Short Communication

Prognostic significance of immunohistochemically detected breast cancer node metastases in 218 patients

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Axillary lymph node metastases detected by immunohistochemistry in standard node-negative patients with breast carcinomas (13 out of 129 infiltrating ductal carcinomas and 37 out of 89 infiltrating lobular carcinomas) do not have any prognostic significance in patients followed up for a long time (respectively 24 and 18 years). Moreover, their pejorative significance in the literature is debatable since the groups and events taken into account are heterogeneous.

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Previous studies on the prognostic significance of axillary node metastases detected by immunohistochemical stainings (IHM) in invasive breast cancer have focused on a variable number of cases with different histological sampling techniques and statistical methods. The prognostic significance of such metastases is still debated and their clinical management is controversial. In our two previously published groups (Trojani et al, 1987a,b) of patients (grouped together in the present study under the name ‘study 1’ – 1987), nodal metastases detected by immunohistochemistry were associated with shorter metastasis-free probability (MFP) and overall survival probability (OSP) in the infiltrating ductal carcinoma node-negative group of patients (IDC, median follow-up: 10 years, Trojani et al, 1987a) but not in the infiltrating lobular carcinoma node-negative group of patients (ILC, median follow-up: 6.5 years, Trojani et al, 1987b). In the same two groups of patients with a longer follow-up (median follow-up: 15.6 years in the IDC group and 9.3 years in the ILC group, ‘study 2’ – 1992, de Mascarel et al, 1992), these IHM were still associated in the IDC group with a shorter MFP, but survival was not different between patients with or without metastases. In the ILC group there was still no difference in MFP and OSP between patients with or without metastases.

The aim of the present study (‘study 3’ – 2001) was to use longer follow-up to assess the prognostic significance of metastases detected by immunohistochemical stainings in these two IDC and ILC groups of patients with node-negative breast carcinomas.

MATERIALS AND METHODS

Patients

From 1965 to 1984, 2768 patients with distant metastasis-free breast cancer underwent surgery at Institut Bergonie. They were prospectively included in our clinical, histological and biological database and followed up at our institution. In 1987 129 node-negative patients were selected with infiltrating ductal carcinomas (IDC) operated on between 1965 and 1977 (Trojani et al, 1987a) and 89 node-negative patients with infiltrating lobular carcinoma (ILC) operated on between 1965 and 1984 (Trojani et al, 1987b).

All slides of tumours and lymph nodes were reviewed by a senior pathologist (IM) and the distribution of clinical and pathological criteria are summarised in Table 1. All the patients were treated by Patey type mastectomy and axillary node dissection (only five patients without IHM in the IDC group and one in the ILC group received a brief course of chemotherapy). In the IDC and ILC groups, respectively 24 and 30 patients received radiotherapy. Among the 129 patients with IDC (median follow-up: 24 years), 26 had distant metastases (20%) and 67 died (52%). Among the 89 patients with ILC (median follow-up: 18 years), 16 had distant metastases (18%) and 37 died (41.5%).

Macroscopic lymph node processing: macroscopic serial sectioning

The mean number of lymph nodes analysed in each case was 14 (range 2–29). Since 1965 all axillary lymph nodes have been examined at our institute by macroscopic serial sectioning. After fixation in Bouin-Holland, each node is macroscopically cut entirely into 1–1.5 mm thick slices perpendicular to the long axis (one to nine slices, mean: four). All slices of one node are placed together in as many numbered cassettes as necessary and paraffin-embedded. The number of cassettes (paraffin blocks) required to analyse each entire node ranged from one (90% of the cases) to three. Each block is examined on one haematoxylin-eosin-safran (HES) stained slide. Thus, in 90% of the cases all the slices of one node were situated on one HES slide (Figure 1A).

Immunohistochemical stainings

Immunostaining was performed on the original diagnostic HES-stained slides of the axillary nodes. These were the same sections in which metastases were considered to be negative by routine HES examination. They were successively destained and re stained by a three-stage immunoperoxidase procedure with a cocktail of five monoclonal antibodies against epithelial cell antigens (Trojani et al, 1987a,b). IHM were found in 37 ILC (41%) and in 13 IDC.
(10%). They were detected in only one lymph-node per axillary node dissection in the IDC group and in one (26%), two (6%), three (6%) or four (3%) lymph nodes per dissection in the ILC group. In all the cases IHM were unequivocal but morphologically different according to the histological type. In IDC, they corresponded to small tumour cell clusters in the subcapsular sinuses ranging from 0.01 to 0.2 mm in size, whereas in ILC, they corresponded to a variable number of isolated tumour cells with an irregular distribution, sometimes throughout the entire node sections. These isolated cells were neither counted nor measured.

**Table 1** Distribution of clinical and pathological criteria in the infiltrating ductal carcinoma (IDC) and in the infiltrating lobular carcinoma (ILC) patient groups (218 patients)

|                      | Infiltrating ductal carcinoma (n=129) | Infiltrating lobular carcinoma (n=89) |
|----------------------|--------------------------------------|--------------------------------------|
|                      | No. (%)                              | No. (%)                              |
| Age                  |                                      |                                      |
| ≤50 years            | 34 (27)                              | 27 (30)                              |
| >50 years            | 94 (73)                              | 62 (70)                              |
| Clinical tumour size |                                      |                                      |
| non-palpable tumour  | 2 (2)                                | 4 (55)                               |
| 1 – 20 mm            | 65 (50)                              | 27 (30)                              |
| 21 – 50 mm           | 43 (33)                              | 47 (53)                              |
| >50 mm               | 15 (12)                              | 10 (11)                              |
| TX                   | 4 (3)                                | 1 (1)                                |
| Grade                |                                      |                                      |
| I                    | 24 (19)                              | ND*                                  |
| II                   | 59 (46)                              | ND*                                  |
| III                  | 42 (32)                              | ND*                                  |
| Not specified        | 4 (3)                                | 89                                   |
| Obvious peritumoral emboli |                        |                                      |
| Absent               | 102 (79)                             | 84 (94)                              |
| Present              | 24 (19)                              | 2 (2)                                |
| Not specified        | 3 (2)                               | 3 (4)                                |

ND=not done.

**RESULTS**

The distribution of distant metastases and deaths in relation to the presence or the absence of node metastases detected by immunohistochemistry in the two groups is summarised in Table 2. In neither group were there any significant differences in MFP (Figures 2 and 3) or OSP between patients with and without IHM.

**DISCUSSION**

Analysis of our study

The relatively small number of patients and events requires caution in the interpretation of these results. Nevertheless, our ILC group is the largest published to date. In 1987, events taken into account to calculate survival probabilities were different from now and included distant metastases and loco-regional recurrences for recurrence-free probability and only deaths from cancer for survival. However, the results are the same when taking into account the same events as those in 1987. Furthermore, as regards patients who received radiotherapy vs those who did not, it has been proved that radiotherapy decreases loco-regional recurrences but has no influence on MFP or OSP in node-negative patients (Rutqvist et al., 1993). Lastly, although the serial macroscopic sectioning method is now recommended (Fitzgibbons et al., 2000), our study is the only series to date with serial macroscopic sectioning and with such a long follow-up. In our series, serial macroscopic sectioning and IH stainings on the original sections may explain the small size of IHM, all of which were occult metastases. They could be termed micrometastases in the IDC group because they were much smaller than 2 mm in size. On the other hand, the use of such a term is debatable in the ILC group since they corre-
spond to a variable number of isolated tumour cells which were irregularly distributed, sometimes throughout the entire lymph node sections. Finally, our results underline the importance of length of follow-up in assessing the prognostic significance of metastases detected by immunohistochemistry, since the difference in MFP between patients with and without IHM was no longer statistically significant in the IDC group. Whatever the cases, differences in MFP at 10 years may still be clinically relevant even if no differences are subsequently apparent, although they do not justify using such a single criterion to initiate chemotherapy. On the contrary, in ILC the difference between true node-negative and IHM was not significant at any time point. Thus, these differences in frequency, histological pattern and variability of prognostic significance according to histological type suggest that a difference in nature might exist between IHM in IDC and IHM in ILC.

Analysis of other studies

Some authors have attempted to summarise studies on axillary micrometastases (Dowlatshahi et al, 1997), but the complexity and heterogeneity of the methodologies used have made the task difficult. We analysed the results of the 11 published series regarding the prognostic significance of metastases detected by immunohistochemistry (Byrne et al, 1987, 1992; Trojani et al, 1987a,b; Sedmak et al, 1989; Chen et al, 1991; Galea et al, 1991; Noël et al, 1991; de Mascarel et al, 1992; Elson et al, 1993; Hainsworth et al, 1993; Nasser et al, 1993; McGuckin et al, 1996; Cote et al, 1999) by comparing size and type of populations, histological tumour types, lymph node processing, immunohistochemical stainings and statistical analyses (Table 3). Only one series was prospective (Cote et al, 1999). Contrary to our study, a standard macroscopic technique was used in all the other studies, i.e. each lymph node was examined on one 2 – 3 mm thick slice transected in the major axis (Figure 1B). The mean number of lymph nodes per axillary node dissection was variable, and immunohistochemical stainings were performed either on original destained slides (Byrne et al, 1987; Trojani et al, 1987a,b; Noël et al, 1991; Byrne et al, 1992) or on slides from recuts of each block (Sedmak et al, 1989; Chen et al, 1991; Galea et al, 1991; Elson et al, 1993; Hainsworth et al, 1993; Nasser et al, 1993; McGuckin et al, 1996; Cote et al, 1999). The latter approach cannot distinguish between metastases that would be identifiable at deeper levels without

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**Table 2** Distribution of events (distant metastases or deaths) in our studies according to the presence or the absence of metastases detected by immunohistochemistry (IHM) in the infiltrating ductal carcinoma (IDC) and infiltrating lobular carcinoma (ILC) groups

| Study 1, 1987a | Study 2, 1992 | Study 3, 2001 |
|---------------|---------------|---------------|
| **IHM** | **IHM** | **IHM** |
| **Pos** | **Neg** | **P-value** | **Median** (years) | **Pos** | **Neg** | **P-value** | **Median** (years) | **Pos** | **Neg** | **P-value** | **Median** (years) |
| IDC | 4 | 9 | 0.002 (S) | 5 | 15 | 0.01 (S) | 5 | 21 | 0.07 |
| ILC | 3 | 5 | 0.9 | 4 | 5 | 0.07 | 7 | 9 | 0.6 |
| **Deaths** | **IHM** | **IHM** | **IHM** |
| IDC | 3 | 7 | 0.02 (S) | 5 | 42 | 0.8 | 6 | 61 | 0.7 |
| ILC | 2 | 2 | 0.5 | 7 | 10 | 0.08 | 19 | 18 | 0.07 |

M=median follow-up (years); S=significant difference. *In 1987 study: 122 IDC and 91 ILC; in 1992 and 2001 studies 129 IDC and 89 ILC.

**Figure 2** Metastasis-free survival according to presence or absence of node metastases detected by immunohistochemistry (IHM) in IDC group.
Table 3  Frequency and prognostic significance of metastases detected by immunohistochemical staining (IHM) in the literature

| Author (date)          | IHS method | Histological type | IHM | Follow-up: median years | Recurrences | Survival |
|------------------------|------------|-------------------|-----|-------------------------|-------------|----------|
| Trojani et al (1987)   | Original slides | 150 122 IDC      | 13 (11) | 10 | DFP 0.002 U SS 0.02 |
|                        |            | 2 ILC             | 8 (38) | DFP NS U SS 0.01 |
|                        |            | 9 ILC             | 8 (38) | 6.5 DFP NS U SS 0.01 |
| Byrne et al (1987)     | Original slides | 40 NSp           | 4 (10) | 5 | DFP NS U OS NS |
| Sedmak et al (1989)    | Recuts     | 45 3 DCIS         | 9 (20) | 10 | NSp U NSp 0.05 |
|                        |            | 1 medullary 41 IDC |         |               |
| Galea et al (1991)     | Recuts     | 98 NSp           | 9 (9)  | 14 | NSp NS U NSp NS |
| Chen et al (1991)      | Recuts     | 80 8 DCIS 68 IDC  | 21 (28) | 3.2 Distant <0.05 U ND |
|                        |            | 4 ILC             | 2 (50) |              |
|                        |            | 31 (18.5)        |         |               |
| Noel et al (1991)      | Original slides | 168 NSp         | 9 (9)  | 10 | Local Death NS U Local Death |
|                        |            | 35 IDC 129 IDC    | 5 (13) | 3.6 | NSp <0.001 U |
| Byrne et al (1992)     | Original slides | 39 35 IDC 4 ILC  |         |               |
| de Mascarel et al (1992) | Original slides | 218 129 IDC    | 13 (10) | 15.6 | DFP <0.01 M |
|                        |            | 89 ILC           | 37 (41) | 9.3 | DFP NS U SS |
| Hainsworth et al (1993) | Recuts    | 343 NSp         | 41 (12) | 6.5 | DFI NS U OS |
|                        |            | 89 ILC           | 37 (41) | 9.3 | DFI NS U OS |
|                        |            | 31 (18.5)        |         |               |
| Elson et al (1993)     | Recuts     | 97 NSp           | 20 (20.6) | 5.7 | NSp NS U NSp NS |
| Nasser et al (1993)    | Serial recuts | 159 140 IDC      | 22 (14) | 10 | DFS NS U OS |
|                        |            | 4 colloid 3 medullary |         |               |
|                        |            | 12 ILC           |         |               |
| McGuckin et al (1996)  | Serial recuts | 208 163 IDC      | 41 (25) | 7.6 | DFS <0.005 M |
|                        |            | 29 ILC           | 11 (38) |              |
|                        |            | 16 others        | 1 (6)  |              |
|                        |            | 51 ILC           | 20 (39) |              |
|                        |            | 90 others        | 16 (18) |              |
|                        |            | 12 ILC           |         |              |
|                        |            | 736              |         |               |
|                        |            | 595 ILC          |         |               |
|                        |            | 51 ILC           |         |               |
|                        |            | 90 others        |         |               |
|                        |            | 18 ILC           |         |               |
|                        |            | 376              |         |               |
|                        |            | 16 others        |         |               |
|                        |            | 51 ILC           |         |               |
|                        |            | 90 others        |         |               |
|                        |            | 18 ILC           |         |               |
|                        |            | 218 129 IDC      | 13 (10) | 24 | DFP NS U OS |
|                        |            | 89 ILC           | 37 (41) | 18 | DFP NS U OS |

*In the post-menopausal group of patients less than 65 years old. U=univariate analysis; M=multivariate analysis; NS=not significant; NSp=not specified in the original study; ND=not done in the study; DFP=disease-free probability; DFI=disease-free interval; SS=specific survival (deaths from breast cancer); OS=overall survival; MFP=metastasis-free probability.

Figure 3  Metastasis-free survival according to presence or absence of node metastases detected by immunohistochemistry (IHM) in ILC group.
immunohistochemistry and cases that are detectable only with immunohistochemical staining. The percentages of IHM according to the histological tumour type have been studied in only four reports (Trojani et al., 1987a,b; Byrne et al., 1992; McGuckin et al., 1996; Cote et al., 1999). When specified, definitions of events taken into account to calculate survival probabilities were heterogeneous and median follow-up was variable. All these differences in methodologies explain why neither the detection rates of these IHM nor their prognostic significance are comparable. IHM were associated with poorer prognosis in five studies (Sedmak et al., 1989; Byrne et al., 1992; Hainsworth et al., 1993; McGuckin et al., 1996; Cote et al., 1999). This prognostic significance is debatable due to the small number of patients (Sedmak et al., 1989; Byrne et al., 1992), the short median follow-up (Hainsworth et al., 1993), and to the fact that IHM were detected not only by immunohistochemistry but by a combination of morphological analysis on haematoxylin-stained slides and immunohistochemistry (McGuckin et al., 1986). In the study by Cote et al. (1999), IHM were associated with a shorter survival by univariate analysis in the under 65-year-old post-menopausal group of patients corresponding to 7% (53 out of 736) of the patients in their series. Multivariate analysis was performed on groups of patients stratified according to oestrogen receptor (ER) status (progesterone receptor status not specified), so the prognostic significance (value of risk) of IHM vs ER status is debatable. Furthermore, the relative importance by multivariate analysis of IHM vs tumour size, grade and vascular invasion was not specified. Lastly, perioperative chemotherapy was not effective in the group of patients in whom IHM were found.

CONCLUSION
On the whole, the pejorative significance of breast axillary node metastases detected by immunohistochemistry is debatable. It has to be proved before using immunohistochemical stainings as a standard in the sentinel lymph node technique. In conclusion: (1) our results emphasise the importance of length of follow-up in assessing the significance of metastases detected by immunohistochemistry; (2) in the literature there is no firm evidence underlining the prognostic significance of such metastases, and (3) more prospective and concordant studies are necessary to confirm or not the prognostic significance of metastases detected by immunohistochemistry. Therefore, a standard methodology is required in the pathological assessment of axillary lymph nodes.

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