CLINICAL, HISTOLOGICAL AND IMMUNOLOGICAL STUDIES IN HUMAN BREAST CANCER

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To Susan

DECLARATION OF ORIGINALITY

I declare that the contents of this thesis were composed entirely by myself. In addition, all the work described herein was performed by myself with the exception of the routine staining of the histological sections and the oestrogen receptor estimations which were carried out by members of the staffs of the Departments of Pathology and Clinical Surgery.

ABSTRACT

Part 1 (Volume 1) A clinico-pathological investigation into the significance of metastases and reactive changes in regional lymph nodes draining breast cancer.

In the introduction, an extensive review of the literature is presented. The original work, which involved review of clinical records, histological techniques, radio-isotope scanning and data processing, is then described. The principal findings were: 1. Lower axillary sampling was only useful for indicating prognosis in primary breast cancer if more than one node was found. However, a trial of sampling versus clearance demonstrated that, if done correctly, sampling was equally capable of detecting metastases. 2. Nodal metastases were shown to correlate significantly with tumour size, tumour border, histological grade, oestrogen receptor status and duration of symptoms. 3. A grading system incorporating all the major histological reactive changes was shown to be an effective prognostic index, but favourable reactive changes were found to be associated with favourable tumour factors, suggesting that these nodal reactions may merely reflect the nature of the tumour rather than represent an effective host response. Furthermore, sinus histiocytosis, a reactive change which is widely recognised to be a favourable prognostic factor, was shown to be induced by breast biopsy. 4. The prognostic significance of lymphocytic infiltration of breast cancer was found to be dependent on reactive changes in the regional nodes. 5. The inaccuracy of clinical examination in detecting axillary metastases or assessing reactive changes was confirmed. Axillary lymphoscintigraphy was found to be no more effective than clinical examination in detecting nodal metastases, but a computer-based combination analysis of several parameters related to metastases was a significant improvement.

Part 2 (Volume 2) An investigation into some of the properties of monocytes and macrophages in breast cancer patients and normal subjects.

In the introduction, a review of the literature is presented. The original work, which involved the identification and characterisation of monocytes and macrophages using rosetting techniques and immunoperoxidase staining, is then described. The principal findings were: 1. Peripheral blood monocytes from breast cancer patients were activated in terms of lysozyme content and Fc (IgG) receptor expression. 2. Tumour-infiltrating macrophages showed depressed lysozyme and alpha-1-antitrypsin contents and defective phagocytosis of IgG-coated red blood cells. 3. A higher macrophage content was found in tumours with a poor prognosis when compared to tumours with a good prognosis. These findings suggest that tumour-infiltrating macrophages are selectively depressed, accounting for their lack of anti-tumour activity in vivo. 4. Studies on normal monocytes revealed that the third component of complement can stimulate lysozyme synthesis, and that phagocytosis may induce the release of alpha-1-antitrypsin. Both of these findings may represent important regulatory mechanisms in monocyte/macrophage physiology.

Publications from Thesis

The contents of this thesis have not yet appeared in any formal publications, but several communications to scientific societies have been given. These are listed below.

Scottish Society for Experimental Medicine

i) A comprehensive grading system for lymph node reactions in breast cancer. (Abstract - Scot Med J. 1982; 27: 90).

British Association for Surgical Oncology

i) The effect of reactive changes in axillary nodes on the prognostic significance of lymphocytic infiltration of primary breast cancer. (Abstract - Clin Oncol, in press).

British Association for Cancer Research

i) A computer programme to assist the pre-operative diagnosis of axillary lymph node metastases in breast cancer. (Abstract - Brit J Cancer. 1982; 46: 488).

ii) Lysozyme content of breast cancer-associated macrophages. (Abstract - Brit J Cancer, in press).

Surgical Research Society

i) Evidence for depressed activation of macrophages infiltrating human breast cancer. (Abstract - Brit J Surg. 1982; 69: 687).

ii) Macrophage content and prognostic factors in human breast cancer. (Abstract - Brit J Surg, in press).

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PART 1

A clinico-pathological investigation into the significance of metastases and reactive changes in regional lymph nodes draining breast cancer.

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INTRODUCTION

Aim

It is clear from the available literature that the axillary lymph nodes draining breast cancer can yield valuable data pertaining to prognosis. In particular, the presence and degree of metastatic involvement are of paramount importance, but in addition, the histological appearances of non-involved nodes have significance.

Two recent developments have made further study of these factors important. Firstly, the treatment of breast cancer has become increasingly conservative from a surgical point of view, and it must be established whether limited axillary sampling can provide sufficient material for prognostic purposes. For the same reason, the accuracy of clinical examination must be critically assessed, and non-operative techniques for the detection of nodal tumour deposits must be developed and examined.

Secondly, our increasing knowledge of immunology has made it possible to define lymph node microarchitecture in more precise terms, and this may provide a useful index of host response to breast cancer. To investigate such a hypothesis, "reactive changes" in lymph nodes must be related to prognosis, and to other factors which are thought to influence the disease process.

With these thoughts in mind, the aim of the work described in this thesis was three fold.

1. To investigate the prognostic significance of regional lymph node metastases in breast cancer, and the relationship of these

metastases to other prognostic factors.

2. To assess methods of detecting metastases and reactive changes in regional nodes draining breast cancer.

3. To determine the prognostic significance of reactive changes in regional nodes in breast cancer, and the relationship of these changes to other prognostic factors.

In order to put existing knowledge of these factors into perspective, an extensive literature review has been carried out. This constitutes the major part of the introduction to the thesis, and is divided into two parts, one concerning nodal metastases and the other reactive changes. At the end of the introduction, some unanswered questions are posed in the light of the literature review, and the section on original work describes how answers to these questions have been sought.

LITERATURE REVIEW I

Regional lymph node metastases in breast cancer

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Introduction

Metastases to the regional lymph nodes have long constituted a central issue in discussions concerning the behaviour and treatment of breast cancer. They occur in about 40% of patients treated by mastectomy[1], and their presence is associated with a poor prognosis[2]. In this review, the present state of knowledge with regard to the significance of these metastases in the natural history of the disease will be examined, and an attempt will be made to determine the optimum available methods for their detection and treatment.

The Anatomy of the Lymphatic Drainage of the Breast

The lymph vessels which drain the breast originate from a plexus in the interlobular connective tissue and the walls of lactiferous ducts, which communicates with an overlying cutaneous lymphatic plexus, especially around the nipple in the subareolar plexus[3]. These intra-mammary lymph vessels form the "trunks of Rouviere"[4] which curve round the anterior border of the axilla to pierce the axillary fascia. They end largely in the pectoral lymph nodes lying along the lower border of pectoralis minor in association with the lateral thoracic vessels, but some pass directly to the subscapular nodes which are grouped around the subscapular vessels.

Although lymph nodes are occasionally found within breast tissue[5], the pectoral nodes are accepted as being the most proximally situated to the breast. From the upper part of the breast, a few lymph vessels pass to the apical nodes which are situated partly posterior to the upper portion of pectoralis minor, and partly above its upper border. Another group of lymph glands which drain the breast are the interpectoral or Rotter's nodes[6]. These glands lie between the pectoral muscles, and although some lymph reaches them via the axillary fascia, animal experiments using high resolution autoradiography have demonstrated lymphatic channels which penetrate pectoralis major[7]. Such channels may form an alternative route from the breast to Rotter's nodes.

The axillary lymph nodes which are described above communicate with central nodes embedded in the axillary fat, and

each group drains to the apical nodes. Lymph from the apical nodes then enters the venous circulation via the subclavian trunk.

In studies of the axillary lymph nodes associated with radical mastectomy specimens, it has been common practice to divide them into three levels. Level 1 consists of the axillary contents lateral and inferior to pectoralis minor, level 2 includes that tissue behind pectoralis minor, and level 3 comprises the remaining area above and medial to the muscle[8-10]. It may be assumed that because level 1 contains those lymph nodes most proximal to the breast, lymphatic tumour spread can only reach levels 2 and 3 by first traversing level 1. It is clear, however, that alternative routes of lymphatic drainage may bypass the lower nodes, and such an assumption cannot be made from first principles.

Finally, it remains to consider the internal mammary nodes. These lie along the internal thoracic vessels, and studies using intravital injections of dye and radioactive colloidal gold have shown that they drain about 25% of the breast[11]. In the past it has been taught that internal mammary nodes drain the medial side of the breast, and axillary nodes the lateral[12]. There is no evidence, however, to support this; indeed, the work of Turner-Warwick[11] and Hultborn[13] has demonstrated that the anatomical distribution of colloid in the regional nodes is not affected by varying the quadrant of the breast into which it is injected. It is fair to conclude, then, that any part of the breast may drain to either the axillary or to the internal mammary nodes, although the larger proportion of lymph is directed laterally.

The Significance of Lymph Node Metastases in Breast Cancer

i. Nodal metastases and the natural history of breast cancer

The presence of metastatic deposits in the regional lymph nodes confers a poor prognosis on a patient with breast cancer. This dictum has long been accepted[2,14,15], and, in absolute terms, 40%-50% of patients with lymph node metastases should be expected to survive for five years, whereas in patients without this stigma, the range will be 70%-80%[2,16].

Nevertheless, the significance of nodal metastases in the natural history of breast cancer is obscure, and it is only now that we are beginning to understand their meaning. For many years, since Virchow proposed that regional lymph nodes formed an effective barrier to the spread of malignant disease[17], it was thought that a tumour spread locally in the first instance, and only became a systemic problem when the neoplastic cells overcame the physical hurdle of the lymph nodes (Figure 1).

Recently, however, this doctrine has been challenged. As long ago as 1869 it was recognised that cancer cells similar to those seen in the primary tumour could be found in the peripheral blood[18], and this has frequently been confirmed[19,20]. In the 1960's, the Fishers showed that, in rats, labelled tumour cells could pass easily between the systemic and lymphatic circulations[21,22], and communications between the venous and lymphatic systems have been shown to exist within lymph nodes[23].

These observations do not refute the barrier theory of lymph nodes, however, and there are studies which testify to their effectiveness in trapping various substances such as

Figure 1 Diagrammatic representation of the theory of progressive spread in breast cancer.

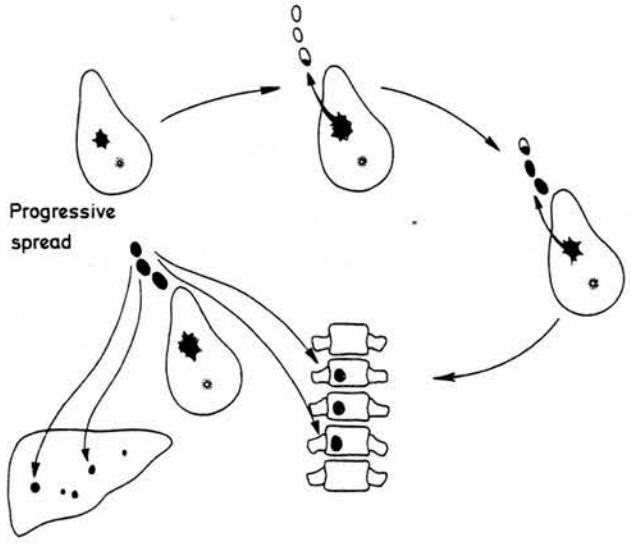
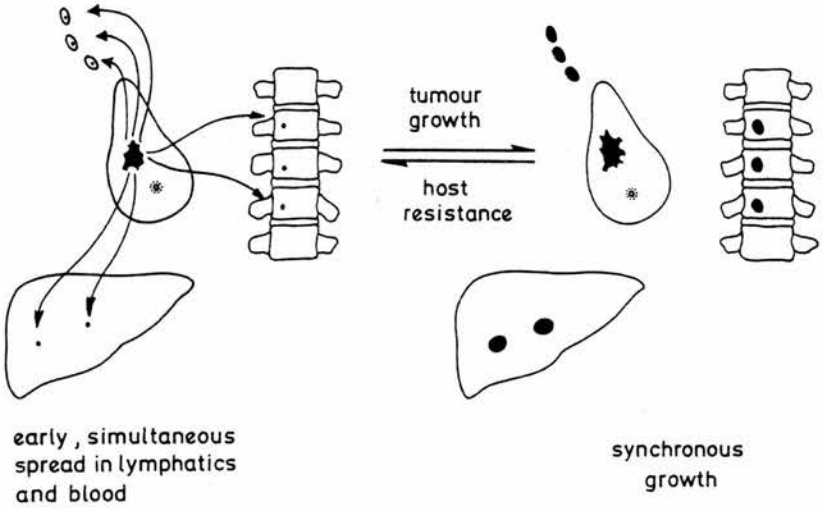


Figure 2 Diagrammatic representation of the theory of early dissemination and synchronous growth in breast cancer.



india ink[24], colloidal gold[25], bacteria[26], viruses[27], and red blood cells[28,29]. Not satisfied with this evidence, however, the Fishers have performed a series of experiments to test the hypothesis that lymph glands can also trap tumour cells. In an important study, again carried out in rats, they injected malignant cells into the afferent lymphatics of the popliteal node, and into the dependant paw[30]. In both cases, they recovered cells from the efferent lymph, which seemed to indicate that transmigration was occurring across the nodes. However, Engzell challenged the reliability of the histological identification of single tumour cells in this system[31]. To overcome such criticism, the experiments were repeated using ^{51}Cr-labelled tumour cells[32,33], and this work produced two very interesting results. Firstly, it confirmed that tumour cells did pass through the popliteal node, although some were trapped for a variable length of time. Secondly, it was found that only 50% of the injected activity could be recovered from the efferent lymph or from the popliteal node after sacrifice of the animal. The latter observation strongly supports the theory of lymphaticovenous communication within lymph nodes.

All this evidence points to the conclusion that lymph nodes cannot trap tumour cells effectively, and that ready communication exists between the lymphatic and venous systems. Nevertheless, lymph node metastases at the time of mastectomy increase the likelihood of distant metastases developing and if we are to accept the experimental evidence, an explanation for this phenomenon must be invoked. A reasonable hypothesis makes the assumption that by the time a patient reaches mastectomy

for breast cancer, her disease has already become widely disseminated. Obvious lymph node deposits, therefore, merely represent an aggressive form of neoplasia, or breakdown of a favourable balance between the tumour and its host (Fig. 2).

If such a theory is to be tenable, there must be clinical evidence to lend support. Initially, skeletal scintiscanning seemed to provide important information. Galasko reported that 24% of patients with apparently operable breast cancer had scintigraphic evidence of bony metastases[34,35], and this appeared to be confirmed by the fact that 83% of these patients died of their disease within five years[36]. In concordance with Galasko, Campbell and his colleagues found positive bone scans in 35.8% of patients with primary breast cancer. At 18 months, 85.7% of these patients had obvious disseminated disease as compared with 11.4% of the subjects whose scans had been negative[37].

These results certainly strengthen the thesis of early systemic spread, but unfortunately they have not been substantiated by other workers. Forrest found that only 8% of patients with operable disease had positive bone scans, and that only half of these developed metastases in two years[38,39]. Five other studies have reported similarly disappointing results[40-44], and although bone scanning is undoubtedly the most sensitive method of demonstrating symptomatic bony metastases[45], its role in the detection of early systemic spread is yet to be established.

We must therefore turn to other clinical evidence to find support for the hypothesis. Even if no axillary metastases can be histologically demonstrated after complete

axillary dissection, some patients still develop distant metastases[46], and one study has shown a 5% annual recurrence rate in such patients[47]. When the regional lymph nodes do contain tumour, moreover, their removal by axillary clearance[48], radical mastectomy[46] or extended radical mastectomy[49] does not necessarily protect the patient against disseminated disease, even if only a very small proportion are actually involved[1]. Evidence from randomised trials, which will be examined later, also points in the same direction; no studies have shown that excision or irradiation of regional lymph node metastases can appreciably reduce the incidence of systemic breast cancer.

Surely, then, tumour dissemination must be able to occur without prior spread to the lymph nodes. There is, however, one more obstacle to overcome in this argument. It has been well established that the frequency of nodal involvement increases proportionally with the duration of symptoms[50-53], and public education in self-examination seems to reduce the number of patients presenting with pathological stage 2 breast cancer[54]. In addition, the HIP screening programme study has shown that women with breast cancer are less likely to have nodal involvement if their disease is detected at a screening evaluation[55].

Such studies strongly suggest that the longer a carcinoma is present in the breast, the more likely it is for lymph node metastases to occur. Superficially, this seems to favour the original idea that tumour spreads in an orderly fashion to the regional nodes. There is an alternative explanation, however. The longer a tumour exists, the more chance there may

be of a breakdown in the delicate tumour-host balance, and therefore of developing nodal metastases. Cancers which are identified at an early stage would thus be less likely to display these stigmata of neoplastic aggression.

If the tumour-host relationship is already unfavourable at the time of presentation, metastases scattered throughout the body will be actively increasing in size, but may only be detectable in the lymph nodes because of their relative accessability. The majority of patients with lymph node metastases will therefore display florid disseminated cancer within a few years.

Having said this, it must not be denied that the tumour-host balance may change favourably in the course of the disease as well as unfavourably. Indeed, there is some evidence that established lymph node metastases may regress. Palpable axillary nodes may disappear without surgical intervention after simple mastectomy[56,57], although whether this is a phenomenon relating to metastases or to reactive changes is not clear. When radical mastectomy was randomly compared to simple mastectomy, 40% of patients with impalpable nodes had axillary metastases proven histologically after the more extensive procedure. However, only 15% of clinically similar patients undergoing simple mastectomy required further treatment for axillary metastases[58]. In addition, there is experimental evidence in mice that regional lymph node cells may have a specific ability to destroy tumour cells[59].

Such unpredictable fluctuation would explain why some "node positive" patients may have a prolonged survival[60,61],

just as some "node negative" patients can die from the disease within a surprisingly short space of time. Simple pathological node status can therefore give some idea of prognosis in large groups of women, but is of little value in predicting the outcome for an individual patient. It is therefore important to determine whether other factors may be taken into account to obtain more precise prognostic information.

ii. The significance of the extent of nodal involvement by tumour.

Accepting that the mere presence of lymph node metastases indicates a poor prognosis, is it possible to refine this knowledge by examining the extent of nodal disease?

Certainly, the absolute number of lymph node metastases identified in a mastectomy specimen helps to narrow the field. Many studies have shown conclusively that the prognosis of breast cancer deteriorates with increasing numbers of involved axillary nodes[1,16,62-68]. There is some confusion, however, over the most convenient way in which to express this phenomenon.

In a study examining the effect of adjuvant chemotherapy, Fisher and his colleagues reported that, "Analysis of the data relative to the number of tumour-containing nodes revealed a distinct difference between those with 1 to 3, or those with 4 or more involved"[63]. This statement was not accompanied by details of the analysis, but it became accepted by many workers that 4 was a "magic number" and that a dramatic

worsening of prognosis mysteriously occurred when 4 or more nodes were invaded by tumour. As a result, several studies have analysed the effect of quantitative lymph node tumour involvement using only two categories - those above and below 4[16,63-66,68]. From such studies it has been postulated that patients with only 1 - 3 nodes involved are at particularly low risk within the "node positive" group as a whole. In fact, one authority goes as far as to state that patients with less than 4 nodal metastases are effectively "node negative."[68]

A sharp cut-off point unique to four involved lymph nodes is difficult to explain. Luckily, there are at least two studies where this distinction has not been made, and which allow the validity of this concept to be tested. Smith and his co-workers have looked at 385 patients undergoing radical mastectomy, and have related their 10 year survival to the number of involved nodes[67]. It is significant that no striking deterioration occurred when 4 glands were found to be invaded (Table I). Similarly, the American College of Surgeons have investigated the number of involved lymph nodes in terms of 5 year survival and recurrence rates[1]. This study was much larger as it comprised part of a national survey, and it included 20,547 patients who had undergone mastectomy, mostly radical or modified radical. The results are displayed graphically in Figure 3, and, again, no sharp demarcation point is seen.

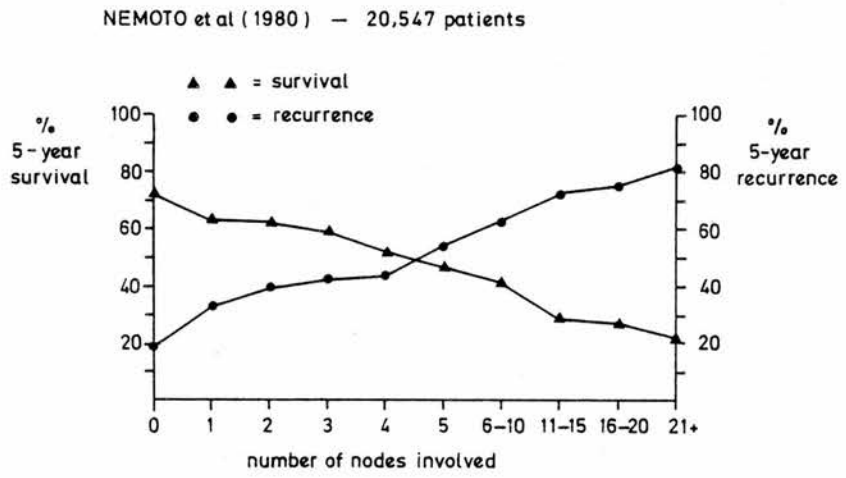
Although it seems that no abrupt deterioration occurs with 4 involved nodes the question is not completely resolved because the original thesis states that 1 - 3 involved nodes confers a much better prognosis than 4 <u>or more</u>. It is

Table 1

Smith 1977

Number of nodes involved	10 year survival
1	44%
2	58%
3	39%
4	33%
5	29%
6 - 10	9%
11 - 43	14%

Figure 3 Relationship between number of lymph nodes involved by metastatic tumour, recurrence of disease and survival rates at five years after treatment. Data from Nemoto et al.



relatively simple to examine this using the data from the American College of Surgeons survey. In table 2, the 5 year survival and recurrence rates have been arranged in 8 ways to demonstrate how the differences between all the possible subgroups vary. It is evident that no dramatic change occurs as more involved nodes are moved out of the "bad prognosis" group into the "good prognosis" group. Equally, no matter which cut-off point is used, there will always be a striking difference between the "good prognosis" group and the "bad prognosis" group, due to the cumulative effect of more involved nodes producing a proportionately worse prognosis.

Analysis of the available data, therefore, cannot support the thesis that having 1 - 3 involved lymph nodes is an especially privileged state. Indeed, reference to Figure 3 shows that the difference between one involved node and no involved nodes is quite definite, and that progressive worsening occurs as more nodes are found to be invaded by tumour. If the number of involved lymph nodes is to be used as a guide to the behaviour of an individual tumour,therefore, absolute numbers must be taken into account, at least until the numbers become large.

The position of lymph node metastases within the axilla has also been proposed as a prognostic factor. It has been suggested that nodal involvement at level 3 carries a worse outlook than metastases confined to level 1[8,9,69]. However, in the study of Smith and his colleagues, the survival curves for those having a single level involved did not vary between the three levels[67]. Similarly, the importance of

Table 2

Nemoto et al[1]

No. of involved nodes	5 yr. survival	5 yr. recurrence
1	63%	33%
2 +	48%	54%
1 - 2	63%	36%
3 +	43%	58%
1 - 3	62%	37%
4 +	40%	62%
1 - 4	61%	38%
5 +	37%	66%
1 - 5	59%	40%
6 +	35%	69%
1 - 10	56%	44%
11 +	28%	75%
1 - 15	54%	46%
16 +	26%	78%
1 - 20	53%	47%
21 +	22%	82%

radical mastectomy for the removal of Rotter's interpectoral nodes has been stressed[70], but Kay found that patients in whom these glands were the only ones involved had a remarkably favourable prognosis[71].

It is probable that the level of axillary metastatic disease is an indirect indicator of prognosis, operating through the number of nodes involved.

So far, we have confined our attention to axillary metastases, but the internal mammary chain must not escape scrutiny. W.S. Handley first drew attention to internal mammary node involvement[72], and R.S. Handley, by employing internal mammary node biopsy, showed that about 35% of patients with axillary metastases would also have deposits in these glands[73-75]. This has been confirmed by Donegan[76], and both authors found that survival was significantly worse in patients exhibiting metastases in both sites.

Biopsy, however, cannot be totally reliable, and more definitive information comes from studies in which extended radical mastectomy was employed. There are five reports in which the relative frequencies of axillary and internal mammary metastases are documented[48,77-80] and the combined results are shown in table 3.

The overall incidence of tumour deposits in the internal mammary nodes was 21%. Of patients with axillary metastases, 35% also had tumour in the internal mammary nodes. However, in patients with no axillary disease, only 8.5% had internal mammary metastases. In these studies, when survival and recurrence rates are considered, patients with axillary

Table 3

References 49,77-80

Ax - axillary
IM - internal mammary
\- - no metastases
\+ - metastases present

-IM -Ax	735
-IM +Ax	520
+IM -Ax	69
+IM +Ax	284

Table 4

	Medial tumours	Lateral tumours
-IM -Ax	64	169
-IM +Ax	12	115
+IM -Ax	12	9
+IM +Ax	26	36

metastases only show a very similar pattern to those with internal mammary metastases only, although when both are present the outlook is very much worse.

It would seem, therefore, that knowledge of the internal mammary node status gives us added prognostic information. This is only to be expected, as we already know that the number of involved glands is a powerful factor in this respect. Occult internal mammary metastases may thus provide a rationale whereby some patients with absent or minimal axillary disease have unexpectedly rapid progression of their tumour.

It might be logical to assume that the position of the tumour within the breast would give us a clue as to the likelihood of internal mammary involvement, and table 4 shows the combined results from two studies in which suitably detailed information was available[73,79]. These figures show that internal mammary metastases are commoner with medial tumours, the overall percentage being 32% as opposed to 14% with lateral tumours. In patients with no axillary metastases 15% of those with medial tumours had internal mammary metastases in contrast to 5% in those with lateral tumours. Conversely, the medial tumours were associated with axillary metastases in only 32% of cases where no internal mammary metastases were present, while this was the case in 60% of patients with lateral tumours. All these differences are highly significant by chi-squared testing.

Evidently, lateral and medial tumours most commonly metastasise to the axillary lymph nodes, but both may also spread to the internal mammary chain. In lateral tumours, medial dissemination is negligible when the axillary nodes are

clear, but in medial tumours, a small but substantial number of patients will have internal mammary metastases only. This does not indicate that medial tumours are particularly aggressive per se; the slight preponderance of cases in which medial tumours show both axillary and internal mammary extension is balanced by the fact that lateral tumours exhibit a higher frequency of axillary disease. A medial tumour with 3 involved internal mammary nodes and 3 involved axillary nodes may be equivalent to a lateral tumour with 6 involved axillary nodes.

To resolve this problem completely, one would require to determine the total number of internal mammary and axillary nodes which contained tumour. This is hardly necessary, however, as medial tumours do not carry a worse prognosis that lateral tumours[81-83]. Evidence for this greatly outweighs suggestions to the contrary[84,85].

Having established that the number of involved lymph nodes is important, it is of interest to discover whether the extent of tumour involvement in individual nodes has any bearing on the prognostic problem. Three independant studies have reported that extranodal extension of metastases identifies a group of patients at high risk of early recurrent breast cancer[86-88]. Interestingly, one of these studies demonstrated that this factor only operates when small numbers of nodes are involved, and that its significance fades in the face of widespread axillary disease[88]. There is also some tentative evidence that the presence of tumour in the efferent paranodal vessels may have grave implications[89,90], but this work has yet to be confirmed.

If large axillary metastases indicate unfavourable disease, does the converse hold? Saphir and Aromin, in 1948, demonstrated that serial section of axillary lymph nodes could pick up microscopic metastatic deposits which would have otherwise gone unnoticed[91]. Two more recent studies have shown that such occult metastases can be detected in 22 - 24% of patients who would have been classified as "node negative" by more limited sectioning techniques[62,92]. However, it was also found that the presence of these tiny foci of tumour had no effect of 5 year survival, and the patients in question appeared to have a similar prognosis to those in whom no axillary metastases whatsoever could be found.

When patients with proven axillary tumour are studied, those with small metastases seem to have a better prognosis. Fourteen-year survival has been found to be significantly better in patients whose axillary metastases were less than 2 mm in diameter[93], and other workers have confirmed that, alone, these "micrometastases" are virtually equivalent to no metastases[94].

It has been pointed out that when micrometastases only are present, it is very unusual for more than 1 or 2 lymph nodes to be involved[93]. This observation has led to the suggestion that the good prognosis associated with 1 - 3 lymph nodes is related to a high incidence of micrometastases in such cases[95]. The reverse argument may also hold, and Fisher has shown by multivariant analysis that the apparently favourable effect of metastases less than 2 mm in diameter was related to the number of nodes involved rather than to the size of the tumour deposits[94]. In the same paper, however, a group of

patients was identified in whom axillary metastases were less than 1.3 mm in diameter. These patients' prognosis was independant of the number of nodes involved, and was similar to those with no axillary metastases. Various methods to increase the probability of detecting small metastases in lymph nodes have been proposed[96,97], but in practical terms, such meticulous searching seems to be of little value.

Whichever cut-off point is used, there is good evidence that minute deposits of tumour within lymph nodes do not appreciably affect prognosis. Moreover, careful sectioning of lymph nodes reveals a much higher rate of tumour involvement than is usually quoted. These observations strongly support the hypothesis of tumour seedlings lying dormant or growing very slowly in patients with a favourable tumour-host relationship.

In summary, the extent of lymph node involvement by tumour is evidently a powerful prognostic factor in breast cancer, and it is clear that, for accurate staging, the number of involved regional lymph nodes should be determined as closely as possible. In addition, the size of individual metastases should be recorded, especially if only a few nodes are invaded.

iii. The relationship between lymph node metastases and other factors of prognostic significance.

We have seen that the presence of regional lymph node metastases indicates a balance between the tumour and the host which favours tumour growth. It would be reasonable to

suppose, therefore, that nodal involvement will be related to other factors which reflect the aggressiveness of the tumour, and the patient's resistance to it. There are many aspects of breast cancer which have been related to pathological lymph node status, and the main ones are listed in table 5. In order to discuss these relationships more easily, each will be considered separately.

Tumour size It is a constant finding that the likelihood of metastatic invasion of axillary lymph nodes increases proportionately with tumour size[98-103]. As a rough guideline, 37% of tumours under 2 cm in diameter would be expected to have associated lymph node metastases, whereas the figures are 50% and 63% for tumours of 2 - 5 cm and over 5 cm in diameter respectively[99]. There is also no doubt that the number of involved nodes is likely to be higher as the tumour increases in size[103].

In terms of the natural history of breast cancer, this phenomenon is likely to be related to two factors. Firstly, the larger tumours have probably been present for a long time, and breakdown of tumour-host synergism is more likely to have occurred. Secondly, large tumours may represent a faster growing, more aggressive population, again favouring neoplastic growth. Certainly, thymidine-labelled studies have confirmed that faster growing tumours are more likely to be associated with lymph node metastases[139].

Tumour size itself has a significant bearing on prognosis. In general terms, the larger the tumour the worse the prognosis, irrespective of lymph node status[99]. However,

Table 5

Prognostic factors	Relationship to positive nodal status	References
1. Tumour size	+	98-103
2. Tumour grade		
a. Nuclear grade	+ (with poorer grade)	104,105
b. Histological grade	+ (with poorer grade)	103,106-108
3. Tumour type		
a. Ductal	+	105
b. Colloid	-	105
c. Papillary	-	105
d. Medullary	N	105
e. Lobular	N	109
f. In-situ	-	110,113
4. Oestrogen receptor activity	N	114-124
5. Tumour contour	+	126-128
	N	125
6. Blood vessel invasion	+	103,129,130
7. Elastosis	?	100
8. Lymphocytic infiltration of tumour	+	100,131
9. Reactive lymph node changes		
a. Sinus histiocytosis	+	104,105
b. Paracortical hyperplasia	-	132
c. Germinal centre formation	+	94
d. Lymphocyte depletion	+	132
10. Nipple involvement	+	133,134
11. Premenopausal status	+	103
12. Pregnancy and lactation	+	135
	N	136
13. Males	+	137
	N	138

\+ positive correlation
\- negative correlation
N no correlation
? not known

although this relationship holds throughout the greater part of the range, there is an interesting variation. Fisher has reported that patients with tumours greater than 6 cm in diameter and no involved lymph nodes have a better prognosis than might be expected on size alone. In addition, there is a suggestion that tumours smaller than 1 cm but with involved nodes, have a worse prognosis than might be expected[99]. It is likely that small, node positive tumours tend to be fast growing and aggressive, whereas large, node negative tumours are slow-growing neoplasms which have existed for a long time in an environment which favours the host.

Tumour grade It has been established for many years that histological examination of the primary tumour can give some indication of its likely behaviour[140], and various grading systems have been used to predict prognosis. Nuclear grading, using pleomorphism, size, mitoses, nucleoli, and chromatin clumping has been shown to exert an effect of prognosis[141], and glandular differentiation is known to be a favourable factor[103,108].

Various attempts have been made to combine nuclear characteristics with degree of gland formation in a comprehensive histological grading system[106,108,142,143], and perhaps the most widely accepted is the Bloom and Richardson technique[106]. In the original paper it was reported that the three grades could identify three separate groups, and this has been confirmed by independant workers[131]. However, such a degree of accuracy is not always equalled; using the same system, Gorski confirmed

that grade one tumours have a particularly good outlook, but could not show that grades two and three were separate in this respect[107].

One problem with all histological grading techniques is their reliance on subjective observation, and it has been repeatedly shown that a considerable degree of inter- and intra-observer variation exists within these systems[144-146]. Accepting this limitation, however, it is possible to gain some idea of the behaviour of a breast tumour on histological grounds alone. If grade represents the aggressiveness of the neoplasm, then the frequency of lymph node metastases would be expected to correspond, and this does seem to be the case. Reference to table 6 demonstrates that, in two separate grading systems, the better differentiated tumours are associated with a lower frequency of lymph node metastases than the more anaplastic ones.

It will also be noticed that, although a relationship between histological grade and nodal status does exist, they are not entirely interdependant. Grade has some effect on prognosis regardless of whether nodal metastases are present.

Tumour type Tumour type is a histological term which refers to specific patterns of neoplasm as opposed to simple grading characteristics. In breast cancer, the commonest type comprises a heterogeneous group of tumour patterns which are not recognised to have any special or constant features. They are referred to as "infiltrating ductal carcinomas" and it is to these tumours that grading systems are normally applied. The other types have highly individual histological appearances, and tend

Table 6 5 year survival related to histological grade and pathological node status. The percentages refer to survival, and the numbers in brackets refer to the absolute number in each group.

Bloom and Richardson[106]				Fisher[108]			
Grade	5 year survival			Grade	5 year survival		
	Total	Node +	Node -		Total	Node +	Node -
I	75% (362)	66% (147)	86% (145)	I	100% (16)	100% (2)	100% (14)
II	49% (640)	33% (265)	68% (324)	II	72% (174)	55% (85)	95% (89)
III	32% (407)	19% (86)	64% (236)	III	62% (397)	45% (235)	85% (162)

to behave in a specific manner[147]. It is therefore common practice to omit such tumours from studies of tumour grade.

In general, it is found that specific tumour types which carry a good prognosis are also associated with a low incidence of metastases to the regional lymph nodes[105,109].

An interesting facet of this observation involves in-situ carcinoma where the neoplastic cells appear to be confined to the ducts or lobules of the breast. These tumours are considered to be in a pre-malignant state, and accordingly prognosis after mastectomy is extremely good[112]. It would also be expected that lymph node metastases would not occur in this situation, but, surprisingly, they are sometimes found. The incidence is low, varying between 1% and 12%[113,127], but why they should occur at all is obscure. In some cases, the original diagnosis may be disproven by more careful sectioning, and a small focus of invasion found[111]. Rosen, however, has reported on eight patients with lymph node metastases in whom exhaustive sectioning of the breast tissue after mastectomy revealed nothing but intralobular or intraductal carcinoma[148]. Some light is shed on this problem by electron micrographic evidence that cells from in-situ lesions can appear to penetrate the basement membrane of ducts[149], but the question is not yet answered.

Oestrogen receptor activity The use of cytoplasmic oestrogen receptor estimations to help select patients with breast cancer for endocrine therapy is well established[150,151]. It has also recently emerged that patients with appreciable amounts of receptor in their primary tumour have an improved disease-free

survival over those whose receptor levels are low[115,118,119,152]. Surprisingly, however, oestrogen receptor activity does not appear to be related to pathological node status[114-124]. This is difficult to explain, especially as some other factors which are associated with nodal metastases are similarly related to oestrogen receptor status. For example, poor histological grade or unfavourable tumour type have frequently been associated with low levels of receptor[117,153-156], and thymidine labelling has shown that fast-growing tumours tend to be receptor negative[157].

The reason why oestrogen receptor positive tumours behave in a less aggressive fashion is uncertain, but it may be reasonable to assume that receptor activity is a biochemical expression of neoplastic differentiation. It is strange, therefore, that its effect on prognosis is not mirrored by nodal status at the time of mastectomy.

As a sideline to this issue, two recent studies have reported that, in oestrogen receptor positive tumours with lymph node metastases, the receptor activity is significantly higher in the nodal deposits[158,159]. This observation is related to the fact that neoplastic epithelial cellularity tends to be greater in lymph node metastases than in the primary cancer[159]. It has been suggested, therefore, that nodal metastases should be sampled for receptor assay whenever possible, as they may provide a more accurate measure of the tumour's ability to bind oestrogen[158].

Tumour contour A spiculated border is thought to be a sinister feature in a breast cancer. Three studies have shown that

survival rates are higher when a tumour exhibits a well-delineated border, both on mammographic and naked-eye appearances[126,128,160]. It has also been reported that lymph node metastases are commoner with an irregular or infiltrating pattern of tumour[126-128], but a careful study by Stewart and her colleagues could not confirm this observation[125].

Blood vessel invasion Blood vessel invasion is determined by histological examination of a primary tumour using an elastic stain to delineate veins and arteries. The phenomenon is associated with a poor prognosis in breast cancer[103,129,130] and is related to the presence of lymph node metastases[129,130]. Again, however, it can be shown to exert an influence on the disease process independently of node status[130].

Elastosis Focal elastosis within breast tumours is believed to correlate both with a good prognosis and with oestrogen receptor status[120,159-163]. Fisher makes a passing comment that pathologically negative nodes are associated with absent elastica[100], but there are no satisfactory studies relating nodal status to focal elastosis. It is reported that elastosis within lymph node metastases is very rare[164].

Lymphocytic infiltration Lymphocytic infiltration around and within a breast tumour was once believed to represent a favourable host response to the tumour[165]. This theory is now given little credence, except in the unusual case of medullary carcinoma[143], and a dense chronic inflammatory infiltrate is

generally found in the more poorly differentiated tumours[131]. It is therefore hardly surprising to find it associated with a high incidence of metastases in the draining lymph nodes[100].

Reactive changes in lymph nodes There are several histological phenomena seen in the regional lymph nodes draining breast cancer which are believed to represent an immunological response to the tumour. The most widely recognised of these are sinus histiocytosis[100,104,105], germinal centre formation[94,132] and paracortical hyperplasia[132]. Both sinus histiocytosis and paracortical hyperplasia are associated with a good prognosis, whereas germinal centre formation tends to be less favourable. In addition, lymph nodes which show signs of "immunological exhaustion" in the form of lymphocyte depletion and fibrosis, are seen in patients with a poor outlook[132].

True to form, lymph node metastases appear less commonly in association with favourable prognostic factors. Only about 25% of patients with appreciable sinus histiocytosis in their axillary lymph nodes will have co-existing metastases[105,132] whereas the figure is closer to 50% in those showing lymphocyte depletion. This subject is considered in greater detail in the second literature review.

Nipple involvement Careful sectioning and histological examination of the nipple from a breast containing invasive carcinoma will reveal tumour in a high percentage of cases (24% - 50%)[133,134]. This may be invasive or purely intraductal, but both patterns are associated with a high incidence of lymph

node metastases[133,134]. Whether this is related to the dense lymphatic plexus around the areola, or whether the nipple involvement purely represents more aggressive disease is not known.

Menopausal studies Menopausal status itself does not appear to be related to the finding of lymph node metastases, but a recent study has demonstrated that pre-menopausal patients under 45 years of age are more likely to have pathological stage 2 disease[103]. It was also found that these younger patients had a higher rate of recurrence within 2 years of treatment, but this was not independent of nodal involvement.

Pregnancy and lactation Breast cancer arising during pregnancy or lactation is unusual, and it is therefore difficult to study large numbers of patients. Holleb and Farrow managed to collect 117 women who presented with breast cancer during pregnancy or in the immediate post-partum period[135]. They found that 72% had axillary metastases, and that 5 year survival was 17% for those with involved nodes and 65% for the others. Comparing these figures with expected values, it would seem that patients who are pregnant or lactating have a higher incidence of nodal metastases, and that those who do have an unusually poor prognosis.

However, these findings are not universal, and Maier has reported that only 47% of 79 patients who were pregnant or in the post-partum period had nodal metastases at the time of first treatment. This compared with 47% of 647 women under 40 years of age who were not pregnant[136].

Hopefully, the prospective collection of information on such patients will provide a conclusive answer in the future.

Males It is well recognised that breast cancer in males has a particularly poor prognosis, but there is some debate as to whether lymph node metastases are commoner at presentation than is the case in women. Crichlow reported that, of 43 cases, 60% had nodal involvement[137], and Heller gave a figure of 54%[138]. However, the latter report stated that the incidence was similar to that in a comparable consecutive group of women.

There is no argument, however, that men with lymph node tumour deposits do badly; the 10 year survival rates in these two studies were 11% and 4.3% respectively.

Conclusion Not surprisingly, most factors which are known to indicate a poor prognosis tend to be associated with lymph node metastases. However, in at least one situation - that of oestrogen receptor activity - this is not the case, and even the other factors are by no means absolutely dependent on nodal status. Presumably this represents a wide variation in the relative importance of different aspects of the tumour and the host response in arriving at the balance which exists at the time of mastectomy.

Because of this relative or absolute independence of prognostic factors, it should be possible to combine them to produce a more accurate prediction than lymph node status alone can provide. This has been performed retrospectively[101,162] but, as yet, no attempt has been made to test a multifactorial prognostic index prospectively. Nodal status appears to be the

most important prognostic factor, and this has been demonstrated by multiple regression analysis in comparison with other pathological findings[168]. If prognostic factors are to be combined effectively, however, it is vital that interrelationships are carefully established.

The Detection of Lymph Node Metastases in Breast Cancer

i. Histology

As in so many aspects of surgical pathology, the definitive diagnosis of lymph node metastases in breast cancer lies with the histopathologist. Although even microscopic examination can miss minute foci of tumour, we have already seen that these are of very little importance, and exhaustive sectioning of lymph nodes is probably of no value.

There is another problem, however, which may confront the pathologist, and which must be borne in mind when cells of mammary origin are seen in the regional nodes. Inclusions of benign breast tissue occasionally occur within lymph nodes[169-171]. These may resemble normal breast ducts[171] or may consist of cystic structures with features suggesting apocrine or sweat gland origin[46,169,170]. The histiogenesis of such lesions is not certain, but embryonic inclusion or embolisation have been suggested[172]. Axillary lymph node tumour deposits of apparent origin in the breast are occasionally found without a concomitant breast tumour[173], and it may be possible that benign inclusions can undergo malignant change.

Despite this rare problem, however, histopathology is reliable, and microscopic examination of the regional lymph nodes remains the standard against which all other methods of detecting metastases must be tested.

ii. Clinical examination

Clinical examination of the axillary lymph nodes can give some indication of prognosis in breast cancer, and various clinical staging systems have been developed[174]. Perhaps the most widely used is the international TNM system[175], and this has five categories of palpable lymph nodes (Table 7). Ten year survival will be about 60% for those with no palpable axillary nodes (N_0), 50% for those with freely mobile palpable nodes (N_1), and 20% in patients with fixed palpable nodes (N_2)[176].

It can be seen from such data that clinical examination predicts prognosis in the same way as histological examination. However, in 1960, McNair and Dudley showed that axillary nodes could be palpated in 37% of female patients with no clinically evident breast pathology[177]. They also pointed out that clinicians differed in their opinion about nodal status in 40% of cases. In a direct comparison between clinical and pathological staging, Wallace and Champion demonstrated that a pre-operative diagnosis of involved axillary nodes was correct in only 45% of patients, and that a "node negative" diagnosis was correct in 75%[178]. Similar discrepancies have been found in at least four other studies[101,179-181].

All this evidence suggests that clinical examination of the axilla is not a useful investigation in breast cancer. However, one study has reported that the finding of clinical stage N_{1b} is just as accurate in predicting two year recurrence as histological involvement of nodes[182]. Unfortunately, the numbers in this study were small, and a direct comparison between clinical and pathological findings was not made. A

Table 7

N_0	-	No palpable homolateral axillary lymph nodes
N_{1a}	-	Moveable homolateral axillary lymph nodes but not suspected to contain metastases
N_{1b}	-	Moveable homolateral axillary lymph nodes suspected to contain metastases
N_2	-	Homolateral axillary lymph nodes fixed to one another or to other structures, and suspected to contain metastases
N_3	-	Homolateral supraclavicular or infraclavicular lymph nodes suspected to contain metastases or oedema of the arm

Classification of palpable homolateral axillary nodes in the international TNM system[175]. Contralateral nodes are considered to be distant metastases.

careful analysis of the reasons for the various degrees of clinical node enlargement, and their prognostic significance related to the presence or absence of histological tumour deposits has yet to be made.

At present, we must accept that, whatever else it might tell us, clinical examination is not a reliable indicator of metastatic disease in the axilla. We must therefore look to other techniques if we wish to obtain accurate lymph node staging pre-operatively.

iii. Investigative techniques for pre-operative staging of lymph nodes.

Several methods have been proposed and tried in the pre-operative detection of lymph node metastases, and we shall consider five of these in turn.

Needle aspiration In Stockholm, needle aspiration biopsy and subsequent cytological examination has become the routine method of establishing the diagnosis of primary breast cancer[183]. Percutaneous aspiration can therefore be used to confirm metastatic disease in enlarged axillary lymph nodes[184]. However, as nodal tumour deposits are by no means always palpable, this method cannot be used to "screen" the axilla.

Mammography Conventional X-rays can be used to visualise the axillary lymph nodes[185], but although it is possible to identify

gross metastatic disease, radiographic axillary studies cannot preclude nodal involvement by tumour[5].

Computerised tomography There is one report in which increased density in the area of the internal mammary nodes was seen in six out of 46 post-mastectomy patients using computerised tomography[186]. There was no attempt at histological confirmation of metastases, however, and the number of patients who would be expected to have internal mammary node involvement is about twice this number. Computerised tomography does not yet seem to be particularly useful in this context.

Lymphangiography It is possible to visualise axillary lymph nodes on X-ray by the injection of contrast into the lymphatic vessel on the dorsum of the hand. This technique has been tried in an attempt to identify nodal metastases in breast cancer, but has proved unsuccessful. Fatty deposits, which are common in axillary lymph nodes, can closely mimick foci of tumour on lymphangiograms[187].

Lymphoscintigraphy In 1953 it was shown that lymph nodes could selectively concentrate radio-labelled colloidal material which had been introduced into the interstitial tissues[188]. Simultaneously, two groups of workers reported that the internal mammary nodes could be visualised using this technique[189,190], and since that time there has been great interest in utilising lymphoscintigraphy to detect internal mammary metastases. Antimony sulphide colloid has been shown to be satisfactory[191,192] and is the most widely used material.

In a series of papers Ege has described her experience with internal mammary lymphoscintigraphy[193-195], and although the results are hampered by a lack of histological confirmation, they are worth considering in detail. Five hundred miCu of isotope-labelled colloid was administered bilaterally into the rectus sheath by subcostal injection, and scintigraphic images of the parasternal regions obtained using a gamma camera. A normal scan was defined as the localisation of isotope in the upper intercostal spaces on the same side as the tumour[193]. An abnormal scan, which was taken to represent metastatic disease, was characterised by absence of isotope in the upper parasternal region, or by the presence of an isotope "blush" presumably related to obstruction of lymph flow[196]. In 439 patients, internal mammary lymphoscintigraphy results were related to histological evidence of metastases in the axillary lymph nodes. The results are shown in Table 8 alongside the expected incidence of internal mammary metastases as reported in five studies of extended radical mastectomy.

From this comparison, it appears that internal mammary lymphoscintigraphy may well reflect the presence of metastases, a view supported by the poor prognosis which accompanies an abnormal scan especially when axillary metastases are present[195,196]. Nevertheless, it would be reassuring to have some data providing histological confirmation of this assumption. The only available information comes from Osborne and his colleagues, who carried out internal mammary biopsy in 15 breast cancer patients who had undergone lymphoscintigraphy[197]. They found that four abnormal scans were confirmed histologically, and that biopsy revealed metastases in only one out of eleven

Table 8

	Internal mammary lymphoscintigraphy and axillary histology	Histology from extended radical mastectomy specimens
-IM -Ax	146 (33%)	735 (46%)
-IM +Ax	175 (39%)	520 (32%)
+IM -Ax	28 (7%)	69 (5%)
+IM +Ax	90 (21%)	284 (17%)

IM - internal mammary
Ax - axillary
- - "node negative"
+ - "node positive"

patients with normal scans. These numbers are very small, however, and a larger study is required, especially as biopsy may miss the presence of metastases in the internal mammary chain.

Quite apart from its value in detection of metastases, lymphoscintigraphy has been used to map out the internal mammary nodes in order to plan radiotherapy fields[198,199]. There is no evidence, however, that such precision is in any way advantageous.

Lymphoscintiscans have also been employed to study the axillary lymph nodes in breast cancer. In the original reports by Boak and Agwunobi, 100miCu of technetium-labelled antimony sulphide colloid was injected subcutaneously around the nipple, and gamma camera scans were performed three hours later[200]. It was found that patients with breast cancer had depressed uptake in the ipsilateral axillary nodes when compared to patients with benign breast disease; this confirmed previous animal experiments[201]. It was suggested that the depressed ability of regional lymph nodes to accumulate colloid in the presence of breast cancer might be used as a diagnostic tool, but, although confirmatory work has been published by the same group[202], independent studies have not been able to reproduce these results[203,204].

However, there have been reports which suggest that axillary lymphoscintigraphy may be of value in the detection of metastatic tumour. Black and his colleagues studied 30 breast cancer patients using intradermal injections of labelled colloid around the nipple[205]. Of 27 satisfactory scans, nine

were abnormal in that there was depressed axillary uptake on the side of the lesion, and all of these patients had gross nodal metastases proven histologically. In the 16 patients with normal scans, three had axillary metastases, but in two they were only micrometastases. In a similar study by Osborne, in which technetium-labelled liposomes were used, 24 patients were scanned; there was one false positive result and two false negatives[206]. Lymphoscintigraphy has also been used to detect groin lymph node metastases in malignant melanoma with the same degree of success[207].

These results are indeed promising, but there is at least one dissenting voice. Peyton and his colleagues have shown that a node containing tumour can take up colloid, and their results using the technique in breast cancer patients were poor[208].

The place of lymphoscintigraphy in the diagnosis of nodal metastases is unresolved, and careful analysis of its accuracy in terms of histological correlation is required. This must be done on much larger numbers of patients than has previously been reported, and a superiority over clinical examination must be demonstrated before its routine use can be contemplated. Meanwhile, other scintiscanning agents are being tried, and claims have been made that labelled DTPA can differentiate between benign and malignant breast disease[209,210]. Should this prove to be generally useful, it might be possible to modify the method to scan the axilla for foci of tumour.

iv. Operative and pathological methods of obtaining lymph nodes for histological examination

Considerable debate rages over the question of how extensive an operation is required to obtain an adequate sample of nodes. It has been claimed that radical mastectomy, which involves the removal of both pectoral muscles as well as total axillary clearance, is the only method of obtaining a true picture of the axillary lymph node status[70]. On the other hand, modified radical mastectomy, which preserves pectoralis major, has been proposed as an equally comprehensive procedure[211].

One group of workers performed a series of 50 radical mastectomies in two stages; the first stage involved simple mastectomy and axillary clearance, and in the second stage they removed both pectoral muscles[212]. In 36 patients, extra lymph nodes were found in the second-stage specimen, and in eight of these metastases were discovered. This study purported to show that modified radical mastectomy is an unreliable guide to lymph node status, but it suffered from one major fault. The pectoralis minor muscle was left intact, and it is recognised practice to divide this in order to obtain adequate access to the axilla[213-215]. Nemoto and Dao have reported that the mean number of lymph nodes from a series of 121 consecutive radical mastectomies was 23.4, whereas the mean yield from a subsequent series of modified operations was 25.7[216]. Similarly, other studies have noted no striking difference between the number of nodes found in these two types of procedure[215,217].

The position within the axilla at which metastases are likely to be found is another important factor when deciding on a sampling procedure. In 342 cases of radical mastectomy, Berg found metastases situated at the apex in only 9%, and of these, only one case had no tumour deposits lower in the axilla[8]. Likewise, Auchincloss has demonstrated that involved lymph nodes tend to be found in the lower two thirds[218]. Such evidence has prompted the belief that lower axillary sampling may be sufficient to obtain pathological node status[219,220], and this concept is worth considering further.

In the Cardiff-St. Mary's trial comparing radical mastectomy with simple mastectomy and pectoral node biopsy, Forrest reported that although nodes were identified slightly less frequently in the conservatively-treated group, the incidence of metastatic involvement of axillary glands was marginally higher. On the other hand, 10.5% of patients considered to be node negative by the sampling procedure subsequently developed enlarged axillary glands requiring radiotherapy[221]. A study by Davies and his colleagues showed that histology of the lower axillary lymph nodes in axillary clearance specimens failed to detect metastases in 14% of cases who transpired to have tumour deposits on further examination[222].

It would seem from this evidence that lower axillary lymph node sampling will miss the presence of metastases in 10 - 15% of patients. However, the high incidence of failure to find any lymph nodes in the Cardiff-St. Mary's trial suggests that an adequate search for nodes had not always been carried out. Also, in the study by Davies, the procedure of separating

lower axillary nodes from a clearance specimen is rather an artificial one, and if a thorough search for lymph nodes is made during lower axillary sampling, these results might be improved.

It will be argued that limited sampling cannot be expected to allow determination of the total number of involved nodes, a factor which is of some importance. However, Fisher and Slack have shown that the number of glands actually examined from a radical mastectomy specimen has no bearing on the prognosis[64], and as up to 13 nodes can be obtained from simple mastectomy and sampling[219], an adequate lower axillary dissection might provide useful quantitative prognostic information. To assist the intra-operative identification of lymph nodes, disulphine blue dye given as a pre-operative intra-mammary injection can be helpful[223].

Various other forms of nodal biopsy have been proposed as adjuncts to mastectomy or as an indication of operability[224-229]. None of these, however, can give a reliable indication of the state of the axilla. In particular, apical node biopsy may easily miss an axilla which is heavily involved by tumour[8].

When a radical or modified radical mastectomy has been performed, a careful search must be made to identify the lymph nodes within it. The literature reports a strikingly wide variation in the average number of nodes found in the axillary contents; one report mentions eight as the mean total[230], whereas, at the other end of the scale, 50 is the number quoted[70].

The reason for this is not clear, but it has been suggested that the method used to isolate the nodes is of critical importance[70]. A search can be made by manual palpation of the

specimen, serial section, or clearing the fat to render the nodes visible[231]. A radiograph of the excised axillary contents may also assist the dissector[232]. However, in an extensive study of 2,000 radical mastectomy specimens from 46 different institutions, Fisher and Slack could not show that the number of nodes found bore any relationship to the methods used. They concluded that the diligence of the pathologist must be the most important factor, with variation in anatomy and surgical technique as secondary causes for the wide discrepancies[64].

In conclusion, until more information is available on thorough lower axillary sampling, the method of choice in detecting regional lymph node metastases appears to be total axillary clearance with a careful search through the specimen. Although radical mastectomy may pick up a few extra nodes, these are unlikely to be of significance, and the modified operation is almost certainly sufficient. Internal mammary biopsy may be a useful adjunct, especially in medial tumours.

The Treatment of Lymph Node Metastases in Breast Cancer

i. Methods of treatment

Having examined the significance of lymph node metastases in breast cancer and the available methods of detecting them, we come now to the vexed question of how they should be treated.

There is little doubt that breast carcinoma requires some form of local therapy. In a review of patients with untreated breast cancer, Bloom reported that 73% had marked ulceration of the breast at death, and that 21% had extensive destruction of the chest wall. He also pointed out that all untreated patients will die from or with obvious tumour, and that their five year survival from onset of symptoms was 20%[233]. This data can only lead us to conclude that the available local treatments for breast cancer can improve survival and morbidity, although nothing can be said about its effect on the incidence of metastatic disease.

It still remains to determine the optimal form of local therapy for cancer which is apparently confined to the breast and regional nodes. Although evidence now exists that primary radiotherapy may be useful[234-236], such treatment has not been subjected to randomised clinical trials[237]. Mastectomy, therefore, remains the treatment of choice in breast cancer.

As an adjunct to this operation, the regional lymph node bearing areas may be excised, irradiated, or left alone. Before embarking on an examination of the relative merits of

various approaches, it may be useful to define the meaning of the terms which will be used.

Simple mastectomy - removal of the entire breast without removal of the pectoral muscles. This operation may be supplemented by sampling the lower axillary lymph nodes[221], but axillary clearance is avoided.

Modified radical mastectomy - introduced by Patey in 1948[213], this operation involves removal of the breast and the axillary contents. Pectoralis major is left intact, but pectoralis minor may be removed or divided to facilitate access to the axilla[215]. The interpectoral (Rotter's) nodes may be left in this procedure, but a method of excising them through a fenestration in pectoralis minor has been described[238].

Radical mastectomy - although often attributed to Halstead[239], this was first described by Moore in 1867[240]. The breast, pectoralis major, pectoralis minor and the entire axillary contents are removed in the manner of a classical cancer operation.

Extended radical mastectomy - first described by Urban in 1955[241], this procedure combines radical mastectomy with excision of the internal mammary node chain.

Adjuvant radiotherapy - this is normally given in the form of X-rays to the axillary, supra- and infra-clavicular and parasternal node-bearing areas. It can be used a s an adjuvant

to simple mastectomy[242], or to radical mastectomy[243]. The usual dose is about 4,500 rads, but there is wide variation in the literature.

Chemotherapy - the role of chemotherapy lies with the treatment of systemic disease and cannot be regarded as specific for lymph node disease. Some workers have, however, attempted to ablate lymph node metastases using bleomycin emulsions, but this is still at an experimental stage[244].

ii. Uncontrolled studies

Of all the reported series, the best results come from Haagensen who has reported an overall ten year survival of 60% in 1,000 patients treated by radical mastectomy[245]. These results have been used to support the notion that radical mastectomy is the mainstay of breast cancer treatment[246], but such a view is not generally accepted[2]. The conclusion drawn from this series is difficult to condone, as the patients were very carefully selected using criteria of operability which are by no means universal[46]. It is certain that these patients belonged to a prognostically favourable group, and as such, they cannot be compared meaningfully to other studies. For instance, another large series of patients treated by radical mastectomy displayed a ten year survival rate of only 54.6%, probably because they were not so carefully selected[247].

Haagensen, however, is quite adamant that a more

radical and more meticulous technique gives better results[248], and he categorically states that removal of involved lymph nodes can effect a cure in breast cancer[70]. If this is so, then radical mastectomy per se should produce superior results to less ablative procedures, and this has never been demonstrated.

In 1948, Patey reported that a modified radical mastectomy was as satisfactory as the full Halstead-type procedure[213], and in 1967 longer follow-up revealed no difference in survival or disease recurrence[48]. This finding has received support from the Mayo Clinic, where no difference in five year survival could be detected between patients treated by radical or modified radical mastectomy, irrespective of pathological node status[217]. These reports are by no means controlled studies, but they do suggest that radical removal of axillary and interpectoral nodes along with both pectoral muscles has no striking advantage over the modified procedure.

To look towards the other extreme, there have been attempts to demonstrate the superiority of extended radical over conventional radical mastectomy. Both Sugarbaker[49] and Caceres[78] reported improved overall five year survival rates in patients treated by the extended procedure, but these findings were not confirmed by a properly controlled study[249].

In the present climate of the controlled randomised clinical trial, it is easy to dismiss these uncontrolled studies as worthless, but is must be remembered that the knowledge gleaned from them has contributed much to our understanding of the disease.

iii. Controlled trials comparing surgical procedures

It is difficult to obtain information pertaining solely to the effect of surgical treatment on breast cancer, as most of the randomised trials comparing different surgical approaches to the regional lymph nodes are compounded by the application of radiotherapy. This stems from the concept proposed by McWhirter that simple mastectomy should be supplemented by radiation to eliminate residual regional metastases[242]. Nevertheless, it is possible to draw some conclusions from a few studies.

The Cardiff-St. Mary's trial compared simple mastectomy and node sampling with radical mastectomy in patients with histologically uninvolved nodes, and after follow-up for three to nine years, no difference could be detected in survival rate, duration of life or incidence of metastatic disease[250]. In this trial, patients with involved nodes were similarly randomised, but also received adjuvant radiotherapy, and although there was a slight trend in favour of the conservative treatment, no statistically significant difference was seen. This is hardly a fair comparison of surgical treatments, however, as the patients treated by simple mastectomy had a smaller dose of radiation. A similar difficulty is encountered with the Edinburgh trial where simple mastectomy with radiotherapy to the axillary, supraclavicular and parasternal regions was compared to radical mastectomy alone[251]. The 12 year follow-up data showed a significant prolongation of survival in the patients treated by the radical operation[252], but it is

impossible to disentangle the relative effects of surgery and radiotherapy. This problem arises again in other studies where different surgical techniques were compared, but with radiotherapy as an adjunct to the conservative approach[58,253,254].

The Hammersmith trial is of interest, as it compared simple mastectomy with radical mastectomy, both groups receiving similar radiotherapy to the axilla and to the clavicular and parasternal regions. The five year survival rates were 65% for the conservatively treated group and 70% for the radical group, but this difference was not significant[255].

In 1976, the results were published of a large multinational trial comparing radical mastectomy with extended radical mastectomy with no radiotherapy given in either group. Overall there was no difference in five year or ten year survival[72].

Finally, the Guy's trial comparing wide local excision of the tumour with radical mastectomy in clinical stages 1 and 2 is worth considering in this context. Both groups received radiotherapy, and no difference was found in survival or distant recurrence rates[256]. This study has fallen into disrepute owing to a high local recurrence rate attributed to inadequate irradiation, but it is of interest that the incidence of generalised disease was not affected by removal of the axillary nodes. Unfortunately it is not possible to determine the effect that metastatic involvement of the lymph nodes may have had on the outcome in the two groups. This problem may be overcome in the future by combining local tumour excision with axillary sampling[257], and a recent trial of

quadrantectomy combined with axillary clearance and radiotherapy has demonstrated no difference from radical mastectomy[258].

From the limited information available, it seems that surgical excision of regional nodes in breast cancer cannot alter the course of systemic disease. However, it is also important to examine its effect on local disease. Again, data are scanty, but Forrest reported that in patients with histologically negative nodes, those treated by simple mastectomy had a 13% axillary recurrence rate as opposed to a 4% rate in the radically-treated group[250]. This almost certainly represents axillary metastases which were missed by the more conservative procedure. When radiotherapy is brought into the picture, the difference in local recurrence rates between the two types of operation is less[250], although the Edinburgh trial has demonstrated a superiority of radical mastectomy over simple mastectomy in this respect.

Comparison of extended radical with radical mastectomy revealed that parasternal recurrence was only seen in the group treated by the lesser operation. However, this occurred in only 4% of cases, which was less than might be expected from the rate of internal mammary involvement in patients treated by extended radical mastectomy[80].

It appears, then, that more extensive surgical procedures may spare a small proportion of patients the inconvenience of tumour recurrence in the regional nodes (Table 9). However, the advantage of radical surgery, even in this respect, is minimal, and it is therefore important to look at the question of morbidity.

Table 9

Study	No. of patients with local recurrence in lymph nodes	P
Forrest 1977[250]		
Node - simple Mx	10/75	
radical Mx	3/79	N.S.
Node + simple Mx and XRT	2/49	
radical Mx and XRT	2/40	N.S.
Burn 1976[255]		
Simple Mx and XRT	8/76	
Radical Mx and XRT	4/76	N.S.
Langlands 1980[252]		
Simple Mx and XRT	30/242	
Radical Mx	7/256	< 0.001
Veronesi 1981[80]		
Radical Mx	15/375	
Extended radical Mx	0/342	< 0.001

Mx - mastectomy
XRT - X-ray therapy

Arm oedema and functional disability following radical mastectomy are well recognised[259,260], but it is important to make comparisons within randomised trials. Burn reported that the incidence of stiff shoulder and arm swelling was the same in patients treated by simple or radical mastectomy, but both groups were also treated with radiotherapy[255]. On the other hand, analysis of arm elevation and arm diameter in patients treated within the Cardiff-St. Mary's trial showed a distinct advantage for simple mastectomy, both in irradiated and non-irradiated groups[261]. Such studies are sadly few, and more intensive investigation is required in this area.

Having examined the effect of different surgical approaches to the regional lymph nodes on generalised disease, local recurrence and morbidity, we must now subject the various trials of adjuvant radiotherapy to similar scrutiny.

iv. Controlled trials designed to evaluate adjuvant radiotherapy

When examining the treatment of node-bearing areas by adjuvant radiotherapy, our chief interest lies in its effect on generalised recurrence and survival. There are many trials which yield information of importance in this respect, but, again, some are difficult to interpret owing to variation in the types of surgery and radiotherapy.

Three trials have compared simple mastectomy and radiotherapy with radical or extended radical mastectomy alone [252-254]. Two of these could demonstrate no difference in survival at five[254] or at ten[253] years, but the Edinburgh trial

reported a significant advantage for patients treated by radical mastectomy only, both in terms of absolute survival, and length of survival after the detection of distant metastases[252]. In none of these studies was it possible to analyse the results according to pathological node status.

Fortunately, there are several randomised studies in which the surgical procedure was constant. Both the Manchester[254] and the Kings/Cambridge[262] trials looked at the effect of radiotherapy with simple mastectomy, the former in clinical stage 1 patients only, and the latter in all operable cases. In neither study was there any difference in survival between the irradiated groups and the controls. Easson, writing in 1968, could demonstrate no difference in ten year survival rates between patients given radiotherapy after radical mastectomy and those having surgery alone[243]. Histological node status had no effect on the distribution of these results. Similarly, Fisher found no advantage or disadvantage attached to adjuvant radiotherapy in the NSABP (protocol B-04) trial after five years follow-up[265] even after protocol violations had been analysed[266].

Wallgren, however, has recently reported promising results from the Stockholm trial which compared pre-operative radiotherapy, post-operative radiotherapy and no adjuvant treatment in patients undergoing modified radical mastectomy[264]. In this study, the patients given pre-operative treatment had significantly improved survival over the controls, although those receiving post-operative irradiation were not advantaged. Lastly, the Oslo trial, which was designed to evaluate radiotherapy with radical mastectomy, has also produced some

hopeful results. The first report suggested that distant metastases actually appeared earlier in node-positive patients when they were given radiotherapy[267]. However, the latest bulletin included the data pertaining to cobalt-60 which produced a higher dose of radiation than the conventional X-ray technique used previously, and the cobalt-treated patients had a statistically significant survival advantage over the controls[268]. This sounds exciting, but there are two major flaws in the arguement. Firstly, the advantage was evident only at five years after treatment, and after this time the survival curves on the life table converge. Secondly, the controls used in the analysis included the controls from the earlier part of the trial when X-rays were being used, so that the groups used for comparison were not entirely contemporaneous.

Finally, a recent trial from America has suggested that adjuvant radiotherapy is deleterious to disease-free survival when combined with adjuvant chemotherapy[269]. This is an important finding and must be taken seriously, although the numbers of patients are small, and follow-up time short.

The controversy over adjuvant radiotherapy has been prolonged, bitter, and, above all, unresolved[270-272]. In a review of controlled trials in 1977, Stewart concluded that simple mastectomy and radiotherapy was as effective in terms of survival and recurrence rates as radical mastectomy with or without radiotherapy[273]. In addition, she pointed out that post-operative radiotherapy did not prolong survival following radical mastectomy. However, the Edinburgh trial would now suggest that radical mastectomy may be slightly superior to

simple mastectomy and radiotherapy[252]. To confuse matters further, moreover, the Stockholm and Oslo trials provide tentative evidence that radiotherapy may be a useful adjunct to radical or modified radical mastectomy[274].

Is it possible to resolve the dilemma by combining the available trial results? Such a technique has been tried in the past by Stjernsward, who brought together data from five trials, and, using the Mantel-Haenzel procedure, showed a statistically significant decrease in five year survival for those receiving radiotherapy[275,276]. This controversial publication was based on the combination of results from trials in which different forms of local surgery and radiotherapy had been used, and it rightly produced a series of articles criticising its validity[277-279].

The concept of randomised controlled trials arose from the need to study contemporaneous populations, and to eliminate selection bias. Combining results from trials which are different in design and in temporal and geographical situation cannot, therefore, have any significance which is appropriate for statistical analysis. Nevertheless, it is important to examine the results of the available trials to determine whether there is an obvious collective trend. Table 10 shows the five year survival data from trials including adjuvant radiotherapy as an option, and table 11 shows the combined results.

No claims are made for the statistical validity of this data, but reference to tables 10 and 11 shows that no striking difference in five year survival exists between

Table 10

A - Trials in which the surgical treatment was not constant

Trial	Therapy	5 year survival
Kane 1968[253]	Extended radical Mx Simple Mx and XRT	138/206(67%) 144/219(66%)
Lythgoe 1978[254]	Radical Mx Simple Mx and XRT	68/129(53%) 53/139(38%)
Langlands 1980[252]	Radical Mx Simple Mx and XRT	192/256(75%) 163/242(67.5%)

B - Trials in which a constant surgical procedure was used

Trial	Therapy	5 year survival
Lythgoe 1978[254] (clinical satge 1)	Simple Mx - no XRT - XRT	 208/306(68%) 217/305(71%)
Kings/Cambridge[262] 1980	Simple Mx - no XRT - XRT	 420/600(70%) 434/594(73%)
Wallgren 1980[264] (assuming equal numbers in each group)	Modified radical Mx - no XRT - XRT pre-op - XRT post-op	 85/116(73%) 91/116(82%) 87/116(75%)

C - Trials in which pathological node status was available

Trial	Therapy	5 year survival		
		N-	N+	Total
Easson 1968[243]	Radical Mx - no XRT - XRT	377/467(81%) 353/464(76%)	139/283(49%) 117/243(48%)	465/750(62% 410/707(58%
Fisher 1976[265]	Radical Mx - no XRT - XRT	77/97(79%) 46/62(74%)	63/133(47%) 67/136(49%)	140/230(61% 113/198(57%
Host 1977[268]	Radical Mx & oophorectomy - no XRT - XRT - Co-60	319/359(89%) 153/172(89%) 149/171(87%)	140/184(76%) 78/109(72%) 82/95 (86%)	459/542(85%) 231/281(82%) 231/266(87%)

N- uninvolved nodes
N+ involved nodes
Mx mastectomy
XRT X-ray therapy

Table 11

	5 year survival	
A - comparable groups only		
no XRT	1777/2944	(70%)
XRT	1814/2584	(70%)
B - by nodal status (all comparable studies)	**Node negative**	**Node positive**
no XRT	773/922 (84%)	342/600 (57%)
XRT	701/869 (81%)	344/583 (59%)
C - overall combination		
no XRT	2175/3135	(69%)
XRT	2174/3183	(68%)

patients given adjuvant radiotherapy and those treated by surgery alone.

It will be argued that statistically significant results have been seen in a few individual trials. However, very large numbers are required for adequate trials[280], and it has been estimated that 600 patients would have to be given each type of treatment to demonstrate with certainty ($P < 0.05$) that one treatment will induce a 10% greater survival than the other[281]. We can be fairly certain, therefore, that adjuvant radiotherapy has had no major effect on the course of disease in the general population suffering from breast cancer, irrespective of node status.

There is ample evidence, however, that radiotherapy consistently lowers the incidence of local recurrence after surgery[243,250,256,262,263,265,282,283]. In some of these studies local recurrence was not further specified, but it is interesting to look at those trials in which nodal and chest wall recurrence were examined separately. After radical mastectomy, Fisher found that radiotherapy decreased the incidence of both chest wall and nodal recurrence in patients who had involved axillary nodes at operation[265]. Host found exactly the same, although statistical significance existed only for nodal recurrence[268]. Interestingly, Forrest noted that simple mastectomy with axillary radiotherapy was as effective as radical mastectomy and radical radiotherapy in controlling axillary node recurrence, but that the conservatively-treated group had more chest wall recurrence[250].

The case for radiotherapy as prophylaxis against local recurrence seems strong, but the argument does not end

here. Local recurrence per se is quite a rare phenomenon, and its appearance usually heralds the onset of metastatic disease elsewhere[243,267,284-286]. This suggests that the appearance of local disease is purely a manifestation of actively enlarging systemic metastases. As such, it would not be expected to be a significant cause of death in breast cancer, especially if it can be controlled by radiotherapy when it appears. This does seem to be the case[287,288], and Easson reported that delayed irradiation controlled local recurrence until death in a similar proportion of cases as did prophylactic radiotherapy[243].

It is evident, therefore, that by holding back radiotherapy, the number of patients who will eventually require it will be reduced. Whether it is particularly desirable to do this, however, depends on the morbidity attached to the procedure.

In the Cardiff-St. Mary's trial, it was found that patients receiving adjuvant radiotherapy were more likely to develop arm oedema and restriction of arm elevation in both the simple and radical mastectomy groups[250]. Similarly, Watson reported that, after radical mastectomy, arm swelling and impairment of function was commoner in irradiated patients[259]. However, in both of these studies, only patients with involved axillary nodes were given radiotherapy, and it is not possible to differentiate between the effects of the two factors. De Schryver, on the other hand, in an interim report of the Stockholm trial, demonstrated an increased incidence of arm oedema and restriction of arm movement in patients given pre- or post-operative radiotherapy compared to those treated by modified radical mastectomy alone[289].

Radiation to the chest wall can also lead to pneumonias[290], and after Co-60 treatment radiological pulmonary abnormalities were seen in 87% of cases[291]. It is also well known that both benign[292] and malignant[293] lymphangiomatous lesions can arise in lymphoedematous arms following radical mastectomy. In a review of 57 cases of lymphangiosarcoma occurring in this situation, it was noted that 91% of the patients had also been given radiotherapy[294].

In conclusion, there is no convincing evidence that treating the regional node-bearing areas by radiotherapy at the time of mastectomy confers any benefit on the patient suffering from breast cancer. Any argument for irradiation as prophylaxis against local recurrence can be countered by the undoubted morbidity which is needlessly inflicted on many patients.

v. Summary

In summarising the overall picture, it appears that no therapy directed specifically at regional lymph node metastases has any favourable influence over the natural history of breast cancer. This fits well with the concept of early systemic spread, and has, of course, contributed substantially to it. Equally, it is impossible to state that ablation of healthy axillary nodes is detrimental, and we have already seen that axillary clearance is probably the most accurate staging investigation available.

Precise prognostic information is important because it is of great value in planning trials of treatment regimens.

Throughout this review it has been emphasised that, in most cases, breast cancer appears to be a systemic disease by the time treatment has commenced. It is therefore logical that some form of systemic therapy should be used from the outset. There is now good evidence from two prospective, randomised trials that adjuvant chemotherapy can improve disease-free survival rates[295-300]. These studies were carried out only in women with histologically-proven lymph node involvement, and although there is some evidence that patients free of axillary metastases may benefit from such treatment[301], other studies do not confirm this[63,302].

In the initial trials of adjuvant chemotherapy it is important to identify patients at high risk of early recurrence. There are two reasons for this. Firstly, the chemotherapy carries a high morbidity in terms of physical toxicity and psychiatric or social disturbance[303-307]. Secondly, it has been pointed out that "node negative" patients have a good prognosis anyway, and that a trial to improve their survival would require very large numbers. It is therefore important to obtain accurate staging, as it would be unethical and unproductive to treat women whose outlook is favourable.

While it may not be justifiable to subject patients to the extra morbidity of radical or extended radical mastectomy purely for staging purposes, there is no evidence that the modified radical procedure causes more disability than a simple mastectomy. There is a great need, therefore, to compare lower axillary node sampling with total axillary clearance, not so much to estimate their relative therapeutic values, but more to determine whether there is a significantly greater morbidity

attached to the more extensive procedure. In addition, it is still necessary to confirm that axillary clearance gives better prognostic information than an adequate sample. For this to be done, a careful assessment must be carried out within the confines of a randomised, controlled trial.

LITERATURE REVIEW II

Reactive changes in lymph nodes draining breast cancer.

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Introduction

Tumour immunology has been widely investigated in humans[308,309] and in animals[310]. In breast cancer, host defense mechanisms in the modification of tumour progression are suggested by the propensity of the disease to recur after lying dormant for many years[60], and the occasional occurrence of spontaneous remission[311]. The regional lymph nodes in breast cancer are relatively accessable, and in recent years their morphology has excited a great deal of interest in terms of immunological function[312]. This story is not new, however.

In 1898, Halstead noted that certain proliferations in axillary nodes in patients with breast cancer were associated with a good prognosis[239] and in 1906 Schindler reported that, in carcinoma of the uterine cervix, lymph node metastases were less common in the presence of sinus cell hyperplasia[313]. Since that time, a great deal of work has been done relating prognosis to "reactive changes" in breast cancer, but before examining this, it is important to establish the histological structure of a normal lymph node.

Cottier and his colleagues have admirably described the microarchitecture of the lymph node and its possible variations[314,315]. There are basically three topographical areas which may undergo "reactive change," and these are termed the medullary sinuses, the paracortex and the cortex (Figure 4).

The medullary sinuses may become filled by histiocyte-like cells to give the appearance of "sinus histiocytosis" (Figure 5). This is the reactive change which is

Figure 4 Diagrammatic representation of lymph node structure.

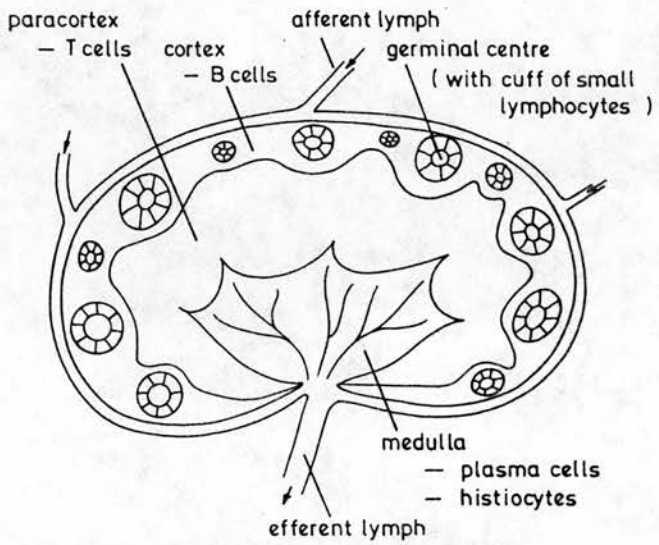
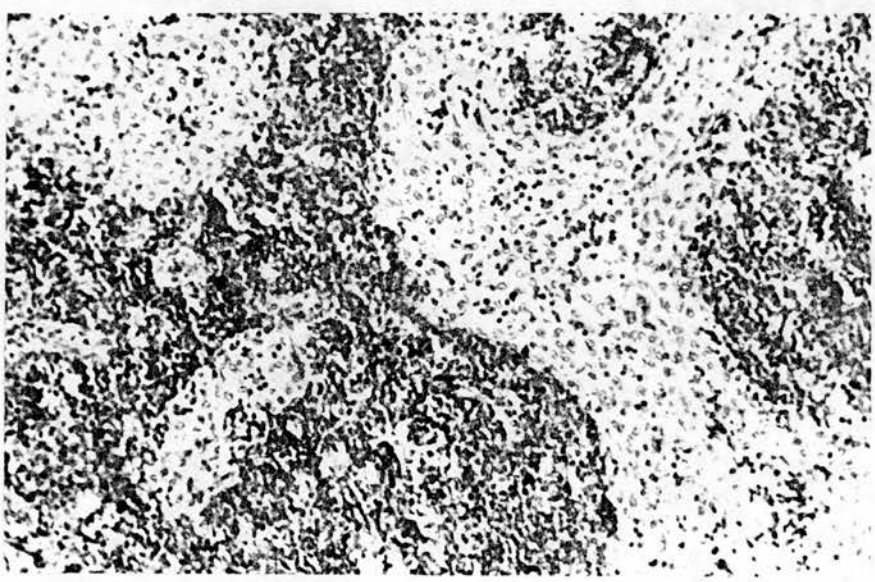


Figure 5 Sinus histiocytosis (x 50)



most often reported as being associated with neoplasia, especially breast cancer[316].

The paracortex is characterised by a "starry sky" appearance which is caused by the endothelial cells of the post capillary venules scattered throughout an otherwise uniform sheet of lymphocytes (Figure 6). These lymphocytes are thymus dependant, and the paracortex undergoes hyperplasia in the early stages of an immune response.

The cortex may display lymphoid follicle or germinal centre formation (Figure 7). As will be discussed later, germinal centre formation almost certainly represents B-cell proliferation and hence active antibody synthesis. Lymphoid follicles are a little more obscure, but there is evidence that serial section of these follicles usually reveals germinal centres[317].

Finally, two other features may be seen in lymph nodes. These are fibrosis, in which areas of the lymph node are replaced by fibrous tissue or hyaline material (Figure 8), and fatty replacement, where fat cells infiltrate the nodal structure (Figure 9).

All these "changes" may occur in lymph nodes which are not draining tumours and to determine their relative frequencies, it is important to consider information from post-mortem studies[316,318-321]. Sinus histiocytosis would appear to be very rare in nodes not draining tumours[316,317-320] and there is no marked variation with age or site[320]. Prominence of the paracortex is also relatively unusual, occurring in only 9.6% of axillary nodes from a series of 487 autopsies[320]. It

Figure 6 Paracortical hyperplasia (x 16)

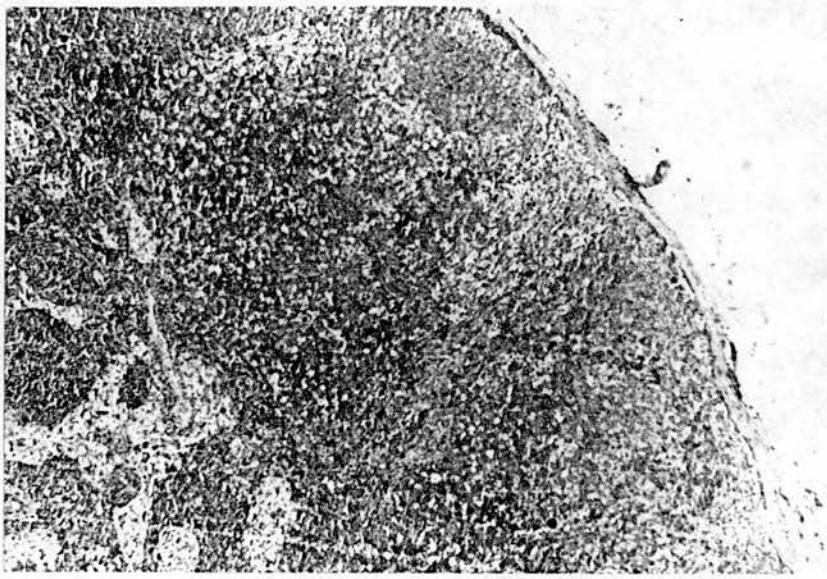


Figure 7 Germinal centre formation (x 16)

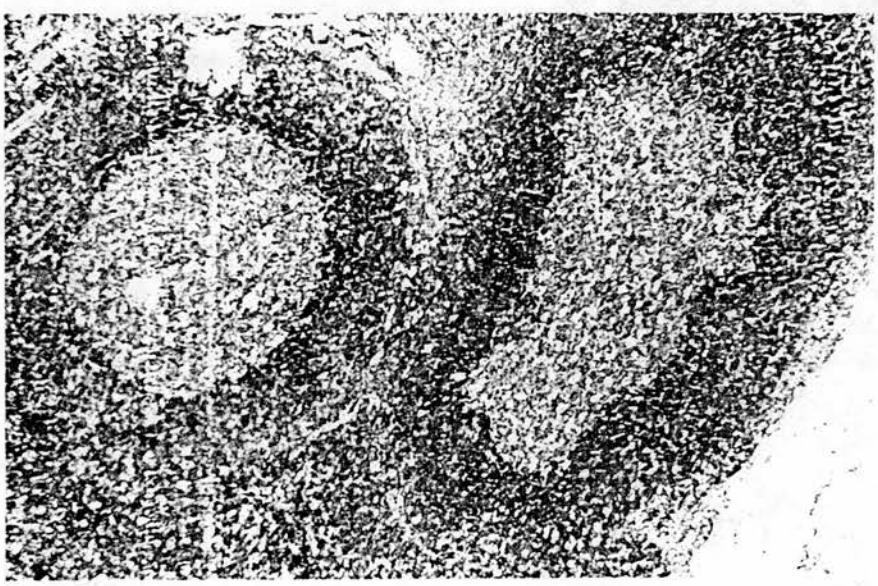
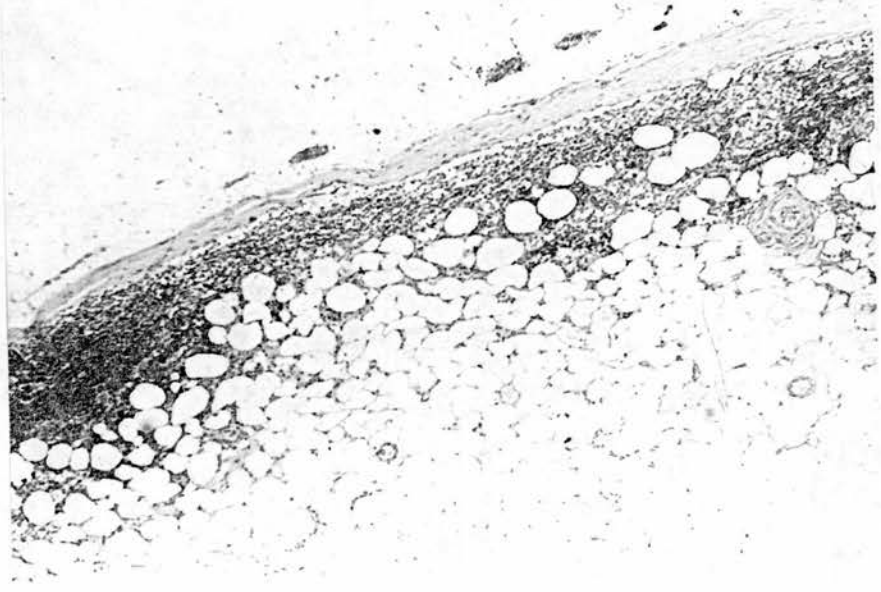


Figure 8 Fibrosis (x 16)

Figure 9 Fatty replacement (x 16)



does not have a predilection for any particular node groups, but does tend to decrease with age [321]. Germinal centres, however, are quite common, especially in nodes which are often exposed to antigenic stimuli such as those in the mesenteric and cervical areas[321]. This feature also decreases in frequency with advancing years[320,321]. Fibrosis and fatty replacement are characteristic features of normal nodes which are not often stimulated, for example those in the axilla or the popliteal fossa[321].

In the following two sections, sinus histiocytosis, paracortical hyperplasia, germinal centre formation and lack of stimulation in the form of fibrosis and fatty replacement will be considered separately.

The Functional Significance of Reactive Changes

i. Sinus histiocytosis

The precise definition of sinus histiocytosis varies slightly between authors, and, indeed, between papers by the same authors. However, the generally accepted definition, as propounded by Black[322], is filling of the medullary sinuses by large cells with eosinophilic cytoplasm and irregular vesicular nuclei. Black also emphasises that sinuses containing hypocellular lymph, inflammatory cells or red blood cells cannot be described as showing sinus histiocytosis[323], but that a syncytial pattern comprising elongated cells with a fibrillary or finely vacuolated appearance is acceptable[316].

Although the nature of sinus histiocytes is not fully understood, there is some evidence that they correspond to macrophages. Injected colloid is rapidly taken up by regional lymph nodes[200,201], animal experiments have shown that such colloid is taken up by sinusoidal cells[324-328], and the presence of foreign material can lead to the accumulation of histiocytes in draining nodes[329,330]. Ultrastructural studies have provided additional evidence by demonstrating that nodal histiocytes display features characteristic of macrophages[325,331]. In breast cancer patients, in-vitro maturation of monocytes correlates positively with sinus histiocytosis in axillary nodes and inversely with prognosis[332,333].

It is tempting to suggest that sinus histiocytes represent macrophages presenting antigen to lymphocytes, and certainly this reactive change is associated with expansion of

the paracortex[132,334,335]. However, sinus histiocytosis does not appear to be related to T-cell percentages[335] or basal thymidine uptake[336], and no correlation with dermal delayed hypersensitivity in breast cancer patients can be demonstrated[337].

It is therefore most likely that sinus histiocytes are macrophages, but their relationship to the immunological function of lymph nodes is obscure.

ii. Paracortical hyperplasia

The term "paracortical area" was coined in 1967 by Oort, who showed that sensitisation of guinea-pigs with oxazolone caused an increase in the size of this region in nearby lymph nodes[338]. Previous work by Scothorne had shown that skin homografting in rabbits caused an increase in the paracortical area of regional nodes, although it was not described as such[334,339].

Since these original studies, the significance of the paracortex has become much clearer, particularly through work done on the rat. There exists a population of small lymphocytes which recirculates between the blood and the lymphatics, passing through the paracortex of the lymph node via the post-capillary venules[340-342]. In addition, the nodal paracortex, along with the white sheath of the spleen, contains the major portion of labelled immigrant thoracic duct lymphocytes, and is specifically depleted upon chronic drainage of thoracic duct lymph[343]. As the same pattern is seen in neonatally

thymectomised animals[344-346] it would seem that the paracortex contains largely thymus derived lymphocytes.

To corroberate these findings, recent work has shown that T-cells identified by the sheep red cell rosetting technique[347] comprise a larger percentage of lymph node lymphocytes when expansion of the paracortex is evident[348,335], and immunofluorescence using heteroantisera has demonstrated a preponderance of T-cells in the paracortical area[349]. There is also evidence from studies using monoclonal antibodies that the helper-inducer subsets of T lymphocytes predominate in the paracortex[350].

It is therefore safe to say that paracortical hyperplasia represents an accumulation of T-cells, and may be expected during a cell-mediated immune response.

iii. Germinal centre formation

Germinal centres are spherical or oval structures surrounded by a dense cuff of small lymphocytes. Large lymphoid cells, many of which are in mitosis, predominate in the centre, and there are considerable numbers of macrophages. The germinal centre is supported by a network of dendritic cells, but these cannot be seen without special staining techniques[314,315].

It appears that germinal centre formation represents B-cell proliferation. Experiments have shown that cortical follicular cells are not depleted by thymectomy in mice[351], and stimulation with pneumococcal antigen causes germinal centre

formation, even in previously thymectomised animals[352,353]. Interestingly, thymectomy reduces the number of mitoses in germinal centres[344], but as T-cells are known to co-operate with B-cells in the production of antibody[354], this is perhaps not surprising.

Such observations are supported by immunofluorescence studies which have repeatedly demonstrated that immunoglobulin in lymph nodes is closely related to germinal centres[317,355,356], and recently, B-cell associated antigens have been found on the surface of cells in and around germinal centres[349]. It has also been shown that immunoglobulin is absent from the mantle of small lymphocytes surrounding the centre[357], suggesting that the transformation of B-cells to plasma cells may be taking place within the centre.

Despite all this information, the precise relationship of germinal centre formation in the cortex to plasma cell proliferation in the medulla is not fully understood[358]. It is reasonable, however, to assume that germinal centres in a node represent active antibody formation.

iv. Lack of stimulation

Little is known of why lymph nodes should become fibrosed or replaced by fat. Hyaline deposition and fibrosis of sinuses has been reported in lymph nodes of animals which have been antigenically overstimulated[359]. However, in humans, fibrosis is a common feature in normal axillary nodes, especially

in older women[320]. Likewise, fatty relacement seems to be a normal feature in nodes from the popliteal fossa or the axilla, where antigenic stimulation is uncommon[321].

It is probable, therefore, that fibrosis and fatty replacement represent lack of stimulation in lymph nodes.

The Relationship between Reactive Changes and Prognosis in Breast Cancer

i. Sinus histiocytosis

As we have seen, sinus histiocytosis is unusual in the absence of tumour[316,319,320]. There is also a variation depending on the type of tumour, and it appears to be uncommon in the regional nodes of gastric cancers[318,360,361] and colonic cancers[318,362,363].

Sinus histiocytosis is often seen in axillary lymph nodes draining breast cancer, however, and before studying its effect on prognosis, it is important to examine the grading systems used to quantify this change. When Black first introduced the concept of grading sinus histiocytosis, he used a 5-tier system, scoring the degree of histiocytosis on a scale of 0 - 4[322,323]. This was subsequently contracted to a 3 or 2 tier system, and as most workers outside Black's group have adopted his criteria in one of these forms, the main features are outlined in Table 12.

As can be seen, this is a highly subjective system, and indeed, a study of its reproducibility showed that equal assessments by the same observer on two different occasions varied between 70% and 47%[144]. Nevertheless, a great deal of work has been done using these criteria, and deserves close attention.

In 1953, Black and his colleagues reported that 5 year survival in breast cancer patients improved with increasing sinus histiocytosis in the axillary nodes[322], and

Table 12 Black's criteria for grading sinus histiocytosis in the 5, 3 or 2 tier systems.

0	-	No sinus histiocytosis
1	-	Sinusoids contain only a few scattered histiocytes
2	-	Sinusoids contain histiocytes forming a layer 3 - 6 cells in thickness
3	-	Sinusoids compactly filled with sinus histiocytes
4	-	Sinusoids very prominent and filled with histiocytes which extend into the cortical region

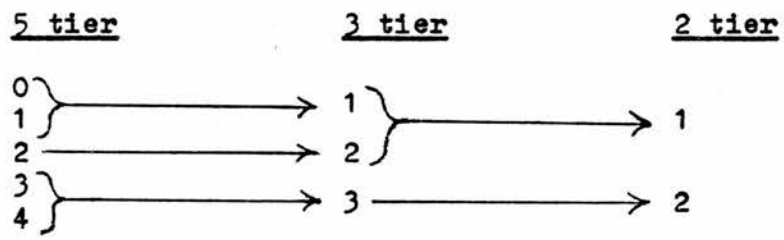


Table 13

Study	Minimum years of follow up	%age showing some sinus histiocytosis
Black 1953[322]	5	59%
Black 1955[364]	5	51%
Black 1956[323]	5	48%
Black 1958[318]	5	36%
Cutler 1963[104]	5	26%
Cutler 1969[105]	10	31%
Silverberg 1970[368]	10	26%
Fisher 1975[100]	0	78%

confirmed these findings in two subsequent studies[323,364]. They concluded that sinus histiocytosis must represent a favourable host response, but this was challenged by Berg, who pointed out that the degree of this reactive change was diminished in nodes containing metastatic tumour[365]. Undaunted, Black's group showed that sinus histiocytosis held its prognostic advantage irrespective of the presence or absence of nodal metastases[104,318], and produced convincing results to the same effect from actuarial analysis of 900 Norwegian breast cancer patients[105].

From the preceding paragraph, it is evident that a lot of the work on sinus histiocytosis has come from a single nucleus of researchers, and it is therefore important to look at the findings of others.

Several independent studies have confirmed the findings of Black and his co-workers, and thereby strengthen the case for sinus histiocytosis as a favourable prognostic factor[230,362,366-369]. However, some groups have been unable to reproduce these results[370,371] and perhaps the most worrying study is that conducted by Fisher using the patients entered into protocol 4 of the NSABP trial. When the patients were entered, various histopathological discriminants were recorded, including sinus histiocytosis[100]. In 1980, follow-up of 681 of these women who had been treated solely by radical mastectomy could demonstrate <u>no</u> advantage for those with sinus histiocytosis in their axillary nodes[372].

A possible reason for this last finding is suggested by the discrepancy shown in table 13. In Fisher's study, the percentage of patients showing <u>some</u> histiocytosis was much higher than in any of the previous studies. This may represent a

lower threshold for the reporting of sinus histiocytosis, which would, of course, compromise its significance. Alternatively, it may represent a true change in incidence of this reactive change over the years, and a lessening of its prognostic value.

While on the subject of incidence, it is interesting to consider a study comparing sinus histiocytosis in British and Japanese breast cancer patients[373]. In Japan, the disease carries a better prognosis than in Britain or America[374], and it was therefore an important finding that sinus histiocytosis was more common in Japanese women. However, the incidence in British patients was only 4% compared to 57% in the Japanese. Either these British women had abnormally reduced sinus histiocytosis, or the criteria used were completely out of line with other studies!

Overall, the weight of evidence supports sinus histiocytosis as a favourable prognostic factor in breast cancer. Whether this has changed in recent times requires further active research.

ii. Germinal centre formation

Germinal centre formation in regional lymph nodes draining cancer is a common phenomenon, and in breast cancer it is found most often in the nodes nearest the tumour[336,369]. Because of the frequency and distribution of germinal centres, some studies have been directed towards their prognostic significance.

Tsakraklides and his colleagues classified axillary nodes from breast cancer patients as showing germinal centre predominance if the majority of nodes from a patient showed "increased numbers of germinal centres in the cortex or throughout the node." They found that such patients had a worse prognosis than those showing expansion of the "deep cortex", but better than those lacking any signs of nodal stimulation[132]. Similarly, Hunter found germinal centres to constitute a poor prognostic factor[369], and Harveit has shown that a combination of germinal centres in the cortex and plasma cells in the medullary cords is associated with early demise in breast cancer[230]. Finally, using actuarial analysis, Fisher has looked prospectively at the effect of germinal centre formation in 681 women undergoing radical mastectomy for invasive tumour. Although an effect was not noted in the early stages of follw-up[375], a significant association with poor prognosis became evident after 5 years[372].

The criteria which have been used for deciding on significant germinal centre formation are vague, but it does seem that its presence bodes ill for breast cancer patients.

This does not necessarily hold for other forms of cancer, however. Certainly, melanoma[376] and laryngeal cancers[377] follow the same pattern, but in colonic carcinoma[378], gastric carcinoma[360,361] and squamous carcinoma of the skin[379], it seems to have favourable implications. Of interest is the finding that germinal centre formation is much more common in colonic cancer than in breast cancer[362,363]. Why these differences in prognostic significance and frequency should exist is quite obscure.

iii. Paracortical hyperplasia

The relationship between paracortical hyperplasia and prognosis in cancer was first noted by Tsakraklides' group when they found that expansion of the "deep cortical areas" correlated with a favourable outlook in carcinoma of the uterus[380] and breast[132]. Since then, comparatively little has been done in this field. A relationship between paracortical hyperplasia and good prognosis has been shown by other workers in breast cancer[230,381], colorectal cancer[378], gastric cancer[382] and squamous cancer[379]. In these studies, however, it has not been possible to separate the effect of this reactive change from an association with the absence of nodal metastases.

In renal carcinoma, the paracortical areas of the draining lymph nodes are depleted when compared to nodes draining benign gastric ulcers[383], but no information is available on the prognostic significance of this finding.

In short, paracortical hyperplasia is generally agreed to be a favourable factor in cancer, although more evidence would be welcome.

iv. Lack of stimulation

Fibrosis of lymph nodes has been described as "lymphocyte depletion" by Tsakraklides and his colleagues, and has been related to a poor 5 year survival in breast cancer[132].

The same has been found for squamous cell carcinoma[379], but Fisher could detect no prognostic significance attached to lymphocyte depletion in 303 breast cancer patients followed up for an average of 2 years[375].

Fatty replacement has never been specifically studied as a prognostic factor, but it should be remembered that it constitutes a normal feature in unstimulated nodes, as indeed does fibrosis[320,321].

v. Mast cells

Mast cells have recently excited some interest, and one group of workers has related increased numbers of mast cells in axillary nodes with improved survival[384,385]. However, it is known that mast cells in lymph nodes are more likely to be found when there is hyperplasia of the thymus dependant areas[386]. It is likely, therefore, that an increase in mast cell numbers reflects paracortical expansion.

vi. Available grading systems

Unfortunately, there is no universally accepted method of grading reactive changes in lymph nodes, and the available systems all have severe deficiencies.

Firstly, there is no usable system which takes into account all the changes known to have prognostic significance. Cottier and his colleagues, in a WHO memorandum, have outlined

a method of combining all the possibilities[314], but this procedure is far too complicated for routine purposes. Some attempts have been made to combine reactive changes in a simpler fashion, however. Sinus histiocytosis and germinal centre formation have been studied simultaneously, and greater prognostic accuracy claimed[369]. Other studies have tried to develop "host defense factors" by taking into account various nodal changes and lymphoid infiltrates in the tumour[387-389], but these are highly complex and idiosyncratic. Perhaps the most successful attempt at a comprehensive grading system comes from Tsakraklides, who classified individual nodes as "lymphocyte predominant", "germinal centre predominant", "unstimulated" or "lymphocyte depleted". Unfortunately, sinus histiocytosis was ignored, and "lymphocyte predominance" does not correspond exactly to paracortical hyperplasia.

The second problem with presently available systems arises from the subjective nature of the definitions used. This is a major problem with sinus histiocytosis (q.v. page 82), and other reactive changes suffer in the same way. For example, Tsakraklides defines lymphocyte predominance as "increased numbers of lymphocytes throughout the cortical and medullary regions"[132]. Such vagueness is detrimental as grading systems are therefore difficult to reproduce or quantify. To rectify this, an absolute frame of reference should be devised for each reactive change.

The third weakness in previous studies lies in the method for selecting the appropriate nodes for examination. In one of the early papers on sinus histiocytosis, Black writes:-

> "While most of the nodes from the same case tended to present a similar appearance, some variability was encountered. In such instances, the case was rated according to the finding in the lymph node showing the most pronounced changes."

It is clearly unsatisfactory to assume that the intensity of a particular reaction in one axillary node reflects the state of all the others. In addition, it is known that different reactive changes of quite different prognostic significance can co-exist in the same patient, and indeed in the same node[314,315].

The approach taken by Tsakraklides is better, as he classified each patient as "the pattern that was present in the majority of the nodes." Even this is nebulous, however, and a method of quantifying the degree of each reactive change in each lymph node and then combining them is required to circumvent this problem.

The Relationship between Reactive Changes and Other Factors of Prognostic Significance

Underlying most studies there is a tacit assumption that reactive changes represent a host response to the tumour which affects the course of the disease. Of course, another possibility exists. The prognosis associated with particular reactions may be due to the reaction merely reflecting the nature of the primary neoplasm rather than altering its behaviour. For example, germinal centres may be unfavourable because they represent the manner in which a lymph node reacts to a particularly aggressive neoplasm.

One method of resolving this problem is to determine whether reactive changes are related to other factors of prognostic significance. Very little specific work has been done on this, but some observations have been made "en passant."

Sinus histiocytosis has been correlated with a low incidence of nodal metastases[318,362,368,390], and a favourable nuclear grade in the tumour[362,368]. It has also been reported that sinus histiocytosis is common in nodes draining in-situ tumours when compared to invasive tumours[391,392]. Germinal centre formation has been associated with poor nuclear grade[363], and paracortical hyperplasia with the absence of nodal metastases[381]. Nodes showing fibrosis or hyaline change have been reported as showing a high rate of metastatic involvement in breast cancer[390].

In general, unfavourable reactions are associated with other unfavourable prognostic features. However, a clear

idea of the relationship between the various types of reactive changes and prognostic factors such as tumour size, tumour grade, oestrogen receptor status and delay before presentation has yet to be established.

The Development of Reactive Changes in Lymph Nodes Draining Tumours

The temporal sequence of events in the regional nodes draining neoplasia has been studied using transplantable animal tumours.

After implantation of sarcoma in rats, the paracortical areas of the nodes expand at 5 days, followed by marked germinal centre formation at 10 days and subsequent depletion and shrinkage of the paracortex[393]. Similar results are found with rat hepatoma, and, interestingly, nodes distant from the transplantation site mirror the changes in regional nodes, but at a later time[394].

When a tumour is implanted, increased numbers of immunoblasts are seen in the node[331,395], but such an increase is not seen in the thoracic duct lymph until the primary tumour has been excised[393]. This suggests that the tumour may exert a cytostatic influence on nearby nodes.

The pattern of events in lymph nodes regional to a skin homograft in rabbits shows a similar sequence of paracortical expansion preceding germinal centre formation[396], suggesting some similarity to the response to a tumour.

It is, of course, difficult to extrapolate from transplanted tumours in animals to those arising spontaneously in humans, but it seems from the available evidence that paracortical hyperplasia is an earlier response than germinal centre formation.

Functional Aspects of Regional Lymph Nodes in Cancer

Even when the questions pertaining to the relationship between reactive changes and the primary tumour are answered, we may still be left with a "chicken and egg" situation. For instance, are favourable reactive changes favourable because they represent a passive host response associated with a favourable tumour, or is a favourable tumour favourable because it induces or permits an actively favourable immunological response? To try to answer these questions it is necessary to ask whether regional lymph nodes are required for a host response to a tumour, and whether different reactive changes are associated with differing degrees of tumour immunity.

i. The functional role of regional lymph nodes in tumour immunity

To assess the functional importance of regional lymph nodes it is necessary to examine the cytotoxic ability of nodal cells. It is also important to ask whether the integrity of regional lymph nodes affects the host's response to the tumour.

Firstly, it has been shown that some cells from lymph nodes draining breast cancer actively take up thymidine, both spontaneously[336] and when stimulated with PHA[397]. Not all nodes from the same patient display the same degree of uptake, however[397]. Those nearest the tumour were most active[336],

suggesting that stimulation increases with proximity to tumour.

Unfortunately, these studies do not examine the effect of tumour on lymphocytes. Using a mixed lymphocyte-target interaction test[398] Vanky and colleagues found that human tumour-draining lymph node cells did not respond to tumour cells by increasing DNA synthesis, although the same cells did respond to PHA and foreign lymphocytes[399]. On the other hand, regional node lymphocytes have been shown to respond to autologous tumour extract by displaying increased leukocyte migration inhibition[400].

Turning to animal studies, direct tests of autochthonous or syngeneic cytotoxicity demonstrate that regional lymph node cells do have the ability to kill tumour cells in vitro[59,401], although not all workers have been able to show this[402]. However, there is some suggestion that the degree of cytotoxicity varies with the duration of tumour growth, for in rats, maximum cytotoxicity resides with regional lymph node cells at two weeks after tumour implantation, but at six weeks these nodes become anergic despite the development and maintenance of cytoxicity in distant nodes[401]. It is also known that the passive transfer of immunity to lymphosarcoma in mice, which can be achieved using regional lymph node cells, is only possible for a short time following implantation of the original tumour[403].

When regional lymph nodes are removed, a slight increase in homograft survival is seen[404], and a considerable amount of work has been done to try to establish whether the same principle applies to tumours. In mice, it has been reported

that removal of lymph nodes near a transplanted tumour increases the incidence of metastatic disease[405], and decreases the animal's ability to resist subsequent tumour implantation[406,407]. However, it has also been shown that although ablation of regional lymph nodes is deleterious to subsequent tumour immunity in the case of a spontaneous transplantable tumour, the same is not true of a methylcolanthrene-induced tumour[408]. This finding highlights the heterogeneity of experimental tumour models, and several groups of workers have been unable to demonstrate any adverse effects from the removal of regional lymph nodes in animal systems[409-412].

In humans, it is possible to examine this problem by asking whether destruction of axillary nodes in breast cancer has any detrimental effect, and radiotherapy has come under particularly close scrutiny in this respect. It is true that adjuvant radiotherapy does cause lymphopenia[413-416] which may last up to one year[417], but the significance of this finding is open to debate[418]. Some workers have found a selective depletion of T-cells[417], whereas others have noted that B-cells are most affected[416]. Transformation of increased DNA synthesis in peripheral blood lymphocytes as a response to mitogens has been shown to be diminished by irradiation by some workers[414,417], but not by others[413,416].

The most important information, however, comes from randomised trials of treatment regimes in breast cancer. In the previous literature review it was demonstrated that adjuvant radiotherapy had no effect on five year survival of breast cancer patients (page 63). Similarly, excision of nodes does not

alter the course of the disease.

The role of regional lymph nodes in the response to tumour is far from clear, and it would be unwise to read too much into animal experiments utilising transplantable neoplasms. However, the evidence to date suggests that regional lymph node cells can be capable of responding to and killing autologous tumour cells, although such ability may be restricted to the early stages of tumour growth. Certainly, in human breast cancer, there is no evidence that maintaining the integrity of the regional lymph nodes confers any benefit, at least by the time that the tumour has presented clinically.

ii. The functional significance of reactive changes in tumour immunity

Three reactive changes in lymph nodes draining breast cancer have a significant bearing on prognosis - sinus histiocytosis, paracortical hyperplasia and germinal centre formation (q.v.). It is therefore of interest to try to assign immunological functions to these changes in terms of a host response to tumour.

It has been shown that non-metastasising experimental tumours are accompanied by sinus histiocytosis and paracortical hyperplasia in the draining lymph nodes, whereas implantation of metastasising tumours gives rise to germinal centre formation[419]. In humans, cells from lymph nodes showing sinus histiocytosis or paracortical hyperplasia have an elevated degree of cytotoxicity

against autologous breast tumours[420] or human mammary cancer cell lines[421].

Turning to sinus histiocytes in particular, there is more information on their significance in tumour immunity. Tumour extract can induce an increased rate of mitosis in sinus histiocytes when injected locally in rats, suggesting that tumours may affect local macrophage proliferation[422]. In addition, it has been shown that breast cancer tissue applied to microabrasions on the patient's own skin is more densely infiltrated by lymphocytes when sinus histiocytosis is prominent in the axillary nodes[423], and there is some evidence that macrophages from regional nodes may induce peripheral lymphocytes to become cytotoxic towards autologous tumour tissue[424]. There is also evidence that factors in regional lymph nodes may render macrophages more cytotoxic to tumour cells[425-427]. This is important, as it is known that macrophages infiltrate tumours, and increased numbers of macrophages have been associated with improved prognosis[428,429]. Finally, in breast cancer patients with involved nodes, physical macrophage-lymphocyte contact appears to be less pronounced than in those with negative nodes[430]. If sinus histiocytes are macrophages, therefore, some immunological rationale does exist behind their prognostic significance.

Paracortical hyperplasia, another favourable reaction, represents an accumulation of T-cells in the lymph node, and it is therefore pertinant to ask whether T-cells influence tumour growth. T-cell function, as measured by PHA stimulation or delayed hypersensitivity, is undoubtedly

reduced in breast cancer[337,431-436], and the depression of PHA stimulation may be dependant on a serum factor[337,431-433]. It has also been demonstrated that T-cell function decreases with the extent of the disease[337,437], but whether this be cause or effect is unknown. Autologous human tumour extracts can stimulate thymidine uptake in peripheral lymphocytes in the absence of mitogens, but this appears to be restricted to tumours with a favourable prognosis[438]. In general, the percentage of lymphocytes with T-cell characteristics in lymph nodes draining breast cancer is smaller than that seen in normal nodes[439,440], and it would appear that depression of T-cell percentages and function occurs in the presence of tumours, but whether this influences or merely reflects tumour growth has not yet been established.

Finally, it remains to consider germinal centre formation, and why a clear indication of antibody formation should be related to poor prognosis. Breast cancer patients certainly have abnormal immunoglobulin profiles; IgA levels in peripheral blood[441] and in regional lymph nodes[356] are raised, and there are reports of IgM in nodes being associated with metastases[442] and a poor prognosis[443]. It should be stressed, however, that these studies do not show that the immunoglobulin in question is actually synthesised in the regional nodes.

There is now a considerable body of evidence to suggest that cancer patients have abnormally high levels of circulating immune complexes[444-446], and that high levels of these complexes indicate a poor prognosis[447,448]. Why this

should be is not clear, but it is known that serum factors in cancer patients reduce T-cell percentages[449,450], and inhibit stimulation of peripheral lymphocytes by autologous tumour[451-453] or PHA[337,431-433]. In addition, immune complexes can block tumour cytotoxicity in experimental animal systems[454], and, in breast cancer patients, the blocking activity of serum can be correlated with immune complex levels[455]. It is possible therefore, that antibody formation by regional lymph nodes in breast cancer patients can block potentially cytotoxic cells by forming immune complexes. This might account for the poor prognostic significance of germinal centre formation.

Overall, it is impossible to say whether reactive changes in regional lymph nodes have prognostic signficance because they influence tumour growth or because they merely reflect the nature of the tumour. However, there is at least some evidence to suggest that they may play a functional role in a tumour-host interaction.

Lymphocytic Infiltration of Primary Breast Cancer, and its Relationship to Reactive Changes in Regional Nodes

i. The significance of lymphocytic infiltration in tumours

Apart from reactive changes in regional lymph nodes the other histological parameter of a host response is lymphocytic infiltration of the tumour itself. This was first noted by MacCarty in 1922, who suggested that it was associated with longer survival in carcinoma of the breast, stomach and rectum[456,457]. However, these results do not stand up to statistical analysis, and Greengough, writing in 1925, did not regard lymphocytic infiltration as a favourable sign[142].

Black's group have done a considerable amount of work on lymphoid infiltrate in tumours and have repeatedly found it to be a favourable prognostic factor both in breast cancer[323,364,458] and in stomach cancer[361]. However, a great number of other studies could not confirm this finding[131,167,372 381,459-461], and, indeed, some have shown that lymphocytic infiltration is associated with tumours of poor histological grade[131,372].

This puzzling discrepancy may be partly resolved by examining exactly what the different studies were scrutinising. Black used a 0 - 4 grading system for lymphocytic infiltration, and found that tumours graded 2 - 4 comprised less than 10% of the total. This is an incidence of marked lymphocytic infiltration which is much lower than that reported in other studies, and must surely represent greater selectivity. In addition, a later publication by Black emphasised the importance of perivascular

infiltration[458], a factor ignored by other workers.

There is another facet to this argument, however. A special type of breast cancer termed "medullary" was first described by Geshickter[462], and defined by Foote and Stewart as, "a tumour made up of large, oval, rounded or polygonal cells with abundant basophilic cytoplasm and large vesicular nuclei, which are arranged in broad masses"[463]. This type of tumour is nearly always associated with a dense lymphocytic infiltrate and a good prognosis[143,464,465]. It is wrong to assume, however, that the favourable outlook of this tumour is necessarily due to the lymphocytic infiltrate, and even more erroneous to extrapolate from this rare neoplasm to the lymphoid component of an ordinary invasive ductal breast carcinoma. Possibly, Black's densely infiltrated tumours were largely medullary, whereas other studies included all types.

At present, therefore, lymphocytic infiltration seems to have no prognostic significance per se, unless associated with the special medullary type of carcinoma.

ii. Functional aspects of lymphocytic infiltration in breast cancers

It is rather disappointing to find that lymphocytes in tumours have such a poor relationship to prognosis, but functional studies of these cells give us some clue as to why this is the case. Three separate experiments have shown that the ability of lymphocytes from a human tumour to attach to or kill

autologous tumour cells in culture is less than that of lymphocytes from lymph nodes or peripheral blood[466-468]. This is corroborated by a recent study which has demonstrated that lymphocytes derived from breast cancers show greatly reduced antibody-dependant cytotoxicity and natural killer ability when compared to peripheral lymphocytes[469]. There is also evidence that some tumour lymphocytes are anergic in response to mitogens[470], although whether this is related to a serum factor is unknown.

In general, it would seem that a tumour has the ability to depress the function of infiltrating lymphocytes or to repel those lymphocytes which are functionally competent.

iii. The relationship between lymphocytic infiltration and reactive changes in regional lymph nodes

As reactive changes and lymphoid infiltrate both represent some form of host response to a tumour, it is of interest to determine whether a relationship exists between them.

Little specific work has been done in this field, but from various sources it is clear that germinal centre formation is related to lymphocytic infiltrate[363,372,458]. However, as both of these parameters increase with deteriorating tumour grade, it is impossible to ascribe a direct relationship between the two from existing data.

Clinical Assessment of Axillary Nodes in Breast Cancer with respect to Reactive Changes

It is widely held that palpable axillary lymph nodes in the absence of metastatic deposits constitute a favourable prognostic factor[471]. This has never been adequately demonstrated, however, and the evidence to suggest that such a phenomenon might exist is scanty.

In 1969, Cutler reported that patients with bilaterally palpable nodes had a better prognosis than those with either no palpable nodes or homolaterally palpable nodes[472]. This was supported by the finding of increased sinus histiocytosis in the homolateral nodes in patients with bilaterally palpable nodes[473]. It has also been claimed that patients with "non-suspicious" homolateral nodes have an improved prognosis[474], and that such palpability is associated with enlarged nodes showing sinus histiocytosis at the apex of the axilla[475].

However, these studies are weak, as no uniformity of reporting clinical assessment was present, and they certainly cannot support the statement that "when nodes are enlarged without the presence of metastases, they are by definition reactive"[476].

There is an interesting study which suggests that palpable axillary nodes which regress after mastectomy may have favourable implications[56,57]. Unfortunately, the numbers in this study are small, and there is no histological data to support the contention that regressing nodes represent favourable reactive changes.

In short, there is no satisfactory evidence that clinical enlargement of axillary nodes in the absence of nodal metastases is a good prognostic sign. Indeed, it is known that about 30% of women with no demonstrable breast pathology will have palpable axillary nodes[177], and there is no justification for assuming that reactive changes can render lymph nodes palpable.

DISCUSSION AND CONCLUSIONS FROM THE LITERATURE

From the available literature, it is possible to draw certain conclusions concerning metastases and reactive changes in lymph nodes draining breast cancer. For the purposes of this thesis, it is useful to list these conclusions, and from them to formulate the questions which are addressed by the original studies to be described.

1. The significance of axillary lymph node metastases.

It is currently believed that breast cancer becomes disseminated in blood and lymphatics at an early stage, and that metastases grow synchronously at a rate determined by the balance between the tumour and the host. Axillary lymph node metastases may appear to arise earlier than other metastases, but only because they are in a more accessible position. Their prognostic importance, therefore, is not that they represent an early stage of tumour spread (Figure 1, page 9), but that they reflect the state of other metastases scattered throughout the body (Figure 2, page 9).

2. The significance of the extent of metastatic involvement of regional nodes.

The number of involved lymph nodes varies inversely with the expected duration of disease-free survival and no abrupt change in prognosis occurs when a specific number is reached (Pages 14 - 17). In addition, the sizes of individual nodal metastases are important, as a minute deposit of tumour is

of no prognostic significance.

This relationship between the extent of axillary metastatic disease and prognosis is of immense importance when selecting patients for adjuvant therapy on the basis of recurrence risk. It is clearly inappropriate to include a patient with a single micrometastasis in the same category as a patient with fifteen involved nodes, and yet this is still being done in many trials of adjuvant chemotherapy.

3. The relationship between nodal metastases and other prognostic factors.

Although the presence of involved lymph nodes is the most powerful prognostic factor available, there are many others which are not entirely dependent on nodal status (pages 24 - 36). It is therefore important to establish the interrelationships between nodal metastases and other prognostic factors so that a meaningful prognostic index can be constructed. There also exists the possibility of combining parameters known to relate to lymph node metastases in order to predict their presence pre-operatively.

4. Pre-operative methods of axillary staging.

Although clinical examination of the axilla is a useful method of predicting prognosis in breast cancer, it is highly inaccurate as a method of detecting nodal metastases (pages 38 - 40). For its resolution, this apparent paradox requires careful study of the relationship between clinical findings, prognosis, and the extent of axillary metastatic

disease. There is also a need to develop more accurate methods of pre-operative nodal staging so that extensive surgery can be avoided. Axillary lymphoscintigraphy holds the greatest promise in this field (pages 41 - 45) and requires careful analysis.

5. Operative methods of axillary staging.

At present, the only sure way of establishing nodal status is surgical extirpation and histological examination (pages 38 - 40). As extensive ablative surgery is now considered unnecessary (pages 54 - 58), a compromise between therapeutic and investigative procedures must be made. Modified radical mastectomy appears to be satisfactory, as axillary node status can be firmly established without the morbidity attached to more radical procedures. However, lower axillary sampling may be preferable, and it is important to determine whether this lesser procedure can give adequate prognostic information.

6. The treatment of regional lymph nodes in breast cancer.

There is no evidence that treatment aimed at the destruction of the regional lymph nodes has any effect on survival in breast cancer (pages 50 - 68). Adjuvant radiotherapy or radical surgery do lower the incidence of local recurrence (pages 56 - 57, 64 - 65), but this advantage must be viewed in the light of the proven morbidity caused by these two therapeutic modalities (pages 56 - 58, 65 - 66).

7. The prognostic significance of reactive changes.

Reactive changes in regional lymph nodes draining breast cancer certainly relate to prognosis (pages 82 - 88). However, there is no satisfactory method of grading which encompasses all types of reactive change which are of importance. Existing grading systems are not only limited in scope, but are also highly subjective, and therefore difficult to reproduce and quantify (pages 88 - 90).

The prognostic significance of reactive changes suggests that they represent some form of host response, favourable or otherwise, directed against the tumour. Alternatively, however, they may merely reflect the aggressiveness of the neoplasm. It is thus important to establish the nature of the relationship between reactive changes and the primary tumour, as this will help to determine whether reactive changes have independant prognostic significance.

8. The functional significance of reactive changes.

Paracortical hyperplasia and germinal centre formation have been convincingly shown to represent enlargement of the T-cell and B-cell nodal compartments respectively (pages 78 - 80). However, sinus histiocytosis is still a mystery, and although some evidence suggests that the cells which comprise this reaction are macrophages (pages 77 - 78), definitive studies have yet to be done.

Despite the finding that these reactive changes relate to immunological criteria, at least in some instances, there is <u>no</u> evidence that regional nodes draining breast cancer

are of functional importance in the natural history of the disease by the time mastectomy is performed (pages 94 - 97). Probably, reactive changes represent an immunological "fait accompli" at the time of mastectomy, and therefore indicate the likely course of events.

9. The relationship between reactive changes and lymphocytic infiltrate.

Infiltration of breast cancers by lymphoid cells is now thought to have little bearing on the likely course of the disease in most cases (pages 101 - 103). However, it is not known whether reactive changes in lymph nodes can affect the prognostic significance of lymphocytic infiltrate in tumours (page 103). As both may represent a host response to the tumour, this question deserves close attention.

10. Clinical assessment of reactive changes.

The evidence that palpable axillary lymph nodes in the absence of metastases have any favourable significance is poor (pages 104 - 105). Further investigation is required in this area, and an analysis of the causes of palpable enlargement of axillary lymph nodes in breast cancer would be of great interest.

The above conclusions show that many problems remain unresolved. To introduce the next section of this thesis, seven questions are now posed. These are listed in an order corresponding to that in which the pertinant studies are subsequently documented.

i. Can limited axillary lymph node sampling provide useful prognostic information in terms of nodal metastatic involvement?

ii. Is there a relationship between metastatic involvement of axillary nodes and features of the primary tumour known to affect prognosis?

iii. Can a histological grading system based on immunological criteria provide useful prognostic information?

iv. Is there a relationship between reactive changes in axillary lymph nodes and aspects of the primary tumour known to affect prognosis?

v. Are reactive changes related to the prognostic significance of lymphocytic infiltration in the primary tumour?

vi. Is clinical assessment of axillary lymph nodes of any value?

vii. Is it possible to predict the histological appearances of the axillary nodes?

ORIGINAL WORK

1. MATERIALS AND GENERAL METHODS

i. Patients studied

Two main groups of patients were studied:

1. A series of 238 patients treated for invasive breast cancer by simple mastectomy and lower axillary node sampling between 1974 and 1977. It should be noted that patients with histologically negative nodes had been randomised to receive adjuvant radiotherapy, whereas those with positive nodes all had radiotherapy and had been randomised to receive chemotherapy (5-Fluorouracil only).

2. A series of 100 patients treated for invasive breast cancer by simple mastectomy and total axillary clearance (a "Patey" type modified radical mastectomy) during the years 1980 and 1981. This cohort was derived from 279 subjects entered into a trial of total axillary clearance versus lower axillary sampling.

The patients in these two groups were consecutive with the exception of the following exclusions:

a. Any patient who had a mastectomy in the presence of detectable metastases. All patients underwent superstaging investigations including chest and pelvic radiographs, bone scanning and liver function tests.

b. Any patient who had a primary breast tumour of a special type i.e. lobular, medullary or colloid. Pure tubular tumours were included, as it was felt that they could be accomodated in conventional grading systems.

All of these patients were assessed clinically by at least two experienced observers, and an agreed stage was recorded pre-operatively using the international TNM system (q.v. pages 38 - 40). Each woman was seen at the follow-up clinic at 3-monthly intervals for the first year after mastectomy, and at yearly intervals thereafter. In the retrospectively studied group, follow-up data was analysed in terms of major recurrence, defined as the appearance of metastatic disease excluding homolateral axillary recurrence.

Various other groups of patients were used for individual studies, but these are documented in the appropriate sections.

ii. Method for obtaining lymph nodes

From the 100 patients treated during 1980 and 1981, nodes were obtained by dissecting the clearance specimen in the following manner.

Firstly, the specimen was X-rayed in a Faxitron 43805N system before it was detached from the breast. The dose of X-irradiation used varied according to the thickness of the specimen, but voltage was within the range 10 - 20 KV for 2 minutes at 3 mA.

The radiographs demonstrated the position of the lymph nodes within the fibro-fatty tissue, regardless of the presence or absence of tumour tissue (Figure 10). This allowed the nodes to be easily identified, and the axillary contents could be cut from the remainder of the breast in the knowledge

that no nodes would be missed. The clearance specimen was then placed on a cork board next to an X-ray box so that the specimen radiograph could be used as a guide to dissection. During the dissection a careful map of the distribution of the nodes was made (Figure 11), and the nodes themselves were labelled individually or in closely associated groups.

The patients treated by mastectomy and node sampling between 1974 and 1977 were studied retrospectively, and the histological sections of their lymph nodes were obtained from the files of the Department of Pathology.

In all cases, the lymph nodes were fixed in Carson's fluid, bisected in their longitudinal axes, and embedded in paraffin wax. A 5μ thick section of the cut surface was then made, and stained using Haematoxylin and Eosin.

iii. Grading system for lymph nodes

All the lymph nodes were examined histologically and graded for the presence of metastases and/or reactive changes.

If metastases were present, they were graded as "micrometastases" when the tumour deposit was less than 2 mm in maximum diameter, and "macrometastases" when greater. This definition was used as previous studies have shown that such "micrometastases" carry a much better prognosis than more extensive involvement (q.v. pages 23 - 24).

For grading reactive changes, an entirely new system was devised. Previous studies have shown that sinus histiocytosis

Figure 10 Radiograph of axillary clearance specimen.

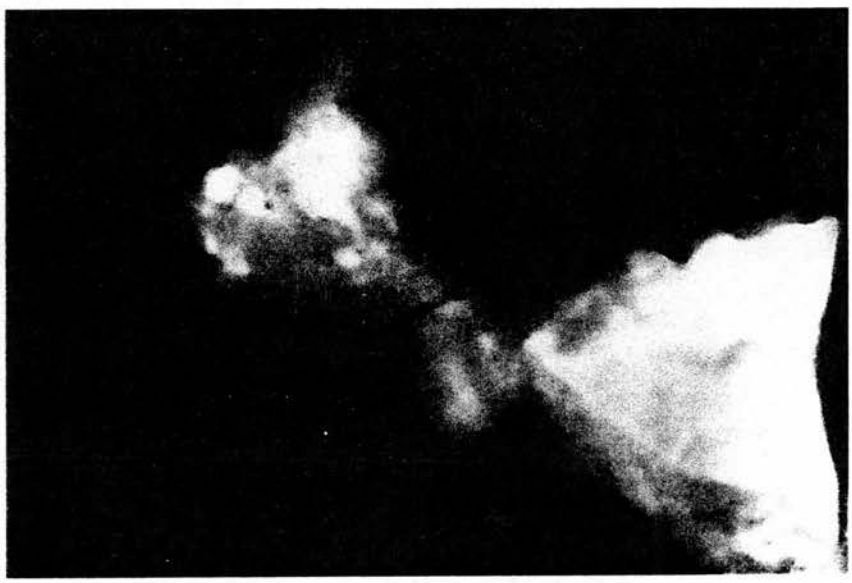
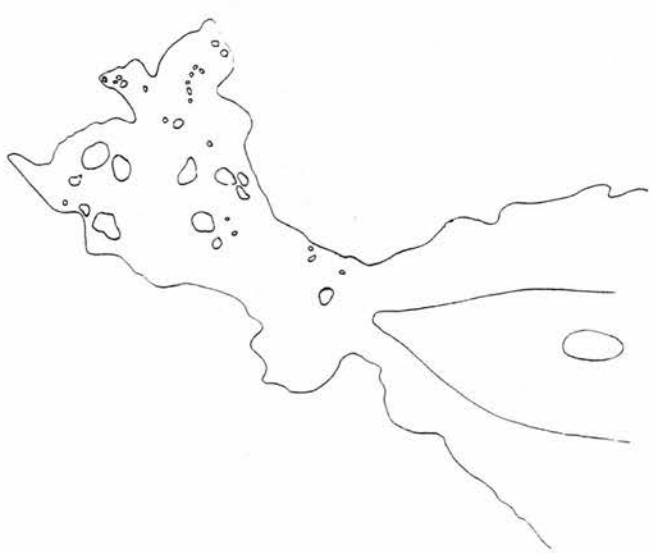


Figure 11 Map of the distribution of lymph nodes made during dissection of specimen shown above.



and paracortical expansion have favourable effects on prognosis in breast cancer, and that germinal centre formation and lymphocyte depletion are unfavourable (q.v. pages 82 - 88). However, most of these studies concentrated on the changes in isolation, without regard for the fact that all of them can co-exist in the same axilla, and indeed in the same lymph node.

As was stressed in literature review II there is no grading system which takes all the reactive changes known to have prognostic significance into account. In addition, all the published methods of grading are highly subjective and extremely difficult to reproduce or quantify. It was felt, therefore, that a simple, objective, quantifiable and, above all, comprehensive system is crucial if the available knowledge of the significance of reactive changes is to be used to best advantage.

Accordingly, the reactive changes were classified as sinus histiocytosis, germinal centre formation, paracortical hyperplasia, fibrosis or fatty replacement. The definition and significance of these categories has been scrutinised in detail in literature review II. In every lymph node each change was graded and 0, 1 or 2 according to the criteria laid down in table 14. For each individual patient, a mean score for each reactive change could be calculated by dividing the total score by the number of nodes found. When these scores had been obtained, it was then possible to ascribe a "predominant reaction" to each patient by selecting the reaction with the highest mean score.

The reproducibility of this system was tested as follows. One hundred lymph nodes from 32 patients were set

Table 14 Criteria for scoring lymph node reactions

S H	:	Sinus histiocytosis (figure 5, page 72)
0	-	No sinus histiocytosis present. This category includes "degenerate" sinus histiocytosis where the medullary sinuses contain large numbers of red blood cells, a few vacuolated unconnected histiocytes, or fibrous/hyaline material
1	-	Appreciable sinus histiocytosis, where the medullary sinuses contain cells with abundant cytoplasm and large, pale, often irregular nuclei
2	-	Where sinus histiocytosis occupies 50% or more of the cross-sectional area of lymphoid tissue
P C H	:	Paracortical hyperplasia (figure 6, page 74)
0	-	Paracortex not evident
1	-	Well defined areas of paracortex exhibiting the "starry sky" appearance caused by endothelial cells
2	-	Paracortex occupying more than 50% of the cross-sectional area of lymphoid tissue
G C F	:	Germinal centre formation (figure 7, page 74)
0	-	No germinal centres present
1	-	A few germinal centres present
2	-	Well defined germinal centres present, and occupying more than 50% of the cross-sectional area of lymphoid tissue
F	:	Fibrosis (figure 8, page 75)
0	-	No fibrosis or hyalinisation
1	-	Appreciable amounts of fibrosis or hyaline material within the lymphoid tissue
2	-	More than 50% of the lymphoid tissue replaced by fibrosis or hyaline material
F R	:	Fatty replacement (figure 9, page 75)
0	-	No fatty replacement
1	-	Significant fatty replacement
2	-	Where fat has replaced over 80% of the cross-sectional area of lymph node, leaving only a rim of lymphoid tissue

Notes 1. If a metastatic deposit was present in the lymph node, this did not exclude grading of the reactive changes unless they were uninterpretable. The extent of each reactive change was expressed as a percentage of the uninvolved portion of the node.

2. The presence of one reactive change in a lymph node did not necessarily exclude any of the others.

aside at random and graded by the author. They were then graded independantly by another observer, and again by the author two months later. The percentage concordance for each reactive change is shown in table 15. Overall, the inter-observer agreement was 73% and the intra-observer agreement was 79% for individual nodes. However, for picking out the predominant reaction in each patient, these were much higher (94% and 97% respectively). This compares very favourably with other histological grading systems[144-146].

In all the studies to be described, in which metastases or reactive changes are compared with recurrence rates or with features of the primary tumour, the lymph nodes were assessed blindly. In this way, any subjective component of the grading systems could not be exposed to bias in a particular direction.

iv. Grading system for tumours

The histological grade of the primary breast tumours was assessed using the Bloom and Richardson system[106], the criteria for which are laid out in table 16. It is well established that grade 1 tumours carry a much improved prognosis over grade 3 irrespective of other factors[106,107,131].

The size of the primary neoplasm was measured in each instance, and the greatest dimension utilised in analysis. In addition, naked eye examination of the lesion was used to categorise its border as "spiculated" or "rounded," and its position within the breast was noted. Where available, the

Table 15

Percentage concordance for grading reactive changes in lymph nodes

	Inter-observer agreement	Intra-observer agreement
Sinus histiocytosis	94%	92%
Germinal centre formation	99%	98%
Paracortical hypertrophy	79%	87%
Fibrosis	93%	98%
Fatty replacement	96%	97%
Whole node	73%	79%
Predominant reaction for each patient	93.8%	96.8%

Table 16

Criteria used in Bloom and Richardson grading.

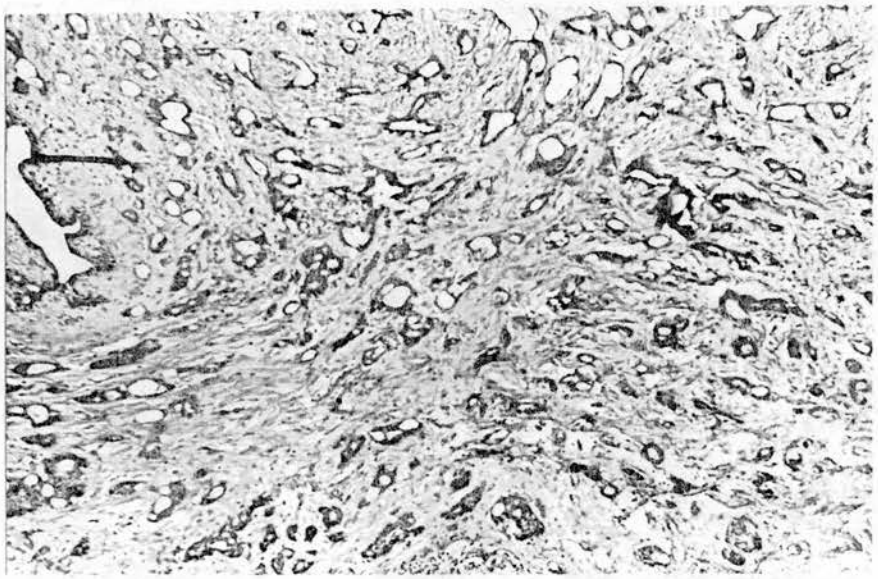
Differentiation	
1	The majority of neoplastic cells form acinar or tubular structures (Figure 12)
2	Moderate tubule formation (Figure 13)
3	No tubule formation, neoplastic cells forming sheets or strands (Figure 14)
Pleomorphism	
1	The nuclei of the neoplastic cells are rounded, and uniform in size (Figure 15a)
2	Moderate pleomorphism (Figure 15b)
3	Marked pleomorphism of nuclei (Figure 15c)
Hyperchromatic and mitotic nuclei	
1	Occasional hyperchromatic or mitotic figure in a high power field (x 750)
2	No more than 3 but always more than 1 figure per high power field
3	More than 3 figures per high power field

A composite score is then obtained in the following way

Total score	Composite score
3, 4, 5	1
6, 7	2
8, 9	3

Figures 12 - 14 Different degrees of tubule formation in invasive breast cancer (x 16).

12



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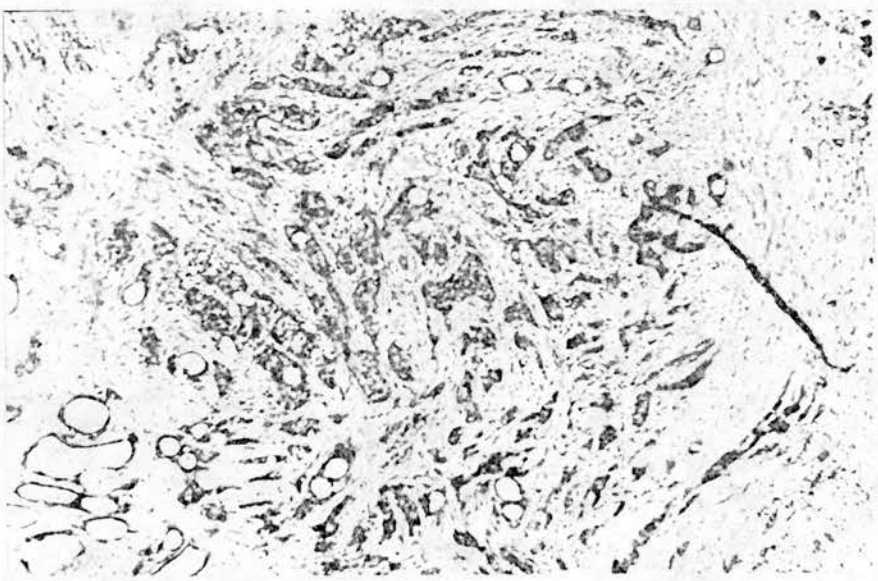

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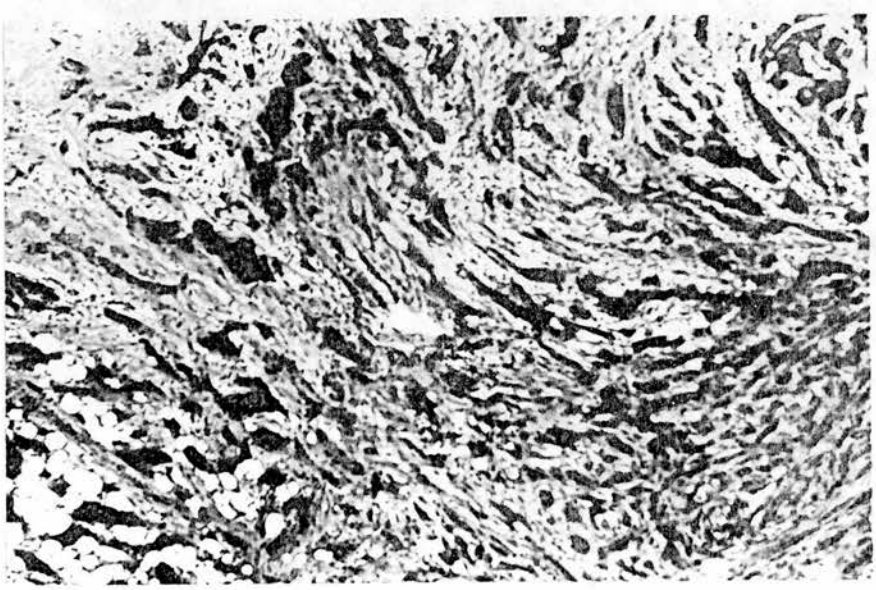
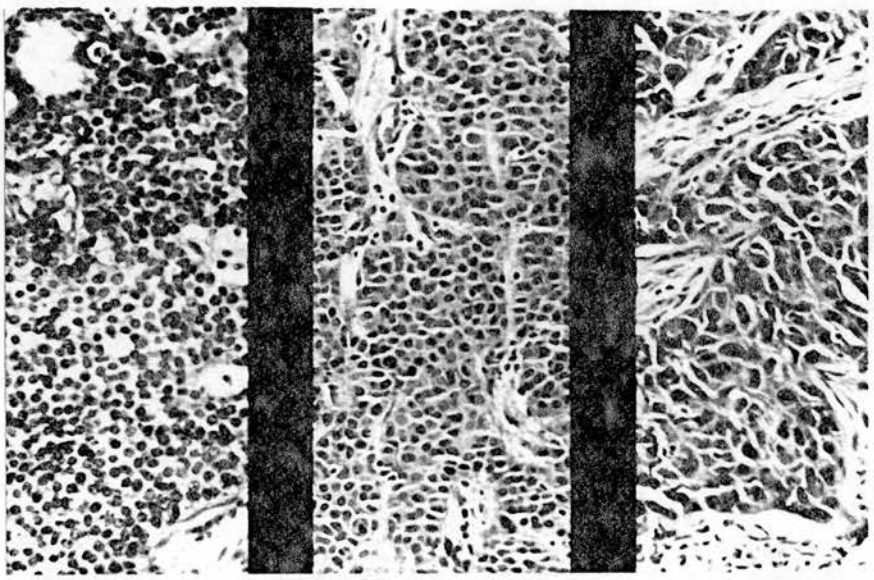
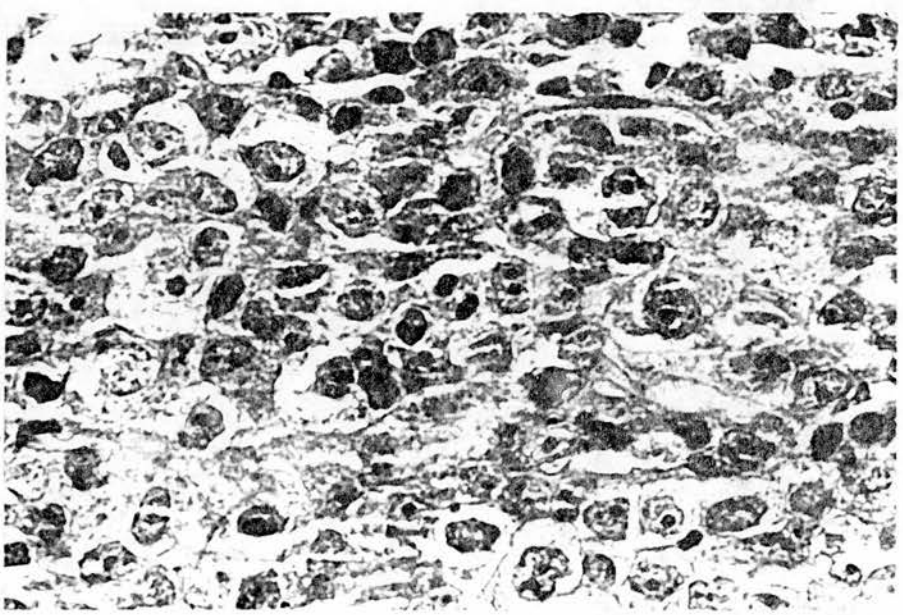


Figure 15 Different degrees of nuclear pleomorphism in breast cancer (x 50).



a b c

Figure 16 An example of a mitotic figure (x 160).



delay between the patient's first awareness of the tumour and presentation to the out-patient clinic was recorded, and termed duration of symptoms.

Oestrogen receptor estimations were carried out on most of the primary tumours, and any tumour containing less than 5 fmols receptor-bound oestrogen/mg cytosol protein was classified as "receptor negative". The method used for this assay is outlined in the appendix (pages 241 - 242).

v. Lymphoscintigraphy

Axillary lymphoscintigraphy was performed using the method described by Black and his colleagues[205].

In summary, this consisted of an intradermal injection of 0.05 miCu of ^{99}Tc-labelled rhenium sulphide colloid in 0.1 ml of buffer delivered to either side of the nipple in both breasts. When the study commenced, the injection was given via a hypodermic needle, and to prevent undue discomfort, 2% lignocaine was first given subcutaneously below the injection site. However, in the last 50 patients, the colloid was administered by gas-powered injection gun[477] equipped with a spacer to ensure intradermal dispersion (Figure 17). It was found that local anaesthesia was not required for this procedure, and use of the gun enabled multiple administrations to be carried out speedily and accurately.

Four hours after the injection, each patient was scanned using a Scintag-Vertholt gamma-camera, and permanent records taken of the emission pattern from both axillae.

Figure 17 Gas-powered injection of radiolabelled colloid.

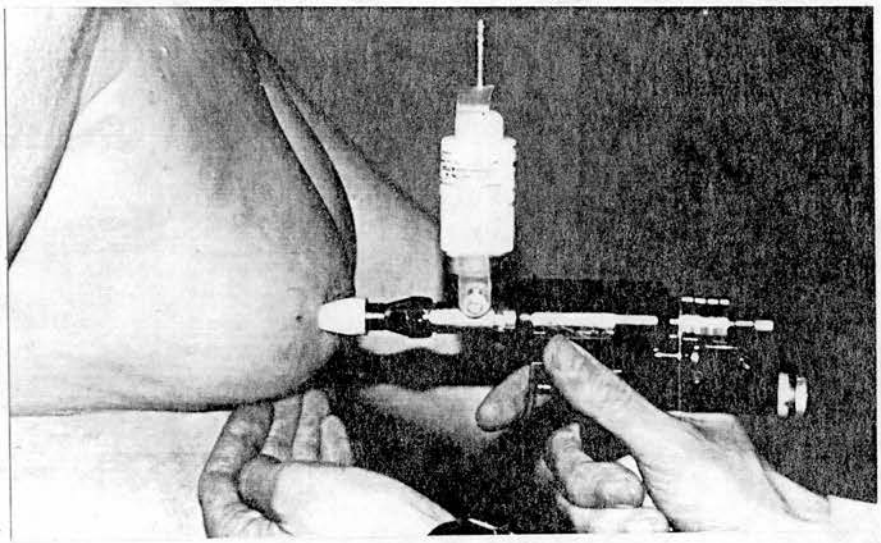
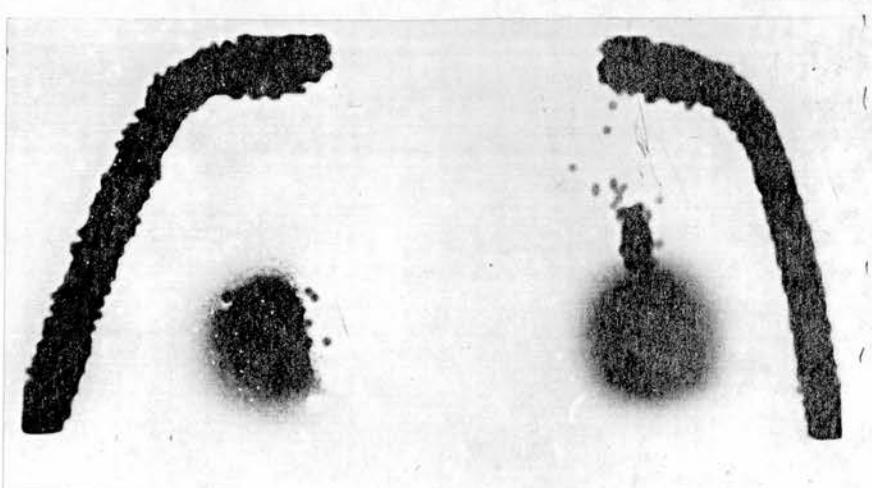


Figure 18 Negative lymphoscintiscan.

Figure 19 Positive lymphoscintiscan (depressed uptake on right).



When equal uptake was seen in the two axillary node groups the scan was taken as normal (Figure 18). An abnormal scan was classified as one where the uptake was depressed or absent on the side of the tumour (Figure 19). If uptake was absent on both sides, the scan was deemed unreadable.

vi. Data storage and retrieval

Data was stored on an 8-inch floppy disc using Cromemco Data Base Management System version 3.05 in a Cromemco "System Three" micro-computer. The data base structure for the retrospective group of 238 patients is shown on pages 244 - 245 of the Appendix. The information on the 100 patients in whom axillary clearance was carried out was stored in a different file, the data base of which is illustrated on pages 246-247.

It will be noted that all of the information on the first data base was not utilised. This is due to the fact that the system was devised for various purposes not all pertinant to the present study. The second data base, however, was specifically designed for the research described in this thesis.

viii. Statistical analysis

As most of the data were not normally distributed, the statistical tests employed were largely non-parametric. These consisted of Wilcoxon's rank sum test[478], Wilcoxon's signed rank test[478], Kendall's rank correlation test[479], the

chi-squared test[480], Fisher's exact test[481] and McNemar's test[480]. Student's t test[480] was used where data were appropriate. The log rank test[482] was used to calculate the significance of the difference between recurrence rates.

The specialised programmes used to perform these analyses are detailed in the appendix (pages 248 - 259).

2. STUDIES ON LYMPH NODE METASTASES IN BREAST CANCER

2 i. The importance of the number of nodes obtained at lower axillary sampling

Introduction

The number of axillary lymph nodes involved by metastatic tumour is well established as an important prognostic factor in breast cancer[1,16,62-68]. However, all the published data on this subject is derived from studies of radical or modified radical mastectomy specimens. As simple mastectomy is now gaining favour[2,250,255], it is important to establish whether lower axillary sampling can give useful prognostic information.

Method

The study group consisted of 238 consecutive patients treated for invasive breast cancer by simple mastectomy and lower axillary sampling (q.v. page 113). For each patient, the number of nodes found at the sampling procedure, and the number of these nodes involved by metastatic tumour was determined. From clinical records, it was also possible to obtain the date at which each patient had last reported to the follow-up clinic, and the date of any recurrence of the disease.

This information was used to calculate major recurrence rates, and the differences between several groups were examined using the log rank test.

Results

Of the 238 patients, lymph nodes were found in 206 cases. In 67 of these cases, only one node was dissected out and submitted for histological examination, whereas more were found in the others. The exact distribution of the number of nodes is shown in Table 17.

When the recurrence rate of patients with proven nodal metastases was compared to that of patients with nodes apparently free of tumour, there was a striking difference in the expected direction (Figure 20). Likewise, recurrence rates increased with the number of positive nodes found and, although small numbers obscured differences which may have existed between individual groups, a very clear distinction existed between one involved node and more than one (Figure 21).

Interestingly, the number of negative nodes found also had a significant bearing on prognosis. As can be seen from Figure 21, in patients who had no axillary metastases detected, recurrence rates fell with increasing numbers of negative nodes. In addition, patients who did have axillary tumour deposits had an improved prognosis if uninvolved nodes had also been found (Figure 22).

These results may suggest that limited axillary sampling can give fairly accurate quantitative prognostic information. However, some of the other findings were less encouraging.

It will be seen from Figure 20 that patients in whom no lymph nodes were found occupied an intermediate position between those with nodal metastases and those without, the

Table 17 Distribution of number of nodes found at lower axillary sampling.

Number of involved nodes found in patients with axillary metastases	Number of patients*	Number of uninvolved nodes found in patients with no axillary metastases	Number of patients
1	64 (31)	1	34
2	16 (7)	2	21
3	9 (4)	3	14
4	3 (1)	4	13
5	3 (1)	5	7
6	3 (2)	6	5
7	2 (1)	7	5
8	1 (1)	8	4
		9	2

*Figures in brackets indicate the number of patients in whom negative nodes were also found.

Figure 20 Comparison between recurrence rates for patients with negative nodes (N), for patients with positive nodes (P), and for patients in whom no nodes were found (?).

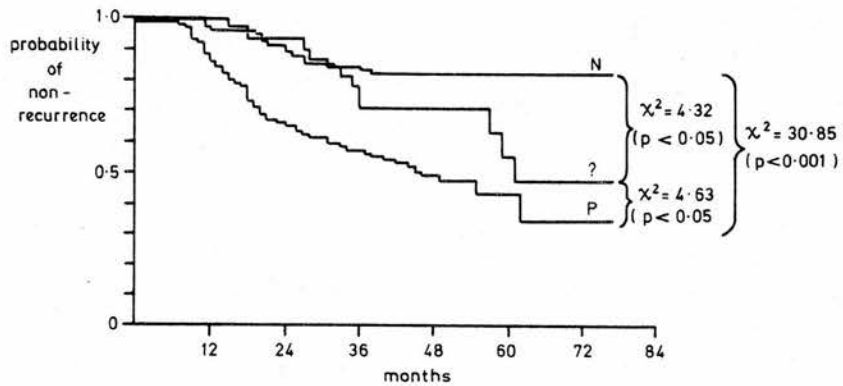


Figure 21 Comparison between recurrence rates for patients with one positive node (P1) and for those with more than one (P>1), and comparison between recurrence rates for patients with one negative node (N1) and for those with more than one (N>1).

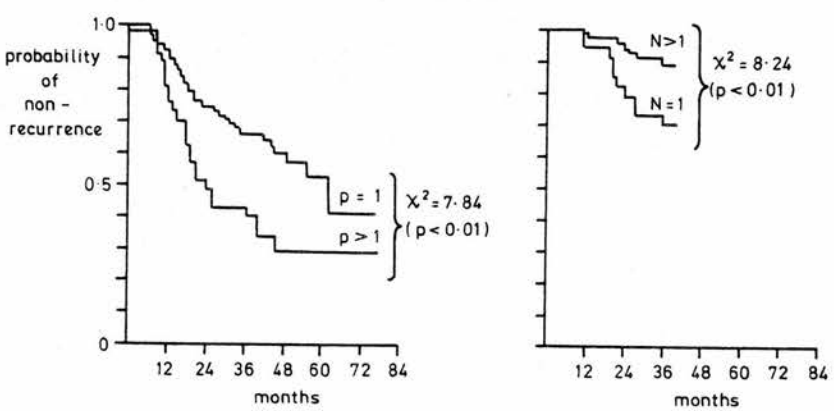


Figure 22 Comparison between recurrence rates for patients with positive nodes only (P alone) and in patients with positive and negative nodes (P + N).

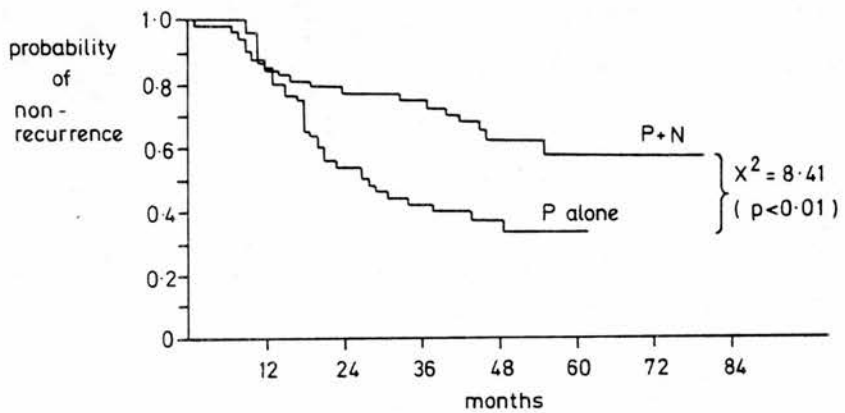
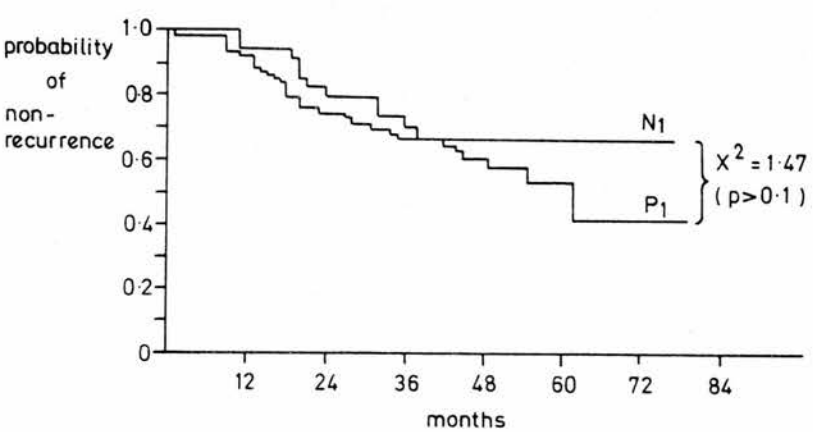


Figure 23 Comparison between recurrence rates for patients in whom only one positive node was found (P_1) and for those in whom only one negative node was found (N_1).



difference being statistically significant in both directions. Further, when the recurrence rate of patients in whom only one positive node had been found was compared with that of patients in whom only one negative node had been found, there was no significant difference (Figure 23). Neither of these two groups differed in their rate of recurrence from the patients in whom the surgeon failed to find any nodes.

Discussion

It is now well established through the results of several controlled clinical trials that simple mastectomy without clearance of the axilla is equally effective in the treatment of carcinoma of the breast as are the more extensive procedures (q.v. pages 54 - 58). Simple mastectomy has been shown to have an advantage over radical mastectomy in terms of morbidity[261], and it is an easier operation to perform. There is, however, a major disadvantage of mastectomy in which only the breast is removed. Histological information on the regional lymph nodes is absent, and it is known that clinical diagnosis of axillary metastases is highly inaccurate, with an error rate of about 30%[101,178-181]. Because of this problem, the operation of simple mastectomy has been combined with lower axillary or "pectoral" lymph node biopsy[483] in an attempt to obtain satisfactory histological staging without resorting to axillary clearance.

Unfortunately, this operation too has some drawbacks. Firstly, both in this series and in previous reports

a substantial number of the procedures failed to identify nodes for histological examination. The results of the present study provide ample evidence that patients in whom no nodes were found occupy a position halfway between those with positive nodes and those with negative nodes in terms of prognosis. In other words, to obtain any useful prognostic information, it is very important that the surgeon takes care to identify lymph nodes at operation and submits them as such to the pathologist. It is only too easy to dissect out a portion of lower axillary fat in the assumption that it contains nodes when in fact it does not.

The second disadvantage of simple mastectomy is that only a few nodes can be examined. The number of involved axillary nodes has been shown to be a very powerful predictive factor, but the relevant studies were exclusively concerned with radical or modified radical mastectomies. It is possible to isolate up to 13 nodes from lower axillary sampling[219], and it is therefore of great interest to determine whether the number of nodes found at this procedure can give useful prognostic information. This study has demonstrated that the number of involved nodes from a sampling can indeed provide a differential guide to the likelihood of recurrence, and must therefore reflect the total axillary situation to some extent. Nevertheless, there is an important caveat to this statement.

When patients in whom one positive node was found were compared with those in whom one negative node was found, no difference in recurrence rates could be detected, and both groups were identical in this respect to the group in which no

nodes at all were detected. This finding clearly indicates that if limited node sampling is to be of any value, more than one gland must be isolated at operation.

This point is emphasised by another intriguing discovery. Not only is the number of positive nodes of prognostic significance, but the number of negative nodes has similarly important implications. When no axillary metastases are present, the patients likelihood of recurrence varies inversely with the total of negative nodes found. Moreover, if positive nodes are accompanied by negative nodes in the sample, the prognosis is considerably improved.

These results are not in keeping with the experience of Fisher and Slack[64] who reported that the number of negative nodes from axillary clearance specimens had no effect on prognosis. It must therefore be concluded that this phenomenon is a product of the limited sampling technique. With sampling, the finding of more than one negative node must tend to eliminate those patients who do have positive nodes which have not been detected by the surgeon, and the finding of negative nodes along with the positive must tend to eliminate those patients who have more involved nodes than the number actually found.

Lower axillary sampling cannot be as accurate a staging procedure as axillary clearance, for metastases can appear exclusively in nodes which are outwith the reach of the lesser procedure[222]. Nevertheless, the present study provides evidence that, if at least more than one node is found, sampling can give useful information. It must be stressed, however, that the finding of only one node is of no value whatsoever, and for

adequate histological staging a thorough search for nodes in the lower part of the axilla must be carried out.

2 ii. The distribution of metastatic disease within the axilla in breast cancer

Introduction

In considering the validity of lower axillary sampling, it is important to establish the likely distribution of axillary nodal metastases in a patient with breast cancer. Previous studies have suggested that tumour deposits are most likely to occur in the lower parts of the axilla[8,218], but a careful analysis of the proportion of nodes involved at various levels has not been reported.

Method

The lymph nodes from 100 patients undergoing total axillary clearance for breast cancer were studied (q.v. page 113). Each clearance specimen was carefully dissected and mapped as described on page 115. The diagram of each clearance was divided into equal thirds from the base to the apex. It was then possible to classify the nodes as belonging to one of three levels, level 1 being the third closest to the breast, and level 3 being the apical region.

The lymph nodes were examined histologically, and a note was taken of those containing metastatic deposits. If the deposit was less than 2 mm. in maximum diameter, this was described as a micrometastasis after Fisher[94].

Results

Of the 100 patients studied, 43 had metastatic

disease demonstrated in their axillary lymph nodes. Of these, 5 (11.6%) had no metastases in the level 1 nodes, although in two cases the tumour deposits in the remainder of the axilla were micrometastases only. In only one instance was metastatic disease found exclusively in level 3, and this comprised a single micrometastasis.

The mean numbers of metastases in level 1, 2 and 3 were 2.26, 2.23 and 2.00 respectively, and the mean numbers of nodes found were 5.17, 9.29 and 8.84 (Figure 24). No significant differences existed between the number of metastases, but the number of nodes found in the lowest third of the axilla was significantly less than in either of the upper two thirds ($P<0.001$ by Student's t test).

When all the lymph nodes from patients with metastases were grouped together, 41% of those from level 1 had tumour deposits, compared with 22% and 23% in levels 2 and 3. This difference is highly significant by chi-squared testing ($P<0.001$, Table 18).

Discussion

From these results, it is clear that metastatic disease does not always spread in an orderly fashion from the lower axillary nodes to the higher. This is hardly surprising, as it is known that lymphatic routes from the breast to the axilla can by-pass the lowest nodes (q.v. pages 6 - 7).

In the present study, 11.6% of patients with metastases had none in the lower third of the axilla, which compares closely with the 14% found by Davies and his colleagues[222]. However, it must be stressed that two of the five patients

Figure 24 Distribution of nodes and nodal metastases in the three axillary levels.

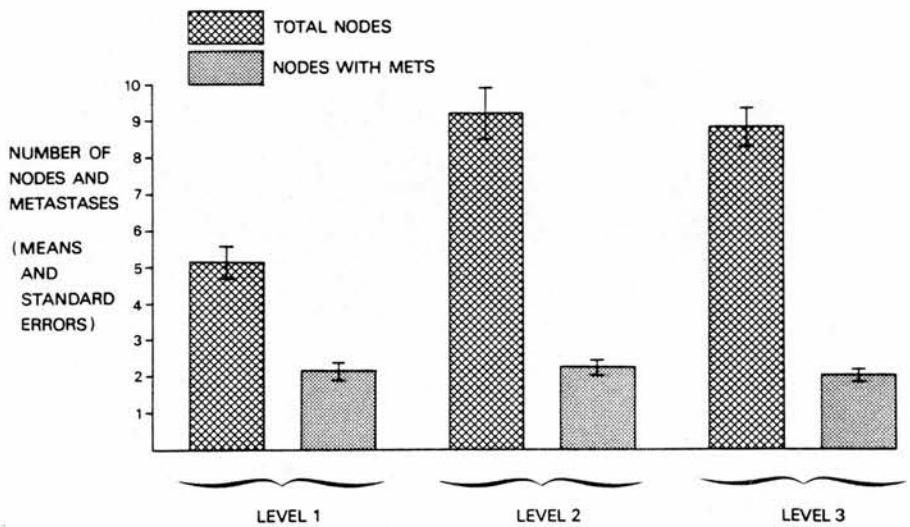


Table 18 The distribution of nodal metastases in 43 patients with axillary tumour deposits.

	Level 1	Level 2	Level 3
Number of nodes with metastases	91 (41%)	94 (22%)	86 (23%)
Number of nodes with no metastases	129 (59%)	328 (78%)	294 (77%)

in whom the lowest third of the axilla was spared had micrometastases only. Furthermore, a much higher proportion of nodes in the lower axilla were involved than in the upper two thirds.

It would seem therefore, that sampling the lower third of the axilla is unlikely to miss significant metastatic disease. In addition, the proportion of involved nodes is greater in the lower reaches, and the likelihood of detecting tumour deposits by lower axillary sampling is therefore high.

There will be occasions, however, when a strict sample of the lowest third of the axilla is not sufficient to detect nodal metastases. If the surgeon can feel an obviously enlarged lymph node beyond his sample, he should therefore be prepared to explore further. In this context, it is comforting to know that exclusive involvement of the apical nodes is extremely unusual.

2 iii. A randomised trial comparing lower axillary sampling with total axillary clearance

Introduction

In Literature Review I, it was pointed out that simple mastectomy is just as effective as radical mastectomy in terms of survival in breast cancer (q.v. pages 54 - 56), and, furthermore, the radical operation carries a significantly higher morbidity (q.v. pages 56 - 58).

However, there is some doubt as to whether lower axillary sampling can provide accurate histological staging of the axilla (q.v. pages 46 - 49), and the present study was therefore set up to determine the efficacy of careful sampling in this respect.

Patients and Methods

Between January 1980 and January 1982, 279 patients with operable breast cancer (T_0-T_3, N_0-N_2, M_0 by UICC criteria) were randomised to be treated either by simple mastectomy and lower axillary sampling or by simple mastectomy and total axillary clearance (a "Patey" type modified radical mastectomy).

In each patient treated by the sampling procedure, the surgeon attempted to identify and remove at least four lymph nodes from the axilla without transgressing the lower border of pectoralis minor. These nodes were then fixed, processed and examined histologically as previously described (q.v. pages 114 - 115). In the patients treated by total axillary clearance, pectoralis minor was divided, and the entire axilla was cleared of fat and lymphatic tissue from the axillary vein downwards,

care being taken to preserve the long thoracic nerve and the thoracodorsal neurovascular bundle. Immediately ofter removal, the clearance specimen was carefully dissected and the nodes fixed, processed and examined histologically as outlined in the general methods section (q.v. pages 114 - 115).

Results

In total, 139 patients were allocated to the sampling group, and 140 to the clearance group. Of these, 15 had to be excluded from the final analysis, because,for a variety of reasons, they received the wrong treatment option. This left 131 patients treated by sampling, and 133 treated by clearance.

The mean number of lymph nodes found in the sample group was 4.9 (standard error 0.2), with a range of 1 to 11, and a median value of 4. The mean number in the clearance group was 21.3 (standard error 0.8), with a range of 5 to 45, and a median value of 19. As these data were normally distributed, Student's t test was used to examine the difference between these two groups, and this was found to be highly significant ($P<0.001$).

The number of patients with involved nodes was then examined, and the distribution of the number of nodal metastases is shown in Figure 25. As can be seen, 75 patients (57%) in the sample group, and 80 patients (60%) in the clearance group had no metastases - a non-significant difference by chi-squared testing.

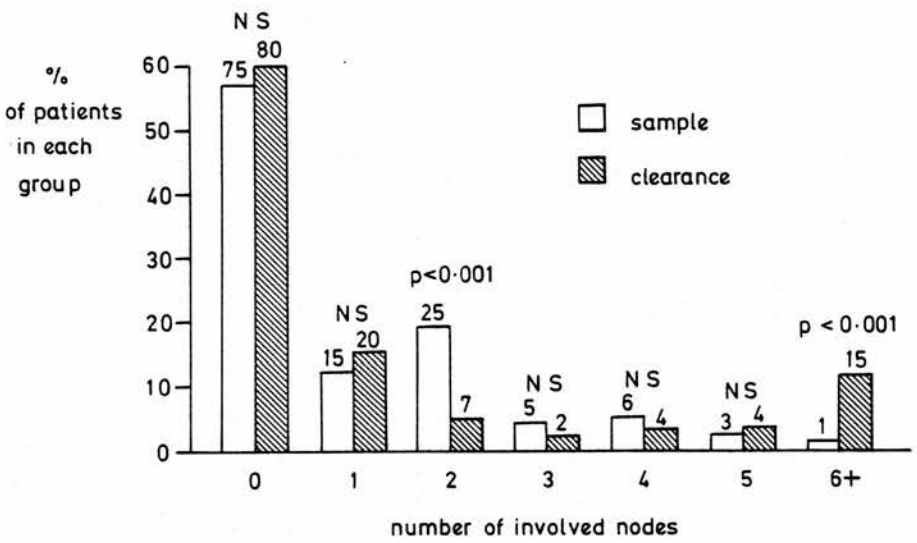
Turning to those patients with axillary node involvement only, the mean number of tumour-bearing nodes in

the sampling group was 2.3 (standard error 0.2), with a range of 1 to 6 and a median value of 2. In the clearance group, the mean was 4.8 (standard error 0.8), with a range of 1 to 25 and a median value of 2. Clearly, these data were not normally distributed, and Wilcoxon's rank sum test was employed for the purposes of comparison. No significant difference could be detected between the two groups using this method ($P<0.1$). However, as illustrated in Figure 25, significantly more patients in the sampling group had two involved nodes, and significantly fewer had six or more, Fisher's exact test being used to calculate the probability values.

Discussion

Existing evidence suggests that lower axillary sampling may fail to detect metastatic involvement of axillary lymph nodes. The Cardiff-St. Mary's trial showed that 10% of patients classified as node negative by sampling developed "recurrent" axillary tumour, suggesting that residual disease had been left behind at the original operation[221]. Further to this observation, Davies and his colleagues found that examination of the nodes from the lower part of excised axillary clearance specimens missed metastatic involvement of the axilla in 14% of cases[222]. However, it may be argued that neither of these findings is particularly useful in practical terms. In the Cardiff-St. Mary's trial, no nodes were found in a substantial proportion of patients, indicating that a careful search had not always been performed, and the study by Davies suffers from the fact that the methods employed for

Figure 25 The distribution of the numbers of nodal metastases in lower axillary samples and total axillary clearances.



obtaining lower axillary nodes are not strictly comparable to a lower axillary sample.

In section 2i (q.v. pages 128 - 136), it was shown that sampling must yield more than one node for the provision of useful prognostic information, but, as described in section 2ii (q.v. pages 137 - 140), careful mapping of nodes in axillary clearance specimens demonstrated that glands from the lower third do give a good indication of the metastatic status of the axilla as a whole. In view of these findings, the present study was carried out for two reasons. Firstly, to determine whether, by lower axillary sampling, the surgeon can consistently obtain more than one node, and secondly, to find out whether the incidence and degree of metastatic involvement of axillary nodes detected by sampling is comparable to that detected by total axillary clearance.

The results show that it is indeed feasible to obtain more than one node at axillary sampling, if adequate care is taken. Furthermore, the incidence of metastatic disease in the patients treated by sampling was almost identical to that in the clearance group, and although the total number of nodes found in a sample was considerable less than that in a clearance, the numbers of <u>involved</u> nodes were markedly similar, and the percentage involvement was in fact much higher in the samples. Certainly, more of the clearances revealed very large numbers of involved nodes. However, it is probable that a small increment in the number of involved nodes has more prognostic significance in the samples than in the clearances, and the

percentage involvement may also prove to be informative. Only prolonged follow-up of these patients will finally show whether the number of involved nodes found at sampling can provide comparable information to similar data from total axillary clearance.

2 iv. The relationship between axillary metastases and the primary tumour

Introduction

As emphasised in Literature Review I, lymph node metastases are related to aspects of the primary tumour which are of poor prognostic significance (q.v. pages 24 - 36). Because the relationship between prognostic factors is important if they are to be used in concert, a detailed analysis of the correlation between nodal metastases and prognostic aspects of the primary breast tumour has been carried out.

Method

100 patients undergoing mastectomy and total axillary clearance for invasive breast cancer were studied (q.v. page 113). The presence and number of metastatic nodal deposits were compared to the following prognostic factors pertaining to the primary tumour:

a) Tumour size

b) Tumour grade

c) Tumour contour

d) Oestrogen receptor level

e) Position of tumour

f) Duration of symptoms

(see pages 119 - 124 for methods of tumour assessment).

Correlation between these parameters and the presence or number of nodal metastases was examined using Kendall's rank correlation test, the Wilcoxon rank sum test, or chi-squared analysis.

Results

Rank correlation was performed to compare the number of nodal metastases with tumour size, tumour grade, oestrogen receptor level and duration of symptoms. As can be seen in Table 19, a significant positive correlation was obtained for size, grade and duration of symptoms, but not for oestrogen receptor level. Figures 26 - 28 illustrate the significant results.

Wilcoxon's rank sum test was used to test the difference between the number of metastases found with spiculated and rounded tumours, medial and lateral tumours, and oestrogen receptor negative and positive tumours. Table 20 demonstrates that spiculated tumours are associated with higher numbers of metastases than rounded tumours, but that position or oestrogen receptor status had no effect.

Tumour contour, position and oestrogen receptor status were also compared to "all or none" nodal status using chi-squared analysis. Again, spiculated tumours were significantly associated with nodal metastases; position and receptor status were not (Table 21).

Discussion

The two established prognostic factors, tumour size and grade, have a clear positive association with nodal metastases. In the case of size, this is already well established, and the present study merely confirms previous findings[98-103]. A relationship between tumour grade and the presence of nodal tumour has also been shown previously[106,108], but a correlation between

Table 19 Correlation between number of nodal metastases and prognostic factors. Significance calculated using Kendall's rank correlation.

	Kendall correlation coefficient	P
Tumour size	+0.26	< 0.01
Tumour grade	+0.22	< 0.05
Oestrogen receptor level	+0.13	> 0.1
Duration of symptoms	+0.25	< 0.01

Figure 26 Relationship between number of axillary metastases and tumour size.

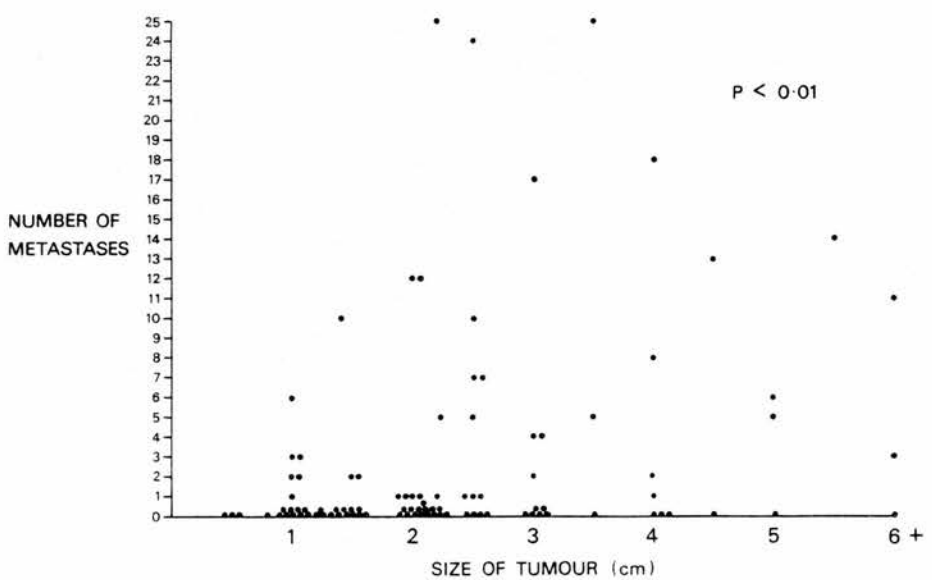


Figure 27 Relationship between number of axillary metastases and tumour grade.

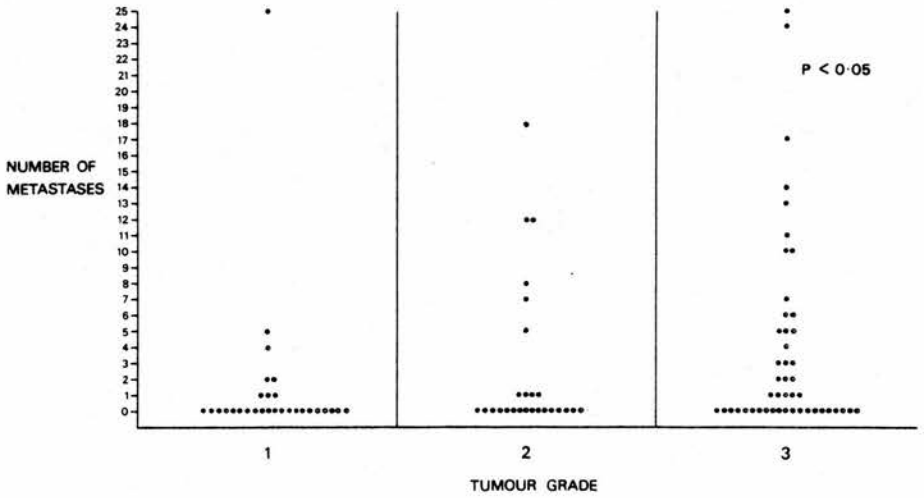


Figure 28 Relationship between number of axillary metastases and duration of symptoms.

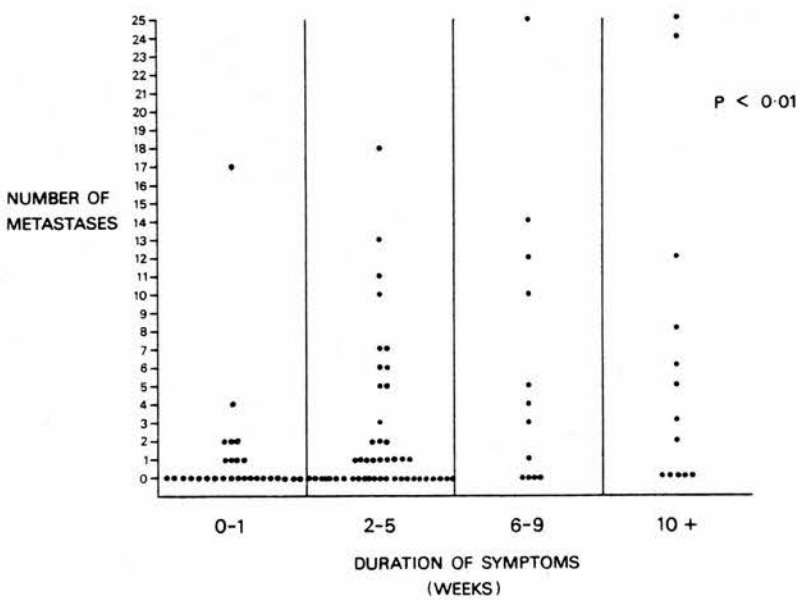


Table 20

	Median number of metastases	Interquartile range	P
Tumour contour			
Spiculated	1.0	0 - 5.0	< 0.01
Rounded	0	0 - 1.0	
Tumour position			
Medial	0	0 - 1.0	> 0.1
Lateral	0	0 - 4.5	
Oestrogen receptor status			
Negative	0	0 - 2.5	> 0.1
Positive	0	0 - 4.0	

Table 21

	Number with no metastases	Number with metastases	P
Tumour contour			
Spiculated	21	32	< 0.001
Rounded	31	11	
Tumour position			
Medial	14	10	> 0.1
Lateral	43	33	
Oestrogen receptor status			
Negative	13	9	> 0.1
Positive	38	33	

grade and number of metastases has not been demonstrated before.

Spiculated tumours have been shown to indicate a poor prognosis[126,128,160], but a study by Stewart and her colleagues could not demonstrate an association between tumour border and nodal metastases[125]. The results presented here, however, do indicate that spiculated breast cancers are more often associated with metastases than are rounded tumours, and that the numbers of nodal deposits tend to be higher.

Duration of symptoms is also a prognostic factor in breast cancer as it provides a measure of the length of time which the disease has been present (q.v. page 12). It may seem strange that a difference of a few weeks should affect the likelihood of extensive nodal involvement (see Figure 28), but this probably reflects a patient's level of awareness of a lump in her breast.

Finally, two factors, tumour position and oestrogen receptor level, are not related to axillary metastases. This is not surprising in the former instance, as medial tumours are not currently believed to carry a particularly poor prognosis[81-83]. However, it is difficult to reconcile the fact that tumours with low oestrogen receptor levels behave aggressively[115,118,119] with the failure to find a relationship between this parameter and nodal metastases. A more extensive study of this question is described in the next section.

In general, unfavourable prognostic factors related to the primary tumour have been found to increase the probability of axillary lymph node metastases occurring. This tends to

confirm the hyposthesis that, rather than constituting an independant indicator of prognosis, nodal tumour spread reflects the potential of the tumour as balanced by the host.

2 v. An extended study of the relationship between oestrogen receptor status and node status

Introduction

Several recent studies have demonstrated that the oestrogen receptor status of the primary tumour has an influence on prognosis in breast cancer. Patients with oestrogen receptor rich tumours have a consistently lower rate of recurrence and a higher survival rate than those whose tumours have little or no receptor[115,118,119,152]. This phenomenon is independent of endocrine treatment, and must therefore reflect the aggressiveness of the neoplasm.

It is somewhat surprising therefore, that no investigators have been able to demonstrate a relationship between oestrogen receptor status and the presence or absence of axillary lymph node metastases[114-124]. However, numbers in individual studies are small, and it was decided to combine the relevant data from Edinburgh with that available in the literature to determine whether such a relationship could be found.

Method and Results

Oestrogen receptor level and histological node status were reviewed in 283 patients treated for breast cancer between 1973 and 1980 in Edinburgh. Of the 216 patients who had oestrogen receptor positive tumours, 46% displayed metastatic deposits in their axillary nodes, whereas the same was true of 57% of the 67 patients with oestrogen receptor negative neoplasms. This difference was not, however, statistically significant.

A search of the literature revealed six reports in which patients were classified as having oestrogen receptor negative or positive primary breast cancer, and as having histologically negative or positive axillary nodes[115,119,121-124]. These data were combined with the Edinburgh results as shown in Table 22. In this aggregate, 48% (446) of oestrogen receptor positive tumours and 57% (352) of oestrogen receptor negative tumours were associated with lymph node metastases. The chi-squared value for these figures is 6.15, giving a probability value of less than 0.02.

Discussion

This study has demonstrated that a relationship does exist between oestrogen receptor status and the presence of regional lymph node metastases in breast cancer. The aggregate analysis which has been employed suffers from the fact that different methods and cut-off points for receptor analysis were used in the various studies, but as no strict definition of "receptor negative" exists, there is no alternative but to accept the small discrepancies.

The finding that breast tumours with low levels of oestrogen receptor activity are more often associated with nodal tumour deposits is of importance in understanding the biology of breast cancer. Oestrogen receptor negative tumours undoubtedly carry a poor prognosis per se[115,118,119,152], and it may be cogent to think of receptor status as a biochemical measure of differentiation. Such a view is supported by the finding that

Table 22 Association between oestrogen receptor status and node status in seven separate studies.

SOURCE	RECEPTOR STATUS	NODE STATUS N-	NODE STATUS N+	DEFINITION OF RECEPTOR NEGATIVE
Edinburgh	OER+	117(54%)	99(46%)	5 fmol/mg cyt pr
	OER-	29(43%)	38(57%)	
Knight[115] Cancer Res 37 : 4669	OER+	46(51%)	45(49%)	10 fmol/mg wt wt
	OER-	25(46%)	29(54%)	
Lippmann[122] Cancer 46 : 2829	OER+	52(61%)	33(39%)	10 fmol/mg wt wt
	OER-	30(41%)	44(59%)	
Furmanski[119] Cancer 46 : 2795	OER+	109(52%)	102(48%)	3 fmol/10 mg wt wt
	OER-	98(48%)	105(52%)	
Blamey[121] Cancer 46 : 2765	OER+	56(50%)	56(50%)	5 fmol/mg cyt pr
	OER-	53(56%)	41(44%)	
Samaan[124] Cancer 47 : 554	OER+	44(38%)	70(62%)	7 fmol/mg cyt pr
	OER-	24(29%)	60(71%)	
Howat[123] Lancet 1 : 1317 (1981)	OER+	61(60%)	41(40%)	?
	OER-	38(52%)	35(48%)	
Aggregate	OER+	485(52%)	446(48%)	
	OER-	297(43%)	352(57%)	

$chi^2 = 6.13$ ($P<0.02$)

oestrogen receptor negative tumours are usually poorly differentiated histologically[117,153-156]. It is therefore not surprising to find this association between oestrogen receptor status and lymph node status, and it again reinforces the belief that lymph node metastases represent an unfavourable balance between the tumour and the host; a balance which depends on the neoplastic potential of the tumour as well as the host resistance to it.

3. STUDIES ON REACTIVE CHANGES IN AXILLARY NODES IN BREAST CANCER

3 i. Reactive changes and prognosis in breast cancer

Introduction

In Literature Review II, it was stressed that there exists no adequate grading system for reactive changes in breast cancer which encompasses all the features known to have prognostic significance (q.v. pages 82 - 90). To circumvent the problems discussed in the review an entirely new system for grading reactive changes has been devised, and its effectiveness in assessing prognosis tested.

Method

The system developed for grading reactive changes is outlined on pages 115 - 119 in the General Methods section. To recap briefly, each lymph node was examined histologically for sinus histiocytosis, paracortical hyperplasia, germinal centre formation, fatty replacement and fibrosis. Each parameter was given a score of 0, 1 or 2 according to the area of the lymph node occupied by that particular change (Table 14, page 118). By adding the scores in all the nodes from one patient and dividing by the number of nodes available for examination, a mean score for each reactive change was derived for the individual patient.

For the purposes of analysis, the reactive change with the highest score in any patient was ascribed to that

patient as the "predominant reaction." If fatty replacement or fibrosis was predominant, then the nodes were described as "unstimulated."

164 patients in whom evaluable lymph node sections were available formed the study group. These patients were obtained from those treated for invasive breast cancer by simple mastectomy and lower axillary node sampling between 1974 and 1977 (q.v. page 113).

Results

Of the 164 patients studied, 79 (48.5%) had metastatic deposits in some nodes, although all had evaluable reactive change. In terms of predominant reactive change, 26 (16%) showed sinus histiocytosis, 56 (34%) showed paracortical hyperplasia, 44 (27%) showed germinal centre formation, and 38 (23%) had nodes which were unstimulated.

Figure 29 shows the overall results, the chi-squared values representing the differences between each category and all the other categories combined. Paracortical hyperplasia conferred a highly significant advantage, whereas germinal centre formation was significantly associated with a poor prognosis. Sinus histiocytosis showed a borderline advantage, whereas lack of stimulation appeared to have no effect.

When the results were analysed according to the presence or absence of nodal metastases, the same pattern held for paracotical hyperplasia and germinal centre formation (Figures 30 and 31). Indeed no patient with paracortical

Figure 29 Comparison between recurrence rates according to predominant reactive change in axillary lymph nodes.

PC - paracortical hyperplasia

SH - sinus histiocytosis

GC - germinal centre formation

US - unstimulated

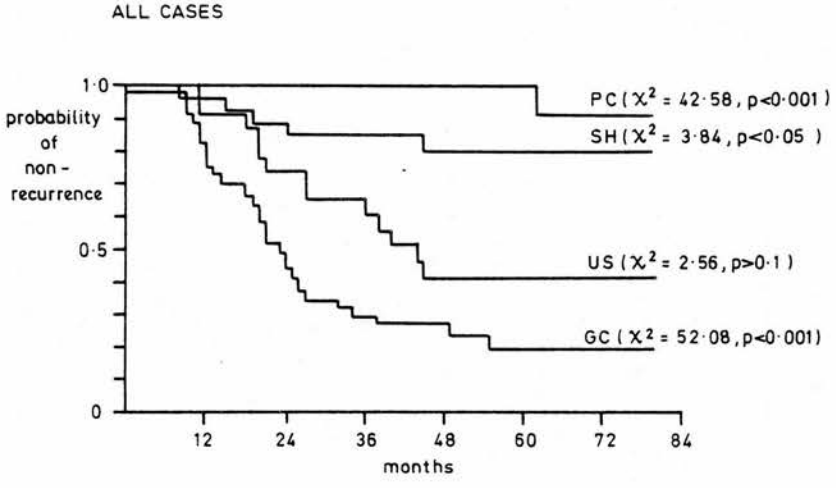


Figure 30 Comparison between recurrence rates according to predominant reactive change in the presence of nodal metastases.

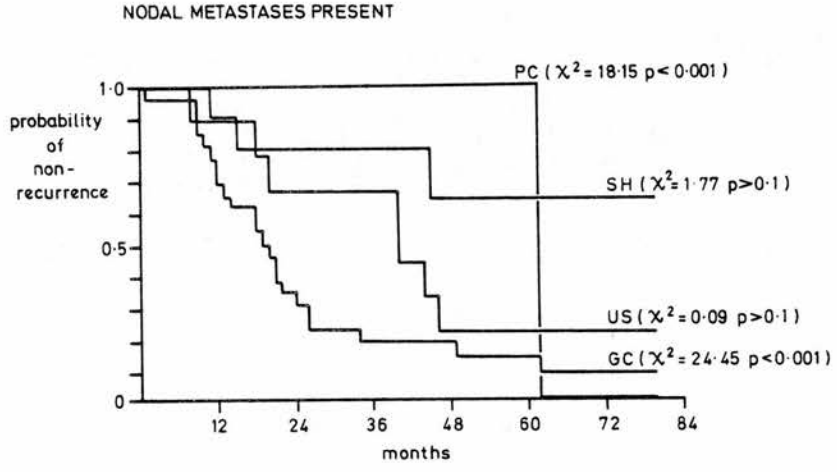
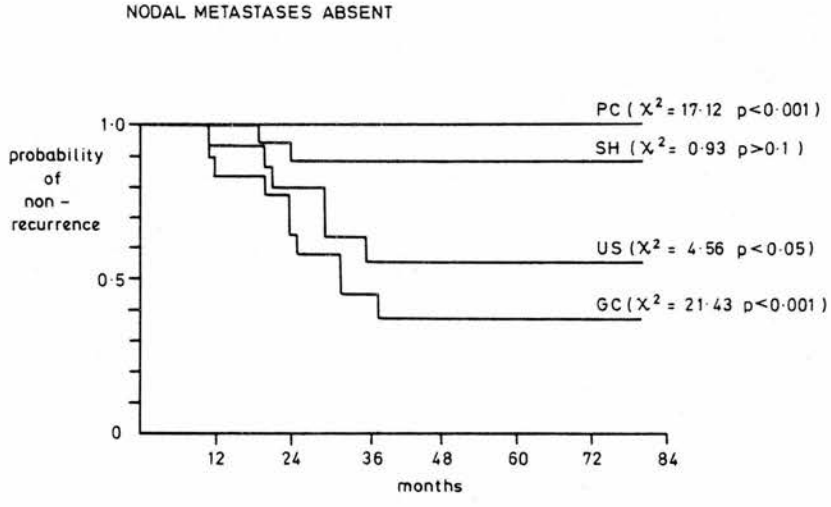


Figure 31 Comparison between recurrence rates according to predominant reactive change in the absence of nodal metastases.



hyperplasia in the "node negative" group had a major recurrence during follow-up. Sinus histiocytosis lost any significance when the patients had been split into these two groups, but, interestingly, lack of stimulation in the patients without metastases had a detrimental effect.

The association between the predominant reactive changes and the presence of metastases was also examined, and the results are shown in table 22. Sinus histiocytosis, paracortical hyperplasia and lack of stimulation were apparently associated with absence of metastases whereas the reverse was true for germinal centre formation. However, the only association to attain statistical significance by chi-squared analysis was that for paracortical hyperplasia.

Discussion

This new system for grading reactive changes is unique in that it takes into account all the changes which are known to have prognostic significance in breast cancer. It also acknowledges the fact that different reactions can occur in the same patient, and indeed within the same lymph node. A quantitative assessment of each reactive change is carried out for each patient, and from this the reaction which predominates can be identified. The strength of this method lies in its ability to balance prognostically opposing reactive changes in the same patient.

From this study, it is apparent that patients in whom paracortical hyperplasia predominates have an extremely good prognosis, and those showing germinal centre formation

Table 22 Relationship between the presence of lymph node metastases and predominant reactive change in lymph nodes.

Predominant Reaction	Metastases Present	Metastases Absent	P
Sinus histiocytosis			
Yes	9 (34.5%)	17 (65.5%)	> 0.1
No	70 (50.5%)	68 (49.5%)	
Paracortical hyperplasia			
Yes	20 (35.5%)	36 (64.5%)	< 0.05
No	59 (54.5%)	49 (45.5%)	
Germinal centre formation			
Yes	26 (59%)	18 (41%)	> 0.1
No	53 (44%)	67 (56%)	
Unstimulated			
Yes	16 (43%)	21 (57%)	> 0.1
No	63 (50%)	63 (50%)	

have a markedly worse outlook, regardless of metastatic node status.

Sinus histiocytosis, on the other hand, has its favourable effect destroyed when the patients are grouped according to node status, and this is probably due to a combination of sample size reduction and an association with metastatic disease in axillary nodes. A further difficulty with the interpretation of sinus histiocytosis is discussed later.

Lack of stimulation of nodes, using the present method, appears to have poor prognostic implications, but only in patients with no lymph node metastases. Possibly, its slight effect is overridden by the distinctly unfavourable presence of nodal tumour.

The immunological significance of these reactive changes and their possible role in tumour growth have been dealt with in Literature Review II (q.v. pages 77 - 81, 94 - 100) and will not be further discussed. Suffice it to say that the method of grading here described offers a new and simple guide to prognosis in breast cancer. However, we are still faced with the question of whether these reactions are influenced by the primary tumour, and therefore reflect its behaviour rather than that of the host. This cannot be answered by the present study, and is investigated in a later section (3 iv).

3 ii. The distribution of reactive changes within the axilla in breast cancer

Introduction

It is of interest to determine the distribution of reactive changes within the axilla in breast cancer for two reasons. Firstly, it might be expected that changes directly related to the tumour would be more prominent in the lower nodes. Secondly, if lower axillary sampling is employed, it is important to know whether it can demonstrate reactive changes occurring throughout the whole axilla.

Method

100 patients undergoing mastectomy and total axillary clearance were studied. The axillary nodes were dissected and mapped as previously described (q.v. pages 114 - 116).

The nodes were divided into three groups according to the level at which they were found, level 1 being nearest to the breast and level 3 being the apical third. Each node was then scored as 0, 1 or 2 for sinus histiocytosis, paracortical hyperplasia, germinal centre formation, fatty replacement and fibrosis as outlined in table 14 (page 118). A mean score for each level was then calculated for every patient by dividing the total score by the number of nodes.

It was then possible to compare the mean scores for each reaction in the three different levels, and significance was estimated using the Wilcoxon signed rank test for paired data.

Results

The median values for the reactive changes in the three levels are shown in table 23. For sinus histiocytosis, the value was significantly higher in level 3 that in level 2 ($P<0.05$) or in level 1 ($P<0.01$). No difference existed between levels 1 and 2. Paracortical hyperplasia decreased steadily from level 1 to 3, with significant differences between levels 1 and 2, and between 2 and 3 ($P<0.05$ and $P<0.01$ respectively). Similarly, germinal centre formation decreased in an apical direction with highly significant differences between the three levels ($P<0.001$ in each case). Fibrosis and fatty replacement did not differ between levels.

Discussion

Paracortical hyperplasia and germinal centre formation are undoubtedly more prominent in lymph nodes nearest to the tumour-bearing breast. This suggests that they are indeed related to events within the breast, and they are unlikely to be missed at lower axillary sampling.

Sinus histiocytosis is decreased slightly in lower axillary nodes, and this makes its possible connection with the primary neoplasm a little less likely. However, the differences between the levels are small, and its presence in the axilla would almost certainly be noted from lower axillary sampling.

Finally, fibrosis and fatty replacement appear to be evenly distributed throughout the axilla, suggesting that the tumour is unlikely to be exerting an influence on these parameters. This is hardly surprising, as lack of stimulation has been noted to be a normal feature in axillary nodes[321].

Table 23 Comparison of reactive changes in the three levels of the axilla.

MV - median value

IQR - interquartile range

	Level 1 MV(IQR)	Level 2 MV(IQR)	Level 3 MV(IQR)
Sinus histiocytosis	0.5 (0.25-0.77)	0.5 (0.27-0.8)	0.6 (0.39-1)
Paracortical hyperplasia	0.35(0-0.45)	0.26(0-0.32)	0 (0-0.21)
Germinal centre formation	0.5 (0.14-0.75)	0.14(0-0.5)	0 (0-0.37)
Fatty replacement	0.23(0-0.66)	0.25(0-0.5)	0.15(0-0.4)
Fibrosis	0 (0-0.28)	0.13(0-0.33)	0 (0-0.25)

3 iii. The relationship between different reactive changes and metastases in the same axilla

Introduction

It has been suggested in previous studies that reactive changes may be related to metastases within the axilla. Particularly, sinus histiocytosis has been associated with absence of nodal tumour deposits[318,362,368,390], and fibrosis with their presence[390]. However, the relationship between the different types of reactive change has never been critically examined.

In this study, an attempt has been made to elucidate these problems.

Method

100 patients undergoing mastectomy and total axillary clearance were studied (q.v. page 113). The axillary nodes were dissected and scored for reactive changes as previously described (q.v. pages 114 - 119). The mean scores for each reaction were then used for correlation with the number of metastatic deposits and with the scores for the other reactions. Kendall's rank correlation test was employed.

Results

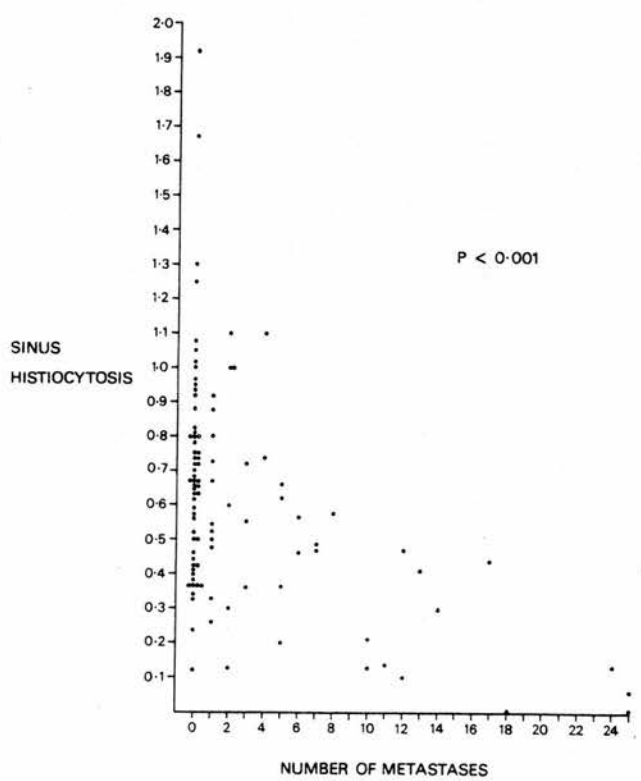
A significant negative correlation was found between the number of metastatic deposits and sinus histiocytosis, paracortical hyperplasia, fibrosis and fatty replacement (Table 24). Germinal centre formation was not related to metastatic disease.

Table 24 Relationship between reactive changes and metastases in nodes of the same axilla

	Kendall correlation coefficient	P
Sinus histiocytosis	-0.32	< 0.001*
Paracortical hyperplasia	-0.21	< 0.05
Germinal centre formation	+0.02	> 0.1
Fibrosis	-0.14	< 0.05
Fatty replacement	-0.16	< 0.05

*Illustrated in Figure 32

Figure 32



When the different reactive changes were compared with one another, sinus histiocytosis was found to increase in parallel with paracortical hyperplasia, and to vary inversely with fibrosis (Table 25, Figure 33). Germinal centre formation correlated positively with paracortical hyperplasia and negatively with fibrosis (Table 25). Fatty replacement and fibrosis were also closely associated (Table 25). There were no other significant relationships.

Discussion

In Literature Review I, it was stressed that lymph node metastases represent a balance between the tumour and the host which favours the tumour. The present study supports this view as sinus histiocytosis and paracortical hyperplasia, both reactions associated with a good prognosis[132,230,322,323,364,366-369,381], have been shown to vary inversely with the number of metastases.

These observations have been recorded previously (q.v. page 33), but the negative correlation between nodal tumour and fibrosis and fatty replacement is novel information. Remembering that the latter features are seen in "normal" axillary nodes[321], it is probable that tumours which are not sufficiently antigenic to provoke morphological changes in nodal microarchitecture have a low malignant potential.

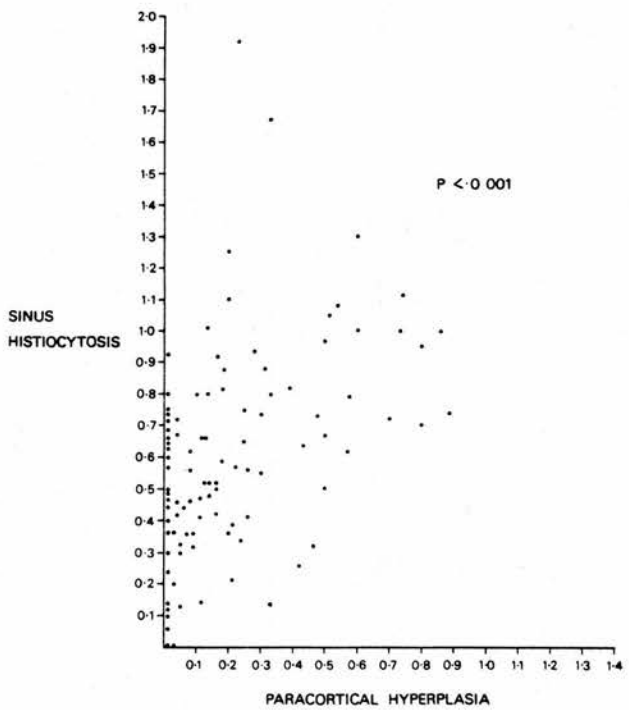
Turning to the interrelationship between reactive changes, it seems clear that sinus histiocytosis and paracortical hyperplasia are closely associated. This makes assessment of

Table 25 Relationship between different reactive changes.

	Kendall correlation coefficient	P
Sinus histiocytosis vs. Paracortical hyperplasia	+0.34	< 0.001*
Sinus histiocytosis vs. Germinal centre formation	+0.09	> 0.1
Sinus histiocytosis vs. Fibrosis	-0.17	< 0.05
Sinus histiocytosis vs. Fatty replacement	+0.08	> 0.1
Paracortical hyperplasia vs. Germinal centre formation	+0.14	< 0.05
Paracortical hyperplasia vs. Fibrosis	-0.08	> 0.1
Paracortical hyperplasia vs. Fatty replacement	-0.008	> 0.1
Germinal centre formation vs. Fibrosis	-0.15	< 0.05
Germinal centre formation vs. Fatty replacement	-0.07	> 0.1
Fibrosis vs. Fatty replacement	+0.30	< 0.001

*Illustrated in Figure 33

Figure 33 Relationship between sinus histiocytosis and paracortical hyperplasia.



their relative importance in prognosis difficult; it is impossible to say whether sinus histiocytosis has favourable prognostic implications merely because it paralells paracortical hyperplasia or vice versa. No published studies have combined these two parameters in an attempt to determine which is the more powerful.

Paracortical hyperplasia is also positively correlated with germinal centre formation, and as these have opposing prognostic significance (q.v. pages 82 - 90), it is critical that any assessment of reactive changes in breast cancer should take account of their relative extents.

Fibrosis and fatty replacement go together, presumably because they both indicate lack of stimulation. For the same reason, it is not surprising that fibrosis displays a negative correlation with sinus histiocytosis and germinal centre formation.

It is tempting to ascribe functional roles to these morphological relationships. Sinus histiocytes may represent macrophages presenting antigen to T-cells accumulating in the paracortex. In turn, the association between paracortical hyperplasia and germinal centre formation may be explained on the basis of T-B cell co-operation[354].

These speculations cannot be resolved on morphological grounds, however. The real importance of establishing the relationships between reactive changes is the recognition that they are not mutually exclusive and that some display a degree of interdependance.

3 iv. The relationship between reactive changes and the primary tumour

Introduction

In Literature Review II, it was indicated that very little is known of the relationship between reactive changes in axillary lymph nodes and features of the primary tumour known to affect prognosis. As this relationship is important both in the understanding of the biology of breast cancer and in the compilation of prognostic indices, a detailed analysis has been carried out.

Method

100 patients undergoing mastectomy and total axillary clearance for invasive breast cancer were studied (q.v. page 113). Reactive changes in the axillary lymph nodes were graded according to previously described criteria (Table 14, page 118), and a mean score for each reaction was calculated for every patient. The mean values ascribed to the reactions were then compared to the following prognostic factors pertaining to the primary tumour:

a) Tumour size

b) Tumour grade

c) Oestrogen receptor level

d) Duration of symptoms

e) Tumour contour

f) Position of tumour

(See pages 119 - 124 for methods of tumour assessment.)

Correlation between these parameters and the "reactions" of sinus histiocytosis, paracortical hyperplasia, germinal centre formation, fibrosis and fatty replacement, were carried out using Kendall's rank correlation test or Wilcoxon's rank sum test.

Results

Tumour size was found to correlate positively with germinal centre formation, and negatively with paracortical hyperplasia, fibrosis and fatty replacement (Table 26, Figures 34 - 36). Tumour grade also displayed a positive association with germinal centre formation, and a negative association with fibrosis (Table 27, Figure 37).

When oestrogen receptor levels in the primary tumour were compared to the reactive changes, germinal centre formation was noted to decrease significantly as levels increased (Table 28, Figure 38). None of the reactive changes were related to menopausal status.

Duration of symptoms correlated negatively with sinus histiocytosis and paracortical hyperplasia (Table 29), but no relationship could be demonstrated between tumour contour or tumour position and any of the reactive changes (Tables 30 and 31).

Table 26 Correlation between tumour size and reactive changes in lymph nodes.

	Kendall's correlation coefficient	P
Sinus histiocytosis	-0.06	> 0.1
Paracortical hyperplasia	-0.13	< 0.05*
Germinal centre formation	+0.16	<0.05*
Fibrosis	-0.25	<0.001*
Fatty replacement	-0.18	<0.05*

*Statistically significant - illustrated in following figures

Figure 34 Relationship between germinal centre formation and tumour size.

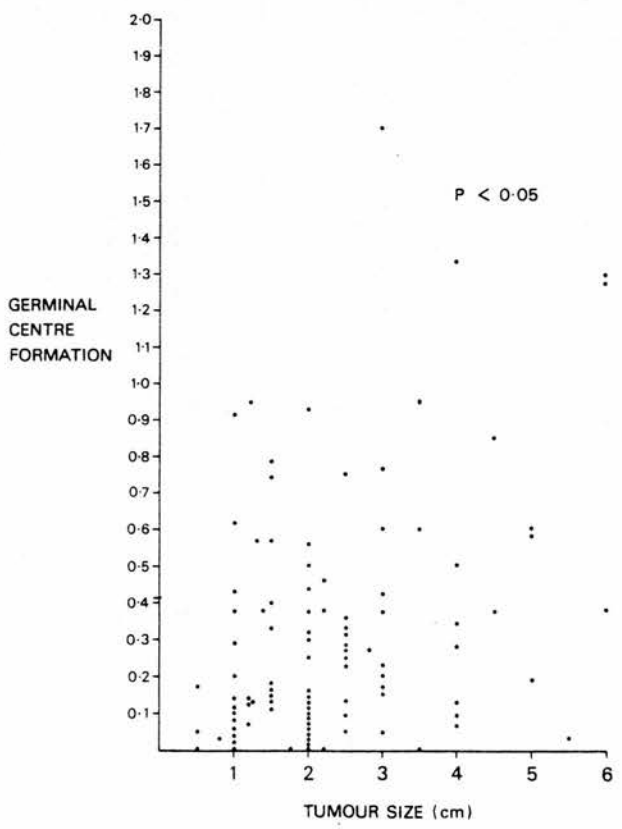


Figure 35 Relationship between paracortical hyperplasia and tumour size.

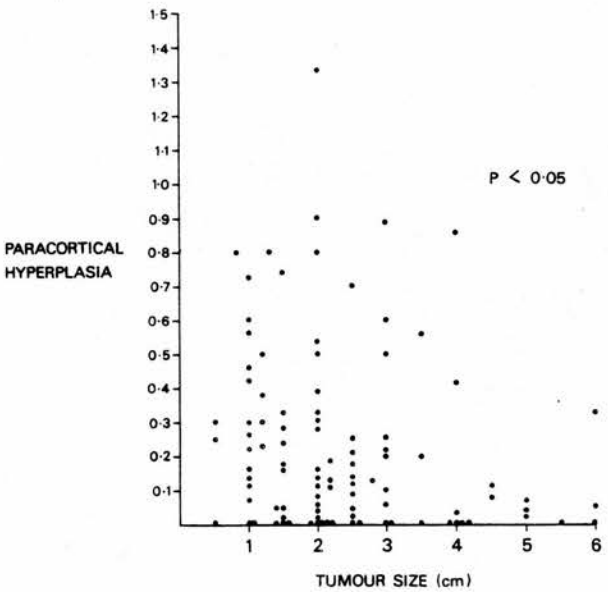


Figure 36 Relationship between fibrosis, fatty replacement and tumour size.

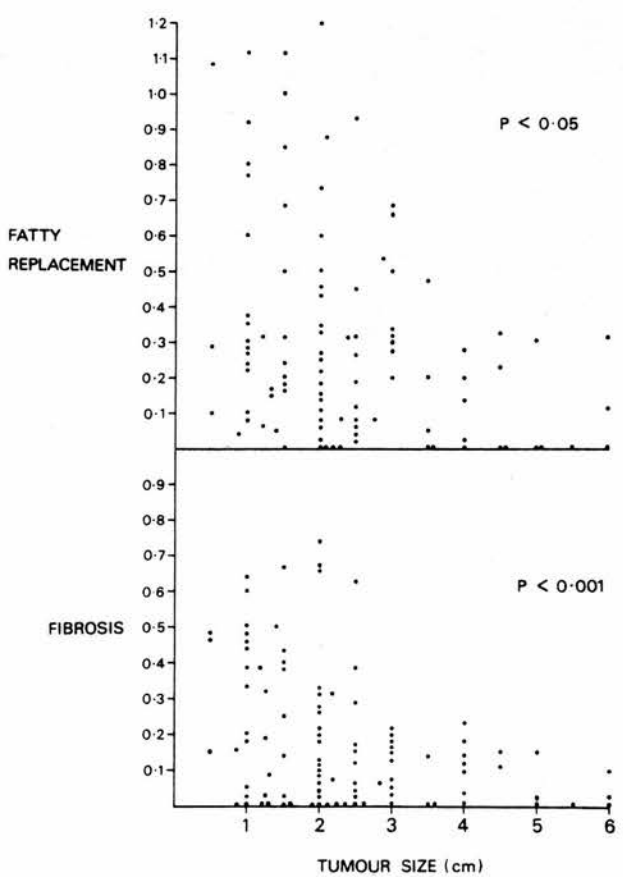


Table 27 Correlation between tumour grade and reactive changes in lymph nodes.

	Kendall correlation coefficient	P
Sinus histiocytosis	-0.04	> 0.1
Paracortical hyperplasia	+0.04	> 0.1
Germinal centre formation	+0.38	< 0.001*
Fibrosis	-0.29	< 0.001*
Fatty replacement	-0.13	> 0.1

*Statistically significant - illustrated in following figure

Figure 37

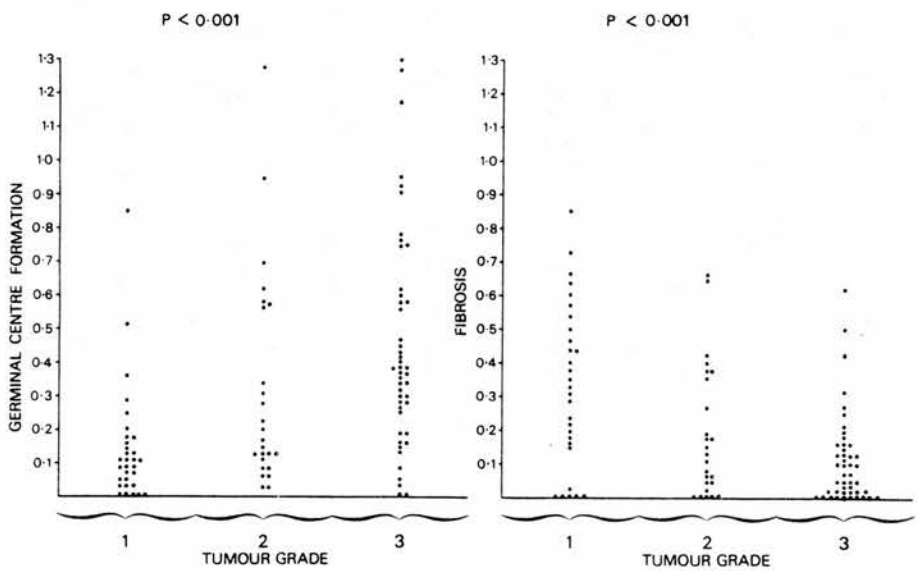


Table 28 Correlation between oestrogen receptor levels and reactive changes in lymph nodes.

	Kendall correlation coefficient	P
Sinus histiocytosis	-0.03	> 0.1
Paracortical hyperplasia	+0.05	> 0.1
Germinal centre formation	-0.22	< 0.01*
Fibrosis	+0.05	> 0.1
Fatty replacement	+0.02	> 0.1

*Statistically significant difference - illustrated in following figure

Figure 38

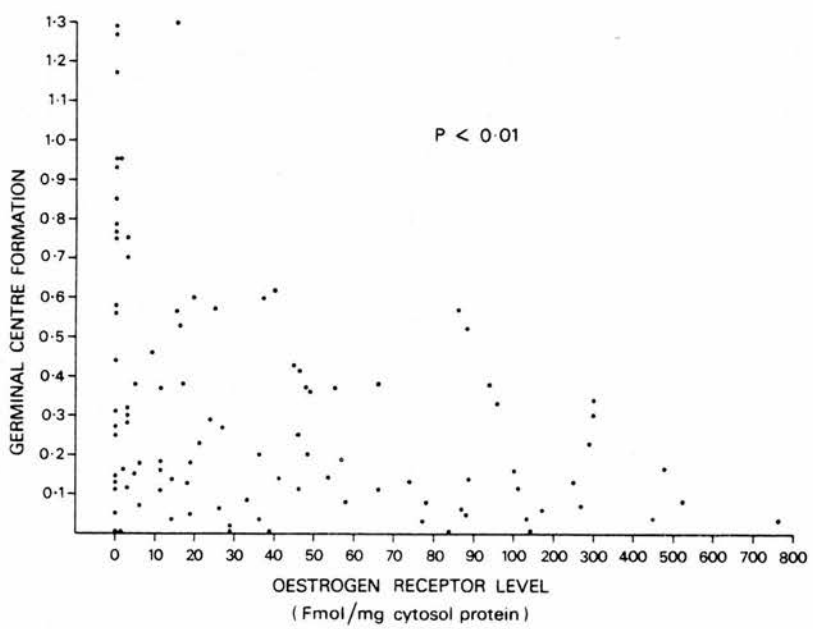


Table 29 Correlation between duration of symptoms and reactive changes in lymph nodes.

	Kendall correlation coefficient	P
Sinus histiocytosis	-0.21	< 0.01
Paracortical hyperplasia	-0.22	< 0.01
Germinal centre formation	+0.007	> 0.1
Fibrosis	-0.008	> 0.1
Fatty replacement	+0.03	> 0.1

Table 30 Relationship between tumour contour and reactive changes in lymph nodes.
(MV - median value; IQR - interquartile range)

	Spiculated MV(IQR)	Rounded MV(IQR)	P*
Sinus histiocytosis	0.57(0.36-0.74)	0.59(0.39-0.80)	> 0.1
Paracortical hyperplasia	0.12(0-0.31)	0.15(0-0.39)	>0.1
Germinal centre formation	0.21(0.08-0.38)	0.20(0.11-0.56)	>0.1
Fibrosis	0.14(0-0.27)	0.13(0-0.32)	>0.1
Fatty replacement	0.21(0.05-0.35)	0.27(0.07-0.45)	>0.1

*Significance calculated using Wilcoxon's rank sum test

Table 31 Relationship between tumour position and reactive changes in lymph nodes.
(MV - median value; IQR - interquartile range)

	Medial MV(IQR)	Lateral MV(IQR)	P*
Sinus histiocytosis	0.67(0.39-0.88)	0.56(0.36-0.74)	>0.1
Paracortical hyperplasia	0.20(0.05-0.31)	0.12(0-0.32)	>0.1
Germinal centre formation	0.31(0.09-0.51)	0.18(0.09-0.43)	>0.1
Fibrosis	0.03(0-0.32)	0.15(0.04-0.30)	>0.1
Fatty replacement	0.21(0.02-0.32)	0.25(0.06-0.47)	>0.1

*Significance calculated using Wilcoxon's rank sum test

Discussion

In general, it appears that unfavourable reactive changes are associated with features in the primary tumour which have poor prognostic implications. Germinal centre formation, which is known to relate to early disease recurrence[369,372], has been shown in this study to be associated with large tumours, tumours of poor histological grade and tumours with low oestrogen receptor levels. Conversely, paracortical hyperplasia, a favourable factor[132,230,381], is associated with small tumours and short duration of symptoms. Sinus histiocytosis seems to have little relationship with the primary tumour, although it is associated with short duration of symptoms. Interestingly, however, fibrosis of lymph nodes correlates positively with two favourable features in the neoplasm - small size and low histological grade.

It would seem, therefore, that reactive changes cannot be regarded as entirely independent of the primary breast tumour. Presumably, an aggressive neoplasm can evoke a humoural immune response which becomes manifest as germinal centre formation in the regional nodes. Thus, such a reactive change reflects the nature of the primary tumour rather than the host's response to it. Of course, it is conceivable that the immune response modifies tumour growth, and it may be that large, poorly differentiated, oestrogen receptor negative tumours behave aggressively despite stimulation of B-cell proliferation and antibody formation, or even <u>because</u> of it.

Small, well differentiated tumours, on the other hand, appear to have little effect on the regional nodes.

Although small tumours are associated with paracortical hyperplasia, fibrosis is the main feature seen in concert with favourable cancers. This finding suggests that tumours with low malignant potential are poorly antigenic, and tend not to evoke an immune response in regional nodes.

In conclusion, the present investigation has shown that reactive changes in axillary nodes in breast cancer are not exclusively governed by the host, but display some degree of dependancy on the tumour.

3 v. The effect of breast biopsy on reactive changes

Introduction

In Literature Review II, it was established that three lymph node reactions, sinus histiocytosis, paracortical hyperplasia and germinal centre formation, have prognostic implications in breast cancer (q.v. pages 82 - 90). However, most of the original studies were done in America, at a time when immediate mastectomy following frozen section diagnosis was in vogue. As many centres, including Edinburgh, have abandoned this policy in favour of a more considered approach, it was felt necessary to establish whether breast biopsy carried out some days before mastectomy could alter the histological pattern of the draining lymph nodes.

Method

Fifty patients who had been treated for breast cancer by simple mastectomy and lower axillary node sampling immediately after biopsy and frozen section diagnosis were randomly selected from the files of the Department of Pathology. A corresponding group, matched for age and the presence of nodal metastases were then chosen from patients in whom "Tru-cut" or excision breast biopsy had preceded mastectomy by between two and five days. Both groups of patients were treated between 1974 and 1977, and both originated from the Edinburgh area.

The histological sections of the axillary lymph nodes were graded for sinus histiocytosis, germinal centre formation and paracortical hyperplasia according to previously

described criteria (q.v. pages 115 - 119). In every patient a mean lymph node score for each reaction was calculated by dividing the total score by the number of nodes.

Results

The mean age in the "frozen section" group was 55.5 years (±9.4) and 55.1 years (±9.6) in the "previous biopsy" group. The mean number of nodes examined was 3.9 (±1.7) and 3.2 (±1.9) respectively. In both groups, eleven patients had histological evidence of lymph node metastases, although nodal architecture was also discernable.

When the number of patients exhibiting no reaction in any of their lymph nodes was compared to the number in whom some reaction was seen, it was noted that the incidence of sinus histiocytosis was significantly increased in those women who had been subjected to biopsy a few days prior to mastectomy. Such a difference was not seen with paracortical hyperplasia or germinal centre formation (Table 32).

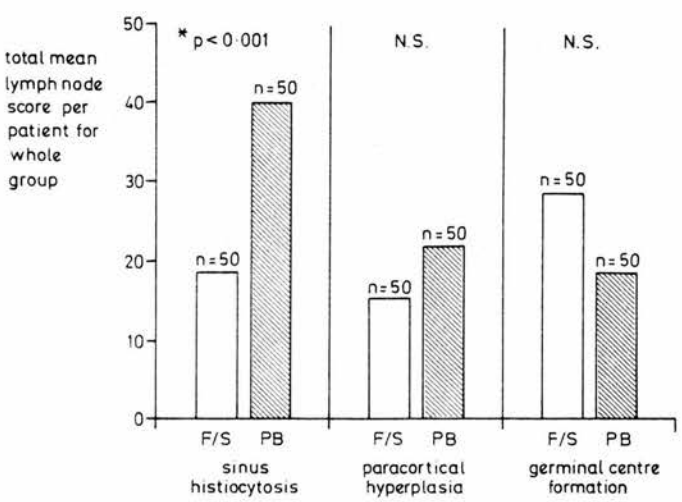
In order to investigate the effect of biopsy on the intensity of the various reactions, the mean lymph node scores were compared using Wilcoxon's rank sum test. Again, a highly significant association between previous biopsy and sinus histiocytosis was found (Figure 39). It was not possible to detect such a difference in germinal centre formation or paracortical hyperplasia.

No association between number of nodes examined and any mean lymph node score could be found using Kendall's rank correlation test.

Table 32

	Sinus histiocytosis		Paracortical hyperplasia		Germinal centre formation	
	+ve	-ve	+ve	-ve	+ve	-ve
Frozen section biopsy	28	22	23	27	33	17
Previous biopsy	48	2	32	18	31	19
	chi^2=19.79 (P<0.001)		chi^2=2.59		chi^2=0.04	

Figure 39 Comparison between previous biopsy (PB) and frozen section biopsy (F/S) in the degree of reactive change. The vertical axis represents the total of the mean lymph node scores in the whole group.



(significance calculated using Wilcoxon's Rank Sum Test)

Discussion

Sinus histiocytosis was first proposed as a favourable prognostic factor by Black and his colleagues in 1953[322], and although some workers have been unable to confirm this finding [370-372], the general consensus of opinion is in agreement with the original tenet[230,362,366-369]. In addition to sinus histiocytosis, expansion of the paracortical or thymus-dependant area of regional lymph nodes has been correlated with a good prognosis in breast cancer, and B-cell proliferation characterised by germinal centre formation is now recognised as an unfavourable risk factor (q.v. pages 85 - 86).

Although the functional relationship between tumour growth and these three changes in lymph nodes has yet to be elucidated, their bearing on prognosis should still be a useful tool in identifying high risk patients. However, it is important to know whether breast biopsy can alter the microscopic architecture of the regional lymph nodes, and the present study has shown that sinus histiocytosis in axillary nodes is greatly increased by prior breast biopsy.

Why this phenomenon should occur is not clear, as the functional nature of sinus histiocytosis remains a mystery. However, colloid taken up by lymph nodes appears first in sinusoidal cells[324-328] suggesting that thet may represent scavenging macrophages. Moreover, a recent study has reported increased uptake of radio-labelled colloid in axillary lymph nodes following breast biopsy[200], suggesting activation or accummulation of phagocytic cells consequent on surgical trauma.

It is also interesting to speculate as to whether changing attitudes to breast biopsy have altered the incidence and thereby the significance of sinus histiocytosis. Fisher, investigating patients treated by mastectomy in 1975, found the incidence of sinus histiocytosis to be 78%[100], a much higher value that quoted in earlier reports (Table 13, page 83). He also found that the reaction had no prognostic value[372]. It is possible that a swing away from frozen section diagnosis has contributed to these disparate findings.

In conclusion, it must be stressed that this report does not necessarily discredit sinus histiocytosis as a prognostic factor in breast cancer. Indeed, there is no doubt that it does occur without previous biopsy. However, the results do suggest that the prognostic significance of sinus histiocytosis should be regarded with extreme caution in patients who have had a breast biopsy some days prior to mastectomy.

3 vi. The influence of axillary nodes on the prognostic significance of lymphocytic infiltration in breast cancer

Introduction

Lymphocytic infiltration in breast cancer is a histological indication of host response which has excited a great deal of interest. Despite this, its significance as a prognostic factor is unresolved, although it is now generally agreed to be of little importance except in the unusual case of medullary carcinoma (q.v. pages 101-102).

However, the relationship between lymphocytic infiltration of the primary tumour and reactive changes in axillary lymph nodes has not been closely examined. The present study, therefore, is aimed at establishing whether any such relationship exists, and whether histology of regional nodes influences the prognostic significance of lymphocytic infiltration.

Method

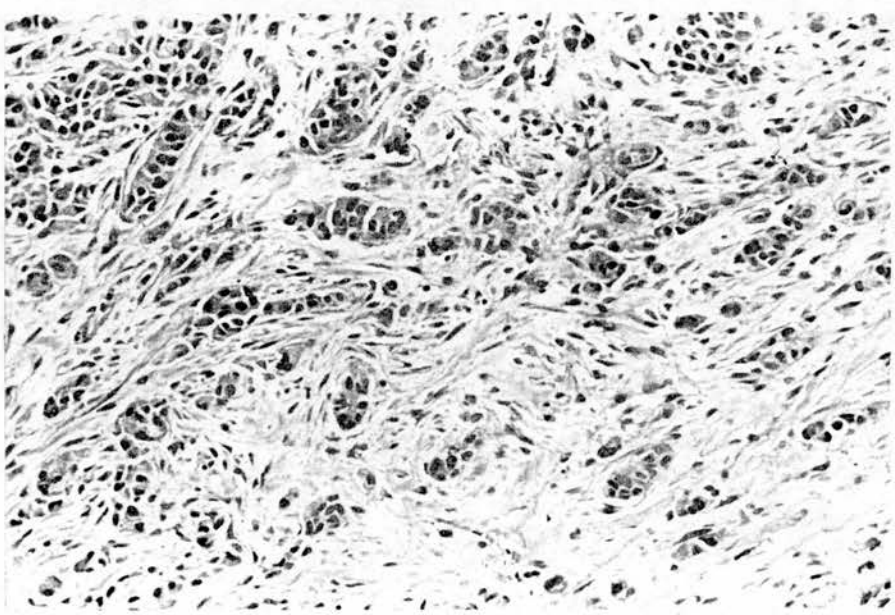
Two groups of patients were studied. The relationship between reactive changes and lymphocytic infiltration was examined using the 100 women who had undergone total axillary clearance (q.v. page 113). To evaluate the influence of axillary node histology on the prognostic significance of lymphocytic infiltrate, the group of 164 patients with evaluable lymph node sections (q.v. page 159) were scrutinised.

Lymphocytic infiltration of the tumour was assessed on histological sections and graded 1 - 4 as described in table

Table 33 Grading system for lymphocytic infiltration of breast tumours (modified from Hamlin[387]).

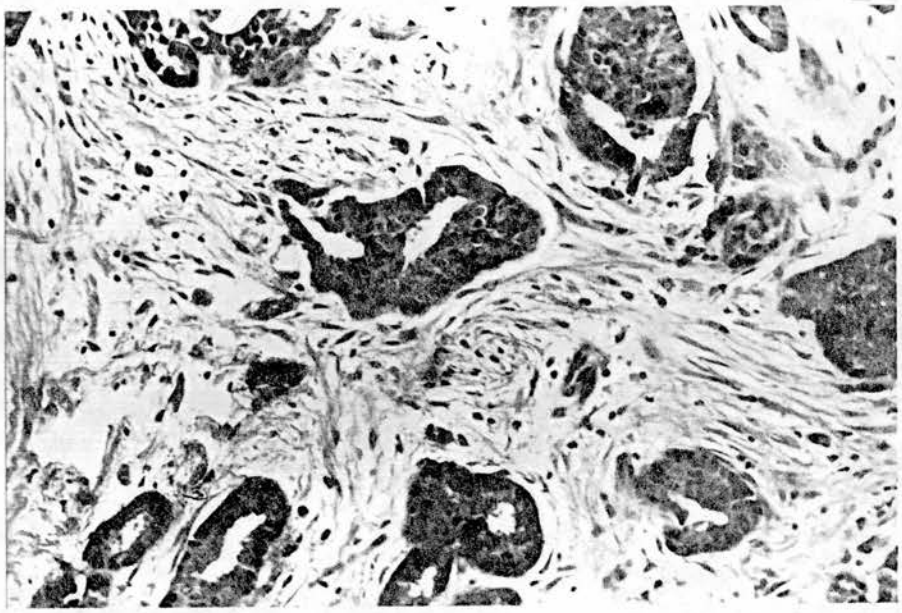
I	-	Virtually no lymphocytes detectable in the tumour section (Figure 40)
II	-	Lymphocytes present, but scanty (Figure 41)
III	-	Definite lymphocytic infiltration, confined to the tumour border (Figure 42)
IV	-	Lymphocytic infiltration not only at the border, but also present between the strands of tumour cells (Figure 43)

Figure 40 Grade I lymphocytic infiltration (x 50)

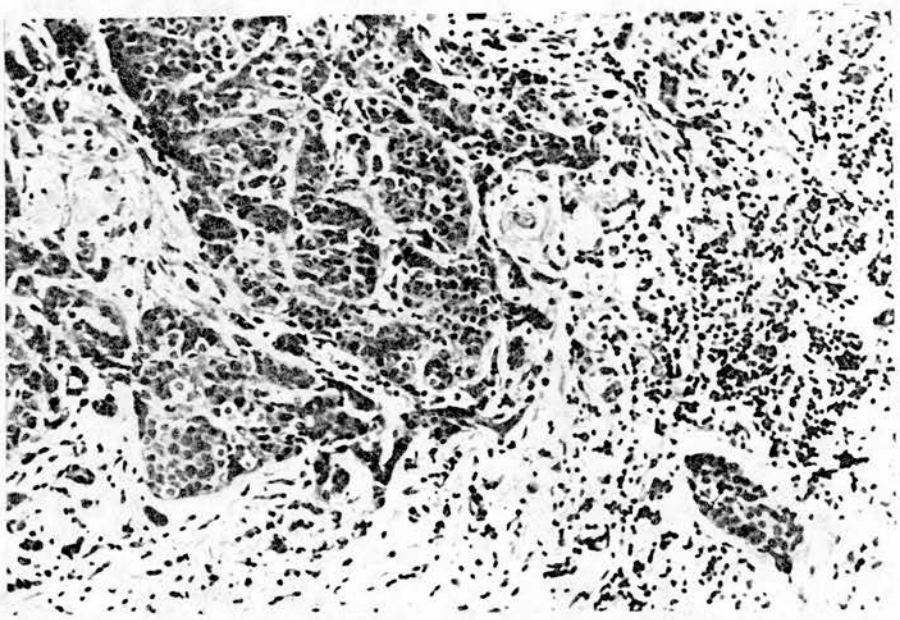


Figures 41 - 43 Grades II - IV lymphocytic infiltrate (x 50)

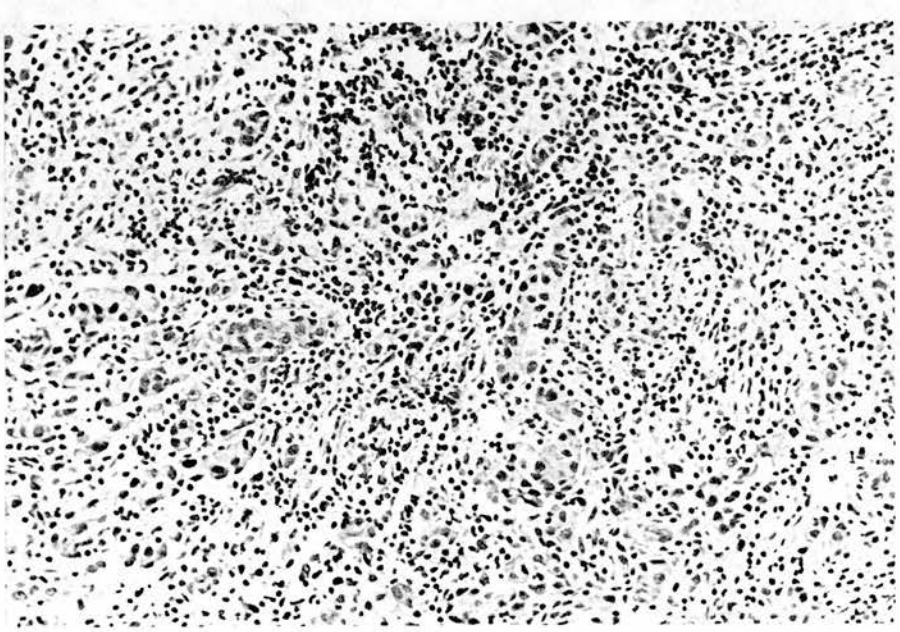
II



III



IV



33. Tumours were also graded for their degree of differentiation using the Bloom and Richardson technique (q.v. pages 119 - 121).

Reactive changes were scored as previously described (q.v. pages 115 - 119). For correlating reactions with lymphocytic infiltration the mean score for each patient was utilised, and compared with the four levels of infiltration using Kendall's rank correlation.

In order to look at the effect of lymphocytic infiltration on prognosis, grades 1 and 2 were considered to be "minimal" and grades 3 and 4 "marked." These two groups were then compared using log rank analysis of major recurrence rates, and further examined when subdivided according to the predominant reaction in the lymph nodes.

Results

A significant positive correlation between lymphocytic infiltration and germinal centre formation was found, whereas an inverse relationship with fibrosis was evident (Figure 44). The other reactive changes did not relate to lymphoid infiltration (Table 34).

When lymphocytic infiltrate was compared with tumour grade, a significant positive correlation was found to exist (Figure 45). Accordingly, the relationship between infiltrate and germinal centre formation and fibrosis was re-examined within each tumour grade. This manoevre destroyed the correlation in the case of fibrosis, but not germinal centre formation (Table 35).

Table 34 Relationship between lymphocytic infiltration and reactive changes.

	Kendall correlation coefficient	P
Sinus histiocytosis	+0.07	> 0.1
Paracortical hyperplasia	+0.11	> 0.1
Germinal centre formation	+0.24	< 0.01*
Fibrosis	-0.21	< 0.05*
Fatty replacement	-0.06	> 0.1

*Significant difference, illustrated in Figure 44.

Figure 44

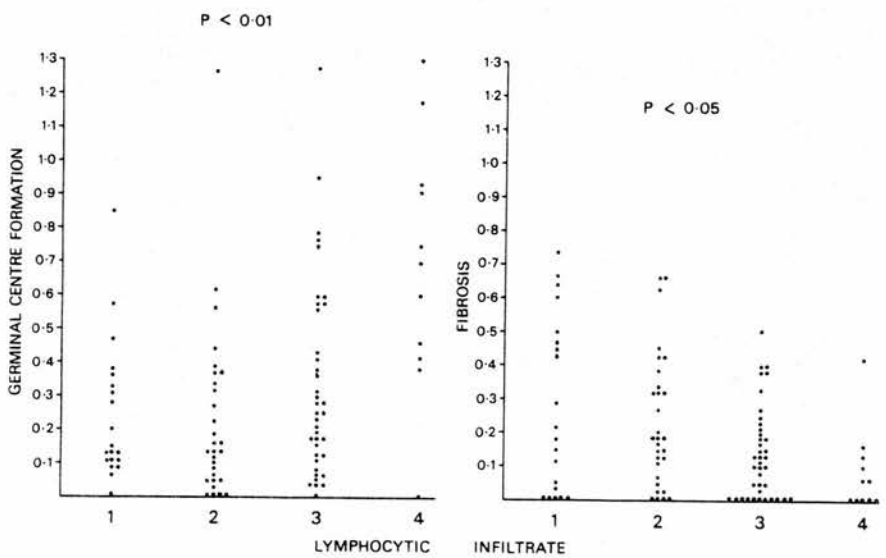


Figure 45 Correlation between lymphocytic infiltrate and tumour grade.

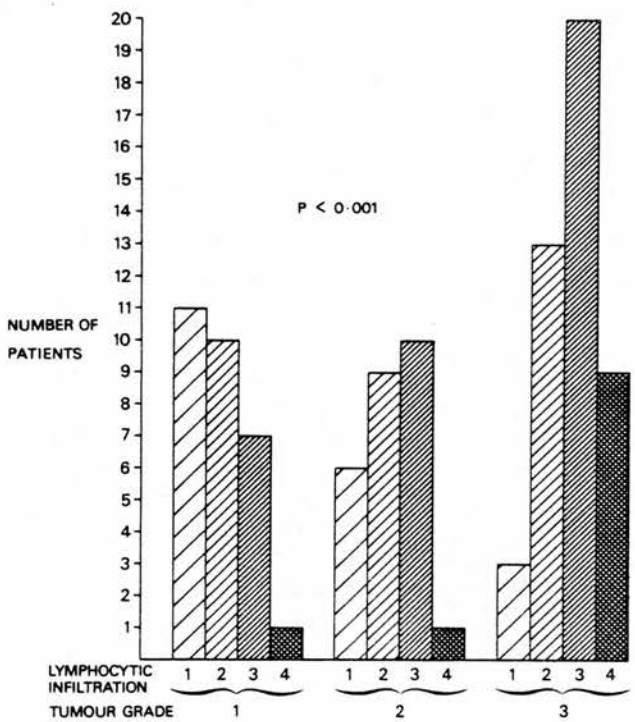


Table 35 Correlation between lymphocytic infiltration and fibrosis and germinal centre formation according to tumour grade.

	Kendall correlation coefficient	P
Fibrosis		
Grade 1	-0.14	> 0.1
Grade 2	-0.16	> 0.1
Grade 3	-0.02	> 0.1
Germinal centre formation		
Grade 1	+0.23	< 0.05
Grade 2	+0.21	< 0.05
Grade 3	+0.27	< 0.05

When lymphocytic infiltration was examined in the 164 patients treated between 1974 and 1977, 72 (44%) displayed "marked" infiltration, and 92 (66%) showed "minimal." Log rank analysis could demonstrate no significant difference in recurrence rates between these two groups (Figure 46).

The patients were then subdivided into groups according to predominant reactive change, and the difference between "marked" and "minimal" lymphocytic infiltration was again examined. Sinus histiocytosis, paracortical hyperplasia and lack of stimulation had no significant effect. However, in the germinal centre predominant group, patients with marked lymphocytic infiltration (n=30) had a higher recurrence rate than those showing minimal infiltration (n=14). The reverse was true for patients in whom germinal centre formation was not predominant, with those showing marked infiltration (n=42) having a better prognosis than those showing minimal infiltration (n=78) (Figure 47).

Finally, the prognostic effect of lymphocytic infiltrate was examined in patients grouped according to tumour grade or metastatic node status. In these instances, lymphocytic infiltrate had no discriminating effects.

Discussion

The present study has shown that lymphocytic infiltrate in breast tumours is associated with germinal centre formation in axillary lymph nodes. This phenomenon may be due in part to an association of both factors to tumour grade, but it does have some autonomy. There is no evidence, however, that lymphocytes in the neoplasm originate from regional nodes, and indeed

Figure 46 Comparison between recurrence rates in patients with marked and minimal lymphocytic infiltrate of primary breast cancer.

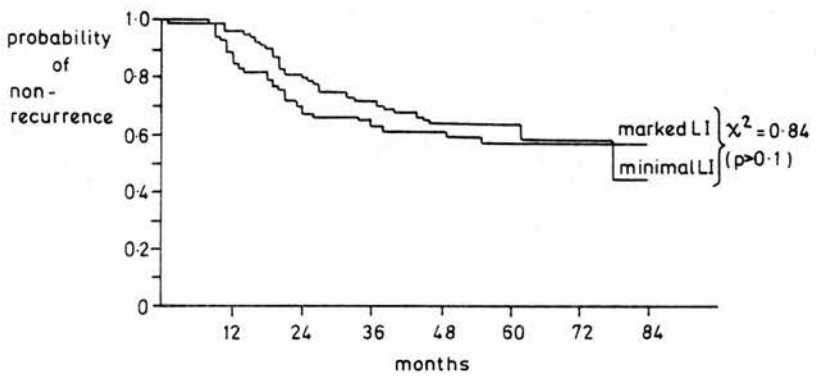
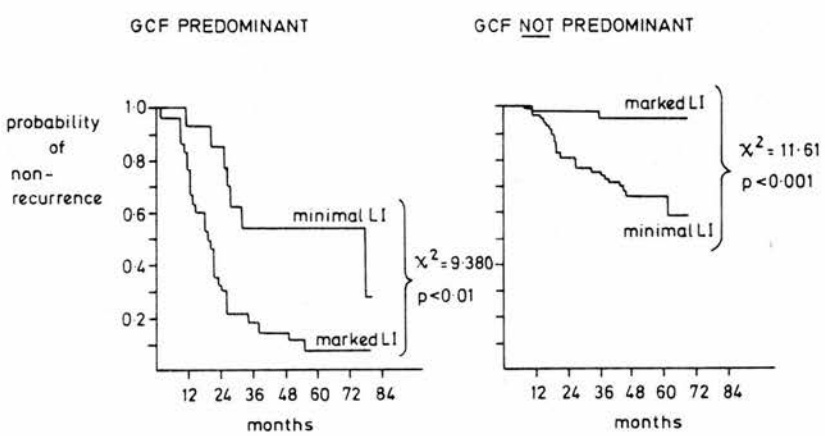


Figure 47 The effect of germinal centre formation in regional nodes on the prognostic significance of lymphocytic infiltration of primary breast cancer.



previous studies have shown that T-cells predominate in tumour infiltrates[484]. It is unlikely, therefore, that lymph nodes showing a predominant B-cell response should be the site of origin of intra-tumoural lymphocytes.

A more probable explanation is that tumours which evoke a local cellular response are also likely to stimulate a humoural response in nearby lymph nodes. This view is supported by the finding that both are influenced by tumour differentiation.

When the effect of lymphocytic infiltration on prognosis was examined, an overall effect could not be detected, a finding which has been reported on many previous occasions (q.v. pages 101 - 102). However, the presence of a predominantly germinal centre reaction in the axillary nodes conferred adverse prognostic significance on marked lymphocytic infiltrate. Conversely, in the remainder of the patients, in whom germinal centre formation was not a predominating factor, marked lymphocytic inflitration was favourable.

As germinal centre formation is positively correlated with tumour grade, this observation may be thought to represent a favourable effect of lymphocytic infiltrate on tumours of favourable grade. This does not appear to be the case however, and another explanation must be invoked.

It is conceivable that germinal centre formation in regional nodes may be exerting a restraining effect on tumour infiltrating lymphocytes. This is not entirely fanciful, as serum factors have been shown to inhibit T-cell function in breast cancer (q.v. pages 99 - 100). It has also been suggested

that immune complexes may be responsible for this[455], and there is no doubt that breast cancer patients have elevated levels of such complexes[444-446].

Whatever the reason, it is clear that the effect of lymphocytic infiltration in primary breast cancer on prognosis can only be assessed in the light of events within the regional nodes.

4. STUDIES ON THE PRE-OPERATIVE ASSESSMENT OF AXILLARY LYMPH NODES IN BREAST CANCER

4 i. The significance of palpable axillary nodes in breast cancer

Introduction

Clinical assessment of the axillary lymph nodes is a powerful tool for predicting the outcome in women with breast cancer, and it is well established that patients with palpable nodes have a high risk of recurrence and premature death (q.v. page 38). The most likely explanation for this phenomenon is that nodal enlargement represents the presence of tumour involvement. However, clinical diagnosis of axillary metastases is known to be wrong in about one third of cases[101,178-181], and, to compound the problem, some workers have suggested that palpable axillary nodes which do not contain tumour actually improve prognosis[474].

The present study was therefore undertaken to assess the relative prognostic accuracy of histological and clinical node staging, and to determine the prognostic significance of non-metastatic enlargement of the axillary glands.

Patients and Method

The study group consisted of the 238 patients treated for invasive breast cancer by simple mastectomy and lower axillary lymph node sampling between 1974 and 1977 (q.v.

page 113). Each patient was clinically staged by at least two experienced observers according to the international TNM system[175] (Table 7, page 39). The differences in major recurrence rates between various groups were examined using the logrank test.

Results

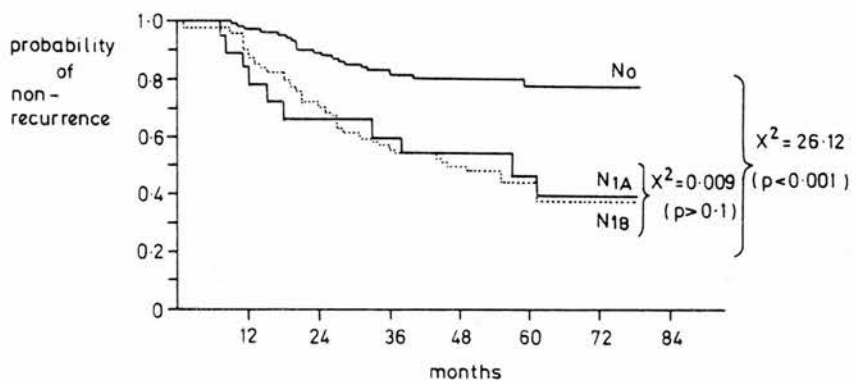
Of the 238 patients, 101 had histological evidence of nodal metastases, 105 had negative nodes, and in 32 cases no nodes were found. 134 patients had been classified as clinical stage N_0, 19 as N_{1a}, 79 as N_{1b}, and 6 as N_2. The distribution of patients with histologically negative or positive nodes in each of the clinical categories is shown in table 36. The correct diagnosis was made in 66% of N_0 patients, in 46% of N_{1a} patients, in 69% of N_{1b} patients, and in 100% of N_2 patients. Overall, the false positive rate was 27% and the false negative rate was 39%, indicating that clinical examination was wrong in about one third of cases. Put another way, the sensitivity of clinical examination as a diagnostic test for axillary metastases was 61%, and its selectivity was 73%.

When the major recurrence rate of the patients classified as N_{1a} was compared to that of the N_{1b} patients, no significant difference could be found, although a highly significant difference existed between both of these two groups and the N_0 category of patients (Figure 48). Because of this, and because the N_2 group was so small, all patients with palpable axillary nodes were considered together for the purposes of the remainder of the study.

Table 36 The relationship between clinical staging and the presence of histologically proven nodal metastases.

	N_0	N_{1a}	N_{1b}	N_2
Metastases absent	77(66%)	6(46%)	22(31%)	0(0%)
Metastases present	39(34%)	7(54%)	49(69%)	6(100%)

Figure 48 Comparison between recurrence rates in patients classified as N_0, N_{1a} and N_{1b}.



As expected, the major recurrence rate of women with histologically proven metastases was significantly higher than that of women with negative nodes. Surprisingly, however, the difference between patients with palpable and impalpable nodes was almost identical, despite the proven inaccuracy of clinical staging (Figure 49).

When the recurrence rates were examined in two separate groups according to histological node status, it was found that patients with palpable nodes had a significantly worse prognosis. This was true in the presence or in the absence of axillary metastases (Figure 50).

Discussion

The clinical staging of axillary lymph nodes in breast cancer is known to be inaccurate (q.v. pages 38 - 40). The present study has confirmed previous findings, and it has also examined the efficacy of dividing palpable axillary nodes into various categories using the international TNM staging system. This system recognises 5 categories of clinical node stage - N_0, N_{1a}, N_{1b}, N_2 and N_3 (Table 7, page 39), but as "N_3" patients usually have inoperable disease, none were available for study. In terms of concordance with histological data, no value accrues from subdividing the N_1 stage into "a" and "b", as 54% of N_{1a} patients actually had regional metastases compared with 69% of N_{1b} patients. On the other hand, N_2 seems to be a more useful category, as all the 6 patients so classified did have tumour deposits in their nodes.

Figure 49 Comparison between recurrence rates in patients with histologically positive or negative nodes, and similar comparison between patients with palpable or impalpable nodes.

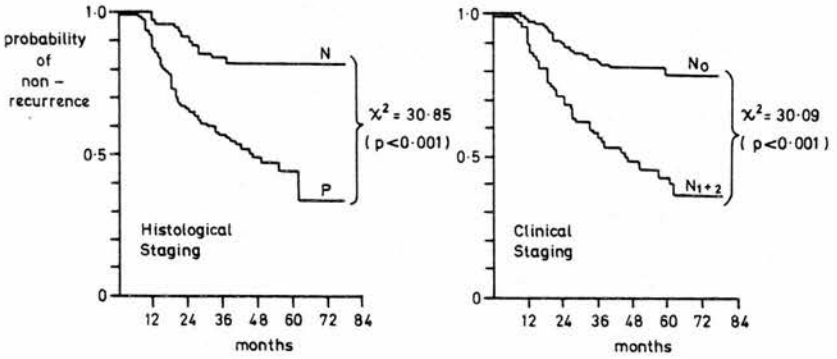
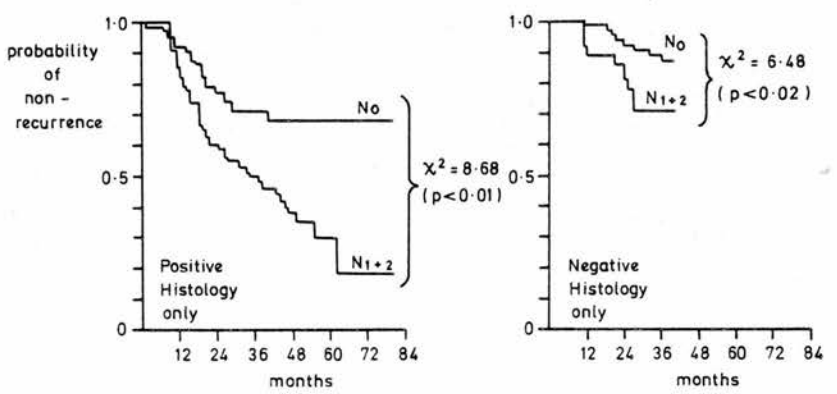


Figure 50 Comparison between recurrence rates in patients with palpable or impalpable nodes, divided into histologically negative or positive groups.



The N_{1a} patients form a potentially interesting group, as it has been suggested that "non-suspicious" palpable nodes constitute a favourable prognostic factor. The present study does not confirm this, however, for patients who fell into the N_{1a} group had a significantly higher recurrence rate than the N_0 patients, and a similar prognosis to the N_{1b} patients. This is not surprising, as we have already seen that all patients with palpable, mobile lymph nodes had the same risk of harbouring regional metastases, regardless of their clinical stage.

Despite the high error rate in the pre-operative detection of axillary tumour deposits, patients with palpable glands had the same risk of developing a major recurrence of their disease as did those with histologically proven metastases. This discovery is partly explained by the fact that, in both histologically positive and negative subgroups, those patients with palpable nodes had a poorer outlook. The reason for this latter phenomenon must remain conjectural within the confines of this study, but in the histologically positive group, it is possible that patients with impalpable nodes have fewer involved nodes, or a higher proportion with micrometastases only. In the histologically negative group, some of the patients with palpable nodes may have had metastases which were not found at operation. This is not likely to represent a large proportion, however, as studies have shown that lower axillary sampling misses metastatic disease in only 10% of cases[221,222]. Alternatively, some other cause of lymph node enlargement may have sinister implications for prognosis in breast cancer. An analysis of the histological appearances in palpable axillary nodes is presented in the next section.

In conclusion, the present study has shown that the "N_{1a}" category of the TNM system has no special significance, and palpable nodes do not appear to confer an advantage on breast cancer patients who do not have histological evidence of axillary metastases. In addition, although clinical staging of axillary nodes in breast cancer is highly inaccurate, it gives just as useful prognostic information as crude "positive or negative" histological staging. This would suggest that, for node microscopy to be of any added value, the <u>extent</u> of axillary involvement must be taken into account.

4 ii. The cause of palpable axillary nodes in breast cancer

Introduction

In the previous section, it was shown that breast cancer patients with palpable axillary nodes had a worse prognosis that those with impalpable nodes. This was true irrespective of the presence or absence of metastatic deposits demonstrated by histological examination. In the present study, an attempt has been made to define the cause of palpable enlargement of axillary nodes in such patients.

Patients and Methods

Two groups of breast cancer patients were studied. The first consisted of 206 consecutive patients treated by mastectomy and node sampling between 1974 and 1977 from whom axillary lymph nodes were available for microscopic examination. The second group comprised 100 patients treated by mastectomy and axillary clearance in whom all the nodes were examined histologically (q.v. page 113). In the latter group, all the nodes were individually dissected out immediately post-operatively and those which were readily palpable in the clearance specimens before dissection commenced were separately labelled. Pre-operatively, each patient had been clinically staged by at least two experienced observers according to the international TNM system[175].

The lymph nodes were all examined histologically for metastatic deposits and for evidence of reactive changes. Tumour foci were classified as micrometastases if less than 2 mm in

maximum diameter, and as macrometastases if greater than this. The reactive changes were classified as sinus histiocytosis, germinal centre formation, paracortical hyperplasia, fibrosis and fatty replacement, and scored as 0, 1 or 2 according to criteria previously described (q.v. pages 115 - 119).

Results

The node sampling procedure yielded between 1 and 13 nodes per patient, giving a mean value of 3.12. Axillary clearance, on the other hand, obtained between 5 and 54 nodes per patient, with a mean of 22.23. In the sample group, 101 patients (49.03%) had tumour deposits detected in their nodes, and in the clearance group 43 patients (43%) had metastases. Combining the two groups, it was found that 33.5% of the N_0 patients and 60% of the N_1 and N_2 patients had metastases, constituting a highly significant tendency for palpable nodes to be associated with axillary tumour (Table 37). Nevertheless, as a diagnostic test for axillary metastases, clinical examination is not particularly accurate, with a sensitivity of 65% and selectivity of 62% in this instance.

When only patients with axillary tumour were considered, it was discovered that 21.5% of those with impalpable nodes had micrometastases only, compared with 2% of patients with palpable nodes (Table 38). The number of involved nodes was also examined in the clearance specimens, and patients with palpable nodes demonstrated a significant tendency to have more axillary metastases than those with no palpable nodes (Table 39). In addition, patients with palpable nodes had significantly

Table 37

	Clinical stage	
	N_0	N_1 and N_2
Metastases present	51 (33.5%)	93 (60%)
Metastases absent	101 (66.5%)	61 (40%)

$chi^2 = 22.11$
$(P<0.001)$

Table 38

	Clinical stage	
	N_0	N_1 and N_2
Micrometastases only present	11 (21.5%)	2 (2%)
Macrometastases present	40 (88.5%)	91 (98%)

$chi^2 = 12.85$ (using Yate's correction)
$(P<0.001)$

Table 39

	Clinical stage	
	N_0	N_1 and N_2
Number of metastases		
Median	1.5	5
Interquartile range	1 - 4	2 - 12

P<0.002

(Wilcoxon rank sum test)

Table 40

	Number of metastases		
	Macro	Micro	
Clinical stage			
N_0 Median	2	1	N.S.
Interquartile range	1 - 7	1 - 1	
N_1 and N_2			
Median	4	2	P<0.001
Interquartile range	2 - 9	1 - 3	

(Wilcoxon rank sum test)

larger numbers of macrometastases than micrometastases whereas this was not true of patients with impalpable nodes (Table 40).

Again using the clearance specimens only, it was possible to analyse the degree of metastatic involvement and the nature of the reactive change in the actual nodes which were palpable in the axilla. No palpable nodes were found in the undissected clearance specimens of patients classified as N_0 but readily palpable nodes were detected in 60 out of the 63 patients classified as N_{1a}, N_{1b} or N_2. From these 60 patients, a total of 120 individually palpable nodes were isolated, and, of these, 57 (47.5%) contained macrometastases, and none contained isolated micrometastases. When the 120 palpable nodes were compared with all the other nodes obtained from the clearance specimens, a significantly greater proportion harboured macrometastases but not micrometastases (Table 41).

The individually palpable nodes which did not contain metastases were then compared to all the other tumour-free nodes with respect to sinus histiocytosis, germinal centre formation, paracortical hyperplasia, fibrosis and fatty replacement, and the differences examined using the Wilcoxon rank sum test. The palpable nodes demonstrated significantly greater germinal centre formation and fatty replacement, whereas the impalpable displayed significantly higher levels of paracortical hyperplasia (Table 42).

Discussion

In the previous study, it had been shown that although clinical assessment of the axilla in breast cancer is

Table 41

	Palpable nodes (n = 120)	Impalpable nodes (n = 2223)	chi^2	P
Metastases	57 (47.5%)	285 (15%)	109.84	<0.001
No metastases	63 (52.5%)	1938 (85%)		
Macrometastases	57 (47.5%)	244 (11%)	135.65	<0.001
No macrometastases	63 (52.5%)	1979 (89%)		
Micrometastases	0 (0%)	41 (2%)	1.31	>0.1
No micrometastases	120 (100%)	2187 (98%)		

Table 42

	Palpable nodes (n = 63)	Impalpable nodes (n = 1938)	P
Sinus histiocytosis			
Median	1	1	
Interquartile range	0 - 1	0 - 2	>0.1
Germinal centre formation			
Median	1	0	
Interquartile range	0 - 2	0 - 1	<0.05
Paracortical hyperplasia			
Median	0	1	
Interquartile range	0 - 0	0 - 1	<0.001
Fibrosis			
Median	1	1	
Interquartile range	0 - 1	0 - 1	> 0.1
Fatty replacement			
Median	1	1	
Interquartile range	0 - 2	0 - 1	<0.05

wrong in about one third of cases, the presence of palpable nodes carries exactly the same prognostic disadvantage as histological proof of nodal metastases. This was partly explained by the finding that patients with palpable nodes had a higher major recurrence rate within both the histologically "node positive" and "node negative" groups. The present study provides information which is relevent to this phenomenon.

Firstly, it is well established that micrometastases confer a much lower risk of recurrence than do larger nodal tumour deposits (q.v. pages 23-- 24). This study has demonstrated that micrometastases are unlikely to render lymph nodes palpable; "node positive" patients classified clinically as N_0 were much more likely to have micrometastases only than patients classified as N_1 or N_2, and no individually palpable node was found to contain only a micrometastasis.

Secondly, many workers have shown that prognosis deteriorates with increasing numbers of involved lymph nodes (q.v. page 14), and in the present study "node positive" patients with palpable nodes had greater numbers of metastatic deposits than did those with impalpable nodes. It is also a fact that the number of macrometastases outweighed the number of micrometastases in patients classified as N_1 or N_2, but not in "N_0" patients.

Lastly, we are faced with the question of why palpable nodes should confer a poor prognosis on patients with no evidence of metastases. This finding may be regarded as controversial, as some studies have suggested the reverse. However, the notion that non-malignant enlargement of axillary nodes has a favourable effect on the prognosis of breast cancer

or that it indicates a reactive change in the nodes is based on somewhat flimsy evidence (q.v. pages 104 - 105), and it has been shown that about 37% of women without any demonstrable breast pathology will have palpable nodes[177]. It seems unlikely that this could be caused by reactive changes.

In the present study, it has been shown that the actual nodes which are palpable tend to display fatty replacement or germinal centre formation if they are not involved by tumour. Fatty replacement is often the "natural state" of axillary lymph nodes, as demonstrated by Luscieti and colleagues[321], and cannot therefore be regarded as any form of "change" associated with breast cancer. Germinal centre formation, however, is a well recognised reaction in breast cancer, and is now known to be a poor prognostic factor (q.v. pages 85 - 86). Sinus histiocytosis and paracortical hyperplasia, both of which have been associated with a good prognosis, were <u>not</u> related to palpability in lymph nodes, and, indeed, paracortical hyperplasia was significantly associated with non-palpable nodes. It is clear, therefore, that favourable reactive changes are <u>not</u> associated with palpable nodal enlargement.

In conclusion, it has been demonstrated that palpable axillary nodes in breast cancer are associated with factors of poor prognostic significance, either in terms of the extent of metastatic involvement, or of the type of reactive change. However, it is not possible to rely on clinical examination for the accurate diagnosis of axillary metastases in the individual patient.

4 iii. Lymphoscintigraphy in the prediction of axillary lymph node histology

Introduction

In Literature Review I, a section is devoted to the use of lymphoscintigraphy in breast disease (q.v. pages 41 - 43). At least two groups have reported that this technique can detect metastases in axillary nodes[205,206], and a great deal of work has been done on the assumption that the same holds true for internal mammary nodes[192-196].

However, the accuracy of the method has been challenged[208], and so the present study has been carried out to compare lymphoscintigraphic findings with histological evidence of metastatic deposits in axillary nodes. In addition, a similar comparison has been made with respect to reactive changes in the lymph nodes.

Method

100 patients undergoing mastectomy and axillary clearance for invasive breast cancer were studied. These patients were not consecutive, as scanning facilities were not always available, but no criteria other than operability were used in their selection.

The techniques used for the execution and interpretation of the lymphoscintigraphy are outlined on pages 124 - 126. All the nodes dissected from the clearances were examined histologically and scored for metastases and reactive changes as previously described (q.v. pages 115 - 119).

Results

Of the 100 patients studied, 15 had scans which were "uninterpretable" owing to complete lack of colloid uptake. Twenty-one had abnormal or "positive" scans, where uptake was absent or depressed in homolateral nodes, and 64 had normal or "negative" scans, characterised by equal uptake or greater uptake by the homolateral nodes.

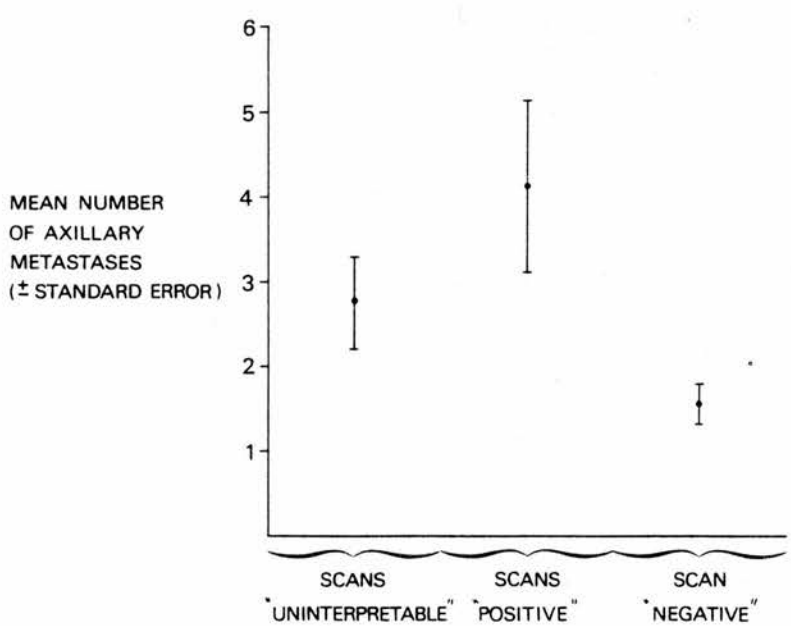
Nine (60%) of the 15 patients with no uptake on either side had metastases, with a mean value of 2.78 (standard error 0.55) metastases per patient. Of the 21 patients with "positive" scans, 14 (66.7%) had metastases with a mean value of 4.14 (standard error 0.99) metastases per patient. In the remaining group of 64 patients, who had "negative" scans, 16 (25%) had metastases, with a mean value of 1.56 (standard error 0.26) metastases per patient. When patients with micrometastases only were excluded from the "histologically positive" group, only 11 (17.2%) of patients with "negative" scans had significant metastatic disease in their nodes. No patient in the other two groups had micrometastases exclusively.

Chi-squared testing, using Yate's correction where applicable, demonstrated that significantly more patients with positive scans had metastases in their axillary nodes. This was true whether micrometastases were taken into account or not (Table 43). Interestingly, patients with no colloid uptake on either side were significantly more likely to have metastases than those with "negative" scans ($chi^2=5.36$, $P<0.05$), and were not significantly different from those with "positive" scans. When only patients with metastases were considered,

Table 43 Comparison of lymphoscintiscans and histological data in the diagnosis of axillary metastases.

Scans	Histology		chi^2	P
	Node positive	Node negative		
Micrometastases included				
Node positive	14 (66.7%)	7 (33.3%)	10.27	< 0.01
Node negative	16 (25%)	48 (75%)		
Micrometastases excluded				
Node positive	14 (66.7%)	7 (33.3%)	16.34	< 0.001
Node negative	11 (17.2%)	53 (82.8%)		

Figure 51 Comparison between numbers of axillary metastases in patients with uninterpretable scans, positive scans and negative scans.



Student's t test demonstrated significantly greater numbers of metastatic deposits in patients with "positive" or "uninterpretable" scans compared to those with "negative" scans ($P < 0.001$, Figure 51).

Finally, the scores for sinus histiocytosis, paracortical hyperplasia, germinal centre formation, fibrosis and fatty replacement were examined, and no differences could be found between patients with "positive" or "negative" scans. However, when a comparison was made between those with scans where uptake was absent bilaterally or homolaterally and those in whom some uptake was seen in homolateral nodes, a difference was found. Using Wilcoxon's rank sum test, both sinus histiocytosis and paracortical hyperplasia were found to be significantly depressed in those where uptake was absent (table 44).

To estimate the value of lymphoscintigraphy as a diagnostic test for axillary metastases, its sensitivity (correct positive rate) and selectivity (correct negative rate) were compared to those of clinical examination in the same patients (table 45). This revealed an improved selectivity for lymphoscintigraphy (87% as opposed to 73%), but its sensitivity was considerably less at 47% compared with 67%. Application of McNemar's test for paired binary data showed that there was no significant difference between the accuracy of these two tests ($P > 0.1$).

Table 44 Comparison of reactive changes in patients with and without colloid uptake in homolateral nodes. (MV:median value, IQR:interquartile range)

	Uptake in homolateral nodes MV (IQR)	No uptake in homolateral nodes MV (IQR)	P*
Sinus histiocytosis	0.75 (0.5-1.0)	0.18 (0-0.33)	< 0.001
Paracortical hyperplasia	0.16 (0-0.40)	0 (0-0.16)	< 0.05
Germinal centre formation	0.25 (0.10-0.50)	0.20 (0-0.50)	> 0.1
Fibrosis	0 (0-0.20)	0 (0-0.33)	> 0.1
Fatty replacement	0.25 (0-0.60)	0.16 (0-0.60)	> 0.1

*Significance calculated using Wilcoxon's rank sum test

Table 45 Comparison of sensitivity and selectivity between lymphoscintigraphy and clinical examination in identical patients.

Histology	Lymphoscintigraphy		Clinical examination	
	Node positive	Node negative	Node positive	Node negative
Node positive	14	16	20	10
Node negative	7	48	13	40

	Sensitivity	Selectivity
Lymphoscintigraphy	47%	87%
Clinical examination	67%	73%

Discussion

The present study has demonstrated that depressed or absent uptake of rhenium sulphide colloid in axillary nodes draining breast cancer is associated with metastatic deposits in those nodes. The reason for this association may be partially explained by replacement of nodal structure by tumour. This view is supported by the finding that increased numbers of involved nodes make suppression of uptake more likely.

However, other factors may also operate, and study of the reactive changes in the nodes provides a further explanation. Sinus histiocytosis and paracortical hyperplasia have been shown to correlate negatively with metastatic disease (q.v. pages 168 - 173). These changes also seem to relate to colloid uptake in lymph nodes, and it is known that particulate matter localises in sinus histiocytes[324-328]. It is probable, therefore, that axillary metastases are accompanied by a depression in the phagocytic capacity of lymph node cells, which is mirrored by an inability to concentrate colloidal particles. It is impossible to say whether this functional change represents cause or effect, but the finding that bilateral failure of uptake is also associated with metastatic disease does suggest the former.

Despite the fact that lymphoscintigraphy does offer a method of detecting axillary metastases, however, it is important to establish whether it constitutes an improvement over existing techniques. In this study, it was not possible

to show that lymphoscintigraphy is any more accurate than clinical examination, for although its selectivity was slightly better, sensitivity was worse.

In short, axillary lymphoscintigraphy does provide an indication of tumour involvement, probably as a result of functional derangement within the nodes. However, as it stands, the technique is not sufficiently accurate to be of added value over clinical examination in routine practice.

4 iv. A computer programme to assist the prediction of axillary nodal metastases from other parameters in breast cancer

Introduction

Although clinical impression is often wrong, it remains the single most accurate method of predicting the presence of axillary lymph node metastases in breast cancer. However, there are other parameters which are associated with nodal tumour, and which can be established before axillary dissection.

In a previous section (2 iv, pages 147 - 153), it has been shown that size, histological grade and contour of the primary tumour correlate with axillary metastatic disease, and that duration of symptoms is also related. Accordingly, these features, along with clinical findings, have been used in combination analysis in an attempt to improve the diagnosis of axillary nodal tumour.

Method

Information from 100 patients undergoing mastectomy and total axillary clearance (q.v. page 113) was used to create the data base for a computer programme. To do this, the patients were divided into three groups, those with no axillary metastases, those with 1 - 3 involved nodes and those with more than 3, and the percentage of each of these groups displaying each individual parameter was calculated. These percentages are shown in table 46.

Table 46

	Number of nodes involved		
	0	1 - 3	more than 3
1. Nodes clinically negative	68%	45%	13%
2. Nodes clinically positive	32%	55%	87%
3. Tumour rounded	54%	35%	15%
4. Tumour spiculated	46%	65%	85%
5. Tumour < 2 cm	46%	35%	9%
6. Tumour 2 - 2.9 cm	32%	40%	39%
7. Tumour ⩾ 3 cm	22%	25%	52%
8. Grade I	37%	25%	13%
9. Grade 2	28%	15%	26%
10. Grade 3	35%	60%	61%
11. Symptoms ⩽ 3 weeks	74%	55%	39%
12. Symptoms > 3 weeks	26%	45%	61%

The programme was written in "Basic" language, and utilised Bayesian probability theory to calculate the probability of a patient being "node negative" or "node positive" from a given combination of clinical information, tumour size, tumour border, histological grade and duration of symptoms (Figure 52). A subroutine was incorporated to calculate the probability of "node positive" patients having 1 - 3 or more than 3 nodes involved.

The programme was run on a Cromemco "System Three" micro-computer, and tested using the 100 patients from whom the data base was derived. In addition, a further 59 independent patients, who were not biased by "belonging" to the data base were studied.

Results

In the initial group of 100 patients, clinical examination was correct in the diagnosis of axillary node metastases in 67% of cases, and accurately predicted their absence in 70%. That is to say, the sensitivity of clinical examination was 67% and the selectivity was 70%. In contrast, the computer programme had a sensitivity of 70% and a selectivity of 90% in the same patients.

With the 59 patients who were not involved in the data base construction, similarly improved results were seen. The sensitivity and selectivity of clinical examination were 52% and 67% respectively, whereas the computer achieved 69% and 87%.

Overall, the accuracy of clinical examination was 65%, and computer prediction was correct in 80%. This difference

Figure 52 **Basic language computer programme for calculating the probability of axillary lymph node involvement in breast cancer.**

>>

```
 60 ON ESC GOTO 730
 70 ON ERROR GOTO 730
 80 @"THIS IS A PROGRAMME TO CALCULATE THE PROBABILITY OF LYMPH"
 90 @"NODE INVOLVEMENT FROM OTHER PARAMETERS IN BREAST CANCER"
110 @"INSERT THE APPROPRIATE NUMBERS, EACH FOLLOWED BY A RETURN"
130 A=1 : B=1 : C=1
140 A1=0.57 : B1=0.2 : C1=0.23
150 @"1 NODES IMPALPABLE              11 T0-1"
160 @"2 NODES PALPABLE                12 T2"
170 @"                                13 T3-4"
180 @"3  ROUNDED TUMOUR"
190 @"4  SPICULATED TUMOUR            14 HISTORY =< 3 WEEKS"
200 @"                                15 HISTORY > 3 WEEKS"
210 @"5  <2 CM"
220 @"6  2-2.9 CM"
230 @"7  >=3 CM"
240 @
250 @"8  GRADE 1"
260 @"9  GRADE 2"
270 @"10 GRADE 3"
280 @
290 @"0  INFORMATION COMPLETE"
310 INPUT X
320 IF X=0 THEN GOTO 530
330 IF X=1 THEN A=0.68 : B=0.45 : C=0.13
340 IF X=2 THEN A=0.32 : B=0.55 : C=0.87
350 IF X=3 THEN A=0.54 : B=0.35 : C=0.17
360 IF X=4 THEN A=0.46 : B=0.65 : C=0.85
370 IF X=5 THEN A=0.46 : B=0.35 : C=0.09
380 IF X=6 THEN A=0.32 : B=0.4 : C=0.39
390 IF X=7 THEN A=0.22 : B=0.25 : C=0.52
400 IF X=8 THEN A=0.37 : B=0.25 : C=0.13
420 IF X=9 THEN A=0.28 : B=0.15 : C=0.26
430 IF A=10 THEN A=0.35 : B=0.6 : C=0.61
440 IF X=11 THEN A=0.28 : B=0.2 : C=0.09
450 IF X=12 THEN A=0.65 : B=0.75 : C=0.7
460 IF X=13 THEN A=0.07 : B=0.05 : C=0.21
470 IF X=14 THEN A=0.74 : B=0.55 : C=0.39
480 IF X=15 THEN A=0.26 : B=0.45 : C=0.61
490 A1=A1*A : B1=B1*B : C1=C1*C
500 Y=A1+B1+C1
510 Q=B1+C1
520 GOTO 310
530 INTEGER P1,P2,P3
540 P1=100*A1/Y : P2=100*B1/Y : P3=100*C1/Y
550 INTEGER P4,P5
560 P4=100*B1/Q : P5=100*C1/Q
580 @"THE PROBABILITIES OF NODAL INVOLVEMENT ARE AS FOLLOWS"
600 @"NODE NEGATIVE       ";P1;"%"
630 @"NODE POSITIVE       ";P2+P3;"%"
640 IF P>(P2+P3)THEN GOTO 730
660 @"NODE POSITIVE 1-3   ";P4;"%"
680 @"NODE POSITIVE >3    ";P5;"%"
730 END
```

was highly significant by McNemar's test for paired binary data ($P<0.001$). All these percentage values are derived from the figures in table 47.

When the accuracy of the subroutine for estimating the number of involved nodes was tested, it was found to be correct in only 33 cases out of 50 (66%).

Finally, the cases in which the computer made an error were analysed. In 10, the lymph nodes were free of tumour, and in 22, metastases were present. In the latter group, 19 (86%) had three or fewer lymph nodes involved.

Discussion

Bayes' theory (Figure 53) provides a means whereby the probabilities of a series of events occurring can be calculated, given a pattern of parameters each of which has a specific frequency distribution throughout the events in question. This approach has been used in computer assisted diagnosis, where the events are represented by diseases, and the parameters by symptoms and signs[485,486].

In the present study, Bayesian analysis has been used to compute the probability of axillary lymph node involvement from various clinical and pathological parameters known to be associated with nodal metastases. The data base was derived from 100 patients, and is represented by lines 330 - 480 in the programme (Figure 52). Each figure represents the probability of each parameter occurring in a patient with no axillary metastases (A), 1 - 3 metastases (B), or more than 3 metastases (C).

Table 47 Comparison between histological evidence of nodal metastases and diagnoses reached by clinical examination or computer analysis.

Group 1
100 patients included in the data base

Histology	Clinical		Computer	
	Node Positive	Node Negative	Node Positive	Node Negative
Node positive	29	14	30	13
Node negative	17	40	6	51

Group 2
59 independant patients

Histology	Clinical		Computer	
	Node Positive	Node Negative	Node Positive	Node Negative
Node positive	15	14	20	9
Node negative	10	20	4	26

Figure 53

Bayes' Theory

$$P\,D_1\,(Sa\text{-}z) = \frac{PD_1 \times PSa(D_1) \ldots\ldots PSz(D_1)}{\sum_{n}^{1} PD_{1-n} \times PSa(D_{1-n}) \ldots\ldots PSz(D_{1-n})}$$

In real terms, therefore, the computer does not actually decide whether a patient does or does not have metastases, but merely gives a percentage probability for each possibility. For the purposes of this study, the larger of the two percentages given for "node negative" or "node positive" has been taken as the "answer." It must be realised, however, that the nearer the "answer" is to 50%, the less likely it is to be correct.

Accepting this limitation, however, the computer is markedly more accurate than clinical examination, both in terms of sensitivity and selectivity. This holds true not only for the patients from whom the data base was constructed, but also for the independent group; an important finding as the programme must be able to operate prospectively to be of any value.

The computer was most inaccurate in those patients with 1 - 3 involved nodes, which is perhaps to be expected. Unfortunately, the computer's ability to discriminate between different numbers of involved nodes, even where a correct diagnosis has been made, was not great. This was probably due to relatively small numbers of patients in the "1 - 3 positive" and the "more than 3 positive" subgroups from which the data base was constructed.

It is to be hoped that improving the data base with information from larger numbers of patients will increase the accuracy of the programme. Nevertheless, its use definitely improves on clinical assessment and may prove extremely valuable in predicting the presence of axillary lymph node metastases in breast cancer.

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION AND CONCLUSIONS

To structure this discussion, the questions posed on page 111 will be considered in turn, and the extent to which each has been answered will be considered.

i) Can limited axillary lymph node sampling provide useful prognostic information in terms of nodal metastatic involvement?

This question is examined in the studies described in sections 2 i - iii (pages 128 - 146). From a careful analysis of major recurrence rates it has been shown that useful information cannot be obtained from only one node. However, when more than one node can be found, the value of this staging operation is greatly increased. In addition, study 2 ii demonstrates that nodes in the lower third of the axilla fairly accurately reflect the metastatic status throughout. The trial of lower axillary sampling versus total axillary clearance, described in section 2 iii, shows that more than one node can be consistently found at sampling, and that the numbers of involved nodes obtained by these two procedures is eminently comparable.

The conclusion which can be drawn is that lower axillary sampling must be carried out meticulously, and the surgeon must identify and remove several lymph nodes. If this is done, the sampling procedure is capable of providing useful prognostic information. Whether the number of involved nodes carries the same significance as the number from a total axillary clearance can only be answered by long term follow-up of the patients in the randomised trial of sample versus clearance.

It should be remembered, however, that even total axillary clearance can give only an approximation to regional node status, as the breast drains directly to supraclavicular and internal mammary nodes as well. It is probable, therefore, that sampling, if done properly, will prove to be quite adequate for prognostic purposes. In the present climate of increasingly conservative surgery for breast cancer, this is an important consideration.

ii) Is there a relationship between metastatic involvement of axillary nodes and features of the primary tumour known to affect prognosis?

In study 2 iv (pages 147 - 153) tumour size, grade, and contour were shown to correlate with the presence of nodal metastases in the expected direction. Thus, metastases were more likely when the tumour was large, of poor grade and spiculated.

Surprisingly, this did not hold for oestrogen receptor status with small numbers of patients. However, as demonstrated in study 2 v (pages 154 - 157), an association between nodal tumour spread and receptor negative tumours does exist.

These observations suggest that nodal metastases are not an independent measure of tumour invasion, but are rather the result of neoplastic aggression as measured by factors known to have poor prognostic significance. This is important for two reasons. Firstly, it supports the hypothesis that nodal status reflects the balance between a tumour and the host. Secondly, it provides a method whereby the presence

of nodal metastases may be predicted before axillary dissection is carried out. Study 4 iv (pages 224 - 231) has shown that Bayes' theory can be used to estimate the probability of nodal involvement from clinical findings, features of the primary tumour and length of history. This approach is more accurate than clinical impression alone, and may provide a method of selecting patients for axillary dissection.

iii) Can a histological grading system based on immunological criteria provide useful prognostic information?

Study 3 i (pages 158 - 164) has shown that a careful appraisal of reactive changes within lymph nodes can identify groups of patients at high and low risk, and that paracortical hyperplasia and germinal centre formation are the most important factors. Study 3 ii (pages 165 - 167) demonstrates that these two parameters are more prominent in the lower parts of the axilla, and this strengthens the hypothesis that both represent a reaction to the tumour.

In estimating reactive change in lymph nodes it is important to take all the different types into account and to establish which is the predominant feature. Study 3 iii (pages 168 - 173) emphasises this point by showing that they can co-exist and are in fact related to a certain extent.

Sinus histiocytosis does not appear to have much prognostic significance in the system used in study 3 i, and there are two possible reasons for this. Firstly, study 3 v (pages 184 - 188) has shown that breast biopsy can increase the incidence of sinus histiocytosis, and as most of the patients

studied in this group had been subjected to biopsy before surgery, the significance of this change must have been diminished. Secondly, study 3 iii demonstrates a strong positive correlation between sinus histiocytosis and paracortical hyperplasia. The effect of sinus histiocytosis may well be obscured by that of paracortical hyperplasia, as the latter change is more likely to occupy a substantial portion of the cross-sectional area of a node.

iv) Is there a relationship between reactive changes in axillary lymph nodes and aspects of the primary tumour known to affect prognosis?

This question is addressed mainly by study 3 iv (pages 174 - 183). Germinal centre formation is associated with factors in the primary tumour of poor prognostic significance, and the converse is true of paracortical hyperplasia, although to a lesser extent.

This observation would suggest that reactive changes reflect the potential of the tumour as well as that of the host, and it may well provide the main explanation for their prognostic significance. It is of great interest that fibrosis relates to favourable prognostic factors in the tumour, as this implies that tumours of low malignancy are less likely to evoke a response in regional nodes.

The hypothesis that reactive changes derive their prognostic significance from a reflection of tumour potential is supported by the finding that removal of lymph nodes at mastectomy has no detrimental effect on the disease process

(q.v. pages 54 - 56). If the nodal changes represented an active response against the tumour, their removal should be deleterious.

v) Are reactive changes in regional lymph nodes related to the prognostic significance of lymphocytic infiltration in the primary tumour?

This problem is dealt with in section 3 vi (pages 189 - 199) and again, the answer is in the affirmative. It appears that germinal centre formation exerts an influence over the prognostic significance of lymphocytic infiltration, and when this nodal reaction is absent, infiltration of the primary tumour has a markedly favourable prognostic effect.

This observation suggests that germinal centre formation may have some influence over tumour growth which is independant of the neoplasm. It is obvious that the relationship between tumour and regional nodes is very complex, and some host factors must play a part.

vi) Is clinical assessment of axillary lymph nodes of any value, and can the cause of palpable enlargement of axillary nodes be defined?

From sections 4 i and 4 ii (pages 200 - 215) it can be seen that palpable axillary nodes are associated with lymph node metastases, but not invariably. Clinical assessment of the axilla is wrong in about one third of cases, with roughly equal false positive and false negative rates.

Despite this, however, the presence of palpable axillary nodes has the same prognostic significance as the presence of histologically proven nodal metastases. This paradox is partly explained by the increased tendency for large numbers of involved nodes to be palpable. In addition, it appears that palpable, uninvolved lymph nodes do not confer a favourable prognosis, and are certainly not associated with favourable reactive changes.

vii) Is it possible to predict the histological appearance of the axillary nodes?

As demonstrated in study 4 ii, clinical examination can predict metastases in axillary nodes, but not accurately. Lymphoscintigraphy held out some hope in this direction, but study 4 iii (pages 216 - 223) has shown that it is little better than clinical examination.

A more promising approach outlined in section 4 iv (pages 224 - 231) utilises combination analysis of several factors known to correlate with nodal involvement. This technique is clearly more accurate than clinical assessment alone, and although it still carries a substantial error rate, it is to be hoped that a more extensive data base will improve its performance.

It is possible to envisage a situation where patients are selected for axillary dissection from the likelihood of metastatic disease in their nodes. The surgical procedure could then serve two functions, as a method of obtaining

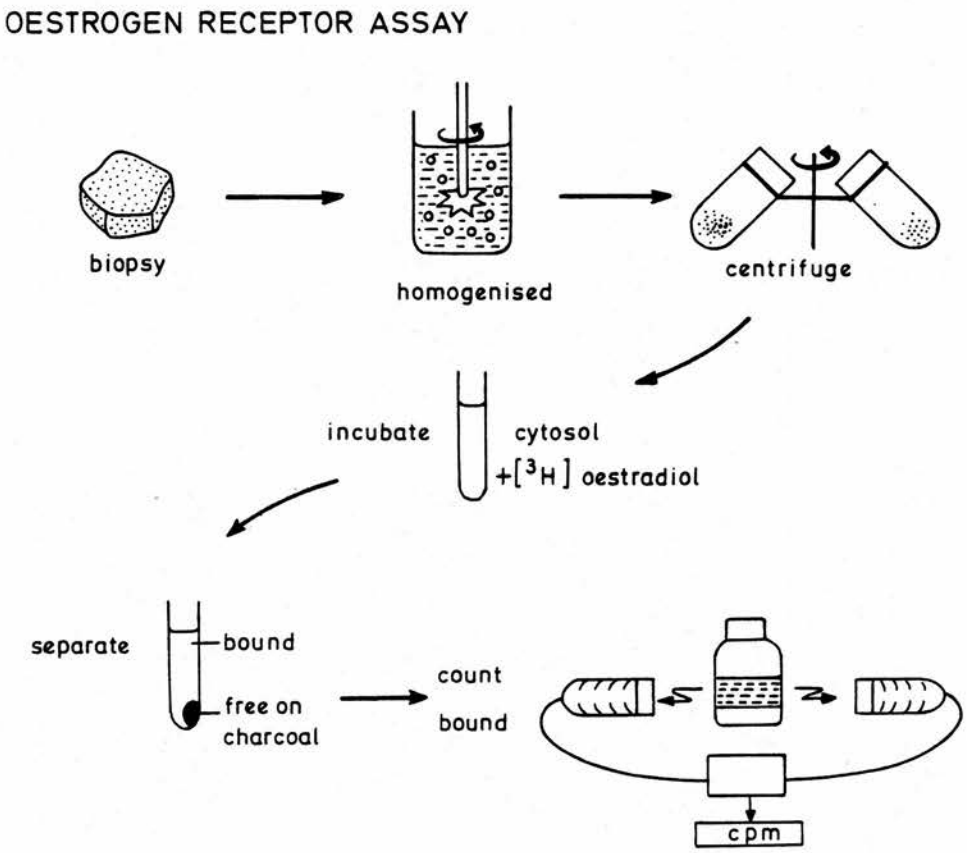
detailed information on the extent of nodal involvement, and as prophylaxis against axillary "recurrence," without subjecting a large proportion of patients to unnecessary morbidity.

APPENDIX

1. Oestrogen receptor analysis

Oestrogen receptor analysis was performed according to the method of Hawkins and his colleagues[487]. The assay was carried out on fresh tissue, transported from the operating theatre on ice to the nearby laboratory. The (supernatant) cytosol was prepared by homogenisation of the tumour tissue in Tris-buffer without any thiol reagent, followed by low-speed centrifugation at 4°C. Portions of supernatant were incubated overnight at 4°C with a fixed concentration of ^{3}H oestradiol-17B (0.030nM) and varying concentrations of non-radioactive oestradiol-17B (0.031, 0.092, 0.153, 0.214, 0.276 and 61.2 nM) in a total of 1.2 ml. Dextran-coated charcoal suspension was used to separate the bound and free fractions. This process is summarised in Figure 54. The concentration of receptor was then calculated by Scatchard analysis[488], and expressed as fmols/mg cytosol protein. Protein was estimated using the protein-dye binding method of Bradford[489].

Figure 54


OESTROGEN RECEPTOR ASSAY
biopsy
homogenised
centrifuge
incubate
cytosol
+[3H] oestradiol
separate
bound
free on
charcoal
count
bound
cpm

2. Data base structure

The data base structure for the 238 patients treated between 1974 and 1977 is shown on page 244 and an example of a single entry is shown on page 245. This data file was called 7477.DAT.

The data base structure for the 100 patients treated by total axillary clearance is shown on page 246 and an entry example on page 247. This file was called MXCL.DAT.

2

System Specifications for Data Base -- A:7477.MST
Data File -- 7477.DAT

Field #	Description	Type
1	IDENTIFICATION	A32
2	DATE OF TREATMENT	A6
3	TREATMENT	A32
4	TNM	A8
5	NODE HISTOLOGY	A3
6	ER (PROTEIN)	A4
7	NUMBER OF NODES	A6
8	DATE LAST SEEN	A6
9	DATE FIRST RECURRENCE	A6
10	DATE DEATH	A6
11	YEARS SINCE LMP	A1
12	DATE OF LOCAL RECURRENCE	A6
13	COMPUTER SCORE	A4
14	SITE OF GEN RECURRENCE	A10
15	CLINICAL TUMOUR SIZE	A5
16	LI,NODE REACTION	A5
17	HISTOLOGY GRADE	A3
18	EMPTY 4	A7
19	SCORE	A1
20	EMPTY 5	A9
21	NODE REACTION SCORE	A10

Sort #	Fields	File Name
1	IDENTIFICATION	7477.SRT

F

1	- IDENTIFICATION	ALLAN MARY	29091
2	- DATE OF TREATMENT	760608	
3	- TREATMENT	MXNS	
4	- TNM	T2AN0 M0	
5	- NODE HISTOLOGY	N	
6	- ER (PROTEIN)	18	
7	- NUMBER OF NODES	P0N1	
8	- DATE LAST SEEN	801201	
9	- DATE FIRST RECURRENCE	N	
10	- DATE DEATH		
11	- YEARS SINCE LMP	0	
12	- DATE OF LOCAL RECURRENCE		
13	- COMPUTER SCORE	2	
14	- SITE OF GEN RECURRENCE		
15	- CLINICAL TUMOUR SIZE	4.5	
16	- LI,NODE REACTION	3 PC	
17	- HISTOLOGY GRADE	322	
18	- EMPTY 4		
19	- SCORE	0	
20	- EMPTY 5		
21	- NODE REACTION SCORE	1005150 0	

2

System Specifications for Data Base -- A:MXCL.MST
Data File -- MXCL.DAT

Field #	Description	Type
1	NAME	A26
2	AGE	A2
3	TIME FROM BIOPSY (DAYS)	A2
4	TYPE OF BIOPSY (T or B)	A1
5	ESR	A2
6	?EBSC (Y or N)	A1
7	DUR. OF SYMPT. (WEEKS)	A3
8	SURGEON(PF UC DN DL or SN)	A2
9	T	A3
10	N	A3
11	LMP	A1
12	ER (fmol/mg pr.)	A3
13	POSITION OF TUMOUR	A4
14	CONTOUR OF TUMOUR	A1
15	SIZE OF TUMOUR(CM)	A3
16	ELASTOSIS	A1
17	LI	A1
18	GRADE (NUC TUB MIT)	A3
19	GRADE (B AND R)	A1
20	GRADE (FISHER)	A1
21	NO OF NODES FOUND	A2
22	MEAN SH	A4
23	MEAN SH AT I II AND III	A12
24	MEAN GCF	A4
25	MEAN GCF AT I II AND III	A12
26	MEAN PCH	A4
27	MEAN PCH AT I II AND III	A12
28	MEAN F	A4
29	MEAN F AT I II AND III	A12
30	MEAN FR	A4
31	MEAN FR AT I II AND III	A12
32	MAIN OVERALL "REACTION"	A2
33	NO OF METASTASES	A2
34	METS AT I II AND III	A6
35	NO OF MACROMETS	A2
36	MACROMETS AT I II AND III	A6
37	NO OF MICROMETS	A2
38	MICROMETS AT I II AND III	A6
39	TOTAL GC'S	A2
40	EMPTY1	A4
41	EMPTY2	A4
42	EMPTY3	A4

Sort #	Fields	File Name
1	NAME	MXCL.SRT

1

1	- NAME	ABELL JOAN
2	- AGE	51
3	- TIME FROM BIOPSY (DAYS)	13
4	- TYPE OF BIOPSY (T or B)	T
5	- ESR	12
6	- ?EBSC (Y or N)	N
7	- DUR. OF SYMPT. (WEEKS)	5
8	- SURGEON(PF UC DN DL or SN)	UC
9	- T	T3A
10	- N	N1B
11	- LMP	2
12	- ER (fmol/mg pr.)	0
13	- POSITION OF TUMOUR	RUOQ
14	- CONTOUR OF TUMOUR	L
15	- SIZE OF TUMOUR(CM)	4.5
16	- ELASTOSIS	1
17	- LI	1
18	- GRADE (NUC TUB MIT)	121
19	- GRADE (B AND R)	1
20	- GRADE (FISHER)	1
21	- NO OF NODES FOUND	13
22	- MEAN SH	0.62
23	- MEAN SH AT I II AND III	1.0 0.200.33
24	- MEAN GCF	0.85
25	- MEAN GCF AT I II AND III	0.400.602.00
26	- MEAN PCH	0.08
27	- MEAN PCH AT I II AND III	0 0.2 0
28	- MEAN F	0.15
29	- MEAN F AT I II AND III	0.4 0 0
30	- MEAN FR	0.23
31	- MEAN FR AT I II AND III	0.400.200
32	- MAIN OVERALL "REACTION"	GC
33	- NO OF METASTASES	0
34	- METS AT I II AND III	0 0 0
35	- NO OF MACROMETS	0
36	- MACROMETS AT I II AND III	0 0 0
37	- NO OF MICROMETS	0
38	- MICROMETS AT I II AND III	0 0 0
39	- TOTAL GC'S	55
40	- EMPTY1	
41	- EMPTY2	
42	- EMPTY3	

3. Statistical analysis

The statistical tests which were most often used were Wilcoxon's rank sum test, Wilcoxon's signed rank test, Kendall's rank correlation test, and the log rank test. The chi-squared test, Fisher's exact test, McNemar's test and Student's t test were also used, but as the programmes for these were not specially modified, they are not listed here.

Wilcoxon's rank sum test

As with all the tests, the programme for this was written in "Basic" language, and run on a Cromemco "System Three" micro-computer. The programme is listed on pages 249 - 250. It is linked to the data file MXCL.DAT (page 246) by line 230, and the data to be analysed is selected by altering line 300. In the example given, the data stored in bytes 66 - 69 has been chosen, and this corresponds to field 22, or mean sinus histiocytosis score. To select the comparison quantity, lines 310 - 330 are employed. In this case, bytes 46 - 48 are chosen, corresponding to field 12, or oestrogen receptor value. Line 315 excludes those patients whose recptor value is unknown, and lines 320 - 330 split the patients into the two groups to be compared - receptor rich and receptor poor.

The rest of the programme carries out the calculation on the data fed into it from the file, and gives a value of t at infinite degrees of freedom by using an approximation suggested by Colquhoun[489] (lines 1070 - 1160).

```
>>LIST 10,690

  10 ON ERROR GOTO 1215
  20 ON ESC GOTO 1215
 150 X=0 : Y=0 : R=0 : M=0 : N=0
 160 C=0 : D=0
 165 A1=0 : B1=0
 180 DIM A(100) : DIM B(100)
 190  FOR I=1 TO 100
 200  A(I)=0 : B(I)=0
 210  NEXT I
 230 OPEN\1,186\"A:MXCL.DAT"
 240 DIM A$(185)
 250 N1=1
 260 IF N1=101 THEN GOTO 420
 270 GET\1,N1\A$(-1)
 280 N1=N1+1
 300 G=VAL(A$(66,69))
 310 V=VAL(A$(46,48))
 315 IF A$(46,46)="?"THEN GOTO 260
 320 IF V<5 THEN GOTO 360
 330 IF V>4 THEN GOTO 390
 360 A(X)=G
 361 @A(X)
 370 X=X+1
 380 GOTO 260
 390 B(Y)=G
 391 @"            ";B(Y)
 400 Y=Y+1
 410 GOTO 260
 420 CLOSE
 450 @"CALCULATION IN PROGRESS"
 460  FOR I=1 TO X-1
 470  S=0
 480   FOR J=1 TO X-I
 500   IF A(J)<=A(J+1)THEN 520
 510   T=A(J) : A(J)=A(J+1) : A(J+1)=T : S=S+1
 520   NEXT J
 530  IF S=0 THEN 550
 540  NEXT I
 550  FOR I=1 TO Y-1
 560  S=0
 570   FOR J=1 TO Y-I
 580   IF B(J)<=B(J+1)THEN 600
 590   T=B(J) : B(J)=B(J+1) : B(J+1)=T : S=S+1
 600   NEXT J
 610  IF S=O THEN 630
 620  NEXT I
 630 M=1 : N=1 : R=0
 640 IF R=X+Y THEN 910
 650 IF M>X THEN 810
 660 IF N>Y THEN 860
 670 R=R+1
 680 IF A(M)<B(N)THEN C=C+R : M=M+1 : GOTO 640
 690 IF A(M)>B(N)THEN D=D+R : N=N+1 : GOTO 640
```

```
>>LIST 700,1220

  700 O2=0 : O1=0 : O=0
  710 E=0
  720 IF A(M)=A(M+1)THEN O=O+1 : M=M+1 : E=E+1
  730 IF B(N)=B(N+1)THEN O1=O1+1 : N=N+1 : E=E+1
  740 IF E>0 THEN 710
  750 O2=O+O1+2 : P=0
  760  FOR I=1 TO O2
  770  P=P+R : R=R+1
  780  NEXT I
  790 C=C+(P*(O+1))/O2 : D=D+(P*(O1+1))/O2
  800 M=M+1 : N=N+1 : R=R-1 : GOTO 640
  810  FOR I=N TO Y
  820  R=R+1
  830  D=D+R
  840  NEXT I
  850 GOTO 910
  860  FOR I=M TO X
  870  R=R+1
  880  C=C+R
  890  NEXT I
  900 GOTO 910
  910 @ : @ : M=MAX(X,Y)
  911 A1=0 : B1=0
  960  FOR I=1 TO M
  970  IF I>X THEN 1000
  980  @"",A(I),
  985  A1=A1+A(I)
  990  GOTO 1010
 1000  @"","",
 1010  IF I>Y THEN 1030
 1020  @B(I)
 1025  B1=B1+B(I) : GOTO 1040
 1030  @""
 1040  NEXT I
 1050 @ : @"SUM OF RANKS",C,D
 1060 @ : @"SIZE OF RANK",X,Y
 1065 @ : @"MEANS",A1/X,B1/Y
 1066 @ : @"MEAN RANK VALUES",C/X,D/Y
 1070 IF X>Y THEN GOTO 1090
 1080 F=X*(X+Y+1)/2 : GOTO 1100
 1090 F=Y*(X+Y+1)/2
 1100 V=SQR((X*Y*(X+Y+1)/12))
 1110 IF C>D THEN GOTO 1130
 1120 H=(C-F)/V : GOTO 1140
 1130 H=(D-F)/V
 1140 H=ABS(H)
 1150 @
 1160 @"t=",H,"(At infinite degrees of freedom)"
 1215 CLOSE
 1220 END
```

Wilcoxon's signed rank test

This test, for dealing with paired data, was carried out by the programme listed on pages 253 - 255. Here, the data file linkage is located at line 140, and the two items to be compared are specified in lines 200 and 210.

Kendall's rank correlation test

In the programme for this test, listed on pages 256 - 257, the data file is linked by line 100. The two items of data to be correlated are specified by lines 140 and 145.

This programme gives the Spearman correlation coefficient as well as the Kendall correlation coefficient, but as tied ranks were relatively common in the data which was analysed, the Kendall value was thought to be more useful. Lines 1001 - 1005 contain a subroutine to estimate the significance of the correlation directly.

The log rank test

This test was used to analyse major recurrence rates in the group of patients treated between 1974 and 1977, and the programme for the test (page 258) serves two functions. Firstly, it is designed to give a printout from which actuarial tables can be constructed, and an example of this is given on page 259. Secondly, it calculates the expected number of recurrences in the two groups to be compared, taking into account the numbers in the groups, and the overall recurrence rate. It then performs chi-squared analysis on the discrepancy between the

expected and observed numbers of recurrences in the two groups.

Turning to the programme itself, line 50 links it to the data file 7477.DAT and lines 160 - 170 calculate the interval to first recurrence in each patient. Patients to be excluded, in this case those in whom node histology is unknown, are picked out by line 260. Line 265 then splits the remaining patients into two groups for comparison, in this case node positive and node negative.

The rest of the programme carries out the calculation, line 630 printing the probabilities of non-recurrence at monthly intervals, and line 710 carries out the chi-squared test.

```
>>LIST 10,500

   10 ON ERROR GOTO 1640
   20 ON ESC GOTO 1650
   70 REM F,G,H AND K ARE DIMENSION TO 50 AND THIS SECTION
   80 REM INPUTS F AND G, THEN CALCULATES DIFFERENCE.
   90 DIM F(100),G(100),H(100),K(100)
  100  FOR I=1 TO 100
  110  F(I)=0 : G(I)=0 : H(I)=0 : K(I)=0
  120  W=0 : Z=0
  130  NEXT I
  140 OPEN\1,186\"A:MXCL.DAT"
  150 DIM A$(185)
  160 I=0
  170 I=I+1 : IF I=101 THEN 300
  180 GET\1,I\A$(-1)
  200 V=VAL(A$(78,81))
  210 V1=VAL(A$(70,73))
  220 F(I)=V : G(I)=V1
  230 @F(I),G(I)
  240 W=W+F(I) : Z=Z+G(I)
  245 @W,Z
  246 @
  250 GOTO 170
  300 CLOSE
  310 @"CALCULATION IN PROGRESS"
  320  FOR I=1 TO 100
  330  H(I)=F(I)-G(I)
  340  K(I)=H(I)
  350  N1=N1+1
  360  NEXT I
  370 REM NOW NEGATIVE AND POSITIVE DIFFERENCES ARE SORTED.
  380 REM NEGATIVE DIFFERENCES BECOME "A" AND POSITIVES "B"
  390 DIM A(100),B(100)
  400  FOR J=1 TO N1
  410  IF H(J)>=0 THEN 470
  430  H(J)=H(J)*(-1)
  440  X=X+1 : I=X
  450  A(I)=H(J)
  460  GOTO 490
  470  Y=Y+1 : I=Y
  480  B(I)=H(J)
  490  NEXT J
  500 REM THIS IS A BUBBLE SORT WHICH RANKS A AND B
```

```
>>LIST 620,1080

  620  FOR I=1 TO X-1
  630  S=0
  640   FOR J=1 TO X-I
  660   IF A(J)<=A(J+1)THEN 680
  670   T=A(J) : A(J)=A(J+1) : A(J+1)=T : S=S+1
  680   NEXT J
  690  IF S=0 THEN 710
  700  NEXT I
  710  FOR I=1 TO Y-1
  720  S=0
  730   FOR J=1 TO Y-I
  740   IF B(J)<=B(J+1)THEN 760
  750   T=B(J) : B(J)=B(J+1) : B(J+1)=T : S=S+1
  760   NEXT J
  770  IF S=O THEN 810
  780  NEXT I
  790 REM THIS SECTION ATTRIBUTES A RANK VALUE TO A AND B
  800 REM THE SUM OF RANKS FOR A=C AND FOR B=D
  810 M=1 : N=1 : R=0
  820 IF R=X+Y THEN 1150
  830 IF M>X THEN 1030
  840 IF N>Y THEN 1090
  850 R=R+1
  860 IF A(M)<B(N)THEN C=C+R : M=M+1 : GOTO 820
  870 IF A(M)>B(N)THEN D=D+R : N=N+1 : GOTO 820
  880 REM THIS SECTION IS NECESSARY IF THE SAME NUMBERS ARE
  890 REM PRESENT IN BOTH COLUMNS
  900 O2=0 : O1=0 : O=0
  910 E=0
  920 IF A(M)=A(M+1)THEN O=O+1 : M=M+1 : E=E+1
  930 IF B(N)=B(N+1)THEN O1=O1+1 : N=N+1 : E=E+1
  940 IF E>0 THEN 910
  950 O2=O+O1+2 : P=0
  960  FOR I=1 TO O2
  970  P=P+R : R=R+1
  980  NEXT I
  990 C=C+(P*(O+1))/O2 : D=D+(P*(O1+1))/O2
 1000 M=M+1 : N=N+1 : R=R-1 : GOTO 820
 1010 REM THIS SUMS THE ADDITIONAL RANKS FOR COLUMN B WHEN
 1020 REM COLUMN A IS FINISHED (NOT REQUIRED FOR PAIRED WILCOXON)
 1030  FOR I=N TO Y
 1040  R=R+1
 1050  D=D+R
 1060  NEXT I
 1070 GOTO 1150
 1080 REM LIKEWISE SUMS RANKS FOR B WHEN COLUMN A IS FINISHED
```

```
>>LIST 1090,1650

 1090   FOR I=M TO X
 1100   R=R+1
 1110   C=C+R
 1120   NEXT I
 1130 GOTO 1150
 1140 REM PRINTING OF RESULTS SECTION
 1150 @ : @ : M=MAX(X,Y)
 1200 F1=0 : G1=0
 1210 @"","FIRST NUMBER","SECOND NUMBER","DIFFERENCE"
 1220   FOR I=1 TO N1
 1240   @"",F(I),G(I),K(I)
 1250   F1=F1+F(I) : G1=G1+G(I)
 1260   NEXT I
 1270 @ : @
 1280 @"    RANKED","NEGATIVE DIFFS","POSITIVE DIFFS"
 1290   FOR I=1 TO M
 1300   IF I>X THEN 1330
 1310   @"",A(I),
 1320   GOTO 1340
 1330   @"","",
 1340   IF I>Y THEN 1360
 1350   @B(I) : GOTO 1370
 1360   @""
 1370   NEXT I
 1380 A=0 : B=0 : E=0 : F=0
 1390 @ : @"SUM OF RANKS",C,D
 1400 @ : @"SIZE OF RANK",X,Y
 1415 @ : @"MEAN OF FIRST GROUP",W/100
 1416 @ : @"MEAN OF SECOND GROUP",Z/100
 1420 A=X+Y
 1430 B=(A*(A+1))/4
 1440 E=SQR((A*(A+1)*(2*A+1))/24)
 1450 IF C>D THEN GOTO 1480
 1460 F=(C-B)/E
 1470 GOTO 1490
 1480 F=(D-B)/E
 1490 F=ABS(F)
 1500 @ : @"U=",F,"(IF U>1.96, THEN P<0.05)"
 1640 CLOSE
 1650 END
```

```
>>LIST 40,530

   40 N=0 : D=0 : U=0 : T1=0 : P=0 : Q=0
   45 N1=0
   50 ON ERROR GOTO 1040
   55 ON ESC GOTO 1040
   60 DIM A(101),B(101),D(101)
   70  FOR I=1 TO 101
   80  A(I)=0 : B(I)=0 : D(I)=0
   90  NEXT I
  100 OPEN\1,186\"A:MXCL.DAT"
  110 DIM A$(185)
  120 N1=N1+1
  130 GET\1,N1\A$(-1)
  140 V=VAL(A$(54,56))
  145 V1=VAL(A$(82,86))
  150 N=N+1
  160 A(N)=V : B(N)=V1
  165 @A(N),B(N)
  170 IF N1=100 THEN GOTO 220
  180 GOTO 120
  220 CLOSE
  230 @ : @ : @"CALCULATION IN PROGRESS"
  240 @ : @ : @ : @ : ON ESC GOTO 1050
  250  FOR I=1 TO N-1
  260  S=0
  270   FOR J=1 TO N-I
  280   IF A(J)<=A(J+1)THEN GOTO 320
  290   T=A(J) : A(J)=A(J+1) : A(J+1)=T
  300   T=B(J) : B(J)=B(J+1) : B(J+1)=T
  310   S=S+1
  320   NEXT J
  330  IF S=0 THEN 380
  340  NEXT I
  380 N1=0
  390  FOR I=1 TO N
  400  IF A(I)<>A(I+1)AND N1=0 THEN A(I)=I : GOTO 530
  410  IF A(I)=A(I+1)THEN N1=N1+1 : GOTO 530
  420  X=0
  430   FOR J=1 TO N1+1
  450   X=X+I+J-N1-1
  460   NEXT J
  470  T1=T1+(N1+1)*N1/2
  480  X=X/(N1+1)
  490   FOR J=1 TO N1+1
  500   A(I-J+1)=X
  510   NEXT J
  520  N1=0
  530  NEXT I
```

```
>>LIST 540,1050

  540  FOR I=1 TO N-1
  550  S=0
  560   FOR J=1 TO N-I
  570   IF B(J)<=B(J+1)THEN 610
  580   T=B(J) : B(J)=B(J+1) : B(J+1)=T
  590   T=A(J) : A(J)=A(J+1) : A(J+1)=T
  600   S=S+1
  610   NEXT J
  620  IF S=0 THEN 650
  630  NEXT I
  650 N1=0
  680  FOR I=1 TO N
  690  IF B(I)<>B(I+1)AND N1=0 THEN B(I)=I : GOTO 810
  700  IF B(I)=B(I+1)THEN N1=N1+1 : GOTO 810
  710  X=0
  720   FOR J=1 TO N1+1
  730   X=X+I+J-N1-1
  740   NEXT J
  750  X=X/(N1+1)
  760  U=U+(N1+1)*N1/2
  770   FOR J=1 TO N1+1
  780   B(I-J+1)=X
  790   NEXT J
  800  N1=0
  810  NEXT I
  820  FOR I=1 TO N
  830  D(I)=A(I)-B(I)
  840  NEXT I
  850  FOR I=1 TO N
  860  D=D+D(I)*D(I)
  870  NEXT I
  880 R=1-6*D/(N*N*N-N)
  890  FOR I=1 TO N-1
  900   FOR J=1 TO N-I
  910   IF B(I)=B(I+J)THEN 940
  920   IF A(I)>A(I+J)THEN Q=Q+1
  930   IF A(I)<A(I+J)THEN P=P+1
  940   NEXT J
  950  NEXT I
  960 R1=(P-Q)/(SQR(N*(N-1)/2-T1)*SQR(N*(N-1)/2-U))
  970 R2=(ABS(P-Q)-1)/(SQR(N*(N-1)*(2*N+5)/18))
  980 @ : @ : @ : @ : @"SPEARMAN CORRELATION COEFFICIENT =  ";R
  990 @"KENDALL  CORRELATION COEFFICIENT =  ";R1
 1000 @"P - Q DIVIDED BY STANDARD ERROR  =  ";R2
 1001 IF R2<1.645 THEN@"NOT SIGNIFICANT p> 0.1"
 1002 IF R2>=1.645 AND R2<1.96 THEN@"p > 0.05 and p < 0.1"
 1003 IF R2>=1.96 AND R2<2.576 THEN@"p > 0.01 and p < 0.05"
 1004 IF R2>=2.576 AND R2<3.291 THEN@"p > 0.001 and p < 0.01"
 1005 IF R2>=3.291 THEN@"p < 0.001"
 1010 @ : @ : @"NUMBER OF PAIRS INSERTED    =  ";N
 1040 CLOSE
 1050 END
```

```
>>

  10 SFMODE : X=0.90000001 : IF X<>0.9 THEN RUN
  30 ON ESC GOTO 670
  40 ON ERROR GOTO 670
  50 OPEN\1,170\"A:7477.DAT"
  60 DIM A$(169)
  70 DIM A(239),B(239),C(239)
 100 E1=0 : E2=0
 110 P1=1 : P2=1 : R3=0 : R4=0 : S1=0 : S2=0
 140  FOR J=1 TO 238
 150  GET\1,J\A$(-1)
 160  Y1=VAL(A$(32,33)) : M1=VAL(A$(34,35))
 170  Y2=VAL(A$(91,92)) : M2=VAL(A$(93,94))
 180  X=(Y2-Y1)*12+(M2-M1)
 190  IF A$(97,97)="N"OR A$(97,97)="S"THEN Y=0 : GOTO 260
 200  Y3=VAL(A$(97,98)) : M3=VAL(A$(99,100))
 210  Y=(Y3-Y1)*12+(M3-M1)
 260  IF A$(78,78)="?"THEN Q=2 : GOTO 290
 265  Q=0 : IF A$(78,78)="P"THEN Q=1
 290  A(J)=X : B(J)=Y : C(J)=Q
 310  NEXT J
 320  FOR I=1 TO 84
 330  N1=0 : N2=0
 340  T1=0 : T2=0 : R1=0 : R2=0
 350   FOR J=1 TO 238
 355   IF C(J)=2 THEN GOTO 470
 360   IF C(J)<>1 THEN GOTO 420
 370   IF B(J)=I THEN R1=R1+1
 380   IF A(J)>=I AND B(J)=0 THEN T1=T1+1
 390   IF A(J)>=I AND B(J)>=I THEN T1=T1+1
 400   N1=N1+1
 410   GOTO 470
 420   IF B(J)=I THEN R2=R2+1
 430   IF A(J)>=I AND B(J)=0 THEN T2=T2+1
 440   IF A(J)>=I AND B(J)>=I THEN T2=T2+1
 450   N2=N2+1
 470   NEXT J
 480  IF S1=0 THEN S1=T1
 500  T1=(T1+S1)/2
 510  IF T1=0 THEN 530
 520  P1=P1*(T1-R1)/T1
 530  S1=2*(T1-S1/2)-R1
 540  IF S2=0 THEN S2=T2
 560  T2=(T2+S2)/2
 570  IF T2=0 THEN 590
 580  P2=P2*(T2-R2)/T2
 590  S2=2*(T2-S2/2)-R2
 600  E1=E1+(R1+R2)*T1/(T1+T2)
 610  E2=E2+(R1+R2)*T2/(T1+T2)
 620  IF R1+R2=0 THEN 640
 630  @I,P1,P2
 640  R3=R3+R1 : R4=R4+R2
 650  NEXT I
 660 @ : @
 670 @"OBS RECURRENCE ",R3,R4
 680 ON ERROR GOTO 720
 690 @"EXP RECURRENCE ",E1,E2
 700 @"TOTAL NUMBER   ",N1,N2
 710 @ : @"CHI-SQUARED =    ",(R3-E1)*(R3-E1)/E1+(R4-E2)*(R4-E2)/E2
 720 CLOSE
```

>>

MONTHS	PROBABILITY OF NON-RECURRENCE	
	POSITIVE	NEGATIVE
1	0.990099	1
7	0.980148	1
8	0.970146	1
9	0.93014	1
10	0.920084	1
11	0.87964	0.971428
12	0.859418	0.961904
13	0.839196	0.961904
14	0.818974	0.961904
15	0.798751	0.961904
16	0.78864	0.961904
17	0.778529	0.961904
18	0.727975	0.961904
19	0.707754	0.952236
20	0.687532	0.932803
21	0.66716	0.913369
23	0.656895	0.913368
24	0.646631	0.893725
25	0.646631	0.883904
26	0.625939	0.883903
27	0.615506	0.854108
28	0.605072	0.854107
31	0.594639	0.854107
32	0.594638	0.843877
33	0.584205	0.843876
34	0.573773	0.843876
36	0.573771	0.833458
37	0.562841	0.833457
38	0.551695	0.821961
40	0.54008	0.821961
42	0.528077	0.821959
44	0.514361	0.821959
45	0.500268	0.821959
46	0.485768	0.821959
49	0.468417	0.821959
55	0.439142	0.82196
62	0.341555	0.821957
78	0	0.82195

OBS RECURRENCE	53	18
EXP RECURRENCE	29.8927	41.1073
TOTAL NUMBER	101	105

CHI-SQUARED = 30.8512

END

4. Reference numbers for figures

Figure number	Reference number (Department of Medical Illustration, University of Edinburgh)
1	19213/B
2	19213/C
3	19213/A
4	18393/C
5	20054/L
6	20054/K
7	20054/J
8	20054/I
9	20054/H
10	20070/B
11	20070/A
12	20054/G
13	20054/F
14	20054/E
15	20054/D
16	20210/A
17	20054/B
18	20054/A
19	20054/C
20	19098/F
21	19098/E
22	20054/EE
23	20054/DD

24	20215/A
25	20848
26	20215/C
27	20215/M
28	20215/B
29	19098/H
30	19098/G
31	19098/B
32	20215/F
33	20215/G
34	20215/I
35	20215/N
36	20215/H
37	20215/L
38	20215/K
39	20054/FF
40	20210/C
41	20210/B
42	20210/D
43	20210/E
44	20215/J
45	20215/E
46	20054/CC
47	20054/BB
48	20054/AA
49	19098/C
50	19098/D
51	20215/D

52	-
53	18477
54	19465/J

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