axillary lymph node metastasis.
N3	Metastasis in ipsilateral infraclavicular lymph node(s), or in clinically apparent* ipsilateral internal mammary lymph node(s) and in the presence of clinically evident axillary lymph node metastasis; or metastasis in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement:
N3a	Metastasis in ipsilateral infraclavicular lymph node(s) and axillary lymph node(s).
N3b	Metastasis in ipsilateral internal mammary lymph node(s) and axillary lymph node(s).
N3c	Metastasis in ipsilateral supraclavicular lymph node(s).

Continuation of table 1.5: TNM Staging system for breast cancer (from Singletary *et al.*, 2002a,b).

Regional lymph nodes- Pathologic (pN)†:	
pNX	Regional lymph nodes cannot be assessed (eg, previously removed or not removed for pathologic study).
pN0	No regional lymph node metastasis histologically, no additional examination for isolated tumor cells ‡:
pN0(i-)	No regional lymph node metastasis histologically, negative IHC.
pN0(i+)	No regional lymph node metastasis histologically, positive IHC, no IHC cluster > 0.2 mm.
pN0(mol-)	No regional lymph node metastasis histologically, negative molecular findings (RT-PCR).
pN0(mol+)	No regional lymph node metastasis histologically, positive molecular findings (RT-PCR).
pN1mi	Micrometastasis (> 0.2 mm, none > 2.0 mm).
pN1	Metastasis in one to three axillary lymph nodes and/or in internal mammary nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent §:
pN1a	Metastasis in one to three axillary lymph nodes.
pN1b	Metastasis in internal mammary nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent §.
pN1c	Metastasis in one to three axillary lymph nodes and in internal mammary lymph nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent §, II.
pN2	Metastasis in four to nine axillary lymph nodes, or in clinically apparent* internal mammary lymph nodes in the absence of axillary lymph node metastasis:
pN2a	Metastasis in four to nine axillary lymph nodes (at least one tumor deposit > 2.0 mm).
pN2b	Metastasis in clinically apparent* internal mammary lymph nodes in the absence of axillary lymph node metastasis.
pN3	Metastasis in 10 or more axillary lymph nodes, or in infraclavicular lymph nodes, or in clinically apparent* ipsilateral internal mammary lymph nodes in the presence of one or more positive axillary lymph nodes; or in more than three axillary lymph nodes with clinically negative microscopic metastasis in internal mammary lymph nodes; or in ipsilateral supraclavicular lymph nodes:
pN3a	Metastasis in 10 or more axillary lymph nodes (at least one tumor deposit > 2.0 mm), or metastasis to the infraclavicular lymph nodes.
pN3b	Metastasis in clinically apparent* ipsilateral internal mammary lymph nodes in the presence of one or more positive axillary lymph nodes; or in more than three axillary lymph nodes and in internal mammary lymph nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent §.
pN3c	Metastasis in ipsilateral supraclavicular lymph nodes.

Continuation of table 1.5: TNM Staging system for breast cancer (from Singletary *et al.*, 2002a,b).

Distant metastasis (M):	
MX	Distant metastasis cannot be assessed.
M0	No distant metastasis.
M1	Distant metastasis.

Abbreviations:

IHC, immunohistochemistry; RT-PCR, reverse transcriptase polymerase chain reaction.

* "Clinically apparent" is defined as detected by imaging studies (excluding lymphoscintigraphy) or by clinical examination.

† Classification is based on axillary lymph node dissection with or without sentinel lymph node dissection. Classification based solely on sentinel lymph node dissection without subsequent axillary lymph node dissection is designated (sn) for "sentinel node" (eg, pN0(i+)(sn)).

‡ Isolated tumor cells are defined as single tumor cells or small cell clusters not greater than 0.2 mm, usually detected only by immunohistochemical or molecular methods but which may be verified on hematoxylin and eosin stains. Isolated tumor cells do not usually show evidence of metastatic activity (eg, proliferation or stromal reaction).

§ "Not clinically apparent" is defined as not detected by imaging studies (excluding lymphoscintigraphy) or by clinical examination.

II If associated with more than three positive axillary lymph nodes, the internal mammary nodes are classified as pN3b to reflect increased tumor burden.

Table 1.6: TNM Stage grouping for breast cancer (from Singletary *et al.*, 2002a,b).

Stage Grouping	TNM classification		
0	Tis	N0	M0
I	T1*	N0	M0
IIA	T0	N1	M0
	T1*	N1	M0
	T2	N0	M0
IIB	T2	N1	M0
	T3	N0	M0
IIIA	T0	N2	M0
	T1*	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
IIIC	Any T	N3	M0
IV	Any T	Any N	M1

*T1 includes T1mic.

1.3.6 Prognostic and predictive factors in breast cancer

The clinical course of breast cancer may vary from indolent slowly progressive one to a course associated with rapid progression and metastatic spread. Therefore, assessment of certain prognostic and predictive 'markers' or 'factors' in the pathological examination of breast tumours, in order to predict disease outcome, is becoming increasingly important in understanding the natural history of breast cancer, planning treatment strategies and counselling patients (Henderson and Patek, 1998; Clark, 2000; Slooten *et al.*, 2001; Morabito *et al.*, 2003; Cianfrocca and Goldstein, 2004; Esteva and Hortobagyi, 2004).

A prognostic factor is defined as any measurement or feature of the patient or tumour available at the time of diagnosis or surgery that is associated with outcomes, such as disease-free or overall survival, in the absence and independent of systemic therapy. Whereas, a predictive factor is any measurement or feature in which its presence or absence signals resistance or sensitivity to a particular therapy independent of prognosis; it may or may not have prognostic value as well (Henderson and Patek, 1998; Clark, 2000; Bundred, 2001; Slooten *et al.*, 2001; Cianfrocca and Goldstein, 2004; Esteva and Hortobagyi, 2004).

Several established prognostic factors and a few predictive factors are used routinely in the clinical management of breast cancer. Through the increased understanding of breast cancer biology, numerous other novel markers have been identified in recent years. These are in general either chronological; indicators of how long the cancer has been present, or biological; indicators of the metastatic potential behaviour of a tumour (Bundred, 2001;

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Cianfrocca and Goldstein, 2004; Esteva and Hortobagyi, 2004), and include the following:

1.3.6.1   Age

Breast cancer tends to be more aggressive with poor prognosis in younger patients than in older. Recent studies have suggested that a young age of less than 35-40 years is associated with the worst prognostic pattern such as excess of high-grade tumour and axillary lymph-node metastasis, with the highest rates of necrosis, vascular invasion and proliferation, and lower ER expression levels, and subsequently, a poorer survival rate. This poor outcome improves as age increases and is best in patients over 75 years of age. In addition, patient age is important for predicting response to chemotherapy and hormonal therapy, as menopausal status is an age-dependent factor (Kroman et al., 2000; Zavagno et al., 2000a; Bundred, 2001; Sundquist et al., 2002; Morabito et al., 2003).

1.3.6.2   Tumour size

Tumour size is a time-dependent prognostic factor that correlates directly with survival. The best measure of tumour size is maximum pathological size assessment since radiological and clinical assessments may be inaccurate (Bundred, 2001; Pinder, 2001). Patients with small tumours have a better prognosis with a better long-term survival than those with larger tumours. The 20-year relapse-free survival rates for patients with tumours ≤ 10 mm in diameter have been reported to be around 88%; falling to 72% and 59% for lesions measuring 11-30 mm and 31-50 mm in diameter, respectively. Nevertheless, 15-20% of tumours of ≤ 10 mm in size have nodal metastasis, compared

with 40% for lesions of more than 15 mm (Carter *et al.*, 1989; Bundred, 2001; Pinder, 2001; Rampaul *et al.*, 2001a; Mirza *et al.*, 2002; Cianfrocca and Goldstein, 2004).

1.3.6.3 Histological type/ grade

The histopathological characteristics of the breast tumour have prognostic significance. Favourable prognosis of certain histological subtypes of invasive breast carcinoma, which based on architectural pattern of the tumour, is well established. Tubular, mucinous, papillary carcinomas, invasive cribriform, medullary, infiltrating lobular and tubulo-lobular types, together with rare tumours such as; adenoid cystic carcinoma, adenomyoepithelioma and low-grade adenosquamous carcinoma have all been reported to have a better prognosis than carcinoma of no special type (ductal NST) (Bundred, 2001; Rampaul *et al.*, 2001a; Cianfrocca and Goldstein, 2004).

The histological grading of breast cancer, as determined by the combination of glandular (tubule) formation, nuclear grade and mitotic rate (Table: 1.4), correlates strongly with prognosis. Patients with grade I breast cancer have the best survival rate with 85% 10-year survival, compare with less than 45% for those patients with grade III tumours. This histological grading potentially provides an overview of a number of molecular events that are reflected in histological morphology, which includes detail of cell morphology (nuclear pleomorhism), with a measurement of differentiation (tubule formation), and an assessment of proliferation (mitotic frequency) (Elston and Ellis, 1991; Pinder, 2001; Rampaul *et al.*, 2001a; Mirza *et al.*, 2002).

1.3.6.4 Axillary lymph node status

The presence or absence of axillary nodal metastases is the single most important prognostic factor for the disease-free and overall survival in breast cancer. It is a time-dependent factor and has a direct correlation with survival from breast cancer. Clinical assessment of nodal status is unreliable; as palpable nodes may be enlarged because of benign reactive changes or secondary to biopsy whilst nodes bearing tumour deposit may be impalpable. Thus, careful histological examination should be carried out for all excised axillary lymph nodes (Pinder, 2001; Rampaul et al., 2001a). A large number of studies with histologically confirmed lymph-node involvement have shown that on average 10-year survival is reduced from 75% for patients with no nodal involvement to 25-30% for those with metastatic disease in the locoregional nodes. Prognosis worsens the greater the number of lymph nodes involved and metastasis to the higher axillary lymph nodes level, particularly those at the apex, carries a worsened outcome (Carter et al., 1989; Bundred, 2001; Pinder, 2001; Rampaul et al., 2001a; Cianfrocca and Goldstein, 2004). Node positivity is reasonably taken as a marker of metastatic potential. However, this is only a qualitative, not a quantitative difference, as many patients with positive lymph nodes never develop distant metastases, while many with negative nodes do. Axillary lymph nodes status has no predictive value for response to therapy (Henderson and Patek, 1998; Morabito et al., 2003).

1.3.6.5 Lymphatic/ vascular invasion

Lymph-vascular invasion has been shown to be a significant prognostic factor in invasive breast cancer, particularly with respect to local recurrence and systemic relapse, and hence poorer overall survival (Pinder et al., 1994; Pinder, 2001; Rampaul et al., 2001a;

Mirza *et al.*, 2002; Cianfrocca and Goldstein, 2004). The presence of lymph-vascular invasion, also, correlates closely with loco-regional lymph-node involvement, tumour grade, and size (Pinder *et al.*, 1994).

1.3.6.6   Hormonal receptors

In breast cancer, the degree of oestrogen (ER) and progesterone (PR) hormone receptors expressions are considered prognostic and predictive factors (Henderson and Patek, 1998; Cianfrocca and Goldstein, 2004).

The oestrogen and progesterone receptors are steroid receptors located in the cell nucleus. Hormone is believed to diffuse into or be transported to the nucleus where a steroid-receptor complex is formed with receptor dimerization. Some of the genes regulated by steroid receptors are involved in cell-growth control, and currently, it is believed that these effects are the most relevant to oestrogen receptor, which influence the behaviour and treatment of breast cancer (Rampaul *et al.*, 2001a).

As prognostic factor, it has been reported that survival of women with ER-positive cancers is longer, and with node negative breast cancer, ER status was a significant predictor of longer survival (Bundred, 2001). Nevertheless, oestrogen receptors are of limited value in predicting long-term survival, and any survival advantage of ER-PR-positivity is lost after 8-10 years of follow-up (Pichon *et al.*, 1996; Leinster, 1998). This is due to its close relationship with histological grade (Rampaul *et al.*, 2001a), and largely because women tend to survive longer after first relapse, following a better response to hormone therapy (Bundred, 2001).

The value of these steroid receptors as a predictive factor for response to systemic endocrine therapy is well established. Approximately 30% of unselected patients with breast cancer will respond to endocrine therapy. However, with ER-positive tumours, a response in about 50-60% of patients is seen compared with less than 10% in patients with ER-negative tumours. Also, it has been suggested that the prediction of hormonal manipulation can be further refined by combining ER and PR assay, and that ER-PR-positive tumours carry a 78% response rates compared to less than 10% in patient with ER-PR-negative tumours, whilst ER-positive/PR-negative tumours have an intermediate respond rate of about 40% (Bundred, 2001; Rampaul et al., 2001a).

1.3.6.7 Human epidermal growth factor receptor-2

The presence of human epidermal growth factor receptor-2 (HER-2) gene amplification and/or protein overexpression have been shown to have both prognostic and predictive value in human breast cancer (Henderson and Patek, 1998; Winston et al., 2004)

The HER-2 proto-oncogene (also known as c-erbB2 or neu) is located on chromosome 17q21 and encodes a 185-kD transmembrane glycoprotein receptor ($p185^{HER2}$) that shares intrinsic tyrosine kinase activity homologous to type I subfamily of the epidermal growth factor receptors (Ross et al., 2003; Cianfrocca and Goldstein, 2004; Esteva and Hortobagyi, 2004). Immunohistochemistry (IHC) analysis and Fluorescent in situ hybridisation (FISH) are the most widely assays used to detect and measure HER-2 protein expression and gene amplification, respectively (Winston et al., 2004).

HER-2 plays an important role in the pathogenesis of breast cancer and is associated with adverse prognostic significance, independent of all other prognostic variables. HER-2/neu

gene amplification and/or protein over-expression has been identified in 40 to 60% of ductal carcinoma *in situ* that associated with more aggressive forms, and in 20-30% of invasive breast cancers, with most studies linking this to worse prognosis, both in axillary node positive and negative breast cancer, and poorer disease free and overall survival (Henderson and Patek, 1998; Walker, 2000; Bundred, 2001; Ross *et al.*, 2003; Cianfrocca and Goldstein, 2004; Esteva and Hortobagyi, 2004; Winston *et al.*, 2004). Also, it has been associated consistently with high tumour grade, DNA aneuploidy, high cell proliferation, negative oestrogen and progesterone receptors, *p*53 mutation, topoisomerase IIα amplification, and alternations in a variety of other molecular biomarkers related to invasiveness and metastasis in breast cancer (Ross *et al.*, 2003; Winston *et al.*, 2004).

HER-2 status has attracted great interest as a potential predictor of endocrine and chemotherapeutic response, and more directly for the selective use of trastuzumab therapy; a humanized monoclonal antibody against HER-2 oncoprotein (Cianfrocca and Goldstein, 2004; Esteva and Hortobagyi, 2004; Winston *et al.*, 2004).

Previous trials have shown HER-2 tumours to be inversely related to ER status and to predict resistance to hormonal therapy, specifically to the common antiestrogen agent tamoxifen. For example, women with HER-2 positive cancer, who were treated with a single agent tamoxifen adjuvant therapy, had a worse outcome than untreated control women. However, in others studies HER-2 status failed to predict tamoxifen resistance in oestrogen receptor positive cases (Bundred, 2001; Rampaul *et al.*, 2001b; Ross *et al.*, 2003; Cianfrocca and Goldstein, 2004; Esteva and Hortobagyi, 2004).

In addition, HER-2- positive tumours appeared to be resistance to alkylator-based chemotherapy (cyclophosphamide/ methotrexate/ 5-fluouracil; CMF), but sensitive to anthracycline-containing chemotherapy regimens. Compared to adjuvant CMF regimens, the addition of doxorubicin to the chemotherapy regimens improved the clinical outcome of patients with HER-2 positive tumours sufficiently to render their prognosis similar to that of the more favourable prognosis HER-2 negative group. Patients with HER-2 positive tumours who did not receive doxorubicin had a significantly worse prognosis. (Walker, 2000; Bundred, 2001; Ross et al., 2003; Cianfrocca and Goldstein, 2004; Esteva and Hortobagyi, 2004). The predictive value of HER-2- positivity on response to taxane-based chemotherapy regimens has been controversial, with some studies suggesting increased response to paclitaxil rather than resistance (Ross et al., 2003; Cianfrocca and Goldstein, 2004).

The optimal use of HER-2 status, as a predictive factor, is for the selection of the humanized monoclonal anti-HER-2 antibody (trastuzumab or Herceptin®) as a direct target therapy against HER-2 positive tumours. Trastuzumab-based therapies either in combination with chemotherapy or as a single agent therapy, offered significant benefit and survival advantage, over chemotherapy alone, in advanced metastatic disease and relapsed-cases following conventional chemotherapy, respectively. In early-stage breast cancer, clinical trials are ongoing to determine its efficacy and safety in the adjuvant setting (Bundred, 2001; Ross et al., 2003; Cianfrocca and Goldstein, 2004; Esteva and Hortobagyi, 2004).

1.3.6.8   Tumour proliferation markers

Tumour proliferation plays an important role in the clinical behaviour of breast cancer, and many of prognostic factors are directly or indirectly related to proliferation, such as cell-cycle regulators and growth factors or angiogenesis, respectively (Diest *et al.*, 2004).

Cellular proliferation takes place through a defined process, in which several phases can be recognised. From the resting (G0) phase, and after appropriate stimuli, cells join the active cycling population that enter the first gap (G1) phase. Both phases have a highly variable duration. In G1, the cell prepares for the synthesis (S) phase, in which DNA synthesis and doubling of the genome take place. The S phase is then followed by a period of apparent inactivity, the second gap (G2) phase, in which the cell prepares for further separation of chromatids during the mitotic (M) phase. After the M phase, each daughter cell may enter G0 phase or move on to the G1 phase to repeat the cell cycle. The inter-phase, including G1, S, and G2 phases, forms the largest part of the cell cycle, but cells in these phases cannot be morphologically recognised. However, cells in the mitotic phase can easily be identified because of the typical appearance of the chromosome sets during the different sub-phase of the M phase (Diest *et al.*, 2004).

In invasive breast cancer, the prognostic values of various proliferation assays, including the S-phase fraction (SPF), thymidine labelling index (TLI), mitotic rate and Ki-67/ MIB1 index, have been shown to be associated with prognosis in the majority of studies. However, technical difficulties and lack of standardization in measurements, have limited their clinical usefulness. Nevertheless, mitosis counting and the Ki-67/MIB-1 proliferation index are considered to be the most practical methods to assess proliferation (Bundred, 2001; Cianfrocca and Goldstein, 2004; Diest *et al.*, 2004).

Mitosis counting, the oldest form of assessing proliferation, provides the most reproducible and independent prognostic value. It is the most well established component of the histological grading system in breast cancer (Morabito *et al.*, 2003; Diest *et al.*, 2004). Patients with tumour of increased mitotic index had a significant poor prognosis for overall and disease-free survival (Mirza *et al.*, 2002; Diest *et al.*, 2004).

Ki-67 protein expression is strictly correlated to cell proliferation and to the active phases of the cell cycle (G1, S, G2 and mitosis), but absent from the resting cells (G0), which makes it an excellent marker for assessing the growth fraction of a given tumour cell population, and in which the Ki-67/ MIB1 monoclonal antibodies have been widely used for its immunohistochemical staining (Querzoli *et al.*, 1996; Scholzen and Gerdes, 2000; Bundred, 2001; Mirza *et al.*, 2002; Morabito *et al.*, 2003).

The Ki-67 labelling index (Ki-67- positive tumours cells) has been shown to have strong correlations with other biological and histopathological markers of invasive breast cancer, and also, with the clinical course and outcome of the disease (Querzoli *et al.*, 1996; Scholzen and Gerdes, 2000; Bundred, 2001).

The Ki-67 proliferation indices have been found to correlate directly with histological grade, tumour size, axillary lymph node status, vascular invasion, p53 and HER-2 overexpression, DNA and Thymidine labelling index score; and inversely with oestrogen and progesterone receptor status (Elston and Ellis, 1991; Querzoli *et al.*, 1996; Morabito *et al.*, 2003.). Tumours with high Ki-67 proliferation index (>20%) were associated with a higher probability of disease-relapse and death, when compared with tumours of low proliferation rates (Veronese *et al.*, 1993). Similarly, in patients with early-stage pT1

tumour or node negative breast cancer, increased Ki-67 was a significant prognostic factor for overall survival and/or disease-free survival (Querzoli *et al.*, 1996; Mirza *et al.*, 2002). Therefore, Ki-67 labelling index is a promising prognostic marker of cell-cycle proliferation in breast cancer.

1.3.6.9 Other markers

A number of other potential prognostic and predictive features, related to different tumour characteristics or indicative of the biological processes of cancer cells such as cell-cycle regulators (p53, c-myc, cyclins), proteases (urokinase, cathepsin D), and metastasis proteins (laminin 67 kDa receptor, nm23), have shown promising results in breast cancer, yet their role in patient management is at present not entirely clear (Bundred, 2001).

1.3.7 The Nottingham Prognostic Index

The value of prognostic factors lies in a better quantifying of risk of recurrence and in defining low-risk patients, for whom adjuvant therapy is not indicated, and high-risk groups, who would most benefit from treatment. Currently, the Nottingham Prognostic Index (NPI; Table 1.7); based on tumour size, histological grade, and lymph node status, is the most practical integral measure available to assess individual patient's prognosis, and thereby, stratify appropriate adjuvant therapy for patients with invasive primary operable breast cancer. In several independent prospective studies, the Nottingham Prognostic Index has been found to give valuable prognostic information, and due to its simplicity, it is suitable for routine clinical use (Haybittle *et al*, 1982; Todd *et al*., 1987; Galea *et al*., 1992; Brown *et al*., 1993; Fisher *et al*., 2001; Rampaul *et al*., 2001a).

Table 1.7: Nottingham Prognostic Index (NPI) (from Galea *et al*., 1992, Gendy and Rainsbury, 2001a).

Nottingham Prognostic Index (NPI):

NPI = (0.2 x tumour size /cm) + lymph node stage + tumour grade

Factor:

Involved nodes	Tumour grade	Score /factor
0	I	1
1-3	II	2
>3	III	3

NPI	Prognosis	Survival (15 years)
< 3.4	Good	80%
3.4-5.4	Moderate	42%
> 5.4	Poor	13%

1.4   Management of breast cancer

Breast cancer management is a rapidly evolving field. Optimal management with improved outcomes of patients with breast cancer have been achieved through a co-ordinated multimodality approach that require inputs from surgeons, radiologists, pathologists, oncologists, radiotherapist and psychologists with special interest in breast diseases, as well as, general practitioners, breast-care nurses and the women herself. Several studies have shown a better outcome with such multidisciplinary specialist treatment, which may relate to sufficient workloads and more frequent use of appropriate systemic therapy (Sainsbury *et al.*, 1995; Gillis and Hole, 1996; Blichert-toft *et al.*, 1997; Golledge *et al.*, 2000).

The purpose of the multidisciplinary approach is to design appropriate individualized treatment plans for all patients who require coordinated multi-specialty care. It lies in having a clear appreciation of the objectives of treatment and knowing its limitations and unwanted effects, in the context of individual patient. The objectives that need to be considered include cure, local control, survival, and cosmetic, social and psychological consequences of the diagnosis and treatment of breast cancer (Webster, 2001).

1.4.1 Clinical presentation and assessment of breast cancer

In the UK, about 5 % of patients with breast cancers will present with either locally advanced disease or with symptoms of metastatic disease, but in developing World this figure is near 20% (Sainsbury, 2004). The widespread use of screening mammography, which started as UK NHS Breast Screening Programme (NHSBSP) in the late 1980s, has enable the diagnosis of small impalpable breast cancer and ductal carcinoma *in situ* (Pinder, 2001).

The preoperative diagnosis of breast cancer can be established using the 'triple assessment', which is a combination of clinical assessment that include both history and physical examination, imaging with mammography and/or ultrasonography, and cyto-histological conformation using fine-needle aspiration cytology (FNAC) and/or core biopsy. The diagnostic accuracy, when all three methods agree, exceeds 99%. But when doubt exists after the triple assessment, then an open biopsy is indicated (Gendy and Rainsbury, 2001b).

A comprehensive history and review of systems are required to reveal the extent and characters of the breast lesions (such as hard painless palpable lumps, nipple discharge, retraction and rash) and help identifying patients with advanced and metastatic diseases (such as axillary mass, peau d'orange, ulceration or fungating breast mass, symptoms of metastasis, such as skeletal pain), as well as, to reveal aetiological and risk factors (such as previous history of breast disease and management, family history of breast and ovarian cancer) and current co-morbidities and medications that may influence treatment decisions (Chianakwalam and Bates, 2001; Gendy and Rainsbury, 2001b).

Breast cancers develop most frequently in the upper outer quadrants, but any portion of the breast, including the axillary tail, may be involved (Sainsbury, 2004). Physical signs suggestive of malignancy include firm breast mass with poorly defined margins, skin nodules, tethering, distortion and ulceration, nipple retraction, fixation to underlying structures, and palpable axillary or supraclavicular nodes. Any other symptomatic regions should be examined as required to identify or exclude metastatic spread. In a specialist breast clinic, clinical examination has an overall sensitivity for cancers of over 80%, but this value tends to be much lower in premenopausal women with nodular breast (Chianakwalam and Bates, 2001; Gendy and Rainsbury, 2001b).

Mammographic examination is one of the most effective methods for the detection of breast cancer, with a sensitivity of about 90% and a specificity of about 87%. Standard mammography comprises a craniocaudal and mediolateral oblique views of the breast, which may be augmented by coned compression and magnification views in certain cases. Suggestive mammographic appearance of malignancy includes; a speculated mass, isolated density, asymmetry, architectural distortion, stellate lesions and calcification (Blamey *et al.*, 2000; Chianakwalam and Bates, 2001; Gendy and Rainsbury, 2001b). Despite the recent controversy on the value of mammographic screening, as a result of suboptimal randomisation or inadequate methodology in some previous trials, regular screening has been shown to reduce breast cancer mortality, for example, by approximately 27% in the "UK Trial of Early Detection of Breast Cancer", with the greatest reduction of about 33% observed in women aged 60-69 years (Alexander *et al.*, 1999; UK Trial of Early Detection of Breast Cancer group, 1999; Gotzsche and Olsen, 2000; Nystrom *et al.*, 2002; Tabar *et al.*, 2003; Freedman *et al.*, 2004). Screen-detected cancers are significantly smaller, more likely to be non- invasive (*in situ*) tumours, and

any invasive cancers are more likely to be of better histological grade and type, and are less likely to be node positive than symptomatic cancers (Nisbet and Borthwick-Clarke, 1999; Blamey *et al.*, 2000; Sainsbury, 2001). Mammography is required before planning surgery to identify extensive tumours or any additional cancers in either breast. Also, it is used for localization of impalpable lesion for FNAC, core biopsy or surgical excision, in addition, as surveillance of breast-cancer survivors post-treatment, and for other high-risk patients (Gendy and Rainsbury, 2001b).

The use of ultrasound as an adjunct to mammography in the diagnosis of breast cancer is well established. It may be the only modality to suggest malignancy in about 3% of patients with palpable breast lumps when mammography and FNAC are normal. It has a sensitivity of about 75% and a specificity of about 90% for the diagnosis of malignancy. On ultrasound, malignant tumours are usually of mixed echogenicity with irregular outline and cast distal hypoechoic shadows. Ultrasonograhy is a more valuable diagnostic tool in young women of less than 35 years of age, where mammography is less sensitive. It can be used for localizing impalpable breast lesions, for accurate measurement of breast cancer before selecting appropriate surgical procedure or for monitoring tumour response to primary systemic therapy (Chianakwalam and Bates, 2001; Gendy and Rainsbury, 2001b).

A preoperative tissue-diagnosis can be obtained in the majority of patients using alternative biopsy techniques that include fine-needle aspiration cytology and/ or core-needle biopsy, rather than excisional biopsy. Fine-needle aspiration cytology and core biopsy are both fast and simple techniques, requiring only local anaesthesia and leave minimal scarring compared with excisional biopsy. These techniques can accurately

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diagnose the breast lesions in more than 90% of patients with palpable breast tumours (Singletary, 2001).

Routine preoperative investigations include a full blood count, liver function test, with alkaline phosphatase and calcium levels, in addition to, chest radiography. Extensive imaging and other special testing such as liver ultrasonography and isotope bone scanning are not required in a clinically negative history and physical examination, which are sufficient to designate a case as M0. However, appropriate specific tests are indicated for patients with symptoms suggestive of metastatic disease or as a follow-up to the first round of tests if they are abnormal (Gendy and Rainsbury, 2001b; Webster, 2001; Singletary et al., 2002a,b).

At present, there are no reliable serum tumour markers for breast cancer. CA 15-3 shows some promise in a few specific circumstances, but it is of limited value in clinical practice. It is raised in 70-80% of metastatic breast cancer, but only in 30% of early disease that confined to the breast. However, CA 15-3 is occasionally of value in assessing response and monitoring treatment of patients with metastatic disease and in lesions that are otherwise not evaluable. CA27-29 and carcinoembryonic antigen (CEA) may also be used to monitor response to treatment and detect relapse in breast cancer (Petersen et al., 2001; Webster, 2001; Emens and Davidson, 2003).

1.4.2   Surgical treatment

In patients with early-stage breast cancer, surgery remains the first treatment modality to achieve local control, and in locally advanced breast cancer with fungating tumour, 'toilet mastectomy' may be required to control symptoms such as bleeding, discomfort, and pain.

In the elderly, breast cancer has been considered to be a more indolent disease compared to younger women. However, the omission of primary surgery in those elderly patients with operable breast cancer who were fit for the procedure has been shown to result in earlier subsequent therapeutic intervention and increased mortality (Bates *et al.*, 2001).

The surgical treatment of breast cancer has evolved into two methods; either mastectomy or breast conservation, with or without reconstruction. Both should be combined with an axillary procedure.

1.4.2.1   Mastectomy

Mastectomy, removing all breast tissue, is considered to be the standard surgical treatment to which other treatments need to be compared. It is indicated for large tumours in relation to the breast size, multifocal primary tumours, central tumours beneath or involving the nipple in a small breast, following local recurrence in patients with previous breast conservation surgery, for lack of patient's commitment to undergo radiotherapy after breast conservation, or for patient preference. Other clinical and pathological factors that may influence the selection of mastectomy over wide local excision, due to their impact on loco-regional recurrence following breast conservation surgery, include

incomplete initial excision, presence of extensive *in situ* component or lymph-vascular invasion, poor histological grade and younger age (Table: 1.8; Sainsbury *et al.*, 2000).

The radical Halsted mastectomy, that excises the breast, axillary lymph nodes, and both pectoralis major and pectoralis minor muscles, is no longer indicated as it causes excessive morbidity with no survival advantages. Modified radical ('Patey') mastectomy is the procedure more commonly performed. It dissects the whole breast and associated structures including a large portion of skin, the centre of which overlies the tumours and also the nipple, and all axillary fat, fascia and lymph nodes '*en bloc*', with the pectoralis minor muscle either divided or retracted to access the axilla. Simple mastectomy involves only the removal of the breast tissue, with no dissection of the axilla, except for the region of axillary tail that usually has few lymph nodes of the low anterior group attached to it. Simple mastectomy is often followed by radiotherapy to axilla, because no pathological staging of the axillary lymph nodes is performed with this procedure (Sainsbury, 2004).

Following mastectomy, common immediate complications include haematoma and seroma formation, infection and flap necrosis. Factors associated with increased long-term risk of loco-regional recurrence in the skin flap include large tumour > 4cm in pathological examination, grade III carcinoma, axillary lymph nodes involvement and lymphatic-vascular invasion (Sainsbury *et al.*, 2000; Sainsbury, 2004).

1.4.2.2 Breast conservation surgery

Breast conserving surgery with breast radiotherapy is now the preferred method of local treatment for most women with early-stage breast cancer and, to some extent, for those patients with larger tumours that have been downstaged through neoadjuvant therapy.

Many randomised controlled studies have shown no significant differences in disease-free survival or overall survival after breast conservation surgery combined with radiotherapy compared to mastectomy, when both included axillary dissection (Blichert-Toft, 1992; Calais et al., 1994; Fisher et al., 1995; Van Dongen et al., 2000; Veronesi et al., 2002; Poggi et al., 2003; Kroman et al., 2004).

Breast conserving surgery is suitable for clinically and mammographically unifocal tumours of $\leq$ 4cm in diameter, or of > 4cm in large breast, and with no sign of local advancement (i.e., T_1, T_2 < 4cm) or extensive nodal involvement (i.e., N_0, N_1) or metastases. There is no age limit for breast conservation therapy. However, patients who have had prior radiotherapy that have included the breasts in its field are not suitable for breast conservation, as well as, those with collagen vascular diseases who could be negatively affected by combined radiotherapy (Sainsbury et al., 2000; Singletary, 2001).

Breast conservation surgery can be achieved with wide local excision, which consist of excision of the tumour with about 1 cm of the surrounding normal tissue and skin if necessary, or quadrantectomy, which is a more extensive resection of a whole quadrant of the breast. The aim is to excise all tumour tissue, preferably in one piece, with adequate margins, while maintaining acceptable cosmetic results, and to confirm histologically that the margins are free of tumour. Otherwise, a higher incidence of local recurrence is anticipated. Any margin < 5mm should be regarded with suspicion and any clearance < 1mm should be regarded as incomplete. Further local re-excision, if feasible, or mastectomy is indicated for margins with incomplete excision. Other risk factors for loco-regional recurrence of breast cancer following breast conserving surgery are shown in Table 1.8 (Sainsbury et al., 2000; Singletary, 2001; Webster, 2001).

Table 1.8: Risk factors for local recurrence of breast cancer after breast conservation (from Sainsbury *et al.*, 2000).

Factor	Relative risk
- Involved resection margins	x 3-4
- Extensive *in situ* component	x 3
- Patient's age < 35(versus age > 50)	x 3
- Lymphatic or vascular invasion	x 2
- Histological grade II or III (versus grade I)	x 1.5

1.4.2.3   Breast reconstruction

Breast reconstruction after breast cancer surgery is a safe and acceptable procedure. It aims to restore the normal shape and, to some extent, consistency of the breast after excisional surgery (quadrantectomy or large wide local excision) or after mastectomy. Evidences on psychological and cosmetic benefits and on cost advantages are well documented, particularly, for immediate reconstruction. Moreover, breast reconstruction does not appear to interfere with subsequent oncological adjuvant therapy or detection of loco-regional recurrence and, also, does not compromise survival (Johnson *et al.*, 1989; Sandelin *et al.*, 1998; Malata *et al.*, 2000; Sainsbury, 2001; Singletary, 2001; Grotting *et al.*, 2003; Langstein *et al.*, 2003).

Breast reconstruction can be performed either as an immediate procedure at the time of initial breast surgery or as a delayed procedure. It can be achieved using either implant-based reconstruction (with silicone-gel or saline-filled implant) or autologous tissue

reconstruction (such as the latissimus dorsi or transverse rectus abdominis myocutaneous flaps; either with 'pedicle' or 'free' flap), or a combination of both techniques. In addition, skin-sparing mastectomy, which preserves the original breast skin envelope and natural peripheral landmarks including the inframammary fold, may be considered for women with normal-size breasts and minimal ptosis that have small primary tumours situated deep in breast parenchyma without skin involvement. It can provide superior cosmetic result, with reduced breast scarring and preserved breast sensation, without compromising oncological safety and outcome. The nipple-areolar complex can be created, under local anaesthesia, using a small skin flaps that have been raised from the surrounding breast mound, and aided with medical-grade pigments tattooing. Implant-based breast reconstructions, however, are not recommended for women who have received or will receive radiotherapy. Many women may also need surgery to the contra-lateral breast such as reduction mammoplasty, mastopexy or augmentation to achieve symmetry (Malata *et al.*, 2000; Sainsbury, 2001; Singletary, 2001; Grotting *et al.*, 2003).

External prosthesis could be worn or attached to the chest wall under bra. Artificial nipple-areolar complex may, also, be created using colour-matched silastic prosthesis. However, they may feel unnatural and may associate with patient unsatisfaction (Sainsbury, 2001).

1.4.2.4 Axillary management

In breast cancer, axillary node status is crucial for staging, prognosis, and frequently directs the use of adjuvant systemic therapy in the management of early disease. It ranges from sampling; which should probably have four or more lymph nodes, to a level III clearance; which should yield an average of 20 or more lymph nodes. It aims for the

histological assessment of nodal metastasis, and the additional clearance allows regional control to minimize the risk of axillary recurrence, reduces the incidence of long-term complications, when compared with axillary node sampling and radiotherapy, in patients with primary breast cancer and a possible survival advantage (Kjaergaard *et al.*, 1985; Steele *et al.*, 1985; O'Dwyer, 1991; Cabanes *et al.*, 1992; Kutiyanawala *et al.*, 1998; Carpenter, 2001; Webster, 2001).

Axillary lymph node dissection remains the gold standard for both staging and prognosis. It is a major operation that requires general anaesthesia, and is either performed in continuity with a mastectomy or through a separate incision with a wide local excision. This procedure, however, has several sequelae and complications, including seroma, wound infection, pain, numbness or paraesthesia, reduced shoulder movements, chronic lymphoedema, in addition to, the emotional distress that associate with these short and long-term complications (O'Dwyer, 1991; Chetty *et al.*, 2000; Webster, 2001; Posther *et al.*, 2004). The acute complication rate can be as high as 20- 30%, and long-term morbidities such as chronic lymphoedema can reach 20% to 30%. Recurrence rates following axillary clearance are 1-3% (Boolbol and Borgen, 2001; Carpenter, 2001).

The high morbidity has been accepted as a necessary consequence of the need to obtain axillary node status and to achieve regional disease control. However, metastatic nodal disease is demonstrated in about 40%-46% of patients, but in tumours less than 1 cm in size, the incidence of axillary metastasis falls to 22%; the remaining patients receive no benefit from axillary dissection and are exposed to the morbidity associated with axillary dissection. Without any axillary treatment, only 21% of patients will develop axillary metastatic tumour in their lifetime (Carpenter, 2001; Humzah, 2001).

The improvement in early diagnosis has led to an increasing number of axillary dissection cases in which axillary nodes are found to be negative (Zavagno *et al.*, 2000b). It has been suggested that axillary lymph node dissection could be avoided in group of patients with clinically negative axillary node for whom the risk of nodal involvement is so low, including patients with pure tubular or mucinous carcinoma of ≤ 15mm, tumour of ≤ 5mm, or with negative lymphovascular invasion (Chua *et al.*, 2001). Nodal metastasis are seen in less than 1% of cases with pure tubular carcinoma < 1cm in diameter, and in about 3% of patients with microinvasive carcinoma (Singletary, 2001). Therefore, a selective policy for the management of the axilla in patients with operable breast cancer has also been found to be associated with no increase in axillary recurrence or mortality rate compared with routine axillary clearance. Patients who are node negative after axillary sampling can avoid axillary clearance or radiotherapy (Chetty *et al.*, 2000).

Sentinel lymph node biopsy, a major development in the field of surgical oncology, has rapidly emerged within the last decade as an alternative staging method to axillary lymph node dissection to detect occult lymph-node metastases in patients with clinically node-negative invasive breast cancer (Boolbol and Borgen, 2001; Humzah, 2001; Kelley *et al.*, 2004). It is based on the observation that specific areas of the breast drain by way of efferent lymphatics to a specific lymph node (sentinel lymph node), and then to other lymph nodes in the basin. As the sentinel node is the first node to receive the lymphatic flow from the primary tumour, it is assumed that if the sentinel node is correctly identified and is free of neoplastic cells, then the other axillary nodes are also negative. It is a minimally invasive technique that avoids the morbidity associated with formal axillary clearance, by excluding negative axillae from unnecessary dissection (Zavagno *et al.*, 2000b; Carpenter, 2001; Humzah, 2001; Singletary, 2001; Kelley *et al.*, 2004).

The sentinel lymph node can be located by injecting a tracer material, such as blue dye or radiolabelled particles, deep into or around the primary tumour (peritumoural), or superficial into the skin over the tumour site (subdermal, intradermal) or into the subareolar tissue. The blue dye allows the direct visualization of the lymphatic channels that lead to the marked lymph node in the axilla, whereas, the radioactive tracer facilitates the detection of the radioactive nodes prior to the surgical exposure of tissues by a hand-held gamma probe (Singletary, 2001; Krag and Harlow, 2003; Kelley *et al.*, 2004; Noguchi, 2004). The identification rates have been demonstrated to be superior with a combination of both techniques, rather than either method alone (Carpenter, 2001; Kelley *et al.*, 2004; Posther *et al.*, 2004). In addition, preoperative lymphoscintigraphy may be performed to visualize the sentinel lymph node before surgery. However, its value is still a matter of debate (Singletary, 2001; Kelley *et al.*, 2004; Noguchi, 2004).

In North America and Europe, the sentinel lymph node biopsy has become a commonplace in the management of patients with clinically node-negative breast cancer, and published data on its efficacy are promising (Flett *et al.*, 1998; Zavagno *et al.*, 2000b; Humzah, 2001; Kelley *et al.*, 2004; Posther *et al.*, 2004). In a recent meta-analysis using variety of techniques, and where sentinel node biopsies were followed by axillary dissections, 84% of sentinel nodes were identified with a false negative rate of 5%, but other series reported 100% accuracy for tumours less than 1cm. The identification rates were also higher with injection around intact tumours (96%), with invasive cancer (95%), and in clinically negative axilla (96%; Carpenter, 2001).

The histological assessment of the sentinel nodes can be more detailed when compared with an axillary clearance specimen, allowing understaging such as with micrometastases

to be corrected (Zavagno *et al.*, 2000b). This is usually carried out using techniques of serial sectioning of the retrieved lymph-node specimen, with Haematoxylin-Eosin and immunohistochemical staining (Carpenter, 2001). Molecular detection technique, based on reverse transcriptase-polymerase chain reaction, has also been incorporated at some centres. However, the clinical significance of micrometastases identified by such techniques remains to be determined (Posther *et al.*, 2004).

The internal mammary sentinel node biopsy has also been found to be feasible without serious additional complications. It improves nodal staging by identifying higher-risk subgroups of patients with internal mammary nodal metastases, that occurs in approximately 20% of patients and which has been shown to have an adverse effect on survival. Such patients might benefit from altered adjuvant treatment regimens (Van der Ent *et al.*, 2001). However, the practicability of this procedure is still in the investigation stage (Kelley *et al.*, 2004; Noguchi, 2004).

Factors that could affect the identification of sentinel node and associate with high false-negative rates include large tumour, previous surgery, palpable lymph nodes, increasing patient age and the surgeon's sentinel lymph node biopsy experience. Other problems that need to be resolved are the potential adverse outcomes relating, for example, to regional recurrence and survival following its use (Carpenter, 2001; Singletary, 2001; Krag and Harlow, 2003; Kelley *et al.*, 2004; Posther *et al.*, 2004). When strict criteria are used, the sentinel node biopsy, without further axillary dissection after a negative histological investigation, is believed to be a safe accurate predictor of nodal status. Therefore, it may considered to be the standard of care for the management of patients with early-stage diseases (Carpenter, 2001; Roumen *et al.*, 2001; Smillie *et al.*, 2001; Posther *et al.*, 2004).

1.4.3 Radiotherapy in breast cancer

Radiation therapy is an essential part of the management of breast cancer. It is indicated as a part of the primary treatment if breast conserving surgery has been performed, and in selective cases following mastectomy. It may be given to the breast, axilla, chest wall, and supra- and infra-clavicular fossa and internal mammary node chain. In patients with advanced disease, local radiotherapy may also be used as a palliative therapy to relief symptoms such as painful bone metastases (Webster, 2001; Kurtz, 2002).

Breast conservation surgery, as an alternative to mastectomy, is only made possible by the use of adjuvant radiotherapy. Disease-free and overall survival after breast conserving surgery, combined with radiotherapy, was equivalent to that following mastectomy (Blichert-Toft, 1992; Fisher et al., 1995; Van Dongen et al., 2000; Veronesi et al., 2002; Poggi et al., 2003; Kroman et al., 2004). In addition, recent studies have demonstrated a significant benefit of adjuvant radiotherapy in reducing locoregional recurrence following conserving surgery, as well as, mastectomy by approximately one to two-thirds. Moreover, post-mastectomy locoregional radiotherapy in high-risk patients, combined with appropriate systemic therapy, significantly reduced breast cancer recurrence and mortality and improved overall survival. The observed 20% absolute reduction in locoregional relapse was associated with an absolute improvement of 5% in long-term breast cancer-specific survival. This was offset in the older trials by an increased inter-current mortality, caused by excess cardiovascular deaths. However, recent results with newer radiotherapy techniques have demonstrated a clear overall survival improvement, without excess cardiovascular morbidity (Fisher et al., 1995; Overgaard et al., 1997;

Ragaz *et al.*, 1997; Overgaard *et al.*, 1999; Early Breast Cancer Trialists, 2000; Julien *et al.*, 2000; Holli *et al.*, 2001; Fisher *et al.*, 2002a; Fisher *et al.*, 2002b; Kurtz, 2002).

In general, a dose of 40-50 Gy (in 25 2-Gy fractions over 5-6 weeks, starting within 8 weeks following surgery in patients not receiving chemotherapy, or after completion of chemotherapy) has been recommended as the standard adjuvant radiotherapy of large volumes including the whole breast after breast conservation, chest wall and nodal areas. Additional radiation boost of 10-20 Gy to smaller volumes may be required to the tumour bed site, either by fractionated external beam irradiation or by means of radioactive implants; using electrons or iridium wire implant, in cases with higher risk of loco-regional recurrence such as close resection margins (< 1cm), extensive *in situ* disease, lymph-vascular invasion, and high grade tumour (Sainsbury *et al.*, 2000; Henry and Ash, 2001; Kurtz, 2002).

After mastectomy, adjuvant radiotherapy to the chest wall and nodal groups is also recommended in women considered to be at higher risk of locoregional recurrence of ≥ 20% at 10 years, including node-positive patients with large tumours (> 5cm) or with chest wall, deep margin, or skin involvement, and all patients with four or more positive axillary lymph nodes. Whereas, patients with T1-2 tumours and 1-3 positive nodes are generally associated with < 15% risk of locoregional recurrence. Other risk factors, in addition, include lymph-vascular invasion, high histological grade, extension of cancer cells beyond lymph node capsule, or gross tumour multicentricity (Sainsbury *et al.*, 2000; Henry and Ash, 2001; Kurtz, 2002).

Radiotherapy to the axilla is avoided after axillary lymph node clearance, but may be used in patients with known residual disease after axillary sampling or in patients with large number of involved nodes and with gross extranodal disease. In these circumstances, adjuvant chemotherapy will also have an important role in preventing local recurrence, as well as, distant metastasis (Dawson and Taylor, 1996; Kurtz, 2002).

Radiation therapy is associated with both early and late complications. Immediate skin reactions normally resolve within a few weeks, but occasionally, severe skin reaction with subsequent cutaneous radionecrosis, skin telangiectasia, thickening of the skin of the breast or chest wall, and cosmetic deformity of the residual breast tissue may develop later. Other less common or rare complications include lymphoedema, brachial plexopathy, shoulder stiffness, osteoradionecrosis of the ribs, radiation pneumonitis with pulmonary fibrosis, increased cardiovascular deaths, due to cardiac and great vessels damage particularly in women with left-sided breast tumours, and secondary malignancy and sarcoma of the chest wall. Recent developments in radiation techniques, by using modern linear accelerators with reduced radiotherapy portals and doses, may minimize these side effects (Dawson and Taylor, 1996; Sainsbury *et al.*, 2000; Sainsbury, 2001; Webster, 2001; Kurtz, 2002).

1.4.4   Hormonal therapy in breast cancer

The aim of endocrine therapy is to decrease the hormonal growth stimulation of hormone-sensitive breast cancer cells. It is indicated as adjuvant therapy or as treatment for advanced systemic disease, and occasionally, as sole treatment in patients who are unfit or unwilling to have surgery (Webster, 2001; Stokes and Chan, 2003; Sweetland, 2004).

The current options for hormonal manipulation include anti-oestrogens such as tamoxifen, aromatase inhibitors, progestogens such as megestrol acetate, in addition to, suppression or ablation of ovarian function, medically by luteinizing hormone releasing hormone analogues, or surgically by laparoscopic or open surgery, or by radiation therapy (Webster, 2001; Stokes and Chan, 2003; Sweetland, 2004).

The selection of hormonal therapy depends on the patient's menopausal status which in turn determines the source of oestrogen production; in premenopausal women by the ovaries, and in postmenopausal women via the peripheral aromatase enzyme that is required for the peripheral conversion of adrenal androgens into oestrogens in the muscle, liver and fat tissue including the breast (Stokes and Chan, 2003; Pritchard, 2003).

Ovarian ablation in premenopausal women was associated with a positive effect on both disease-free and overall survival in patients with node-positive as well as node-negative tumours (Pritchard, 2003). The Early Breast Cancer Trialists' Collaboration Group (EBCTCG, 1992) showed a reduction in the annual rates of recurrence by 26% and death by 25% in patients less than 50-years of age, with further highly significant 15-years recurrence-free and overall survival improvements among those patients allocated to ovarian ablation   (EBCTCG, 1996). Medical castration with luteinizing hormone

releasing hormone analogues goserelin (Zoladex), as well, produced significant reduction in recurrence with a trend toward improvement in breast cancer survival and/ or overall survival, irrespective of the additional adjuvant tamoxifen therapy or chemotherapy (Pritchard, 2003). Ablation therapy precipitates the menopause, whereas, the reversible effect of luteinizing hormone releasing hormone analogues makes them suitable for women who want to remain fertile (Sweetland, 2004).

Tamoxifen, a non-steroidal anti-oestrogen, has been the gold standard for nearly 30 years. It is recommended for both premenopausal and postmenopausal women in the adjuvant and advanced metastatic treatment of hormone-receptor positive tumours (Stokes and Chan, 2003). It has a demonstrable effect on both recurrence-free and overall survival with an overall reduction in the death rate, which is higher in women over 50 years. In the Early Breast Cancer Trialists' Collaboration Group (EBCTCG), adjuvant tamoxifen reduced the annual rates of recurrence by 25% and death by 17% (EBCTCG, 1992). In the 1998 EBCTCG overview, the proportions of reductions in recurrence and mortality during 10 years of follow-up for the 5 years adjuvant tamoxifen were 47% and 26%, respectively. The greatest benefit is achieved in patients who are oestrogen positive with involved nodes. Patients who are oestrogen receptor negative obtain no similar benefit. The proportional reduction in the contralateral breast cancer was 47% for the 5 years adjuvant tamoxifen (EBCTCG, 1998a).

The aromatase inhibitors have recently become established in the management of postmenopausal women with locally advanced and metastatic breast cancer; as a first-line therapy with a superior antitumour activity compared to tamoxifen and as a second-line therapy in patients whose disease has progressed during tamoxifen therapy. The third-

generation aromatase inhibitors anastrozole, letrozole (reversible non-steroidal) and exemestane (irreversible steroidal) have been shown to have a favourable impact on survival with better safety profile compared to megestrol acetate therapy as a second-line therapy (Stokes and Chan, 2003; Buzdar, 2004; Campos; 2004; Clemons *et al.*, 2004).

In current practice, postmenopausal women with hormone-sensitive disease typically receive 5 years of anti-oestrogens adjuvant treatment in the form of tamoxifen. Alternatively, anastrozole is considered in patients whom they have a relative or absolute contraindication to the use of tamoxifen. Although more recent emerging evidence on anastrozole, as initial adjuvant endocrine treatment of postmenopausal women with ER positive breast cancer, has shown its superiority over standard tamoxifen therapy for 5 years in terms of disease-free survival, as well as, a better tolerability and safety profiles, the data so far regarding the overall survival benefit , however, has not yet been reported. Moreover, impressive reductions in risk of recurrence have been demonstrated in trials evaluating the switch from tamoxifen to aromatase inhibitors; either letrozole after 5 years or exemestane after 2-3 years of tamoxifen, respectively (Pritchard, 2003; Campos; 2004; Clemons *et al.*, 2004).

The third-generation aromatase inhibitors have shown lower incidences of hot flashes and endometrial side-effects such as vaginal discharge and bleeding or endometrial cancer, as well as, deep vein thrombosis, ischemic cerebro-vascular and thrombo-embolic events, with a better tolerability and fewer withdrawals from treatment compared to standard tamoxifen or megestrol acetate. However, musculo-skeletal side-effects, reduced bone density and fractures were more common in women treated with aromatase inhibitors (Pritchard, 2003; Buzdar, 2004; Campos; 2004; Clemons *et al.*, 2004).

1.4.5   Chemotherapy in breast cancer

Systemic chemotherapy is indicated as adjuvant therapy, as a treatment for advanced metastatic disease, and to some extent, it is considered in patients with large non-operable breast cancer as preoperative primary (neoadjuvant) therapy to improve operability with breast-conserving surgery (Webster, 2001).

Commonly used chemotherapeutic agents in breast cancer include alkylating agents (cyclophosphamide), antimetabolites (5-fluorouracil, methotrexate, capecitabine, gemcitabine), anthracycline cytotoxic antibiotics (doxorubicin, epirubicin), taxanes (paclitaxel and docetaxel), and vinca alkaloids (vinorelbine). Polychemotherapy is significantly better than single-agent chemotherapy. However, in endocrine-resistance metastatic disease the optimal schedule of chemotherapeutic agents as concurrent versus sequential remains controversial, and decision must be individualized (Aapro, 2001; Webster, 2001; Cardoso and Piccart, 2003; Goldhirsch *et al.*, 2003; Bernard-Marty *et al.*, 2004; Nowak *et al.*, 2004).

The aim of adjuvant chemotherapy is to increase the cure rate after the primary loco-regional surgery. It is most frequently used in premenopausal patients, in patients with aggressive disease and with hormonal-receptor negative tumours (Webster, 2001). Four to six courses (3-6 months) of adjuvant systemic polychemotherapy (>2 agents) provide the optimal benefits and substantially improve disease-free and overall survival, irrespective of lymph node, hormone receptor or menopausal status up to age of 70 years, thought the data are inconclusive for those over the age of 70 years, and in most tumour subsets, except for small node negative tumours of less than 1 cm in diameter (Aapro, 2001).

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In the Early Breast Cancer Trialists' Collaboration Group (EBCTCG), polychemotherapy reduced the annual rates of recurrence by 28% and death by 16%. The greatest benefits of chemotherapy were for women with node positive disease and for younger women. But in women aged 50-69, it also reduced the rates of recurrence and mortality when combined with tamoxifen, being better than chemotherapy alone or tamoxifen alone (EBCTCG, 1992). In the 1998 EBCTCG update, polychemotherapy with a combination of cyclophosphamide, methotrexate and 5-fluorouracil (CMF) or with an anthracycline-based regimen for 3 to 6 months produced significant proportional reductions in recurrence and mortality, both among women aged less than 50 years and those aged 50-69 years (35% and 20% reduction in recurrence, with 27% and 11% reduction in mortality for both age groups, respectively). The anthracycline-containing regimens, compared with CMF alone, produced somewhat greater effects on recurrence and mortality (72% vs 69% 5-year survival). Long-term treatment for more than six months, however, did not provide any additional benefit (EBCTCG, 1998b).

The reduction in recurrence with adjuvant polychemotherapy emerged chiefly during the first 5 years, whereas the difference in survival increased throughout the first 10 years; typically producing an absolute improvement in 10-years survival for young women (<50 years) with early breast cancer of about 7% in node-negative and 11% in node-positive disease, and to a lesser extent, for postmenopausal women of about 2% in node-negative and 3% in node-positive early breast cancer, regardless of the added use of tamoxifen. Moreover, after 10 years follow-up, the anthracycline-based chemotherapy was associated with a 4% absolute risk reduction for recurrence and death above that seen with CMF (11% and 16% relative improvements in relapse and death, respectively). However, in node-negative disease, the absolute advantage of anthracycline over CMF is smaller (1.7

at 5 years). This advantage of anthracycline-based chemotherapy has been found almost exclusively when three-drug regimen was used; either epirubicin-containing (CEF) or doxorubicin-containing (CAF) regimen (EBCTCG, 1998b; Cardoso and Piccart, 2003).

In the adjuvant setting, currently used regimens include anthracycline-based chemotherapy such as four courses of AC, which have been shown to be equivalent to six cycles of classical CMF. The CEF, CAF regimen and to some extent FEC have yielded superior result. The CMF regimen is used less frequently but still valid for lower risk patients. The taxanes (paclitaxel and docetaxel) are being incorporated into adjuvant regimens on the basis of their antitumour activity in advanced breast cancer and the absence of cross-resistance with doxorubicin (Shapiro and Recht, 2001). Recent results support the use of taxanes as adjuvant chemotherapy for women with early breast cancer and involved lymph nodes, independent of hormone-receptor status. The docetaxel-containing regimen (TAC) proved to be superior to FAC in randomized clinical trials, and others trials have shown improvement in disease-free survival with or without significant improvement in overall survival in taxanes-treated patients (Aapro, 2001; Cardoso and Piccart, 2003; Goldhirsch et al., 2003; Nowak et al., 2004).

Primary systemic therapy, especially chemotherapy, is considered the standard of care for non-operable locoregional advanced breast cancer, and provides additional opportunity for breast-conserving surgery in patient presenting with large unifocal breast cancer. It allows clinical downstaging of the primary tumour, provides in-vivo chemo-sensitivity assessment of the tumour response to a specific chemotherapy regime and offers unique advantages in understanding the tumour biology, and has been shown to improve survival

in patients with the best clinical response (Calais *et al.*, 1994; Kaufmann *et al.*, 2003; Garces and Cance, 2004).

Randomized controlled clinical trials in patients with operable breast cancer, which include stage T1-3, N0, M0 or T1-3, N1, M0, have shown that primary polychemotherapy; using CMF, AC, FAC, or FEC regimes, increases the proportion of patients who subsequently shown to have metastatic-free axillary lymph nodes or who become candidates for breast-conserving surgery. The reported absolute differences in the rate of successful breast-conservation surgery between patients who received primary chemotherapy compared to those who received adjuvant chemotherapy range from 5% to 36%. In addition, primary chemotherapy offers the same disease-free and overall survival benefits as adjuvant chemotherapy with the same drug combinations (Fisher *et al.*, 1997; Kaufmann *et al.*, 2003). Recent trials with neoadjuvant taxanes-containing regimens have been shown to produce superior clinical and pathological response rates, compared to non-taxane containing group (Garces and Cance, 2004; Nowak *et al.*, 2004). However, despite the potential advantages, preoperative chemotherapy has not been advocated as being preferable over adjuvant chemotherapy in patients with early breast cancer, because of a lack of significant survival advantage (Goldhirsch *et al.*, 2003).

In patients with metastatic breast disease, chemotherapy is indicated as a palliative measure to ensure prolonged disease control without compromising quality of life. Treatment should be tailored according to patient and tumour characteristics, including patient's performance status, tolerability, tumour burden and aggressiveness. The most active cytotoxic drugs include the anthracyclines and the taxanes, followed by alkylating agents, antimetabolites (capecitabine, gemcitabine), and vinca alkaloids. Unless

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symptoms or signs of life-threatening disease exist, the single used agents can produce objective response rates of 20%-80%. However, the rare complete responses are short lived and progression of disease is almost inevitable (Bernard-Marty et al., 2004).

Side-effects of chemotherapy are significant. Doxorubicin and cyclophosphamide are more likely to cause alopecia and vomiting, whereas cyclophosphamide, methotrexate, and fluorouracil are more likely to cause nausea, myelosuppression, and ovarian failure, with its subsequent menopausal symptoms including hot flashes, vaginal dryness, depression, sleep disturbance, increased risk of osteoporosis. Doxorubicin directly damages the myocardium and can cause cardiomyopathy. Both taxanes (paclitaxel and docetaxel) can cause hypersensitivity reactions, peripheral neuropathy, myalgia, arthralgia and fatigue. Peripheral neuropathy is seen, as well, with vinca alkaloids. The increased incidence of secondary leukaemia, commonly of acute myeloid leukaemia, and myelodysplasia have been documented in patients treated with adjuvant alkylating and anthracyclines-based agents, and are dependent on the administered dose (dose intensity and cumulative dose) and the duration of treatment (Aapro, 2001; Shapiro and Recht, 2001; Webster, 2001; Cardoso and Piccart, 2003; Nowak et al., 2004).

Most acute side-effects can be controlled or reduced by modern measures, including drugs like corticosteroids and ondansetron for nausea and vomiting and scalp cooling for alopecia, and resolve on completion of treatment (Sainsbury, 2001; Webster, 2001). Whereas, longer-term side-effects are to be carefully considered on individual patient basis. In the adjuvant setting, if both chemotherapy and hormonal therapy are recommended, the chemotherapy is given first to minimize the toxicity and improve the efficacy of each therapeutic modality (Pritchard, 2003).

1.4.6   Biological therapy in breast cancer

Trastuzumab (Herceptin®) is a humanized monoclonal antibodies with anti-tumour activity targeted against the epidermal growth factor family oncogene (Her-2/*neu*). It is a novel therapeutic option for patients with aggressive forms of advanced metastatic Her-2/*neu*-positive breast cancer (Ross *et al.*, 2003; Vogel and Franco, 2003).

It suppresses tumour growth when used as a single agent or in combination with other chemotherapeutic agents. Single-agent trastuzumab therapy produces objective benefits in 15-20% of patients with Her-2 positive tumours. Trastuzumab in combination with cytotoxic chemotherapy, either anthracycline plus cyclophosphamide or taxans, has been shown to improve overall survival, with a higher rate of objective response and a longer duration of response, as well as, to increase time to disease progression and time to treatment failure, in synergistic rather than an additive manner. Ongoing trials of trastuzumab in combination with various chemotherapy agents are encouraging and showing significant clinical activity over chemotherapeutic regimens alone (Leonard *et al.*, 2002; Ross *et al.*, 2003; Vogel and Franco, 2003).

Trastuzumab therapy is generally well tolerated. However, mild side-effects may include fever and chills; which are generally seen with first infusion dose, in addition to, diarrhoea, nausea, headache, rash and rhinitis. Other severe adverse events include cardiac toxicity, especially occurring when used in combination with anthracycline-based chemotherapy regimens. In the absence of pre-existing cardiac disorders or prior anthracycline therapy, intrinsic cardiotoxicity with single-agent trastuzumab appears to be rare (Leonard *et al.*, 2002; Ross *et al.*, 2003; Vogel and Franco, 2003).

1.4.7 Follow-up

Increased incidence of breast cancer together with improved management and reduced mortality have created a large population of breast-cancer survivors at risk of disease-relapse (local recurrence and/ or distant metastasis), or development of a second new primary breast tumours, in addition to, therapy-related complications, whom they require follow-up medical care and psychological support (Emens and Davidson, 2003; Hurria and Hudis, 2003).

Data suggest that screening for disease relapse is likely to be most useful in the first 5 years after primary therapy, with more frequent evaluation during the first 2 years when relapse rates are highest. After mastectomy, local recurrence is most common in the first 2-3 years and decreases with time, but after breast conservation, it occurs at a fixed rate each year. Patients with carcinoma of one breast are also at high risk of cancer in the contralateral breast; about 0.6% a year develop second primary tumour (Sainsbury et al., 2000). In addition, late recurrence, metastatic relapse and death from breast cancer are well documented, even 20 years or more after initial diagnosis and primary treatment (Louwman et al., 2001; Emens and Davidson, 2003).

Therefore, all patients should be followed on regular basis for at least 5 years. The recommended strategy includes; history and physical examination (every 3-6 months for 3 years, every 6-12 months for 2 years, and annually thereafter), monthly self-breast examination, annual mammography of the preserved and contralateral breast (Hurria and Hudis, 2003). Mammography may be difficult to interpret after breast conservation due to scarring, and in this situation, magnetic resonance imaging (MRI) is useful to differentiate

it from cancer recurrence (Sainsbury *et al.*, 2000). Patients on adjuvant tamoxifen, based on the recommended surveillance strategy, are also advised to have annual gynaecological examination, reserving specific test such as endometrial biopsy and/ or transvaginal ultrasound to those who developed abnormal vaginal bleeding or discharge (Emens and Davidson, 2003; Hurria and Hudis, 2003).

Although it is important to be aware of the potential short and long-term primary therapy-related complications of breast cancer including the psychological impact, the available data support a conservative approach with clinically directed follow-up, based on careful history and physical examination, in which further diagnostic-tests are to be guided by clinical information. Current data on early diagnosis of systemic-relapse, prior to the onset of clinical signs or symptoms, by aggressive or routine serological or radiological testing (with the exception of mammography) have failed to impact overall survival, and so should not be conducted routinely. Instead, such women should be educated about the signs and symptoms of breast-cancer recurrence, and encouraged to report any new persistence symptoms when they develop to their medical personnel (Emens and Davidson, 2003; Hurria and Hudis, 2003).

1.5 The systemic and local inflammatory response in breast cancer

Breast cancer is a heterogeneous disease with diverse biological and clinical manifestations, in which the clinical evolution is much less predictable. Established prognostic factors, which are used as a guide to decide appropriate adjuvant therapy, are mainly including tumour-associated characteristics such as the primary tumour size, histological grade, lymph-node status, hormone-receptor status and oncogene over-expression. Nevertheless, outcome remains highly variable even when these factors are taken into account.

It has long been thought that disease variability in cancer patients, including breast cancer, is not solely determined by the characteristics of the tumour but also by the host-response factors (Hart and Saini, 1992).

The tumour-host interaction is a complex relationship that has yet to be fully understood (Figure 1.5). The host-immune responses can be divided into innate or adaptive immunity that can act within the local/regional microenvironment or systemically (Figure 1.6). The cellular mediators of the innate immunity include macrophages, granulocytes, natural killer cells, and non-MHC-restricted and gamma/delta T lymphocytes. In addition, soluble factors including natural antibodies, complement components and C reactive protein play a role in the innate immunity. The adaptive immune response is mediated by the tumour antigen-specific T- lymphocytes that include cytotoxic effector CD8+ and MHC class I restricted cells, as well as, helper CD4+ T- lymphocytes of MHC class II molecules (Figure 1.7; Whiteside, 2003).

However, there is now increasing evidence that both the local inflammatory response, within the tumour microenvironment, and the systemic inflammatory response, as evidenced by increased circulating concentrations of acute phase proteins such as C-reactive protein, play pivotal roles in malignant disease development, progression and metastatic formation in a variety of common solid tumours (Figure 1.5 & 1.6; Witz, 2001; Coussens and Werb, 2002). The basis of the tumour-host response is not clear. However, it appears to be a driven response of the tumour behaving as "wound that does not heal" (Dvorak, 1986).

Local inflammatory response	Pro-inflammatory mediators	Systemic inflammatory response
Tumour inflammatory infiltrates such as: T-lymphocytes, macrophages	such as IL-6, IL-10	Circulating acute phase proteins such as: C-reactive protein, albumin.
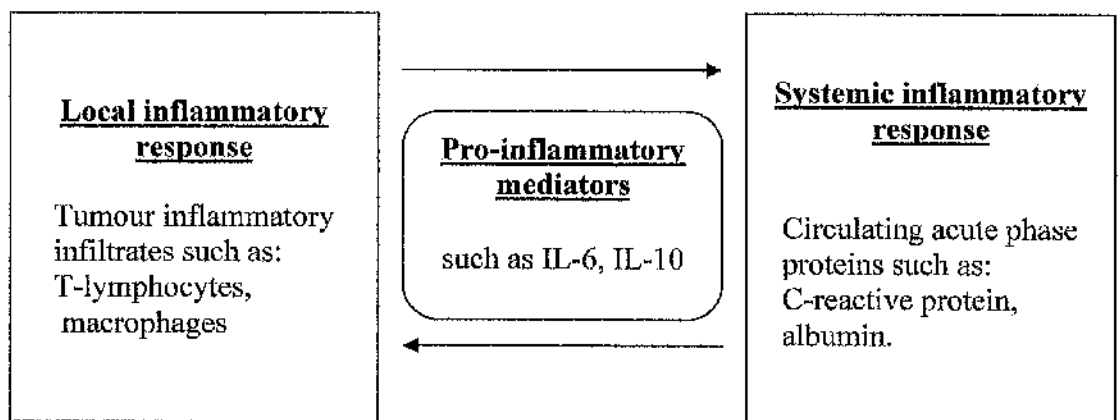
Figure 1.5: The tumour-host relationship.
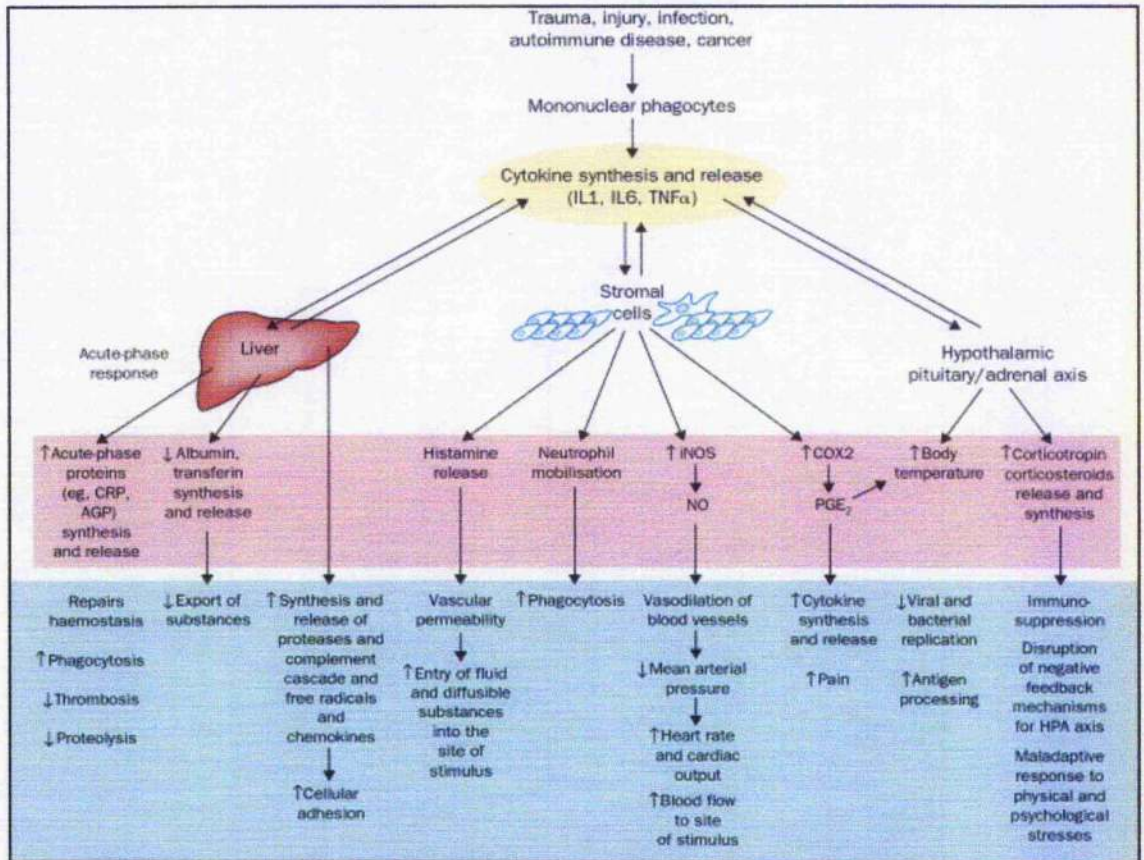
Figure 1.6: Components of the inflammatory response (from Slaviero *et al.*, 2003).
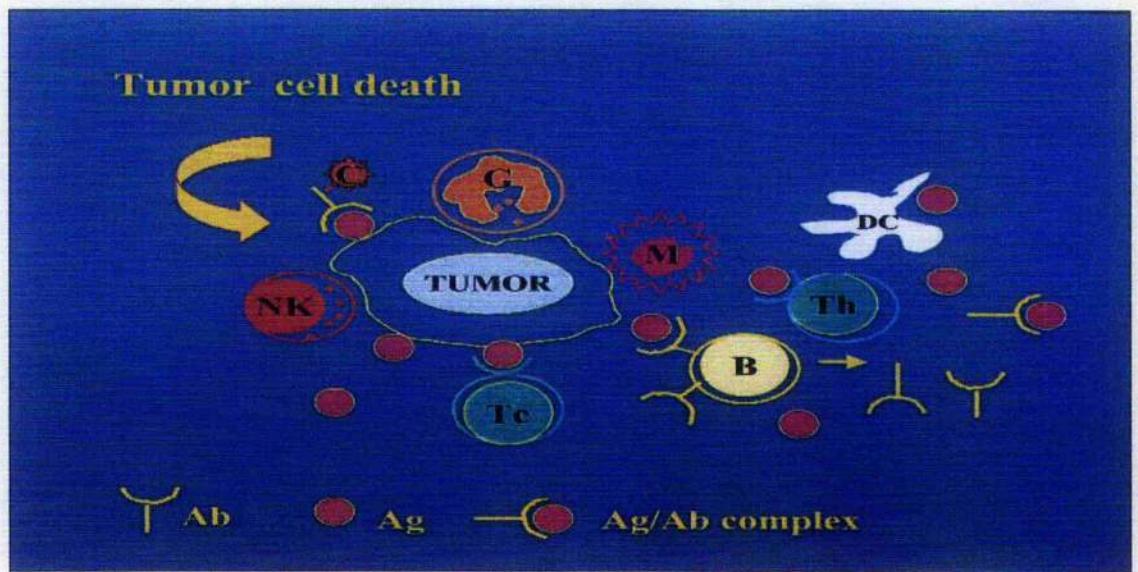
Figure 1.7: Immune responses to cancer cells (B: B-cells, C: complements, DC: dendritic cells, G: granulocytes, M: macrophages, NK: natural killer cells, Tc: T-cytotoxic lymphocytes, Th: T-helper lymphocytes; from Whiteside, 2003).

1.5.1 Systemic inflammatory response

The systemic inflammatory response (acute-phase response) is the wide-ranging patho-physiological disturbances in homoeostasis that result from tissue damage, infection, immunological disorders, or malignant disease. It involves a large number of systemic changes in the biosynthetic, metabolic and catabolic profiles of many organs, along with behavioural and nutritional changes, which is characterized clinically by the presence of fever, leucocytosis, anaemia, muscle wasting, anorexia and fatigue (Figure 1.6). The function of the acute-phase response is to restore the body's homoeostasis by removing the cause of the initial disturbance. However, in certain conditions of chronic or recurring inflammation and cancer, the response can persist and may contribute to many deleterious consequences (Gabay and Kushner, 1999; Slaviero et al., 2003).

A complex cascade of phagocyte-derived endogenous mediators, especially cytokines, induces the acute-phase reaction (Figure 1.8). These secreted pro-inflammatory cytokines, including interleukins 1 and 6 (IL-1 & IL-6) and tumour necrosis factor α (TNF-α), circulate from the initial site of disturbance to distant organs, such as the liver, resulting in the stimulation of expression of acute-phase proteins. Circulating concentrations of acute-phase proteins increase (positive acute-phase proteins), or decrease (negative acute-phase proteins), by at least 25% during inflammatory disorders (Figure 1.8; Gabay and Kushner, 1999; Slaviero et al., 2003).

Other gene products such as phospholipase-A_2, cyclooxygenase-2, and inducible nitric oxide synthase are upregulated during the cytokine cascade to generate small mediator molecules, including platelet activating factor, leukotrienes, prostanoids, and nitric oxide, that contribute to the inflammatory response (Figure 1.6; Slaviero et al., 2003).

The major pathway for the transcriptional activation of acute-phase proteins and other proteins, such as cyclooxygenase-2, by cytokines, involves Janus kinases (JAK) and signal transducers and activators of transcription (STATs, particularly STAT3). Another pathway involves the activation of mitogen-activated protein kinases, which in turn induce the expression of various nuclear factors including CCAAT enhancer binding proteins (C/EBP), such as Nuclear Factor-κB (Slaviero et al., 2003).

C-reactive protein and albumin are the classical (positive and negative; respectively) acute-phase proteins in humans (Figure 1.8). These measurements may be a useful indicators in predicting the net effect of various local and systemic inflammatory response mediators, including cytokines (Gabay and Kushner, 1999).
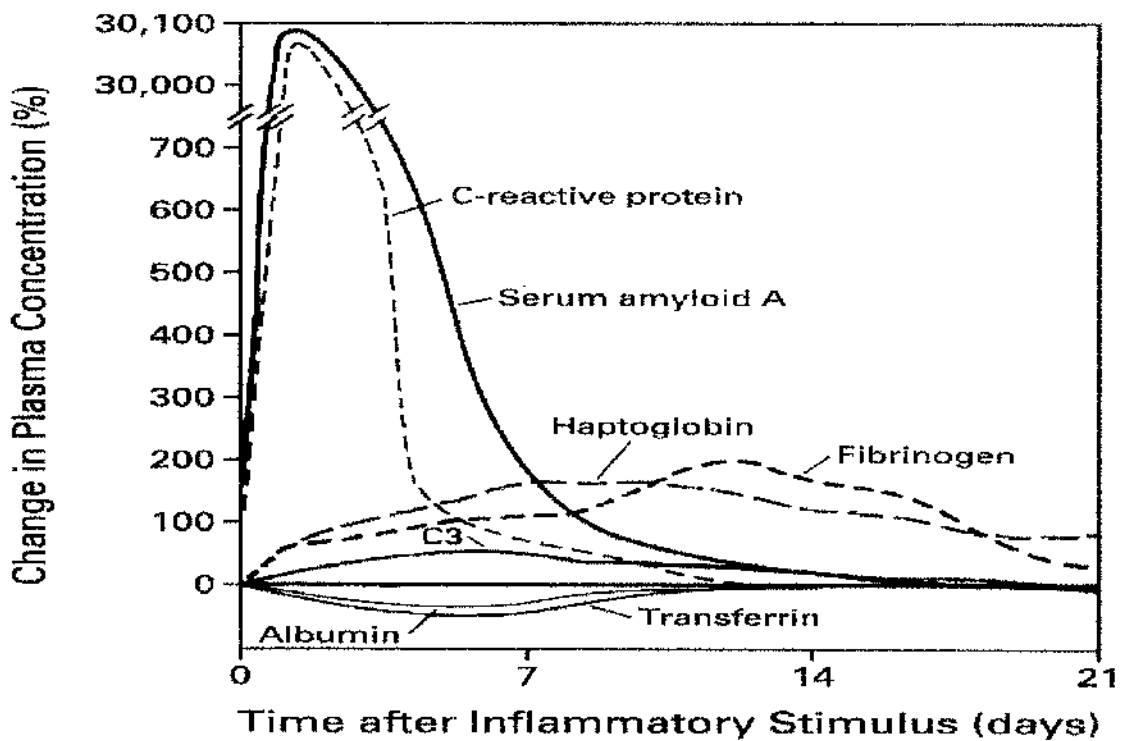
Figure 1.8: Characteristic patterns of change in plasma concentrations of some acute-phase proteins after a moderate inflammatory stimulus (from Gabay and Kushner, 1999).

1.5.1.1 C-reactive protein

C-reactive protein is a non-specific positive acute-phase protein synthesized by hepatocytes in response to trauma, inflammation and malignant processes. Its serum concentrations are extensively used in routine clinical practice to monitor the systemic inflammatory response, and therefore the extent and activity, as well as, the prognosis of many underlying diseases (Kolb-Bachofen, 1991; Pepys, 1996).

C-reactive protein belongs to the pentraxin family of proteins and consists of five identical non-glycosylated subunits, encoded by a single gene on chromosome 1. The monomers, each of molecular mass 23 027, are non-covalently associated in a very stable disc-like configuration with cyclic pentameric symmetry, which is resistant to proteolysis. C-reactive protein is primarily upregulated by the local and systemic release of the pro-inflammatory cytokines, in particular interleukin-6. In the presence of calcium, C-reactive protein undergoes specific ligand-binding to phosphocholine in autologous phospholipids and microbial polysaccharides. Complexed or aggregated C-reactive protein efficiently activates the classical complement pathway and can thereby opsonize ligands for phagocytosis. C-reactive protein neutralizes the potent inflammatory mediator, platelet-activating factor, which contains phosphocholine and has down-regulatory effects on neutrophil function. C-reactive protein may thus contribute to host defence, modulation of inflammation and lipid metabolism (Pepys, 1996).

The plasma half-life and fractional catabolic rate of C- reactive protein appears to be constant under virtually all conditions. Therefore, its plasma concentration is determined primarily by its synthesis rate. In normal healthy individuals the serum C-reactive protein

concentrations are barely detectable with a median level of 0.8 mg/l and with 99% of apparently healthy individuals having C-reactive protein concentrations less than 10 mg/l. Higher values are abnormal and objectively reflect the presence, extent and activity of underlying organic diseases and disorders. The rapid increase in concentrations may exceed 200 mg/l within 24 hours of an acute event. High serum concentration can be maintained for a prolonged period if the stimulus or underlying condition persists. After effective treatment or cessation of the stimulus the value can fall rapidly and almost to near-normal values within 2-3 days (Kolb-Bachofen, 1991; Pepys, 1996).

In recent years, there has been a considerable interest in the role of systemic inflammatory response, as evident by raised serum C-reactive protein concentration, in patients with malignant disease. Pro-inflammatory cytokines are usually produced by activated white blood cells (Gabay and Kushner, 1999; Whiteside, 2003), and may act as autocrine growth factors for tumours (Dunlop and Campbell, 2000). However, there is some evidence that cancer cells may also produce significant amounts of pro-inflammatory cytokines (Balkwill and Mantovani, 2001; Whiteside, 2003). Higher C-reactive protein concentration at presentation has been found to correlate with the overall tumour load and has been demonstrated to have stage independent prognostic value in a variety of cancers, notable of gastrointestinal carcinoma (McMillan et al., 1995; Goransson et al., 1996; Pepys, 1996; Nozoe et al., 1998; Fujita et al., 1999; Nielsen et al., 2000; Nozoe et al., 2001).

In breast cancer, few studies have been conducted with conflicting results. However, the majority of studies were based on small number of patients (Coombes et al., 1977a,b; Lee et al., 1982), included patients with operable early-stage and inoperable advanced-stage

diseases (Coombes *et al.*, 1977a,b; Drahovsky *et al.*, 1981), were retrospective (Weinstein *et al.*, 1984), and used non-specific assay techniques with limited sensitivity (Mortensen and Rudczynski, 1982) and limited follow-up.

Coombes and co-workers (1977 a,b) first showed, in a study of 51 patients with breast disease, that C-reactive protein was abnormally elevated ($\geq$ 10 mg/l) in 14 / 16 (87%) patients with overt metastatic breast cancer, but rare in patients with apparently localised disease. They reported that high pre-operative C-reactive protein concentrations, with other biochemical markers abnormalities such as carcinoembryonic antigen (CEA), can delineate patients with primary tumour that associated with poorer prognosis on histological ground or some other patients with no clinical or investigational evidence of metastasis but who have micrometastasises, in whom relapses might occurred sooner than others (3/7 patients in this study), suggesting that C-reactive protein with other biochemical markers could be of a value in the initial staging of patients, in the early detection of metastasis, in assessing prognosis and, therefore, could be used to select patients for adjuvant systemic therapy in addition to local therapy.

Similarly, Drahovsky and co-workers (1981) reported, in a study of 287 patients with different stages breast cancer, that there were 153 (53%) patients who had a positive C-reactive protein value of > 2 mg/l. A significant increase in the frequency of elevated C-reactive protein concentration was seen with advanced stages of disease (61.2% and 72.3% in stage III and IV respectively vs. 45.1% and 47.2% in stage I and II). C-reactive protein concentrations were also significantly elevated in early stages with active disease. C-reactive protein appeared to reflect tumour mass with a clearly positive correlation to

TNM staging, as well as, tumour activity (recurrence and/or metastasis) at the time of determination; i.e. independent of TNM stages.

However, Hillyard and co-workers (1982), in their study of 53 patients with benign breast masses and 196 patients with breast cancer and which aimed to test whether the biochemical changes can aids the staging of breast cancer, showed a considerable overlap between the concentrations of fifteen plasma proteins, including C-reactive protein, and the various clinical stages, and were often normal even in the metastatic disease. The mean C-reactive protein concentrations were raised in less than 50 % of patients with overt metastases.

As a part of a major study to identify prognostic factors for breast cancer, Mortensen and Rudczynski (1982) studied 297 patients with different stage breast cancer, and with a clinical follow-up period of 3-48 months post-surgery, to evaluate the role of serum C-reactive protein concentration and the extent of lymphocytic infiltration of the primary tumour as prognostic markers. Elevated C-reactive protein concentrations ($\geq$ 10 mg/l) were present in a higher proportion of patients with stage IV disease (24/57; 42%), when compared to patients in stages I (23/106; 22%), II (25/115; 22%) and III (4/19; 21%). There was no difference in C-reactive protein concentrations among patients with stage I-III disease. Although the serum C-reactive protein concentrations at the time of initial surgery and the presence of a localized lymphocytic infiltration within the primary breast tumour correlated with each other, neither measure was a useful in predicting the disease-free interval following surgery. C-reactive protein concentrations had no correlation with the size of the primary breast tumour, the histopathological grade or the lymph node involvement.

In the therapeutic monitoring of 179 patients with metastatic breast cancer, Williams and co-workers (1990) found a highly significant association between the therapeutic response according to the International Union Against Cancer (UICC) criteria and the alterations in the serum concentration of C-reactive protein and other tumour-related markers, including carcinoembryonic antigen, ferritin, orosomucoid and the erythrocyte sedimentation rate, after 6months of primary endocrine therapy. Suggesting that the design of an objective measurement of response, as a 'therapeutic index', that combined these tumour-related markers had the potential to replace the existing and largely subjective UICC criteria and so could be used to direct systemic endocrine therapy.

In a prospective study, C-reactive protein concentration with other tumour markers such as CEA and erythrocyte sedimentation rate (ESR) were observed to have no significant elevation in primary breast cancer (100 patients), compared to normal control (56 patients) or benign breast disease (100 patients). Therefore, appeared to have no role either in screening or in the differential diagnosis of breast cancer. On the other hand, C-reactive protein concentration was significantly elevated (>10mg/l) in 43/85 (51%) patients with systemic (metastatic) breast cancer. However, the sequential changes of C-reactive protein concentration in the 65 patients who survived beyond 3 months showed no significant correlation with the International Union Against Cancer (UICC) criteria-assessed therapeutic response; indicating that C-reactive protein would not appear to be of a clinical value in measuring response to therapy in advanced breast cancer (Roberston *et al.*, 1991).

In another prospective study of 85 patients with newly diagnosed metastatic breast cancer, the pre-treatment serum concentrations of C-reactive protein and other tumour markers

did not predict therapeutic response. However, the pre-treatment serum concentrations of C-reactive protein, ferritin, human milk fat globule membrane 1 and 2 above the mean + 2SD of the normal control group appeared to be prognostic and correlated significantly with longer post- metastases survival (Albuquerque et al., 1995).

More recently, in 77 patients with locally advanced non-metastatic breast cancer undergoing neo-adjuvant chemotherapy, Heys and co-workers (1998) demonstrated that pre-treatment concentrations of acute phase proteins did not indicate which patients will respond to chemotherapy, as assessed either by the clinical response or by the histological destruction of the tumour. C-reactive protein concentrations were not related to patient survival.

Zhang and Adachi (1999) in a study of 46 Japanese patients with metastatic breast cancer, observed a strong correlation between IL-6 concentrations and C-reactive protein concentrations. IL-6 concentrations correlated with the extent of metastases dissemination, being significantly higher in liver and pleural metastases. In these patients, higher IL-6 serum concentrations were associated with a poorer response to chemotherapy or chemo-endocrine therapy and correlated with a shortened survival, and therefore, representing a poorer prognostic predictor in patients with metastatic breast carcinoma.

O'Hanlon and colleagues (2002) in a recent study of 92 patients with breast cancer and 31 patients with benign breast disease, reported a significantly higher C-reactive protein concentration in patients with stage IV disease, T_4 ulcerating tumours, and during recurrence, but it was of no benefit in predicting recurrence.

In conclusion, C-reactive protein concentration appears to reflect the presence of systemic metastatic disease. However, the majority of studies were small or included patients unlikely to progress (primary operable) and patients who had already progressed (metastatic).

1.5.1.2 Albumin

Human albumin, the major negative acute-phase protein, is a single polypeptide consisting of 585 amino acids with a molecular weight of approximately 66 kDa. It is the most abundant of hepatic secretory proteins with a total albumin pool size of 4-5 g/kg of body weight, of which 40-45% is in the intravascular space and other 60% is in the interstitial space. The serum albumin concentration is normally around 40 g/l (Margarson and Soni, 1998).

The main functions of albumin are maintenance of colloid osmotic pressure, as a result of its relatively low molecular weight compared to the other major intravasular proteins such as immunoglobulin (accounting for 75-80% of the colloid osmotic pressure of human plasma), binding and transport, free radical scavenging, platelet function inhibition and antithrombotic effects, and its effects on capillary membrane permeability (Margarson and Soni, 1998).

The serum albumin level remains a traditional standard factor by which to assess a patient's nutritional status. In disease status, a decrease in serum albumin concentration is almost an inevitable finding, with marked persisting changes generally associated with poor prognosis (Margarson and Soni, 1998). It appears to be primarily mediated in the acute phase response by the altered energy and protein metabolism, that associate with its increased demand for specific amino acids for mediator and acute-phase protein synthesis and immune and antioxidant defences, which promotes the degradation and progressive loss of the available body vital protein components, including albumin and body cell mass. The modest albumin pool-size, relative to that of body cell mass, may be reflected

in its loss becoming noticeable at an earlier stage (Fearon *et al.*, 1999; McMillan *et al.*, 2001a).

The association between reduced serum albumin concentrations and/or elevated C-reactive protein concentrations with severity of illness and poor outcome has long been recognised. More recently, in malignant diseases, low albumin concentrations (< 35 g/l) and/or elevated C-reactive protein concentrations were found more often in patients with advanced inoperable/ metastatic cancers than in patients with early stage operable cancers, most probably reflecting disseminated disease with more pro-inflammatory cytokine-mediated metabolic events, and subsequent poorer prognosis (Goransson *et al.*, 1996; Fiorenza *et al.*, 2000; McMillan *et al.*, 2001a).

In locally advanced non-metastatic breast cancer, Heys and co-workers (1998) in a study of 77 patients undergoing neo-adjuvant chemotherapy, reported that reduced pre-treatment serum albumin concentration, progressive lymph node involvement, and advanced tumour stage were independent prognostic indicators for poor survival.

Similarly, low baseline serum albumin concentration of < 35 g/l in 180 consecutive patients with different stage breast cancer was a powerful prognostic variable of overall survival, accounting for approximately 10% of the overall variation in patient survival time. In mid- and lower-stage breast cancer, the overall 5-year survival of patients with hypoalbuminaemia was 60% compared with 85% in patients with a normal albumin. Whereas, patients with stage IV lesions who fell in the lowest quartile serum albumin concentration had a median survival of 14.8 months compared with 22 months for the rest of patients in this stage (Lis *et al.*, 2003).

In summary, there is some evidence that an elevated C-reactive protein and a low albumin concentration may identify patients with breast cancer at a higher risk of poor outcomes and premature death. However, there is little data on the relationship between these systemic acute-phase proteins and the local inflammatory response in patients with breast cancer.

1.5.2 Immune-cellular infiltration

Locally, the immune-cellular infiltration within the tumour microenvironment of many human malignant neoplasms, including breast cancer, was observed as early as the middle of the nineteenth century, and since that time, discrepancies have been existing on the functional and prognostic significance of this phenomenon.

Breast cancer, among other solid tumours and their metastases, have been documented to be largely orchestrated by a heterogeneous populations of immune cells, consisting of variable proportions of helper, suppressor, and cytotoxic T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils and mast cells. All of which are capable of producing an assorted array of cytokines, growth factors and proteases, and are believed to represent a marker of the host immune response to neoplastic growth (Chin et al., 1993; O'Sullivan and Lewis, 1994; Goedegebuure and Ederlein, 1995; Wong et al., 1998; Witz, 2001; Coussens and Werb, 2002; Yu and Rak, 2003).

In breast cancer, T- lymphocytes have been demonstrated to be a major component of the tumour-associated leucocytes (TALs) population, of which both the CD8+ (T suppressor/cytotoxic) and the CD4+ (T helper/inducer) subsets have been reported to predominate (Chin et al., 1993; Ogmundsdottir, 2001; Georgiannos et al., 2003). However, the relationship between patient's prognosis and the extent of lymphocytic infiltrate and /or the degree of phenotypic difference remains the subject of considerable debate (Hadden, 1999).

Previous investigators have indicated that an intense lymphocytic infiltrate is associated with a favourable prognosis. The assumption for this was based on that lymphoid cells

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infiltration of breast cancer has often been interpreted as an indication of an active immune response against the tumour and, thus, a favourable prognostic sign (Ogmundsdottir, 2001). However, others studies found no relationship, or even, claim that patients with intensely infiltrated cancers had worse prognosis (Stewart and Tsai, 1993; Ogmundsdottir et al., 1995). These contradictory results may be related to the small and limited number of tumours analysed or due to the differences in the type of analysis performed.

In general, each histological type differs in its biological properties and clinical progression, and the immune response between tumour-types may also vary. It is of interest that the medullary subtype of breast carcinomas is characterized by marked lymphoid infiltration, with increased numbers of activated cytotoxic lymphocytes (CD8+), and generally has a more favourable prognosis than other histological subtypes (Yakirevich et al., 1999; Ogmundsdottir, 2001).

In contrast, primary breast cancers with markers of poor prognosis, such as high-grade tumour, tumour size of more than 2 cm, and with three or more positive lymph nodes, were found to exhibited increased lymphocytic infiltrate (Griffith et al., 1990; Vgenopoulou et al., 2003). It was suggested, in a significant subgroup of breast cancer patients, that a high degree tumour-infiltrating lymphocytes might create a favourable environment for the malignant cells and may even facilitate cancer development and growth and, therefore, correlated with poor prognosis (Stewart and Tsai, 1993).

A higher proportion of CD8+ (cytotoxic type) compare to CD4+ cells (T helper type) has been observed in some studies (Ogmundsdottir, 2001), with a significantly higher CD8+

T cells in progesterone-positive tumour than that in progesterone-receptor negative tumours (Chin *et al.*, 1993). Also in patients with early disease stage or those who were alive with no residual disease compared with late stage or those dead of disease (Marrogi *et al.*, 1997). On the other hand, T helper/inducer lymphocyte subset predominated in other studies, especially in large tumours, and they usually increased with the size of the tumours. It has been reported that there is elevated CD4+ T cells with a declined CD8 expression in oestrogen-receptor negative tumours, and increased CD4/CD8 ratio in lymph node metastases (Griffith *et al.*, 1990; Chin *et al.*, 1992; Chin *et al.*, 1993; Wong *et al.*, 1998). However, in patients with primary operable breast cancer (n = 77), neither the density of lymphocytic infiltration nor the ratio of helper to suppressor lymphocytes were related to the Nottingham Prognostic Index or with improved survival or reduced rates of cancer recurrence in the immediate 3-years period following tumour resection (Griffith *et al.*, 1990).

The relationship between the extent of lymphoid-cell infiltration within the primary tumour and the serum C- reactive protein concentration, as a systemic inflammatory response, has been evaluated in 297 breast cancer patients using radial immunodiffusion technique. Although, the presence of lymphoid-cell infiltration has found to be correlated with C- reactive protein concentration, neither measures showed any obvious association with the length of disease-free interval following surgery (Mortensen and Rudczynski, 1982).

Tumour-associated macrophages, derived from the circulating monocytes, are another major component of the cellular leucocytic infiltrates within the breast tumour microenvironment (O'Sullivan and Lewis, 1994; Lewis *et al*, 1995; Lee *et al*, 1996; Leek

and Harris, 2002). In breast cancer, recent studies have indicated that the pro-tumour role of tumour-associated macrophages is dominant over the anti-tumour activities, and the evidence from both experimental and clinical studies suggests, in most cases, that these cells can play an active role in enhancing tumour progression and metastasis. In addition to tumour cells, activated macrophages secret a wide range of growth factors, digestive enzymes, angiogenic cytokines and hypoxia inducible factors, such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), tumour necrosis factor (TNFα), thymidine phosphorylase (TP), transforming growth factor-β (TGF-β), platelet-derived growth factor, interleukin-6 (IL-6), urokinase plasminogen activator and tissue-type plasminogen activator (t-PA), which may promote tumour growth, facilitate tumour-cell invasion and migration, and enhance tumour angiogenesis (O'Sullivan and Lewis, 1994; Lewis et al, 1995; Leek et al, 1996; Leek and Harris, 2002).

A high tumour-associated macrophages density has been demonstrated to be associated with established prognostic factors indicating aggressive phenotype breast cancer. For example, in series of less than 80 patients with breast cancer, diffuse inflammation of macrophages, and to lesser extent T-lymphocytes, was most frequently associated with larger tumour size, higher tumour grade or mitotic activity index, tumour necrosis or/ and positive c-erbB-2 expression (Lee et al, 1996; Pupa et al, 1996; Lee et al, 1997; Jonjic et al, 1998).

Similarly, the prognostic significance of tumour-associated macrophages in breast cancer has been reported in association with increased tumour angiogenesis, as well as, poor prognosis (Steele et al, 1984; Leek et al, 1996). In a cohort of nearly 100 patients with breast cancer, increased tumour-associated macrophage density correlated significantly

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with high vascular grade, as assessed by quantitative CD68+ and CD31+ immuno-staining; respectively, and independently associated with reduced relapse- free and overall survival (Leek *et al*, 1996).

Other cellular infiltrates such as B-lymphocytes, natural killer cells, granulocytes, eosinophils, mast cells, or plasma cells are scarce in malignant breast tumours, and their involvement in cytotoxicity or tumour progression is limited (O'Sullivan and Lewis, 1994).

In summary, the relationship between the tumour, cellular immune response, and outcome in patients with primary operable breast cancer remains unclear.

1.5.3 Angiogenesis

Invasion and metastasis in breast cancer, as most of other solid tumours, depend on the activation of angiogenesis (or neovascularization); a multi-step process leading to the formation of new blood vessels from an existing vascular endothelium in a growing tumour beyond 1-2 mm in size (Gasparini, 1995; Uzzan et al., 2004).

Angiogenesis is an active process, derived by hypoxia and regulated by a large number of pro-angiogenic growth factors, cytokines and proteases, such as vascular endothelial growth factor, platelet-derived endothelial cell growth factor, and basic fibroblast growth factor, as well as, anti-angiogenic molecules, which are produced by tumour cells and stromal cells, including infiltrating inflammatory cells (Gasparini, 1995; Yu and Rak, 2003; Coradini and Daidone, 2004; Esteva and Hortobagyi, 2004; Uzzan et al., 2004). Either within the primary tumour or its metastases, angiogenesis is a necessary process required both at the beginning and at the end of the development of distant metastasis. Furthermore, it has been implicated in the phenomenon of dormant micrometastases (Gasparini, 1995).

Within a given tumour type, the angiogenic activity is believed to be heterogeneous (Gasparini, 1995; Uzzan et al., 2004). Nevertheless, intratumoural microvessel density (MVD) or vascular grading, a surrogate marker of tumour angiogenesis, which has been determined by panendothelial markers and immunohistochemical techniques in several studies, has been shown to have a prognostic value and associate with clinical outcome (Gasparini et al., 1995; Hansen et al., 2000a,b; Gasparini et al., 2001; Uzzan et al., 2004).

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Angiogenesis correlates significantly with other clinico-pathologic factors, including axillary node metastasis, large tumour-size, high malignancy grade, histological type and oestrogen receptor-negative tumour. It has, also, been shown to have independent prognostic value for both relapse-free survival and overall survival in all patients with breast cancer, including those with node-negative and node-positive disease (Gasparini *et al.*, 1995; Hansen *et al.*, 2000a,b; Gasparini *et al.*, 2001; Coradini and Daidone, 2004; Esteva and Hortobagyi, 2004; Uzzan *et al.*, 2004). Patients with highly vascularized primary tumours are at high risk of early recurrence and metastasis, at any time (Gasparini *et al.*, 2001).

Conflicting results, however, have been observed with some other studies, most probably resulting from the application of different methods, including different staining markers and assessment techniques (Uzzan *et al.*, 2004).

With the standardization of intratumoural vascular profile, angiogenesis may prove to be a novel biological marker that can be added to other demonstrated prognostic and predictive factors in breast cancer.

1.5.4   Interleukin-6

Cytokines and growth factors are powerful modulators of the immune response in cancer development and malignant disease progression, and have been the focus of numerous investigations.

Interleukin-6, as a key member of the interleukin family, is a multifunctional pro-inflammatory cytokine, which plays a central role in regulating the immune and inflammatory responses, via the synthesis of most acute-phase proteins such as the C-reactive protein. Elevated serum interleukin-6 production is often seen in infectious diseases, chronic inflammatory diseases, and certain malignant diseases. It is produce by immune and immune-accessory cells, as well as, many non-immune cells including; polymorphonuclear neutrophils, monocytes / macrophages, B and T lymphocytes, mast cells, endothelial and mesothelial cells, fibroblasts, keratinocytes, some nerve cells and certain tumour cells (Gabay and Kushner, 1999; Kovacs, 2001).

The biological activities of interleukin-6 are initiated by its binding to a specific receptor complex on the target cells. This is composed of an 80 kDa component receptor (IL-6R) that binds interleukin-6 with low affinity, and a signal-transducing component of the 130 kDa (gp130), which is required by the complex for the high-affinity binding of interleukin-6 (Kovacs, 2001; Corcoran and Costello, 2003).

The interleukin-6 receptor is present in two forms: a membrane-bound and a soluble form. The soluble receptors as 'carrier' protect cytokines from proteolytic activation and thereby increase their half-life in the blood. The soluble interleukin-6 receptor as an agonist enhances the biological effect of released interleukin-6. The soluble form of

gp130 is a common signal transducer not only for Interleukin-6 but also for other Interleukin-6 related cytokines. It is a potent endogenous antagonist that inhibits the effects of the interleukin-6/ soluble interleukin-6 receptor complex. This inhibitory activity of soluble gp130 is enhanced by soluble interleukin-6 receptor (Kovacs, 2001).

About 70% of secreted interleukin-6 forms a complex with the soluble interleukin-6 receptor in the blood and binds directly to membrane gp130. The other 30% has only a transient existence in the blood, or binds to the membrane-bound receptor. Almost every cell expresses gp130 on the membrane, in contrast to interleukin-6 receptor, which exists only on specific cells (Kovacs, 2001).

The interleukin-6 signalling complex can diversify its action by engaging other signalling pathways. Recent studies in prostate and breast cancer cells have shown that the interleukin-6 with its gp130 subunit appear to induce activation of certain members of the epidermal growth factor receptor family, through the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3'-kinase (PI3K) pathways (Qiu et al., 1998; Badache and Hynes, 2001; Corcoran and Costello, 2003).

Elevated serum values of interleukin-6, soluble interleukin-6 receptor and soluble gp130 have been reported in 20-40% of patients with certain malignant disease (Kovacs, 2001). In patients with colorectal and prostate cancer, increased serum interleukin-6 levels have been shown to reflect the disease's status and significantly correlate with cancer stage. It is believed to be associated with malignant transformation and tumour progression, by a paracrine or autocrine mechanism (Qiu et al., 1998; Chung and Chang, 2003).

In the breast, limited numbers of studies, mostly with smaller series of selected patients, have shown paradoxical results on the significance of interleukin-6 and its related protein-receptors.

Breast tissue of normal, benign and neoplastic origin expresses a wide range of cytokines, including interleukin-6 (Green *et al.*, 1997; Kuang *et al.*, 1998).

In early-stage breast cancer, interleukin-6 has been correlated with good prognosis. Karczewska and his co-workers (2000), in a study of 75 patients with breast cancer, showed that the expression of interleukin-6 and its receptor subunits (interleukin-6 receptor and gp130) in breast cancer tissue and their cultured cells were associated with earlier stages of the disease in term of tumour size and lymph node involvement only (with no correlation to overall grade, nuclear grade, mitotic index, menopausal status, or oestrogen or progesterone receptor status), and proved to be a favourable prognostic factor for overall and disease free survival.

In another study of 149 patients with invasive breast cancer, who received no anti-neoplastic treatments before surgery, the majority were found to express at least low levels of immunoreactive interleukin-6. The expression was directly related to oestrogen and progesterone receptor-positive cells, and inversely associated with the tumour histological grade and Ki67 positivity. However, no association was demonstrated between interleukin-6 and tumour size, nodal status, or p53 protein expression (Fontanini *et al.*, 1999).

However, contradictory results have been reported in patients with advanced breast cancer, in which higher serum interleukin-6 levels were demonstrated to correlate with

the extent of metastatic dissemination and were associated with worse prognosis (Zhang and Adachi, 1999; Salgado et al., 2003).

Serum interleukin-6 concentration was significantly higher in patients with metastatic breast cancer compared to those with loco-regional operable disease (Benoy et al., 2002), and with a significant higher values detected in those patients with more metastatic deposits and with dominant metastatic visceral disease (such as liver metastasis or pleural effusion) than in those with one metastatic site or with dominant metastatic bone or soft tissue disease (Zhang and Adachi, 1999; Salgado et al., 2003).

Salgado and his colleagues (2003), in their study of 96 unselected consecutive patients with progressive metastatic breast cancer and before the initiation of systemic therapy, found no correlation between soluble interleukin-6 receptor and age, menopausal status, performance status, tumour grade, body-mass index, histology and hormone receptor status.

Serum interleukin-6 concentration was demonstrated to have a strong correlation with C-reactive protein concentrations and to be an independent prognostic factor for advanced and metastatic breast cancer, with significantly poorer survival rate in patients with higher interleukin-6 values than in patients with lower interleukin-6 values (Zhang and Adachi, 1999; Bachelot et al., 2003; Salgado et al., 2003).

Moreover, in recurrent and metastatic breast cancer, higher serum interleukin-6 concentrations were found to predict a poorer response to chemo-endocrine therapy, and are now believed to be link to drug resistance (as with anthracycline resistance). Patients unresponsive to chemo-endocrine therapy were shown to have significantly higher serum

interleukin-6 levels than those who responded to chemo-endocrine therapy (Zhang and Adachi, 1999; Yokoe *et al.*, 2000).

A similar relationship has also been described for endocrine therapy with medroxyprogesterone acetate with low pre-treatment interleukin-6 levels and less increased interleukin-6 levels during treatment being associated with response to treatment (Nishimura *et al.*, 2000).

Tumour-derived interleukin-6 may influence tumour-related angiogenesis, decrease cell adhesion, promote cell migration by activating the mitogen-dependent protein kinase pathway, and inhibit the activation of proteases involved in apoptosis and thereby increase chemotherapeutic resistance (Asgeirsson *et al.*, 1998; Benoy *et al.*, 2002; Bachelot *et al.*, 2003).

It has been suggested that as tumours evolve toward a metastatic phenotype and interfere with other endogenous or exogenous factors, interleukin-6 activity on cancer cells and their environment might actually shift from growth inhibition and differentiation to proliferation and anti-apoptosis, this could explain the favourable prognosis associated with the presence of interleukin-6 in early-stage breast cancer and the poor survival associated with the high serum interleukin-6 in patients with metastatic breast cancer (Bachelot *et al.*, 2003). However, the net effects of this prime pro-inflammatory mediator with respect to both local and systemic inflammatory responses and other pathological prognostic factors within the primary breast cancer have not been adequately investigated.

1.5.5 Cyclo-oxygenase-2

The tumour microenvironment may include up-regulation of other mediators of the inflammatory response, which may impact tumour-cell growth and survival-signalling pathways (O'Byrne and Dalgleish, 2001). A weight of epidemiological, pharmacological, genetic and expression studies suggested a key role for Cyclooxygenase-2 (COX-2) in the pathogenesis of a variety of epithelial human cancers, including breast cancer (Howe *et al.*, 2001; Howe and Dannenberg, 2003).

The COX enzyme-system, the two isoenzymes COX-1 and COX-2, are prostaglandin synthases with intrinsic cyclooxygenase and peroxidase activities, which mediate the conversion of arachidonic acid into prostaglandins (Figure 1.9).
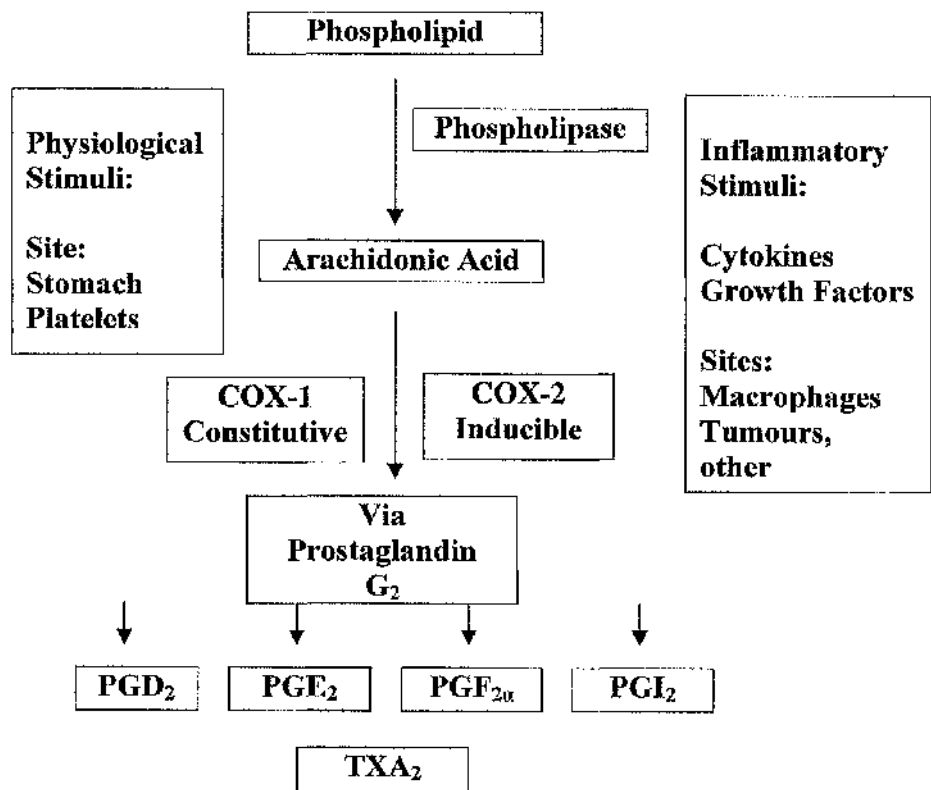
Figure 1.9: Prostaglandin synthesis via arachidonic acid (from Davies, 2003).

Cyclooxygenase-derived prostanoids contribute to many normal physiological processes including haemostasis, platelet aggregation, kidney and gastric function, reproduction, pain and fever (Howe et al., 2001; Howe and Dannenberg, 2003).

Despite the similar enzymatic activities of both enzymes, the *COX-1* and *COX-2* genes have distinct properties, and differing expression patterns. Both are 72-kDa proteins and localize to endoplasmic reticulum and nuclear envelope in the cell. COX-2 is glycosylated yielding an additional 74-kDa species. While COX-1 is constitutively expressed, COX-2 'the inducible isoenzyme', is normally expressed in part of the kidney and brain but is over-expressed predominantly in various pathological status including cancer; in the tumour-epithelial cells, as well as, in the tumour-associated fibroblasts, vascular endothelial cells, and inflammatory mononuclear cells. Induction of COX-2 occurs by the upregulation of transcription and by the stabilization of COX-2 mRNA (Howe et al., 2001; Singh and Lucci, 2002; Howe and Dannenberg, 2003).

The exact mechanisms by which COX-2 expression is upregulated in breast carcinoma and contribute to carcinogenesis are still unclear, even though previous studies revealed that COX-2 is expressed in response to growth factors, tumour promoters, cytokines and several oncogenes such as *HER-2/neu*, *v-src*, *v-Ha-ras*, and *Wnt* genes (Howe et al., 2001; Singh and Lucci, 2002; Howe and Dannenberg, 2003).

Mounting evidence suggests that COX-2 expression contributes to several processes during tumour development and progression. Enhanced prostaglandins synthesis, the most obvious consequence of COX-2 overexpression, has been shown to stimulate mitogenesis of mammary epithelial cells, fibroblasts, and osteoblasts. Indirectly, it may stimulate

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mammary cellular proliferation and tumour growth, via stromal aromatase induction that lead to increased oestrogen biosynthesis. However, prostaglandins may have antiproliferative effects, which mediate immune suppression, by inhibiting T and B cell proliferation, dendritic cells development and function, cytokines synthesis, and diminishing the cytotoxic activity of natural killer cells, allowing tumours to avoid immune surveillance that might otherwise limit their growth. In addition, COX-2 overexpression-related effects may result in inhibition of apoptosis, as well as, increased production of mutagens, such as malondialdehyde (MDA) that lead to DNA damage, thereby contributing to carcinogenesis. Also, it contributes to the production of several proangiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor, transforming growth factor β-1, platelet-derived growth factor, and endothelin-1, leading to increased angiogenesis. COX-2 may enhance cancer cells invasion and metastasis by stimulating expression or activity of matrix metalloproteinases and by increasing adhesion to extracellular matrix. All of these characteristics may contribute to tumorigenicity, although the exact molecular mechanisms by which COX-2 causes these effects are unknown (Howe et al., 2001; Singh and Lucci, 2002; Howe and Dannenberg, 2003).

The expression of COX-2 in human breast cancer has been evaluated in several studies using different methods of detection and different approaches, including reverse-transcriptase polymerise chain reaction (RT-PCR), western blot, and immunohistochemistry (IHC). Preliminary analyses, however, revealed conflicting data regarding the frequency of COX-2 expression and its possible prognostic significance in breast cancer, most probably reflecting different COX-2 expression techniques and

smaller sample size (Costa *et al.*, 2002; Half *et al.*, 2002; Davies *et al.*, 2003; Yoshimura *et al.*, 2003).

In contrast, larger recent studies have investigated the role of COX-2 with focus on associations of COX-2 overexpression with other clinico-pathological parameters and possible prognostic role. In the majority, COX-2 positive tumours correlated with several poor prognostic characteristics and associated with an unfavourable outcome (Ristimaki *et al.*, 2002; Denkert *et al.*, 2003; Spizzo *et al.*, 2003).

In a recent large-scale analysis of COX-2 expression in 1576 invasive breast cancer, using a monoclonal anti-COX-2 antibody immunohistochemistry, moderate to strong expression of COX-2 protein was observed in 37.4% of the tumour. COX-2 immunoreactivity localized exclusively to the cytoplasm of the tumour cells, and was not elevated in stromal cells. Elevated COX-2 expression was associated with a large tumour size, a high histological grade, a ductal histological type, with axillary node metastases, a negative hormone receptor status, a high proliferation rate (identified by Ki-67), high p53 expression, and the presence of HER-2 oncogene amplification, along with unfavourable distant disease-free survival. Interestingly, the association with unfavourable outcomes was especially apparent in a subgroup of patients with oestrogen positive receptor, low p53 expression, and no HER-2 amplification (Ristimaki *et al.*, 2002).

In another set of 221 primary breast carcinomas, Denkert and co-workers (2003) have investigated the expression of COX-1 and COX-2, using IHC with monoclonal antibody. COX-2 was found to be expressed in 36% of breast carcinoma, with a significant increased COX-2 expression in tumours > 20mm, in high-grade lesions, and in tumours

with lymph node metastasis and vascular invasion. COX-1 expression was observed in 45% of tumour, but was significantly increased in smaller tumours (<20mm) and in tumours without lymph node metastasis. A significant association was observed between COX-2 expression and decreased disease-free survival and overall survival in univariate analysis, with only borderline significance for disease-free survival in multivariate analysis. Elevated COX-1 expression, however, had no significant influence on patient prognosis.

Similarly, a moderate to strong cytoplasmic COX-2 staining was observed in 40.6% of breast cancer samples, using IHC technique with tissue microarry (TMA) and semiquantitative analysis in 600 samples of 200 patients. Increased COX-2 expression corresponded to higher tumour grade and stage, HER-2 amplification, lymphovascular invasion and a high Ki-67 proliferation index, with inverse relationship to oestrogen and progesterone receptor content of tumours. COX-2 overexpression, in contrast, had no significant association with disease-free survival or overall survival (Wulfing et al., 2003).

Witton and co-workers (2004) from our laboratory reported that COX-2 overexpression, which was observed in 21.2% of 179 patients with primary breast cancer, was not associated with tumour size, grade, high NPI, ER negativity, or HER 1-4 expression. High COX-2 expression was associated with reduced disease-free survival and disease-related survival in ER-negative but not ER-positive disease.

Together, these observations suggest that high COX-2 expression may serve as an additional unfavourable prognostic marker that may associate with adverse disease

outcome in COX-2 positive breast cancer. However, the magnitude of the effect is likely to be relatively small.

1.5.6   Hypothesis and aims

It would appear from previous studies in patients with different stages of breast cancer that the information of the inter-relationship between the tumour-based factors and the host-response and the relationship with disease outcome is limited.

It may be hypothesised that the local environment of cytokines, proteases, angiogenic and growth factors, and its subsequent systemic inflammatory response, may stimulate tumour growth, progression and metastasis, resulting in poor outcome.

Therefore, the aims of this thesis were to examine:

1. The prognostic significance of the systemic inflammatory response in patients with metastatic breast cancer.

2. The relationship between standard tumour-based clinico-pathological factors and the systemic inflammatory response, as evidenced by C-reactive protein and albumin concentrations, measured prior to surgery, and their prognostic significance in patients with invasive primary operable (loco-regional) breast cancer.

3. The inter-relationship between the local inflammatory response, as evidenced by tumour infiltration of T-lymphocyte sub-populations and macrophages, systemic inflammatory response, as evidenced by C-reactive protein and albumin concentrations measured prior to surgery, and tumour-based clinico-pathological

factors, including tumour proliferative activity and microvessel density, and their prognostic significance in patients with invasive primary operable breast cancer.

CHAPTER 2: EVALUATION OF AN INFLAMMATION-BASED PROGNOSTIC SCORE (GPS) IN PATIENTS WITH METASTATIC BREAST CANCER.

2.1   Introduction

Breast cancer is the second most common cause of cancer-related death among women in the Western world (CancerStats, www.cancerresearchuk.org; Jemal *et al.*, 2003). Approximately 10% of newly diagnosed breast cancer patients have locally advanced and/or metastatic disease at the time of presentation (Li *et al.*, 2003; Sant, 2001). In addition, more than 40% of patients, who are diagnosed with early-stage breast carcinoma, will eventually experience later recurrence and/or metastatic disease (Early Breast Cancer Trialists' Collaborative Group, 1998b).

Metastatic breast carcinoma exhibits a great deal of variability in its clinical presentation and behaviour. The prognosis is generally poor with a median overall survival of approximately 2 to 3 years (Ali *et al.*, 2003; Bernard-Marty *et al.*, 2004). Current therapies are palliative, aiming at improving or maintaining quality of life, controlling symptoms, and prolonging survival. Nevertheless, specific subgroups of patients exist for which, depending on the site of metastasis and treatment given, survival may range from a few months to several years (Nomura, 1998; Insa *et al.*, 1999). This prognostic uncertainty has driven the search for well standardised laboratory based parameters which have prognostic value.

Previous studies have established the prognostic importance of the systemic inflammatory response, as evidenced by an elevated circulating C-reactive protein concentration, in patients with advanced solid tumours (O'Gorman *et al.*, 2000; Mahmoud and Rivera,

2002; Maltoni *et al.*, 2005) including breast cancer (Albuquerque *et al.*, 1995; Zhang and Adachi, 1999). Recently, it has been shown that, using established cutoffs, a combination of an elevated C-reactive protein and hypoalbuminaemia, the Glasgow Prognostic score (GPS) has prognostic value, independent of stage and performance status, in patients with inoperable non-small cell lung cancer (Forrest *et al.*, 2003; 2004; 2005). However, there is no information on the prognostic value of this combination in patients with metastatic breast cancer.

The aim of the present study is to examine the relationship between the GPS and survival in patients with metastatic breast cancer.

2.2   Patients and methods

Breast cancer patients presenting, with metastatic relapse, to a single oncology clinic in the Beatson Oncology centre between February 2002 and November 2004 and who had a measurement of C-reactive protein and albumin undertaken were included in the study. At this time no patients showed clinical evidence of infection or other inflammatory conditions.

All patients had confirmed metastatic disease on the basis of either clinical findings or imaging. Patients were group according to whether they soft tissue and/ or bone metastases, visceral metastases and visceral and soft tissue and/ or bone metastases. Oestrogen receptor (ER) status was considered as positive when nuclear staining was seen in ≥ 10% of cancer cells.

Patients who developed other malignancies, and patients with C-reactive protein measured during a course of taxane therapy were excluded from the study since they may produce a hypersensitivity reaction and elevated concentrations of interleukin-6 (Tsavaris *et al.*, 2002; Williams *et al.*, 2003) which is a primary mediator of C-reactive protein (Gabay and Kushner, 1999). In total, 96 out of 295 (33%) patients with metastatic disease were eligible for the study.

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

Routine laboratory measurements of C-reactive protein and albumin concentrations were carried out. The coefficient of variation was less than 5% as established by routine quality control procedures. The limit of detection of the assay is a C-reactive protein concentration of less than 6 mg/l.

The GPS was constructed as previously described (Forrest *et al.*, 2003). Briefly, patients with both an elevated C-reactive protein (>10 mg/l) and hypoalbuminaemia (<35 g/l) were allocated a score of 2. Patients in whom only one of these biochemical abnormalities was present were allocated a score of 1. Patients in whom neither of these abnormalities was present were allocated a score of 0.

Statistics

Grouping of the variables was carried out using standard thresholds. Survival (cancer-specific) analysis was performed using the Cox proportional hazard model. Deaths up to 31st May 2005 have been included in the analysis. Multivariate survival analysis was performed using a stepwise backward procedure to derive a final model of the variables that had a significant independent relationship with survival. To remove a variable from the model, the corresponding *P*-value had to be greater than 0.10. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

2.3 Results

The baseline clinicopathological characteristics of the patients with metastatic breast cancer (n=96) are shown in Table 2.1. The majority of patients were over 50 years of age (78%), and received active treatment (96%). C-reactive protein and albumin concentrations were measured prior to systemic therapy in 35 patients and during systemic therapy in 61 patients. In all, 72 (75%) patients had not received cytotoxic chemotherapy for metastatic disease prior to the measurement of C-reactive protein and albumin concentrations.

In all, 92 (96%) patients received active treatment in the form of chemotherapy and/ or endocrine therapy. In those patients in whom Her-2 status was assessed, 14 out of 47 patients were Her-2 positive and 11 of the 14 patients received Herceptin therapy. The majority of patients had C-reactive protein (53%) and albumin (94%) concentrations in the normal range; the GPS was elevated in 47% of patients. Of the 6 patients with hypoalbuminaemia, all had an elevated C-reactive protein concentration.

The minimum follow-up was 7 months; the median follow-up of the survivors was 16 months. During this period 51 patients died of their cancer. On univariate analysis, an elevated C-reactive protein ($P<0.01$), hypoalbuminaemia ($P<0.05$), the GPS ($P<0.001$) and treatment ($P<0.05$) were associated with poor cancer-specific survival. On multivariate analysis of the GPS and treatment, only the GPS (HR 2.26, 95% CI 1.45–3.52, $P<0.001$) remained significantly associated with cancer-specific survival.

The relationship between clinicopathological characteristics and an inflammation-based prognostic score (GPS) in patients with metastatic breast cancer is shown in Table 2.2.

The median survival in these patients was 24 months, 13 months and 1 month for a GPS of 0, 1 and 2 respectively (Figure 2.1).

2.4 Discussion

In the present study, a simple inflammation-based prognostic score (GPS) based on standard laboratory measurements of C-reactive protein and albumin, was an independent predictor of survival in patients with metastatic breast cancer. This work is consistent with previous work in patients with inoperable non-small cell lung cancer (Forrest *et al.*, 2003; 2004; 2005) and improves on the prediction of survival using an elevated C-reactive protein alone (Albuquerque *et al.*, 1995; Zhang and Adachi, 1999). If these results are confirmed in larger studies, the GPS may be useful in the assessment of advanced breast cancer patients at diagnosis and the stratification of patients entering randomised trials.

In the present study, performance status was not recorded in the majority of patients. However, almost all the patients received active treatment and this would suggest that they had similar good performance status. Moreover, performance status is subjective (Ando *et al.*, 2002) and the GPS has been shown to be a prognostic factor, independent of performance status in patients with inoperable non-small cell lung cancer (Forrest *et al.*, 2003; 2004). Other recognized prognostic factors, which have been shown to affect survival post relapse, such as oestrogen receptor status and disease free interval, did not reach statistical significance. This was probably due to the small number of patients in the present study. In contrast, an increasing GPS, (in particular 0 vs 1) was associated with a halving of survival. These results may point to the strength of the systemic inflammatory response (the GPS) in predicting survival in the patient with metastatic breast cancer.

It has been previously shown that, in patients with inoperable non-small cell lung cancer at diagnosis, approximately three-quarters of patients had an abnormal GPS (1 or 2) and that these patients had a significantly poorer outcome (Forrest *et al.*, 2003; 2004). It was of interest that, in the present study, although the proportion of patients with an abnormal GPS was less (47%), those metastatic breast cancer patients with a GPS of 2 had a similarly poorer survival. It may be that the prognostic value of the GPS is independent of tumour type in patients with advanced cancer (Elahi *et al.*, 2004).

The mechanisms by which a systemic inflammatory response (the GPS) might impact on survival in advanced cancer patients are still not well defined. It may reflect the pro-inflammatory cytokine activity, in particular interleukin-6 (McKeown *et al.*, 2004), which not only stimulates breast tumour growth (Kurebayashi, 2000), but also produce profound catabolic effects on host metabolism (McMillan *et al.*, 1998; Kotler, 2000). In this way, the presence and magnitude of a chronic systemic inflammatory response may produce progressive nutritional and functional decline, ultimately resulting in reduced survival. Indeed, this concept is consistent with the observation in the present study that all patients with hypoalbuminemia had an elevated elevated C-reactive protein concentration.

At the time of diagnosis, there are well-established prognostic factors on which to base the prediction of likely survival in cancer patients. In contrast, predicting survival of patients with advanced disease is more problematic. As a result, clinicians often overestimate survival (Christakis and Lamont, 2000; Glare *et al.*, 2003). The results of the present study suggest that the GPS may be useful in the assessment of survival in patients with metastatic breast cancer. It is simple to use and based on routinely available, well-standardised measurements.

Table 2.1: Clinico-pathological characteristics in patients with metastatic breast cancer, univariate survival analysis.

	Patients (n= 96)	Hazard ratio (95% CI)	P-value
Age (≤50/ >50years)	21/ 75	0.70 (0.38-1.31)	0.266
Prior cytotoxic chemotherapies			
(0/ ≥1)	72/ 24	1.42 (0.76-2.65)	0.266
Metastatic site			
(Non-visceral/ visceral/ both)	43/ 14/ 39	1.08 (0.79-1.48)	0.625
Oestrogen receptor status			
(positive/ negative)	63/ 27	1.17 (0.65-2.12)	0.594
Disease free interval			
(>2/ ≤2 years)	67/ 29	1.16 (0.63-2.11)	0.633
White cell count			
(≤11/ >11 x10^9/l)	74/ 3	1.13 (0.15-8.34)	0.904
Haemoglobin			
(≥12/ <12 g/dl)	46/ 30	1.75 (0.90-3.42)	0.100
C- reactive protein			
(≤10/ >10 mg/l)	51/ 45	2.50 (1.40-4.48)	0.002
Albumin concentration			
(≥35/ <35 g/l)	90/ 6	3.41 (1.33-8.72)	0.011
GPS			
(0/ 1/ 2)	51/ 39/ 6	2.26 (1.45-3.52)	<0.001
Treatment			
(Herceptin/ active/ supportive)	11/ 81/ 4	2.29 (1.02-5.19)	0.046

Table 2.2: The relationship between clinico-pathological characteristics and an inflammation-based prognostic score (GPS) in patients with metastatic breast cancer.

	GPS 0 (n= 51)	GPS 1 (n=39)	GPS 2 (n=6)	P-value
Age ($\leq$50/ >50years)	10/ 41	10/ 29	1/ 5	0.751
Prior cytotoxic chemotherapies (0/ $\geq$1)	36/ 15	32/ 7	4/ 2	0.409
Metastatic site (Non-visceral/ visceral/ both)	24/ 9/ 18	16/ 5/ 18	3/ 0/ 3	0.684
Oestrogen receptor status (positive/ negative)	27/ 18	30/ 9	6/ 0	0.061
Disease free interval (>2/ $\leq$2 years)	35/ 16	27/ 12	5/ 1	0.756
White cell count ($\leq$11/ >11 x10^9/l)	40/ 1	28/ 2	6/ 0	0.580
Haemoglobin ($\geq$12/ <12 g/dl)	26/ 14	18/ 12	2/ 4	0.334
Treatment (Herceptin/ active/ supportive)	9/ 42/ 0	2/ 33/ 4	0/ 6/ 0	0.044
Survival (months)	23.8 (20.2- 27.5)	12.7 (5.1-20.3)	1.2 (0.7-10.4)	<0.001
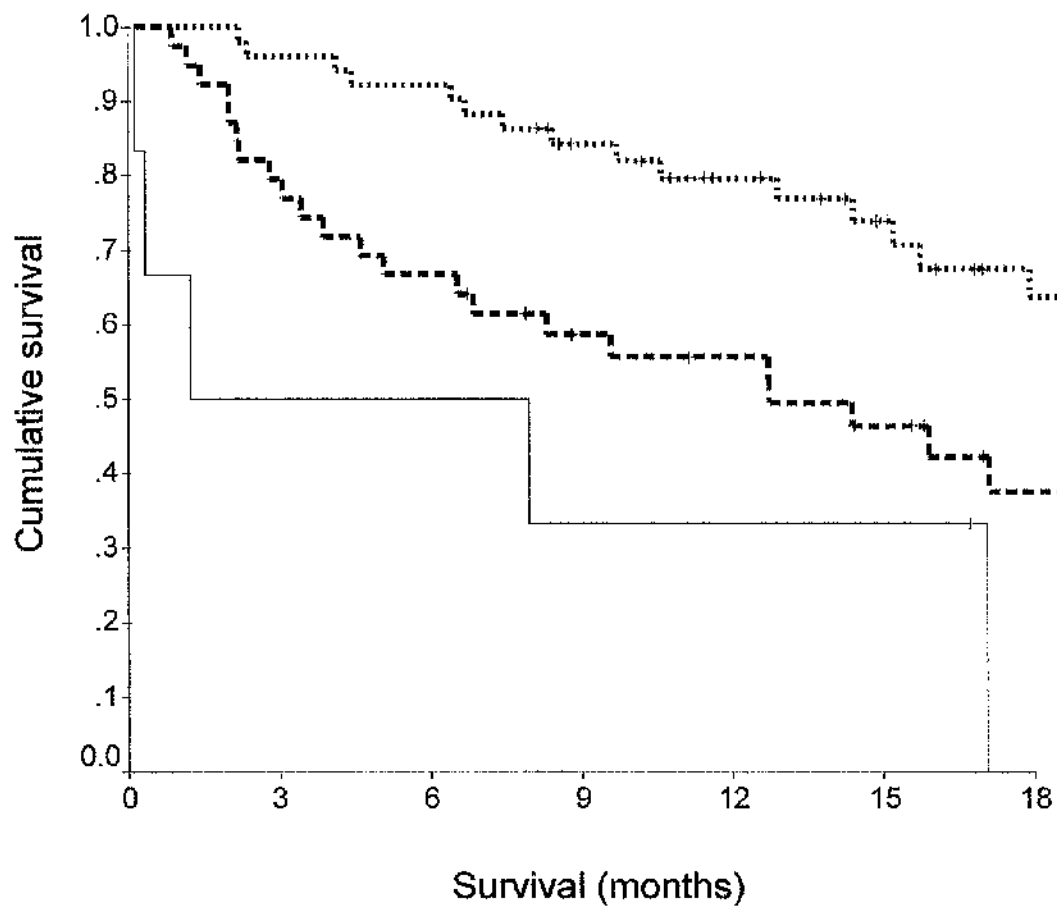
Figure 2.1: The relationship between an inflammation-based prognostic score (GPS, 0, 1, 2 from top to bottom) and survival in patients with metastatic breast cancer.

CHAPTER 3: EVALUATION OF THE RELATIONSHIP BETWEEN THE SYSTEMIC INFLAMMATORY RESPONSE AND CANCER SPECIFIC SURVIVAL IN PATIENTS WITH PRIMARY OPERABLE BREAST CANCER.

3.1 Introduction

Breast cancer is the commonest female malignancy and is a major cause of morbidity and mortality in the Western World. For example, in the UK, there are approximately 42,000 new patients each year and almost 13,000 deaths attributable to the disease (CancerStats, 2003).

It is increasingly recognised that variations in outcome in cancer patients are not solely determined by the characteristics of the tumour but also by the host-response factors (Balkwill and Mantovani, 2001; Coussens and Werb, 2002). However, the tumour-host interaction is complex and has yet to be fully understood. It is now accepted that the host systemic inflammatory response can be assessed by examining the changes in the concentrations of acute phase proteins, such as elevated circulating concentrations of C-reactive protein and low concentrations of albumin (Gabay and Kushner, 1999). It is of interest that, either singly or combined, these factors have been shown to be stage independent prognostic factors in patients with a variety of advanced cancers (McMillan *et al.*, 2001b; Mahmoud and Rivera, 2002; Forrest *et al.*, 2003; Crumley *et al.*, 2006a).

There is also some evidence that the systemic inflammatory response has prognostic value in patients with metastatic breast cancer. For example, previous studies have reported that the presence of elevated circulating concentrations of C-reactive protein (Williams *et al.*, 1990; Albuquerque *et al.*, 1995; Zhang and Adachi, 1999; Chapter 2) and

low concentrations of albumin (Heys *et al.*, 1998; Chapter 2) are associated with poorer survival.

However, few studies have examined the prognostic value of the systemic inflammatory response in patients with primary breast cancer (Mortensen and Rudczynski, 1982; Lis *et al.*, 2003). Those studies appear to have produced conflicting results with regard to whether C-reactive protein or albumin concentrations have independent prognostic value. For example, Mortensen and Rudczynski (1982) studied almost 300 patients with a follow-up period of 3-48 months post-surgery and reported that the presence of an elevated C-reactive protein concentrations was not an independent prognostic factor. In contrast, Lis and coworkers (2003) reported that, in almost 200 patients with a follow-up period of 6-84 months, albumin was a stage independent prognostic factor. However, in both these studies the median follow-up was limited and approximately 20% of patients studied had advanced disease.

Therefore, the prognostic value of C-reactive protein and albumin in patients with primary operable breast cancer remains unclear. The aim of the present study was to examine the relationship between clinico-pathologic status, C-reactive protein and albumin concentrations, measured prior to surgery, and cancer specific survival in patients with invasive primary operable breast cancer.

3.2 Patients and methods

Three hundred patients presenting with invasive primary operable breast cancer to two hospitals (Western Infirmary, Glasgow and Wishaw General Hospital, Lanarkshire) in the West of Scotland between June 2001- July 2003 were prospectively included in the study.

The extent of deprivation was derived from the 1991 census, using the postcode of residence at diagnosis (Carstairs and Morris, 1990; Carstairs and Morris, 1991). The results are presented by amalgamating the seven categories into three groups: affluent (categories 1 and 2), intermediate (categories 3–5) and deprived (categories 6 and 7).

Clinico-pathological data including age, deprivation category, histological type, tumour size, and grade, lymph node status, oestrogen (ER) and progesterone (PR) receptor status. The type of surgery and the use of adjuvant treatment (chemotherapy, hormonal therapy and radiotherapy) were recorded.

Routine pre-operative laboratory measurement of C-reactive protein, albumin and white cell count were carried out. At this time no patients showed clinical evidence of infection or other active chronic inflammatory conditions such as rheumatoid arthritis or crohn's disease. The coefficient of variation for these measurements was less than 10% as established by routine quality control procedures. The limit of detection of C-reactive protein concentration assay was 6 mg/l, with the upper limit of normal values being ≤ 10 mg/l.

The study was approved by the local Research Ethics committees.

Statistics

As appropriate, data are presented as median and range, and comparisons between patient groups were carried out using the Chi-Square-test or Mann-Whitney U test. Statistical analysis was based on the seven individual deprivation categories. Grouping of the laboratory variables was carried out using standard thresholds (Goldwasser and Feldman, 1997; O'Gorman *et al.*, 2000; McMillan *et al.*, 2001b; Maltoni *et al.*, 2005).

The correlation between C-reactive protein, white cell counts and albumin concentrations was performed using the Spearman's Rank correlation. Relapse-free, cancer-specific, overall survival analyses of the group variables were performed using the Cox proportional hazard model. Deaths up to the end of May 2006 were included in the analysis. Multivariate survival analyses, including all covariates that were significant on univariate analysis, were performed using a stepwise backward procedure to derive a final model of the variables that had a significant independent relationship with survival. To remove a variable from the model, the corresponding P-value had to be greater than 0.10. Analysis was performed using SPSS software version 13.0 (SPSS Inc., Chicago, IL, USA).

3.3 Results

The baseline clinico-pathological characteristics of the patients with primary operable breast cancer (n=300) are shown in Table 3.1. Two hundred and thirty three patients (78%) patients were over 50 years of age; 82 (27%) were in the most deprived categories 6 and 7.

Of the 300 patients, 254 (85%) patients had ductal carcinoma, 122 (41%) had a tumour greater than 2 cm, and 130 (43%) had axillary lymph node involvement. The majority of patients had tumour grade II/ III (81%) disease. Sixty two (21%) had oestrogen receptor negative tumours. Twenty out of 300 (7%) patients had evidence of pre-existing co-morbidity, such as liver dysfunction, cardiovascular disease or diabetes mellitus. In one patient this was severe enough to interfere with planned adjuvant treatment. In all, 288 (96%) patients received adjuvant treatment in the form of endocrine therapy and/or chemotherapy.

The majority had a white cell count, albumin and C-reactive protein concentrations in the normal range (96%, 100% and 88% respectively) prior to surgery. White cell count was correlated with C-reactive protein (r_s= 0.13, P=0.023) but not albumin concentration (r_s= 0.03, P=0.587). Albumin was correlated with C-reactive protein concentration (r_s= -0.19, P=0.002).

The minimum follow-up was 35 months; the median follow-up of the survivors was 46 months. During this period, 37 patients relapsed and 25 died of their cancer; a further 14 patients died of intercurrent disease.

On univariate survival analysis, age (*P*<0.10), tumour type (*P*<0.10), tumour size (*P*<0.0001), grade (*P*<0.01), lymph node involvement (*P*<0.01), hormone receptor status (*P*<0.0001), albumin (*P*≤0.001), loco-regional treatment (*P*<0.0001) and systemic treatment (*P*<0.0001) were significantly associated with relapse-free survival (Table 3.1). On multivariate analysis of these significant covariates, age (HR 5.02, 95% CI 1.49-16.93, *P*=0.009), tumour size (HR 2.34, 95% CI 1.18-4.62, *P*=0.015), albumin (HR 3.65, 95% CI 1.71-7.78 *P*=0.001), loco-regional treatment (HR 2.56, 95% CI 1.17-5.59, *P*=0.019) and systemic treatment (HR 2.26, 95% CI 1.54-3.32, *P*<0.0001) were significant independent predictors of relapse-free survival.

On univariate survival analysis, tumour size (*P*<0.0001), grade (*P*<0.01), lymph node involvement (*P*≤0.001), hormone receptor status (*P*<0.0001), albumin (*P*<0.01), loco-regional treatment (*P*<0.0001) and systemic treatment (*P*<0.0001) were significantly associated with cancer-specific survival (Table 3.1). On multivariate analysis of these significant covariates, tumour size (HR 2.53, 95% CI 1.15-5.55, *P*=0.021), albumin (HR 4.44, 95% CI 1.60-12.28, *P*=0.004), loco-regional treatment (HR 3.55, 95% CI 1.30-9.67, *P*=0.013) and systemic treatment (HR 2.67, 95% CI 1.56-4.57, *P*<0.0001) were significant independent predictors of cancer-specific survival.

On univariate survival analysis, age (*P*<0.10), tumour size (*P*<0.0001), grade (*P*<0.05), lymph node involvement (*P*<0.05), hormone receptor status (*P*<0.01), albumin (*P*≤0.001), loco-regional treatment (*P*<0.01) and systemic treatment (*P*<0.001) were significantly associated with overall survival (Table 3.1). On multivariate analysis of these significant covariates, age (HR 4.19, 95% CI 1.26-13.91, *P*=0.019), albumin (HR 3.33, 95% CI 1.60-6.90, *P*=0.001), tumour size (HR 2.48, 95% CI 1.36-4.55, *P*=0.003)

and systemic treatment (HR 2.10, 95% CI 1.45-3.05, $P<0.0001$) were significant independent predictors of overall survival.

Patients were then grouped according to albumin concentrations (>43/ ≤43g/l) as shown in Table 3.2. A lower serum albumin concentration (≤43g/l) was associated with deprivation ($P<0.05$), hormonal receptor negative tumours ($P<0.01$) and significantly poorer 3-year relapse free (85% vs 93%, $P=0.001$) cancer specific (87% vs 97%, $P<0.0001$) and overall survival (84% vs 94%, $P=0.001$) rates.

3.4 Discussion

Predicting recurrence and survival following potentially curative surgical resection for primary operable breast cancer, is conventionally based on standard clinico-pathological criteria such as age, tumour size and grade, nodal status and hormonal receptor status. However, it was of interest that other host-related factors, such as the systemic inflammatory response, have previously been shown to be associated with poor survival following a potentially curative resection for a variety of cancers including gastro-oesophageal, pancreatic, colorectal, and urinary bladder cancers (McMillan *et al.*, 2003; Hilmy *et al.*, 2005; Jamieson *et al.*, 2005; Crumley *et al.*, 2006b).

In the present study, albumin but not C-reactive protein was a significant, stage independent, predictor of survival in patients with primary operable breast cancer. The relationship between albumin concentrations and outcome are therefore consistent with those previously reported in patients with advanced breast cancer (Heys *et al.*, 1998; Chapter 2).

Previous studies have shown that elevated C-reactive protein concentrations have prognostic value in patients with a variety of primary operable tumours (Ikeda *et al.*, 2003; McMillan *et al.*, 2003; Hilmy *et al.*, 2005; Jamieson *et al.*, 2005) and also in patients with advanced breast cancer (Williams *et al.*, 1990; Albuquerque *et al.*, 1995; Zhang and Adachi, 1999; Chapter 2). It was therefore was unexpected that C-reactive protein, either as a continuous or categorical variable, was not a significant prognostic factor in the present study.

The basis of this observation is not clear. However, an elevated C-reactive protein concentration (>10mg/l) was seen in only 12% of patients, a lower proportion than previously seen in both primary operable disease (approximately 20-40%) and in advanced cancer (approximately 40-80%). This may, in part, reflect the relatively small number of events relating to relapse-free survival, cancer specific and overall survival in the present study.

It was of interest that, of the two acute phase proteins examined in the present study, albumin, generally considered to be a relatively insensitive measure of the systemic inflammatory response, had independent prognostic value, even with values within the normal range. It has been previously shown that small reduction in albumin concentrations are associated with certain comorbid conditions such as liver dysfunction, cardiovascular disease or diabetes mellitus and poorer survival in the general population (Goldwasser and Feldman, 1997). In the present study only 7% of patients had such comorbidity; only one patient had planned adjuvant treatment altered, and therefore this would suggest that there may be an interaction between breast cancer and albumin concentrations which might impact on the survival of these patients.

The mechanism by which a low serum albumin might impact on relapse-free, cancer-specific and overall survival is not clear. It may reflect the biological functions of circulating albumin including binding and transporting of hormones and growth factors, inhibition of platelet function and thrombosis (Margarson and Soni, 1998). Furthermore, albumin in the breast cancer cell cytosol may inhibit tumour growth (Soreide *et al.*, 1991), and tumour cell proliferation by modulating the activities of autocrine growth regulatory factors (Laursen *et al.*, 1990).

In the present study, conventional prognostic factors, including tumour size, histological grade, nodal status and hormonal receptor status were significant on univariate analysis. However, including potentially curative loco-regional treatment and systemic treatment that based on hormonal-receptor status in the multivariate analysis, nodal status was not independently significant. This probably reflects the close association between nodal status and the treatment received and their relative impact on relapse and survival.

Nevertheless, lower albumin concentrations prior to surgery, even those within the normal range, may be a sensitive measure of those patients likely to recur and therefore be used to identify high risk patients and alter their treatment accordingly. Furthermore, a follow-up measure of albumin, after the systemic inflammatory response of treatment has resolved, may be of a value to establish whether treatment of the primary tumour has resulted in recovery of albumin concentrations (McMillan *et al.*, 2003; Hilmy *et al.*, 2005; Jamieson *et al.*, 2005). Clearly, further studies are required to establish the prognostic value of pre-operative albumin concentrations and to determine whether longitudinal albumin concentrations may also be of value.

In summary, the results of the present study suggest that pre-operative albumin concentration predicts survival, independent of tumour-based factors, in patients undergoing potentially curative surgery for primary operable breast cancer.

Table 3.1: The clinical and pathological characteristics of patients with invasive primary operable breast cancer and survival (Relapse-free, Cancer-specific, Overall survival), univariate survival analysis.

	Patients (n=300)	Relapse-free Hazard ratio (95% CI)	survival P-value	Cancer-specific Hazard ratio (95% CI)	survival P-value	Overall Hazard ratio (95% CI)	survival P-value
Age (≤50/ >50 years)	67/233	2.52 (0.89-7.11)	0.081	1.56 (0.54-4.54)	0.416	2.60 (0.93 - 7.33)	0.070
Deprivation (1-2/ 3-5/ 6-7)*	39/179/82	0.93 (0.75-1.14)	0.485	1.01 (0.79-1.31)	0.915	1.10 (0.90-1.36)	0.360
Type (Special type/ Lobular/ Ductal)	11/35/254	3.09 (0.83-11.55)	0.094	14.97 (0.33-677.82)	0.164	1.23 (0.59-2.60)	0.583
Size (≤20/ 21-50/ >50 mm)	178/117/5	3.29 (1.83-5.94)	<0.0001	3.56 (1.74-7.26)	<0.0001	2.86 (1.62-5.06)	<0.0001
Grade (I / II / III)	56/148/95	2.23 (1.33-3.74)	0.002	2.85 (1.46-5.58)	0.002	1.74 (1.07-2.82)	0.026
Involved lymph node (0/ 1-3/ >3)	169/92/38	1.90 (1.25-2.88)	0.003	2.37 (1.44-3.93)	0.001	1.52 (1.01-2.30)	0.046
Hormonal-receptor status (ER+ PR-/ ER+ PR- or unknown / ER- PR- or unknown)	116/122/62	2.61 (1.66-4.13)	<0.0001	2.76 (1.57-4.85)	<0.0001	1.94 (1.26-2.97)	0.002
White cell count (10^9/l)**	7.0 (3.4-17.4)	0.97 (0.83-1.15)	0.755	0.94 (0.76-1.16)	0.559	1.04 (0.89-1.21)	0.619
White cell count (<8.5/ 8.5-11/ >11 $x10^9$/l)	230/53/13	0.81 (0.41-1.60)	0.547	1.07 (0.52-2.21)	0.857	1.36 (0.81-2.29)	0.247
Albumin concentration (g/l)**	44 (35-52)	0.88 (0.78-0.99)	0.035	0.82 (0.71-0.94)	0.006	0.88 (0.78-0.98)	0.026
Albumin concentration (>43/ ≤43 g/l)	155/114	3.39 (1.61-7.12)	0.001	5.01 (1.85-13.57)	0.002	3.23 (1.58-6.59)	0.001
C-reactive protein concentration (mg/l)**	≤6 (≤6-66)	0.96 (0.88-1.04)	0.293	0.97 (0.89-1.06)	0.518	0.97 (0.90-1.04)	0.391
C-reactive protein (≤10/ >10 mg/l)	265/35	0.40 (0.10-1.68)	0.211	0.62 (0.15-2.65)	0.522	0.60 (0.19-1.95)	0.395
Loco-regional treatment (Mastectomy or conservation surgery + radiotherapy/ mastectomy + radiotherapy)	221/79	3.70 (1.94-7.06)	<0.0001	5.51 (2.43-12.48)	<0.0001	2.40 (1.27-4.51)	0.007
Systemic treatment (ER-based treatment) (hormonal/ hormonal + chemotherapy/ chemotherapy/ none)	150/86/52/9	2.03 (1.43-2.88)	<0.0001	2.59 (1.67-4.01)	<0.0001	1.93 (1.37-2.73)	<0.0001

*: Individual deprivation categories were used in the statistical analysis, **: median (range).

Table 3.2: The clinico-pathological characteristics of patients with invasive primary operable breast cancer according to albumin concentrations.

	Albumin >43 g/l (n= 155)	Albumin ≤43 g/l (n= 114)	(P-value)
Age (≤50/ >50 years)	35/ 120	26/ 88	0.965
Deprivation (1-2/ 3-5/ 6-7)*	26/ 90/ 39	13/ 68/ 33	0.011
Type (Special type/ Lobular/ Ductal)	8/ 17/ 130	3/ 15/ 96	0.524
Size (≤20/ 21-50/ >50 mm)	96/ 57/ 2	62/ 49/ 3	0.384
Grade (I / II / III)	31/ 72/ 52	18/ 65/ 30	0.201
Involved lymph node (0/ 1-3/ >3)	93/ 47/ 15	60/ 35/ 18	0.269
Hormonal-receptor status			
(ER+ PR+/ ER+ PR- or unknown / ER- PR-			
or unknown)	80/ 47/ 28	35/ 54/ 25	0.002
White cell count (10^9/l)**	6.8 (3.6-13.9)	7.0 (3.4–17.4)	0.920
White cell count (<8.5/ 8.5-11/ >11 x10^9/l)	127/ 18/ 8	83/ 27/ 3	0.281
C- reactive protein (mg/l)**	<6 (<6-57)	<6 (<6-66)	0.138
C- reactive protein (≤10/ >10 mg/l)	136/ 19	102/ 12	0.660
Loco-regional treatment			
(Mastectomy or conservation surgery +			
radiotherapy/ mastectomy + radiotherapy)	120/ 35	78/ 36	0.098
Systemic treatment (ER-based treatment)			
(Hormonal/ hormonal + chemotherapy/			
chemotherapy/ none)	74/ 52/ 25/ 3	64/ 25/ 21/ 4	0.185
3 years relapse-free survival rate***	93 (2)	85 (3)	0.001
3 years cancer-specific survival rate***	97 (1)	87 (3)	<0.0001
3 years overall survival rate***	94 (2)	84 (3)	0.001

*: Individual deprivation categories were used in the statistical analysis, **: median (range), ***: 3 year survival rate (SE).

CHAPTER 4: THE RELATIONSHIP BETWEEN THE SYSTEMIC INFLAMMATORY RESPONSE, TUMOUR PROLIFERATIVE ACTIVITY, T-LYMPHOCYTIC AND MACROPHAGE INFILTRATION, MICROVESSEL DENSITY AND SURVIVAL IN PATIENTS WITH PRIMARY OPERABLE BREAST CANCER.

4.1 Introduction

It is now recognised that the development of cancer and its progression is dependent on a complex interaction of the tumour and the host inflammatory response (Balkwill and Mantovani, 2001; Coussens and Werb 2002; Vakkila and Lotze 2004). Recently, the systemic inflammatory response, as evidenced by elevated circulating concentrations of C-reactive protein and hypoalbuminaemia, has been shown to be independently associated with poorer survival in patients with advanced disease (McMillan *et al.*, 2001b; Forrest *et al.*, 2003; Maltoni *et al.*, 2005) including breast cancer (Albuquerque *et al.*, 1995; Zhang and Adachi, 1999; Chapter 2). There is also some evidence that these acute phase proteins have independent prognostic value in primary operable disease (McMillan *et al.*, 2003; Jamieson *et al.*, 2005; McMillan *et al.*, 2007) including breast cancer (Lis *et al.*, 2003; Chapter 3).

In animal models, at least, it would appear that the cell-mediated immune response is more important than humoural immunity in preventing the progression of cancer and there is some evidence that cell-mediated immunity can bring about tumour regression. The principal cells involved in the cell-mediated response are T-lymphocytes and macrophages (O'Sullivan and Lewis, 1994; Lee *et al.*, 1996; Ogmundsdottir, 2001;

Whiteside, 2003; Lee *et al.*, 2006). However, this immune response, in particular the local environment of cytokines, proteases, angiogenic/ growth factors, and the resulting systemic inflammatory response, may, in turn, stimulate tumour growth and metastasis (O'Sullivan and Lewis, 1994; Lewis *et al.*, 1995; Leek *et al.*, 1996; Leek and Harris, 2002; Yu and Rak, 2003; Lewis and Pollard, 2006).

A number of studies have observed that, in breast tumours, there is a diffuse infiltrate of T-lymphocytes and macrophages (Lee *et al.*, 1996; Lee *et al.*, 2006). Also, that there is an association with better outcome in patients with a moderate or marked diffuse inflammatory pattern in the subgroup of high grade cases (Pupa *et al.*, 1996; Lee *et al.*, 2006). Recently, the use of immunohistochemical techniques to reliably identify and assess tumour-infiltrating T-lymphocytes subsets and macrophages has led to renewed interest in the relationship between the tumour inflammatory infiltrate and cancer specific survival in a variety of common solid tumours. With reference to tumour CD4+ T-lymphocytic infiltration a significant association with survival has been shown in renal (Bromwich *et al.*, 2003), prostate (McArdle *et al.*, 2004) colorectal (Canna *et al.*, 2005) and head and neck cancers (Badoual *et al.*, 2006). However, few studies have examined the association between tumour CD4+/ CD8+ T-lymphocytic infiltration and/or CD68+ macrophage infiltration and survival in patients with primary operable breast cancer (Griffith *et al.*, 1990; Wintzer *et al.*, 1991; Leek *et al.*, 1996; Toi *et al.*, 1999; Tsutsui *et al.*, 2005).

Griffith and coworkers (1990), as well as, Wintzer and coworkers (1991) have both reported that disease-free survival and overall survival in breast cancer patients were not influenced by the tumour infiltration of any lymphocyte subset. However, these were

relatively small studies of less than 80 patients. In contrast, different monocyte subsets appeared to either be associated with good or poor disease-free survival (Toi *et al.*, 1999). Furthermore, in studies between 100 -250 cases, there was conflicting evidence as to whether or not CD68+ macrophage infiltration was superior to microvessel density in predicting disease-free survival (Leek *et al.*, 1996; Tsutsui *et al.*, 2005).

Therefore, the inter-relationship between local and systemic inflammatory responses and its prognostic significance in patients with primary operable breast cancer remains unclear. The aim of the present study was to examine the relationship between circulating concentrations of C-reactive protein and albumin, tumour infiltration of T-lymphocyte subpopulations and macrophages and survival in patients who had undergone potentially curative surgical resection for invasive primary operable breast cancer.

4.2   Patients and methods

One hundred and sixty eight patients presenting with invasive primary operable breast cancer to two hospitals (Western Infirmary, Glasgow and Wishaw General Hospital, Lanarkshire) in the West of Scotland between June 2001- December 2002 were studied prospectively.

Clinico-pathological data included the age, deprivation category, histological type and grade (Figure: 4.1 - 4.14), tumour size, lymph node status, and oestrogen (ER) and progesterone (PR) receptor status. The type of surgery and the use of adjuvant treatment (chemotherapy, hormonal therapy and radiotherapy) were recorded.

The extent of deprivation was derived from the 1991 census, using the postcode of residence at diagnosis (Carstairs and Morris, 1990; Carstairs and Morris, 1991). The results are presented by amalgamating the seven categories into three groups: affluent (categories 1 and 2), intermediate (categories 3–5) and deprived (categories 6 and 7).

Routine pre-operative laboratory measurement of C-reactive protein, albumin and white cell count were carried out. At this time no patients showed clinical evidence of infection or other inflammatory conditions. The coefficient of variation for these measurements was less than 10% as established by routine quality control procedures. The limit of detection of C-reactive protein concentration assay was 6 mg/l, with the upper limit of normal values being ≤ 10mg/l.

The study was approved by the local Research Ethics committees.

Immunohistochemistry

For the immuno-histochemistry of CD4+ and CD8+ (T-lymphocytes), CD68+ (tumour associated macrophages), Ki-67 (proliferative index) and CD34+ (microvessel density), blocks from the primary tumour were fixed in 10% buffered formalin in saline and embedded in paraffin wax. One representative block of tumour was selected for each patient. Serial individual sections (4µm) were cut and mounted on slides coated with aminopropyltriethoxysilane and placed in oven at 56 °C for 40 minutes.

Slides were dewaxed in xylene for 4 minutes twice and rehydrated through graded alcohols then rinsed with water.

The following mouse monoclonal antibodies were used: CD4 (Vector, Peterborough, UK) at dilution of 1:10, CD8 (Dako, Cambridgeshire, UK) at dilution of 1:1000, CD68 (Dako, Cambridgeshire, UK) at dilution of 1:200, Ki-67 (Dako, Cambridgeshire, UK) at dilution of 1:500 and CD34 (Novocastra, Newcastle upon Tyne, UK) at dilution of 1:50.

Antigen retrieval for CD4, CD8, CD68 and Ki-67 was carried out by microwaving in Tris EDTA buffer solution (0.555g Sodium EDTA, 0.825g Trisma base in 1.5 litre distilled water; PH: 8) for 5 minutes under full pressure in a plastic pressure cooker in a 850W microwave on full power. For CD34, antigen retrivel was carried out using 1 % trypsin in 1% $CaCl_2$ solution (0.1 g $CaCl_2$, 0.1 g trypsin in 100ml H_2O) at 37°C for 25 minutes.

Staining method for CD4, CD8, CD68 and Ki-67 was as follows:

1. 5% Normal Goat Serum 20 minutes.

2. Primary antibody 30 minutes.

3. Wash in Tris Buffered Saline (TBS).

4. Hydrogen Peroxide blocking agent (Dako S2023).

5. Wash in TBS.

6. Envision (Dako, Cambridgeshire, UK) 30 minutes.

7. Wash in TBS.

8. DAB (Dako, Cambridgeshire, UK) 10 minutes.

9. Wash in water.

Staining method for CD34 was as follows:

1. Blocking solution (15μl horse serum per 1ml TBS) 20 minutes.

2. Primary antibody 60 minutes.

3. Wash in TBS for 5 minutes.

4. Envision (Dako, Cambridgeshire, UK) 30-60 minutes.

5. Wash in TBS for 5 minutes.

6. DAB (Dako, Cambridgeshire, UK) 2-10 minutes.

7. Wash in water for 10 minutes.

Appropriate positive controls were included in each run. Negative controls were stained using antibody diluting fluid alone.

Tissues were counter-stained with haematoxylin for 15 seconds then wash with water, followed by scotts water for another 30 seconds before washing them with water. Then, copper enhancement for CD4, CD8, CD68 and Ki-67 was carried out for 5 minutes then wash with water. Finally, Slides were dehydrated through graded alcohols 70%, 90% and 100% and then xylene. The slides were cleared, mounted with Pertex and appropriate slide covers applied.

IL-6 staining

Previous attempts to reliably identify regions of IL-6 expression in colorectal and bladder cancers were unsuccessful. Different methods of antigen retrieval and staining, with the use of negative and positive controls, have been tried. However, the background staining precluded accurate scoring of IL-6 positive cells in the tumour tissue sections. The reasons for this poor quality of IL-6 immunostaining are unclear. On inspection of previous equivalent IL-6 staining in colorectal carcinoma by Kinoshita and co-workers (1999), it would appear likely that non-specific binding by the primary antibodies was a problem. Therefore, the unsuccessful results of IL-6 immunostaining have precluded further attempts to be undertaken, including this study on breast tumours.

Morphometry

CD4+, CD8+ and CD68+

Quantitative analysis of the lymphoid infiltrate (CD4+ and CD8+) and tumour associated macrophages (CD68+) were performed using a point counting method (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 x 400 magnification and 30 fields were counted per case for CD4+, CD8+ and CD68+ immunopositive cells (Figure: 4.15-4.20).

Ki-67

The percentages of Ki-67-reactive tumour cells were evaluated at x 400 magnification by scoring a minimum of 1000 tumour cells in randomly selected fields (Ki-67 labelling index; Figure: 4.21 and 4.22).

CD34+

Quantitative analysis of the microvessel density was performed by selecting the three most vascular areas (hot spots), where the highest numbers of discrete microvessels were stained, at low power (x40 and x100). Counting of discrete vessels was performed with a magnification of x 200, using a 25- point Chalkley grid as described by Hansen *et al.* (2000a,b; Figure: 4.23 and 4.24).

Only fields containing tumour (including tumour nest and surrounding tissues stroma) were counted. Any normal tissue on the slide was excluded from the analysis. All cases were counted by the author (AA). For the purpose of assessing inter-observer reproducibility, a second observer (JB) and (MH) independently scored the slides for T-lymphocytes (CD4+ and CD8+), and the tumour associated macrophages (CD68+) and CD34+ respectively. The intra-observer intraclass correlation coefficients (ICCC) were as follow; CD4+ = 0.71, CD8+ = 0.93, CD68+ = 0.79, Ki67 = 0.98 and CD34+ = 0.93. The inter-observers ICCC were as follow; CD4+ = 0.69, CD8+ = 0.78, CD68+ = 0.67, and CD34+ = 0.94 (ICCC values $\geq$ 0.6 were considered acceptable and ICCC $\geq$ 0.9 were considered excellent). The observers were blinded to the clinical outcome of the patient.
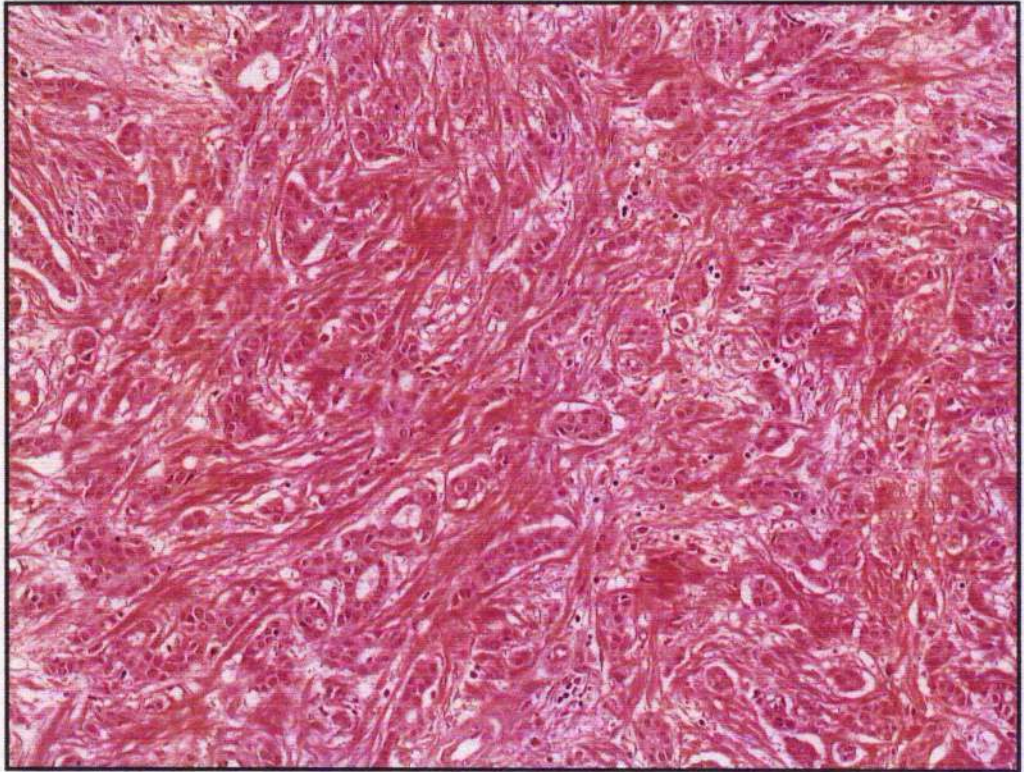
Figure 4.1: Invasive ductal carcinoma- Grade I (x100).
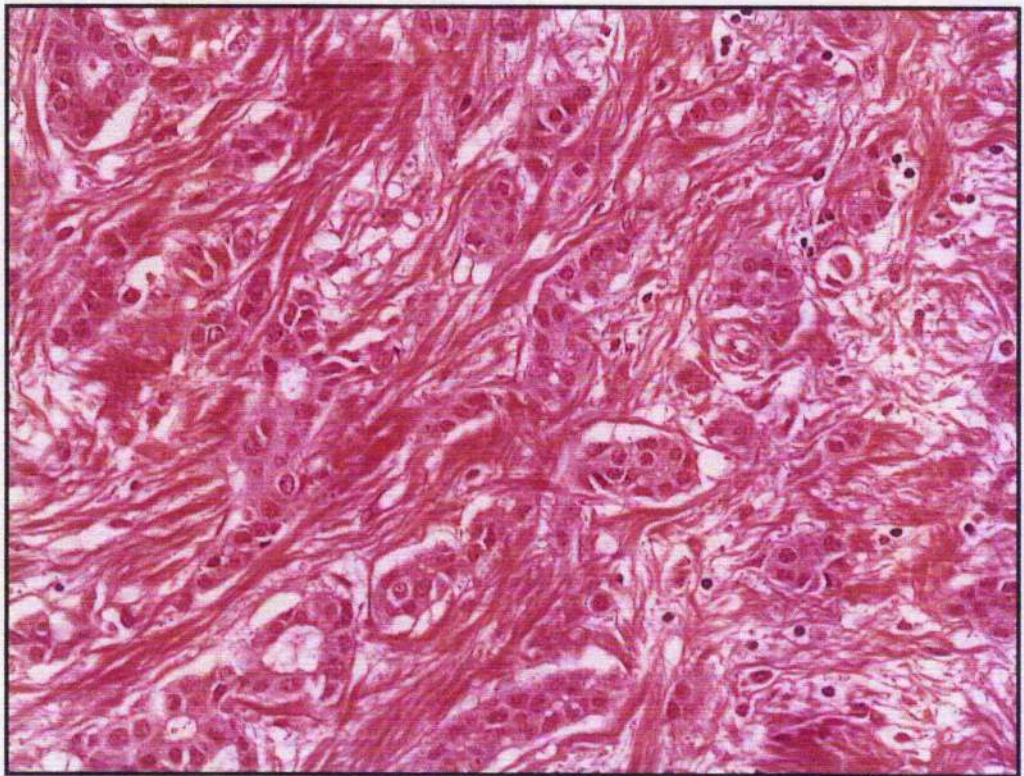
Figure 4.2: Invasive ductal carcinoma- Grade I (x200).
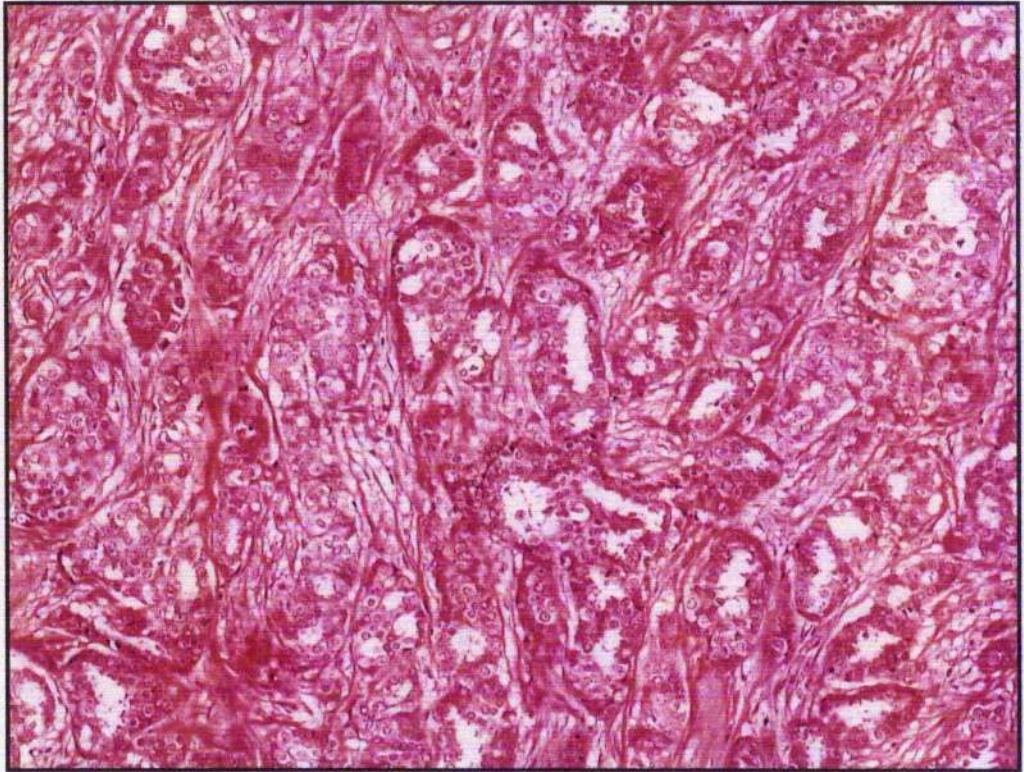
Figure 4.3: Invasive ductal carcinoma- Grade II (x100).
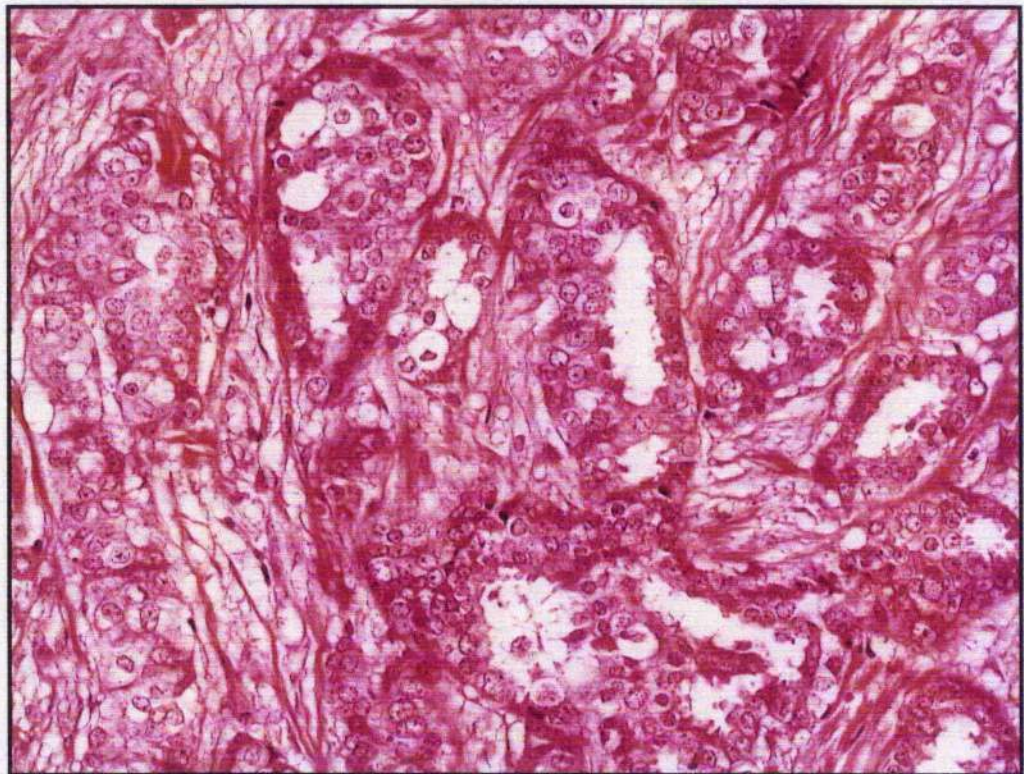
Figure 4.4: Invasive ductal carcinoma- Grade II (x200).
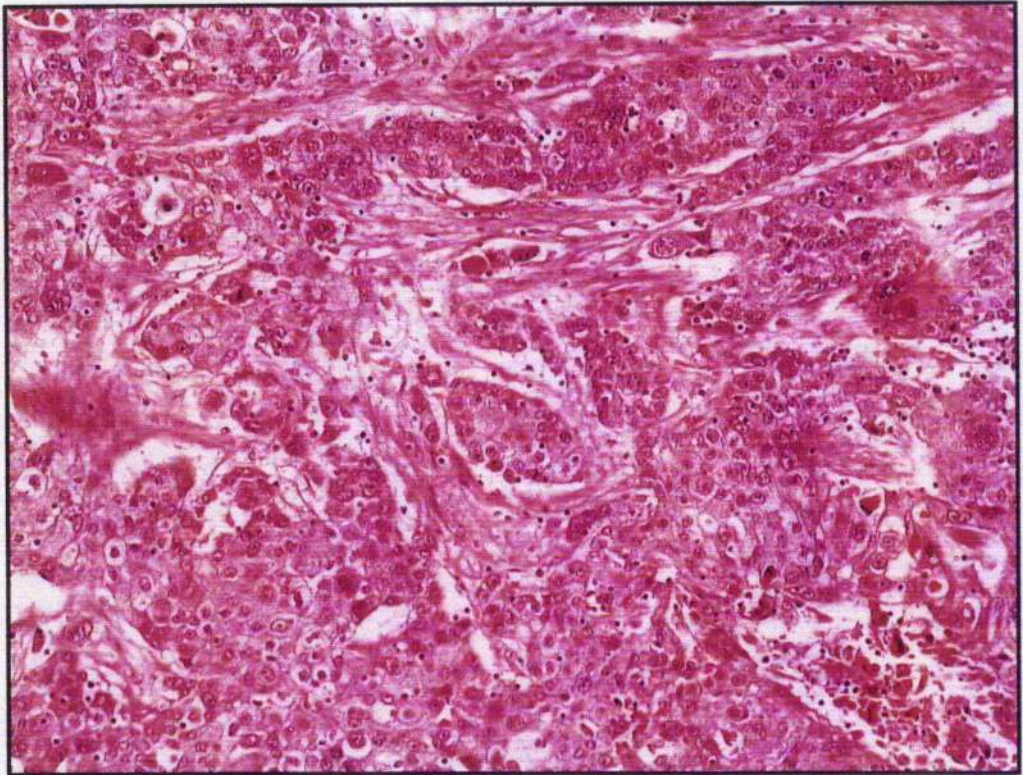
Figure 4.5: Invasive ductal carcinoma- Grade III (x100).
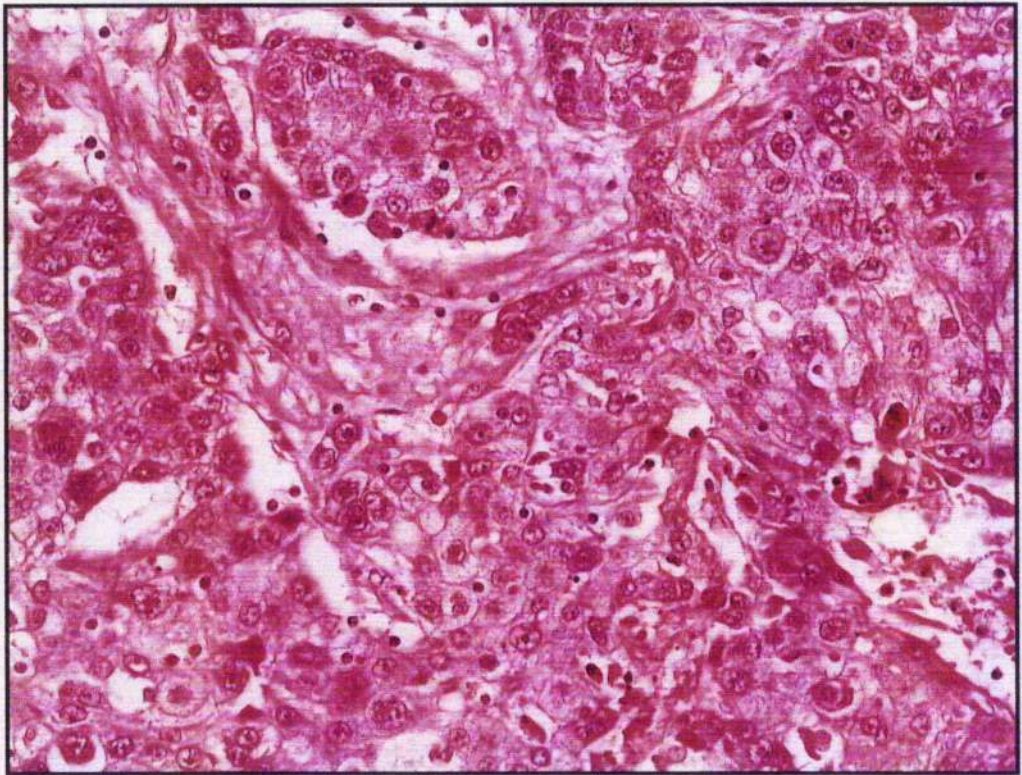
Figure 4.6: Invasive ductal carcinoma- Grade III (x200).
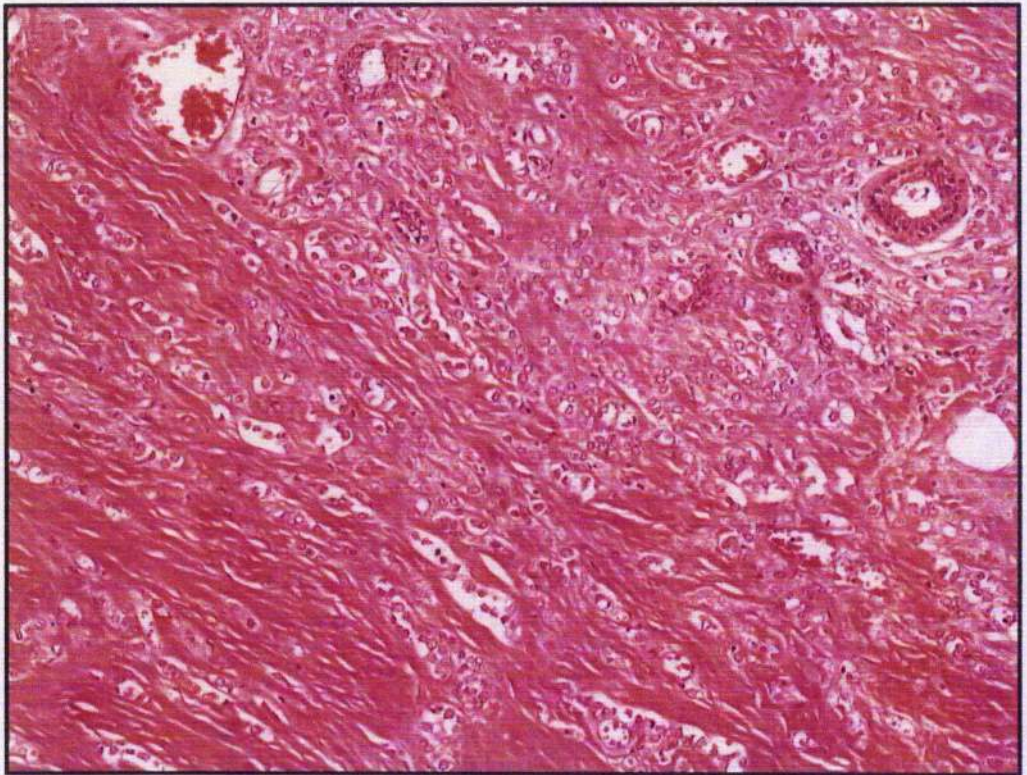
Figure 4.7: Invasive lobular carcinoma- Grade I (x100).
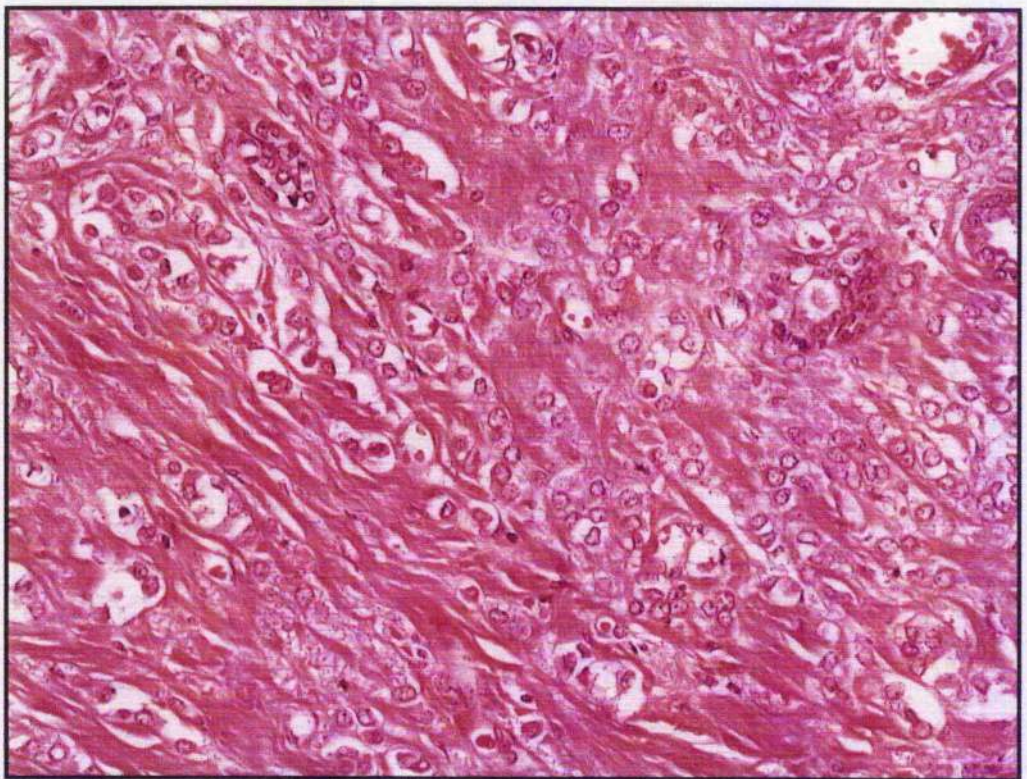
Figure 4.8: Invasive lobular carcinoma- Grade I (x200).
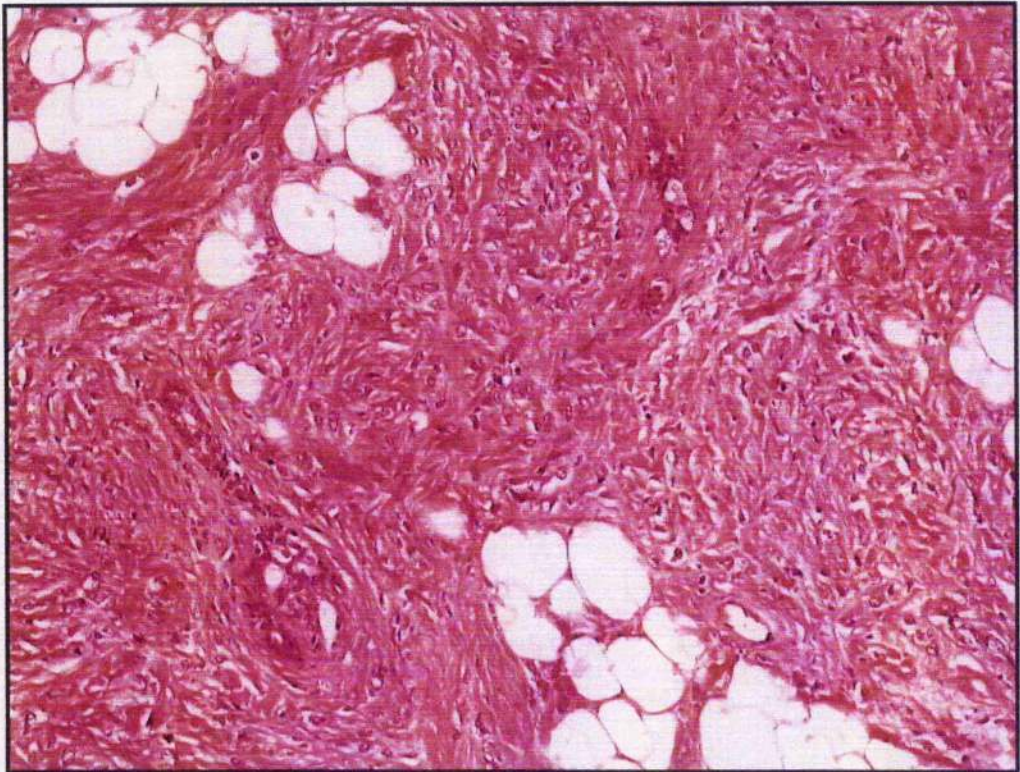
Figure 4.9: Invasive lobular carcinoma- Grade II (x100).
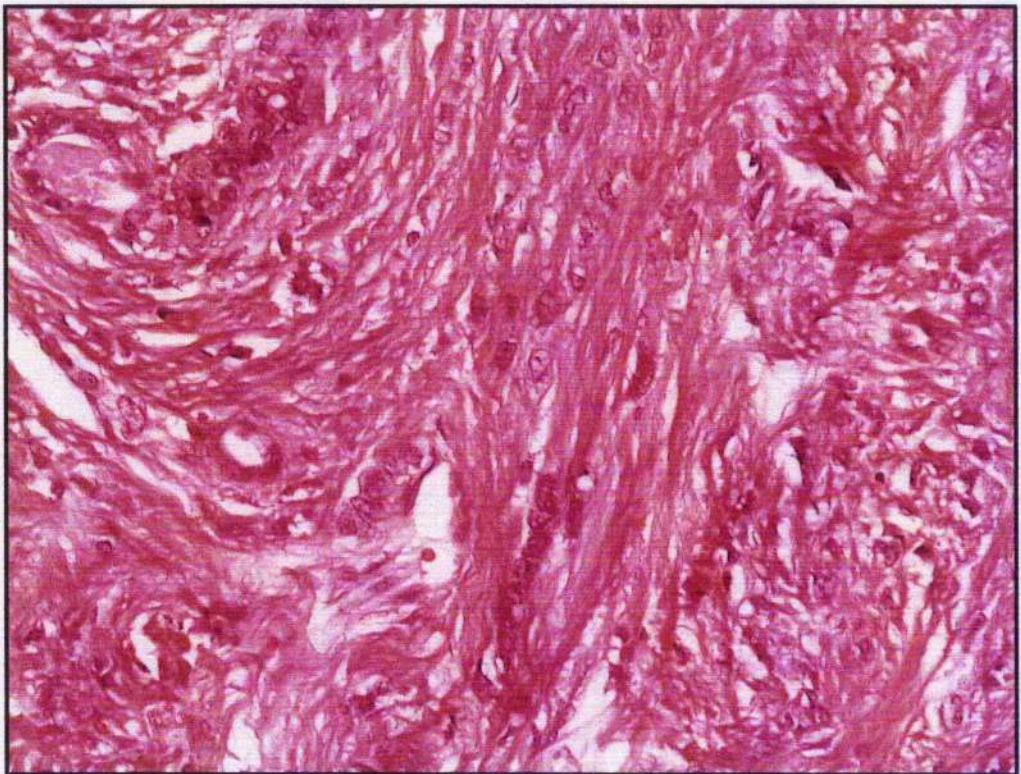
Figure 4.10: Invasive lobular carcinoma- Grade II (x200).
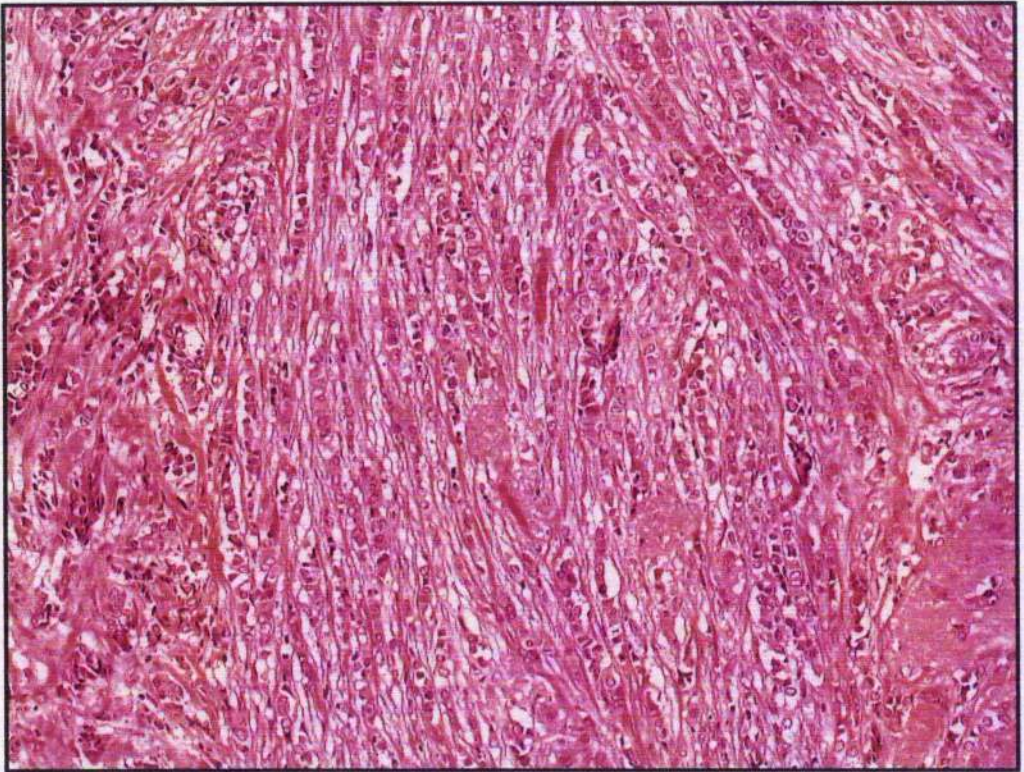
Figure 4.11: Invasive lobular carcinoma- Grade III (x100).
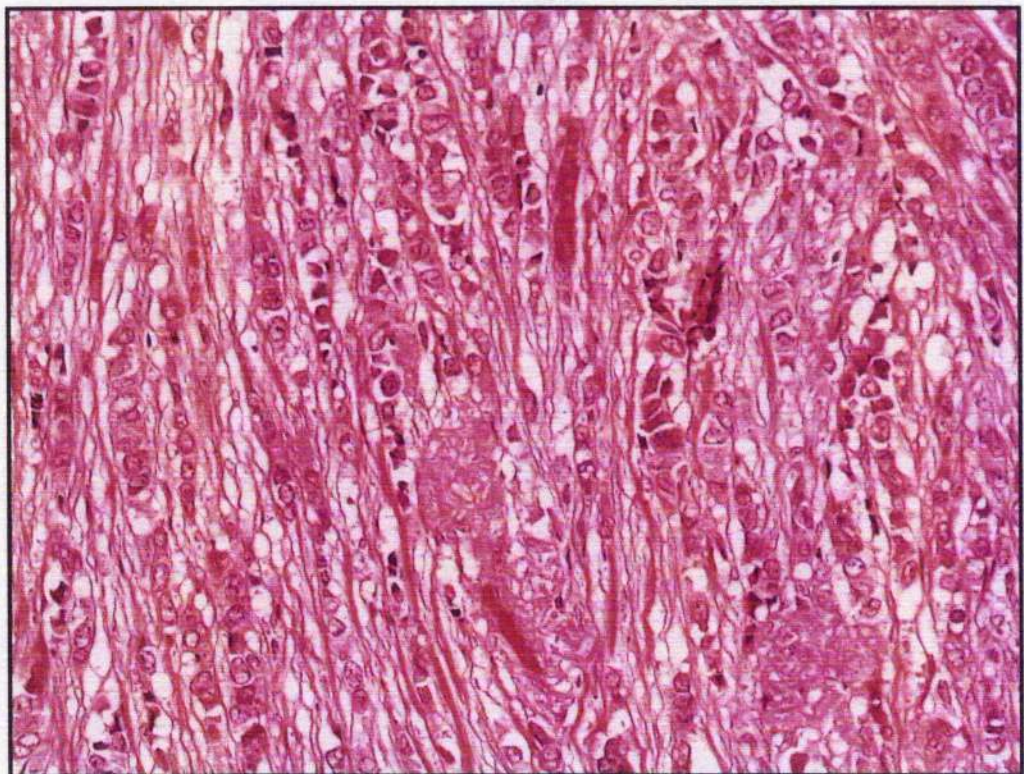
Figure 4.12: Invasive lobular carcinoma- Grade III (x200).
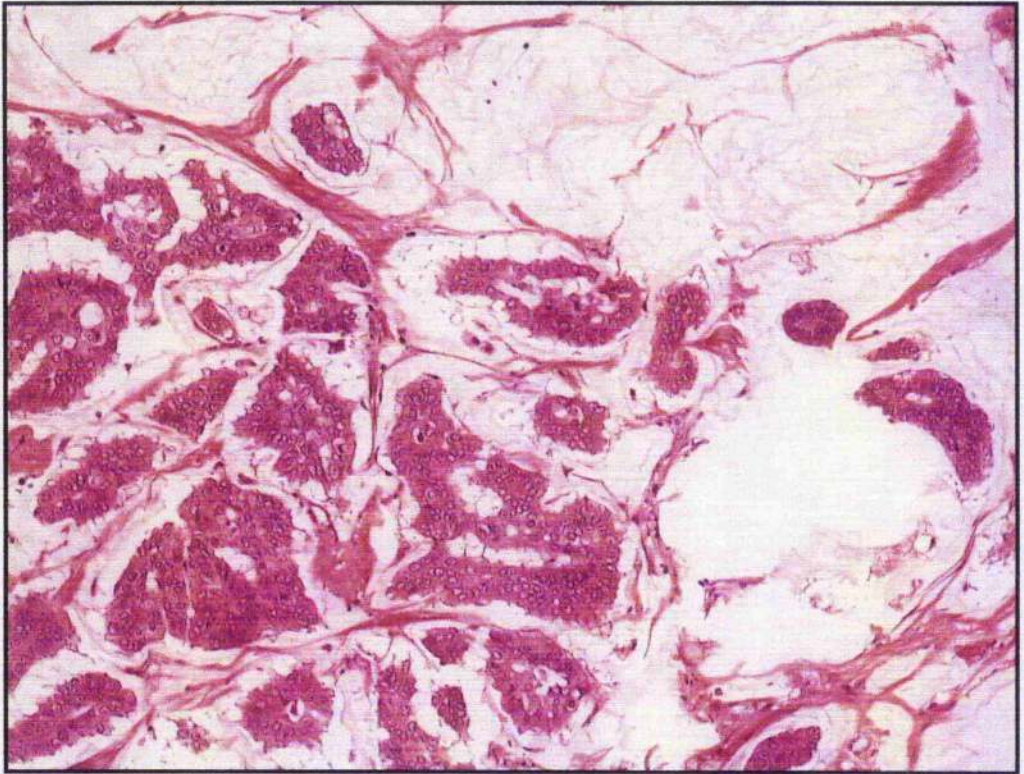
Figure 4.13: Invasive mucinous carcinoma- Grade I (x100).
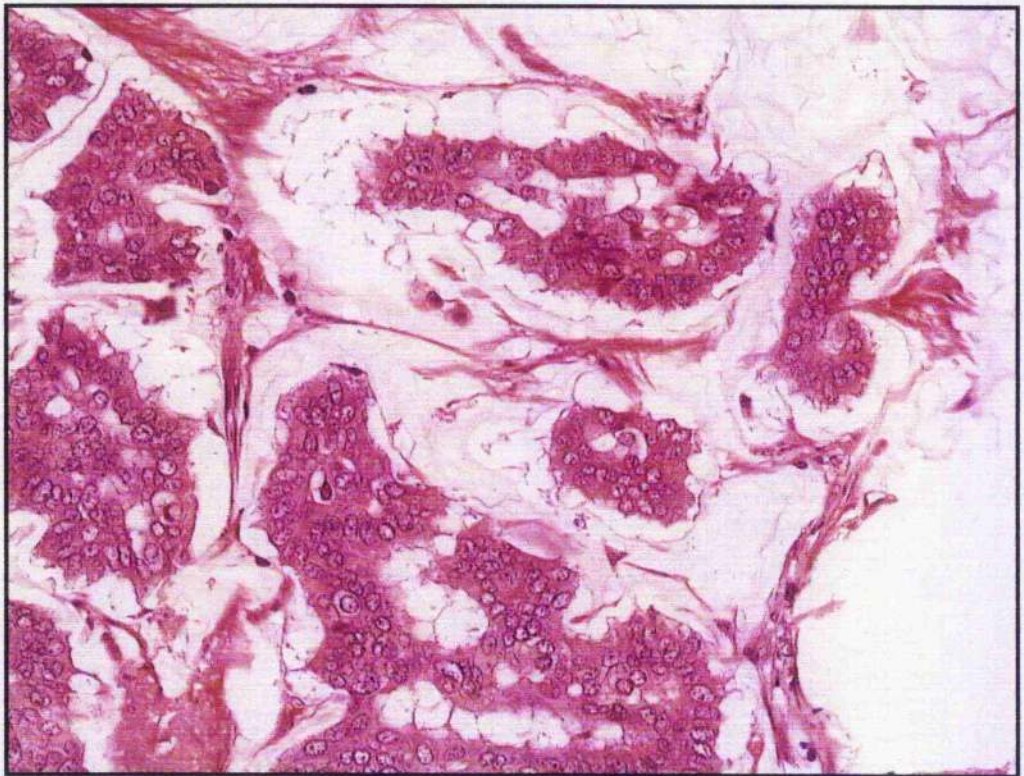
Figure 4.14: Invasive mucinous carcinoma- Grade I (x200).
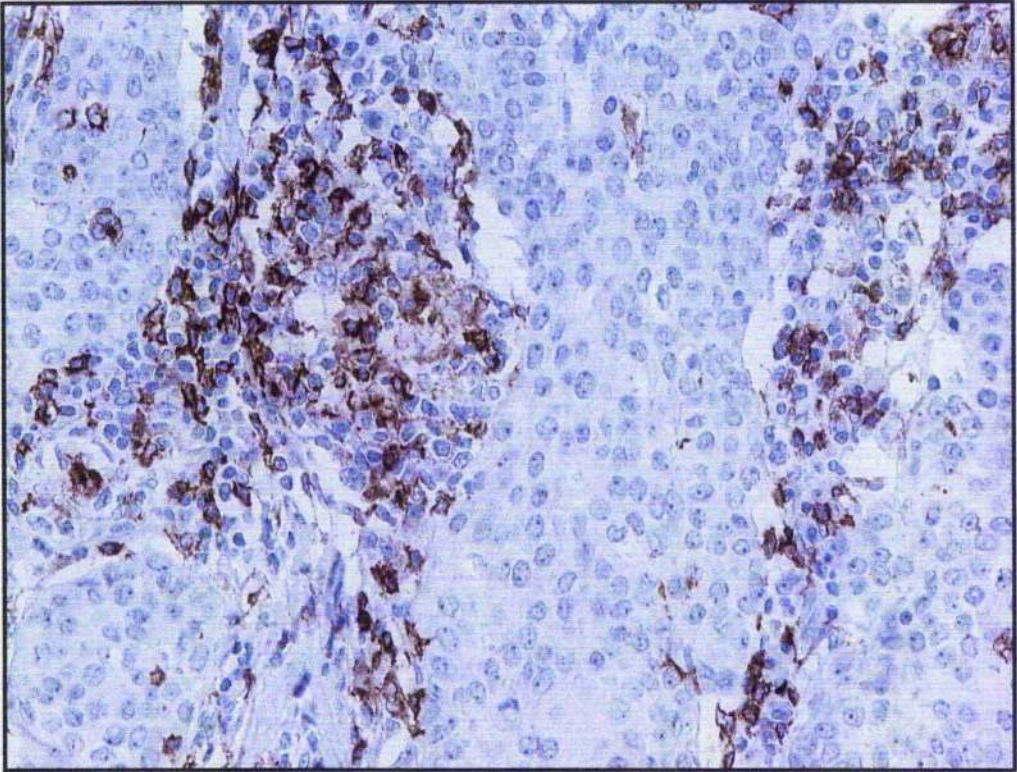
Figure 4.15: CD4+ immunohistochemical staining in invasive breast cancer (x200).
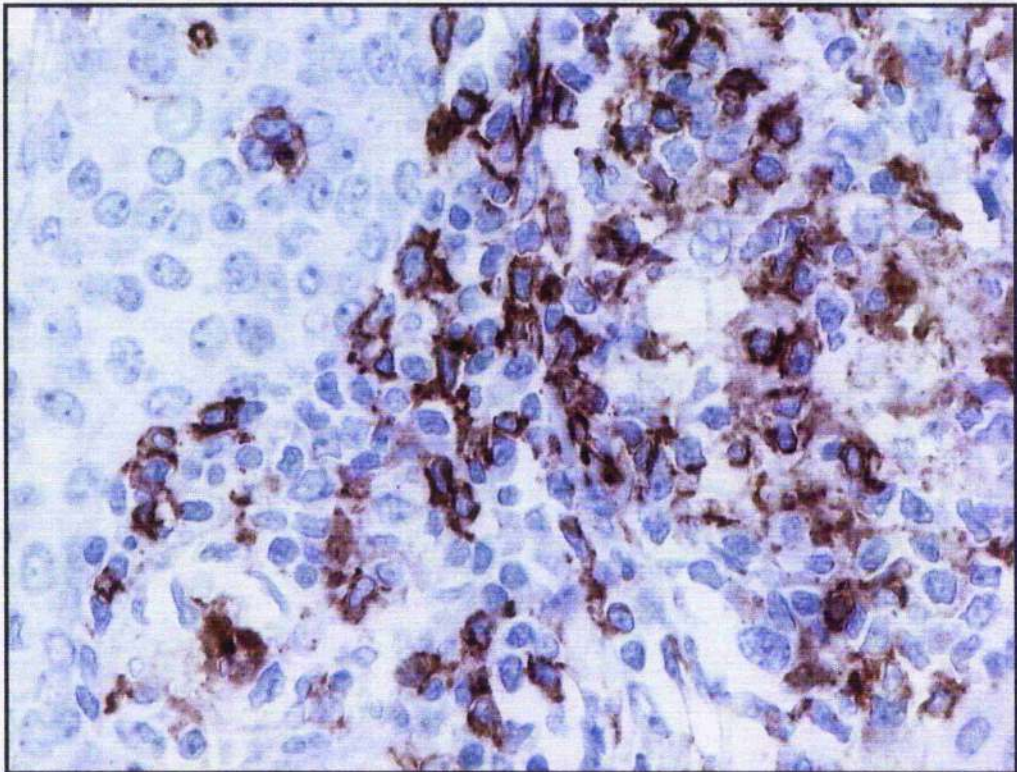
Figure 4.16: CD4+ immunohistochemical staining in invasive breast cancer (x400).
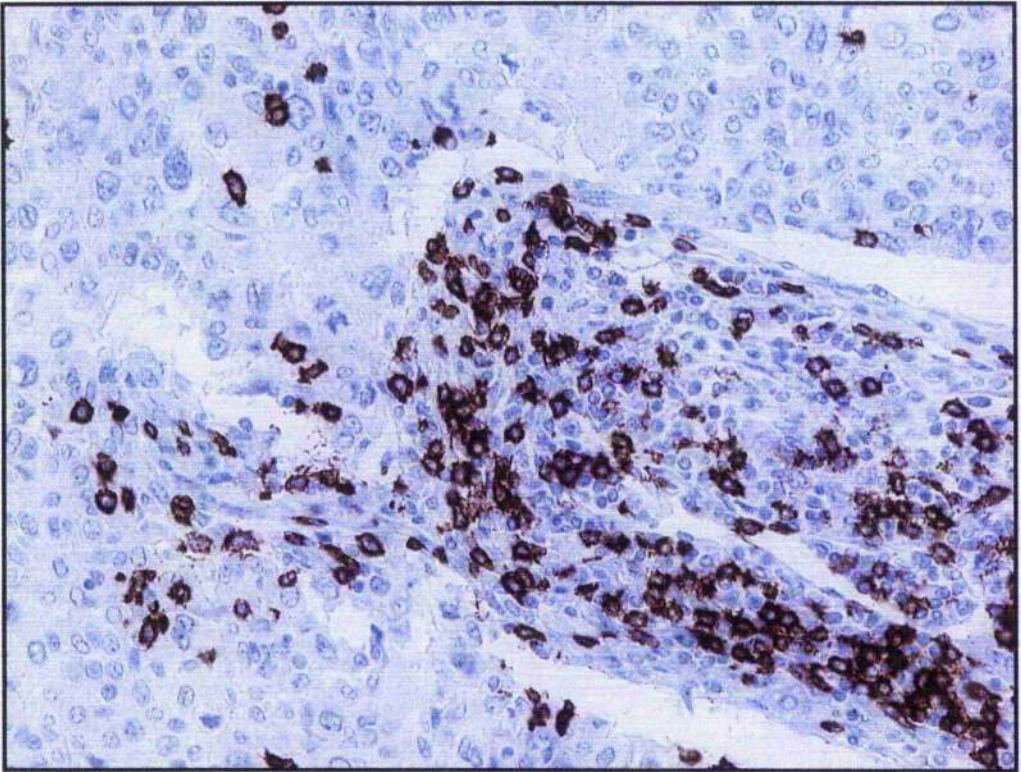
Figure 4.17: CD8+ immunohistochemical staining in invasive breast cancer (x200).
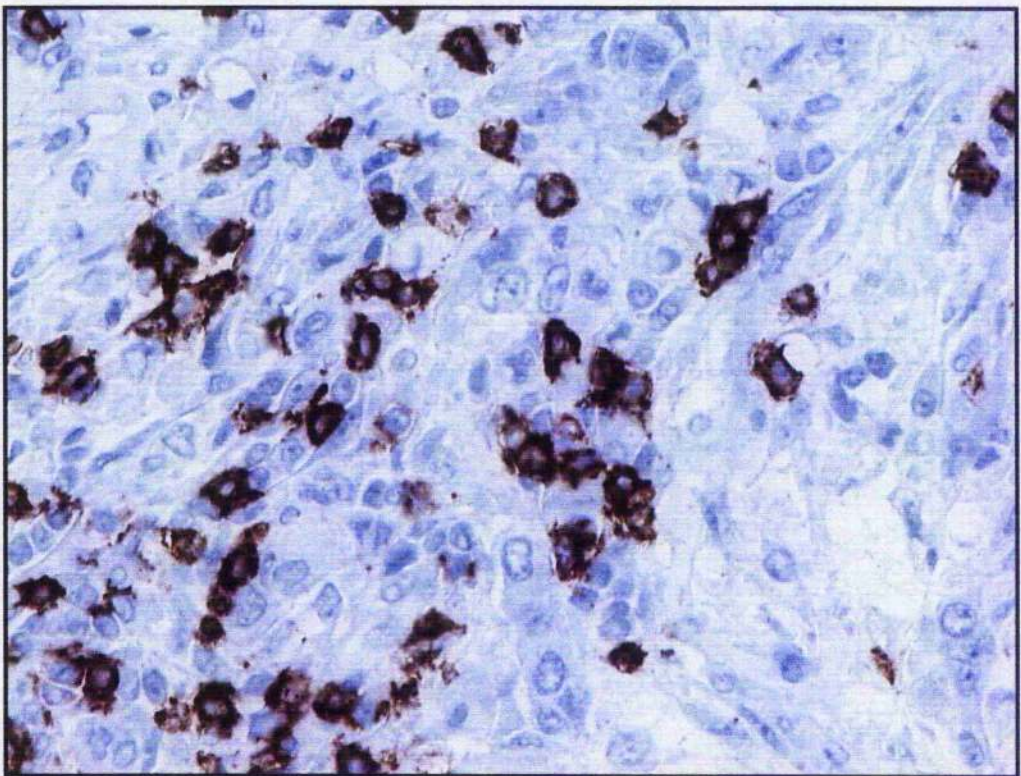
Figure 4.18: CD8+ immunohistochemical staining in invasive breast cancer (x400).
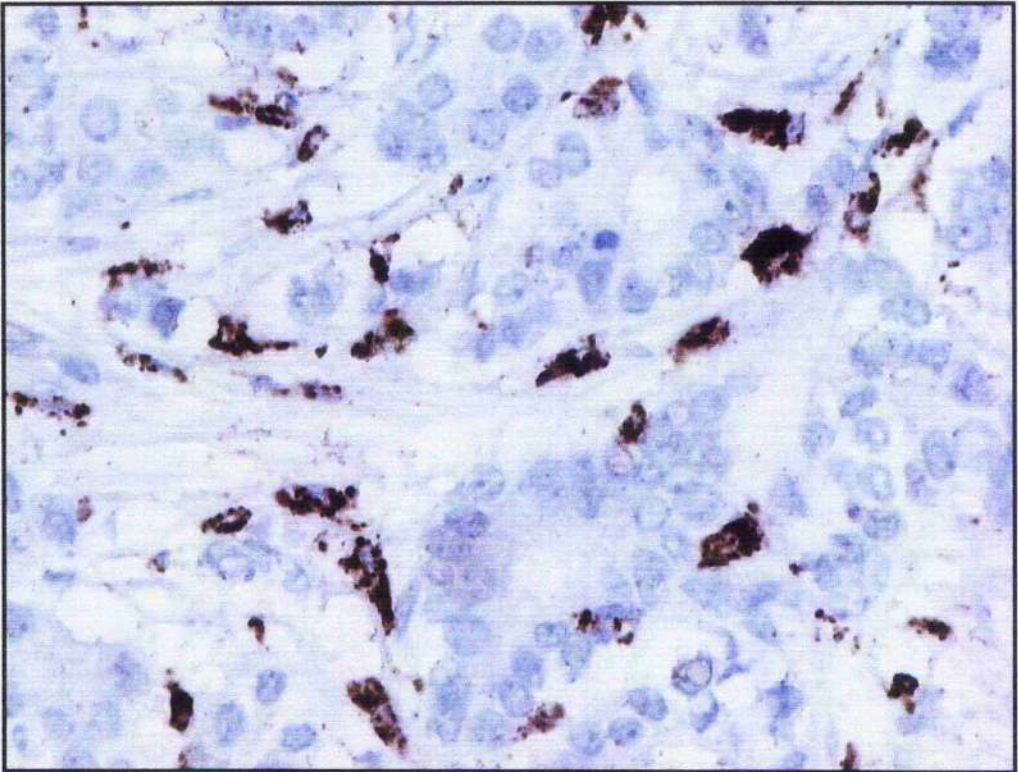
Figure 4.19: CD68+ immunohistochemical staining in invasive breast cancer (x400).
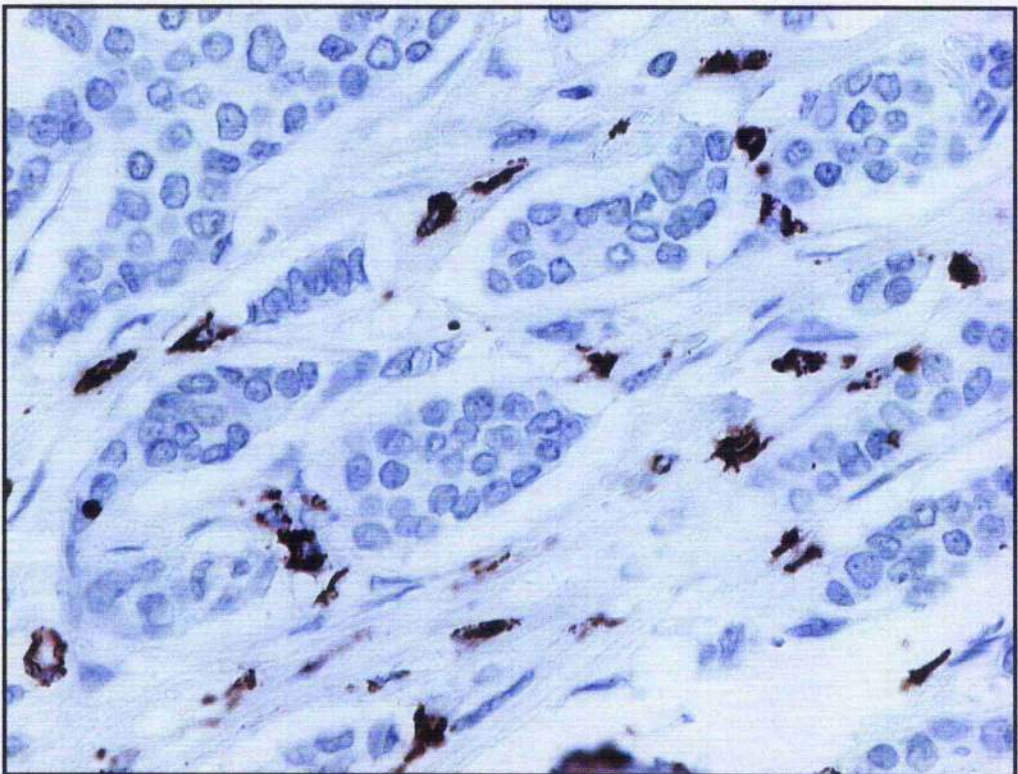
Figure 4.20: CD68+ immunohistochemical staining in invasive breast cancer (x400).
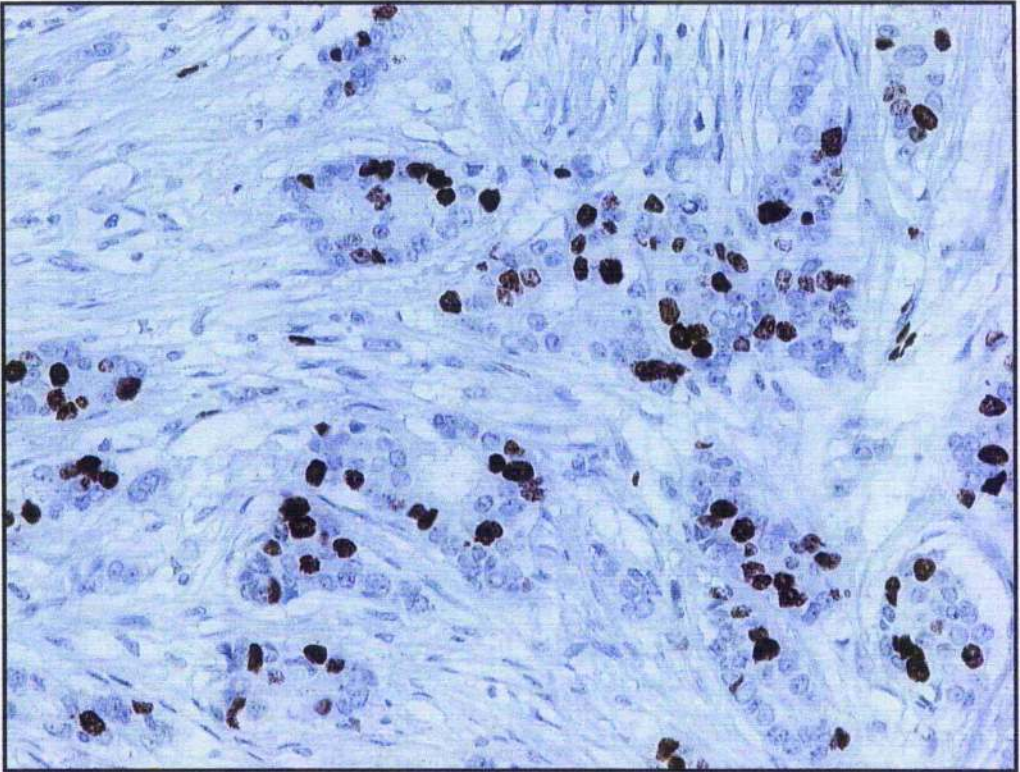
Figure 4.21: Ki67 immunohistochemical staining in invasive breast cancer (x200).
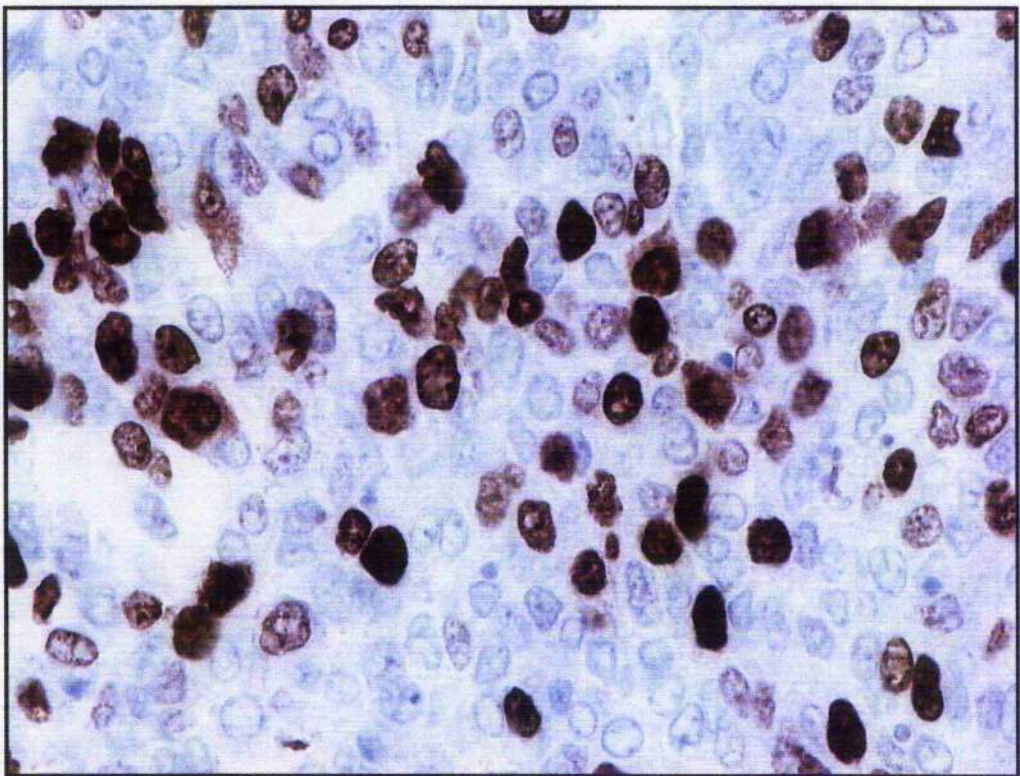
Figure 4.22: Ki67 immunohistochemical staining in invasive breast cancer (x400).
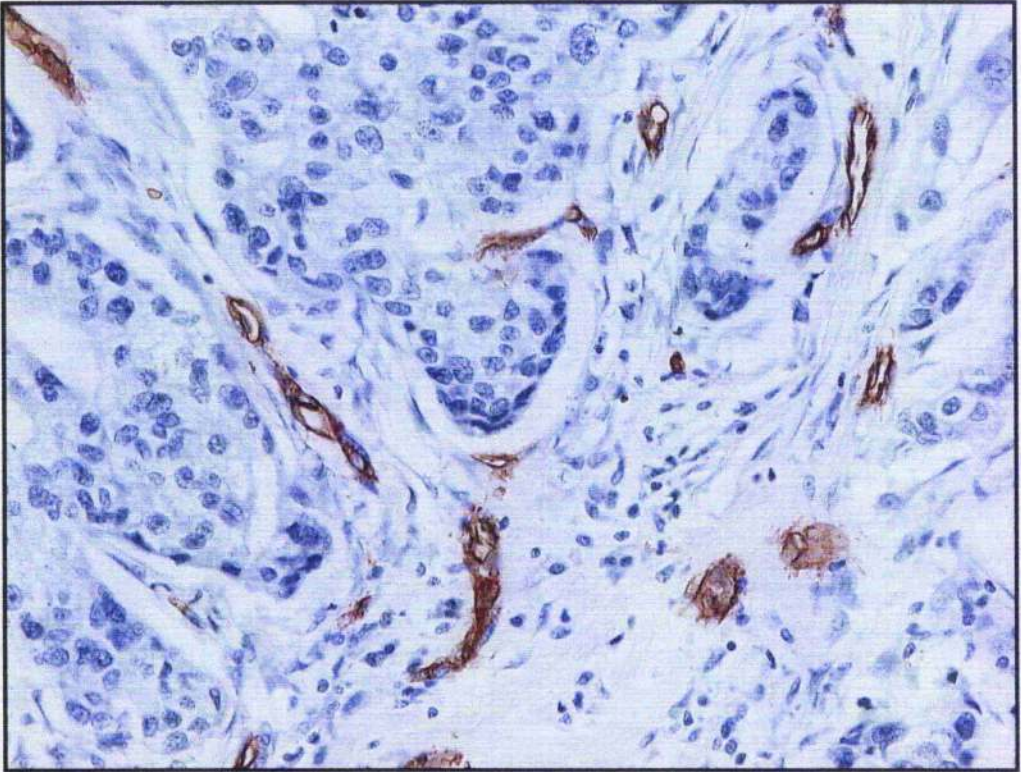
Figure 4.23: CD34+ immunohistochemical staining in invasive breast cancer (x200).
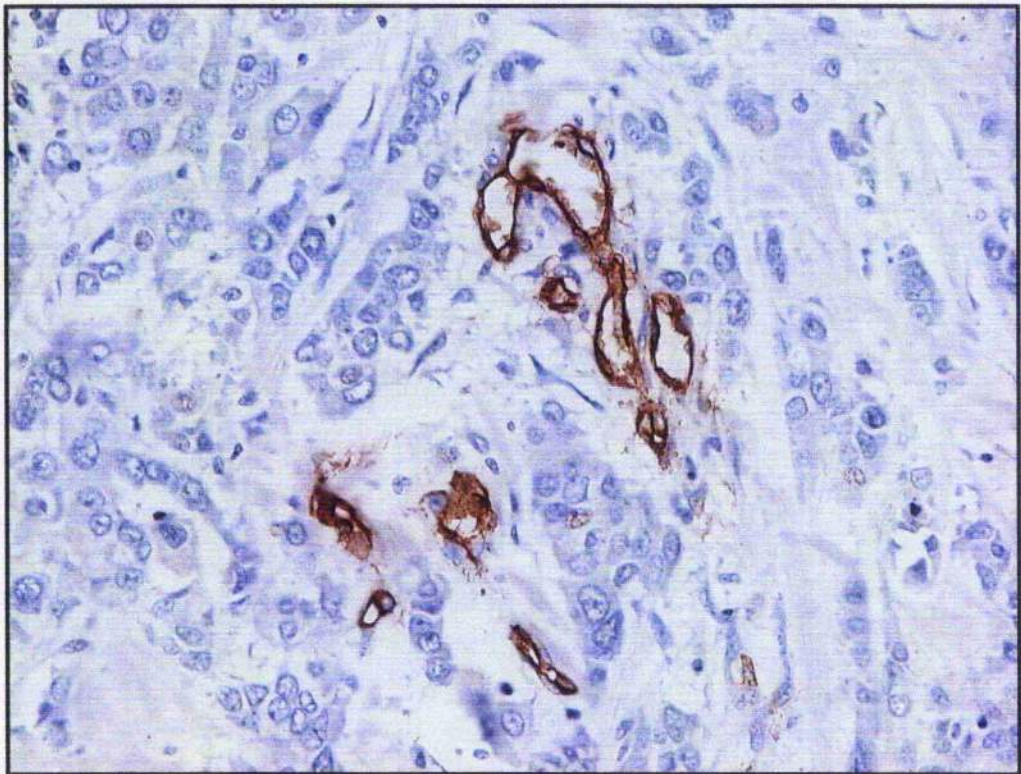
Figure 4.24: CD34+ immunohistochemical staining in invasive breast cancer (x200).

Statistics

Data are presented as median and range. Grouping of the laboratory variables was carried out using standard thresholds (Goldwasser and Feldman, 1997; O'Gorman *et al.*, 2000; McMillan *et al.*, 2001b; Maltoni *et al.*, 2005). For the purpose of analysis, the tumour Ki67 proliferative index, and T-lymphocytes subset populations (CD4+ and CD8+) and tumour associated macrophages (CD68+) were grouped by tertiles, and microvessel density (CD34+) was grouped by vascular grade; based on Chalkley mean count with cut-off points at 5 and 7 as described by (Hansen *et al.*, 2000a,b). The relationships between these and other variables were analysed using the Mantel-Haenszel (X^2) test for trend and Spearman rank correlation as appropriate.

Survival analysis was performed using the Cox proportional hazard model. Multivariate survival analysis was performed using stepwise backward procedure to derive a final model of the variables that had a significant independent relationship with survival. To remove a variable from the model, the corresponding P-value had to be a greater than 0.10. Deaths up to the end of March 2007 were included in the analysis. Analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA).

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4.3   Results

The baseline clinico-pathological characteristics of the patients with primary operable breast cancer (n=168) are shown in Table 4.1. One hundred and thirty six (81%) patients were over 50 years of age, and 49 (29%) were in the most deprived categories 6 and 7.

Of the 168 patients, one hundred and forty two (85%) patients had ductal carcinoma, 100 (60%) had a tumour less than 2 cm and 139 (83%) had a grade II/ III tumour. Ninety four (56%) patients had no axillary lymph node involvement. Thirty five patients (21%) had oestrogen receptor negative tumours.

Prior to surgery the majority had a white cell count, albumin and C-reactive protein concentrations in the normal range (96%, 100% and 85% respectively). C-reactive protein concentration was correlated with albumin concentration (r_s= -0.24, P=0.003) but not white cell count (r_s= 0.13, P=0.100).

In all, 162 (97%) patients received adjuvant treatment in the form of endocrine therapy and/or chemotherapy. The minimum follow-up was 52 months; the median follow-up of the survivors was 60 months. During this period, 20 patients relapsed and 15 died of their cancer. On univariate survival analysis, lymph node involvement (P<0.0001), hormone receptor status (P<0.05), albumin (P<0.01), Ki-67 (P<0.05), microvessel density CD34+ (P<0.05), loco-regional treatment (P<0.0001) and systemic treatment (P≤0.01) were significantly associated with relapse-free survival (Table 4.1). On multivariate analysis of these significant covariates, albumin (HR 3.55, 95% CI 1.15-11.00, P=0.028), loco-regional treatment (HR 5.00, 95% CI 1.71-14.60, P=0.003) and systemic treatment (HR 1.76, 95% CI 1.03-3.02, P=0.038) were significant independent predictors of relapse-free

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survival. When albumin was excluded from the multivariate analysis, only hormone receptor status (HR 1.80, 95% CI 0.96-3.39, P=0.068), and loco-regional treatment (HR 6.79, 95% CI 2.57-17.98, P<0.0001) were independently associated with poorer relapse-free survival.

On univariate survival analysis, lymph node involvement (P<0.01), hormone receptor status (P<0.05), albumin (P<0.01), Ki-67 (P<0.05), microvessel density CD34+ (P<0.05), loco-regional treatment (P<0.0001) and systemic treatment (P<0.01) were significantly associated with cancer-specific survival (Table 4.1). On multivariate analysis of these significant covariates, albumin (HR 5.67, 95% CI 1.24-25.88, P=0.025), loco-regional treatment (HR 6.07, 95% CI 1.63-22.54, P=0.007) and systemic treatment (HR 2.22, 95% CI 1.22-4.07, P=0.010) were significant independent predictors of cancer-specific survival. When albumin was excluded from the multivariate analysis, only loco-regional treatment (HR 8.70, 95% CI 2.41-31.42, P=0.001) and systemic treatment (HR 2.47, 95% CI 1.30-4.71, P=0.006) were independently associated with poorer cancer-specific survival.

The inter-relationships between clinico-pathological characteristics are shown in Table 4.2. In all patients, high tumour grade was positively associated with negative hormonal-receptors tumours (P<0.001), high Ki-67 labelling index (P<0.001) and high expression of CD34+ (P<0.01), CD68+ (P<0.05), CD4+ (P<0.05) and CD8+ (P<0.05). Similarly, Ki-67 labelling index was positively associated with CD34+ (P<0.001), CD68+ (P≤0.001), CD4+ (P<0.001) and CD8+ (P<0.01) T-lymphocytes. Negative hormonal-receptors tumours were directly associated with lower albumin concentrations (P<0.05), high Ki-67 labelling index (P<0.001) and the presence of CD68+ (P<0.05) and CD8+

(*P*<0.05). An elevated C-reactive protein concentration was positively associated with the expression of CD34+ (*P*=0.05) and the presence of CD4+ T-lymphocytes (*P*<0.05).

Microvessel density CD34+ was positively associated with the presence of CD68+ (*P*<0.01) and CD4+ T-lymphocytes (*P*<0.05). Tumour associated macrophages CD68+ were positively correlated with tumour CD4+ (*P*<0.01) and CD8+ (*P*<0.001) T-lymphocytes. Tumour CD4+ T-lymphocytes were also positively associated with CD8+ T-lymphocytes (*P*<0.001).

4.4 Discussion

In the present study, increased tumour grade and Ki-67 labelling index were associated with increased infiltration by CD68+ tumour associated macrophages, CD4+ and CD8+ T-lymphocytes and increased tumour microvessel density in patients with primary operable breast cancer. Furthermore, increased Ki-67 labelling index and microvessel density were associated with poorer relapse free and cancer survival. These results may be consistent with the concept that there is an active immune response to poor tumour cell differentiation and increased proliferative activity in these patients which results in increased angiogenesis (Pupa *et al.*, 1996; Tsutsui *et al.*, 2005; Lee *et al.*, 2006). Alternatively, it may reflect a more passive consequence of increased cytokine excretion from high grade proliferating tumours that attracts macrophages and T lymphocytes and increases microvessel density. Irrespective, these results together with previous studies suggest that these tumour immune-cellular infiltrates, in particular of macrophages, may in fact promote angiogenesis and disease progression (Leek *et al.*, 1996; Tsutsui *et al.*, 2005).

Previous studies have shown that tumour CD4+ T-lymphocyte infiltration was associated with poor outcome, independent of grade or stage, in patients with a variety of cancer including renal and prostate cancer (Bromwich *et al.*, 2003; McArdle *et al.*, 2004). However, in the present study, the extent of tumour lymphocytes and macrophages infiltration was not a significant prognostic marker in determining disease-outcome, consistent with previous studies (Griffith *et al.*, 1990; Wintzer *et al.*, 1991; Vgenopoulou *et al.*, 2003).

Recently, Lee and coworkers (2006) in 700 patients with stage 1 and 2 breast cancer and a median follow-up period of nearly 10 years reported that, on simple staining with haematoxylin and eosin, there was a significant relationship between the extent of both macrophage and lymphocytic infiltration and cancer specific survival. Although, moderate or marked diffuse inflammation was present in only 10% of tumours, only moderate or dense tumour inflammatory infiltrates were associated with a better prognosis in the subset of patients with grade 3 carcinomas.

The apparent discrepancies in the results of the present study and those of Lee and his co-workers (2006) and some other previous studies may reflect methodological differences, including the subsets of immune-cellular infiltrates examined and the way in which the inflammatory infiltrates were assessed. In the present study, the subsets of the tumour cellular infiltrates were identified by immunohistochemistry and the density was assessed using a point counting technique. This approach provided a more objective assessment and circumvents the problem of variation in distribution within an individual tumour. In addition, some previous studies have not included the type of surgery and/ or the adjuvant treatment received in their survival analysis. However, the relatively limited number of events and the relatively short follow-up period in our study should also be taken into account.

In the present study, also consistent with previous works, increased tumour grade, Ki-67 labelling index (Veronese et al., 1993; Scholzen and Gerdes, 2000; Trihia et al., 2003; Tsutsui et al., 2005) and microvessel density (Hansen et al., 2000a,b; Uzzan et al., 2004; Tsutsui et al., 2005) were significantly associated with poorer relapse free and cancer-specific survival. However, none of tumour-based prognostic factors, including

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histological grade, nodal status, hormonal receptor status, Ki67 proliferative index, and CD34+ microvessel density, were independently significant when potentially curative loco-regional and systemic treatment based on hormonal-receptor status, were included in the multivariate survival analysis. This probably reflects the close association between the risk-assessment and the treatment received and their relative impact on relapse and survival. Also, adjuvant chemotherapy, in addition to its direct cytotoxic effect on cancer cells, might attenuate surgery-stimulated tumour cell proliferation and tumour growth possibly occurring at distant dormant or indolent micrometastases, which might follow the excision of primary tumour with its subsequent angiogenic surge that result from the removal of the inhibitors secreted by or in response to the primary tumour, or to appearance of stimulators or growth factors in response to surgical wounding (Retsky *et al.*, 2004). Furthermore, adjuvant chemo-radiotherapy may be effective by virtue of its cellular-immune suppression and modification of specific host-immune related mechanisms, which may persist for few years after adjuvant treatment (Reizenstein *et al.*, 1985; Stewart and Tasi, 1993).

In contrast, the systemic inflammatory response, as evidenced by lower albumin concentrations, was significant on both univariate and multivariate survival analysis. Therefore, the present study examines, for the first time in patients with primary operable breast cancer, the relationship between the preoperative systemic inflammatory response, tumour-based factors and outcome, and suggests that the systemic inflammatory response is more closely related to cancer specific relapse and survival.

In the present study it was of interest that albumin but not C-reactive protein, which are relatively insensitive and sensitive measure of the systemic inflammatory response

respectively (Gabay and Kushner, 1999), had independent prognostic value in patients with primary operable breast cancer. This relationship between albumin, tumour recurrence and cancer specific survival was present even in patients with values within the normal range.

The basis of this observation is not clear but it may be that chronic illness, reflected by a lower albumin (Goldwasser and Feldman, 1997), also impacts on cancer survival. Alternatively, since a lower albumin concentration was directly associated with hormone receptor negative tumours, an unfavourable prognostic sign, it may, in part, reflect the biological functions of circulating albumin that include binding and transporting of hormones and growth factors (Margarson and Soni, 1998), inhibiting growth in the breast tumour-cell cytosol (Soreide *et al.*, 1991) and tumour proliferation by modulating the activities of autocrine growth regulatory factors (Laursen *et al.*, 1990).

Other intracellular signalling systems may play important roles in regulating cancer-cell survival and progression pathways during different tumour stages. For example, growing evidences suggest that the Nuclear Factor- κB with its associated pathways may represent the key cellular mediators that modulate poor genes expression and prognostic markers within the tumour, as well as, the host-cellular immune response in a variety of malignant diseases including breast cancer (Haffner *et al.*, 2006).

In summary, the results of the present study show the interrelationships between systemic and tumour-based factors and cancer specific outcome in patients with primary operable breast cancer. The host inflammatory responses appear to be closely related to poor tumour differentiation and malignant disease progression in primary invasive operable

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disease. Only pre-operative albumin concentration, loco-regional and systemic treatments were independent predictors of relapse-free and cancer-specific survival.

Table 4.1: The clinico-pathological characteristics of patients with invasive primary operable breast cancer, univariate survival analysis.

Clinico-pathological characteristics	Patients n=168	Relapse-free HR (95% CI)	survival P-value	Cancer-specific HR (95% CI)	Survival P-value
Age ($\leq$50/ >50 years)	32/ 136	2.32 (0.54-10.01)	0.258	3.57 (0.47-27.17)	0.219
Deprivation (1-2/ 3-5/ 6-7)*	23/ 96/ 49	1.16 (0.86-1.56)	0.332	1.19 (0.84-1.68)	0.324
Type (Ductal/ Lobular/ Special type)	142/ 20/ 6	0.55 (0.15-2.01)	0.365	0.07 (<0.01-8.76)	0.277
Size ($\leq$20/ 21-50/ >50 mm)	100/ 68/ 0	1.90 (0.79-4.58)	0.154	2.33 (0.83-6.55)	0.109
Grade (I / II / III)	28/ 87/ 52	1.64 (0.82-3.26)	0.161	1.61 (0.73-3.54)	0.234
Involved lymph node (0/ 1-3/ >3)	94/ 52/ 21	2.79 (1.58-4.94)	<0.0001	2.68 (1.38-5.19)	0.004
Hormonal-receptor status					
(ER+ PR+/ ER+ PR- or unknown/ ER- PR- or unknown)	54/ 79/ 35	1.94 (1.04-3.59)	0.036	2.59 (1.22-5.48)	0.013
White cell count (10^9/l)**	7.1 (3.4-13.5)	0.86 (0.68-1.10)	0.231	0.85 (0.64-1.13)	0.272
White cell count (<8.5/ 8.5-11/ >11 x10^9/l)	123/ 34/ 8	0.66 (0.25-1.75)	0.399	0.90 (0.34-2.39)	0.832
Albumin (g/l)**	44 (37-50)	0.77 (0.65-0.91)	0.002	0.71 (0.58-0.86)	0.001
Albumin (>43/ $\leq$43 g/l)	82/ 68	4.72 (1.55-14.35)	0.006	7.98 (1.79-35.66)	0.007
C- reactive protein (mg/l)**	$\leq$6 ($\leq$6-66)	0.96 (0.86-1.06)	0.414	0.97 (0.88-1.08)	0.588
C- reactive protein ($\leq$10/ >10 mg/l)	143/ 25	0.29 (0.04-2.13)	0.221	0.39 (0.05-2.99)	0.367
Ki-67 (tertiles 1, 2, 3)***	6.2/ 15.5/ 37.2	1.96 (1.08-3.54)	0.026	2.60 (1.22-5.51)	0.013
CD34+ ($\leq$5/ 5-7/ $\geq$7)	39/ 74/ 55	2.05 (1.06-3.98)	0.034	2.17 (1.00-4.71)	0.049
% Tumour associated macrophages CD68+ (tertiles 1, 2, 3)***	2.90/ 5.05/ 7.70	1.57 (0.88-2.79)	0.125	1.62 (0.83-3.19)	0.158
% Tumour T-lymphocytes					
CD4+ (tertiles 1, 2, 3)***	0.03/ 0.30/ 1.32	0.95 (0.56-1.62)	0.850	0.96 (0.52-1.79)	0.907
CD8+ (tertiles 1, 2, 3)***	0.27/ 0.73/ 2.23	1.07 (0.62-1.85)	0.802	1.00 (0.54-1.88)	0.993
Loco-regional treatment (Mastectomy or conservation surgery + radiotherapy/ mastectomy + radiotherapy)	125/ 43	6.23 (2.48-15.63)	<0.0001	8.77 (2.79-27.55)	<0.0001
Systemic treatment (ER-based treatment) (hormonal/ hormonal + chemotherapy/ chemotherapy/ none)	80/ 53/ 29/ 5	1.91 (1.17-3.11)	0.010	2.49 (1.41-4.40)	0.002

*: Individual deprivation categories were used in the statistical analysis, **: median (range), ***: median.

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Table 4.2: Inter-relationships* between the clinico-pathological characteristics in patients with invasive primary operable breast cancer.

	Involved lymph node (P-value)	Hormonal-receptor status (P-value)	Albumin (P-value)	C- reactive protein (P-value)	Ki-67 (P-value)	CD 34+ (P-value)	CD68+ (P-value)	CD4+ (P-value)	CD8+ (P-value)
Grade (I / II / III)	0.109	<0.001	0.216	0.653	<0.001	0.006	0.027	0.030	0.035
Involved lymph node (0/ 1-3/ >3)		0.843	0.150	0.554	0.504	0.106	0.079	0.360	0.633
Hormonal-receptor status (ER+ PR+/ ER+ PR- or unknown/ ER- PR- or unknown)			0.047	0.804	<0.001	0.402	0.030	0.128	0.017
Albumin (>43/ ≤43 g/l)				0.362	0.548	0.405	0.193	0.927	0.386
C- reactive protein (≤10/ >10 mg/l)					1.000	0.054	0.252	0.028	0.120
Ki-67 (tertiles 1, 2, 3)						<0.001	0.001	<0.001	0.004
CD34+ (≤5/ 5-7/ ≥7)							0.002	0.048	0.256
% Tumour associated macrophages									
CD68+ (tertiles 1, 2, 3)								0.002	<0.001
% Tumour T-lymphocytes									
CD4+ (tertiles 1, 2, 3)									<0.001

*: Chi-Square-test.

CHAPTER 5: CONCLUSION

In the present studies, the results suggest a potential independent prognostic value of the systemic inflammatory response, as evidenced by lowered albumin concentrations with/ without elevated circulating C-reactive protein concentrations, particularly in patients with advanced metastatic disease, and early-staged breast cancer. Also, the local host-inflammatory response in early-staged invasive disease appears to be closely related to poor tumour differentiation and malignant disease progression.

In patients with advanced metastatic breast cancer, the mechanisms by which a systemic inflammatory response, as evidenced by higher inflammatory-based score (GPS), might impact on survival may reflect the underlying activities of pro-inflammatory cytokines, in particular interleukin-6 (McKeown *et al.*, 2004), which not only stimulate tumour growth (Kurebayashi, 2000), but also produce profound catabolic effects on host metabolism (McMillan *et al.*, 1998; Kotler, 2000). The presence and magnitude of a chronic systemic inflammatory response may result in progressive nutritional and functional decline that ultimately result in reduced survival, consistent with the observation of this study which showed that all patients with metastatic breast cancer who had hypoalbuminemia had an elevated C-reactive protein concentration.

Therefore, the potential value of the systemic inflammatory-based score (GPS) in predicting the advanced breast cancer patient's survival is encouraging but clearly requires to be validated on an independent set of patients with a larger sample size. In addition to, head-to-head direct comparisons with other scoring systems that are used to predict survival in advanced metastatic breast cancer such as performance status.

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Furthermore, a combination of these clinico-pathological factors together with the GPS may allow more accurate prognostication (Chang *et al.*, 2003; Maltoni *et al.*, 2005; Kattan, 2006). Also, the systemic inflammatory-based score may be used to identify patients most likely to benefit from the systemic immune-modulation using anti-inflammatory agents.

In those patients with early-staged breast cancer, there are well-established prognostic factors at the time of diagnosis on which to base the prediction of likely survival. It was of interest that the GPS does not appear to have value in primary operable disease, primarily due to majority of patients having C-reactive protein and albumin concentrations in the normal range ($\leq$ 10 mg/l and $\geq$ 35 g/l, respectively). However, albumin as a continuous or categorical variable (split at 43 g/l) did have prognostic value in early-staged invasive breast cancer, and was superior to tumour-based factors including angiogenesis. Therefore, the addition of these simple well-standardised measurements that reflect the ongoing host-tumour interaction in the routine management of early-staged disease at initial presentation may be useful in identifying those high-risk patients that are likely to relapse and, therefore, adjust or alter their treatment accordingly. Clearly, further studies with large cohort and against conventional prognostic factors and established scores; either in comparison or added to a prognostic score such as the Nottingham Prognostic Index (Hansen *et al.*, 2000b), are required to establish the prognostic value of pre-operative, as well as, post operative (longitudinal) measures of circulating albumin and C-reactive protein concentrations.

Within the tumour microenvironment of primary early-staged invasive breast cancer, increased tumour grade and proliferative activity (Ki-67 labelling index) were associated

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with increased tumour cell-mediated immune infiltrations (CD68+ tumour associated macrophages, CD4+ and CD8+ T-lymphocytes) and increased tumour angiogenesis (microvessel density). These results may be consistent with the concept that there is an active immune response to poor tumour cell differentiation and increased proliferative activity, which may result in increased angiogenesis (Pupa *et al.*, 1996; Tsutsui *et al.*, 2005; Lee *et al.*, 2006). Alternatively, it may reflect a more passive consequence of increased cytokine excretion from high grade proliferating tumours that attracts macrophages and T-lymphocytes and increases microvessel density. Irrespectively, these results together with previous studies suggest that these immune-cellular infiltrates, in particular of macrophages, may in fact promote angiogenesis and disease progression (Leek *et al.*, 1996; Tsutsui *et al.*, 2005) and, therefore, may represent an important target for selective immuno-modulation or immuno-inhibitory therapy in breast cancer.

The results of the present studies, therefore, emphasize the inter-relationship between the tumour-host inflammatory responses and other conventional prognostic factors with outcomes in breast cancer. However, the exact underlying molecular mechanisms of this intimate association are poorly understood.

These findings parallel recent findings in animal models of human cancer. These have established that inflammation is a critical component of both tumour-promotion and tumour-progression (Balkwill *et al.*, 2005; Karin, 2006).

At the biological level, it appears to involve complex network interactions of intracellular and extracellular signals that control multiple underlying processes. In recent years the transcription factor, Nuclear Factor- kappaB (NF-κB), and associated pathways have

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emerged as a critical mediators for the inflammation-associated tumour growth and progression, as well as, an important modulator of the immune response and tumour surveillance that associated with high cell proliferation, poor differentiation and unfavourable prognosis (Rayet and Gelinas, 1999; Hagemann *et al.*, 2005; Wu and Kral, 2005).

Nuclear Factor-κB transcription factor is a key cellular regulatory molecule of the human immune stress response and of the cell survival pathway, which mediates a wide variety of target genes expression that influence developmental, cell-cycle progression and physiological processes; such as cellular proliferation, differentiation and apoptosis, as well as, immunity, inflammation, and acute phase responses, and also other diseases processes including tumour survival pathways; such as tumour growth, invasion, angiogenesis and metastasis (Pahl, 1999; Rayet and Gelinas, 1999; Li and Verma, 2002; Wu and Kral, 2005).

Different signal-transduction pathways selectively activate different NF-κB complexes in a coordinated manner, however, it is not known how these pathways integrate the diverse extracellular signals and maintain specificity in the context of gene transcription. Pleiotropic extracellular stimuli including various physiological, physical, and oxidative stress stimuli, as well as, cytokines, interleukins, growth factors and chemotherapeutic agents interact with cell surface receptors. The intracellular signalling pathways are then transmitted in either a linear or a network manner leading to the activation of NF-κB, mostly through IκB kinase (IKK)-dependent phosphorylation with subsequent degradation and release of its inhibitor (the IκB family of proteins) that result in nuclear translocation of NF-κB and its binding to DNA at specific κB sites which are then

followed by a rapid induction and modulation of expression of variety of encoding genes involved in normal cell survival and physiological pathways and diseases processes (Pahl, 1999; Chen *et al.*, 2001; Wu and Kral, 2005).

Previous studies have implicated deregulation and/or sustained activation of the transcription Nuclear Factor-κB family and its associated pathways in a variety of malignant diseases in which NF-κB is overexpressed (Rayet and Gelinas, 1999). Activation of NF-κB does not appear to be confined to a unique stage during tumour development and progression, and has been postulated to be required for the regulation of different characteristics of the tumour (Haffner *et al.*, 2006). In addition, there is an increasing body of evidence which delineates the role of the Nuclear Factor-κB in linking inflammation and cancer. However, the mechanisms underlying their association remain unclear (Rayet and Gelinas, 1999; Hagemann *et al.*, 2005; Haffner *et al.*, 2006).

In particular, it is still unclear whether the increased production of the pro-inflammatory cytokines is the cause or the result of NF-κB activation, and whether NF-κB activation in the immune system influences mammary cancer (Li and Verma, 2002; Lin and Karin, 2007).

Recent studies in human breast cancer cell lines and animal models of breast cancer have implicated the NF-κB transcription factors signalling systems in mammary carcinogenesis and progression via a number of target genes and mechanisms that are linked to breast tumour cell proliferation, growth, differentiation and pro-survival pathways (cyclin D1, BRCA2, tumour necrosis factor-α), tissue invasion (matrix metalloproteinase, urokinase plasminogen activator, cell adhesion molecules such as ICAM-1), tumour angiogenesis

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and metastasis (VEGF, cyclooxygenase-2, inducible nitric-oxide synthase), resistance to anticancer chemotherapeutic agents and ionizing radiation, such as doxorubicin, taxols, topoisomerase inhibitors, and with γ-radiation (Figure 5.1; Rayet and Gelinas, 1999; Wu and Kral, 2005; Haffner *et al.*, 2006). Furthermore, molecular approaches have documented antagonistic cross-talk between the NF-κB and ER pathways by demonstrating the ability of oestrogen-activated ER to inhibit NF-κB signals. In a specific subclass, NF-κB activity may be implicated in the conversion to less differentiated and hormone-independent tumours; a characteristic of more aggressive and metastatic tumours. For example, a positive correlation has been observed between NF-κB activation and over-expression of epidermal growth factor receptor family members (erbB-2), especially in oestrogen receptor negative tumours (Biswas *et al.*, 2005; Zhou *et al.*, 2005; Biswas and Iglehart, 2006; Haffner *et al.*, 2006). Therefore, it would be of interest to re-examine the prognostic value of the acute-phase proteins (C-reactive protein and albumin) in a cohort of patients with primary operable ER-negative tumours.

At present, there is little evidence of a prominent role of active immunosurveillance in breast cancer. Instead, tumour-cellular infiltrate may be subverted to promote tumour progression and metastasis. For example, tumour-associated macrophages secrete cytokines, such as IL-6 and IL-10, which act on proliferation, survival and metastasis of tumour cells and promote angiogenesis (Haffner *et al.*, 2006). The in-vitro interaction of tumour cells with macrophages, in a JNKII- and NF-κB-dependent manner, induces macrophages to release matrix metalloproteinases and so support tumour cell invasion (Hagemann *et al.*, 2005). Furthermore, NF-κB inhibitors have been found to blunt the action of some of these cytokines in tumour cells. Whether NF-κB inhibitors could also act on tumour-associated macrophages and prevent the secretion of tumour growth and

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invasion promoting cytokines remain to be shown (Haffner *et al.*, 2006). On the other hand, reduced NF-κB signalling in immune cells from patients with cancer may also contribute to tumour progression (Rayet and Gelinas, 1999).

TUMOUR INITIATION

METASTASIS

PROLIFERATION

NF-κB

IL-6 ⇌ IL-10

Acute-phase proteins

CHEMO-RESISTANCE

ANTI-APOPTOSIS

ANGIOGENESIS
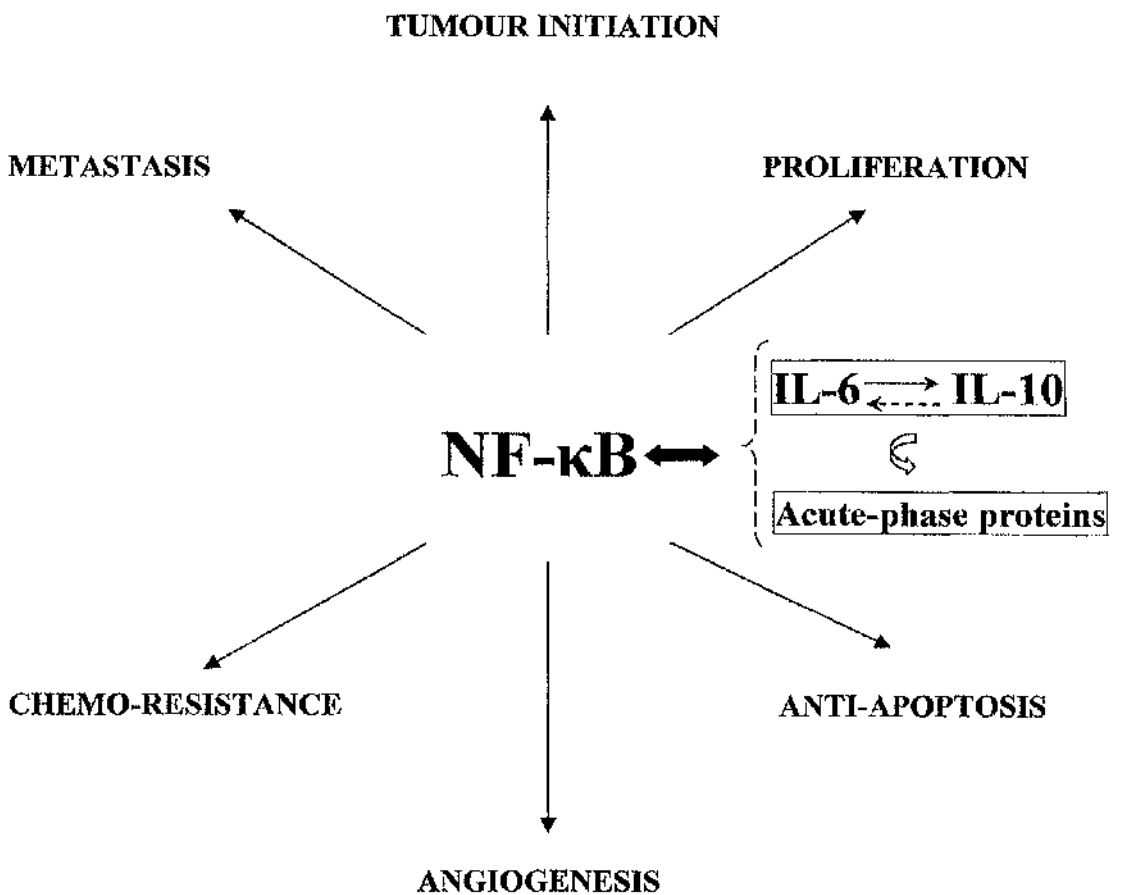
Figure 5.1: Possible link and relationship between NF-κB, cytokines (IL-6 & IL-10), acute-phase proteins and breast cancer promotion/ progression (Balkwill *et al.*, 2005; Wu and Kral, 2005; Lin and Karin, 2007).

The determination of the transcriptional outcome of Nuclear Factor-κB activation remains a challenging task, because the detection of nuclear localization and DNA binding does not always predict which NF-κB target genes are actually transcribed. These target genes are also dependent on its post-transcriptional modifications, as well as, the cell type. Thereby, the analysis of the downstream genes that are regulated by the NF-κB, in addition, is likely to provide important insight into its function. This should be integrated with clinical information on outcomes to establish the prognostic and predictive significance of NF-κB in breast cancer (Li and Verma, 2002; Wu and Kral, 2005; Haffner *et al.*, 2006).

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APPENDICES

Appendix 1: The clinico-pathological characteristics of patients with metastatic breast cancer (Chapter 2).

No.	Age	Prior chemo.	Metastatic site	ER	Her-2	DFI/m.	Lab timing	WBC (x10⁹/l)	HB (g/dl)	CRP (mg/l)	ALB (g/l)	GPS	Treatment	Final status	OS/m.
1	57	0	VISCERAL & NON-VISCERAL	Positive	Negative	27.1	during treatment	6.8	13.5	44	47	1	chemo-endocine +/- supportive	Alive	17.0
2	56	0	VISCERAL & NON-VISCERAL	Positive	Negative	41.1	at diagnosis	4.5	12.2	32	46	1	chemo-endocine +/- supportive	Alive	14.4
3	58	0	VISCERAL	Negative	Positive	29.9	at diagnosis	7.7	13.6	8	43	0	herceptin-based	Alive	21.5
4	58	0	NON-VISCERAL	Positive	Negative	117.3	at diagnosis	17.5	12.9	8	40	0	chemo-endocine +/- supportive	Alive	16.8
5	70	0	VISCERAL	Positive	Positive	0.0	during treatment	6.5	11.9	14	45	1	herceptin-based	Alive	11.1
6	65	0	NON-VISCERAL	—	—	0.0	at diagnosis	10.8	11.9	7	44	0	chemo-endocine +/- supportive	Alive	8.8
7	39	2	VISCERAL	Negative	Positive	0.5	during treatment	—	—	9	44	0	herceptin-based	Alive	12.6
8	50	1	VISCERAL & NON-VISCERAL	Negative	Positive	58.2	during treatment	10.0	12.0	17	36	1	chemo-endocine +/- supportive	Alive	15.8
9	80	0	NON-VISCERAL	Positive	—	37.7	during treatment	5.9	12.2	10	44	0	chemo-endocine +/- supportive	Alive	15.1
10	57	0	NON-VISCERAL	—	—	109.0	during treatment	5.4	13.2	6	45	0	chemo-endocine +/- supportive	BC death	9.7
11	72	0	VISCERAL	Positive	Negative	0.9	at diagnosis	3.9	11.9	6	40	0	chemo-endocine +/- supportive	Alive	11.4
12	79	0	VISCERAL	Negative	—	0.8	at diagnosis	7.7	11.7	36	41	1	chemo-endocine +/- supportive	BC death	15.9
13	58	1	VISCERAL & NON-VISCERAL	Positive	—	65.5	during treatment	—	—	6	47	0	chemo-endocine +/- supportive	Alive	22.1
14	58	0	VISCERAL	Negative	Negative	161.6	at diagnosis	6.3	10.0	6	46	0	chemo-endocine +/- supportive	BC death	31.7
15	34	2	VISCERAL & NON-VISCERAL	Positive	—	25.0	during treatment	7.5	13.3	28	29	2	chemo-endocine +/- supportive	BC death	1.2
16	63	2	VISCERAL & NON-VISCERAL	Positive	—	45.6	during treatment	3.3	9.2	6	38	0	chemo-endocine +/- supportive	Alive	14.9
17	62	0	VISCERAL & NON-VISCERAL	Positive	Negative	55.3	at diagnosis	6.2	13.5	36	41	1	chemo-endocine +/- supportive	BC death	17.1
18	53	0	NON-VISCERAL	Positive	—	43.1	during treatment	6.6	11.4	20	50	1	chemo-endocine +/- supportive	Alive	6.7
19	84	0	NON-VISCERAL	Positive	—	126.8	during treatment	10.8	13.3	16	35	1	chemo-endocine +/- supportive	BC death	4.6
20	53	1	VISCERAL & NON-VISCERAL	Negative	Negative	47.2	during treatment	4.5	12.5	8	44	0	chemo-endocine +/- supportive	Alive	8.1
21	82	0	NON-VISCERAL	Positive	—	132.8	during treatment	4.6	10.2	9	40	0	chemo-endocine +/- supportive	Alive	8.3
22	76	0	VISCERAL & NON-VISCERAL	Positive	—	98.2	at diagnosis	7.0	9.2	63	37	1	chemo-endocine +/- supportive	Alive	14.4
23	50	2	VISCERAL & NON-VISCERAL	Negative	Negative	24.5	during treatment	14.9	11.9	21	35	1	chemo-endocine +/- supportive	BC death	2.8
24	81	0	NON-VISCERAL	Positive	—	101.8	during treatment	6.0	10.2	22	31	2	chemo-endocine +/- supportive	Alive	16.7
25	43	0	NON-VISCERAL	Negative	—	34.8	at diagnosis	4.0	13.4	6	41	0	chemo-endocine +/- supportive	BC death	20.2
26	69	0	NON-VISCERAL	—	—	105.2	at diagnosis	10.6	15.3	6	36	0	chemo-endocine +/- supportive	Alive	14.2
27	84	0	NON-VISCERAL	Positive	—	49.2	during treatment	9.4	9.7	25	43	1	chemo-endocine +/- supportive	BC death	6.8
28	78	5	VISCERAL & NON-VISCERAL	Positive	Positive	66.4	during treatment	5.4	13.4	6	41	0	herceptin-based	BC death	2.4
29	73	0	VISCERAL & NON-VISCERAL	Positive	Negative	0.0	at diagnosis	8.3	13.0	54	33	2	chemo-endocine +/- supportive	BC death	17.1
30	69	0	NON-VISCERAL	Positive	Negative	232.3	at diagnosis	4.2	14.0	17	43	1	chemo-endocine +/- supportive	Alive	28.5
31	74	0	NON-VISCERAL	Positive	—	62.8	during treatment	8.5	12.8	9	46	0	chemo-endocine +/- supportive	BC death	4.4
32	83	0	NON-VISCERAL	Positive	—	60.4	during treatment	8.2	8.2	111	39	1	chemo-endocine +/- supportive	BC death	2.2
33	75	0	NON-VISCERAL	Positive	—	58.3	during treatment	10.2	11.2	28	34	2	chemo-endocine +/- supportive	BC death	0.1
34	34	0	NON-VISCERAL	Positive	Negative	0.6	at diagnosis	5.5	—	29	40	1	chemo-endocine +/- supportive	BC death	21.4

Appendix 1: The clinico-pathological characteristics of patients with metastatic breast cancer (Chapter 2).

No.	Age	Prior chemo.	Metastatic site	ER	Her-2	DFI/m.	Lab timing	WBC (x10^9/l)	HB (g/dl)	CRP (mg/l)	ALB (g/l)	GPS	Treatment	Final status	OS/m.
35	55	0	VISCERAL & NON-VISCERAL	Negative	Negative	10.7	at diagnosis	7.3	-	10	45	0	chemo-endocine +/- supportive	BC death	2.2
36	40	0	VISCERAL & NON-VISCERAL	Positive	-	30.2	during treatment	-	9.8	21	43	1	chemo-endocine +/- supportive	BC death	5.1
37	85	0	NON-VISCERAL	Positive	-	37.0	at diagnosis	-	-	14	38	1	chemo-endocine +/- supportive	BC death	8.3
38	70	0	VISCERAL & NON-VISCERAL	Positive	Negative	12.4	at diagnosis	12.4	12.8	23	47	1	chemo-endocine +/- supportive	Alive	7.9
39	65	0	NON-VISCERAL	Positive	-	152.7	at diagnosis	-	-	15	43	1	supportive	BC death	1.4
40	54	0	VISCERAL & NON-VISCERAL	Positive	Negative	41.0	during treatment	-	-	12	43	1	chemo-endocine +/- supportive	BC death	12.7
41	58	0	NON-VISCERAL	Positive	Negative	38.6	at diagnosis	5.1	10.0	6	42	0	chemo-endocine +/- supportive	Alive	19.2
42	61	0	VISCERAL & NON-VISCERAL	Positive	-	87.3	during treatment	-	-	38	40	1	chemo-endocine +/- supportive	BC death	18.5
43	60	1	NON-VISCERAL	Negative	Positive	39.1	during treatment	6.4	11.7	9	42	0	chemo-endocine +/- supportive	Alive	8.1
44	40	6	VISCERAL & NON-VISCERAL	Positive	Negative	72.0	during treatment	3.5	11.9	6	49	0	chemo-endocine +/- supportive	Alive	17.0
45	61	0	VISCERAL	Negative	Negative	0.7	at diagnosis	-	-	6	46	0	chemo-endocine +/- supportive	Alive	11.6
46	28	0	VISCERAL	Negative	Positive	1.0	at diagnosis	5.4	13.2	6	49	0	herceptin-based	BC death	15.2
47	38	0	NON-VISCERAL	Positive	Negative	30.3	at diagnosis	6.1	14.8	8	53	0	chemo-endocine +/- supportive	BC death	10.6
48	72	0	NON-VISCERAL	Positive	-	89.0	at diagnosis	5.7	13.6	6	45	0	chemo-endocine +/- supportive	Alive	25.6
49	71	1	VISCERAL & NON-VISCERAL	Negative	Negative	73.6	during treatment	6.5	8.1	6	35	0	chemo-endocine +/- supportive	Alive	8.5
50	36	0	NON-VISCERAL	Positive	Positive	17.8	at diagnosis	4.5	13.6	11	48	1	chemo-endocine +/- supportive	Alive	20.7
51	62	2	VISCERAL & NON-VISCERAL	Positive	-	42.1	during treatment	3.8	12.0	10	44	0	chemo-endocine +/- supportive	BC death	4.1
52	36	2	VISCERAL & NON-VISCERAL	Negative	Negative	29.8	during treatment	3.9	9.5	59	36	1	chemo-endocine +/- supportive	BC death	3.9
53	83	0	NON-VISCERAL	Positive	-	386.0	during treatment	6.1	12.5	6	39	0	chemo-endocine +/- supportive	Alive	19.8
54	55	0	VISCERAL & NON-VISCERAL	Positive	-	0.0	during treatment	-	-	6	46	0	chemo-endocine +/- supportive	BC death	6.7
55	56	0	NON-VISCERAL	Positive	-	49.7	during treatment	5.4	14.6	6	49	0	chemo-endocine +/- supportive	Alive	10.8
56	56	0	VISCERAL & NON-VISCERAL	Positive	-	37.0	during treatment	-	-	57	39	1	chemo-endocine +/- supportive	BC death	1.2
57	83	0	NON-VISCERAL	Negative	Negative	35.0	during treatment	6.7	13.7	16	44	1	supportive	Alive	15.6
58	36	2	VISCERAL & NON-VISCERAL	Positive	Negative	56.5	during treatment	3.2	13.4	6	49	0	chemo-endocine +/- supportive	Alive	20.7
59	42	0	VISCERAL & NON-VISCERAL	Positive	-	22.3	during treatment	8.9	12.0	6	48	0	chemo-endocine +/- supportive	Alive	19.8
60	67	0	VISCERAL & NON-VISCERAL	Positive	-	119.2	during treatment	8.3	12.9	52	39	1	chemo-endocine +/- supportive	Alive	19.3
61	67	4	NON-VISCERAL	Negative	Negative	197.2	during treatment	5.1	10.1	6	46	0	chemo-endocine +/- supportive	BC death	8.4
62	63	0	NON-VISCERAL	Positive	-	8.4	during treatment	7.8	11.1	24	39	1	chemo-endocine +/- supportive	Alive	20.2
63	73	0	VISCERAL	-	-	119.6	during treatment	5.2	11.5	9	35	0	chemo-endocine +/- supportive	Alive	21.4
64	65	0	VISCERAL & NON-VISCERAL	-	-	100.8	during treatment	7.5	10.3	6	41	0	chemo-endocine +/- supportive	Alive	13.8
65	71	0	NON-VISCERAL	Positive	-	49.9	during treatment	10.1	13.9	70	36	1	chemo-endocine +/- supportive	BC death	2.0
66	69	0	NON-VISCERAL	Positive	-	20.2	at diagnosis	7.6	14.4	31	44	1	chemo-endocine +/- supportive	Alive	21.4
67	41	1	VISCERAL	Negative	Negative	18.4	during treatment	5.2	12.1	55	41	1	chemo-endocine +/- supportive	BC death	2.2
68	47	1	VISCERAL & NON-VISCERAL	Negative	Positive	41.1	during treatment	5.6	11.1	30	39	1	herceptin-based	BC death	9.6

Appendix 1: The clinico-pathological characteristics of patients with metastatic breast cancer (Chapter 2).

No.	Age	Prior chemo.	Metastatic site	ER	Her-2	DFI/m.	Lab timing	WBC (x10^9/l)	HB (g/dl)	CRP (mg/l)	ALB (g/l)	GPS	Treatment	Final status	OS/m.
69	63	0	NON-VISCERAL	Positive	—	156.4	at diagnosis	8.4	13.3	23	33	2	chemo-endocrine +/- supportive	BC death	0.3
70	51	0	NON-VISCERAL	Negative	—	95.9	at diagnosis	6.6	11.2	6	42	0	chemo-endocrine +/- supportive	BC death	24.0
71	57	0	NON-VISCERAL	Negative	Negative	126.6	during treatment	—	—	6	50	0	chemo-endocrine +/- supportive	BC death	20.7
72	47	2	NON-VISCERAL	Positive	Negative	18.7	during treatment	5.5	14.1	16	44	1	chemo-endocrine +/- supportive	BC death	14.4
73	61	0	NON-VISCERAL	Positive	—	1.0	during treatment	—	—	6	41	0	chemo-endocrine +/- supportive	BC death	12.9
74	74	0	NON-VISCERAL	Positive	—	0.8	during treatment	10.8	12.2	68	46	1	chemo-endocrine +/- supportive	Alive	8.8
75	52	0	NON-VISCERAL	Positive	—	52.2	at diagnosis	3.4	13.9	6	46	0	chemo-endocrine +/- supportive	Alive	23.7
76	58	0	VISCERAL & NON-VISCERAL	Positive	—	16.1	during treatment	5.9	12.3	6	47	0	chemo-endocrine +/- supportive	BC death	7.4
77	58	0	VISCERAL & NON-VISCERAL	Positive	Negative	135.7	at diagnosis	10.1	14.8	14	43	1	chemo-endocrine +/- supportive	BC death	25.6
78	65	0	VISCERAL & NON-VISCERAL	Positive	—	0.6	during treatment	—	—	6	44	0	chemo-endocrine +/- supportive	Alive	16.0
79	59	0	NON-VISCERAL	—	—	-6.3	during treatment	7.0	12.1	10	44	0	chemo-endocrine +/- supportive	BC death	15.7
80	64	0	NON-VISCERAL	Positive	—	114.6	during treatment	—	—	6	42	0	chemo-endocrine +/- supportive	Alive	11.1
81	50	0	NON-VISCERAL	Positive	—	1.1	during treatment	4.7	12.2	6	45	0	chemo-endocrine +/- supportive	Alive	10.2
82	58	0	VISCERAL	Negative	Positive	81.4	at diagnosis	7.3	13.5	6	46	0	herceptin-based	Alive	22.4
83	69	0	VISCERAL & NON-VISCERAL	Negative	Negative	14.7	at diagnosis	6.8	12.6	9	44	0	chemo-endocrine +/- supportive	BC death	6.4
84	82	0	NON-VISCERAL	Negative	—	25.6	during treatment	—	—	83	41	1	supportive	BC death	6.5
85	41	2	VISCERAL	Negative	Positive	0.3	during treatment	5.2	12.9	6	43	0	herceptin-based	BC death	30.0
86	51	1	NON-VISCERAL	Negative	—	36.2	during treatment	6.5	16.0	6	44	0	chemo-endocrine +/- supportive	BC death	23.8
87	72	0	NON-VISCERAL	Positive	—	246.7	during treatment	5.5	12.3	6	40	0	chemo-endocrine +/- supportive	BC death	14.4
88	58	0	VISCERAL & NON-VISCERAL	Positive	Positive	75.1	at diagnosis	—	—	7	38	0	herceptin-based	BC death	55.9
89	55	3	VISCERAL & NON-VISCERAL	Positive	Negative	139.3	during treatment	9.2	10.4	82	30	2	chemo-endocrine +/- supportive	BC death	7.9
90	74	0	VISCERAL & NON-VISCERAL	Positive	—	14.8	at diagnosis	8.1	12.8	18	47	1	chemo-endocrine +/- supportive	BC death	3.4
91	67	0	VISCERAL & NON-VISCERAL	Negative	—	32.5	at diagnosis	8.9	11.7	49	36	1	chemo-endocrine +/- supportive	BC death	3.1
92	69	0	VISCERAL	Positive	Negative	35.7	at diagnosis	—	—	29	38	1	supportive	BC death	0.9
93	45	3	VISCERAL & NON-VISCERAL	Positive	Negative	104.8	during treatment	—	—	23	39	1	chemo-endocrine +/- supportive	BC death	2.0
94	53	2	VISCERAL & NON-VISCERAL	Positive	Positive	31.5	during treatment	5.3	10.7	9	39	0	herceptin-based	Alive	8.8
95	47	1	VISCERAL & NON-VISCERAL	Negative	Positive	0.0	during treatment	4.4	10.9	6	36	0	herceptin-based	BC death	17.9
96	56	0	VISCERAL	Positive	Negative	31.3	during treatment	—	—	22	43	1	chemo-endocrine +/- supportive	BC death	12.7

Appendix 2: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 3).

No.	Age	Deprivation	WBC (x10^9/l)	ALB (g/l)	CRP (mg/l)	Breast Surgery	Axillary Surgery	Histological type	Grade	Size (cm)	Total LN	Involved LN	ER status	PR status
1	56	5	7.23	43	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.5	10	0	negative	unknown
2	42	4	5.32	44	6	conservation surgery	dissection/ clearance	invasive ductal	3	1.6	21	0	positive	unknown
3	80	5	5.29	38	<6	mastectomy	dissection/ clearance	invasive ductal	3	5.0	18	0	negative	unknown
4	52	3	7.01	40	<6	mastectomy	dissection/ clearance	invasive ductal	2	4.0	14	7	positive	unknown
5	60	6	6.39	-	9	mastectomy	sampling	invasive ductal	2	2.2	9	1	positive	unknown
6	71	6	7.57	-	10	mastectomy	dissection/ clearance	invasive ductal	1	2.5	11	0	positive	unknown
7	70	6	8.51	-	10	mastectomy	sampling	invasive ductal	1	1.5	7	0	positive	unknown
8	65	4	8.58	-	20	mastectomy	dissection/ clearance	invasive ductal	2	2.5	18	8	positive	unknown
9	76	5	8.82	-	<6	mastectomy	sampling	invasive ductal	3	1.8	3	0	negative	unknown
10	44	5	8.70	44	<6	mastectomy	dissection/ clearance	invasive ductal	1	2.1	14	0	positive	unknown
11	44	4	9.21	-	<6	conservation surgery	dissection/ clearance	invasive ductal	3	2.1	19	0	negative	unknown
12	58	3	7.06	-	<6	mastectomy	dissection/ clearance	invasive ductal	3	1.0	11	2	negative	unknown
13	56	6	-	-	<6	conservation surgery	dissection/ clearance	invasive ductal	1	2.0	11	2	positive	unknown
14	47	5	10.38	43	<6	mastectomy	dissection/ clearance	invasive ductal	1	1.7	9	0	positive	unknown
15	50	4	7.77	-	<6	mastectomy	dissection/ clearance	invasive lobular	2	2.0	9	0	positive	unknown
16	41	5	12.35	46	10	conservation surgery	dissection/ clearance	invasive ductal	2	2.6	20	0	positive	unknown
17	70	5	6.70	42	<6	mastectomy	dissection/ clearance	invasive lobular	3	3.0	17	8	positive	unknown
18	79	3	5.03	-	<6	mastectomy	sampling	invasive ductal	3	2.9	7	0	negative	unknown
19	40	3	9.61	-	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.0	13	2	negative	unknown
20	68	5	6.19	41	6	conservation surgery	dissection/ clearance	invasive ductal	2	1.1	11	1	positive	unknown
21	59	4	5.83	43	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.0	8	0	negative	unknown
22	42	3	7.59	-	8	mastectomy	dissection/ clearance	invasive lobular	2	4.0	9	6	positive	unknown
23	46	4	4.93	42	6	mastectomy	dissection/ clearance	invasive ductal	3	1.8	19	0	negative	unknown
24	60	6	7.86	42	10	mastectomy	sampling	invasive lobular	2	2.0	8	0	positive	unknown
25	66	6	11.27	-	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.8	8	2	positive	unknown
26	67	5	9.83	47	7	conservation surgery	dissection/ clearance	invasive ductal	2	1.2	8	0	positive	unknown
27	77	3	6.43	40	<6	mastectomy	dissection/ clearance	invasive ductal	1	2.3	7	0	positive	unknown
28	66	5	7.95	-	7	conservation surgery	dissection/ clearance	invasive ductal	3	2.4	11	4	positive	unknown
29	69	5	7.72	-	15	conservation surgery	sampling	invasive ductal	2	2.2	9	0	negative	unknown
30	59	4	5.89	39	6	mastectomy	dissection/ clearance	invasive ductal	2	1.1	14	0	negative	unknown
31	46	4	7.70	44	8	mastectomy	dissection/ clearance	invasive ductal	2	0.8	14	0	positive	unknown
32	44	5	8.48	41	8	conservation surgery	dissection/ clearance	invasive ductal	2	3.0	16	9	negative	unknown
33	64	5	5.73	-	<6	mastectomy	dissection/ clearance	invasive ductal	1	2.0	8	1	positive	unknown
34	76	4	6.23	-	<6	conservation surgery	sampling	invasive ductal	2	1.5	11	0	positive	unknown

Appendix 2: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 3).

No.	Age	Deprivation	WBC (x10⁹/l)	ALB (g/l)	CRP (mg/l)	Breast Surgery	Axillary Surgery	Histological type	Grade	Size (cm)	Total LN	Involved LN	ER status	PR status
35	76	3	5.72	45	<6	mastectomy	sampling	invasive ductal	2	2.0	5	2	positive	unknown
36	59	6	4.85	—	<6	mastectomy	sampling	invasive ductal	2	1.1	17	0	positive	unknown
37	75	3	8.60	37	14	mastectomy	sampling	invasive ductal	2	4.0	9	3	positive	unknown
38	75	5	9.47	40	19	mastectomy	dissection/ clearance	invasive ductal	1	1.5	8	3	positive	unknown
39	61	4	8.27	44	<6	conservation surgery	dissection/ clearance	invasive ductal	3	1.5	15	0	negative	unknown
40	66	5	7.15	43	11	mastectomy	dissection/ clearance	invasive ductal	2	1.4	18	1	positive	unknown
41	55	3	4.62	43	<6	mastectomy	dissection/ clearance	invasive ductal	3	1.8	11	1	negative	unknown
42	61	6	—	42	<6	mastectomy	dissection/ clearance	invasive ductal	1	2.4	—	3	positive	unknown
43	51	5	5.04	—	<6	mastectomy	dissection/ clearance	invasive lobular	1	2.5	15	4	positive	unknown
44	40	6	7.86	42	<6	conservation surgery	dissection/ clearance	invasive ductal	2	2.0	11	1	positive	unknown
45	74	3	7.26	44	<6	mastectomy	dissection/ clearance	invasive ductal	3	3.0	8	0	positive	unknown
46	39	3	4.63	44	<6	mastectomy	dissection/ clearance	invasive ductal	1	2.2	17	0	positive	unknown
47	48	5	10.13	43	10	mastectomy	dissection/ clearance	invasive lobular	3	2.5	14	1	positive	unknown
48	44	4	8.54	41	<6	conservation surgery	dissection/ clearance	invasive ductal	3	1.2	20	0	positive	unknown
49	63	6	6.88	38	8	mastectomy	dissection/ clearance	invasive ductal	3	1.8	7	0	negative	unknown
50	70	5	5.66	38	9	mastectomy	sampling	invasive ductal	3	4.0	14	5	negative	unknown
51	55	5	7.29	45	<6	mastectomy	sampling	invasive ductal	2	2.5	15	0	positive	unknown
52	82	6	5.93	40	8	mastectomy	dissection/ clearance	invasive ductal	2	3.0	22	0	positive	unknown
53	48	4	11.62	44	12	conservation surgery	dissection/ clearance	invasive ductal	3	2.0	6	0	negative	unknown
54	35	3	10.66	43	<6	conservation surgery	dissection/ clearance	invasive ductal	3	2.2	13	0	negative	unknown
55	57	5	5.90	43	6	mastectomy	dissection/ clearance	invasive lobular	1	2.0	11	2	positive	unknown
56	77	6	8.16	39	7	mastectomy	dissection/ clearance	invasive ductal	2	1.6	9	0	positive	unknown
57	50	6	9.96	46	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.0	12	0	positive	unknown
58	52	6	7.35	—	<6	conservation surgery	dissection/ clearance	invasive ductal	3	2.0	12	1	negative	unknown
59	72	6	7.25	—	7	mastectomy	dissection/ clearance	invasive ductal	3	1.7	12	4	positive	unknown
60	53	5	12.92	41	8	conservation surgery	dissection/ clearance	invasive ductal	3	1.5	12	0	positive	unknown
61	50	5	10.06	41	8	mastectomy	dissection/ clearance	invasive ductal	2	1.5	11	0	positive	unknown
62	73	5	5.90	37	10	conservation surgery	—	invasive ductal	1	1.2	—	0	positive	unknown
63	61	5	4.50	44	<6	mastectomy	sampling	invasive ductal	1	0.6	11	0	positive	unknown
64	62	5	6.53	44	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.5	8	1	positive	unknown
65	69	5	9.55	43	17	mastectomy	dissection/ clearance	invasive ductal	2	1.0	15	0	positive	unknown
66	80	6	9.06	43	7	mastectomy	dissection/ clearance	special types	—	4.5	9	0	negative	unknown
67	56	6	10.83	47	<6	conservation surgery	dissection/ clearance	invasive ductal	1	0.9	10	1	positive	unknown
68	60	5	8.27	47	8	conservation surgery	dissection/ clearance	invasive ductal	1	2.0	11	3	positive	unknown

Appendix 2: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 3).

No.	Age	Deprivation	WBC (x10^9/l)	ALB (g/l)	CRP (mg/l)	Breast Surgery	Axillary Surgery	Histological type	Grade	Size (cm)	Total LN	Involved LN	ER status	PR status
69	54	4	5.02	44	<6	conservation surgery	sampling	special types	2	1.0	9	8	negative	unknown
70	45	5	6.57	—	<6	mastectomy	dissection/ clearance	invasive ductal	1	0.2	6	0	positive	unknown
71	41	5	8.94	42	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.2	21	0	negative	unknown
72	44	3	8.44	44	<6	mastectomy	dissection/ clearance	invasive ductal	3	0.6	15	0	negative	negative
73	39	4	6.51	42	8	mastectomy	sampling	invasive ductal	2	4.5	12	6	positive	unknown
74	74	6	6.61	43	<6	conservation surgery	sampling	invasive ductal	2	1.5	5	0	positive	unknown
75	75	6	6.94	40	10	conservation surgery	dissection/ clearance	invasive ductal	2	2.0	7	1	positive	unknown
76	50	4	3.56	42	<6	conservation surgery	dissection/ clearance	invasive ductal	1	0.8	22	0	positive	unknown
77	47	4	7.74	42	6	conservation surgery	dissection/ clearance	invasive ductal	2	2.7	8	0	positive	unknown
78	60	4	13.53	40	9	conservation surgery	dissection/ clearance	invasive ductal	3	2.2	4	0	negative	negative
79	64	6	7.46	39	9	mastectomy	dissection/ clearance	invasive ductal	2	1.8	16	0	positive	unknown
80	53	6	9.70	37	22	mastectomy	dissection/ clearance	invasive lobular	1	2.5	11	0	positive	unknown
81	82	6	7.30	39	8	mastectomy	dissection/ clearance	invasive ductal	1	3.3	9	8	positive	positive
82	73	5	6.42	41	11	mastectomy	dissection/ clearance	invasive lobular	2	1.8	11	0	positive	unknown
83	66	4	7.03	45	6	mastectomy	dissection/ clearance	invasive ductal	2	1.5	11	2	positive	unknown
84	69	6	6.74	44	8	mastectomy	dissection/ clearance	invasive lobular	2	2.0	5	1	positive	unknown
85	74	6	5.43	41	<6	conservation surgery	dissection/ clearance	invasive lobular	2	1.8	6	0	positive	unknown
86	61	4	9.35	46	<6	mastectomy	dissection/ clearance	invasive lobular	2	2.5	16	2	positive	unknown
87	54	5	7.39	40	8	mastectomy	dissection/ clearance	invasive ductal	3	9.0	12	7	negative	negative
88	76	3	6.89	42	6	mastectomy	dissection/ clearance	invasive ductal	2	3.0	17	11	positive	negative
89	65	3	4.84	41	<6	conservation surgery	dissection/ clearance	invasive ductal	2	2.0	6	0	positive	unknown
90	48	7	4.72	42	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.0	8	1	positive	unknown
91	73	5	10.29	44	47	mastectomy	dissection/ clearance	invasive ductal	1	2.9	9	4	positive	unknown
92	45	6	5.73	39	8	mastectomy	dissection/ clearance	invasive lobular	2	1.4	15	12	positive	positive
93	48	3	8.83	40	<6	mastectomy	dissection/ clearance	invasive ductal	2	1.2	9	1	positive	unknown
94	63	3	5.04	—	<6	conservation surgery	dissection/ clearance	invasive ductal	3	0.9	14	0	positive	unknown
95	75	5	9.69	42	<6	mastectomy	dissection/ clearance	invasive ductal	2	3.0	7	5	positive	unknown
96	54	4	7.91	47	<6	mastectomy	dissection/ clearance	invasive ductal	3	7.8	25	25	negative	negative
97	62	4	7.96	46	<6	conservation surgery	dissection/ clearance	invasive ductal	3	2.5	15	0	positive	unknown
98	52	5	7.13	45	<6	conservation surgery	dissection/ clearance	invasive ductal	2	2.4	11	2	positive	unknown
99	53	6	10.56	43	8	conservation surgery	dissection/ clearance	invasive ductal	2	1.7	17	0	negative	negative
100	51	3	6.86	44	8	conservation surgery	dissection/ clearance	invasive ductal	2	2.0	11	4	positive	unknown
101	48	5	8.16	42	<6	conservation surgery	dissection/ clearance	special types	1	0.9	21	0	positive	unknown
102	49	4	10.28	43	<6	mastectomy	dissection/ clearance	invasive ductal	2	3.0	16	1	negative	negative

Appendix 2: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 3).

No.	Age	Deprivation	WBC (x10^9/l)	ALB (g/l)	CRP (mg/l)	Breast Surgery	Axillary Surgery	Histological type	Grade	Size (cm)	Total LN	Involved LN	ER status	PR status
103	57	3	5.13	43	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.5	16	0	positive	unknown
104	40	5	7.05	42	<6	conservation surgery	dissection/ clearance	invasive ductal	2	0.9	8	0	negative	negative
105	54	3	7.94	46	<6	conservation surgery	dissection/ clearance	invasive ductal	2	0.8	11	0	positive	unknown
106	80	4	8.52	42	7	conservation surgery	dissection/ clearance	invasive ductal	2	1.2	10	0	positive	unknown
107	45	4	6.40	43	<6	mastectomy	dissection/ clearance	invasive ductal	3	1.8	4	1	negative	negative
108	49	6	7.04	45	<6	mastectomy	dissection/ clearance	invasive ductal	2	1.3	9	0	positive	unknown
109	67	5	8.30	41	6	conservation surgery	dissection/ clearance	invasive ductal	2	1.3	1	0	positive	unknown
110	65	4	10.75	46	<6	conservation surgery	dissection/ clearance	invasive ductal	2	2.0	12	1	positive	unknown
111	45	5	8.71	42	<6	conservation surgery	dissection/ clearance	invasive ductal	2	0.9	13	0	positive	unknown
112	75	6	4.52	46	8	conservation surgery	dissection/ clearance	invasive ductal	2	4.0	7	0	positive	positive
113	74	6	8.88	42	6	mastectomy	dissection/ clearance	special types	1	3.0	17	0	positive	positive
114	68	3	6.40	45	7	conservation surgery	dissection/ clearance	invasive ductal	1	1.5	14	1	positive	positive
115	69	6	6.50	44	<6	mastectomy	sampling	invasive ductal	3	2.5	4	3	negative	negative
116	64	3	9.06	43	9	mastectomy	dissection/ clearance	invasive ductal	3	3.0	14	0	negative	negative
117	75	5	9.51	39	<6	mastectomy	dissection/ clearance	invasive ductal	1	2.2	13	1	positive	positive
118	71	4	7.31	38	17	conservation surgery	dissection/ clearance	invasive ductal	2	4.0	14	0	positive	positive
119	57	4	7.61	41	<6	mastectomy	dissection/ clearance	invasive lobular	2	4.0	4	0	positive	positive
120	60	5	7.97	44	8	mastectomy	dissection/ clearance	invasive lobular	1	2.2	22	0	positive	unknown
121	73	4	6.18	48	6	mastectomy	dissection/ clearance	invasive ductal	2	3.2	15	1	positive	positive
122	46	5	11.97	47	<6	mastectomy	dissection/ clearance	invasive ductal	2	1.7	15	1	positive	positive
123	56	6	6.69	45	7	mastectomy	dissection/ clearance	invasive ductal	3	0.8	15	0	positive	positive
124	55	4	5.84	44	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.0	9	2	positive	positive
125	45	4	7.04	44	6	conservation surgery	dissection/ clearance	invasive ductal	3	2.0	9	0	positive	positive
126	45	4	7.52	45	<6	conservation surgery	dissection/ clearance	invasive lobular	2	4.0	18	1	positive	positive
127	56	3	9.14	43	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.8	14	4	negative	negative
128	82	6	5.66	46	8	mastectomy	dissection/ clearance	invasive ductal	2	2.5	10	4	negative	negative
129	44	3	7.44	46	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.5	12	0	positive	positive
130	76	5	6.86	39	<6	mastectomy	dissection/ clearance	invasive ductal	1	1.8	6	0	positive	unknown
131	44	3	5.93	45	<6	conservation surgery	dissection/ clearance	special types	1	1.9	14	0	positive	positive
132	71	5	9.06	39	7	mastectomy	dissection/ clearance	invasive ductal	2	2.7	12	0	positive	unknown
133	84	3	8.95	41	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.5	12	1	positive	unknown
134	84	4	7.49	41	10	mastectomy	dissection/ clearance	invasive ductal	2	2.8	14	4	positive	unknown
135	63	5	5.73	39	14	mastectomy	dissection/ clearance	invasive lobular	2	1.8	7	0	positive	unknown
136	39	3	9.33	41	7	conservation surgery	dissection/ clearance	invasive ductal	2	1.0	18	1	positive	unknown

Appendix 2: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 3).

No.	Age	Deprivation	WBC (x10⁹/l)	ALB (g/l)	CRP (mg/l)	Breast Surgery	Axillary Surgery	Histological type	Grade	Size (cm)	Total LN	Involved LN	ER status	PR status
137	46	6	6.95	43	6	mastectomy	dissection/ clearance	invasive ductal	3	1.7	11	2	negative	negative
138	38	5	9.74	36	10	mastectomy	dissection/ clearance	invasive ductal	3	1.4	14	2	positive	positive
139	41	5	7.56	44	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.5	22	1	positive	positive
140	72	6	9.20	40	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.0	13	1	positive	positive
141	48	3	3.60	35	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.0	18	1	negative	negative
142	72	4	7.58	45	7	conservation surgery	dissection/ clearance	invasive ductal	2	1.4	9	0	positive	unknown
143	62	6	6.27	44	<6	mastectomy	dissection/ clearance	invasive ductal	3	1.8	6	1	negative	negative
144	41	3	3.75	44	<6	conservation surgery	dissection/ clearance	invasive ductal	3	2.2	33	1	positive	unknown
145	51	5	13.85	44	<6	mastectomy	dissection/ clearance	invasive ductal	2	1.4	4	0	positive	unknown
146	57	5	5.67	44	20	mastectomy	dissection/ clearance	invasive ductal	3	3.5	10	0	negative	negative
147	51	2	6.76	44	<6	conservation surgery	dissection/ clearance	invasive ductal	1	0.6	11	0	positive	positive
148	76	2	4.96	48	<6	conservation surgery	sampling	invasive ductal	1	0.6	4	0	positive	positive
149	55	4	4.56	42	<6	mastectomy	dissection/ clearance	invasive ductal	1	0.9	16	0	positive	negative
150	67	7	5.92	46	<6	mastectomy	dissection/ clearance	invasive lobular	2	2.0	15	1	positive	negative
151	53	2	—	44	<6	conservation surgery	dissection/ clearance	invasive ductal	3	1.2	17	0	positive	positive
152	77	6	8.48	42	6	conservation surgery	dissection/ clearance	invasive ductal	2	2.0	21	0	positive	positive
153	68	7	7.96	46	<6	mastectomy	dissection/ clearance	invasive ductal	3	3.6	15	5	positive	positive
154	56	6	4.12	45	<6	mastectomy	dissection/ clearance	invasive ductal	2	1.9	11	1	positive	positive
155	57	3	5.77	45	9	conservation surgery	dissection/ clearance	invasive ductal	3	1.9	15	0	negative	negative
156	78	6	6.79	46	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.0	10	0	positive	positive
157	52	3	5.08	50	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.0	20	0	negative	negative
158	57	6	9.44	43	<6	conservation surgery	dissection/ clearance	invasive lobular	2	2.0	26	5	positive	positive
159	60	6	8.15	44	14	conservation surgery	dissection/ clearance	invasive ductal	3	0.6	17	1	negative	negative
160	56	6	5.06	44	28	conservation surgery	dissection/ clearance	special types	2	0.9	15	0	positive	positive
161	53	4	9.11	44	6	mastectomy	dissection/ clearance	invasive ductal	2	1.1	14	0	positive	negative
162	60	3	6.45	45	16	conservation surgery	dissection/ clearance	special types	1	0.9	17	0	positive	positive
163	73	4	6.98	42	<6	conservation surgery	dissection/ clearance	invasive ductal	1	0.8	16	0	positive	positive
164	44	4	7.20	44	<6	conservation surgery	dissection/ clearance	invasive ductal	3	0.9	15	0	negative	negative
165	52	2	8.53	46	57	conservation surgery	dissection/ clearance	invasive ductal	3	1.4	6	0	positive	negative
166	42	3	4.17	42	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.2	22	1	positive	positive
167	61	4	5.84	47	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.6	17	1	positive	negative
168	82	2	4.52	41	66	mastectomy	dissection/ clearance	invasive ductal	3	2.0	10	6	positive	negative
169	70	4	8.79	43	<6	mastectomy	dissection/ clearance	invasive ductal	2	4.2	11	3	positive	positive
170	52	1	8.15	46	<6	conservation surgery	dissection/ clearance	invasive ductal	2	0.5	14	0	negative	negative

No.	Age	Deprivation	WBC (x10^9/l)	ALB (g/l)	CRP (mg/l)	Breast Surgery	Axillary Surgery	Histological type	Grade	Size (cm)	Total LN	Involved LN	ER status	PR status
171	62	1	4.49	42	9	mastectomy	dissection/ clearance	invasive lobular	2	2.1	8	0	positive	negative
172	59	1	4.11	45	<6	conservation surgery	dissection/ clearance	invasive ductal	2	2.0	15	3	positive	positive
173	55	4	5.18	49	10	conservation surgery	dissection/ clearance	invasive ductal	1	0.9	13	0	positive	positive
174	60	6	4.31	41	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.2	29	17	negative	negative
175	41	2	8.40	46	<6	mastectomy	dissection/ clearance	invasive ductal	3	3.5	16	9	negative	negative
176	59	4	7.80	41	15	mastectomy	dissection/ clearance	invasive ductal	3	6.0	22	17	positive	negative
177	60	7	4.65	46	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.7	18	0	negative	negative
178	72	2	4.99	40	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.3	9	0	positive	positive
179	59	2	6.13	45	<6	mastectomy	dissection/ clearance	invasive lobular	2	2.5	15	0	positive	positive
180	52	2	4.88	41	7	mastectomy	dissection/ clearance	invasive ductal	3	1.1	23	2	positive	positive
181	81	2	7.59	46	6	conservation surgery	dissection/ clearance	special types	1	2.1	10	0	positive	positive
182	69	4	8.63	44	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.5	17	0	positive	positive
183	65	2	11.18	44	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.1	22	12	positive	positive
184	69	3	6.32	44	<6	conservation surgery	dissection/ clearance	invasive ductal	1	1.3	8	0	positive	negative
185	40	2	6.25	46	<6	mastectomy	dissection/ clearance	invasive ductal	3	1.5	17	0	positive	positive
186	61	3	6.73	44	<6	conservation surgery	dissection/ clearance	invasive lobular	2	1.4	23	0	positive	positive
187	66	4	5.05	42	<6	mastectomy	dissection/ clearance	invasive ductal	2	1.7	13	0	positive	positive
188	52	6	4.73	42	8	mastectomy	dissection/ clearance	invasive ductal	2	1.3	19	2	positive	positive
189	60	3	4.67	42	<6	conservation surgery	dissection/ clearance	invasive ductal	3	1.3	21	0	positive	unknown
190	70	2		44	6	mastectomy	dissection/ clearance	invasive ductal	3	2.8	15	0	negative	negative
191	63	7	4.68	45	11	conservation surgery	dissection/ clearance	invasive ductal	1	0.7	11	0	positive	unknown
192	67	6	5.91	48	<6	mastectomy	dissection/ clearance	invasive lobular	2	4.0	10	10	positive	positive
193	52	3	8.27	49	6	conservation surgery	dissection/ clearance	invasive lobular	2	1.5	16	0	positive	positive
194	66	7	3.42	43	<6	mastectomy	dissection/ clearance	invasive ductal	3	3.0	24	1	positive	negative
195	34	2	5.57	48	<6	mastectomy	dissection/ clearance	invasive ductal	3	1.1	19	0	positive	negative
196	88	1	7.11	42	<6	mastectomy	dissection/ clearance	invasive ductal	1	2.0	19	0	positive	negative
197	87	5	7.79	45	54	mastectomy	dissection/ clearance	invasive ductal	1	1.2	16	1	positive	negative
198	52	4	7.60	50	6	conservation surgery	dissection/ clearance	invasive lobular	3	2.2	15	1	positive	positive
199	70	6	4.94	44	8	conservation surgery	dissection/ clearance	invasive ductal	2	2.1	18	0	negative	negative
200	57	4	8.04	46	<6	conservation surgery	dissection/ clearance	invasive ductal	2	2.4	18	2	positive	positive
201	51	4	9.31	48	6	conservation surgery	dissection/ clearance	special types	1	1.1	15	0	positive	positive
202	72	6	6.10	47	<6	mastectomy	dissection/ clearance	invasive ductal	2	1.7	15	0	positive	positive
203	72	1	7.03	48	<6	mastectomy	dissection/ clearance	invasive ductal	3	1.9	13	0	negative	negative
204	56	4	8.03	46	23	conservation surgery	dissection/ clearance	invasive ductal	1	0.4	9	0	positive	positive

Appendix 2: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 3).

No.	Age	Deprivation	WBC (x10⁹/l)	ALB (g/l)	CRP (mg/l)	Breast Surgery	Axillary Surgery	Histological type	Grade	Size (cm)	Total LN	Involved LN	ER status	PR status
205	62	7	9.04	46	15	conservation surgery	dissection/ clearance	invasive ductal	3	1.4	16	2	negative	negative
206	58	3	5.80	44	6	conservation surgery	sampling	invasive ductal	2	0.3	4	0	positive	unknown
207	58	6	9.31	47	15	mastectomy	dissection/ clearance	invasive ductal	2	2.5	18	1	positive	positive
208	52	6	4.29	45	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.6	16	0	positive	positive
209	64	3	5.32	45	<6	conservation surgery	dissection/ clearance	invasive ductal	1	0.5	12	0	positive	positive
210	52	7	6.53	47	<6	conservation surgery	dissection/ clearance	invasive lobular	1	1.4	19	0	positive	positive
211	64	4	11.67	45	<6	conservation surgery	dissection/ clearance	invasive ductal	1	0.4	14	0	positive	negative
212	63	1	3.83	42	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.5	19	3	negative	negative
213	39	2	5.86	44	6	mastectomy	dissection/ clearance	invasive ductal	2	2.3	19	0	positive	positive
214	56	5	6.68	38	6	conservation surgery	dissection/ clearance	invasive lobular	3	2.5	17	0	positive	negative
215	44	1	5.84	45	6	conservation surgery	dissection/ clearance	invasive ductal	2	2.6	18	2	positive	positive
216	71	4	9.31	45	<6	mastectomy	dissection/ clearance	invasive ductal	3	4.8	14	0	negative	negative
217	52	4	4.61	44	8	conservation surgery	dissection/ clearance	invasive lobular	3	1.1	16	0	positive	positive
218	62	4	7.68	49	<6	conservation surgery	dissection/ clearance	invasive ductal	2	3.0	18	0	positive	positive
219	58	4	6.30	44	13	conservation surgery	dissection/ clearance	invasive ductal	3	2.6	15	0	positive	positive
220	89	6	5.49	42	9	mastectomy	dissection/ clearance	invasive ductal	2	3.0	10	2	positive	positive
221	51	3	12.36	46	<6	conservation surgery	dissection/ clearance	invasive ductal	3	0.9	21	0	negative	negative
222	51	4	7.85	41	7	conservation surgery	dissection/ clearance	special types	1	1.2	11	0	positive	positive
223	70	6	7.14	44	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.1	13	1	positive	positive
224	54	2	4.81	43	<6	conservation surgery	dissection/ clearance	invasive ductal	1	1.6	15	0	positive	negative
225	81	2	7.05	44	<6	mastectomy	dissection/ clearance	invasive ductal	3	1.6	16	0	positive	positive
226	52	4	4.40	39	22	conservation surgery	dissection/ clearance	invasive ductal	2	1.6	7	1	positive	negative
227	56	5	6.08	47	<6	conservation surgery	dissection/ clearance	invasive ductal	2	3.2	17	0	positive	positive
228	60	2	7.59	43	<6	conservation surgery	dissection/ clearance	invasive lobular	2	0.9	18	0	positive	negative
229	55	4	10.73	46	<6	conservation surgery	dissection/ clearance	invasive ductal	2	0.7	14	0	negative	negative
230	55	2	6.10	43	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.5	10	0	positive	positive
231	63	2	5.37	45	<6	conservation surgery	dissection/ clearance	invasive ductal	2	2.0	23	0	positive	positive
232	51	2	6.49	42	<6	conservation surgery	dissection/ clearance	invasive ductal	3	2.5	9	1	positive	positive
233	63	5	7.43	40	7	conservation surgery	dissection/ clearance	invasive ductal	2	1.2	10	0	positive	positive
234	52	2	7.15	45	<6	conservation surgery	dissection/ clearance	invasive ductal	1	0.7	10	0	positive	positive
235	68	3	4.18	44	<6	mastectomy	sampling	invasive ductal	2	1.5	7	0	positive	negative
236	78	2	7.48	45	<6	mastectomy	dissection/ clearance	invasive ductal	3	3.0	15	2	positive	positive
237	52	5	6.59	44	8	conservation surgery	dissection/ clearance	invasive ductal	2	1.7	14	0	positive	positive
238	46	2	6.68	46	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.6	18	2	positive	positive

Appendix 2: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 3).

No.	Age	Deprivation	WBC (x10⁹/l)	ALB (g/l)	CRP (mg/l)	Breast Surgery	Axillary Surgery	Histological type	Grade	Size (cm)	Total LN	Involved LN	ER status	PR status
239	72	7	8.18	46	<6	conservation surgery	dissection/ clearance	invasive ductal	1	0.9	8	0	positive	negative
240	57	6	8.23	45	<6	conservation surgery	dissection/ clearance	invasive ductal	3	1.9	15	0	positive	negative
241	71	6	5.09	43	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.2	16	0	positive	positive
242	59	5	9.57	44	8	conservation surgery	dissection/ clearance	invasive ductal	2	1.4	15	3	positive	positive
243	61	5	8.42	—	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.9	11	2	positive	positive
244	47	2	11.54	43	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.7	6	0	positive	positive
245	61	3	7.22	43	32	conservation surgery	dissection/ clearance	invasive ductal	2	1.7	13	0	positive	positive
246	63	3	9.02	48	9	conservation surgery	dissection/ clearance	invasive ductal	1	1.2	16	0	positive	positive
247	60	4	7.73	48	<6	conservation surgery	dissection/ clearance	invasive ductal	3	1.2	8	0	positive	positive
248	63	7	5.84	48	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.1	24	0	positive	negative
249	70	7	11.90	45	14	conservation surgery	dissection/ clearance	invasive lobular	2	2.1	15	0	positive	negative
250	64	6	5.81	41	7	mastectomy	dissection/ clearance	invasive ductal	2	3.0	13	2	positive	positive
251	89	2	6.94	44	<6	mastectomy	dissection/ clearance	invasive ductal	2	3.0	10	0	positive	positive
252	62	7	4.82	43	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.4	17	0	positive	positive
253	54	3	4.96	46	<6	conservation surgery	dissection/ clearance	invasive ductal	1	0.7	10	0	positive	positive
254	65	3	8.34	45	<6	conservation surgery	dissection/ clearance	special types	1	0.5	13	0	positive	unknown
255	45	1	6.54	46	<6	mastectomy	dissection/ clearance	invasive ductal	2	1.1	12	0	positive	positive
256	63	4	7.64	46	<6	conservation surgery	dissection/ clearance	invasive ductal	3	2.6	11	0	negative	negative
257	82	5	9.30	43	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.1	11	1	positive	positive
258	56	4	6.12	46	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.8	11	3	negative	negative
259	62	2	7.44	44	11	conservation surgery	dissection/ clearance	invasive ductal	3	3.5	11	1	positive	positive
260	65	6	6.16	44	15	mastectomy	dissection/ clearance	invasive ductal	3	5.0	32	0	negative	negative
261	52	5	7.98	47	11	mastectomy	dissection/ clearance	invasive ductal	3	2.1	16	5	negative	negative
262	83	6	7.63	39	8	mastectomy	dissection/ clearance	invasive ductal	2	2.5	10	0	positive	positive
263	53	4	4.49	46	6	conservation surgery	dissection/ clearance	invasive lobular	2	1.1	12	0	positive	negative
264	46	3	6.12	44	10	mastectomy	dissection/ clearance	invasive ductal	3	2.5	11	1	positive	positive
265	52	3	6.44	44	<6	conservation surgery	dissection/ clearance	invasive ductal	3	1.6	17	1	positive	positive
266	48	6	5.73	50	12	conservation surgery	dissection/ clearance	invasive ductal	2	1.2	17	3	positive	positive
267	46	5	9.12	47	<6	mastectomy	dissection/ clearance	invasive ductal	1	2.5	14	0	positive	positive
268	79	6	6.53	42	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.0	12	1	negative	negative
269	57	6	6.52	38	21	conservation surgery	dissection/ clearance	invasive ductal	2	1.6	10	1	positive	positive
270	37	3	6.47	47	13	conservation surgery	dissection/ clearance	invasive ductal	3	2.4	25	0	negative	negative
271	61	3	6.91	48	<6	mastectomy	dissection/ clearance	invasive ductal	3	1.2	7	2	positive	negative
272	61	6	6.58	41	9	conservation surgery	dissection/ clearance	invasive ductal	3	2.8	22	0	negative	negative

Appendix 2: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 3).

No.	Age	Deprivation	WBC (x10^9/l)	ALB (g/l)	CRP (mg/l)	Breast Surgery	Axillary Surgery	Histological type	Grade	Size (cm)	Total LN	Involved LN	ER status	PR status
273	65	4	6.15	44	6	mastectomy	dissection/ clearance	invasive ductal	1	2.3	18	0	positive	positive
274	47	7	7.63	44	9	mastectomy	dissection/ clearance	invasive lobular	2	1.6	25	11	positive	positive
275	76	4	5.11	40	<6	mastectomy	dissection/ clearance	invasive ductal	2	1.6	24	0	positive	positive
276	67	3	5.35	46	<6	mastectomy	dissection/ clearance	invasive ductal	3	0.1	11	7	positive	negative
277	78	1	8.18	42	33	mastectomy	dissection/ clearance	invasive ductal	2	3.0	10	1	positive	positive
278	48	6	5.77	45	<6	mastectomy	dissection/ clearance	invasive lobular	2	2.9	18	13	positive	positive
279	72	6	7.76	52	6	conservation surgery	dissection/ clearance	invasive ductal	2	1.6	13	2	positive	positive
280	72	7	6.68	48	<6	conservation surgery	dissection/ clearance	invasive ductal	1	1.2	20	1	positive	positive
281	64	3	6.65	41	10	conservation surgery	dissection/ clearance	invasive ductal	2	0.8	14	0	positive	negative
282	80	6	5.65	42	6	mastectomy	dissection/ clearance	invasive ductal	2	3.5	17	17	positive	positive
283	52	5	6.41	44	<6	mastectomy	dissection/ clearance	invasive ductal	1	3.0	18	0	positive	positive
284	69	5	17.42	41	<6	conservation surgery	dissection/ clearance	invasive ductal	3	1.5	18	0	positive	positive
285	68	6	6.54	46	<6	mastectomy	dissection/ clearance	invasive ductal	3	3.0	17	11	positive	positive
286	46	6	3.60	44	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.0	9	1	positive	positive
287	70	4	8.05	46	7	conservation surgery	dissection/ clearance	invasive ductal	2	1.1	16	0	positive	positive
288	75	3	6.76	44	<6	mastectomy	dissection/ clearance	invasive ductal	3	1.4	16	0	negative	negative
289	82	5	3.88	41	<6	mastectomy	dissection/ clearance	invasive lobular	2	5.5	22	16	positive	positive
290	63	3	5.13	47	<6	conservation surgery	dissection/ clearance	invasive ductal	2	2.3	14	2	positive	positive
291	54	6	7.43	44	8	mastectomy	dissection/ clearance	invasive ductal	2	11.0	33	3	positive	positive
292	54	1	7.72	43	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.1	13	3	negative	negative
293	49	1	3.94	44	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.0	10	0	positive	positive
294	68	2	5.38	46	<6	mastectomy	dissection/ clearance	invasive lobular	2	4.0	25	1	positive	negative
295	47	6	8.67	45	<6	mastectomy	dissection/ clearance	invasive ductal	3	0.8	12	0	positive	positive
296	82	4	6.21	44	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.6	22	2	positive	positive
297	55	4	5.07	45	<6	conservation surgery	dissection/ clearance	invasive ductal	2	0.7	9	2	positive	positive
298	42	6	7.04	47	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.5	15	13	positive	positive
299	50	6	6.27	43	<6	conservation surgery	dissection/ clearance	invasive ductal	1	2.1	9	0	positive	positive
300	40	3	7.66	46	<6	conservation surgery	dissection/ clearance	invasive ductal	2	3.2	17	1	positive	positive

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No.	Radiotherapy	Chemotherapy	Hormonal therapy	Locoregional recurrence	Systemic metastasis	Final status	RFS/m.	OS/m.
1	no	CMF	no	breast /chest wall	VISCERAL	Breast cancer related death	17.4	33.5
2	yes	CMF	aromatase inhibitors	None	None	Alive and well	58.5	58.5
3	yes	no	no	None	VISCERAL	Breast cancer related death	14.9	18.6
4	yes	Epi/CMF	tamoxifen	None	NON-VISCERAL	Breast cancer related death	17.1	32.2
5	yes	CMF	tamoxifen	None	None	Alive and well	57.2	57.9
6	no	no	tamoxifen	None	None	Alive and well	55.4	55.4
7	no	no	tamoxifen	None	VISCERAL	Alive with recurrent/metastatic BC	53.8	54.7
8	yes	CMF	tamoxifen	None	None	Alive and well	53.2	53.2
9	yes	no	no	None	None	Alive and well	58.2	58.6
10	no	no	tamoxifen	None	None	Alive and well	48.3	48.3
11	yes	CMF	no	None	None	Alive and well	59.6	59.6
12	no	CMF	no	breast /chest wall	VISCERAL & NON-VISCERAL	Breast cancer related death	21.5	48.3
13	yes	CMF	tamoxifen	None	None	Alive and well	52.9	52.9
14	no	CMF	tamoxifen	None	None	Alive and well	48.3	48.3
15	yes	CMF	tamoxifen	None	None	Alive and well	58.9	58.9
16	yes	CMF	tamoxifen	None	None	Alive and well	51.8	51.8
17	yes	no	tamoxifen	cervical lymph-node	None	Alive with recurrent/metastatic BC	9.1	53.9
18	no	no	tamoxifen	None	None	Breast cancer related death	25.0	25.0
19	yes	Epi/CMF	no	None	VISCERAL & NON-VISCERAL	Breast cancer related death	35.0	42.9
20	yes	no	tamoxifen	None	None	Alive and well	54.8	54.8
21	no	CMF	no	None	None	Alive and well	58.8	60.3
22	yes	Epi/CMF	tamoxifen	None	None	Alive and well	51.2	51.2
23	no	CMF	no	None	None	Alive and well	60.2	60.2
24	no	no	tamoxifen	None	None	Alive and well	55.8	55.8
25	yes	no	tamoxifen	None	None	Alive and well	51.1	51.1
26	yes	no	tamoxifen	None	None	Alive and well	49.7	50.4
27	no	no	tamoxifen	None	None	Alive and well	48.3	48.3
28	yes	no	tamoxifen	None	None	Alive and well	58.3	58.3
29	yes	no	no	None	None	Alive and well	55.8	55.8
30	no	CMF	no	None	None	Alive and well	55.0	55.0
31	no	CMF	tamoxifen	None	None	Alive and well	51.1	51.8
32	yes	Epi/CMF	no	None	None	Alive and well	49.7	49.7
33	no	CMF	tamoxifen --> AI	None	None	Alive and well	58.8	58.8
34	yes	no	tamoxifen	None	None	Alive and well	55.4	56.4

Appendix 2: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 3).

No.	Radiotherapy	Chemotherapy	Hormonal therapy	Locoregional recurrence	Systemic metastasis	Final status	RFS/m.	OS/m.
35	yes	no	tamoxifen	None	None	Alive and well	52.8	52.8
36	no	no	tamoxifen --> AI	None	None	Alive and well	54.2	54.7
37	yes	no	tamoxifen	None	None	Alive and well	55.6	55.6
38	yes	no	tamoxifen	None	None	Breast cancer related death	36.8	36.8
39	yes	Epi/CMF	no	None	None	Alive and well	51.5	51.5
40	no	no	tamoxifen --> AI	None	None	Alive and well	50.7	50.7
41	yes	Epi/CMF	no	None	VISCERAL & NON-VISCERAL	Breast cancer related death	14.7	20.2
42	no	no	tamoxifen	None	None	Alive and well	59.7	59.7
43	yes	Epi/CMF	tamoxifen	None	None	Alive and well	52.0	52.0
44	yes	TACT STUDY	tamoxifen	None	None	Alive and well	50.2	50.6
45	no	no	tamoxifen	None	None	Alive and well	58.3	58.3
46	no	no	tamoxifen	None	None	Alive and well	48.3	48.3
47	yes	Epi/CMF	tamoxifen	None	None	Alive and well	56.9	58.3
48	yes	Epi/CMF	tamoxifen	None	None	Alive and well	48.0	48.0
49	no	CMF	no	None	None	Alive and well	55.6	55.6
50	yes	AC	no	breast /chest wall	VISCERAL & NON-VISCERAL	Breast cancer related death	18.6	21.5
51	no	no	tamoxifen	None	None	Alive and well	50.9	52.0
52	no	no	tamoxifen	None	None	Non-cancer related death	50.8	50.8
53	yes	AC	no	None	None	Alive and well	50.0	50.0
54	yes	Epi/CMF	no	None	None	Alive and well	56.7	56.7
55	yes	CMF	tamoxifen	None	None	Alive and well	54.1	54.8
56	no	no	tamoxifen	None	None	Alive and well	48.0	48.0
57	yes	CMF	tamoxifen	None	None	Alive and well	57.4	57.4
58	yes	Epi/CMF	no	None	None	Alive and well	57.4	57.9
59	yes	no	no	None	None	Alive and well	58.9	58.9
60	yes	no	tamoxifen	None	None	Non-cancer related death	30.9	30.9
61	no	CMF	tamoxifen	None	None	Alive and well	51.1	51.1
62	no	no	tamoxifen	None	None	Breast cancer related death	42.2	42.2
63	no	no	tamoxifen	None	None	Alive and well	53.9	54.4
64	yes	CMF	tamoxifen	None	None	Alive and well	50.6	50.6
65	no	no	tamoxifen	None	None	Alive and well	55.5	55.5
66	yes	no	no	None	None	Other cancer death	36.4	36.4
67	yes	CMF	tamoxifen	None	None	Alive and well	47.7	47.7
68	yes	Epi/CMF	tamoxifen	None	None	Alive and well	52.6	52.6

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No.	Radiotherapy	Chemotherapy	Hormonal therapy	Locoregional recurrence	Systemic metastasis	Final status	RFS/m.	OS/m.
69	yes	Epi/CMF	no	None	None	Alive and well	54.1	54.1
70	no	no	tamoxifen	None	None	Alive and well	59.6	59.6
71	no	Epi/CMF	no	None	None	Alive and well	55.3	55.3
72	no	Epi/CMF	no	None	None	Alive and well	47.3	47.3
73	yes	Epi/CMF	tamoxifen	None	None	Alive and well	48.8	48.8
74	yes	no	tamoxifen	None	None	Non-cancer related death	16.7	16.7
75	yes	no	tamoxifen	None	None	Alive and well	44.8	45.8
76	yes	no	tamoxifen	None	None	Alive and well	46.6	47.2
77	yes	Epi/CMF	tamoxifen	None	None	Alive and well	46.6	46.6
78	yes	no	no	None	VISCERAL	Breast cancer related death	9.7	10.1
79	no	no	tamoxifen	None	None	Alive and well	46.2	46.2
80	no	no	tamoxifen	None	None	Alive and well	45.9	46.6
81	yes	no	tamoxifen	None	None	Alive and well	43.9	43.9
82	no	no	tamoxifen	None	None	Alive and well	46.3	46.3
83	yes	CMF	tamoxifen	breast /chest wall	VISCERAL	Breast cancer related death	12.0	22.7
84	yes	AC	tamoxifen	None	None	Alive and well	46.3	46.3
85	yes	no	tamoxifen	None	None	Alive and well	46.9	47.4
86	no	AC	tamoxifen	None	None	Alive and well	42.5	43.0
87	yes	Epi/CMF	no	breast /chest wall	VISCERAL	Breast cancer related death	14.1	23.7
88	yes	no	tamoxifen	None	VISCERAL	Alive with recurrent/metastatic BC	36.4	45.3
89	yes	no	tamoxifen	None	None	Alive and well	43.5	44.2
90	yes	CMF	tamoxifen	None	None	Alive and well	43.5	44.4
91	yes	no	tamoxifen	None	None	Alive and well	43.0	43.0
92	yes	Epi/CMF	tamoxifen	None	None	Alive and well	45.3	46.2
93	yes	Epi/CMF	tamoxifen	None	None	Alive and well	44.5	44.5
94	yes	no	tamoxifen	None	None	Alive and well	43.2	43.2
95	yes	no	tamoxifen	None	None	Alive and well	42.4	42.4
96	yes	Epi/CMF	no	None	VISCERAL	Alive with recurrent/metastatic BC	31.4	41.7
97	yes	Epi/CMF	tamoxifen	None	None	Alive and well	42.2	42.2
98	yes	Epi/CMF	tamoxifen	None	None	Alive and well	42.1	42.1
99	yes	Epi/CMF	no	None	None	Alive and well	42.2	42.2
100	yes	Epi/CMF	tamoxifen	None	None	Alive and well	42.2	42.2
101	yes	no	tamoxifen	None	None	Alive and well	41.3	42.4
102	yes	TANGO STUDY	no	None	None	Alive and well	41.6	41.6

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No.	Radiotherapy	Chemotherapy	Hormonal therapy	Locoregional recurrence	Systemic metastasis	Final status	RFS/m.	OS/m.
103	yes	no	tamoxifen	None	None	Alive and well	41.4	41.4
104	yes	AC	no	None	None	Alive and well	41.4	42.2
105	yes	no	tamoxifen	None	None	Alive and well	42.0	42.0
106	yes	no	tamoxifen	None	None	Alive and well	42.0	42.0
107	yes	TANGO STUDY	no	None	None	Alive and well	41.1	41.1
108	no	no	tamoxifen	None	None	Alive and well	41.0	41.0
109	yes	no	tamoxifen	None	None	Non-cancer related death	5.9	5.9
110	yes	no	tamoxifen	None	None	Alive and well	43.0	43.0
111	yes	no	tamoxifen	None	None	Alive and well	42.1	42.4
112	yes	no	tamoxifen	None	None	Alive and well	34.9	34.9
113	no	no	aromatase inhibitors	None	None	Alive and well	35.8	35.8
114	yes	no	tamoxifen	None	None	Alive and well	36.0	36.0
115	no	no	no	None	None	Non-cancer related death	34.8	34.8
116	no	Epi/CMF	no	None	None	Alive and well	36.2	36.2
117	no	no	tamoxifen	None	None	Alive and well	36.4	36.4
118	yes	no	tamoxifen	None	None	Alive and well	36.4	36.4
119	no	no	tamoxifen	breast /chest wall	None	Alive with recurrent/metastatic BC	32.4	36.6
120	yes	no	tamoxifen	None	None	Alive and well	36.8	36.8
121	yes	no	tamoxifen	None	None	Alive and well	35.5	36.8
122	no	Epi/CMF	tamoxifen	None	None	Alive and well	37.1	37.1
123	yes	Epi/CMF	tamoxifen	None	None	Alive and well	37.2	37.2
124	yes	Epi/CMF	tamoxifen	None	None	Alive and well	37.5	37.5
125	yes	Epi/CMF	tamoxifen	None	None	Alive and well	37.5	37.5
126	yes	Epi/CMF	tamoxifen	None	None	Alive and well	37.6	37.6
127	yes	Epi/CMF	no	None	VISCERAL	Breast cancer related death	32.0	32.0
128	yes	no	no	None	VISCERAL & NON-VISCERAL	Breast cancer related death	7.0	11.8
129	no	Epi/CMF	tamoxifen	None	VISCERAL	Breast cancer related death	28.8	34.9
130	no	no	tamoxifen	None	None	Alive and well	38.6	38.6
131	yes	no	tamoxifen	None	None	Alive and well	37.6	38.6
132	no	no	aromatase inhibitors	None	None	Alive and well	38.7	38.7
133	no	no	tamoxifen	None	None	Alive and well	39.4	40.1
134	yes	no	tamoxifen	None	None	Alive and well	39.4	39.4
135	no	no	tamoxifen	None	None	Alive and well	39.0	39.4
136	yes	TANGO STUDY	tamoxifen	None	None	Alive and well	39.5	40.2

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No.	Radiotherapy	Chemotherapy	Hormonal therapy	Locoregional recurrence	Systemic metastasis	Final status	Rf-S/m.	OS/m.
137	yes	Epi/CMF	no	None	None	Alive and well	39.6	39.6
138	yes	Epi/CMF	tamoxifen	None	None	Alive and well	39.6	39.6
139	yes	Epi/CMF	tamoxifen	None	None	Alive and well	39.7	40.6
140	no	no	tamoxifen	None	None	Alive and well	40.1	40.1
141	yes	Epi/CMF	no	None	None	Alive and well	40.2	40.2
142	yes	no	tamoxifen	None	NON-VISCERAL	Alive with recurrent/metastatic BC	22.0	40.3
143	yes	AC	no	None	None	Alive and well	40.9	40.9
144	yes	EC + taxol	no	None	None	Alive and well	40.9	41.4
145	no	no	tamoxifen	None	None	Alive and well	41.3	41.3
146	no	Epi/CMF	no	None	None	Alive and well	35.3	35.3
147	yes	no	tamoxifen	None	None	Alive and well	51.6	51.6
148	yes	no	tamoxifen --> AI	None	None	Alive and well	52.7	54.4
149	no	no	aromatase inhibitors	None	None	Alive and well	57.2	57.2
150	no	no	tamoxifen	None	None	Alive and well	49.8	49.8
151	yes	CMF	tamoxifen --> AI	None	None	Alive and well	56.8	56.8
152	yes	no	tamoxifen	None	None	Alive and well	51.6	51.6
153	yes	Epi/CMF	tamoxifen	None	None	Alive and well	58.4	58.8
154	yes	Epi/CMF	tamoxifen	None	None	Alive and well	51.6	51.6
155	yes	CMF	no	None	None	Alive and well	55.8	55.8
156	no	no	tamoxifen --> AI	None	None	Alive and well	52.0	52.0
157	yes	CMF	no	None	None	Alive and well	51.2	51.2
158	yes	Epi/CMF	tamoxifen	None	None	Alive and well	57.2	57.2
159	yes	Epi/CMF	tamoxifen	None	None	Alive and well	57.2	57.2
160	no	no	tamoxifen	None	None	Alive and well	57.9	57.9
161	no	no	tamoxifen	None	None	Alive and well	56.2	56.2
162	yes	no	no	None	None	Alive and well	52.3	52.3
163	yes	no	tamoxifen	None	None	Other cancer death	25.9	25.9
164	yes	Epi/CMF	no	None	None	Alive and well	55.5	56.2
165	yes	CMF	tamoxifen	None	None	Alive and well	57.8	57.8
166	yes	Epi/CMF	tamoxifen	None	None	Alive and well	49.8	49.8
167	no	CMF	tamoxifen	breast /chest wall	VISCERAL & NON-VISCERAL	Breast cancer related death	17.7	26.1
168	yes	no	tamoxifen	None	None	Alive and well	54.8	56.0
169	no	no	tamoxifen	None	None	Non-cancer related death	18.9	19.9
170	yes	Epi/CMF	no	breast /chest wall	None	Alive with recurrent/metastatic BC	35.2	52.0

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No.	Radiotherapy	Chemotherapy	Hormonal therapy	Locoregional recurrence	Systemic metastasis	Final status	RFS/m.	OS/m.
171	no	no	tamoxifen	None	None	Alive and well	58.1	58.1
172	yes	CMF	tamoxifen	None	None	Alive and well	50.2	50.2
173	yes	no	tamoxifen	None	None	Alive and well	57.2	57.2
174	yes	Epi/CMF	no	None	VISCERAL & NON-VISCERAL	Breast cancer related death	26.1	30.0
175	yes	no	no	None	None	Alive and well	55.5	55.5
176	yes	Epi/CMF	tamoxifen	None	VISCERAL	Breast cancer related death	14.7	15.1
177	yes	CMF	no	None	None	Alive and well	49.1	49.8
178	yes	no	tamoxifen	None	None	Alive and well	52.0	52.0
179	no	no	tamoxifen	None	None	Alive and well	50.2	51.1
180	yes	Epi/CMF	tamoxifen	None	None	Alive and well	55.8	55.8
181	yes	no	tamoxifen	None	None	Non-cancer related death	39.2	39.2
182	yes	no	tamoxifen	None	None	Alive and well	56.9	56.9
183	yes	CMF	tamoxifen	None	None	Alive and well	50.4	50.4
184	yes	no	tamoxifen	None	None	Alive and well	50.9	50.9
185	no	Epi/CMF	tamoxifen	None	None	Alive and well	56.9	56.9
186	yes	no	tamoxifen --> AI	None	None	Alive and well	56.9	57.8
187	no	no	tamoxifen	None	None	Alive and well	51.2	51.2
188	no	Epi/CMF	tamoxifen --> AI	None	None	Alive and well	56.8	56.8
189	yes	no	tamoxifen --> AI	None	None	Alive and well	57.6	57.6
190	no	CMF	no	None	None	Alive and well	57.9	57.9
191	yes	no	tamoxifen	None	None	Alive and well	56.7	56.7
192	yes	Epi/CMF	tamoxifen	None	None	Alive and well	57.9	57.9
193	yes	no	tamoxifen	None	None	Alive and well	49.9	51.1
194	yes	CMF	tamoxifen --> AI	None	VISCERAL	Breast cancer related death	8.7	12.2
195	no	no	tamoxifen+LHRH analogues	None	NON-VISCERAL	Breast cancer related death	18.2	41.9
196	no	no	tamoxifen --> AI	None	NON-VISCERAL	Alive with recurrent/metastatic BC	37.2	56.8
197	no	no	tamoxifen	None	None	Alive and well	56.1	56.1
198	yes	TACT STUDY	tamoxifen	None	None	Alive and well	52.2	52.2
199	yes	CMF	no	None	None	Non-cancer related death	30.9	30.9
200	yes	Epi/CMF	tamoxifen	None	None	Alive and well	57.2	57.2
201	yes	no	tamoxifen	None	None	Other cancer death	15.9	21.1
202	no	no	tamoxifen	breast /chest wall	None	Alive and well	31.7	52.7
203	no	no	tamoxifen	None	None	Alive and well	58.8	58.8
204	yes	no	tamoxifen	None	None	Alive and well	56.9	56.9

Appendix 2: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 3).

No.	Radiotherapy	Chemotherapy	Hormonal therapy	Locoregional recurrence	Systemic metastasis	Final status	RFS/m.	OS/m.
205	yes	Epi/CMF	no	None	None	Alive and well	51.3	51.3
206	yes	no	tamoxifen	None	None	Alive and well	56.7	57.6
207	no	no	aromatase inhibitors	None	None	Non-cancer related death	27.9	27.9
208	yes	no	tamoxifen	None	None	Alive and well	50.2	50.2
209	yes	no	tamoxifen	None	None	Alive and well	56.0	56.0
210	yes	no	tamoxifen	None	None	Alive and well	51.3	51.3
211	yes	no	tamoxifen	None	None	Alive and well	56.5	56.5
212	yes	TACT STUDY	no	None	None	Alive and well	51.3	51.3
213	no	CMF	tamoxifen	None	None	Alive and well	57.6	57.6
214	yes	Epi/CMF	tamoxifen	None	None	Alive and well	56.2	56.2
215	yes	FEC + taxotere	tamoxifen	None	None	Alive and well	52.7	52.7
216	no	CMF	no	None	None	Alive and well	57.8	57.8
217	yes	no	tamoxifen	None	None	Alive and well	56.5	56.5
218	yes	no	tamoxifen	None	None	Alive and well	52.2	52.2
219	yes	CMF	tamoxifen	None	None	Alive and well	51.8	51.8
220	no	no	tamoxifen	None	None	Alive and well	46.6	46.6
221	yes	Epi/CMF	no	None	None	Alive and well	46.4	46.4
222	yes	no	no	None	None	Alive and well	46.2	46.2
223	yes	no	aromatase inhibitors	None	None	Alive and well	46.0	46.0
224	yes	no	tamoxifen	None	None	Alive and well	46.0	46.0
225	no	no	aromatase inhibitors	None	None	Alive and well	45.8	45.8
226	yes	Epi/CMF	tamoxifen	None	None	Alive and well	45.7	45.7
227	yes	no	tamoxifen	None	None	Alive and well	45.7	45.7
228	yes	no	tamoxifen	None	None	Alive and well	45.7	45.7
229	yes	Epi/CMF	no	None	None	Alive and well	45.5	46.2
230	yes	no	tamoxifen	None	None	Alive and well	45.3	45.3
231	yes	no	aromatase inhibitors	None	None	Alive and well	45.0	45.7
232	yes	Epi/CMF	tamoxifen	None	None	Alive and well	45.0	45.0
233	yes	no	tamoxifen	None	None	Alive and well	45.0	45.0
234	no	no	tamoxifen	None	None	Alive and well	45.0	45.7
235	no	no	tamoxifen	None	None	Alive and well	44.9	44.9
236	yes	no	tamoxifen	None	None	Alive and well	44.9	44.9
237	yes	no	tamoxifen	None	None	Alive and well	44.8	44.8
238	yes	Epi/CMF	tamoxifen	None	None	Alive and well	44.8	44.8

Appendix 2: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 3).

No.	Radiotherapy	Chemotherapy	Hormonal therapy	Locoregional recurrence	Systemic metastasis	Final status	RFS/m.	OS/m.
239	yes	no	tamoxifen	None	None	Alive and well	44.8	44.8
240	yes	no	tamoxifen	None	None	Alive and well	44.8	44.8
241	yes	no	tamoxifen --> AI	None	None	Alive and well	44.8	44.8
242	yes	Epi/CMF	tamoxifen	None	None	Alive and well	44.8	46.2
243	yes	CMF	tamoxifen	None	None	Alive and well	44.6	44.6
244	yes	Epi/CMF	tamoxifen	None	NON-VISCERAL	Breast cancer related death	24.5	26.4
245	yes	no	tamoxifen	None	None	Alive and well	44.6	44.6
246	yes	no	tamoxifen	None	None	Alive and well	44.1	44.1
247	yes	no	tamoxifen	None	None	Alive and well	44.1	44.1
248	yes	no	tamoxifen	None	None	Alive and well	46.0	46.0
249	yes	no	tamoxifen	None	None	Alive and well	43.9	43.9
250	yes	no	tamoxifen	None	VISCERAL	Breast cancer related death	37.5	40.8
251	no	no	tamoxifen --> AI	None	None	Alive and well	43.6	43.6
252	yes	no	tamoxifen	None	None	Alive and well	43.6	43.6
253	yes	no	tamoxifen	None	None	Alive and well	43.6	43.6
254	yes	no	tamoxifen	None	None	Alive and well	43.6	43.6
255	no	no	tamoxifen	None	None	Alive and well	43.2	43.9
256	yes	AC	no	breast /chest wall	None	Alive with recurrent/metastatic BC	20.8	43.9
257	no	no	tamoxifen	None	None	Non-cancer related death	39.8	39.8
258	yes	Epi/CMF	no	None	None	Alive and well	43.6	43.6
259	yes	Epi/CMF	aromatase inhibitors	None	None	Alive and well	43.6	43.6
260	yes	Epi/CMF	no	None	None	Alive and well	43.4	43.4
261	yes	Epi/CMF	no	None	None	Alive and well	43.1	43.1
262	no	no	tamoxifen	None	None	Alive and well	43.2	43.2
263	yes	no	tamoxifen	None	None	Alive and well	43.2	43.2
264	yes	Epi/CMF	tamoxifen+LHRH analogues	None	None	Alive and well	43.1	43.1
265	yes	Epi/CMF	tamoxifen	None	None	Alive and well	43.1	43.8
266	yes	FEC + taxotere	tamoxifen	None	None	Alive and well	41.3	42.5
267	no	no	tamoxifen	None	None	Alive and well	41.3	42.5
268	no	AC	no	None	VISCERAL & NON-VISCERAL	Breast cancer related death	24.5	28.2
269	yes	TACT STUDY	tamoxifen --> AI	None	None	Alive and well	42.0	42.0
270	yes	Epi/CMF	no	None	None	Alive and well	42.2	42.2
271	yes	Epi/CMF	tamoxifen	None	None	Alive and well	42.2	42.9
272	yes	Epi/CMF	no	breast /chest wall	VISCERAL	Alive with recurrent/metastatic BC	39.8	41.8

Appendix 2: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 3).

No.	Radiotherapy	Chemotherapy	Hormonal therapy	Locoregional recurrence	Systemic metastasis	Final status	RFS/m.	OS/m.
273	no	no	tamoxifen	None	None	Alive and well	40.4	40.4
274	yes	Epi/CMF	aromatase inhibitors	None	None	Alive and well	40.4	40.4
275	no	no	tamoxifen	None	None	Alive and well	39.5	39.5
276	yes	TACT STUDY	tamoxifen	None	None	Alive and well	38.7	38.7
277	no	no	tamoxifen	None	None	Alive and well	39.0	39.0
278	yes	FEC + taxotere	tamoxifen	None	None	Alive and well	38.7	38.7
279	yes	no	tamoxifen	None	None	Alive and well	38.7	38.7
280	yes	no	aromatase inhibitors	None	None	Alive and well	38.0	38.0
281	yes	no	tamoxifen	None	None	Alive and well	37.5	37.5
282	no	no	tamoxifen	None	VISCERAL & NON-VISCERAL	Breast cancer related death	3.0	6.1
283	no	no	tamoxifen	None	None	Alive and well	36.9	36.9
284	yes	no	tamoxifen	None	None	Alive and well	36.9	36.9
285	yes	AC	tamoxifen	None	None	Alive and well	36.4	36.4
286	yes	Epi/CMF	tamoxifen	None	None	Alive and well	37.2	37.2
287	yes	no	tamoxifen	None	None	Alive and well	36.9	36.9
288	no	no	no	None	None	Other cancer death	19.9	19.9
289	yes	no	tamoxifen	None	None	Alive and well	36.2	36.2
290	yes	CMF	tamoxifen	None	None	Alive and well	35.9	35.9
291	yes	AC	tamoxifen --> AI	None	None	Alive and well	35.5	35.5
292	yes	TANGO STUDY	no	None	VISCERAL	Alive with recurrent/metastatic BC	24.8	35.3
293	yes	no	tamoxifen --> AI	None	None	Alive and well	35.5	35.5
294	yes	no	tamoxifen --> AI	None	None	Alive and well	35.3	35.8
295	no	AC	tamoxifen --> AI	None	None	Alive and well	35.5	35.5
296	yes	no	tamoxifen	None	None	Alive and well	35.2	35.2
297	yes	Epi/CMF	tamoxifen	None	None	Alive and well	34.8	34.8
298	yes	Epi/CMF	tamoxifen	None	None	Alive and well	35.5	35.5
299	yes	no	tamoxifen	None	None	Alive and well	35.2	35.2
300	yes	Epi/CMF	tamoxifen	None	None	Alive and well	34.6	34.6

Appendix 3: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 4).

No.	Age	Deprivation	WBC (x10⁹/l)	ALB (g/l)	CRP (mg/l)	Breast Surgery	Axillary Surgery	Histological type	Grade	Size (cm)	Total LN	Involved LN	ER status	PR status
1	42	4	5.32	44	6	conservation surgery	dissection/ clearance	invasive ductal	3	1.6	21	0	positive	unknown
2	80	5	5.29	38	<6	mastectomy	dissection/ clearance	invasive ductal	3	5.0	18	0	negative	unknown
3	52	3	7.01	40	<6	mastectomy	dissection/ clearance	invasive ductal	2	4.0	14	7	positive	unknown
4	71	6	7.57	44	10	mastectomy	dissection/ clearance	invasive ductal	1	2.5	11	0	positive	unknown
5	44	5	8.70	44	<6	mastectomy	dissection/ clearance	invasive ductal	1	2.1	14	0	positive	unknown
6	56	6	—	—	<6	conservation surgery	dissection/ clearance	invasive ductal	1	2.0	11	2	positive	unknown
7	47	5	10.38	43	<6	mastectomy	dissection/ clearance	invasive ductal	1	1.7	9	0	positive	unknown
8	41	5	12.35	46	10	conservation surgery	dissection/ clearance	invasive ductal	2	2.6	20	0	positive	unknown
9	70	5	6.70	42	<6	mastectomy	dissection/ clearance	invasive lobular	3	3.0	17	8	positive	unknown
10	40	3	9.61	—	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.0	13	2	negative	unknown
11	68	5	6.19	41	6	conservation surgery	dissection/ clearance	invasive ductal	2	1.1	11	1	positive	unknown
12	59	4	5.83	43	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.0	8	0	negative	unknown
13	42	3	7.59	—	8	mastectomy	dissection/ clearance	invasive lobular	2	4.0	9	6	positive	unknown
14	60	6	7.86	42	10	mastectomy	sampling	invasive lobular	2	2.0	8	0	positive	unknown
15	66	6	11.27	—	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.8	8	2	positive	unknown
16	67	5	9.83	47	7	conservation surgery	dissection/ clearance	invasive ductal	2	1.2	8	0	positive	unknown
17	77	3	6.43	40	<6	mastectomy	dissection/ clearance	invasive ductal	1	2.3	7	0	positive	unknown
18	66	5	7.93	—	7	conservation surgery	dissection/ clearance	invasive ductal	3	2.4	11	4	positive	unknown
19	69	5	7.72	39	15	conservation surgery	sampling	invasive ductal	2	2.2	9	0	negative	unknown
20	59	4	5.89	39	6	mastectomy	dissection/ clearance	invasive ductal	2	1.1	14	0	negative	unknown
21	46	4	7.70	44	8	mastectomy	dissection/ clearance	invasive ductal	2	0.8	14	0	positive	unknown
22	44	5	8.48	41	8	conservation surgery	dissection/ clearance	invasive ductal	2	3.0	16	9	negative	unknown
23	76	4	6.23	—	<6	conservation surgery	sampling	invasive ductal	2	1.5	11	0	positive	unknown
24	76	3	5.72	45	<6	mastectomy	sampling	invasive ductal	2	2.0	5	2	positive	unknown
25	75	3	8.60	37	14	mastectomy	sampling	invasive ductal	2	4.0	9	3	positive	unknown
26	75	5	9.47	40	19	mastectomy	dissection/ clearance	invasive ductal	1	1.5	8	3	positive	unknown
27	66	5	7.15	—	11	mastectomy	dissection/ clearance	invasive ductal	2	1.4	18	1	positive	unknown
28	55	3	4.62	43	<6	mastectomy	dissection/ clearance	invasive ductal	3	1.8	11	1	negative	unknown
29	51	5	5.04	—	<6	mastectomy	dissection/ clearance	invasive lobular	1	2.5	15	4	positive	unknown
30	74	3	7.26	44	<6	mastectomy	dissection/ clearance	invasive ductal	3	3.0	8	0	positive	unknown
31	39	3	4.63	44	<6	mastectomy	dissection/ clearance	invasive ductal	1	2.2	17	0	positive	unknown
32	48	5	10.13	43	10	mastectomy	dissection/ clearance	invasive lobular	3	2.5	14	1	positive	unknown
33	63	6	6.88	—	8	mastectomy	dissection/ clearance	invasive ductal	3	1.8	7	0	negative	unknown
34	70	5	5.66	38	9	mastectomy	sampling	invasive ductal	3	4.0	14	5	negative	unknown

Appendix 3: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 4).

No.	Age	Deprivation	WBC (x10^9/l)	ALB (g/l)	CRP (mg/l)	Breast Surgery	Axillary Surgery	Histological type	Grade	Size (cm)	Total LN	Involved LN	ER status	PR status
35	55	5	7.29	43	<6	mastectomy	sampling	invasive ductal	2	2.5	15	0	positive	unknown
36	82	6	5.93	40	8	mastectomy	dissection/ clearance	invasive ductal	2	3.0	22	0	positive	unknown
37	48	4	11.62	44	12	conservation surgery	dissection/ clearance	invasive ductal	3	2.0	6	0	negative	unknown
38	35	3	10.66	—	<6	conservation surgery	dissection/ clearance	invasive ductal	3	2.2	13	0	negative	unknown
39	57	5	5.90	43	6	mastectomy	dissection/ clearance	invasive lobular	1	2.0	11	2	positive	unknown
40	77	6	8.16	39	7	mastectomy	dissection/ clearance	invasive ductal	2	1.6	9	0	positive	unknown
41	50	6	9.96	46	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.0	12	0	positive	unknown
42	52	6	7.35	—	<6	conservation surgery	dissection/ clearance	invasive ductal	3	2.0	12	1	negative	unknown
43	53	5	12.92	—	8	conservation surgery	dissection/ clearance	invasive ductal	3	1.5	12	0	positive	unknown
44	50	5	10.06	41	8	mastectomy	dissection/ clearance	invasive ductal	2	1.5	11	0	positive	unknown
45	73	5	5.90	37	10	conservation surgery	—	invasive ductal	1	1.2	—	—	positive	unknown
46	61	5	4.50	44	<6	mastectomy	sampling	invasive ductal	1	0.6	11	0	positive	unknown
47	62	5	6.53	44	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.5	8	1	positive	unknown
48	69	5	9.55	—	17	mastectomy	dissection/ clearance	invasive ductal	2	1.0	15	0	positive	unknown
49	80	6	9.06	43	7	mastectomy	dissection/ clearance	special types	—	4.5	9	0	negative	unknown
50	56	6	10.83	—	<6	conservation surgery	dissection/ clearance	invasive ductal	1	0.9	10	1	positive	unknown
51	60	5	8.27	47	8	conservation surgery	dissection/ clearance	invasive ductal	1	2.0	11	3	positive	unknown
52	41	5	8.94	42	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.2	21	0	negative	unknown
53	44	3	8.44	44	<6	mastectomy	dissection/ clearance	invasive ductal	3	0.6	15	0	negative	negative
54	39	4	6.51	42	8	mastectomy	sampling	invasive ductal	2	4.5	12	6	positive	unknown
55	75	6	6.94	40	10	conservation surgery	dissection/ clearance	invasive ductal	2	2.0	7	1	positive	unknown
56	47	4	7.74	42	6	conservation surgery	dissection/ clearance	invasive ductal	2	2.7	8	0	positive	unknown
57	60	4	13.53	40	9	conservation surgery	dissection/ clearance	invasive ductal	3	2.2	4	0	negative	negative
58	64	6	7.46	39	9	mastectomy	dissection/ clearance	invasive ductal	2	1.8	16	0	positive	unknown
59	53	6	9.70	57	22	mastectomy	dissection/ clearance	invasive lobular	1	2.5	11	0	positive	unknown
60	82	6	7.30	39	8	mastectomy	dissection/ clearance	invasive ductal	1	3.3	9	8	positive	positive
61	73	5	6.42	41	11	mastectomy	dissection/ clearance	invasive lobular	2	1.8	11	0	positive	unknown
62	66	4	7.03	45	6	mastectomy	dissection/ clearance	invasive ductal	2	1.5	11	2	positive	unknown
63	69	6	6.74	44	8	mastectomy	dissection/ clearance	invasive lobular	2	2.0	5	1	positive	unknown
64	74	6	5.43	41	<6	conservation surgery	dissection/ clearance	invasive lobular	2	1.8	6	0	positive	unknown
65	61	4	9.35	46	<6	mastectomy	dissection/ clearance	invasive lobular	2	2.5	16	2	positive	unknown
66	65	3	4.84	41	<6	conservation surgery	dissection/ clearance	invasive ductal	2	2.0	6	0	positive	unknown
67	48	7	4.72	42	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.0	8	1	positive	unknown
68	73	5	10.29	44	47	mastectomy	dissection/ clearance	invasive ductal	1	2.9	9	4	positive	unknown

Appendix 3: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 4).

No.	Age	Deprivation	WBC (x10^9/l)	ALB (g/l)	CRP (mg/l)	Breast Surgery	Axillary Surgery	Histological type	Grade	Size (cm)	Total LN	Involved LN	ER status	PR status
69	45	6	5.73	39	8	mastectomy	dissection/ clearance	invasive lobular	2	1.4	15	12	positive	positive
70	48	3	8.83	40	<6	mastectomy	dissection/ clearance	invasive ductal	2	1.2	9	1	positive	unknown
71	63	3	5.04	—	<6	conservation surgery	dissection/ clearance	invasive ductal	3	0.9	14	0	positive	unknown
72	75	5	9.69	42	<6	mastectomy	dissection/ clearance	invasive ductal	2	3.0	7	5	positive	unknown
73	62	4	7.96	46	<6	conservation surgery	dissection/ clearance	invasive ductal	3	2.5	15	0	positive	unknown
74	52	5	7.13	45	<6	conservation surgery	dissection/ clearance	invasive ductal	2	2.4	11	2	positive	unknown
75	53	6	10.56	43	8	conservation surgery	dissection/ clearance	invasive ductal	2	1.7	17	0	negative	negative
76	51	3	6.86	44	8	conservation surgery	dissection/ clearance	invasive ductal	2	2.0	11	4	positive	unknown
77	48	5	8.16	42	<6	conservation surgery	dissection/ clearance	special types	1	0.9	21	0	positive	unknown
78	49	4	10.28	43	<6	mastectomy	dissection/ clearance	invasive ductal	2	3.0	16	1	negative	negative
79	40	5	7.05	42	<6	conservation surgery	dissection/ clearance	invasive ductal	2	0.9	8	0	negative	negative
80	54	3	7.94	46	<6	conservation surgery	dissection/ clearance	invasive ductal	2	0.8	11	0	positive	unknown
81	80	4	8.52	42	7	conservation surgery	dissection/ clearance	invasive ductal	2	1.2	10	0	positive	unknown
82	67	5	8.30	41	6	conservation surgery	dissection/ clearance	invasive ductal	2	1.3	1	0	positive	unknown
83	65	4	10.75	46	<6	conservation surgery	dissection/ clearance	invasive ductal	2	2.0	12	1	positive	unknown
84	45	5	8.71	42	<6	conservation surgery	dissection/ clearance	invasive ductal	2	0.9	13	0	positive	unknown
85	51	2	6.76	44	<6	conservation surgery	dissection/ clearance	invasive ductal	1	0.6	11	0	positive	positive
86	55	4	4.56	42	<6	mastectomy	dissection/ clearance	invasive ductal	1	0.9	16	0	positive	negative
87	67	7	5.92	46	<6	mastectomy	dissection/ clearance	invasive lobular	2	2.0	15	1	positive	negative
88	53	2	—	44	<6	conservation surgery	dissection/ clearance	invasive ductal	3	1.2	17	0	positive	positive
89	68	7	7.96	46	<6	mastectomy	dissection/ clearance	invasive ductal	3	3.6	15	5	positive	positive
90	56	6	4.12	45	<6	mastectomy	dissection/ clearance	invasive ductal	2	1.9	11	1	positive	positive
91	57	3	5.77	45	9	conservation surgery	dissection/ clearance	invasive ductal	3	1.9	15	0	negative	negative
92	78	6	6.79	46	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.0	10	0	positive	positive
93	52	3	5.08	50	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.0	20	0	negative	negative
94	57	6	9.44	43	<6	conservation surgery	dissection/ clearance	invasive lobular	2	2.0	26	5	positive	positive
95	60	6	8.15	44	14	conservation surgery	dissection/ clearance	invasive ductal	3	0.6	17	1	negative	negative
96	56	6	5.06	44	28	conservation surgery	dissection/ clearance	special types	2	0.9	15	0	positive	positive
97	53	4	9.11	44	6	mastectomy	dissection/ clearance	invasive ductal	2	1.1	14	0	positive	negative
98	60	3	6.45	45	16	conservation surgery	dissection/ clearance	special types	1	0.9	17	0	positive	positive
99	73	4	6.98	42	<6	conservation surgery	dissection/ clearance	invasive ductal	1	0.8	16	0	positive	positive
100	52	2	8.53	46	57	conservation surgery	dissection/ clearance	invasive ductal	3	1.4	6	0	positive	negative
101	61	4	5.84	47	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.6	17	1	positive	negative
102	82	2	4.52	41	66	mastectomy	dissection/ clearance	invasive ductal	3	2.0	10	6	positive	negative

Appendix 3: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 4).

No.	Age	Deprivation	WBC (x10⁹/l)	ALB (g/l)	CRP (mg/l)	Breast Surgery	Axillary Surgery	Histological type	Grade	Size (cm)	Total LN	Involved LN	ER status	PR status
103	52	1	8.15	46	<6	conservation surgery	dissection/ clearance	invasive ductal	2	0.5	14	0	negative	negative
104	59	1	4.11	45	<6	conservation surgery	dissection/ clearance	invasive ductal	2	2.0	15	3	positive	positive
105	60	6	4.31	41	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.2	29	17	negative	negative
106	41	2	8.40	46	<6	mastectomy	dissection/ clearance	invasive ductal	3	3.5	16	9	negative	negative
107	72	2	4.99	40	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.3	9	0	positive	positive
108	52	2	4.88	41	7	mastectomy	dissection/ clearance	invasive ductal	3	1.1	23	2	positive	positive
109	69	4	8.63	44	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.5	17	0	positive	positive
110	65	2	11.18	44	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.1	22	12	positive	positive
111	52	6	4.73	42	8	mastectomy	dissection/ clearance	invasive ductal	2	1.3	19	2	positive	positive
112	70	2		44	6	mastectomy	dissection/ clearance	invasive ductal	3	2.8	15	0	negative	negative
113	67	6	5.91	48	<6	mastectomy	dissection/ clearance	invasive lobular	2	4.0	10	10	positive	positive
114	66	7	3.42	43	<6	mastectomy	dissection/ clearance	invasive ductal	3	3.0	24	1	negative	negative
115	52	4	7.60	50	6	conservation surgery	dissection/ clearance	invasive lobular	3	2.2	15	1	positive	positive
116	70	6	4.94	44	8	conservation surgery	dissection/ clearance	invasive ductal	2	2.1	18	0	negative	negative
117	57	4	8.04	46	<6	conservation surgery	dissection/ clearance	invasive ductal	2	2.4	18	2	positive	positive
118	72	6	6.10	47	<6	mastectomy	dissection/ clearance	invasive ductal	2	1.7	15	0	positive	positive
119	62	7	9.04	46	15	conservation surgery	dissection/ clearance	invasive ductal	3	1.4	16	2	negative	negative
120	58	6	9.31	47	15	mastectomy	dissection/ clearance	invasive ductal	2	2.5	18	1	positive	positive
121	52	6	4.29	45	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.6	16	0	positive	positive
122	52	7	6.53	47	<6	conservation surgery	dissection/ clearance	invasive lobular	1	1.4	19	0	positive	positive
123	63	1	3.83	42	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.5	19	3	negative	negative
124	39	2	5.86	44	6	mastectomy	dissection/ clearance	invasive ductal	2	2.3	19	0	positive	positive
125	56	5	6.68	38	6	conservation surgery	dissection/ clearance	invasive lobular	3	2.5	17	0	positive	negative
126	44	1	5.84	45	6	conservation surgery	dissection/ clearance	invasive ductal	2	2.6	18	2	positive	positive
127	71	4	9.31	45	<6	mastectomy	dissection/ clearance	invasive ductal	3	4.8	14	0	negative	negative
128	52	4	4.61	44	8	conservation surgery	dissection/ clearance	invasive lobular	3	1.1	16	0	positive	positive
129	62	4	7.68	49	<6	conservation surgery	dissection/ clearance	invasive ductal	2	3.0	18	0	positive	positive
130	58	4	6.30	44	13	conservation surgery	dissection/ clearance	invasive ductal	3	2.6	15	0	positive	positive
131	89	6	5.49	42	9	mastectomy	dissection/ clearance	invasive ductal	2	3.0	10	2	positive	positive
132	51	3	12.36	46	<6	conservation surgery	dissection/ clearance	invasive ductal	3	0.9	21	0	negative	negative
133	51	4	7.85	44	7	conservation surgery	dissection/ clearance	special types	1	1.2	11	0	positive	positive
134	54	2	4.81	43	<6	conservation surgery	dissection/ clearance	invasive ductal	1	1.6	15	0	positive	negative
135	52	4	4.40	39	22	conservation surgery	dissection/ clearance	invasive ductal	2	1.6	7	1	positive	negative
136	56	5	6.08	47	<6	conservation surgery	dissection/ clearance	invasive ductal	2	3.2	17	0	positive	positive

Appendix 3: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 4).

No.	Age	Deprivation	WBC (x10⁹/l)	ALB (g/l)	CRP (mg/l)	Breast Surgery	Axillary Surgery	Histological type	Grade	Size (cm)	Total LN	Involved LN	ER status	PR status
137	55	2	6.10	43	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.5	10	0	positive	positive
138	63	2	5.37	45	<6	conservation surgery	dissection/ clearance	invasive ductal	2	2.0	23	0	positive	positive
139	51	2	6.49	42	<6	conservation surgery	dissection/ clearance	invasive ductal	3	2.5	9	1	positive	positive
140	63	5	7.43	40	7	conservation surgery	dissection/ clearance	invasive ductal	2	1.2	10	0	positive	positive
141	52	2	7.15	45	<6	conservation surgery	dissection/ clearance	invasive ductal	1	0.7	10	0	positive	positive
142	68	3	4.18	44	<6	mastectomy	sampling	invasive ductal	2	1.5	7	0	positive	negative
143	78	2	7.48	45	<6	mastectomy	dissection/ clearance	invasive ductal	3	3.0	15	2	positive	positive
144	52	5	6.59	44	8	conservation surgery	dissection/ clearance	invasive ductal	2	1.7	14	0	positive	positive
145	72	7	8.18	46	<6	conservation surgery	dissection/ clearance	invasive ductal	1	0.9	8	0	positive	negative
146	57	6	8.23	45	<6	conservation surgery	dissection/ clearance	invasive ductal	3	1.9	15	0	positive	negative
147	71	6	5.09	43	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.2	16	0	positive	positive
148	59	5	9.57	44	8	conservation surgery	dissection/ clearance	invasive ductal	2	1.4	15	3	positive	positive
149	61	3	7.22	43	32	conservation surgery	dissection/ clearance	invasive ductal	2	1.7	13	0	positive	positive
150	60	4	7.73	48	<6	conservation surgery	dissection/ clearance	invasive ductal	3	1.2	8	0	positive	positive
151	63	7	5.84	48	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.1	24	0	positive	negative
152	70	7	11.90	45	14	conservation surgery	dissection/ clearance	invasive lobular	2	2.1	15	0	positive	negative
153	64	6	5.81	41	7	mastectomy	dissection/ clearance	invasive ductal	2	3.0	13	2	positive	positive
154	89	2	6.94	44	<6	mastectomy	dissection/ clearance	invasive ductal	2	3.0	10	0	positive	positive
155	62	7	4.82	43	<6	conservation surgery	dissection/ clearance	invasive ductal	2	1.4	17	0	positive	positive
156	65	3	8.34	45	<6	conservation surgery	dissection/ clearance	special types	1	0.5	13	0	positive	unknown
157	45	1	6.54	46	<6	mastectomy	dissection/ clearance	invasive ductal	2	1.1	12	0	positive	positive
158	82	5	9.30	43	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.1	11	1	positive	positive
159	56	4	6.12	46	<6	mastectomy	dissection/ clearance	invasive ductal	3	2.8	11	3	negative	negative
160	62	2	7.44	44	11	conservation surgery	dissection/ clearance	invasive ductal	3	3.5	11	1	positive	positive
161	65	6	6.16	44	15	mastectomy	dissection/ clearance	invasive ductal	3	5.0	32	0	negative	negative
162	52	5	7.98	47	11	mastectomy	dissection/ clearance	invasive ductal	3	2.1	16	5	negative	negative
163	46	3	6.12	44	10	mastectomy	dissection/ clearance	invasive ductal	3	2.5	11	1	positive	positive
164	52	3	6.44	44	<6	conservation surgery	dissection/ clearance	invasive ductal	3	1.6	17	1	positive	positive
165	48	6	5.73	50	12	mastectomy	dissection/ clearance	invasive ductal	2	1.2	17	3	positive	positive
166	46	5	9.12	47	<6	mastectomy	dissection/ clearance	invasive ductal	1	2.5	14	0	positive	positive
167	79	6	6.53	42	<6	mastectomy	dissection/ clearance	invasive ductal	2	2.0	12	1	negative	negative
168	57	6	6.52	38	21	conservation surgery	dissection/ clearance	invasive ductal	2	1.6	10	1	positive	positive

Appendix 3: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 4).

No.	CD4+ %	CD4+tert	CD8+ %	CD8+tert	CD68+ %	CD68+tert	Ki67 %	Ki67tert	CD34+ mean	CD34+ Grade	Radiotherapy	Chemotherapy	Hormonal therapy aromatase inhibitors
1	1.10	3	2.23	3	6.3	2	14.47	2	5.00	1	yes	CMF	no
2	0.00	1	1.23	2	9.1	3	27.55	3	7.00	3	yes	no	no
3	0.57	3	1.70	3	4.8	2	14.79	2	5.33	2	yes	Epi/CMF	tamoxifen
4	0.20	2	1.00	2	5.3	2	4.57	1	8.67	3	no	no	tamoxifen
5	1.37	3	1.50	3	3.0	1	2.76	1	6.33	2	no	no	tamoxifen
6	0.03	1	0.83	2	1.8	1	19.98	2	5.33	2	yes	CMF	tamoxifen
7	0.03	1	0.27	1	9.1	3	17.01	2	6.00	2	no	CMF	tamoxifen
8	0.03	1	0.53	2	7.9	3	30.73	3	7.00	3	yes	CMF	tamoxifen
9	0.13	1	0.60	2	6.5	3	23.88	2	7.00	3	yes	no	tamoxifen
10	0.53	2	2.10	3	11.6	3	47.80	3	5.67	2	yes	Epi/CMF	no
11	2.20	3	3.80	3	6.1	2	41.99	3	5.33	2	yes	no	tamoxifen
12	0.63	3	2.20	3	12.9	3	39.65	3	8.33	3	no	CMF	no
13	0.73	3	0.97	2	5.4	2	10.32	1	6.67	2	yes	Epi/CMF	tamoxifen
14	0.97	3	0.57	2	4.2	2	34.75	3	5.67	2	no	no	tamoxifen
15	0.10	1	0.27	1	5.8	2	10.58	1	5.67	2	yes	no	tamoxifen
16	9.10	3	14.03	3	14.2	3	48.04	3	6.33	2	yes	no	tamoxifen
17	0.13	1	0.70	2	1.0	1	3.60	1	4.67	1	no	no	tamoxifen
18	4.13	3	5.03	3	5.3	2	35.42	3	7.67	3	yes	no	tamoxifen
19	4.87	3	5.13	3	11.2	3	22.00	2	7.33	3	yes	no	no
20	0.07	1	0.60	2	2.5	1	5.62	1	7.67	3	no	CMF	no
21	0.37	2	1.27	3	6.1	2	7.27	1	3.67	1	no	CMF	tamoxifen
22	1.20	3	1.73	3	10.3	3	32.32	3	7.67	3	yes	Epi/CMF	no
23	0.03	1	0.10	1	6.0	2	14.22	2	4.67	1	yes	no	tamoxifen
24	0.03	1	0.73	2	4.3	2	23.30	2	5.00	1	yes	no	tamoxifen
25	2.20	3	4.17	3	7.4	3	43.56	3	6.00	2	yes	no	tamoxifen
26	0.07	1	0.57	2	6.7	3	15.06	2	6.67	2	yes	no	tamoxifen
27	0.57	3	1.33	3	3.6	1	10.08	1	4.33	1	no	no	tamoxifen --> AI
28	0.00	1	0.47	2	4.6	2	11.27	1	6.33	2	yes	Epi/CMF	no
29	0.07	1	0.70	2	5.3	2	14.55	2	5.33	2	yes	Epi/CMF	tamoxifen
30	0.53	2	1.45	3	6.4	3	16.02	2	7.67	3	no	no	tamoxifen
31	0.10	1	0.80	2	6.6	3	13.47	2	6.67	2	no	no	tamoxifen
32	0.00	1	0.60	2	0.7	1	0.20	1	6.33	2	yes	Epi/CMF	tamoxifen
33	2.30	3	2.53	3	6.9	3	25.16	3	6.00	2	no	CMF	no
34	0.00	1	0.33	1	1.7	1	31.00	3	10.33	3	yes	AC	no

No.	CD4+ %	CD4+tert	CD8+ %	CD8+tert	CD68+ %	CD68+tert	Ki67 %	Ki67tert	CD34+ mean	CD34+ Grade	Radiotherapy	Chemotherapy	Hormonal therapy
35	0.17	2	1.00	2	4.0	2	8.75	1	5.00	1	no	no	tamoxifen
36	0.13	1	0.73	2	7.7	3	10.94	1	5.00	1	no	no	tamoxifen
37	5.77	3	8.03	3	13.0	3	38.35	3	7.67	3	yes	AC	no
38	0.47	2	1.10	2	12.0	3	60.36	3	7.00	3	yes	Epi/CMF	no
39	0.13	1	0.10	1	7.1	3	13.92	2	9.33	3	yes	CMF	tamoxifen
40	0.00	1	0.13	1	1.5	1	7.11	1	6.67	2	no	no	tamoxifen
41	0.63	3	6.00	3	6.3	2	35.58	3	6.67	2	yes	CMF	tamoxifen
42	1.93	3	5.27	3	8.1	3	60.55	3	6.33	2	yes	Epi/CMF	no
43	2.10	3	2.50	3	6.5	3	24.47	2	6.67	2	yes	no	tamoxifen
44	0.37	2	1.37	3	12.6	3	12.95	2	5.00	1	no	CMF	tamoxifen
45	0.20	2	1.17	2	5.8	2	13.25	2	5.33	2	no	no	tamoxifen
46	2.07	3	5.00	3	2.9	1	4.90	1	5.33	2	no	no	tamoxifen
47	0.43	2	1.80	3	6.5	3	14.52	2	8.00	3	yes	CMF	tamoxifen
48	0.83	3	5.73	3	6.4	3	9.65	1	4.33	1	no	no	tamoxifen
49	0.83	3	6.67	3	10.0	3	29.50	3	9.00	3	yes	no	no
50	0.00	1	0.37	1	2.1	1	0.29	1	5.00	1	yes	CMF	tamoxifen
51	0.60	3	0.97	2	4.3	2	38.99	3	7.00	3	yes	Epi/CMF	tamoxifen
52	0.87	3	3.67	3	6.2	2	52.67	3	8.00	3	no	Epi/CMF	no
53	2.13	3	3.73	3	4.6	2	9.38	1	4.67	1	no	Epi/CMF	no
54	0.23	2	1.17	2	6.7	3	8.59	1	6.00	2	yes	Epi/CMF	tamoxifen
55	0.07	1	0.20	1	3.6	1	30.99	3	5.00	1	yes	no	tamoxifen
56	0.20	2	0.40	1	9.6	3	35.38	3	6.00	2	yes	Epi/CMF	tamoxifen
57	0.33	2	0.37	1	4.0	2	38.19	3	8.67	3	yes	no	no
58	0.40	2	1.67	3	6.7	3	2.71	1	4.33	1	no	no	tamoxifen
59	0.70	3	1.20	2	5.1	2	3.67	1	4.67	1	no	no	tamoxifen
60	1.37	3	0.70	2	6.4	3	12.29	2	6.00	2	yes	no	tamoxifen
61	0.47	2	0.40	1	5.0	2	12.32	2	6.67	2	no	no	tamoxifen
62	0.33	2	1.27	3	7.6	3	38.28	3	6.33	2	yes	CMF	tamoxifen
63	0.13	1	0.77	2	4.5	2	16.35	2	5.67	2	yes	AC	tamoxifen
64	0.17	2	0.27	1	3.4	1	11.05	1	3.33	1	yes	no	tamoxifen
65	0.17	2	0.73	2	1.8	1	11.84	2	3.67	1	no	AC	tamoxifen
66	1.40	3	0.57	2	6.2	2	36.23	3	6.33	2	yes	no	tamoxifen
67	0.03	1	0.13	1	3.7	1	12.49	2	5.35	2	yes	CMF	tamoxifen
68	0.05	1	0.57	2	3.3	1	13.78	2	5.67	2	yes	no	tamoxifen

Appendix 3: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 4).

No.	CD4+ %	CD4+tert	CD8+ %	CD8+tert	CD68+ %	CD68+tert	Ki67 %	Ki67tert	CD34+ mean	CD34+ Grade	Radiotherapy	Chemotherapy	Hormonal therapy
69	0.60	3	1.50	3	3.4	1	12.03	2	6.33	2	yes	Epi/CMF	tamoxifen
70	0.00	1	0.43	1	2.4	1	2.45	1	5.00	1	yes	Epi/CMF	tamoxifen
71	1.17	3	1.47	3	12.8	3	27.88	3	6.00	2	yes	no	tamoxifen
72	0.03	1	0.07	1	3.4	1	9.38	1	5.67	2	yes	no	tamoxifen
73	0.07	1	0.20	1	3.5	1	29.64	3	4.67	1	yes	Epi/CMF	tamoxifen
74	0.23	2	0.10	1	3.0	1	25.33	3	7.00	3	yes	Epi/CMF	tamoxifen
75	0.93	3	2.07	3	3.9	2	25.07	3	6.33	2	yes	Epi/CMF	no
76	0.30	2	1.77	3	2.7	1	5.43	1	5.00	1	yes	Epi/CMF	tamoxifen
77	0.50	2	1.20	2	2.8	1	5.15	1	3.33	1	yes	no	tamoxifen
78	1.57	3	1.70	3	4.7	2	49.57	3	9.67	3	yes	TANGO STUDY	no
79	0.30	2	0.93	2	7.7	3	29.41	3	5.33	2	yes	AC	no
80	0.10	1	0.63	2	3.1	1	15.75	2	4.33	1	yes	no	tamoxifen
81	0.00	1	0.40	1	4.7	2	14.07	2	5.67	2	yes	no	tamoxifen
82	1.13	3	1.27	3	8.5	3	24.61	2	5.33	2	yes	no	tamoxifen
83	0.10	1	1.23	2	6.5	3	8.49	1	6.67	2	yes	no	tamoxifen
84	0.77	3	0.60	2	6.6	3	17.52	2	4.33	1	yes	no	tamoxifen
85	0.00	1	0.10	1	3.2	1	6.60	1	4.67	1	yes	no	tamoxifen
86	0.03	1	0.23	1	—	—	20.70	2	6.67	2	no	no	aromatase inhibitors
87	0.07	1	0.30	1	0.9	1	2.60	1	9.00	3	no	no	tamoxifen
88	0.30	2	0.53	2	14.7	3	40.80	3	7.00	3	yes	CMF	tamoxifen --> AI
89	0.40	2	0.77	2	—	—	22.80	2	7.67	3	yes	Epi/CMF	tamoxifen
90	0.00	1	0.07	1	4.4	2	2.40	1	5.33	2	yes	Epi/CMF	tamoxifen
91	0.43	2	1.17	2	6.3	2	61.10	3	4.33	1	yes	CMF	no
92	0.00	1	0.43	1	3.4	1	14.00	2	5.00	1	no	no	tamoxifen --> AI
93	0.43	2	0.17	1	5.2	2	19.00	2	6.33	2	yes	CMF	no
94	0.57	3	1.43	3	—	—	6.00	1	6.33	2	yes	Epi/CMF	tamoxifen
95	0.50	2	0.80	2	3.6	1	58.40	3	6.00	2	yes	Epi/CMF	tamoxifen
96	0.10	1	0.13	1	—	—	10.90	1	7.33	3	no	no	tamoxifen
97	1.60	3	1.70	3	8.1	3	14.30	2	6.33	2	no	no	tamoxifen
98	3.67	3	4.23	3	8.9	3	5.30	1	8.00	3	yes	no	no
99	1.23	3	1.90	3	1.9	1	2.90	1	4.33	1	yes	no	tamoxifen
100	1.40	3	2.47	3	—	—	9.40	1	9.33	3	yes	CMF	tamoxifen
101	2.37	3	3.57	3	—	—	62.60	3	10.33	3	no	CMF	tamoxifen
102	0.03	1	0.40	1	5.2	2	22.10	2	3.67	1	yes	no	tamoxifen

Appendix 3: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 4).

No.	CD4+ %	CD4+tert	CD8+ %	CD8+tert	CD68+ %	CD68+tert	Ki67 %	Ki67tert	CD34+ mean	CD34+ Grade	Radiotherapy	Chemotherapy	Hormonal therapy
103	0.10	1	0.20	1	5.5	2	2.70	1	5.00	1	yes	Epi/CMF	no
104	0.10	1	0.37	1	2.8	1	3.90	1	6.33	2	yes	CMF	tamoxifen
105	2.00	3	1.40	3	6.8	3	56.80	3	9.33	3	yes	Epi/CMF	no
106	0.90	3	1.33	3	3.3	1	54.90	3	6.67	2	yes	Epi/CMF	no
107	0.00	1	0.10	1	6.8	3	8.90	1	6.33	2	yes	no	tamoxifen
108	0.23	2	0.17	1	3.5	1	22.80	2	5.00	1	yes	Epi/CMF	tamoxifen
109	0.13	1	0.60	2	7.0	3	21.60	2	4.33	1	yes	no	tamoxifen
110	0.43	2	0.27	1	3.5	1	17.60	2	6.67	2	yes	CMF	tamoxifen
111	0.07	1	0.37	1	4.5	2	8.20	1	4.67	1	no	Epi/CMF	tamoxifen
112	4.03	3	1.87	3	—	—	38.20	3	8.67	3	no	CMF	no
113	0.17	2	0.30	1	6.2	2	15.60	2	7.67	3	yes	Epi/CMF	tamoxifen
114	0.53	2	0.43	1	6.2	2	73.80	3	6.33	2	yes	CMF	tamoxifen --> AI
115	0.03	1	0.37	1	3.5	1	3.00	1	5.33	2	yes	TACT STUDY	tamoxifen
116	0.17	2	0.03	1	3.9	2	29.10	3	4.67	1	yes	CMF	no
117	0.23	2	0.57	2	2.0	1	13.50	2	6.67	2	yes	Epi/CMF	tamoxifen
118	0.50	2	0.77	2	7.6	3	19.80	2	8.67	3	no	no	tamoxifen
119	0.00	1	0.20	1	6.2	2	51.50	3	5.33	2	yes	Epi/CMF	no
120	0.30	2	1.73	3	7.6	3	2.00	1	7.33	3	no	no	aromatase inhibitors
121	0.20	2	0.70	2	4.2	2	14.30	2	9.67	3	yes	no	tamoxifen
122	0.17	2	0.23	1	2.4	1	9.80	1	6.33	2	yes	no	tamoxifen
123	0.53	2	0.50	2	11.1	3	47.80	3	7.33	3	yes	TACT STUDY	no
124	0.90	3	0.97	2	5.7	2	17.80	2	6.00	2	no	CMF	tamoxifen
125	0.30	2	0.27	1	2.1	1	13.70	2	5.00	1	yes	Epi/CMF	tamoxifen
126	0.07	1	0.30	1	2.9	1	12.10	2	6.67	2	yes	FEC + taxotere	tamoxifen
127	0.13	1	0.27	1	—	—	62.90	3	8.33	3	no	CMF	no
128	0.33	2	0.70	2	8.4	3	6.00	1	6.00	2	yes	no	tamoxifen
129	0.17	2	2.10	3	7.7	3	4.90	1	7.00	3	yes	no	tamoxifen
130	1.47	3	3.23	3	8.0	3	29.60	3	7.33	3	yes	CMF	tamoxifen
131	0.13	1	0.70	2	4.5	2	12.50	2	3.67	1	no	no	tamoxifen
132	5.03	3	8.07	3	8.4	3	46.10	3	5.67	2	yes	Epi/CMF	no
133	0.20	2	0.07	1	1.7	1	5.10	1	4.33	1	yes	no	no
134	0.27	2	0.30	1	1.7	1	5.60	1	7.00	3	yes	no	tamoxifen
135	1.43	3	3.07	3	6.3	2	22.70	2	6.00	2	yes	Epi/CMF	tamoxifen
136	0.00	1	0.23	1	7.1	3	9.80	1	7.67	3	yes	no	tamoxifen

Appendix 3: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 4).

No.	CD4+ %	CD4+tert	CD8+ %	CD8+tert	CD68+ %	CD68+tert	Ki67 %	Ki67tert	CD34+ mean	CD34+ Grade	Radiotherapy	Chemotherapy	Hormonal therapy
137	2.73	3	4.73	3	10.2	3	8.20	1	6.33	2	yes	no	tamoxifen
138	0.03	1	0.67	2	5.3	2	23.80	2	6.33	2	yes	no	aromatase inhibiors
139	0.17	2	0.87	2	7.2	3	15.40	2	6.00	2	yes	Epi/CMF	tamoxifen
140	0.17	2	0.23	1	2.7	1	3.90	1	7.67	3	yes	no	tamoxifen
141	0.37	2	1.43	3	3.8	2	21.50	2	5.67	2	yes	no	tamoxifen
142	0.10	1	0.23	1	3.5	1	6.40	1	6.67	2	no	no	tamoxifen
143	0.13	1	0.73	2	4.5	2	41.90	3	7.00	3	yes	no	tamoxifen
144	0.33	2	0.87	2	2.4	1	15.90	2	5.67	2	yes	no	tamoxifen
145	0.03	1	0.40	1	1.1	1	9.00	1	5.00	1	yes	no	tamoxifen
146	1.27	3	3.30	3	3.8	2	29.50	3	5.33	2	yes	no	tamoxifen --> AI
147	2.00	3	3.60	3	4.4	2	28.30	3	6.67	2	yes	Epi/CMF	tamoxifen
148	0.63	3	2.23	3	4.5	2	9.90	1	5.33	2	yes	no	tamoxifen
149	0.30	2	0.13	1	2.1	1	11.40	1	9.00	3	yes	no	tamoxifen
150	0.70	3	1.10	2	3.6	1	25.80	3	4.33	1	yes	no	tamoxifen
151	5.57	3	7.07	3	4.6	2	27.70	3	4.33	1	yes	no	tamoxifen
152	2.83	3	2.63	3	4.9	2	25.70	3	9.33	3	yes	no	tamoxifen
153	0.27	2	0.27	1	3.2	1	16.50	2	9.33	3	yes	no	tamoxifen
154	1.03	3	0.63	2	7.3	3	12.60	2	6.67	2	no	no	tamoxifen --> AI
155	0.03	1	0.27	1	1.8	1	11.80	2	7.00	3	yes	no	tamoxifen
156	0.30	2	0.37	1	1.2	1	4.00	1	6.00	2	yes	no	aromatase inhibitors
157	0.97	3	1.03	2	4.7	2	15.50	2	7.00	3	no	no	tamoxifen
158	0.20	2	0.60	2	5.3	2	9.10	1	6.00	2	no	no	tamoxifen
159	0.27	2	0.67	2	3.4	1	31.10	3	8.67	3	yes	Epi/CMF	no
160	0.20	2	0.47	2	6.5	3	27.10	3	7.33	3	yes	Epi/CMF	aromatase inhibitors
161	1.40	3	2.40	3	5.2	2	38.10	3	9.00	3	yes	Epi/CMF	no
162	0.63	3	0.37	1	5.1	2	74.80	3	8.33	3	yes	Epi/CMF	no
163	0.53	2	0.33	1	3.4	1	19.20	2	7.00	3	yes	Epi/CMF	tamoxifen+LHRH analogues
164	0.17	2	0.57	2	3.6	1	25.00	3	7.67	3	yes	Epi/CMF	tamoxifen
165	0.30	2	0.97	2	2.8	1	14.10	2	6.00	2	yes	FEC – taxotere	tamoxifen
166	0.23	2	0.27	1	3.4	1	2.40	1	6.67	2	no	no	tamoxifen
167	0.53	2	0.27	1	4.3	2	31.60	3	8.33	3	no	no	no
168	1.13	3	0.67	2	5.1	2	8.00	1	7.00	3	yes	AC	tamoxifen --> AI

Appendix 3: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 4).

No.	Locoregional recurrence	Systemic metastasis	Final status	RFS/m.	OS/m.
1	None	None	Alive and well	68.7	68.7
2	None	VISCERAL	Breast cancer related death	14.9	18.6
3	None	NON-VISCERAL	Breast cancer related death	17.1	32.2
4	None	None	Alive and well	65.5	65.5
5	None	None	Alive and well	58.4	58.4
6	None	None	Alive and well	63.1	63.1
7	None	None	Alive and well	58.4	58.4
8	None	None	Alive and well	61.9	61.9
9	cervical lymph-node	None	Alive with recurrent/metastatic BC	9.1	64.0
10	None	VISCERAL & NON-VISCERAL	Breast cancer related death	35.0	42.9
11	None	None	Alive and well	64.9	64.9
12	None	None	Alive and well	68.9	70.4
13	None	None	Alive and well	61.3	61.3
14	None	None	Alive and well	66.0	66.0
15	None	None	Alive and well	61.2	61.2
16	None	None	Alive and well	59.8	60.5
17	None	None	Alive and well	58.4	58.4
18	breast /chest wall	None	Alive with recurrent/metastatic BC	66.4	68.4
19	None	None	Alive and well	66.0	66.0
20	None	None	Alive and well	65.2	65.2
21	None	None	Alive and well	61.2	61.9
22	None	None	Alive and well	59.8	59.8
23	None	None	Alive and well	65.5	66.6
24	None	None	Alive and well	62.9	62.9
25	None	None	Alive and well	65.7	65.7
26	None	None	Breast cancer related death	36.8	36.8
27	None	None	Alive and well	60.8	60.8
28	None	VISCERAL & NON-VISCERAL	Breast cancer related death	14.7	20.2
29	None	None	Alive and well	62.1	62.1
30	None	None	Alive and well	68.4	68.4
31	None	None	Alive and well	58.4	58.4
32	None	None	Alive and well	67.0	68.4
33	None	None	Alive and well	65.7	65.7
34	breast /chest wall	VISCERAL & NON-VISCERAL	Breast cancer related death	18.6	21.5

Appendix 3: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 4).

No.	Locoregional recurrence	Systemic metastasis	Final status	RFS/m.	OS/m.
35	None	None	Alive and well	61.0	62.1
36	None	None	Non-cancer related death	50.8	50.8
37	None	None	Alive and well	60.1	60.1
38	None	None	Alive and well	66.8	66.8
39	None	None	Alive and well	64.2	64.9
40	None	None	Alive and well	58.2	58.2
41	None	None	Alive and well	67.5	67.5
42	None	None	Alive and well	67.5	68.0
43	None	None	Non-cancer related death	30.9	30.9
44	None	None	Alive and well	61.2	61.2
45	None	None	Breast cancer related death	42.2	42.2
46	None	None	Alive and well	64.0	64.6
47	None	None	Alive and well	60.7	60.7
48	None	None	Alive and well	65.6	65.6
49	None	None	Other cancer death	36.4	36.4
50	None	None	Alive and well	57.8	57.8
51	None	None	Alive and well	62.7	62.7
52	None	None	Alive and well	65.4	65.4
53	None	None	Alive and well	57.5	57.5
54	None	None	Alive and well	59.0	59.0
55	None	None	Alive and well	54.9	55.9
56	None	None	Alive and well	56.8	56.8
57	None	VISCERAL	Breast cancer related death	9.7	10.1
58	None	None	Alive and well	56.4	56.4
59	None	None	Alive and well	56.1	56.8
60	None	VISCERAL & NON-VISCERAL	Breast cancer related death	46.9	49.0
61	None	None	Alive and well	56.4	56.4
62	breast /chest wall	VISCERAL	Breast cancer related death	12.0	22.7
63	None	None	Alive and well	56.4	56.4
64	None	None	Alive and well	57.1	57.6
65	None	None	Alive and well	52.7	53.1
66	None	None	Alive and well	53.6	54.3
67	None	None	Alive and well	53.6	54.5
68	None	None	Alive and well	53.1	53.1

Appendix 3: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 4).

No.	Locoregional recurrence	Systemic metastasis	Final status	RFS/m.	OS/m.
69	breast /chest wall	NON-VISCERAL	Alive with recurrent/metastatic BC	49.1	56.3
70	None	None	Alive and well	54.7	54.7
71	None	None	Alive and well	53.4	53.4
72	None	None	Alive and well	52.6	52.6
73	None	None	Alive and well	52.3	52.3
74	None	None	Alive and well	52.2	52.2
75	None	None	Alive and well	52.3	52.3
76	None	None	Alive and well	52.3	52.3
77	None	None	Alive and well	51.4	52.6
78	None	None	Alive and well	51.7	51.7
79	None	None	Alive and well	51.5	52.3
80	None	None	Alive and well	52.1	52.1
81	None	None	Other cancer death	44.3	44.3
82	None	None	Non-cancer related death	5.9	5.9
83	None	None	Alive and well	53.1	53.1
84	None	None	Alive and well	52.2	52.6
85	None	None	Alive and well	61.7	61.7
86	None	None	Alive and well	67.3	67.3
87	None	None	Alive and well	59.9	59.9
88	None	None	Alive and well	66.9	66.9
89	None	None	Alive and well	68.5	68.9
90	None	None	Alive and well	61.7	61.7
91	None	None	Alive and well	65.9	65.9
92	None	None	Alive and well	62.2	62.2
93	None	None	Alive and well	61.3	61.3
94	None	None	Alive and well	67.4	67.4
95	None	None	Alive and well	67.3	67.3
96	None	None	Alive and well	68.0	68.0
97	None	None	Alive and well	66.4	66.4
98	None	None	Alive and well	62.4	62.4
99	None	None	Other cancer death	25.9	25.9
100	None	None	Alive and well	68.0	68.0
101	breast /chest wall	VISCERAL & NON-VISCERAL	Breast cancer related death	17.7	26.1
102	None	None	Alive and well	64.9	66.1

Appendix 3: The clinico-pathological characteristics of patients with invasive primary operable breast cancer (Chapter 4).

No.	Locoregional recurrence	Systemic metastasis	Final status	RFS/m.	OS/m.
103	breast /chest wall	None	Alive with recurrent/metastatic BC	35.2	62.2
104	None	None	Alive and well	60.4	60.4
105	None	VISCERAL & NON-VISCERAL	Breast cancer related death	26.1	30.0
106	None	None	Alive and well	65.7	65.7
107	None	None	Alive and well	62.1	62.1
108	None	None	Alive and well	65.9	65.9
109	None	None	Alive and well	67.1	67.1
110	None	None	Alive and well	60.5	60.5
111	None	None	Alive and well	66.9	66.9
112	None	None	Alive and well	68.1	68.1
113	None	None	Alive and well	68.1	68.1
114	None	VISCERAL	Breast cancer related death	8.7	12.2
115	None	None	Alive and well	62.4	62.4
116	None	None	Non-cancer related death	30.9	30.9
117	None	None	Alive and well	67.3	67.3
118	breast /chest wall	None	Non-cancer related death	31.7	54.5
119	None	None	Alive and well	61.4	61.4
120	None	None	Non-cancer related death	27.9	27.9
121	None	None	Alive and well	60.4	60.4
122	None	None	Alive and well	61.4	61.4
123	None	None	Alive and well	61.4	61.4
124	None	None	Alive and well	67.7	67.7
125	None	None	Alive and well	66.4	66.4
126	None	None	Alive and well	62.8	62.8
127	None	None	Alive and well	68.0	68.0
128	None	None	Alive and well	66.6	66.6
129	None	None	Alive and well	62.4	62.4
130	None	None	Alive and well	61.9	61.9
131	None	None	Alive and well	56.8	56.8
132	None	None	Alive and well	56.6	56.6
133	None	None	Alive and well	56.3	56.3
134	None	None	Alive and well	56.1	56.1
135	None	None	Alive and well	55.9	55.9
136	None	None	Alive and well	55.9	55.9

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No.	Locoregional recurrence	Systemic metastasis	Final status	RFS/m.	OS/m.
137	None	None	Alive and well	55.4	55.4
138	None	None	Alive and well	55.2	55.8
139	None	None	Alive and well	55.2	55.2
140	None	None	Alive and well	55.2	55.2
141	None	None	Alive and well	55.2	55.9
142	None	None	Alive and well	55.0	55.0
143	None	None	Alive and well	55.0	55.0
144	None	None	Alive and well	54.9	54.9
145	None	None	Alive and well	54.9	54.9
146	None	None	Alive and well	54.9	54.9
147	None	None	Alive and well	54.9	54.9
148	None	None	Alive and well	54.9	56.3
149	None	None	Alive and well	54.7	54.7
150	None	None	Alive and well	54.2	54.2
151	None	None	Alive and well	56.2	56.2
152	None	None	Alive and well	54.0	54.0
153	None	VISCERAL	Breast cancer related death	37.5	40.8
154	None	None	Alive and well	53.8	53.8
155	None	None	Alive and well	53.8	53.8
156	None	None	Alive and well	53.8	53.8
157	None	None	Alive and well	53.4	54.1
158	None	None	Non-cancer related death	39.8	39.8
159	None	None	Alive and well	53.7	53.7
160	None	None	Alive and well	53.7	53.7
161	None	None	Alive and well	53.5	53.5
162	None	None	Alive and well	53.3	53.3
163	None	None	Alive and well	53.3	53.3
164	None	None	Alive and well	53.3	54.0
165	None	None	Alive and well	51.4	52.6
166	None	None	Alive and well	51.4	52.7
167	None	VISCERAL & NON-VISCERAL	Breast cancer related death	24.5	28.2
168	None	None	Alive and well	52.1	52.1