Tumour Bed Analysis And Local Recurrence After Breast-Conserving Surgery For Breast Cancer

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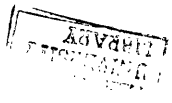

ABSTRACT

This thesis is a study of the causes and significance of local recurrence after breast-conserving surgery for breast cancer. Relevant published literature is reviewed. The core study involved 300 patients with invasive breast cancer who underwent breast-conserving surgery. All patients had tumour bed analysis performed by pathological examination of a shaving taken from the cavity wall. This was excised after the surgeon had performed a wide macroscopic clearance of the tumour. Disease in the cavity shaving was found in 39.3% of cases (tumour bed positivity). Re-excision was performed selectively and all patients received post-operative radiotherapy. At 4.4 years mean follow-up the local recurrence rate was 2.2% for breast-conserved patients and the systemic recurrence rate for all patients was 10.4%. Tumour bed positivity was found to be significantly associated with higher tumour grade, presence of an extensive in-situ component, dense mammographic pattern, casting-type mammographic calcification and absence of mammographic nidus. Non-significant trends were observed between tumour bed positivity and smaller lumpectomy diameter, younger patient age and lobular carcinoma. Tumour bed positivity was found to be significantly associated with poor distant disease-free survival. The implications of these findings are discussed.

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DECLARATION

I declare that this thesis has been composed by myself, and that the work described herein was performed by me or by myself in conjunction with others. Pathological analysis of tumours and cavity shavings described in Chapter 1, and grading of immunohistochemical staining described in Chapter 3 was performed by Dr E Mallon, Department of Pathology, Western Infirmary, Glasgow. The immunohistochemical staining described in Chapter 3 was performed by myself and Miss R Ferrier, Department of Pathology, Western Infirmary, Glasgow. Slides were prepared by Miss R Ferrier. The mammographic features described in Chapter 2 were interpreted by Dr C Cordiner, Department of Radiology, Western Infirmary, Glasgow. Drs G Murray and J Love, Robertson Institute of Biostatistics, University of Glasgow supervised statistical analyses of all work.

This work has not been presented at any previous application for a degree by others or myself.

Papers pertaining to the work described in this thesis that have been published or presented at scientific meetings are listed below.

PUBLISHED MANUSCRIPTS, ABSTRACTS AND PRESENTATIONS BASED ON WORK CONTAINED

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INTRODUCTION

Epidemiology of breast cancer

Globally, breast cancer is the most common cancer affecting women. It accounts for almost 20% of malignancies and over 500,000 new cases of breast cancer are registered every year[1]. In Scotland, between 1981 and 1990, breast cancer accounted for 23.5% of all malignancies in women[2]. An average of 2633 new cases and 1257 deaths from the disease were registered each year and the 5 year relative survival rate was 64.3%. Over this 10 year period the incidence of breast cancer increased by 11.8%. A large part of this increase occurred between 1989 and 1990. Breast screening was introduced in a phased manner to Scotland from 1988 with national coverage by 1991 and in 1993, 21% of all new cases of breast cancer were screen-detected[3]. Within the 50 to 64 age group screening has resulted in an increase in the proportion of cancers with a pathological tumour diameter of 2cm or less (36.7% in 1987 and 59.4% in 1993).

The history of surgery for breast cancer

The earliest documented case of attempted breast cancer surgery is found in the Edwin Smith papyrus that dates to 3000BC[4]. It is attributed to the Egyptian physician, Imhotep and recounts a variety of disease presentations and treatments. Eight cases of breast disorders are documented and case 39 describes treatment of a breast tumour with cauterisation using a fire drill. However, the earliest detailed description of surgical procedures used to treat breast cancer is derived from the works of Aetius, a Byzantine court physician of the 6th century AD and Celsus, the author of *De Medicina* from Provence who lived in the 1st century AD. They documented the practices of many physicians and surgeons who were based in Alexandria after 300BC[5]. One such surgeon, Leonides, advocated excising breast tumours using a combination of cutting and cautery and it is believed that quite radical procedures were attempted at this time. The physician Galen (131AD - 203AD) put forward theories on cancer aetiology based on humoralism. He advocated surgery only when the excision could be extended to healthy surrounding tissue, which was to be cauterised instead of ligated to burn the roots of the disease. He also recommended prescription of special diets and the application of

salves, caustics or ointments to ulcerated tumours, which were most likely to have been the mainstay of his practice[5-7].

Interest in science and advancement of the theories put forward by this early civilisation were noticeable principally by their absence during much of the middle ages. The teachings of Galen remained the basis of breast cancer treatment during the thirteenth, fourteenth and fifteenth centuries. Italian Renaissance surgeons such as Bruno da Longoburgo and Guy de Chauliac advocated surgery only when the tumour was positioned such that wide excision was feasible, sometimes removing the entire breast[5]. The prevailing opinion was however that these procedures represented palliative treatment and success in curing a patient by surgery was rarely claimed.

The discovery of blood circulation by William Harvey in 1628 and the later detailed description of lymph channels gradually altered the prevailing philosophy with regard to breast cancer aetiology and spread. Advances in anatomy and the development of sophisticated surgical tools, coupled with the introduction of formalised surgical training, led to a large increase in the number of surgeons practising breast cancer surgery during the seventeenth and eighteenth centuries. Enthusiasm was frustrated only by

the lack of anaesthesia, the frequent development of sepsis and short post-operative survival. Alexander Monro (1697-1767), professor of anatomy in Edinburgh reported that out of sixty cases of surgically treated breast cancer, only four remained alive without signs of disease after 2 years[8]. A wide variety of surgical procedures were employed at this time. Amputation of the breast was used for small tumours using a variety of surgical instruments specifically designed to expedite the procedure, which would usually last only a few minutes. William Cheselden (1688-1752) and Rene-Jacques Croissant De Gavengot (1689-1759) practised simple excision of the lump. Jean Louis Petit (1674-1750) who became the first surgical director of the French Academy of Surgery, described wide excision including pectoral fascia, en block resection and axillary dissection. Benjamin Bell (1749-1806) of the Royal Infirmary, Edinburgh recommended radical excision of the breast as soon as the diagnosis was made even if the tumour was small and in 1774 Bernhard Perilhe (1735-1804) advocated that radical excision include removal of pectoralis major[5-8]. Surgery however, remained an unpopular treatment for breast cancer. The suffering endured by the patient during the procedure coupled with no observed survival advantage meant that surgery was invariably only sought at very advanced stages of the disease. In 1844 a large survey of

survivors beyond 2 years was carried out by Jean-Jacques-Joseph Leroy d'Etoiles (1789-1860) which concluded that operative treatment of breast cancer was more harmful than beneficial[5].

During the early part of the nineteenth century considerable advances were made in histology and cell biology eventually allowing diagnosis of cancer by microscopy. The introduction of surgical anaesthesia in 1846 and antisepsis in 1867 revolutionised surgery in the mid 19th century. Antisepsis immediately halved the operative mortality to less than 10%. However, despite improved operating conditions, local control of disease was disappointing. In 1874, Sir James Paget reported a series of 235 patients who underwent surgery for breast cancer[6]. Operative mortality was 10% and all cases recurred within 8 years. In 1867 the same year as Joseph Lister reported on antisepsis, Charles Moore (1821-1870), surgeon to the Middlesex and St Lukes hospitals in London, published an important paper detailing the general principals on which he believed the surgical treatment of breast cancer should be based[9]. The paper was titled *"On the influence of inadequate operations on the theory of cancer"* and detailed the case histories of 14 patients who presented to the Middlesex hospital with locally recurrent breast cancer at various times after surgery. From his observations Moore concluded that local

recurrence always occurred at the site of previous operation and that it was due to continuous growth of fragments of tumour remaining in the breast after the initial operation. He proceeded to recommend that the minimum operation for breast cancer should be complete mastectomy including wide skin clearance. In addition, he advocated extending the operation to encompass adjacent tissues if the tumour lay in close proximity to them and en-bloc removal of diseased axillary nodes without dividing the intervening lymphatics. Evidence that radical surgery might improve recurrence-free and overall survival was offered by Samuel Gross (1837-1889), Professor of Surgery at Jefferson Medical College, who reported 200 cases of surgically treated breast cancer[10]. No axillary dissection was performed in 55 patients and all died from recurrence shortly after surgery. Axillary dissection and removal of pectoral fascia was performed in the remaining patients and 3 year survival was 19.4%. The indication for radical surgery was also fuelled by the late 19th century anatomists who described microscopic tumour involvement of pectoral fascia and via lymphatics, pectoral muscle.

In 1882 William Halsted (1852-1922) whilst practising at the Roosevelt Hospital in New York, began to routinely remove pectoralis major en bloc with the breast and axillary nodes. In 1884 he published the

results of the first 50 cases treated this way and reported a local recurrence rate of 6% despite all patients being clinically node-positive at the time of surgery[11]. This figure was ten times less than the local recurrence rates commonly reported at the time. Long follow-up showed that the local recurrence rate increased to 31.9%, which was still a considerable improvement on contemporary figures[12]. In addition, his results for 232 operations were published in 1907 and a 3 year survival rate of 38.3% was reported which was approximately double the observed rate in other series presented at the end of the 19th century[13]. William Meyer described a similar operation to the Halsted mastectomy in 1884[14]. It included resection of pectoralis minor in addition to pectoralis major, which Halsted later adopted. Halsted also performed a series of 119 operations that included supraclavicular node dissection. However, morbidity was high and only 2 of 44 patients who were supraclavicular node-positive were alive after 5 years[13]. This modification of the operation was abandoned although future en bloc resections that included supraclavicular and internal mammary node dissections were envisaged. Such extended radical operations were performed on small series of patients during the early part of the 20th century but were abandoned due to high morbidity and no observed survival advantage over the standard procedure[15]. The

standard Halsted mastectomy involved wide skin excision, en bloc removal of both pectoral muscles, axillary dissection and Thiersh skin graft, and was widely adopted as the routine surgical operation for breast cancer.

The reported survival advantage conferred by radical mastectomy over less radical procedures has been contested[16]. Analyses of the published results of radical mastectomies performed during the early part of this century are fraught with difficulty. Formal staging of breast cancer was not a routine practice and details such as patient age and presence of nodal disease were frequently missing in reported series. In addition follow-up was often unavailable for over 20% of patients. An overview was performed by Lane-Claypon in 1924 who reviewed all literature prior to this date[17]. The early results of 20,000 operations were analysed. Patients lost to follow-up or without histologically proven breast cancer were excluded. Following a non-radical mastectomy, the 3 year survival was 29.2% and following radical mastectomy, the 3 year survival was 43.2%. None of the patients included in this review were randomised to receive either treatment and the results are open to the criticism that selection bias played a large role in the survival differences observed. Nevertheless, the literature testifies to the popularity of radical mastectomy at

this time and it remained the standard operative procedure for breast cancer for three-quarters of a century.

The paradigm for breast cancer surgery put forward by Halsted was based on the theory that breast cancer spreads by direct extension into adjacent tissues. Even bone and liver metastases were explained by lymphatic spread of tumour with uninterrupted microscopic connections to the primary in the breast[13]. This theory was not contested until 1936 when anatomical studies of lymphatics demonstrated the lymphatic drainage of the breast and the presence of normal lymphatic channels between breast tumour and axillary metastases. The theory of cancer spread outwith the breast by embolisation was put forward[18]. In 1906 Handley described a modified radical mastectomy in which skin flaps were raised although most of the pectoral muscles were still excised[6]. He recommended this procedure for older patients or those with early cancers. In 1938 Patey and Duson further modified this procedure and taking into account new scientific evidence relating to tumour spread, recommended preservation of pectoralis major except in advanced cases. Pectoralis minor was divided to allow a complete dissection of the axillary contents[19].

Dissatisfaction with the morbidity caused by radical surgery, the tendency for patients to present earlier with less advanced tumours and the development of radiotherapy all contributed to a growing interest in more surgically conservative methods of breast cancer treatment. In 1937, Geoffrey Keynes presented the results of patients he had treated using interstitial radium with or without very limited surgery and reported survival figures similar to those that would be expected from radical surgery[18]. In 1948 Robert McWhirter suggested that if postoperative radiotherapy was used then radical surgery might be safely substituted with simple mastectomy[20]. The first randomised trial to show that equivalent degrees of local control and survival could be achieved by these two methods of treatment was conducted between 1951 and 1957 in Copenhagen[21]. Other trialists randomised patients undergoing radical mastectomy to postoperative radiotherapy or no further treatment. The first such trial was conducted at the Christie Hospital in Manchester between 1949 and 1955[22]. At 10 years the incidence of local recurrence was reduced by 40% in the radiotherapy group. At 15 years however, a small survival benefit was observed for the non-irradiated group although this was shown to be due to the adverse cardiac effects of radiotherapy and not due to death from breast cancer. A recently published overview of

the eight trials started before 1975, which assessed the value of adjuvant radiotherapy after radical or total mastectomy, has reported that beyond 15 years follow-up there were a reduced number of deaths due to breast cancer in the group of patients who received radiotherapy[23]. This is set against an increased number of cardiac deaths in irradiated patients particularly influenced by the early trials.

During the 1950's and 1960's several investigators reported survival rates from series of patients treated by limited surgery and radiotherapy that were equivalent to those observed after radical surgery[24-27]. These results, along with a growing understanding of the mechanisms of breast cancer spread and the introduction of high energy (super voltage) radiotherapy equipment in the late 1950's generated interest in conservative approaches to breast cancer surgery.

Breast-conserving surgery

The aim of breast-conserving surgery is to achieve maximal cosmesis with minimal physical and

psychological morbidity without compromising overall survival. One of its earliest exponents was Geoffrey Keynes who reported the results of a series of patients treated using radium wire implants to the breast and axilla followed by very limited surgery in 1937[18]. He found a 5 year survival of 71.4% in 85 Stage 1 patients and 29.3% in 92 Stage 2 patients. However, these promising results were slow to make any impact on surgical practice. Thirty years later, Peters et al reported a large group of patients 200 of whom were treated by excisional biopsy and 652 were treated by radical mastectomy[26]. Both groups received radiotherapy and 5 and 10 year survival results were identical. Wise et al reported the results of 96 patients treated by local excision and radiotherapy and found that survival in these patients was not significantly different to 207 comparable patients treated by radical mastectomy with radiotherapy given to those who were node-positive[28]. Local recurrence was observed in 9.4% of the patients treated by breast-conserving surgery. In a study by Taylor et al where sector mastectomy and radiotherapy was performed for 77 patients with tumours less than 5cm, the local recurrence rate was 18.2%[29]. Local recurrence also occurred in 25.8% of patients treated with local excision and radiotherapy in a large retrospective review by Rissanen[27]. Despite this high local recurrence rate, survival at 5 and 10 years was

almost identical to a group of similar patients treated by radical mastectomy and radiotherapy.

The first randomised trial to assess breast-conserving surgery as an alternative to radical mastectomy was started by Sir Hedley Atkins at Guy's hospital in 1961[30]. Over a 10 year period, 370 patients over age 50 were randomised to either radical mastectomy or local excision of the tumour with a 3cm margin of normal tissue followed by radiotherapy. No axillary surgery was performed in the breast-conserving group. Radiotherapy after mastectomy consisted of 30Gy given to the supraclavicular and axillary fields over 3 weeks. After local excision patients received this same treatment over 2 weeks with the addition of 30 Gy over 2 weeks to the ipsilateral breast. Recurrence in the breast occurred in only 6 patients (3%) at 5 years. However, a higher incidence of regional recurrence was observed with a 15.4% axillary recurrence rate. Clinically node-positive patients in the breast-conserving group also had an increased rate of distant recurrence resulting in decreased overall survival compared to those treated by mastectomy. Patients who were clinically node-negative had equivalent distant disease-free and overall survival although more axillary recurrences were seen in the breast-conserving group who had no axillary surgery. The error in clinical node staging within the mastectomy group was

found to be 25%. A second trial was conducted between 1971 and 1975 which randomised 276 patients to the same treatment options but only included those who were clinically node-negative. This second trial reported that regional recurrence, distant recurrence and survival were worse in the breast-conserving group and thus was in conflict with the results of the clinically node-negative subgroup in first trial[31]. The overall results were confusing and did much to dissuade surgeons from practising breast-conserving surgery at this time.

The dose of radiotherapy used in the Guy's trials was by modern standards inadequate and a late analysis of the trial results suggested an explanation for the findings. A comparison of the two trials showed that the main difference between them was that patients who underwent radical mastectomy fared better in the second trial[32]. Analysis revealed that there was a significantly larger group of patients in the second trial with tumours less than 2cm in diameter. The observed differences in the second trial between radical and breast-conserving treatment were limited almost entirely to this group of patients. This analysis suggested that radical surgery may confer a survival benefit for patients with early (localised) disease and inferred that inadequate local treatment especially to the axilla, may adversely affect outcome.

The trial also demonstrated that a low local recurrence rate can be achieved with a wide, 3cm clearance around the tumour. Only 6 local recurrences occurred in the breast-conserving group in the second trial at 5 years (5%).

To-date seven randomised trials have compared mastectomy with breast-conserving surgery plus radiotherapy. One trial failed due to the constraints of informed consent resulting in poor patient accrual[33]. Six trials have published medium to long-term results[34-39]. The protocols of these trials varied slightly and are summarised in *Table 1*. With follow-up ranging from 6 to 13 years these trials have demonstrated no significant difference in overall survival, and distant disease-free survival between the various treatment arms [*Table 2*].

The use of breast-conserving surgery sharply increased during the late 1980's as the early results of these trials were declared. In a survey of 16 radiotherapy departments in the Netherlands, the number of patients treated by breast-conserving surgery more than doubled between 1986 and 1990[40]. By 1990, 36% of all women with newly diagnosed invasive breast cancer were treated in this way. In Scotland the use of breast-conserving surgery increased from 40% of cases in 1987 to 52% in 1993[3]. The encouraging results of randomised trials

comparing breast-conserving surgery and radiotherapy with mastectomy prompted the design of trials assessing the need for post-operative radiotherapy. A course of radiotherapy after breast-conserving surgery usually involves 40 to 50 Gy given in 20 to 25 daily fractions over 5 or 6 weeks with a boost of 10 to 20 Gy over 7 or 14 days to the site of excision. Apart from the inconvenience of such a treatment schedule, radiotherapy may be associated with significant physical and psychological side effects such as fatigue, skin irritation, telangectasia, breast fibrosis, respiratory symptoms, mild dysphagia, anorexia and anxiety[41,42]. Five randomised trials have therefore, compared breast-conserving surgery alone with breast-conserving surgery plus adjuvant radiotherapy[35,43-46]. The protocols are summarised in *Table 3*. With follow-up ranging from 4-9 years no significant difference in overall survival or distant disease-free survival between the various treatment arms has been reported although small non-significant differences have been observed. However, highly significant differences in the incidence of local recurrence were found in all these trials with unacceptably high rates for non-irradiated patients[*Table 4*].

Local recurrence after breast-conserving surgery is a significant concern. It potentially undermines all the

aims of breast-conserving surgery. Psychologically, a patient will lose confidence in her treatment, suffer the anxiety of cancer recurrence and may face the prospect of undergoing a mastectomy. The cosmetic benefits of breast-conserving surgery are therefore lost. In addition, there is also the theoretical concern that in certain patients, inadequate local treatment may allow disease dissemination and adversely affect outcome. When patients with local recurrence are analysed separately, associations between local recurrence and shorter distant disease-free and overall survival have been reported[44,47-49]. The significance of this association is the subject of considerable debate.

Table 1. Randomised trials of breast-conserving surgery plus radiotherapy versus mastectomy (Protocols).

TRIAL	NUMBER OF PATIENTS	TUMOUR SIZE	RADIOTHERAPY
MILAN I *(1973-1980)*	701	≤2cm	50Gy (plus 10Gy boost)
NSABP B-06 *(1976-1984)*	1843	≤4cm	50 Gy (no boost)
WHO *(1972-1980)*	179	≤2cm	45Gy (plus 15Gy boost)
NCI *(1979-1987)*	237	≤5cm	48Gy (plus 15-20Gy boost)
EORTC *(1980-1986)*	878	≤5cm	50Gy (plus 25Gy boost)
DANISH *(1983-1989)*	859	Not defined	50Gy (plus 20Gy boost if margin positive)

Table 2. Randomised trials of breast-conserving surgery plus radiotherapy versus mastectomy (Results).

TRIAL	RANDOMISATION	FOLLOW-UP	OS	DDFS
MILAN I	Quadrantectomy + Radiotherapy V Mastectomy	13 years	*71% V 69%*	*NSSD*
NSABP B-06	Lumpectomy + Radiotherapy V Mastectomy	9 years	*69% V 68%*	*60% V 63%*
WHO	Lumpectomy + Radiotherapy V Mastectomy	10 years	*79% V 80%*	*NSSD*
NCI	Lumpectomy + Radiotherapy V Mastectomy	10 years	*77% V 75%*	*NSSD*
EORTC	Lumpectomy + Radiotherapy V Mastectomy	8 years	*NSSD*	*NSSD*
DANISH	Lumpectomy + Radiotherapy V Mastectomy	6 years	*79% V 82%*	*NSSD*

OS = Overall Survival, DDFS = Distant Disease-Free Survival, NSSD = No statistically significant difference (actual values not available)

Table 3. Randomised trials of breast-conserving surgery plus or minus radiotherapy (Protocols).

TRIAL	NUMBER OF PATIENTS	TUMOUR SIZE	REQUIRED MARGIN	RADIOTHERAPY
MILAN III *(1987-1989)*	567	<2.5cm	2-3cm gross	50Gy (plus 10Gy boost)
NSABP B-06 *(1976-1984)*	1843	≤4cm	1cm gross + pathologically clear	50Gy (no boost)
OCTRF *(1984-1989)*	837	≤4cm	Gross + pathologically clear	40Gy (plus 12.5Gy boost)
SWEDISH *(1981-1988)*	381	≤2cm	2cm pathological clearance	54Gy (no boost)
SCOTTISH *(1985-1989)*	585	<4cm	1cm Gross	50Gy (plus 10-15Gy boost)

Table 4. Randomised trials of breast-conserving surgery plus or minus radiotherapy (Results).

TRIAL	RANDOMISATION	FOLLOW-UP	OS	DDFS	LRR
MILAN III	Quadrantectomy V Quadrantectomy + Radiotherapy	4 years	*NSSD*	*NSSD*	*8.8% V 0.3%**
NSABP B-06	Lumpectomy V Lumpectomy + Radiotherapy	9 years	*68% V 69%*	*59% V 60%*	*43% V 12%**
OCTRF	Lumpectomy V Lumpectomy + Radiotherapy	7.6 years	*76% V 79%*	*N/A*	*35% V 11%**
SWEDISH	Sector resection V Sector resection + Radiotherapy	5 years	*90% V 91%*	*87.1% V 90%*	*18.4% V 2.3%**
SCOTTISH	Lumpectomy V Lumpectomy + Radiotherapy	6 years	*81.6% V 83.2%*	*64% V 79.8%*	*24.5% V 5.8%**

*OS = Overall Survival, DDFS = Distant Disease-Free Survival, LRR = Local Recurrence Rate, * = Statistically significant, NSSD = No statistically significant difference (actual values not available), N/A = Not available*

Local recurrence after breast-conserving surgery

The mechanism of local recurrence

Local recurrence after breast-conserving surgery is defined as disease recurring in the ipsilateral breast. It may arise either due to residual disease left in the breast at the time of surgery for the primary tumour or alternatively due to a second primary tumour developing in the breast. Throughout the evolution of surgery for breast cancer the possibility of leaving behind residual disease has been a cause for concern. All trials of breast-conserving surgery have therefore adopted measures to ensure complete local excision of the tumour. This practice was supported by laboratory-based studies involving serial sectioning or simulated wide local excision of tumours in mastectomy specimens. Several such studies have been performed[50-61]. The incidence of multicentricity in these studies varied according to the detail with which the specimens were examined. When 1 or 2 random samples from each quadrant were analysed, multicentricity was found in 18% of cases[59]. Rosen et al performed quadrantectomy or wide local excision with a 2 cm margin in 203 mastectomy specimens[53]. Only 2 or 3 sections were taken from each

remaining quadrant. Residual microscopic invasive or in-situ disease was found in 26% of specimens containing tumours less than 2 cm in diameter and 38% of those containing tumours larger than 2 cm. Egan developed a standardised method of pathological-radiological whole organ analysis. Multicentricity was found in 69% of 161 specimens when 5mm sections of the whole breast were taken[56]. An important study by Holland et al analysed 282 mastectomy specimens by serial sectioning in a similar way to Egan[57]. All of these patients had invasive breast cancer and were theoretically suitable for breast-conserving surgery with no clinical or mammographic evidence of multifocal disease, no skin or chest wall fixation and histological tumour diameter less than 5cm. Specimens were sectioned at 5mm intervals and the presence of microscopic disease was recorded and mapped on a topogram. Tumour foci were found in 43% of specimens (27% non-invasive and 16% invasive) outside a radial distance of 2cm from the reference tumour. This would represent residual disease had the patients undergone breast-conserving surgery with a 2cm gross clearance margin. An important finding was that the distribution of tumour foci was independent of tumour size. For tumours less than 2cm diameter residual invasive or in-situ disease was found in 59% of specimens at a distance of 1cm, 42% at a distance of 2 cms, 17% at a

distance of 3 cms and 10% at a distance of 4 cms from the macroscopic edge of the tumour. A recent study using similar methods of analysis in 30 mastectomy specimens has corroborated these findings[61]. These results have accurately predicted the incidence of local recurrence observed in clinical trials where radiotherapy was not administered. In the lumpectomy only arm of the NSABP-B06 trial where a 1cm clearance margin was taken around the tumour, the local recurrence rate was 27.9% at 5 years and 53% at 10 years[62,63]. In the quadrantectomy only arm of the Milan III trial where a 2-3cm clearance might be expected, the local recurrence rate was 8.8% at 3 years[43]. Invasive residual disease might be expected to be responsible for early local recurrence whilst residual in-situ disease might declare as a later recurrence. Another interesting finding in Holland's study was that 41% of patients had no evidence of tumour outwith the macroscopic tumour mass suggesting that surgical excision alone may be adequate local treatment for a significant minority of patients.

There is strong support for the view that the large majority of local recurrences represent residual disease. In an early analysis of the lumpectomy patients in the NSABP-B06 trial, all 110 local recurrences occurred in or close to the quadrant of the original tumour. The histological types and grades were

identical in 86%[64]. In an overview of patients treated by quadrantectomy in Milan, 60% of local recurrences were of the same histological type and 79% occurred at or very close to the site of previous excision[47]. Kurtz et al reported that 86% of local recurrences that occurred within 5 years affected the site of previous wide local excision[48]. Only 75% of local recurrences that occurred between 5 and 10 years and 36% of those presenting after 10 years affected the site of previous excision. In a study of 990 patients treated by lumpectomy with a median follow-up of over 5 years, Haffty et al analysed local recurrences in terms of location, histology and DNA flow cytometry[65]. Of 80 recurrences, 59% were classified as representing residual disease and 41% as new primary tumours. The time to local recurrence was significantly shorter in those patients with local recurrence secondary to residual disease (3.6 years) compared with those thought to have a second primary cancer (5.7 years). In the NSABP-B06 trial, the annual rate of local recurrence in the lumpectomy only arm decreased from 8.5% during the first 3 years follow-up to 4.6% in years 4 to 9, whereas in the lumpectomy plus radiotherapy arm the rate of local recurrence was constant at 1.4%[49]. Thus the evidence strongly favours attributing early local recurrence to residual disease. The cause of late local recurrence or recurrence in the

breast distant from the site of previous excision is less clear.

It is difficult to distinguish between local recurrence due to residual disease from that due to a new primary tumour. Previous studies have highlighted the degree to which many breast tumours are multicentric[50-61]. In the Milan II trial which compared quadrantectomy plus radiotherapy (QUART) with tumourectomy plus radiotherapy (TART) local recurrence was defined as tumour relapse within 3cm of the surgical scar[66,67].Recurrence in the breast beyond this was defined as a new primary. Significant differences were observed in the incidence of local recurrence, confirming that a wider excision margin reduces the risk, but there was no significant difference in the incidence of new primaries. In addition, the incidence of contralateral tumours was similar for both QUART and TART groups and was greater than the incidence of new primaries in the ipsilateral breast. Thus the incidence of new primaries was no greater than expected. It may even be less, suggesting that radiotherapy may protect the breast from developing a second primary breast cancer.

Assessing margins of excision

Various methods of assessing completeness of excision have been devised. Patients with evidence of incomplete excision can be selected for subsequent wider excision or mastectomy. In the NSABP-B06 trial, margins were assessed using the technique of India Ink staining[64]. This involved coating the lumpectomy specimen in ink and taking sections from the anterior, posterior, medial and lateral aspects of its inked surface. These were blocked sagitally and excision was deemed incomplete only when tumour had been transected by the inked margin. In this trial, between 12 and 20 blocks were taken from each specimen. In all, 10% of patients were shown to have involved margins and subsequently underwent mastectomy. Only 29% of mastectomy specimens contained residual disease. However, a review of the histological slides of patients thought to have margin involvement, demonstrated a lack of consensus agreement among different pathologists in 69% of cases. Where agreement was reached, residual disease was found in 67% of mastectomy specimens[64]. Despite this policy, local recurrence rates in this trial were high. This method of margin assessment appears therefore, to markedly underestimate the true incidence of residual disease after lumpectomy. Frazier et al used the same

criteria as the NSABP-B06 trial to assess margins[68]. In this study of 87 patients who underwent diagnostic excision biopsy for breast cancer, 75 patients subsequently opted for mastectomy regardless of margin status and 12 patients who wished breast conservation had a re-excision performed for involved margins. Original margin status and incidence of residual disease in the breast could therefore be compared. Re-excision and mastectomy specimens contained residual tumour in 52% of those with involved margins, 32% of those with uninvolved but close margins and 26% of those with clear margins. Close margins have been variably defined as tumour found within 1, 2 or 5mm from the inked surface[69-71]. Gwin et al demonstrated residual disease in 65% of patients with a positive margin, 23% with a close margin and 45% where the margin status was unknown[72]. Five year local recurrence rates have been reported as being between 2 and 21% for patients with positive margins and between 4 and 11% for those with close margins[70,71]. In two studies no local recurrences have been recorded at 5 years for patients with negative margins after initial surgery or re-excision[69,71]. However, in one of these studies over 50% of patients had positive margins initially and therefore required re-excision[71]. In a prospective study of 87 patients only those with a pathologically clear margin of at least 1cm as determined by inking,

were included[73]. No radiotherapy was administered and local recurrence occurred in 16% of patients by 4.5 years. A total of 78% of recurrences affected the original tumour site. The technique of analysing margins by inking has been criticised previously. It has been stated that sections taken from the surface of an irregular, fatty lumpectomy specimen are likely to assess the least pathologic areas of the specimen[74]. Even if sectioning is targeted to apparent stellate extensions of the tumour which are in any case difficult to detect three-dimensionally, areas of in-situ disease will be missed. An almost infinite number of sections would be required to accurately detect disease on the surface of a lumpectomy specimen.

Other methods of assessing the margin of excision include taking multiple biopsies from the cavity remaining in the breast following lumpectomy. Umpleby et al demonstrated that after taking biopsies from the superior, inferior, medial, lateral and deep margins of the cavity wall, residual disease was found in 25% of patients[75]. Frozen section is of little value due to the number of sections required for margin assessment and the difficulty in diagnosing in-situ disease by this method[76]. In summary, analysis of resection margins can indicate that incomplete excision is likely. However, assessing completeness of excision is difficult, labour intensive, and current methods appear

flawed. It is therefore important to also establish the other factors that might accurately predict patients at risk of developing local recurrence.

Risk factors for local recurrence after breast-conserving surgery

Extensive intraduct component

The presence of an extensive intraduct component (EIC) within the tumour was proposed as a risk factor for local recurrence by the Joint Centre for Radiation Therapy (JCRT). EIC is defined as being present if an infiltrating ductal carcinoma has both prominent intraduct carcinoma present within the tumour (also specified as >25%) and intraduct carcinoma present in sections of grossly normal adjacent breast tissue. Tumours which are predominantly intraductal with foci of invasion are also considered to have EIC. Vicini et al demonstrated that in 584 patients treated by local excision and radiotherapy, 5 year local recurrence rates were 21% in 166 patients who had EIC and only 6% in 418 patients without EIC. Patients with EIC

accounted for 58% of all local recurrences. Almost all recurrences occurred at the operation site[77,78]. In an analysis of the series of mastectomy specimens previously reported to show residual disease at varying distances from the macroscopic edge of the tumour, Holland et al looked specifically at the influence of EIC on their observations[57,79]. Of 66 specimens where the tumour exhibited EIC, the incidence of residual disease was significantly greater (74%) when compared with 151 specimens that did not show EIC (42%). Further analysis showed that this difference was predominantly due to a greater incidence of residual intraduct carcinoma in patients with EIC. In addition, 44% of patients with EIC had prominent residual intraduct carcinoma (greater than five low power fields) compared to 3% of patients without EIC. A detailed study using computer graphic three-dimensional mapping of mammary duct systems has shown that the degree and distance of intraduct tumour extension is greater for tumours with EIC[80]. EIC has no effect on the incidence of recurrent tumour within the breast distant from the site of previous excision[81,82].

The significance of EIC in relation to the incidence of local recurrence has been analysed in some of the randomised trials of breast-conserving surgery. EIC was found to be a highly significant predictor for local recurrence in the Milan II trial particularly for

patients in the tumourectomy group where local recurrence was seen in 28% of patients who had EIC[26]. EIC accounted for 18% of all local recurrences in an overview of patients treated by quadrantectomy[47]. No association between local recurrence and EIC was found in the NSABP B-06 trial and this is the major exception to the consensus that EIC is an important predictor of local recurrence[62]. An important difference between these trials was the fact that 10% of patients in the NSABP-B06 trial had a mastectomy because of positive resection margins but were analysed in their original randomised treatment arm. In addition, the reported incidence of tumours with EIC varied. It was reported as being present in 13% of patients in the NSABP-B06 trial but only 6.7% of those in the Milan trials[66,83]. In the JCRT study EIC was present in 28% of patients and in the pathological study by Holland et al EIC was present in 30% of specimens[78,79]. Thus definition and interpretation of what constitutes EIC appears to vary considerably between different institutions.

The type of intraduct carcinoma present may be important. Lindlay et al found that comedo-type intraduct carcinoma was associated with a local recurrence rate of 50%[84]. This is in accordance with studies of pure ductal carcinoma in situ (DCIS). In the NSABP-B17 trial of breast-conserving surgery for DCIS, comedo necrosis and involved margins were the only

independent predictors of local recurrence[85]. Schwartz et al observed that of 70 patients who had wide excision of DCIS, there were 11 recurrences at 4 years. All but one had comedo DCIS as the primary lesion[86]. In addition the presence of EIC can frequently be predicted by identifying typical patterns of microcalifications on pre-operative mammograms[87].

Lymphatic/vascular invasion

Lymphatic and vascular invasion may be identified separately but are often considered together as "vascular" invasion. Vascular invasion is defined as the presence of tumour emboli within an endothelium-lined vascular space. In one large series, this was identified in 23% of breast tumours[88]. Lymphatic invasion was found to significantly predict for local recurrence in the Milan overview of quadrantectomy patients[47]. It was only found in 6.5% of patients but 24% of all local recurrences occurred in this group. In the NSABP-B06 trial, lymphatic invasion was associated with a significantly increased risk of local recurrence in patients undergoing breast-conserving surgery. This association was noted at 3 years follow-up but lost statistical significance with longer follow-up[62,64]. In

an analysis of 518 patients treated at the Institute Curie, 6% of patients had vascular invasion and 13% of all local recurrences occurred in this group[89]. Locker et al found vascular invasion in 24% of tumours, which strongly correlated with local recurrence[90]. Vascular invasion was the most powerful predictor for local recurrence in this series of patients.

Histological type and grade

Other pathological factors reported to be significant predictors for local recurrence include histological type and histological grade of the tumour. It was initially thought that lobular carcinoma would not be suitable for breast-conserving surgery due to its association with multicentricity. Studies have shown however, that local recurrence rates comparable with those for ductal carcinoma can be achieved[91,92]. Lobular carcinoma was not associated with an increased risk of local recurrence in the Milan or NSABP conservation trials[47,62]. The importance of histological grade varies between studies and with the type of grading system used. The NSABP-B06 trial reported that nuclear grade was a significant predictor for time to local recurrence in node positive patients

after long follow up[62]. Where the Bloom and Richardson[93] classification of tumour grade has been adopted the risk of local recurrence has generally been found to increase with increasing grade[84,90,94].

Tumour size

Tumour size has been reported to be a predictor for local recurrence in many studies. Local recurrence was significantly more common in patients with pathological tumour size greater than 2cm compared with smaller tumours in the NSABP-B06, EORTC and Milan trials particularly in those patients who did not receive radiotherapy[38,47,49,62]. Other investigators have found the same observations in non-randomised studies[90]. Some studies have also found difficulty in obtaining pathologically clear margins of resection in patients with large tumours[71,72]. Interestingly, the incidence of widespread microscopic disease around the tumour was not related to tumour size in Holland's series of sectioned mastectomy specimens, although Rosen et al demonstrated that large tumour size was associated with multifocal disease[53,57]. In a study by Vaidya et al, which used similar methods of analysis to Holland et al, multicentricity was related to tumour size[61].

However, although larger tumours were more likely to have surrounding multicentric foci, they were not any more extensive or likely to be any further removed from the macroscopic edge of the tumour. The probability that these foci would be excised with a 1 or 2cm clearance margin around the tumour was not therefore related to tumour size. It is likely that wide local excision of large tumours is at times technically difficult and that tumour size assumes importance when surgical clearance is limited for cosmetic reasons.

Age

A consistent association between young age and increased risk of local recurrence has been reported. Patients younger than 35 years appear to be at particularly high risk. In the Milan overview of quadrantectomy patients, young women were significantly more likely to develop local recurrence in Cox regression analysis[47]. Women over age 70 were very unlikely to develop local recurrence. However, associations have also been reported between young age and multicentricity, multifocality, vascular invasion, high tumour grade and EIC[95-98]. Young age may not be

independently significant when these factors are taken into account.

The presence of EIC, lymphatic invasion, poor histologic grade, large tumour size and young age have therefore all been implicated with increased risk of local recurrence. Locker et al have reported a series of 253 patients treated by breast-conserving surgery and radiotherapy where the local recurrence rate was 21%. They found that the risk of local recurrence could be estimated using a prognostic index calculated from a combination of weighted values attached to tumour stage, tumour size, patient age and the presence of vascular invasion[90].

The significance of local recurrence after breast-conserving surgery

It has been argued that local recurrence is of no significance and that attention given to it in the literature is exaggerated[99,100]. This opinion is based on the fact that in the most recent trials, local recurrence rates are low. This has been attributed to the more widespread use of chemo-endocrine therapy,

which as might have been expected, reduces the incidence of local recurrence[34,38,47,62,101,102]. The results of the breast conserving trials [*Table 2* & *Table 4*] show that overall survival and the risk of developing distant recurrence following surgery for breast cancer has no statistically significant association with either the extent of the surgical procedure or the administration of post-operative radiotherapy. However, when patients who develop local recurrence are analysed separately and compared to those without local recurrence, a clear difference in distant disease-free and overall survival is observed.

In the NSABP B-06 study, a Cox regression model analysis of local recurrence demonstrated that patients who developed local recurrence were 3.41 times more likely to develop distant disease[49]. In addition, at 1, 2 or 5 year cut-offs patients who had early local relapse were significantly more likely to develop distant disease than those who experienced this event late. A similar analysis was performed on results from the OCTRF randomised trial of breast-conserving surgery with or without radiotherapy[44]. This showed that the relative risk of both distant disease and overall mortality were more than doubled in patients who had local recurrence (2.11 and 2.18 respectively). Again, patients who developed local recurrence within one year were at significantly higher risk than those in whom

local recurrence occurred late. Applied to data from the Milan breast-conservation trials and a large group of non-randomised patients treated by quadrantectomy the Cox regression model analysis has shown the risk for distant disease to be 4.62 times greater in patients who had a local recurrence. In this trial the risk was six times greater if local recurrence occurred within 1 year[47]. It has been shown by Kurtz et al that survival relative to the onset of local recurrence varied significantly in a non-randomised study of 1593 patients treated by breast-conserving surgery and radiotherapy with follow-up of 11 years. Patients who developed local recurrence after 5 years had the same overall survival as those who were disease-free[48]. In the group of patients who had local recurrences analysed in terms of location, histology and DNA flow cytometry by Haffty et al, the 5 year survival rate following breast recurrence varied from 89% in those with second primaries to 36% in those with recurrences due to residual disease[65]. Early local recurrence appears therefore to be strongly associated with the development of distant disease.

Some risk factors for local recurrence are risk factors for both local recurrence and distant disease and some are not. In addition, not all patients who get local recurrence are at increased risk of distant disease. EIC, whilst strongly predicting residual disease and

local recurrence does not as might have been predicted correlate with distant disease-free or overall survival[78,81,82]. Lymphatic/vascular vessel invasion and tumour grade however correlate with local recurrence, distant disease and overall survival[47,88,89,90,104,105].

The evidence implicating local recurrence with the development of disseminated disease does not however, translate into significant differences in distant disease-free or overall survival between the treatment arms of randomised trials. This is surprising considering the large differences in the incidence of local recurrence observed. In the NSABP B-06 trial the cumulative incidence of local recurrence at 10 years was 53% in the lumpectomy only group compared with 12% in the group which received adjuvant radiotherapy. However, there was no significant difference in the incidence of distant disease or overall survival between these groups[62]. Four other trials of breast-conserving surgery plus or minus radiotherapy have supported this finding although non-significant differences in distant disease-free survival have been reported in favour of patients who received radiotherapy [*Table 4*]. Preventing local recurrence in the breast by performing a mastectomy has not been shown to lower the incidence of distant disease or improve survival [*Table 2*].

Arguments have been postulated to explain this apparent inconsistency. Firstly, if local recurrence did cause distant disease, predicted differences in outcome would be small and the numbers of patients and duration of follow-up in each of these trials may not be sufficient to detect this. This argument is supported by the long-term results of patients undergoing adjuvant radiotherapy following mastectomy in which a survival benefit conferred by radiotherapy was only seen after 15 years[23]. Similarly an apparent benefit of radical surgery for small tumours was seen in the Guy's trials after 15 years[32]. It has also been suggested that local recurrence after breast-conserving surgery is a marker for distant disease rather than the cause for it[49]. A patient's risk of developing distant disease would thus be pre-determined before treatment, with local recurrence being a manifestation of this risk.

If local recurrence was to influence survival or the incidence of distant disease directly, then it might be expected that such an effect would be more apparent in patients who were node-negative at the time of initial surgery. Such patients would be more likely to have localised disease and would be less likely to receive adjuvant systemic therapy. In the NSABP-B06 trial all patients who were node-positive received chemotherapy[63]. In a recently available re-analysis of the trial, excluding patients from a single clinic

where fraudulent data submission was identified, follow-up is continued beyond 10 years[103]. A 12% difference in distant disease-free survival was observed between node-negative patients in the lumpectomy only and the irradiated group (58% and 70% respectively). In addition, overall survival among node-negative patients varied by 4% (74% and 78% respectively). The differences in distant disease-free and overall survival were increased from the 5 year follow-up figures (8% and 2% respectively). For all patients in this re-analysis of the NSABP-B06 trial, distant disease-free and overall survival at 10 years was 61% and 66% for the mastectomy group, 62% and 71% for the lumpectomy plus radiotherapy group and 55% and 65% for the lumpectomy only group respectively. A study using Bayesian analysis of the NSABP-B06 data estimated the probability that radiotherapy conferred a positive benefit to patients who were lymph node and margin negative[106]. The probability was 65% at 5 years increasing to 87% at 10 years with a relative reduction in annual mortality of 8.2% and 17.5% respectively.

Distant disease-free survival differed by 16% in favour of irradiated patients in the Scottish trial[46]. The local recurrence rates for irradiated and non-irradiated patients were 5.8% and 24.5% respectively. In this trial all oestrogen receptor positive patients received tamoxifen and all oestrogen receptor negative

patients received chemotherapy. Thus, systemic therapy cannot substitute for radiotherapy to achieve local disease control. However the effect of systemic therapy on local recurrence has been widely reported and may be pronounced in the first few years of follow-up. The results of adjuvant tamoxifen and polychemotherapy trials show that the benefit for disease-free survival was two or three fold greater than the benefit for overall survival at 5 years follow-up[102,107]. This was predominantly due to a decreased incidence of local and regional recurrences in patients receiving systemic therapy and most of these were local recurrences. At 10 years however, the benefit for disease-free and overall survival was identical. An explanation for this effect is that adjuvant chemo-endocrine therapy has a preferential effect on soft tissue, as opposed to bony metastases.

In summary, there is no hard evidence to support the theory that local recurrence may directly effect patient outcome by disease dissemination. There is however, considerable indirect evidence to suggest that local control may be critical for patients with early breast cancer for whom local therapy is potentially curative.

To achieve the aims of breast-conserving surgery, local recurrence must be avoided and survival must be no

worse than that after more radical surgery. In order to minimise local treatment for breast cancer without compromising safety, a better insight into the causes of local recurrence is required, with better criteria for patient selection. The aim of this thesis is to further the current understanding of these specific areas.

CHAPTER 1

INTRODUCTION

Incomplete surgical excision of the primary tumour is the primary cause of local recurrence after breast-conserving surgery for breast cancer as outlined earlier. The exact incidence of incomplete excision is unknown. However in the study by Holland et al in which mastectomy specimens from patients who would have been theoretically suitable for breast-conserving surgery were serially sectioned, residual disease was predicted in 42-59% of patients depending on the macroscopic clearance margin[57]. A local recurrence rate within this range has been reported for patients not receiving radiotherapy in a randomised trial[62].

The completeness of surgical excision, both macoscopic and microscopic is difficult to determine due to a combination of factors. In most cases the tumour edge is not uniform or rounded. Eccentric intraductal extension of tumour is common[80]. The lumpectomy specimen also has an irregular edge with a large surface area. Identifying microscopic disease at the surface of this specimen would require an impractical number of tangential sections[74]. The technique of using

India ink staining to assess resection margins is cumbersome and time-consuming. It typically reports margin involvement in approximately 10% of patients of which only half or less than half may have evidence of residual disease in a further resection specimen[64,68]. In addition, the definition of margin positivity may be somewhat subjective[64]. Local recurrence rates after breast-conserving surgery have not been convincingly reduced in trials using this method of margin assessment[35,44,45].

A previous study has described the technique of taking biopsies from the cavity wall remaining in the breast following breast-conserving surgery. In a study of 51 patients where 5 such biopsies were taken in each case, the incidence of residual disease detected was 25%[75].

This study presented in this chapter was designed to answer the following questions:

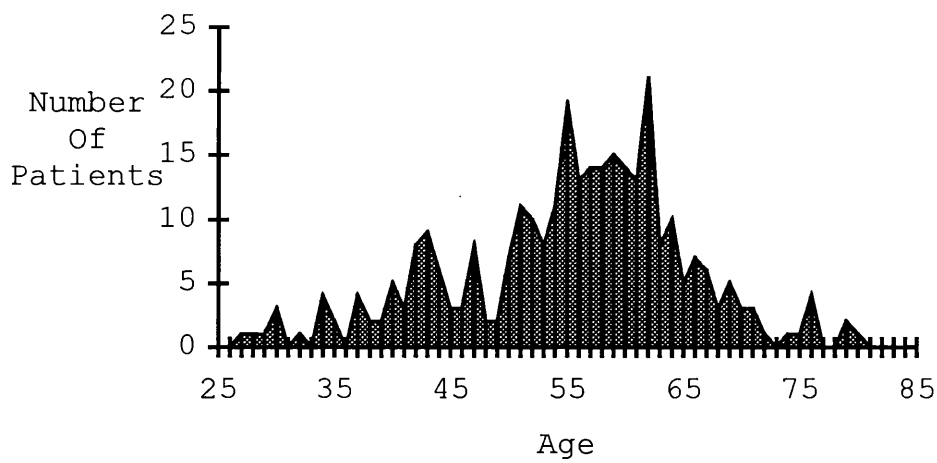
- Is shaving the lumpectomy cavity a practical method for assessing completeness of excision during breast-conserving surgery for breast cancer?
- What is the incidence of residual disease after breast-conserving surgery for breast cancer?
- What are the clinical and pathological risk factors associated with residual disease?

PATIENTS AND METHODS

Patients

Three hundred patients entered the study between March 1988 and September 1992. All had invasive breast cancer clinically less than 2cm in diameter diagnosed by mammography, fine needle aspiration (FNA) cytology or trucut biopsy (Travenol Laboratories, Thetford, UK). The mean age of the patients was 55.3 years (range 27-81, distribution shown in Graph 1). One hundred and seventy four presented symptomatically and 126 were referred through the National Breast Screening Programme (69 with palpable lesions).

Illustration 1. Age distribution.

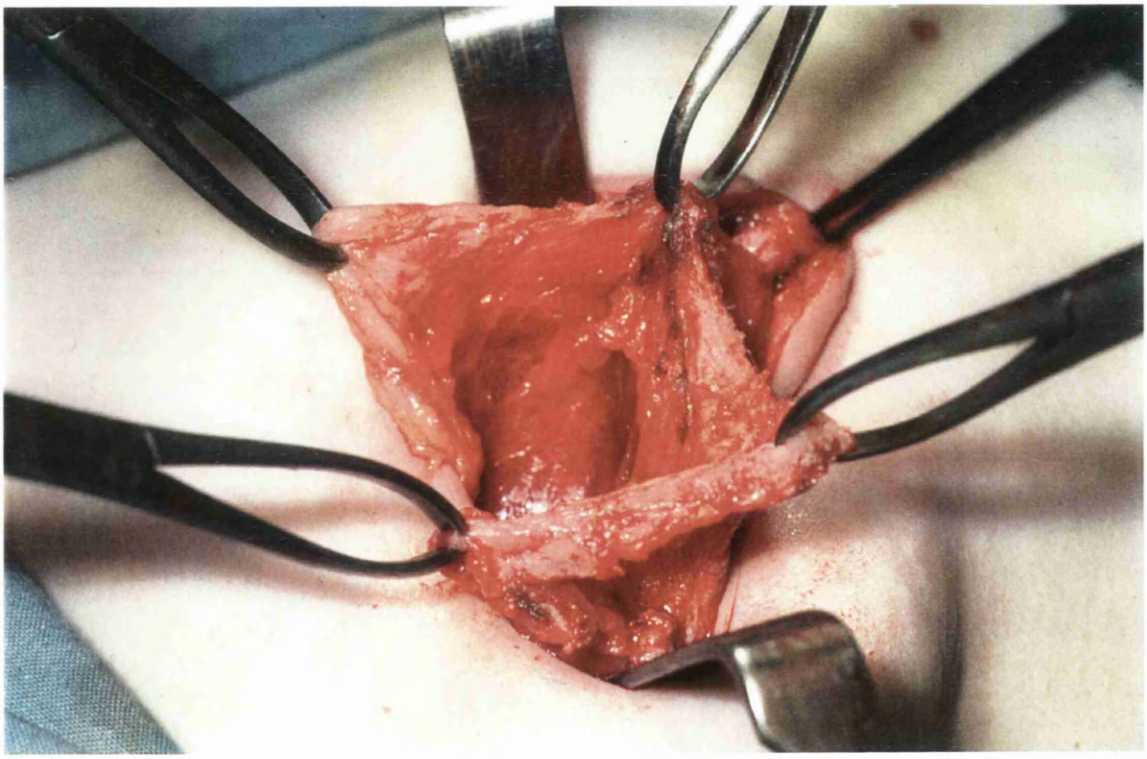


Surgical Management

A wide local excision was performed with the intention of tumour removal with at least 1-2cms of surrounding macroscopically normal breast tissue. The deep extent of the excision was to the pectoral fascia. Tissue forceps were then applied to the breast tissue lining the wall of the cavity and this wall was excised completely using the scalpel (Illustration 1) and labelled "cavity shaving" (CS). In the light of preliminary results, additional random biopsies were taken from the wall of the secondary cavity in 165 patients. These were taken by applying tissue forceps to the superior, inferior, lateral and medial parts of the remaining biopsy cavity wall and excising small (approximately 1cm) pieces of tissue. These were labelled "bed biopsies" (BB). All specimens were submitted for histopathology. In all cases the cavity shaving was only taken after definitive wide local excision of the tumour. In patients with impalpable tumours, definitive surgery was performed following initial wire-guided diagnostic biopsy.

Illustration 2. Taking a Cavity Shaving.

Original in colour.



Cavity Shaving

Pathological Assessment

The maximum diameter of the original lumpectomy specimen and the tumour were recorded. Ductal carcinoma in situ was described as being absent, minimal, moderate or extensive; lymphatic or vascular invasion and Bloom and Richardson grade[90] were noted.

The total number of blocks for the cavity shavings ranged between 20 and 40 depending on the specimen volume. All tissue was processed. One block was taken from each of the bed biopsies. All blocks were serially sectioned and the type and extent of disease assessed.

Further Surgery

The decision to offer further surgery on the basis of CS or BB involvement with disease was pragmatic and related to the pathological findings in each individual case. Factors taken into account were the type of disease (invasive or in situ) and the extent of disease (number of foci and number of blocks involved). Where both CS and BB were positive, further surgery was always recommended. Further surgery was also recommended if the BB was negative but several foci of residual disease were found in the CS. Mastectomy, as opposed to wider local excision, was more likely to be offered in cases where there was evidence of residual

disease involving several blocks, especially when extensive residual in-situ disease was present. Patient choice and cosmetic factors were also taken into account. For instance, mastectomy might be preferred when further wide excision would unacceptably compromise cosmesis in patients with small breasts.

Adjuvant treatment

In general, patients who were premenopausal and node-positive were offered chemotherapy (cyclophosphamide, methotrexate and 5-flourouracil) and patients who were postmenopausal and oestrogen receptor-positive were offered tamoxifen. Intermediate group patients were entered into national clinical trials. In addition all patients were offered postoperative radiotherapy. This consisted of a course of 46Gy in 23 fractions over 35 days using paired glancing megavoltage fields with a boost to the site of local excision of 12 Gy in 4 fractions over 7 days using 6MEV electrons.

Stastistical analysis

Chi squared tests with Bonferoni correction were used to analyse associations of tumour bed positivity with clinical and pathological factors where data formed a

contingency table. The Mann-Whitney test was used to analyse association of tumour bed positivity with patient age, and lumpectomy specimen diameter with tumour diameter where the comparison of two means was required.

RESULTS

Disease was found in the cavity shavings of 118 patients (39.3%) and in the bed biopsies of 17 patients (10.3%). These results and the type of disease found are shown in Table 5. The extent of involvement with disease in both the cavity shavings and bed biopsies varied widely. Seventy five (63.6%) patients with positive cavity shavings and 10 (58.8%) patients with positive bed biopsies had only 1 to 3 foci of disease within all the blocks examined. The remaining patients had four or more foci. Positivity ranged from one focus of ductal carcinoma in situ involving one block to extensive invasive disease involving several blocks.

Table 5. Incidence of disease in Cavity Shavings and Bed Biopsies.

	CAVITY SHAVINGS	BED BIOPSIES
Total	300	165
• Invasive only	28 *(9.3)*	1 *(0.6)*
• Invasive + In-situ	27 *(9.0)*	1 *(0.6)*
• In-situ only	63 *(21.0)*	15 *(9.1)*
TOTAL POSITIVE	118 *(39.3)*	17 *(10.3)*
TOTAL NEGATIVE	182 *(60.7)*	148 *(89.7)*

Values in parentheses are percentages.

No patient with negative cavity shavings had a positive bed biopsy.

Effect of tumour and lumpectomy diameters

All tumours were 25mm or less in diameter as measured pathologically. The median tumour diameter was 13mm and the mean lumpectomy specimen diameter was 59mm. To determine whether cavity shaving or bed biopsy positivity was related to tumour size, the patients were divided into two groups according to tumour diameter. One hundred and forty two patients had tumours less than 10mm in diameter, 158 had tumours measuring 10-25mm in diameter. Cavity shaving positivity and bed biopsy positivity in relation to these size groups is shown in Table 6. No statistically significant association was observed between tumour diameter and cavity shaving or bed biopsy positivity ($p=0.453$ and $p=0.083$ respectively) although a slight trend towards increasing tumour bed positivity with increasing tumour diameter was observed.

Of the patients with positive cavity shavings, 4 or more foci of residual disease were found in 16 of 53 tumours smaller than 10mm (30.2%) and 27 of 65 tumours between 10 and 25 mm (41.5%).

Table 6. Effect of tumour diameter on tumour bed positivity.

TUMOUR DIAMETER (mm)	Positive Cavity Shavings	Positive Bed Biopsies
0-9	53 *(37.3)*	5 *(7.6)*
10-25	65 *(41.1)*	12 *(14.3)*

Values in parentheses are percentages.

The mean diameters of the lumpectomy specimens were correlated with the median tumour diameters in the two groups of patients and then sub-divided into whether or not the cavity shavings were positive or negative. There was no statistically significant correlation between the mean lumpectomy diameter and tumour bed positivity (Table 7, p=0.283). However there was a trend for cavity shaving and bed biopsy positivity to be associated with a smaller lumpectomy diameter for tumours less than 10mm in diameter.

Table 7. Relationship between tumour bed positivity and diameter of lumpectomy specimen.

TUMOUR DIAMETER (mm)	MEAN LUMPECTOMY SPECIMEN DIAMETER (mm)			
	Cavity Shavings		Bed Biopsies	
	Positive	Negative	Positive	Negative
0 - 9	53	58	43	57
10 - 25	61	63	63	63

Association with screen-detected and impalpable tumours

There was no statistically significant difference in cavity shaving positivity between symptomatic and screen-detected tumours or screen-detected palpable and impalpable tumours. However, tumour-bed positivity was less frequent in patients with impalpable screen-detected tumours (Table 8).

Table 8. Incidence of positive Cavity Shavings among patients with symptomatic and screen-detected tumours.

	Total	POSITIVE CAVITY SHAVINGS
Symptomatic	174	75 *(43.1)*
Screen-detected		
• Palpable	69	25 *(36.2)*
• Impalpable	57	18 *(31.6)*

Values in parentheses are percentages.

Effect of patient age

The relationship between cavity shaving positivity and patient age is shown in Tables 9 and 10. The mean age of patients with positive cavity shavings or bed biopsies was lower than those with negative cavity shavings or bed biopsies (non-significant p=0.08, Table 9).

When patients were divided into age groups the greatest incidence of tumour bed positivity was found in the 35 to 49 years group and the smallest incidence in the over 65 group (Table 10). To assess whether the extent

of surgery was influenced by age the mean lumpectomy diameters were compared for different age groups. The mean lumpectomy diameter tended to be larger in older patients (Table 10).

Table 9. Relationship between patient age and tumour bed positivity.

	MEAN AGE (years)
Positive Cavity Shavings	53.9
Negative Cavity Shavings	56.2
Positive Bed Biopsies	51.2
Negative Bed Biopsies	55.2

Table 10. Relationship between patient age groups and cavity shaving positivity and lumpectomy diameter.

	AGE (years)			
	27-34	**35-49**	**50-64**	**Over 65**
Positive Cavity Shavings	4 of 11 *(36.4)*	31 of 59 *(52.5)*	70 of 188 *(37.2)*	13 of 42 *(31.0)*
Positive Bed Biopsies	0 of 6 *(0)*	8 of 34 *(23.5)*	8 of 104 *(7.7)*	1 of 19 *(5.3)*
Median Tumour Diameter (mm)	15	15	10	15
Mean Lumpectomy Diameter (mm)	56	57	59	64

Values in parentheses are percentages.

Effect of tumour grade, lymphatic/vascular invasion and in-situ component.

Cavity shaving positivity was significantly associated with tumour grade (p=0.023, Table 11). The highest incidence of cavity shaving positivity was found with Grade II tumours. There were 42 tumours that were non-ductal and hence ungraded. Of these, 19 were classified as lobular. The incidence of cavity shaving positivity

for lobular carcinomas was 47.4%. Cavity shaving positivity was not significantly related to the presence of lymphatic or vascular invasion within the tumour (Table 11).

Table 11. Relationship between Cavity Shaving positivity and tumour grade and lymphatic/vascular invasion.

		Total	POSITIVE CAVITY SHAVINGS
Tumour Grade	**1**	72	19 *(26.4)*
	2	145	69 *(47.6)*
	3	41	15 *(36.6)*
	Ungraded	42	15 *(35.7)*
Lymphatic/Vascular Invasion	**Present**	52	22 *(42.3)*
	Absent	248	96 *(38.7)*

Values in parentheses are percentages.

To analyse the association between in-situ component and tumour bed positivity, tumours with a moderate or

extensive in-situ component were grouped as EIC and compared with those with minimal or no in-situ component (grouped as no EIC). Fifty-two patients (17.3%) had tumours with EIC. Both cavity shaving and bed biopsy positivity were significantly related to the presence of EIC ($p<0.001$, Table 12). Further analysis showed that EIC was associated with in-situ disease in the tumour bed.

The presence of an in-situ component varied with age. For patients with no EIC the mean age was 56.0 years whereas the mean age of patients with EIC was 51.8 years ($p=0.21$).

Association with axillary lymph node metastases

A total of 253 patients had a level two axillary dissection performed of whom 68 (26.9%) were node positive and 185 (73.1%) were node negative. No significant association was found between tumour bed positivity and axillary nodal status ($p=0.827$) or number of nodes involved (table 13).

Table 12. Relationship between tumour bed positivity and In-situ component of the tumour.

	Total	MODERATE/EXTENSIVE IN-SITU COMPONENT
CAVITY SHAVINGS		
• **Invasive only**	28	2 *(7.1)*
• **Invasive + In-situ**	27	12 *(44.4)*
• **In-situ only**	63	18 *(28.6)*
Total Positive	118	32 *(27.1)*
Total Negative	182	20 *(11.0)*
BED BIOPSIES		
Positive	17	7 *(41.2)*
Negative	148	16 *(10.8)*

Values in parentheses are percentages.

Table 13. Relationship between tumour bed positivity and axillary lymph node status.

	Total	LYMPH NODE POSITIVE		
		Overall	1-3 nodes positive	4 or more nodes positive
CAVITY SHAVINGS				
• **Invasive only**	24	10 *(41.7)*	8 *(33.3)*	2 *(8.3)*
• **Invasive + In-situ**	25	7 *(28.0)*	6 *(24.0)*	1 *(4.0)*
• **In-situ only**	51	12 *(23.5)*	11 *(21.6)*	1 *(2.0)*
Total Positive	100	29 *(29.0)*	25 *(25.0)*	4 *(4.0)*
Total Negative	153	39 *(25.5)*	33 *(21.6)*	6 *(3.9)*
BED BIOPSIES				
Positive	14	3 *(21.4)*		
Negative	133	33 *(24.8)*		

Values in parentheses are percentages.

Inter-surgeon variability and tumour bed positivity

Three surgeons with specialist breast interests (consultant, a staff grade and a senior registrar) performed 79% of the operations. The rate of cavity shaving positivity for these surgeons varied from 38.2 to 45.8% (p=0.67). The remainder of the operations were performed by 2 senior registrars and 2 registrars and the rate of cavity shaving positivity among these surgeons varied from 25 to 38.9% (p=0.61). The variation in tumour and lumpectomy diameter between different surgeons is shown in table 14.

Table 14. Inter-surgeon variability

	Median Tumour Diameter	Mean Lumpectomy Diameter	Tumour Bed Positivity
Surgeon 1	13	57	61/157 *(38.9%)*
Surgeon 2	13	57	22/48 *(45.8%)*
Surgeon 3	12	61	13/34 *(38.2%)*
Others	13	64	22/61 *(36.1%)*

Further surgery

Further surgery was performed on 41 patients because of extensive disease involving cavity shavings, bed biopsies or both. Eight had further wide local excision and 33 patients underwent mastectomy. Further invasive cancer was found in 12 patients and further in-situ disease in 10 patients. Altogether 53.7% of patients who underwent further surgery had evidence of further residual disease.

Further residual disease was more likely to be found if an extensive in-situ component was present in the tumour (66.7%), 4 or more foci were present in the cavity shavings (58.3%) and if both the cavity shavings and bed biopsies were positive (72.7%).

DISCUSSION

This study has demonstrated a 39.3% incidence of tumour bed positivity after conventional breast-conserving surgery for early breast cancer. In 21% of patients residual disease was purely in-situ and in 18.3% there was an invasive component. No significant association between tumour bed positivity and tumour diameter was found. These findings are consistent with those of the pathological study by Holland et al in which residual disease was found beyond 2cm from the primary tumour in 43% of mastectomy specimens. In 27% of specimens this disease was in-situ and in 16% there was an invasive component[57]. Holland et al also found that residual disease was independent of tumour size[57].

The extent of disease within positive cavity shavings was not significantly related to tumour size although 41.5% of 10-25mm tumours with positive cavity shavings had 4 or more foci of residual disease compared to 30% of tumours less than 10mm. Since the spatial orientation of the tumour within the lumpectomy specimen is not known no comment can be made on the incidence of residual disease at precise distances from the tumour edge. However, to assess whether tumour bed positivity was related to lumpectomy size the mean lumpectomy diameter was correlated with tumour bed

positivity for varying tumour size groups. No significant association was found suggesting that a standard surgical procedure was carried out on all patients although there was a trend for tumour bed positivity to be associated with a slightly smaller mean lumpectomy diameter.

The incidence of tumour bed positivity among screen-detected patients was 34.1%. This is an important group of patients who might be expected to have a 30% survival advantage over the non-screened population[108]. They are likely to be node-negative and adequacy of local treatment may be particularly significant for these patients.

Tumour bed positivity was significantly associated with both tumour grade and the presence of a moderate or extensive in-situ component. High tumour grade and an extensive in-situ component (EIC) have generally been found to predict for an increased risk of local recurrence. EIC was originally defined as present if greater than 25% of the tumour consisted of in-situ carcinoma and in-situ disease was present around the tumour. Different definitions of EIC have been used by other investigators. The reported incidence of EIC varies from 6.7% in the Milan trials to 30% in the series of mastectomy specimens studies by Holland et al[47,79]. In this study EIC was defined as absent,

minimal, moderate or extensive. Patients with a moderate in-situ component were grouped with those who had an extensive component (collectively termed EIC) due to the small numbers of patients in the latter category. In all, 17.3% of patients had EIC. It was strongly associated with residual in-situ disease in the tumour bed but did not correlate with residual invasive disease. In the study by Holland et al tumours with EIC were associated with a 59% incidence of disease at a distance greater than 2cm from the tumour edge compared to 29% of those without EIC[79]. As with the present study, this significant difference was attributable to a strong association between EIC and diffuse in-situ disease.

The mean age of patients did not vary significantly with tumour bed positivity. However, the mean age of patients who had a positive tumour bed tended to be younger than those with a negative tumour bed. The mean lumpectomy diameter also tended to be smaller for younger patients possibly due to the greater concern regarding cosmesis in this group. A study analysing tumour bed positivity by documenting disease in re-excision specimens performed for involved or unknown margins found no association between age and residual disease[72]. Other studies have correlated very young age with a high incidence of local recurrence[47]. This has been attributed to the association between young age

and EIC[96]. In this study the mean age for patients with EIC was 4 years less than that of patients with no EIC (not statistically significant).

Previous investigators have suggested that local recurrence is related to axillary lymph node positivity and that inoperable local recurrences tend to occur in patients who are node-positive[86,109]. In this study no significant relationship was found between tumour bed positivity and lymph node status.

The decision to offer further surgery was pragmatic and was influenced by many variables. Analysis of the predictors for further residual disease is therefore limited by this fact. However, an extensive in-situ component, 4 or more foci in the cavity shavings and bed biopsy positivity were all associated with an increased incidence of further residual disease.

In summary, shaving the tumour bed is a practical method to assess completeness of surgical excision. Any disease found in the tumour bed is undoubtedly residual disease. The cavity shaving specimen is easily processed and embedded blocks and sections do not require orientation relative to a cut surface. This study has shown that the incidence of residual disease after conventional breast-conserving for invasive breast cancer is 39.3%. This incidence closely approximates that predicted by studies simulating

lumpectomy in mastectomy specimens. Statistically significant risk factors for tumour bed positivity found in this study are high tumour grade and a moderate or extensive intraduct component. However an increased incidence of tumour bed positivity was also found with young age, lobular carcinoma and small lumpectomy diameter.

CHAPTER 2

INTRODUCTION

Mammography is a mandatory pre-operative investigation in all patients undergoing surgery for breast cancer. For patients considered suitable for breast-conserving surgery mammography has an established role to detect clinically occult multifocal disease[110,111]. Such patients are more likely to require mastectomy. A few studies have assessed mammography as a method of predicting risk for local recurrence[87,112-113]. This is possible if mammographic features are shown to correlate with pathological risk factors for local recurrence. As discussed previously the main pathological risk factors for local recurrence are residual disease at the resection margin, an extensive intraduct component, lymphatic or vascular invasion and high tumour grade. Pre-operative identification of risk factors for local recurrence may influence the extent of primary excision and limit the need for further surgery.

Previous studies have concentrated on the value of post-operative mammography to detect residual disease at the resection margin[112-114]. However, the interpretation of post-operative mammography is difficult and inaccurate due to distortion created by the surgical scar[110,113]. Other investigators have established that certain mammographic features such as mammographic calcifications and absence of mammographic nidus predict for the presence of an extensive intraduct component in the tumour[87,115].

The study presented in this chapter was designed to answer the following questions:

- Can pre-operative mammography predict patients more likely to have microscopic disease in the tumour bed after breast-conserving surgery?
- Can pre-operative mammography identify patients with pathological risk factors for local recurrence?

PATIENTS AND METHODS

Patients

Of the 300 patients with pathological analysis of tumour bed presented in Chapter One, 231 had pre-operative mammograms available for review. The study population consisted therefore of 231 patients who presented between March 1988 and September 1992 with invasive breast cancer suitable for breast-conserving surgery. The mean age of these patients was 56 years (range 27-80). One hundred and twenty patients presented symptomatically while 111 presented through the National Breast Screening Programme.

Mammogram interpretation

Bilateral craniocaudal and oblique mammograms were performed pre-operatively on each patient. The following features were recorded: Wolfe grade[116] (N1, P1, P2 or DY), nidus size (mm), nidus type (round, stellate, absent), tumour calcification (casting, non-casting or absent), maximum diameter of mammographic abnormality (nidus diameter plus diameter of surrounding distorted of breast architecture), nidus to nipple distance and nidus location (quadrant). All

mammograms were reported by a specialist breast radiologist without knowledge of pathological results.

Surgical management and pathological assessment

The surgical management and pathological assessment of specimens has been previously described (Chapter One).

Statistical analysis

The Chi squared test with Bonferroni correction was used to analyse possible associations between mammographic features and pathological factors where data formed a contingency table. The two sample T test was used to analyse possible associations between mammographic features and pathological factors where comparison of two means was required.

RESULTS

Correlation of mammographic features with tumour bed positivity

There was no evidence of residual disease in the cavity shavings for 138 patients. Microscopic foci of residual

invasive disease were found in 44 patients (19%) and in-situ disease in 49 patients (21.2%).

The presence of residual disease in the cavity shavings was correlated with pre-operative mammographic features. Disease in cavity shavings was significantly associated with DY compared with N1 mammographic pattern on Wolfe grade ($p=0.03$, Table 15), casting-type calcification compared with either non-casting or absence of calcification ($p=0.02$ and $p<0.001$ respectively, Table 16) and absence of mammographic nidus compared with either stellate or round nidus ($p=0.05$ and $p<0.001$ respectively, Table 17).

Table 15. Association between Wolfe Grade and cavity shaving positivity.

	Total	POSITIVE CAVITY SHAVINGS
Wolfe Grade		
• **N1** *(no visible ducts)*	33	8 *(24.2)*
• **P1** *(<25% ducts visible)*	50	16 *(32.0)*
• **P2** (>25% ducts visible)	78	34 *(43.6)*
• **DY** *(ducts obscured by density of breast)*	70	35 *(50.0)*

Values in parentheses are percentages

Table 16. Association between mammographic calcification and cavity shaving positivity.

	Total	POSITIVE CAVITY SHAVINGS
Tumour Calcification		
• **Casting**	43	29 *(67.4)*
• **Non-casting**	42	16 *(38.1)*
• **Absent**	146	48 *(32.9)*

Values in parentheses are percentages.

Table 17. Association between type of mammographic nidus and cavity shaving positivity.

	Total	POSITIVE CAVITY SHAVINGS
Type Of Nidus		
• **Round**	98	29 *(29.6)*
• **Stellate**	114	50 *(43.9)*
• **Absent**	19	14 *(73.7)*

Values in parentheses are percentages.

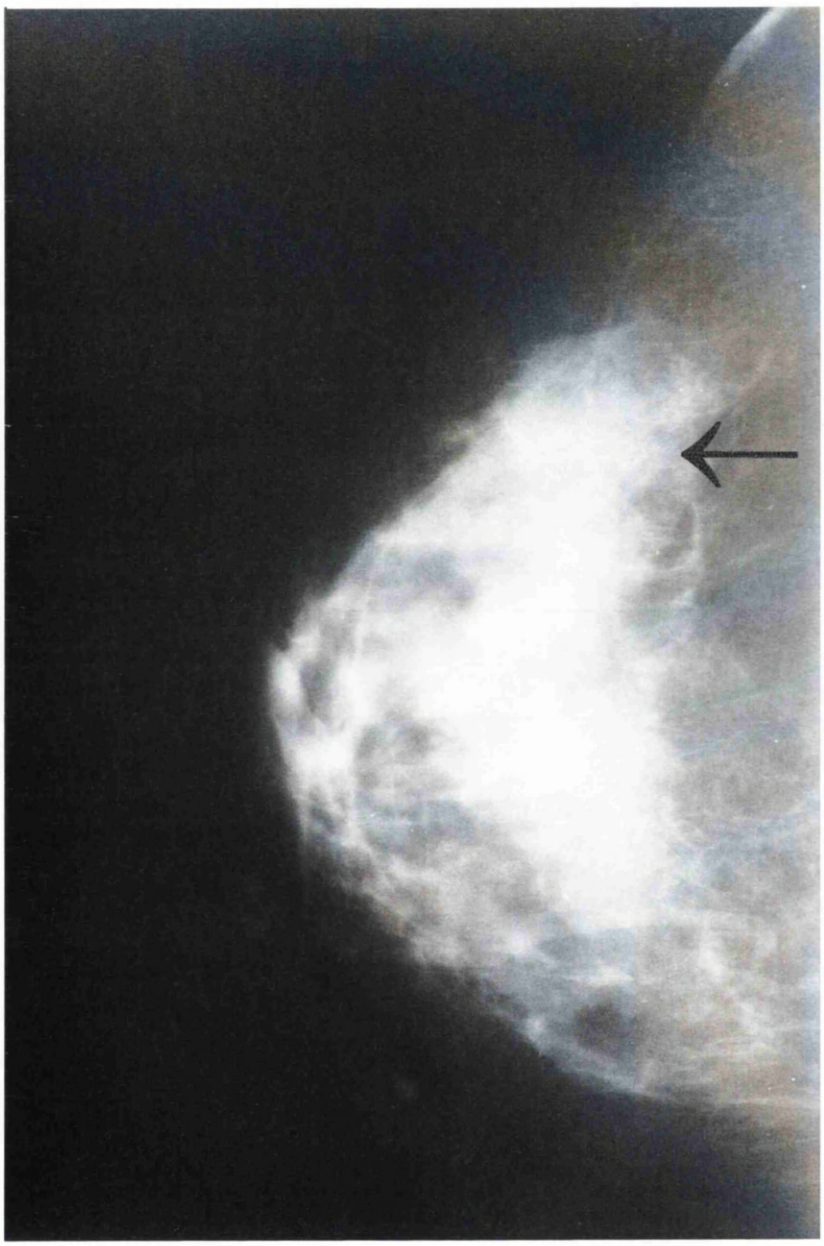
Disease in the cavity shavings was found in 75-88.9% of patients when DY pattern, casting calcification or absent mammographic nidus were identified in combination on the pre-operative mammogram (Table 18 and Illustration 3). In patients with N1 mammographic pattern, absence of calcification and round mammographic nidus, disease in the cavity shavings was found in 13.3% of cases (Illustration 4).

Table 18. Combinations of mammographic features predicting cavity shaving positivity.

	Total	POSITIVE CAVITY SHAVINGS
COMBINATION		
• **Casting calcification + absent nidus**	12	9 *(75.0)*
• **DY pattern + casting calcification**	12	10 *(83.3)*
• **DY pattern + absent nidus**	9	8 *(88.9)*

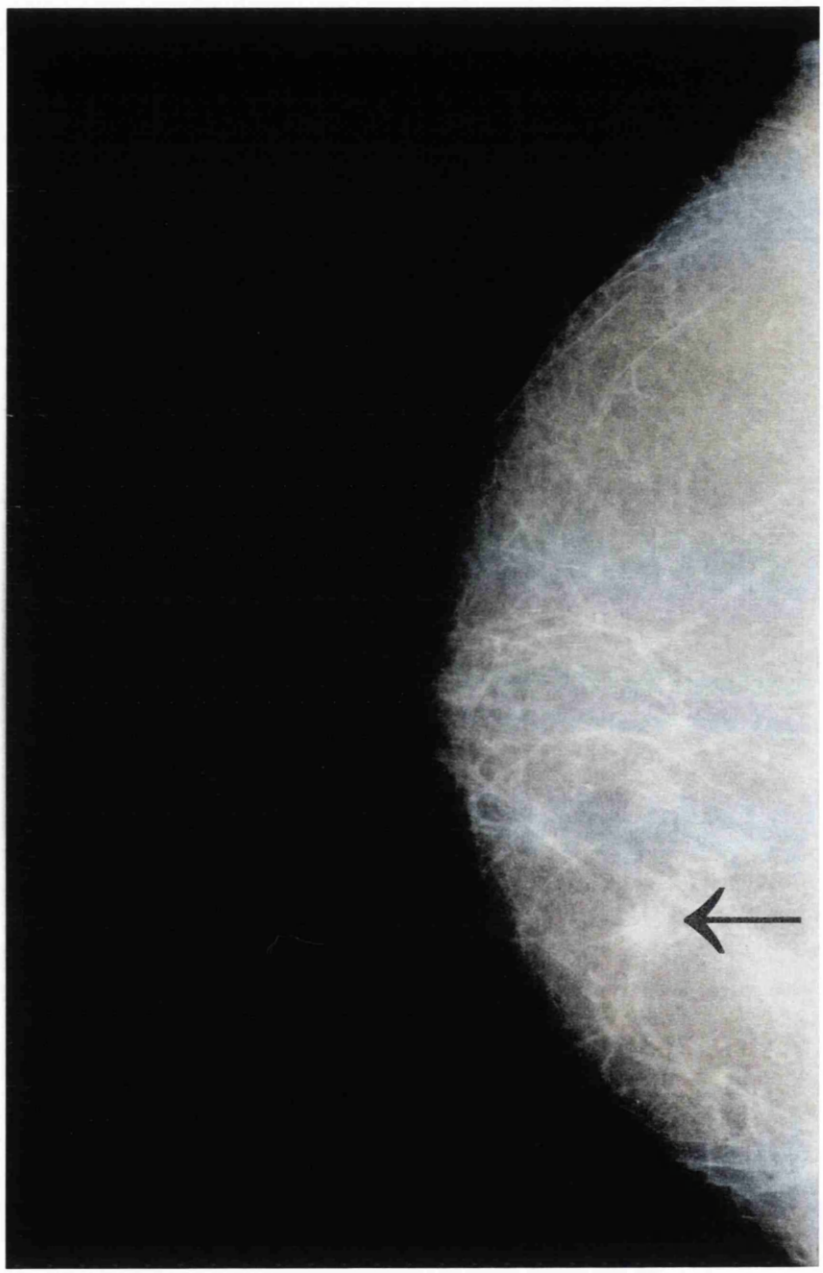
Values in parentheses are percentages.

Illustration 3. Mammographic features predicting cavity shaving positivity.



DY Wolfe pattern, casting - type calcification, no mammographic nidus

Illustration 4. Mammographic features predicting cavity shaving negativity.



NI Wolfe pattern, no calcification, round mammographic nidus

There were no significant associations between disease in the cavity shavings and nidus size, maximum diameter of mammographic abnormality, nidus to nipple distance (Table 19).

Table 19. Association between cavity shaving positivity and nidus size, maximum diameter of mammographic abnormality and nidus to nipple distance.

	NEGATIVE CAVITY SHAVINGS	POSITIVE CAVITY SHAVINGS
• **Nidus size (mean in mm)**	16	16
• **Maximum diameter mammographic abnormality (mean in mm)**	39	37
• **Nidus to nipple distance on craniocaudal film (mean in mm)**	79	77
• **Nidus to nipple distance on oblique film (mean in mm)**	80	77

Correlation of mammographic features with intraduct component of the tumour.

As in Chapter One, tumours with a moderate or extensive intraduct component were compared with those with minimal or no intraduct component. The presence of a moderate or extensive intraduct component was significantly associated with both casting and non-casting mammographic calcification compared with absence of mammographic calcification ($p<0.001$ and $p=0.04$ respectively, Table 20). A significant relationship was also found between the absence of mammographic nidus and a stellate nidus compared with a round nidus ($p<0.001$ and $p=0.02$ respectively, Table 21).

A moderate or extensive intraduct component was found in 32.6% or 70.6% of patients when tumour calcification and stellate mammographic nidus or absence of mammographic nidus were identified together on the pre-operative mammogram (Table 22 and Illustration 4). In patients with absence of calcification and a round mammographic nidus it was found in 8.2% of cases (Illustration 5). There were no significant associations between the degree of intraduct component and Wolfe grade, nidus size, maximum diameter of mammographic abnormality, nidus to nipple distance or nidus location.

Table 20. Association between mammographic tumour calcification and the in-situ component of the tumour.

	Total	MODERATE/EXTENSIVE IN-SITU COMPONENT
Tumour Calcification		
• **Casting**	43	19 *(44.2)*
• **Non-Casting**	42	12 *(28.6)*
• **Absent**	146	20 *(13.7)*

Values in parentheses are percentages.

Table 21. Association between type of mammographic nidus and in-situ component of the tumour.

	Total	MODERATE/EXTENSIVE IN-SITU COMPONENT
Type Of Nidus		
• **Round**	100	11 *(11.0)*
• **Stellate**	114	28 *(24.6)*
• **Absent**	17	12 *(63.2)*

Values in parentheses are percentages.

Table 22. Combinations of mammographic features predicting for in-situ component in the tumour.

	Total	MODERATE / EXTENSIVE IN-SITU COMPONENT
COMBINATION		
• **Tumour Calcification + Stellate Nidus**	43	14 *(32.6)*
• **Tumour Calcification + Absent Nidus**	17	12 *(70.6)*

Values in parentheses are percentages.

Other associations

There were no significant associations between mammographic features and lymphatic/vascular invasion, tumour grade or tumour type (ductal versus lobular).

Wolfe grade was significantly related to age ($p<0.001$) such that a dense mammographic pattern was more common in younger patients (Table 23). The mean maximum diameter of the lumpectomy specimen also varied significantly with Wolfe grade from 64mm in N1 patients

to 54mm in DY patients (N1 versus DY p=0.039, Table 23), whereas the mean tumour diameter did not vary significantly.

Table 23. Association of age, mean lumpectomy diameter and tumour diameter with Wolfe grade.

	WOLFE GRADE			
	N1	**P1**	**P2**	**DY**
Mean Age (years)	60.8	58.0	56.3	51.8
Mean Lumpectomy Diameter (mm)	64	60	57	54
Mean Tumour Diameter (mm)	12	12	12	13

DISCUSSION

This study used pre-operative mammograms to identify mammographic features that correlate with residual disease and other pathological risk factors for local recurrence. It has demonstrated that DY mammographic pattern, casting calcification and absence of mammographic nidus are associated with a significantly increased risk of residual disease (50-73.7%) and a combination of these features predicts residual disease in 75-88.9% of cases. Conversely, patients with N1 mammographic pattern, absence of calcification and round mammographic nidus are at low risk of residual disease (24.2-32.9%) and when all these features are present residual disease is found in 13.3% of cases.

Previous reports correlating residual disease with mammographic features have been small studies relating the appearances of post-operative mammograms to disease found in re-excision specimens[112-114]. In a study of 43 patients, Gluck et al demonstrated that mammographic calcifications detected on post-operative films were associated with residual disease in 69% of re-excision specimens compared with 30.8% with no remaining mammographic calcifications[112]. Other studies have shown however, that the appearances of up to 50% of post-operative mammograms may be inaccurately

interpreted due to biopsy-related distortion or oedema, rendering post-operative mammography an insensitive method of detecting residual disease[110,114].

This study has demonstrated that despite similar tumour diameters, patients with DY pattern (Wolfe grade) had a significantly smaller amount of breast tissue excised than patients with N1 pattern. The Wolfe grading system was originally described as a means to categorise the parenchymal patterns of the breast as they appear mammographically[116]. An N1 pattern exists when no ducts are visible, a P1 pattern is present when less than 25% of the breast is composed of "prominent ducts", a P2 pattern has more than 25% "prominent ducts" and a DY pattern is defined by sheet-like areas of increased density obscuring the underlying duct pattern. Subsequent studies have shown that these patterns are related to the distribution of fibrous and adipose tissue in the breast[117]. Breasts with a N1 pattern are composed primarily of fat and those with a DY pattern are dense and fibrous. It has been previously reported that agreement on the classification of mammographic pattern between radiologists is generally very good. Hence in the present study all mammograms were reported by one consultant breast radiologist[118-120]. The association between Wolfe grade and tumour bed positivity suggests that there may be a tendency to excise a relatively smaller margin of macroscopically

normal breast tissue in patients with dense fibrous breasts in whom the tumour edge may not be easily palpable. This may partly explain the increased risk of local recurrence in younger patients observed in some studies[96].

Tumours with a large intraduct component, lymphatic or vascular invasion and high tumour grade also predict for local recurrence. Previous investigators have shown that the extent of the intraduct component correlates with mammographic calcification and absence of mammographic nidus[87,115]. Healey et al reported that 73% of patients with mammographic calcifications and absent mammographic nidus had tumours with an extensive intraduct component (EIC). Only 8% of patients had tumours with EIC if a mammographic nidus was present without calcifications[87]. The present study has shown that mammographic tumour calcification without a mammographic nidus is associated with a 70.6% incidence of tumours containing a moderate or extensive intraduct component. Only 8.2% of tumours had a large intraduct component if a round mammographic nidus was present without calcification. This study was unable to demonstrate any association between mammographic features and tumour grade or the presence of lymphatic/vascular invasion.

In summary, certain features identified on pre-operative mammograms may predict patients who are likely to have residual disease after breast-conserving surgery and have tumours with a large intraduct component. Combinations of these features can identify small numbers of patients at particularly high or low risk of local recurrence. In high risk patients a wider primary excision may be indicated or alternatively, in some cases a patient may be deemed unsuitable for breast-conserving surgery.

CHAPTER 3

INTRODUCTION

Many biological factors associated with breast cancer have been shown to correlate with outcome after surgery. Three of the most established factors of prognostic interest are c-erbB-2, p53 and the oestrogen receptor.

C-erbB-2

C-erbB-2 is an oncogene located at 17q21.2-12 that codes for a 185 kDa transmembrane protein growth factor receptor (p185). P185 is a member of the type 1 family of growth factor receptors, which also includes c-erbB-3 and c-erbB-4. C-erbB-2 is similar to, but distinct from c-erbB-1, an oncogene located on chromosome 7 that codes for the epidermal growth factor receptor[121]. The p185 protein is a normal constituent of cytoplasmic membranes and has some intracellular tyrosine kinase activity. The natural ligands for this receptor are under investigation. The neu differentiation factor

appears to play an important role and p185 and EGFR may co-operate as heterodimers upon ligand binding[122]. It has been shown that EGF can indirectly activate p185 via activation of EGFR, which mediates tyrosine phosphorylation of p185[123]. It is possible that this interaction between EGF and p185 is limited to a few cells that express the two receptors simultaneously[124]. Experimental evidence suggests that p185 may have an important role in the pathogenesis of breast cancer and overexpression of c-erbB-2 alone is sufficient to support mammary carcinoma in transgenic mice[125].

Amplification and over-expression of the c-erbB-2 oncogene detected by Southern and Northern blotting have been shown to correlate with c-erbB-2 oncoprotein expression evaluated by Western blotting and immunohistochemistry suggesting that immunohistochemistry is a valid method for evaluating c-erbB-2 in archive breast cancer specimens[126].

Moderate to low levels of p185 are found in normal breast although activation of c-erbB-2 has not been found in benign breast lesions. The physiological action of c-erbB-2 in normal breast is unknown although it is believed to be related to the differentiation of the breast[124]. Amplification of the gene with overexpression of p185 is found in approximately 20% of breast cancers. It varies with histological subtype

such that amplification is found in approximately 22% of ductal carcinomas, 7% of lobular carcinomas, 7% of tubular carcinomas, 62% of inflammatory carcinomas and 22% of medullary carcinomas[127].

Over-expression of p185 is found in the great majority (68-90%) of patients with comedo-type DCIS, whilst overexpression is extremely uncommon in other types of DCIS[128]. A significant association with tumours containing an intraduct component has also been found. One study found that 13/21 (62%) of patients staining positively for c-erbB-2 had an intraduct component compared to 31/86 (36%) of those staining negatively[129]. In a larger study, 51/97 (52.6%) of patients who stained for c-erbB-2 had a significant intraduct component compared to 237/412 (33.7%) of those who stained negatively[130]. In the latter study there was concordance of overexpression between the invasive and in-situ components of the tumour in all but two cases, where only the in-situ component stained positively. The increased expression of c-erbB-2 in in-situ breast cancer partly supports the hypothesis that activation of c-erbB-2 is an early event in the development of breast cancer.

An association between poor nuclear or histological grade and overexpression of c-erbB-2 has been found[131-135]. There are also conflicting reports associating

overexpression of c-erbB-2 with young age, oestrogen receptor negative and progestogen receptor negative tumours, high proliferative index and large tumour size[127]. Over-expression is associated with a poor response to endocrine and conventional cytotoxic drug therapy[136-138].

C-erbB-2 was first shown to have clinical significance in 1987 by Slamon et al who reported that amplification was associated with decreased survival in node-positive patients[139]. A large number of groups have now published data concerning the prognostic significance of c-erbB-2[140]. Of these, very few have failed to find any prognostic effect. A minority have found that c-erbB-2 has a prognostic effect in both recurrence-free and survival analyses which is maintained in multivariate analysis[131,132,141]. However, most studies have found that the prognostic effect of c-erbB-2 is stronger for survival than it is for recurrence[128,131,132,136] and some studies have shown an effect on survival but no effect on recurrence[140]. Most studies have shown that the prognostic value of c-erbB-2 is greater for node-positive patients but this may reflect the relatively large number of patients required to show a prognostic effect in node negative groups[132]. Within a large study comparing breast-conserving surgery with mastectomy Sauer et al 1992 found c-erbB-2 to predict for distant disease-free

survival in node-positive and node-negative patients[142].

Two studies have related c-erbB-2 over-expression to local recurrence following surgery for breast cancer. One study found that 1/6 (17%) of c-erbB-2 positive tumours developed chest wall recurrence compared to 6/15 (40%) of c-erbB-2 negative tumours[136]. Another study found that 2/21 (10%) of c-erbB-2 positive patients developed local recurrence after conservation surgery compared to 16/84 (19%) of c-erbB-2 negative patients[129]. In the latter study, local failure rates following mastectomy were equal for c-erbB-2 positive and c-erbB-2 negative patients (both 13%).

p53

The p53 gene was discovered in 1979[143]. It is located on the short arm of chromosome 17 at position 17p13.1 and encodes for a 393 amino acid, 53 kda nuclear phosphoprotein (p53). This protein is present at very low levels in normal tissues that cannot usually be detected by conventional immunohistochemical methods. The normal (wild type) p53 gene inhibits cellular transformation by oncogenes, acting as a tumour suppressor gene, although the mechanism by which it

performs this protective role is not fully understood. The p53 protein appears to act by monitoring the genome and minimising the mutations that arise from exposure to DNA-damaging agents. It may bind to cellular or viral proteins and promote cytoplasmic sequestration[144]. Increased levels of p53 protein are found in tissue exposed to various DNA damaging agents such as UV and gamma radiation. It may block the division of cells that have sustained DNA damage and in some cases trigger cell death by apoptosis[145-147].

Failure of p53 to exert its tumour suppressive function may occur if there is loss of heterozygosity (loss of 1 allele) or homozygous deletion (loss of both alleles) of the p53 gene. This is the most common genetic change detected in a wide range of human cancers. In breast cancer loss of heterozygosity occurs in 42 - 61% of tumours but homozygous deletion is very rare. In addition, mutant forms of p53 are commonly expressed in tumour cells (occurring in 15 - 46% of breast cancers) and some of these have oncogenic properties with the ability to immortalise cells in culture[145-147]. Germ line mutation of p53 is a rare event in patients with sporadic or familial breast cancer except in the Li-Fraumeni syndrome in which inherited p53 mutation leads to a variety of tumours including breast cancer[148].

The wild type p53 protein has a very short half life of approximately 20 minutes. It is thought that point mutations in the p53 gene cause accumulation of the p53 protein by increasing its stability (and hence its half life), and in most cancers p53 mutation is accompanied by increased amounts of p53 protein which is detectable using immunohistochemical staining. Immunostaining of p53 protein in tumours is usually confined to the nucleus. A wide variation in the percentage of breast cancers staining positive for p53 protein has been reported although most large studies have shown definite staining in approximately 20% of tumours[149].

In breast cancer it appears that a second region on 17p13, distinct from p53 may exert an influence over p53 expression. This may partially explain the discrepancy that exists between the loss of heterozygosity, the rate of p53 mutation and the frequency with which p53 protein is detected[142-147]. Increased expression of wild type p53 protein can also occur as a consequence of p53 mutation and excessive amounts of wild type as well as mutant protein may be detected immunohistochemically. In addition, the method of fixation can influence the stability of p53 protein and tumours often show marked heterogeneity of staining for p53 protein, which may cause sampling error[150].

Mutations that result in deletion or truncation of the protein (nonsense and frameshift mutations) do not cause protein accumulation. In addition, immunohistochemical positivity can occur in the absence of mutation. One mechanism for this is the stabilisation of non-mutant p53 protein by cellular oncoproteins such as MDM-2 (mouse double minute 2) [151]. p53 is known to regulate MDM-2 gene transcription and it is believed that interaction between MDM-2 and p53 is necessary to maintain controlled cell growth[152].

In general, however, there is good concordance between immunohistochemical positivity and mutation in conserved regions of the p53 gene. Molecular analysis can be employed to detect gene mutations using rapid PCR-based mutation screening procedures such as single-strand conformation polymorphism. Alternatively DNA sequencing of the entire coding region can be performed. Sequencing is probably the most sensitive method but its use is limited by its expense and need for relatively pure tissue samples.

In breast cancer patients associations have been found between p53 mutations or overexpression of p53 protein and ER negative and PR negative tumours, EGFR positive tumours, high tumour grade, increased proliferation index, metastatic disease, a poor response to chemotherapy and decreased disease-free and overall

survival[153-161]. Studies have shown no association between overexpression of p53 and overexpression of c-erbB-2[142,162].

p53 protein overexpression also appears to be associated with comedo-type DCIS[163,164]. In one study p53 was overexpressed in 34/95 cases of comedo-type DCIS compared to 2/48 cases of non-comedo DCIS[163]. This has led to the suggestion that p53 abnormalities may occur as an early event in breast carcinogenesis and may play a role in the malignant transformation of DCIS.

Oestrogen receptor

In 1896, Beatson observed regression of metastatic breast cancer in premenopausal women following oophorectomy[165]. Over fifty years later a similar beneficial response was reported in postmenopausal women after adrenal gland ablation[166]. However oestrogen receptors were not recognised until 1971 when Jensen et al correlated the response to adrenalectomy with the specific binding of oestradiol in tumour sections[167].

High affinity cytoplasmic oestrogen receptors (ER) bind circulating oestrogen. This hormone-activated receptor complex is translocated into the breast cancer cell nucleus where it promotes specific gene transcription. Oestrogen receptor positivity increases with age. Tumours with poor differentiation, high histological grade, high proliferative index and extensive necrosis are more likely to be ER negative. ER positive tumours are more likely to be well differentiated with low histological grade and low proliferative index[168]. No consistent association between ER positivity and tumour size, vascular or lymphatic invasion, intraduct component or axillary lymph node status has been found.

Many studies have shown a correlation between ER status and distant disease-free and overall survival with ER negative tumours associated with poor outcome[169]. This correlation does not appear to be dependent on adjuvant hormonal therapy. It has also been suggested that ER positivity loses its prognostic power with long follow-up and that it is related to the growth rate of the tumour rather than its metastatic potential[170].

ER status did not affect the incidence of local recurrence in the NSABP B-06 or the NCI trials[37,49]. Other studies have found a small but non-significant association between ER negative tumours and local recurrence[77,94].

C-erbB-2, p53 and ER are therefore, established prognostic factors in breast cancer. Both c-erbB-2 and p53 are strongly associated with comedo DCIS. This is known to predict for margin involvement and local recurrence after breast conserving surgery. In addition all of these factors are associated with high tumour grade, which is again strongly associated with local recurrence. C-erbB-2, p53 and ER can all be assessed in needle biopsy specimens and their status could theoretically therefore be determined preoperatively. If an association was found between tumour bed status and these factors then they may be helpful in preoperative selection of patients for breast-conserving surgery.

This study presented in this chapter was designed to answer the following question:

- Is the incidence of residual disease related to known biological prognostic factors (ER, p53, c-erbB-2)?

PATIENTS AND METHODS

Patients

Of the 300 patients presented in chapter one, 253 had archival tissue fixed in paraffin available for immunohistochemical staining. Oestrogen receptor analysis was performed at the time of surgery for 275 patients.

Statistics

Associations between tumour bed positivity and oestrogen receptor, c-erbB-2 and p53 status were analysed for statistical significance using the chi-squared test with Bonferoni correction.

Antibodies

The c-erbB-2 antibody used was NCL-CB11 (Novocastra Laboratories Ltd, Newcastle upon Tyne) at 1:20 dilution. This monoclonal antibody has been shown to give intense membrane staining for c-erbB-2 protein in paraffin fixed tissue[171]. The p53 antibody used was DO-1 (Donated by David Lane, Biochemistry Department, University of Dundee) at 1:1000 dilution.

Oestrogen receptor assay

ER assays were determined by the dextran-charcoal assay on fresh tumour samples at the time of surgery[172]. ER levels greater than or equal to 20 fmol/mg protein were designated positive.

Immunohistochemical staining

5μm thick sections of tissue were cut and ovened at 60°C for 30 minutes. Sections were soaked in xylene for 10 minutes then washed in alcohol (3 changes), methylated spirits (2 changes) and rinsed in methanol. Sections were then soaked in a solution of methanol and 0.5% hydrogen peroxide (60ml:1ml) for 30 minutes to block endogenous peroxide activity. Slides were then laid out on trays and a blocker solution of 2% bovine serum albumin in phosphate buffer solution was applied and left for 10 minutes. The blocker was then tipped off and the primary antibody was applied to the sections in dilutions as detailed above. Sections were incubated at 4°C for 18 hours after which they were washed with phosphate buffer solution for 30 minutes. Secondary antibody was then applied in two layers for 45 minutes each. The secondary antibodies were biotin conjugated anti-mouse immunoglobulin and ExtrAvidin peroxidase (ExtrAvidin mouse kit, Sigma

immunochemicals, Poole, Dorset) applied at 1:25 dilution in 2% bovine serum albumin/phosphate buffer solution and pooled normal human serum (also at 1:25 dilution). Sections were washed with phosphate buffer solution for 30 minutes between and after the secondary antibody layers were applied. Visualisation was achieved using 3,3'-Diaminobenzidene tetrahydrochloride dihydrate D.A.B (Aldrich Chemicals, Gillingham, Dorset) as a working solution of 1ml 0.04% DAB, 225ml 0.05M Tris buffer (Sigma), 25ml 0.1M imidazole in 0.05M Tris buffer (Sigma) and 125µl hydrogen peroxide (BDH).

Two sections per patient were used, each taken from a different tissue block. For the purposes of this study and according to the criteria of Wright et al and other studies, only definite membrane staining was reported as c-erbB-2 positive[133,164,165]. Previous studies using the NCL-CB11 monoclonal antibody to c-erbB-2 protein have defined positive staining as > 25% of cells with membrane staining[136]. No difference in prognosis was found comparing tumours with no staining against those with weak staining. In this study therefore, c-erbB-2 staining intensity was scored as 0 for no staining or cytoplasmic staining or weak membrane staining (<25% of cells), 1 for >25% cells with membrane staining and 2 for >50% cells with membrane staining. In keeping with previous studies any cells showing nuclear staining were reported as positive for p53[157,161,164]. Staining

intensity was therefore scored as 0 for no staining, 1 for <25% cells with nuclear staining, 2 for >25% cells with nuclear staining and 3 for >50% cells with nuclear staining. The higher score for the 2 sections determined the overall score assigned to each patient. All tumours scoring 0 were termed negative.

RESULTS

The staining scores for 252 tumours stained for c-erbB-2 and p53 and 275 tumours stained for oestrogen receptor are shown in Table 24. All tumours with cells staining were scored as positive for c-erbB-2 and p53.

A total of 70 tumours were scored as positive for c-erbB-2 (27.7%), 59 were positive for p53 (23.4%) and 202 were positive for oestrogen receptor (73.5%).

Relationship between tumour bed positivity and c-erbB-2

No statistically significant relationship was found between tumour bed status and c-erbB-2 status (p=0.65, Table 25). Among patients who were tumour bed positive,

c-erbB-2 staining was more intense among those who had an in-situ component in the cavity shavings.

Table 24. Results staining for c-erbB-2, p53 and oestrogen receptor.

SCORE	C-ERBB-2	P53	OESTROGEN RECEPTOR	
0	182	193	Negative	73
1	62	46	Positive	202
2	8	11		
3		2		

Relationship between tumour bed positivity and p53

No statistically significant relationship was found between tumour bed status and p53 status (p=0.827, Table 26).

Relationship between tumour bed positivity and oestrogen receptor

No statistically significant relationship was found between tumour bed status and ER status (p=0.462, Table

27). A trend for tumour bed positivity to be associated with ER positivity was observed.

Table 25. Relationship between tumour bed positivity and c-erbB-2 positivity.

	Total	C-ERBB-2 POSITIVE
CAVITY SHAVINGS		
• **Invasive only**	24	3 *(12.5)*
• **Invasive + In-situ**	24	12 *(50.0)*
• **In-situ only**	51	14 *(27.5)*
Total Positive	99	29 *(29.3)*
Total Negative	153	41 *(26.8)*
BED BIOPSIES		
Positive	15	3 *(26.7)*
Negative	122	33 *(18.9)*

Values in parentheses are percentages.

Table 26. Relationship between tumour bed positivity and p53 positivity.

	Total	P53 POSITIVE
CAVITY SHAVINGS		
• **Invasive only**	24	7 *(29.2)*
• **Invasive + In-situ**	24	6 *(25.0)*
• **In-situ only**	51	11 *(21.6)*
Total Positive	99	24 *(24.2)*
Total Negative	153	35 *(22.9)*
BED BIOPSIES		
Positive	15	2 *(13.3)*
Negative	122	15 *(12.3)*

Values in parentheses are percentages.

Table 27. Relationship between tumour bed positivity and oestrogen receptor positivity.

	Total	OESTROGEN RECEPTOR POSITIVE
CAVITY SHAVINGS		
• **Invasive only**	27	23 *(85.2)*
• **Invasive + In-situ**	25	18 *(72.0)*
• **In-situ only**	59	45 *(76.3)*
Total Positive	111	86 *(77.5)*
Total Negative	164	116 *(70.7)*
BED BIOPSIES		
Positive	16	14 *(87.5)*
Negative	131	99 *(75.6)*

Values in parentheses are percentages.

DISCUSSION

This study has shown no significant association between tumour bed positivity and c-erbB-2 status, p53 status or ER status. The incidence of positive staining for these biological factors was consistent with previous studies. C-erbB-2 positivity tended to be associated with an in-situ component in the cavity shavings. C-erbB-2 is usually found to be positive in tumours with a large intraduct component and comedo-type DCIS[128-130].

In keeping with the non-significant association between ER status and local recurrence reported in the literature, this study has found no correlation between ER status and tumour bed positivity.

CHAPTER 4

INTRODUCTION

The reported incidence of local recurrence at five years after breast-conserving surgery and radiotherapy ranges from 0.3% after quadrantectomy to between 2.3 and 8% after lumpectomy in randomised trials[43-45,63]. In non-randomised series of patients local recurrence has been observed in as many as 21% of patients[90].

Local recurrence after breast-conserving surgery is associated with a short distant disease-free survival. Patients with local recurrence are 2 to 4 times more likely to develop distant disease than those without local recurrence and this risk increases to six-fold if local recurrence occurs within 1 year of surgery[44,47-49]. As detailed earlier, early local recurrence can be attributed to residual disease left behind at the time of surgery.

The evidence implicating local recurrence with the development of disseminated disease does not however translate into significant differences in survival between the treatment arms of randomised trials[43-45,49]. Currently, the generally accepted explanation of the relationship between local recurrence and systemic

recurrence is that local recurrence is a marker of poor prognosis rather than a cause of it[49].

The study presented in this chapter was designed to answer the following questions:

- What is the outcome in terms of disease recurrence and survival for the patients presented in Chapter 1?
- What factors influenced prognosis?
- Is tumour bed positivity a marker of poor prognosis following breast-conserving surgery?

PATIENTS AND METHODS

Patients and follow-up

The data presented in this chapter relates to the patients presented in Chapter One. All patients were followed up at 3 monthly intervals for the first 5 years at alternate surgical and oncology clinics, and 6 monthly intervals thereafter. Total follow-up time was calculated from the date of operation to the last clinic visit. For patients who had failed to attend the clinic in the past year, their General Practitioner was contacted and a clinic visit was arranged. For patients who had died outwith hospital, the cause of death was obtained from death certificates.

Statistical analysis

Patients were analysed with regard to disease-free, distant disease-free and overall survival (see below). Univariate analyses were performed using Kaplan Meier techniques for estimation of the survival curves and the log rank test for comparison of different patient subgroups. The subgroups analysed were patient age, tumour grade, tumour size, lymphatic/vascular invasion, nodal status, c-erbB-2 status, p53 status, ER status

and tumour bed status. For continuous measurements the cases were split into groupings defined by the quartiles of the measurements. Multivariate analysis was performed for distant disease-free survival using Cox Proportional Hazards Models[175] to allow assessment of the inter-relationship between variables. This was performed in a stepwise manner. The initial model consisted of the variable with the greatest statistical significance. The significance of those variables not in the model was then assessed adjusting for the variable in the model. The most significant of these variables was then entered into the model and the significance of the remaining variables assessed. This was repeated until none of the variables outwith the model were significant after adjustment for those in the model.

The following end points were used :

Disease-free survival

Percentage of patients who are alive and free of loco-regional and distant disease at the time of follow-up.

Distant disease-free survival

Percentage of patients who are free of distant disease at the time of follow-up.

Overall survival

Percentage of patients who have not died as a result of breast cancer at the time of follow-up.

RESULTS

Two patients were lost to follow-up and 298 patients were therefore evaluable. Mean follow-up was 4.4 years (range 2.0 to 7.5 years).

All patients received post-operative radiotherapy to the breast, 232 patients received tamoxifen and 18 patients received chemotherapy. The administration of adjuvant tamoxifen and chemotherapy did not differ significantly relative to tumour bed status ($p=0.62$ and $p=0.59$ respectively, Chi-square test). This is shown in Table 28.

Table 28. Number of patients who received adjuvant treatment relative to tumour bed positivity.

ADJUVANT TREATMENT	ALL PATIENTS		TUMOUR BED NEGATIVE		TUMOUR BED POSITIVE	
Tamoxifen	232	**(76.7)**	139	**(76.4)**	93	**(78.8)**
Chemotherapy	18	**(6.0)**	12	**(6.6)**	6	**(5.1)**

Values in parentheses are percentages.

Site of recurrence

The site of first relapse is shown in Table 29. A total of 24 patients died from breast cancer and 11 died from other causes. Seven patients were alive with distant recurrence at the time of follow-up. One patient with local recurrence as the first site of disease relapse was alive with distant disease. Four patients developed a second primary tumour in the contralateral breast.

Of 267 patients who were treated by breast-conserving surgery (excluding 33 patients treated by mastectomy for extensive tumour bed disease), 24 patients developed distant disease of which 19 died. Nine

patients died of other causes and 3 developed a second primary tumour in the contralateral breast.

Table 29. Frequency of first site of disease recurrence.

TYPE OF RECURRENCE	ALL PATIENTS		PATIENTS TREATED BY BREAST-CONSERVING SURGERY ONLY	
Local	6	**(2.0)**	6	**(2.3)**
Axillary	2	**(0.7)**	2	**(0.7)**
Systemic	31	**(10.4)**	24	**(9.0)**

Values in parentheses are percentages.

There were 6 local recurrences. The clinico-pathological characteristics for these patients are shown in Table 30. The mean age of patients with local recurrence was 41.4 years. Only 2 patients with local recurrence had a positive tumour bed. Both had less than 4 foci of disease in the cavity shavings and did not receive further surgery for tumour bed positivity.

Local recurrence was detected by clinical examination in 5 patients and by mammography in the remaining patient. All affected the site of previous excision. The recurrence was invasive in 5 patients and in-situ in 1 patient. All invasive recurrences except one had the same Bloom and Richardson grade as the original tumour. For one patient the recurrence was grade 3 and the primary tumour, grade 2. Treatment of local recurrence was by mastectomy in 5 patients and wide excision in 1 patient.

Two patients with local recurrence (A and C) were ER negative, no patient was c-erbB-2 positive and 1 patient was p53 positive (F).

All patients were re-staged by bone scan, liver ultrasound and chest x-ray at the time of local recurrence. In all cases these investigations were negative. One patient (patient B in Table 30) subsequently developed distant metastases four and a half years after local recurrence.

Table 30. Patients with local recurrence.

	PATIENT					
	A	**B**	**C**	**D**	**E**	**F**
Age	66	43	28	34	37	39
Cavity shaving	Neg	Neg	Neg	Neg	Inv	In-situ
Bed biopsies	Neg	Neg				
Tumour size(mm)	20	20	15	10	20	12
Tumour grade	3	2	2	1	2	2
Intraduct component	None	None	None	Ext	None	None
Lymphatic/ vascular invasion	No	No	No	Yes	No	No
Axillary lymph node status	Neg	Pos	Pos	Pos	Neg	Neg
Chemotherapy	No	Yes	Yes	Yes	No	No
Tamoxifen	Yes	No	No	No	No	No
Site of recurrence	All at site of previous operation					
Size of recurrence (mm)	20	Diffuse	16	In-situ	17	10

Neg = negative, Pos = positive, Inv = invasive, Ext = extensive

Univariate analysis of disease-free survival

Results are shown in Table 31. Useful predictors of disease-free survival were nodal status, lymphatic/vascular invasion, ER status, tumour grade and tumour bed status.

Univariate analysis of distant disease-free survival

Results are shown in Table 32. All variables apart from c-erbB-2 and p53 had significant or near-significant associations with distant recurrence. The relative hazards for the predictors of distant disease-free survival are shown in Table 33. Patients who were tumour bed positive were twice as likely to suffer distant disease recurrence than patients who were tumour bed negative. The life-table analysis of tumour bed status in relation to distant disease is shown in Illustration 5. Distant disease-free survival was not significantly related to the type of disease found in the tumour bed (Illustration 6).

Univariate analysis of overall survival

Results are shown in table 34. The only variables useful in predicting overall survival were tumour bed status and age at operation.

Table 31. Univariate analysis of disease-free survival.

VARIABLE	LOG RANK TEST
Age	p = 0.377
Tumour Grade	p = 0.039*
Tumour Size	p = 0.115
Nodal Status	p = 0.003*
Oestrogen Receptor Status	p = 0.012*
Lymphatic/Vascular Invasion	p = 0.006*
C-erbB-2 status	p = 0.364
p53 status	p = 0.372
Tumour Bed Status	p = 0.042*

* Statistically significant

Table 32. Univariate analysis of distant disease-free survival.

VARIABLE	LOG RANK TEST
Age	p = 0.035*
Tumour Grade	p = 0.019*
Tumour Size	p = 0.068
Nodal Status	p = 0.007*
Oestrogen Receptor Status	p = 0.020*
Lymphatic/Vascular Invasion	p = 0.002*
C-erbB-2 Status	p = 0.304
p53 Status	p = 0.335
Tumour Bed Status	p = 0.042*

* Statistically significant

Table 33. Relative hazards for variables associated with distant disease-free survival (Univariate analysis).

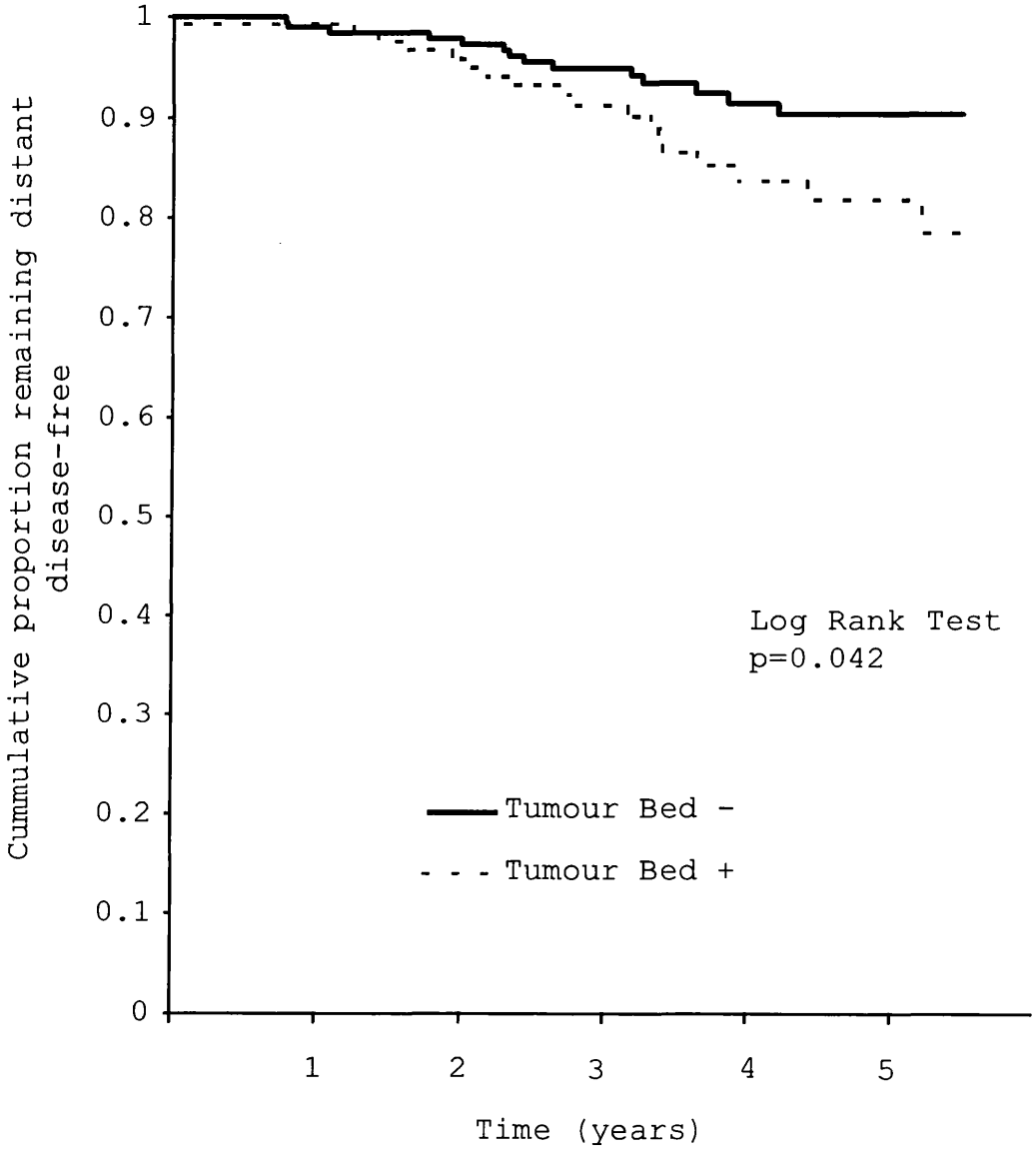
VARIABLE	COMPARISON FOR RELATIVE HAZARD ESTIMATION	RELATIVE HAZARD *
Age	UQ vs LQ	0.86 (0.57 to 1.31)
Tumour Grade	2 vs 1	3.30 (0.95 to 11.5)
	3 vs 1	5.50 (1.45 to 20.9)
	3 vs 2	1.66 (0.73 to 3.76)
Tumour Size	UQ vs LQ	1.66 (1.08 to 2.54)
Nodal Status	Pos vs Neg	2.70 (1.25 to 5.83)
Oestrogen Receptor Status	Neg vs Pos	2.26 (1.10 to 4.66)
Lymphatic/Vascular Invasion	Present vs Absent	2.94 (1.44 to 6.01)
Tumour Bed Status	Pos vs Neg	2.03 (1.00 to 4.15)

* Point estimate for relative hazard (95% confidence intervals for relative hazard in parenthesis).

UQ = Upper Quartile, LQ = Lower Quartile.

Pos = positive, Neg = negative

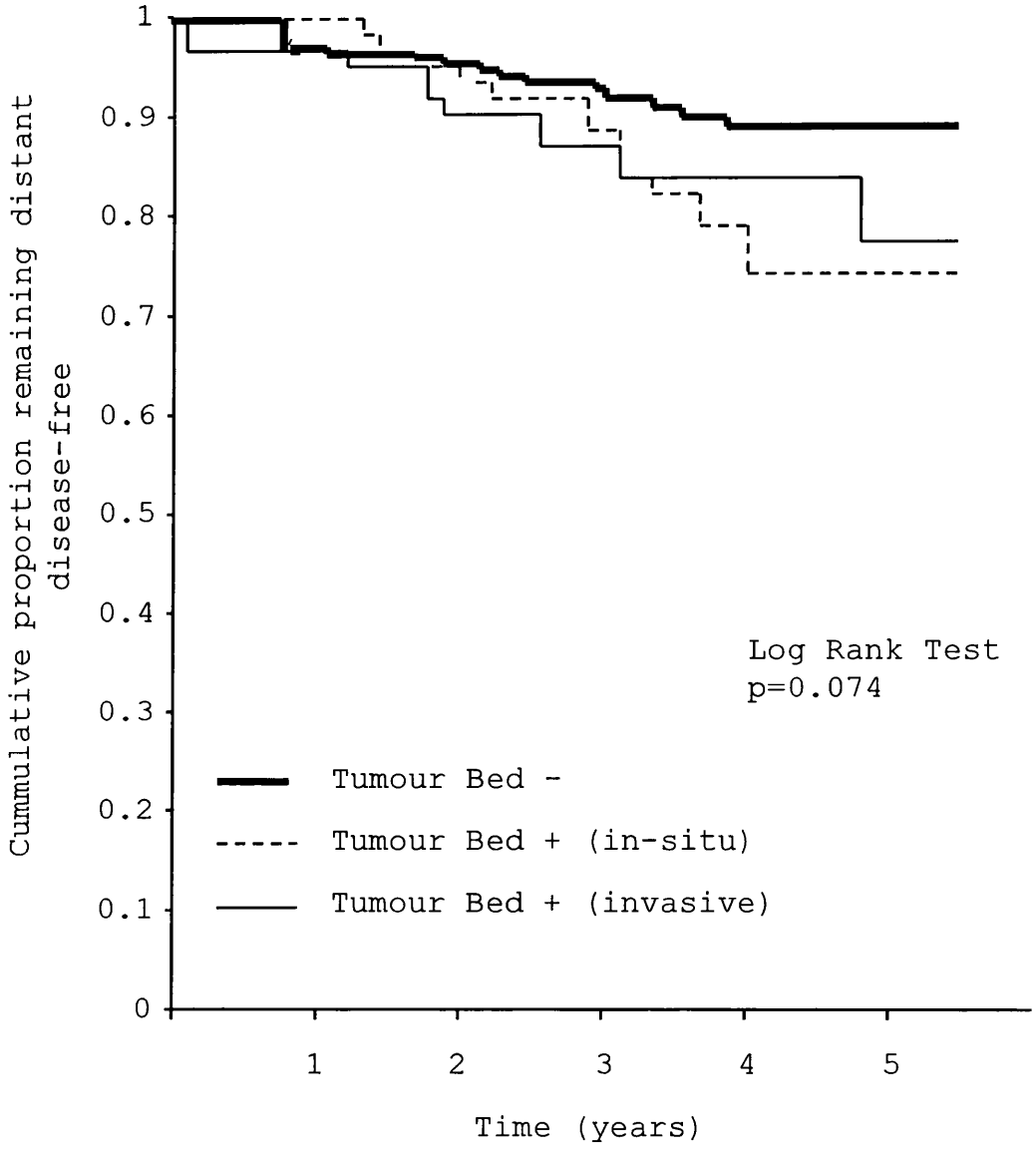
Illustration 5. Life table analysis of tumour bed status and distant disease-free survival



Number at risk

——	178	175	134	87	47
- - -	116	110	79	50	28

Illustration 6. Life table analysis of type of disease in tumour bed and distant disease-free survival



Number at risk

———	178	175	134	86	48
-----	61	58	41	23	13
———	54	50	40	28	16

Table 34. Univariate analysis of overall survival.

VARIABLE	LOG RANK TEST
Age	p = 0.049*
Tumour Grade	p = 0.329
Tumour Size	p = 0.306
Nodal Status	p = 0.167
Oestrogen Receptor Status	p = 0.143
Lymphatic/Vascular Invasion	p = 0.638
C-erbB-2 Status	p = 0.255
p53 Status	p = 0.590
Tumour Bed Status	p = 0.016*

* Statistically significant

Multivariate analysis of distant disease-free survival

Three variables were identified as a set of independently useful predictors of distant disease-free survival; Lymphatic/Vascular invasion, ER status and Tumour Bed status. These factors constituted the final Cox proportional hazards model. After adjusting for these factors, none of the other variables were useful in predicting distant disease-free survival. No statistically significant interaction was found between tumour bed positivity and lymphatic/vascular invasion

(p=0.783, likelihood ratio test) or tumour bed positivity and ER status (p=0.665, likelihood ratio test). Life table analyses of tumour bed status and lymphatic/vascular invasion or tumour bed status and ER status are shown in Illustrations 7 and 8. Patients with ER negative and tumour bed positive tumours appeared to be at particular risk of distant disease with a distant disease-free survival of 49.6%.

Distant disease-free survival relative to further surgery performed for tumour bed positivity

To assess the influence of performing further surgery on the outcome of patients who were tumour bed positive a life table analysis was performed for distant disease-free survival. There was no significant difference in outcome between those who were tumour bed positive and had further surgery and those who were tumour bed positive and had no further surgery (p=0.09, Illustration 9). However those patients who had further surgery tended to be more likely to develop distant disease than those who had no further surgery, regardless of tumour bed status.

The 33 patients who had mastectomy were compared with the 277 patients treated by breast-conserving surgery (plus or minus re-excision). The relative distribution

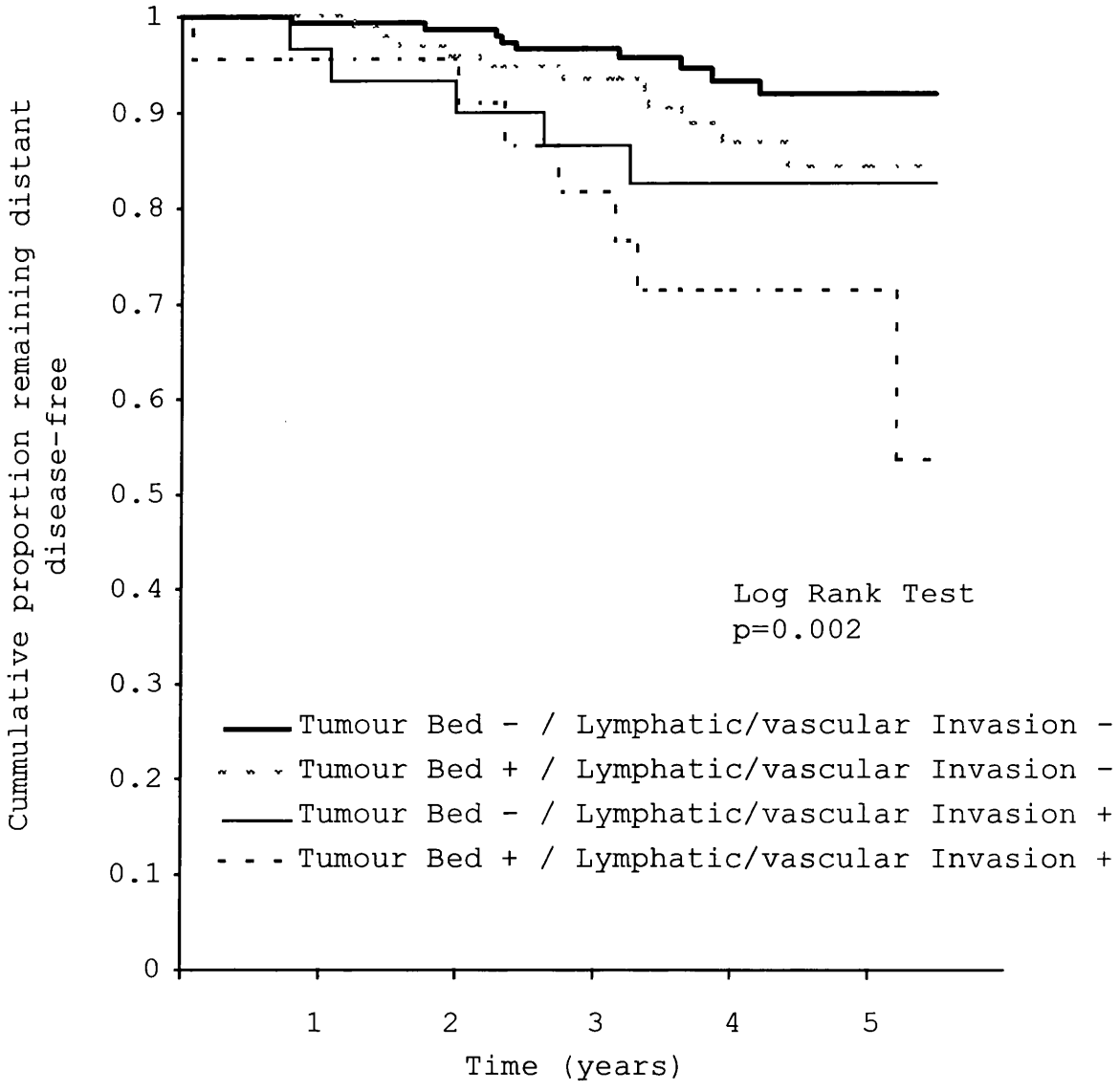
of tumours in terms of size, grade and lymph node status between these two groups is shown in Table 35. Patients who had mastectomy were more likely to be node positive (p=0.029, chi-square) and tended to have higher grade tumours (p=0.053, chi-square). Distant disease-free survival for mastectomy patients was 78.8% versus 91.0% for patients treated by breast-conserving surgery (median 4.5 years for both groups, p=0.037 Log Rank test, Illustration 10).

Table 35. Distribution of tumour size, grade and lymph node status between patients treated by mastectomy and those treated by breast-conserving surgery.

	MASTECTOMY	BREAST-CONSERVING SURGERY
Median tumour diameter (mm)	12	13
Tumour grade		
• **I**	2 *(6.1)*	70 *(25.3)*
• **II**	22 *(66.7)*	123 *(44.4)*
• **III**	5 *(15.2)*	36 *(13.0)*
• **ungraded**	4 *(12.1)*	38 *(13.7)*
Lymph node		
• **Negative**	21 *(66.7)*	222 *(80.1)*
• **Positive**	12 *(33.3)*	55 *(19.9)*

Values in parentheses are percentages.

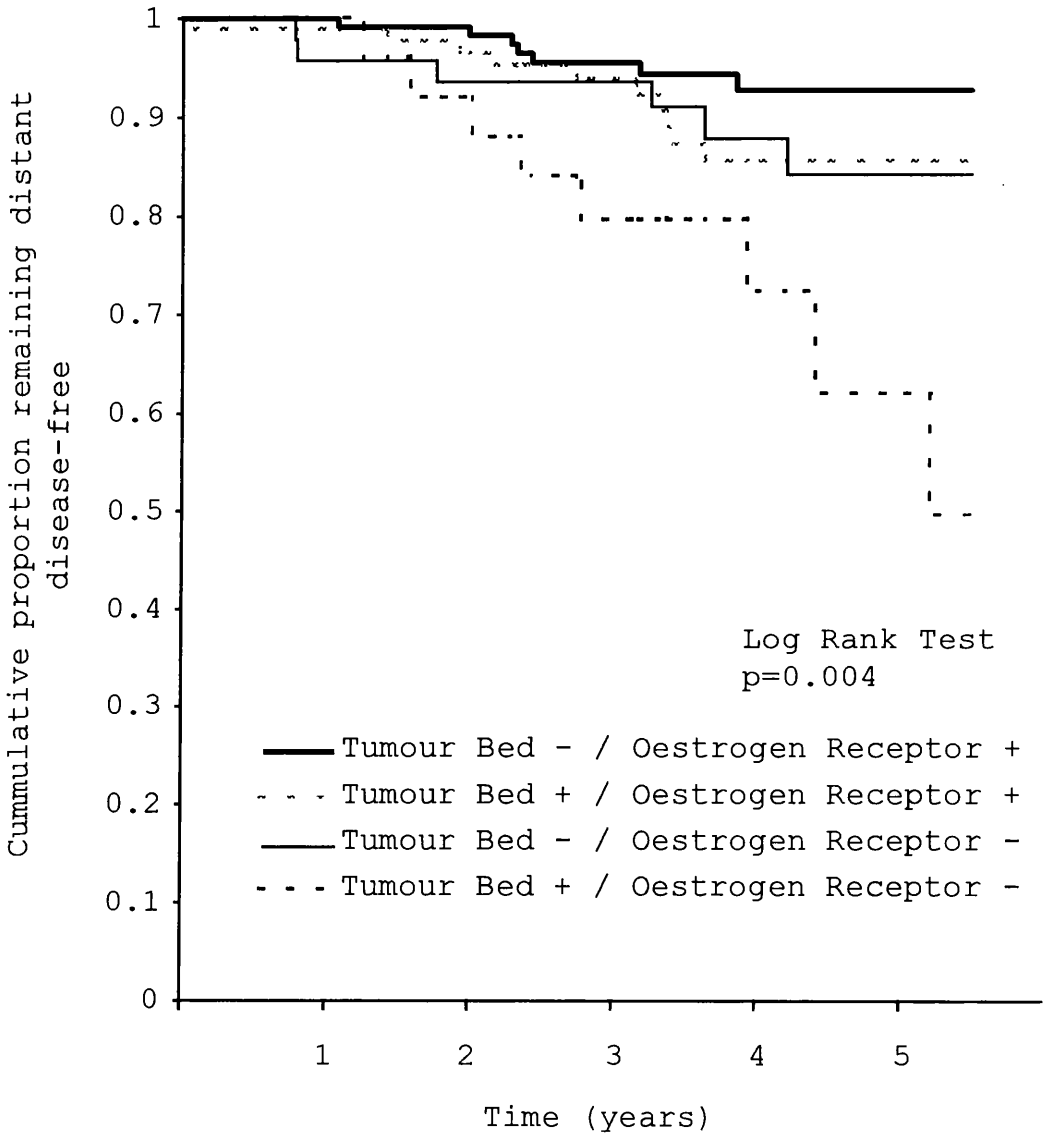
Illustration 7. Life table analysis of tumour bed status and lymphatic/vascular invasion and distant disease-free survival



Number at risk

———	150	149	111	73	41
* * *	95	90	63	42	24
———	29	27	23	14	6
- - -	21	21	16	10	7

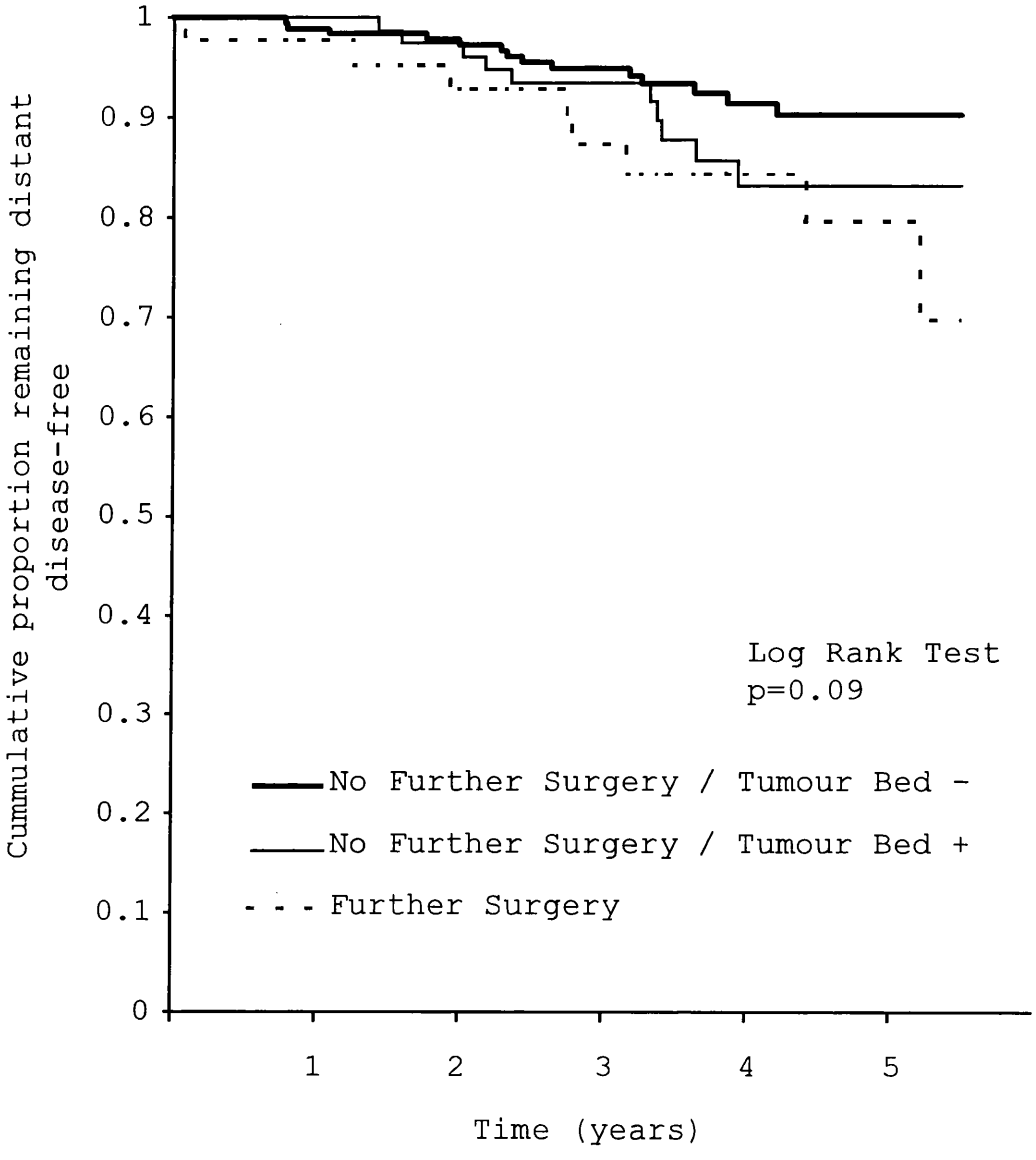
Illustration 8. Life table analysis of tumour bed status and ER status and distant disease-free survival



Number at risk

———	115	113	86	55	35
- - -	44	43	37	25	13
———	83	80	57	38	22
- - -	24	22	17	8	4

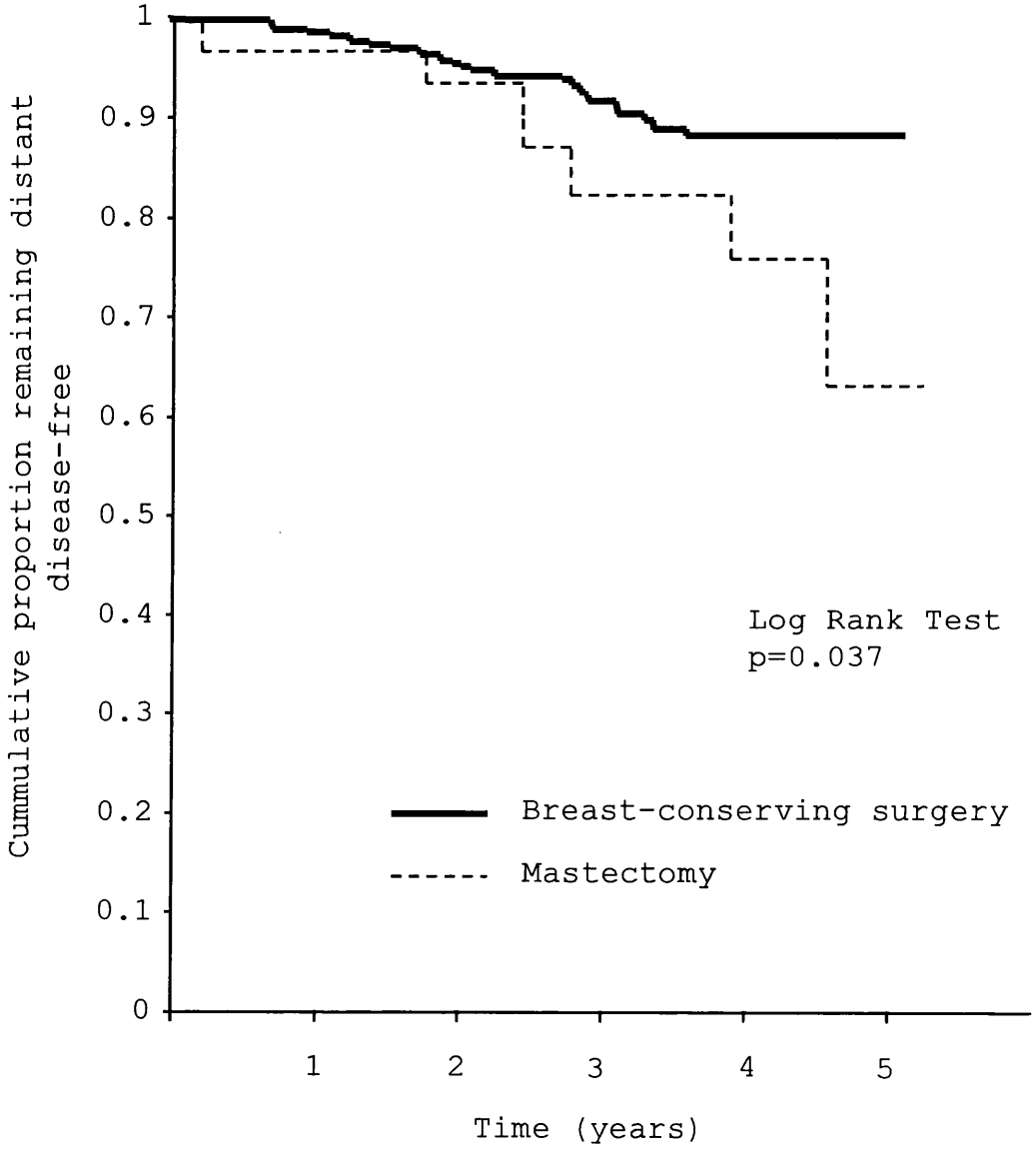
Illustration 9. Life table analysis of further surgery and distant disease-free survival



Number at risk

———	178	175	134	86	48
——	76	74	51	31	18
- - -	39	38	30	21	11

Illustration 10. Life table analysis comparing patients treated by mastectomy with those treated by breast-conserving surgery (distant disease-free survival)



Number at risk

———	263	256	193	124	76
------	32	30	22	15	8

DISCUSSION

A low local recurrence rate of 2% has been observed in this study. This is comparable with the lowest reported rates of local recurrence in the literature (Table 4). All patients in this study received post-operative radiotherapy, 77% received adjuvant tamoxifen and 6% received adjuvant chemotherapy. With short-term follow-up and few local recurrences analysis of factors associated with local recurrence is premature. However, a significant feature of the 6 patients with local recurrence was that their mean age was 41.4 years and only 1 patient was over 43 years old. The association between young age and local recurrence is well recognised. Several investigators have shown that risk of local recurrence decreases with advancing age and that patients aged below 40 years are at the highest risk[43,47,83,90,176,177]. In a regression analysis of patients treated with lumpectomy and radiotherapy in the NSABP-B06 trial, age less than 35 years was the most significant prognostic factor for local recurrence[83]. In Chapter 1 no statistically significant association was found between age and tumour bed positivity although the incidence of tumour bed positivity varied with age and patients between 35 and 50 were more likely to be tumour bed positive. All

local recurrences in this study affected the site of previous excision and hence are likely to represent residual disease. However, only two patients with local recurrence had evidence of residual disease in the cavity shavings and this was very minimal involvement. The rest were tumour bed negative and 2 had negative bed biopsies. Other factors other than residual tumour burden might therefore have an important role in the aetiology of local recurrence in young women. Young age has been reported to be associated with an increased risk of local recurrence regardless of whether or not radiotherapy was administered[43-45]. Resistance to radiotherapy therefore, does not appear to be a concern nor does it explain the association. Young patients with breast cancer are known to have more aggressive tumours with a worse overall outcome regardless of surgical treatment. It has been suggested that the reason for the increased risk of local recurrence in young women is related to high circulating oestrogen levels[178]. It has also been suggested that the chemical environment at the operation site in young women may be very conducive to the culture of very small numbers of residual tumour cells[179].

Follow-up for this study was relatively short and given that all tumours were less than 25mm it is not surprising that overall survival was good. Longer follow-up will allow a more powerful analysis of

variables predicting outcome. However distant disease-free survival is a surrogate measure of overall survival when follow-up is limited. As 5 patients in this study were alive with distant disease it was important to assess variables for a significant relationship with this outcome measure as well as for overall survival. Not surprisingly, some variables were found to significantly predict for distant disease-free survival but not overall survival.

In this study, tumour bed positivity was associated with a short distant disease-free survival. Patients with disease in the tumour bed were twice as likely to develop distant metastasis compared with patients who were tumour bed negative. In addition, multivariate analysis showed that lymphatic/vascular invasion, ER status and tumour bed status were all independently useful predictors of distant disease-free survival. After adjusting for these factors no other variables were significant. This included tumour grade which was the only factor significantly associated with tumour bed positivity in Univariate analysis. The type of disease (in-situ or invasive) found in the tumour bed was also not significantly associated with distant disease-free survival. Failure to find a significant relationship between c-erbB-2 and survival in this series may be due to the relatively small numbers of node positive patients, a group in whom the prognostic

power of c-erbB-2 is most likely to be apparent. Patients who were ER negative and tumour bed positive appeared to have a particularly high risk of distant metastasis with a distant disease-free survival of less than 50% compared to greater than 90% for patients who were ER positive and tumour bed negative.

In reviewing the literature for previous reports of an association between tumour bed disease and patient outcome, the study by Egan et al is of interest[56]. This study analysed 118 mastectomy specimens containing invasive primary cancers and documented the presence of multifocal disease using a detailed pathological-radiological technique. The findings were correlated with patient survival. Patients with unifocal disease had a significantly better prognosis than those with multifocal tumours regardless of tumour stage.

An association between local recurrence and distant disease is well recognised as detailed earlier. Currently, the generally accepted theory to explain this association is that local recurrence is merely a visible manifestation of the original tumour and is thus a marker for distant disease rather than a direct cause of it. Residual disease after breast-conserving surgery is the cause of early local recurrence and hence tumour bed positivity might be expected to also correlate with prognosis as has been demonstrated.

Therefore, the mechanism for the association between tumour bed positivity and distant disease may be the same as that between local recurrence and distant disease.

In the NSABP-B06 trial 10% of patients had a positive resection margin determined by India ink staining and these patients all underwent mastectomy[64]. No significant difference in survival was found between patients receiving breast-conserving surgery alone and those who also received adjuvant radiotherapy despite large differences in local recurrence rates[35]. In this study 41 patients (14%) underwent further surgery determined by extensive disease involvement of the cavity shavings. The distant disease-free survival tended to be worse for those patients who had further surgery compared to those who did not. This finding is consistent with the view that no *causal* relationship exists between residual disease in the tumour bed and distant disease-free survival.

Patients who underwent mastectomy were also analysed separately and compared with those who were treated by breast-conserving surgery. There was a 12% difference in distant disease-free survival with mastectomy patients having a worse prognosis. When pathological features were compared between these two groups, this difference in prognosis could be explained by the fact

that mastectomy patients had tumours with higher grade and a higher incidence of lymph node positivity. In addition, of course, they all had positive cavity shavings.

Taken together, these results favour the hypothesis put forward by Fisher that prognosis is determined by tumour biology and not influenced by local recurrence[49].

In conclusion, tumour bed analysis is a useful method of assessing completeness of surgical excision. A selective re-excision policy for cavity shaving positivity results in a low local recurrence rate. This study has also demonstrated that tumour bed status is an independent marker for distant disease-free survival.

FINAL DISCUSSION

In the treatment of patients with breast cancer, the role of surgery is to achieve local control of disease and allow pathological tumour staging. During this century the surgical strategy to achieve these aims has evolved from radical mutilating dissections of the chest wall to increasingly conservative procedures. Most recently the use of breast-conserving surgery has become widespread. Breast-conserving operations are designed to achieve the same degree of disease control as more radical procedures but with considerably better cosmesis and considerably less psychological morbidity. Several randomised trials have assessed the value and safety of breast-conserving surgery. It has become clear that breast-conserving surgery combined with axillary dissection and post-operative radiotherapy to the residual breast tissue is a safe alternative to radical mastectomy for treating small breast cancers. Five year local recurrence rates after this treatment regime are 2.3 to 8% (table 4) and may be lower if chemotherapy is also given. Breast-conserving surgery alone, without radiotherapy, results in an unacceptably high incidence of local recurrence. Currently therefore, radiotherapy is a mandatory adjunct to breast-conserving surgery. However it also has an

associated morbidity and for many patients it is a significant ordeal and inconvenience.

The large majority of local recurrence after breast-conserving surgery alone (without radiotherapy) occurs within the first 5 years of surgery at the site where the primary tumour was excised. This would appear to indicate that the initial surgical procedure may have been incomplete. This scenario has been previously demonstrated by pathological studies, which showed that microscopic disease frequently extends beyond the macroscopic boundary of the tumour (multifocality). In over 40% of cases multifocality was observed to extend beyond what might be considered an adequate "gross" excision margin by the surgeon.

Currently approximately half of all new cases of breast cancer are treated by breast-conserving surgery. Both breast cancer screening and the use of neoadjuvant chemoendocrine therapy are likely to increase the proportion of tumours suitable for breast-conserving procedures in the future. Measures taken to prevent local recurrence are important for several reasons; local recurrence is difficult to detect, is likely to require mastectomy and in many cases may be inoperable. It is also likely to be associated with extreme patient anxiety. In addition there is indirect evidence supporting the controversial theory that local

recurrence may disseminate and directly effect overall outcome for some patients. Finally, if patients at risk of local recurrence can be identified, then radiotherapy could be administered selectively.

Pathological and clinical risk factors for local recurrence have been investigated extensively. Withholding adjuvant radiotherapy is undoubtedly the most important predictor of increased risk for local recurrence. Another important predictor is margin status. Microscopic disease at resection margins is a strong indicator of risk for early local recurrence. Other major risk factors are young age and the presence of an extensive in-situ component in the tumour. Less consistent associations are found between local recurrence and high tumour grade, lymphatic/vascular invasion and tumour size.

This thesis has presented a novel and simple method for analysing the resection margin. This involves taking a shaving from the wall of the cavity remaining in the breast after the surgeon has performed what he regards as an adequate wide local excision (Chapter 1). This method avoids the difficulties encountered during assessment and interpretation of margin analyses based on sections taken from the lumpectomy specimen surface. In a series of 300 patients, microscopic disease was detected within the cavity shaving in 39.3% (tumour bed

positivity). This incidence of margin positivity is considerably higher than those reported by other studies using conventional methods of margin analysis. However it is consistent with predictions of tumour bed positivity based on pathological studies and is consistent with the reported incidence of local recurrence after breast-conserving surgery if adjuvant radiotherapy is withheld (43%, table 4).

The extent of tumour bed positivity varied widely. In many instances only 1 or 2 foci of in-situ cancer were detected in 1 or 2 sections out of 30 blocks sectioned. In other cases extensive invasive disease involving many blocks was found. Tumour bed positivity consisted of invasive disease in 18.3% of patients and only a third of patients had more than 4 foci of residual disease. All patients received adjuvant radiotherapy. It is not known how much residual disease can be safely treated by radiotherapy. With a selective re-excision policy targeted at patients with evidence of extensive tumour bed positivity, further disease was found in 22 out of 41 patients who had further wider excision or mastectomy. At mean follow-up of 4.4 years this policy resulted in a local recurrence rate of 2% (Chapter 4). This rate compares very favourably with 5-year local recurrence rates reported in the literature.

These advantages of cavity shaving must be weighed against the preparation and reporting time for this technique. It might be argued that a cost benefit analysis comparing standard methods of margin assessment with cavity shaving would be likely to show no difference or even favour cavity shaving. With standard methods, preparation time may be longer as the specimen requires more detailed and accurate preparation and microscopy requires an excision margin to be identified and measurements to be taken. In addition, the fact that cavity shaving can quantify residual disease and that patients with minimum residual disease do not undergo re-operation must be compared with standard methods which cannot quantify residual disease and are thus unable to select patients who do not require re-excision. If long-term follow up supports cavity shaving as a technique resulting in a low rate of local recurrence as this study suggests, then this will also represent a cost benefit.

Factors that might predict risk of tumour bed positivity were assessed. Such factors might alter surgical policy if detected at diagnostic biopsy or pre-operatively by mammography. Thus certain patients might be selected for a wider initial resection. Tumour bed positivity was found to be significantly associated with higher tumour grade, presence of a large in-situ component, dense mammographic pattern, casting-type

mammographic calcification and absence of mammographic nidus. Non-significant trends were also observed between tumour bed positivity and smaller lumpectomy diameter, younger patient age and lobular carcinoma. Of interest, no association was found between tumour bed positivity and tumour size, lymphatic/vascular invasion, axillary lymph node metastases, and oestrogen receptor, c-erbB-2 and p53 status (Chapters 1,2 and 3).

Of the 300 patients who had tumour bed analysis, 165 had bed biopsies taken from the cavity wall remaining after the cavity shaving had been taken. For the 41 patients who had re-excision because of extensive tumour bed positivity, the likelihood of finding further disease was associated with disease in the bed biopsies, 4 or more foci of disease in the cavity shavings and a large intraduct component in the tumour (Chapter 1).

On the basis of these results, a future study might prospectively investigate the value of these criteria to select patients for wider primary excision. Similarly, patients who are tumour bed negative with no intraduct component may not require radiotherapy. A randomised trial of selected patients might assess this. Interestingly, a study similar to this has recently been reported[180]. Radiotherapy was not given to patients with unicentric, clinically T1 tumours that

had no extensive intraduct component or lymphatic vessel invasion and had a negative pathological clearance margin of at least 1cm (determined by inking). After a median follow-up of 4.7 years the local recurrence rate was 16% and the trial was prematurely closed. This study did not state the number of patients who might have been suitable for enrolment but were excluded because of positive margins. As previously stated, inking is known to underestimate margin positivity and 79% of local recurrence in this study affected the site of original excision. Thus, original assessment of margins may have been inadequate. The need for caution in assessing the role for performing breast-conserving surgery without radiotherapy is highlighted. However, even if highly selective criteria are used (including perhaps patient age and tumour grade) a significant percentage of patients may avoid radiotherapy. Another study addressing this issue is the BASO II trial (British Association of Surgical Oncology). This trial was started in 1991 and is currently in progress. Patients who have grade I or special type tumours, 2cm or less in diameter and are axillary node negative are eligible for enrolment. They are randomised to wide local excision plus or minus radiotherapy[181]. The trial did not specify a particular technique for margin analysis, stating only that clear margins should be confirmed by

microscopy. The results of this trial may also, therefore, highlight the necessity of adequate and detailed margin assessment. Analysis of the factors which are associated with tumour bed positivity in a larger number of patients than that presented in this thesis may shed further light on the criteria which would be most useful in selecting patients for whom radiotherapy can be safely omitted.

Tumour bed positivity was also assessed as a prognostic factor for distant disease-free survival. This was of interest because of the strong association reported between local recurrence and distant disease-free survival. Patients who were tumour bed positive were found to be significantly more likely to develop distant disease than patients who were tumour bed negative within the limited follow-up period of this study (Chapter 4). This relationship requires further investigation. Specifically, with larger numbers of patients or longer follow-up it may be possible to show that the strength of this association is related to the extent of tumour bed positivity. Patients who had further surgery because of extensive tumour bed disease tended to have a shorter distant disease-free survival than those who were tumour bed positive but had no further surgery.

In summary, investigations into the causes and methods of preventing local recurrence after breast-conserving surgery is of great interest and is relevant to the current surgical management of patients with breast cancer. Hopefully our understanding of local recurrence has progressed a little with the investigations performed in this thesis.

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