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# PROGNOSTIC RELEVANCE OF IMMUNOLOGIC VARIABLES IN BREAST CARCINOMA

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Much attention has been directed towards the competence of the immune system in relation to the development and clinical course of malignant disease in man. Tumour specific antigens leading to the development of tumour associated immunity as detected for example by the presence of specifically cytotoxic lymphocytes and humoral antibodies have been demonstrated in several human tumours (KLEIN et coll. 1967, HELLSTRÖM et coll. 1968, MORTON et coll. 1968, BUBENIK et coll. 1970 a, b, EILBER et coll. 1970, HELLSTRÖM et coll. 1971, O'TOOLE et coll. 1972). The findings of an increased incidence of malignancies in children with immunologic deficiencies (WALDMANN et coll. 1972, KERSEY et coll. 1973) and in immunosuppressed recipients of renal transplants (MCKHANN 1969, PENN & STARZL 1970, STARZL et coll. 1970) as well as the fact that the disease-free interval or survival may be prolonged by non-specific immunologic stimulants (ISRAEL & HALPERN 1972, CROWTHER et coll. 1973, GUINAN et coll. 1973, GUTTERMAN et coll. 1973 a, b, SOKAL et coll. 1974) suggest that the immunologic status of patients may be of some significance for the development and growth of malignant tumours. Little information is available concerning the relationship between the general immunologic reactivity of patients at the time of the diagnosis of a malignant tumour and prognosis. Previously it was found that the ability of peripheral lymphocytes to respond to PPD in vitro was reduced as the

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disease advanced (GLAS et coll. 1976). Similar findings with regard to PHA reactivity in patients with advanced tumours have been reported (LEHANE & LANE 1974). These observations, taken together with the findings that the PPD or PHA reactivity or both increase in disease-free patients after successful treatment (WATKINS 1973, BARAL et coll. 1977), suggest that the tumour may contribute to an impairment of cell-mediated immunity.

The aim of the present analysis was to assess whether there are any correlations between some clinical and immunologic features in patients with carcinoma of the breast at the time of diagnosis and whether these are of prognostic value.

## Material

The material consisted of 203 consecutive patients with an operable primary carcinoma of the breast without evidence of distant metastases who, during June 1971 to June 1973 entered a randomized clinical trial aiming at establishing the value of pre- and postoperative radiation therapy. Details of this trial have been reported previously (GLAS & WASSERMAN 1974, DE SCHRYVER 1975). In brief, the diagnosis was verified by fine needle aspiration biopsy (FRANZÉN & ZAJICEK 1968). The initial clinical assessment was made jointly by a surgeon and a radiotherapist. The condition of the axilla was determined and two perpendicular diameters of the palpable tumour were measured using a caliper. The patients were then randomly allocated to three treatment groups, pre- or postoperative radiation therapy or radical mastectomy only. The details of the treatment modalities are reported elsewhere (GLAS & WASSERMAN, DE SCHRYVER). Complete peripheral blood status, liver function tests, chest radiography and metastatic bone survey were carried out on all patients before treatment.

## Methods

*Clinical follow-up.* After completion of the primary treatment, the patients were examined at three-month intervals during the first two years and thereafter every four months. Routine radiographic and laboratory examinations were performed when a recurrence was possible. The mean follow-up time (i.e. time elapsing between entry into the trial and the development of a recurrence or the end of the investigation, December 31, 1975) for all patients was 39 months, ranging from 3 to 54 months. The shortest follow-up for disease-free patients was 30 months. Of the 42 patients who developed distant metastases by the end of December, 1975, 12 did so within one year of diagnosis and of the 25 patients who developed a local recurrence, 7 did so within a year of diagnosis. The reappearance of the disease within the operated or irradiated area (chest wall, axilla, supraclavicular fossa) was defined as a local recurrence.

*Microscopic typing and grading.* The slides were reviewed by one pathologist. Only cases with no preoperative irradiation were reviewed (131 patients). The review

**Table 1**

*List of the variables used in the correlative study; with means and standard deviations (SD) or distributions*

| No. | Variable | Mean | SD | Distribution per cent |
|---|---|---|---|---|
| 1 | Age (years) | 54 | 9 | |
| 2 | Tumour size (mm) | 34.4 | 13.0 | |
| 3 | Involvement of axillary nodes (yes/no)* | | | (36/64) |
| 4 | Grade of malignancy (I, II or III) | | | (4, 37, 59) |
| 5 | Distant metastases (yes/no) | | | (21/79) |
| 6 | Local recurrence (yes/no) | | | (13/87) |
| 7 | PHA-reactivity (cpm) | 46 984 | 17 958 | |
| 8 | PPD-reactivity (cpm) | 16 948 | 19 093 | |
| 9 | Lymphocyte counts (number/$\mu$l) | 2 016 | 730 | |
| 10 | Antinuclear antibodies -ANA- (yes/no) | | | (33/67) |
| 11 | Antibodies against smooth muscle -SMA- (yes/no) | | | (31/69) |
| 12 | Antibodies against glomerular elements -GA- (yes/no) | | | (10/90) |
| 13 | Mitochondrial antibodies -MA- (yes/no) | | | (2/98) |
| 14 | Number of autoantibodies (0–4) | | | (42, 42, 13, 3, 0) |

* Axillary nodes were considered as involved only when confirmed by microscopy. Variables 1–4 are considered as 'clinical', 5–6 as 'follow-up' and 7–14 as 'immunologic' variables.

was carried out without knowledge of the clinical course or the laboratory findings. The original hematoxylin-eosin stained sections of the primary tumours and axillary nodes were reexamined. The malignancy grading was carried out as recommended by the International Reference Centre for Breast Tumours (WHO) (SCARFF & TORLONI 1968).

*Immunology. Separation of lymphocytes.* Venous blood was defibrinated by agitation in beakers containing glass pearls. Lymphoid cells were obtained after gelatin sedimentation of the erythrocytes according to the method of COULSON & CHALMERS (1964, 1966).

*Lymphocyte stimulation tests.* $2 \times 10^6$ lymphoid cells were cultured in glass tubes containing 1.0 ml Eagle's Minimal Essential Medium supplemented with Earle's Salts (MEM) with 10% heat inactivated human AB serum, streptomycin and penicillin. The stimulants used were:

**Table 2**

*Product-moment correlations and number of observations*

| Variable | Age | Tumour size | Ax. node inv. | Gr. of mal. | Dist. met. | Local recurr. |
|---|---|---|---|---|---|---|
| Age | 203 | 203 | 131 | 132 | 203 | 203 |
| Tumour size | 0.06 | 203 | 131 | 132 | 203 | 203 |
| Ax. node inv. | −0.06 | 0.24** | 131 | 131 | 131 | 131 |
| Gr. of mal. | −0.18* | 0.26** | 0.12 | 132 | 132 | 132 |
| Dist. met. | −0.04 | 0.32*** | 0.33*** | 0.25** | 203 | 203 |
| Loc. recurr. | −0.02 | 0.23*** | 0.12 | 0.16* | 0.28*** | 203 |
| PHA react. | −0.13 | 0.02 | 0.02 | 0.01 | 0.04 | 0.11 |
| PPD react. | −0.14 | 0.07 | −0.12 | 0.03 | −0.06 | −0.05 |
| Lymph. counts | 0.03 | 0.10 | 0.21* | 0.02 | −0.04 | −0.07 |
| ANA | 0.19* | 0.00 | 0.03 | 0.14 | 0.14* | 0.07 |
| SMA | 0.06 | 0.08 | 0.09 | −0.02 | −0.10 | −0.01 |
| GA | 0.04 | 0.04 | −0.03 | 0.05 | 0.00 | −0.07 |
| MA | 0.02 | −0.08 | 0.04 | 0.10 | −0.07 | −0.05 |
| No. ab. | 0.17* | 0.05 | 0.06 | 0.11 | 0.01 | −0.01 |

The product-moment correlations (r) between every pair of variables are found below the main diagonal. Correlations which are significantly different from zero are indicated by stars. The figures above the main diagonal represent the number of observations included in each calculation. In order to simplify evaluation of the table, the expected correlations between the 'clinical' and 'follow-up' variables are boxed. 'Nonsense correlations' between autoantibodies and number of autoantibodies are placed within parenthesis.

(1) Phytohaemagglutinin (PHA-M, Bacto Phytohaemagglutinin-M Difco Lab., Detroit, Michigan, USA). The contents of the vials were dissolved in 5.0 ml MEM and the cells cultured with this agent at a final concentration of 0.6 mg/ml, previously found to be optimal (GLAS & WASSERMAN 1974).

(2) PPD-tuberculin (PPD, RT 22, Statens Seruminstitut, Copenhagen, Denmark) at a concentration of 1.0 $\mu$g/ml. Cultures were set up with PHA or PPD in parallel with non-stimulated controls. After four days of incubation at 37°C in a humidified 5 % $CO_2$ air atmosphere, to each tube was added 0.4 $\mu$Ci $^{14}C$ thymidine (Radiochemical Centre Amersham, England. Specific activity 54 mCi/mM). Twenty-four hours later the cells were harvested and incorporated radioactivity determined as described previously (GLAS & WASSERMAN). Activity of the control cultures expressed as counts per minute (cpm) was subtracted from the values obtained in the corresponding test cultures. Mean values of triplicate cultures were calculated on an arithmetic basis.

*Autoantibodies.* Sera were collected at the time of diagnosis and stored at −20°C for periods not exceeding 24 months. They were then examined for presence of 4

**Table 2** (*cont.*)

| PHA react. | PPD react. | Lymph. counts | ANA | SMA | GA | MA | No. ab. |
|---|---|---|---|---|---|---|---|
| 125 | 125 | 148 | 201 | 201 | 201 | 201 | 201 |
| 125 | 125 | 148 | 201 | 201 | 201 | 201 | 201 |
| 85 | 82 | 93 | 129 | 129 | 129 | 129 | 129 |
| 85 | 82 | 93 | 130 | 130 | 130 | 130 | 130 |
| 125 | 125 | 148 | 201 | 201 | 201 | 201 | 201 |
| 125 | 125 | 148 | 201 | 201 | 201 | 201 | 201 |
| 125 | 122 | 105 | 125 | 125 | 125 | 125 | 125 |
| 0.11 | 125 | 106 | 125 | 125 | 125 | 125 | 125 |
| −0.07 | 0.08 | 148 | 148 | 148 | 148 | 148 | 148 |
| 0.01 | −0.03 | −0.11 | 201 | 201 | 201 | 201 | 201 |
| 0.05 | −0.05 | −0.07 | −0.02 | 201 | 201 | 201 | 201 |
| 0.00 | −0.17* | 0.08 | 0.05 | 0.25** | 201 | 201 | 201 |
| 0.03 | −0.09 | 0.01 | −0.10 | −0.02 | −0.05 | 201 | 201 |
| 0.04 | −0.13 | −0.08 | (0.60)*** | (0.68)*** | (0.55)*** | 0.09 | 201 |

different autoantibodies, namely: Antinuclear antibodies (ANA), smooth muscle antibodies (SMA), antibodies against glomerular elements (GA) and mitochondrial antibodies (MA). The method employed was that of indirect immunofluorescence as described previously (WASSERMAN et coll. 1975). Antibody titres of 1:10 or higher were considered positive.

*Statistical methods.* The statistical task has essentially been to analyse relations within the set of clinical and immunologic variables and also between these variables and prognosis of the disease as expressed by development of distant metastases or local recurrences. Description of variables is given in Table 1. As a measure of association for each pair of variables, Pearson's product-moment correlation (r) has been chosen. The significance tests for pairs of non-continuous variables were carried out by the chi-square test statistic, and for the continuous variables by the normal approximations. Correlations which are significantly different from zero are indicated by one to three stars, one star indicating the 5, two stars the 1, and three stars the 0.1 per cent significance levels, respectively.

## Results

The variables included in this analysis with their means, standard deviations or distributions are listed in Table 1. Variables 1–4 are hereafter termed 'clinical', 5–6 'follow-up' variables and the remaining are termed 'immunologic'. Table 2 presents estimates of correlations between the variables. The table should be evaluated in the

following way: the product-moment correlations between every pair of variables are found below the main diagonal. The figures above the main diagonal represent the number of observations included in each calculation. For instance, variables 3 and 5, based on 131 observations, show a correlation of 0.33, which means a positive association.

Correlations between tumour size, axillary node involvement, grade of malignancy and the development of local recurrences and distant metastases were found as expected as well as a positive correlation between age and ANA (Table 2). Moreover, a positive correlation existed between peripheral lymphocyte counts and axillary node involvement and between ANA and the development of distant metastases.

The PHA and PPD reactivities did not correlate with any of the clinical or follow-up variables.

Peripheral lymphocyte counts did not correlate to either development of distant metastases or local recurrences.

## Discussion

The general immunologic reactivity of patients, as measured by delayed cutaneous hypersensitivity to some microbial antigens or development of sensitivity to Dinitrochlorobenzene (DNCB), has previously been demonstrated to be of prognostic value in untreated patients with lung carcinoma (STEFANI et coll. 1976), malignant melanoma and sarcomas of bone and soft tissue origin (EILBER et coll. 1975). The present material was analysed to assess whether some immunologic parameters of patients with operable carcinomas of the breast, at the time of the diagnosis, correlated to their prognosis as well as whether any relation existed between the immune status and the size of the primary tumour, axillary lymph node involvement and grade of malignancy. No statistically significant correlations were revealed between the responses of the patients' lymphocytes to PHA and PPD with any of the tumour characteristics. This is surprising in view of the fact that several authors have reported decreased mitogenic responses of blood lymphocytes in patients with malignant tumours (DUCOS et coll. 1970, GARRIOCH et coll. 1970, HAN & TAKITA 1972, KUMAR & TAYLOR 1973, LANDER & BONE 1973, LEHANE & LANE 1974, KNIGHT & DAVIDSON 1975). However, it should be emphasized that such decreased reactivities were most readily demonstrated in patients with advanced disease (GARRIOCH et coll., HAN & TAKITA, LEHANE & LANE, KNIGHT & DAVIDSON). HOLM et coll. (1976) have shown an association between stage, prognosis and the magnitude of the pretreatment mitogenic responsiveness of blood lymphocytes to certain phytomitogens and PPD in Hodgkin's disease. This disease is, however, a neoplasm of the lymphoreticular system. The initial presence of more than one type of autoantibody was associated with a higher incidence of both local recurrences and distant metastases (WASSERMAN et coll. 1975). This correlation could not be confirmed in the present material, which was performed on a larger number of patients. However, an association between ANA and the development of distant metastases was found. Since the frequency of

autoantibodies in the more recently examined patients was lower than in those previously investigated, it is uncertain whether these two series are entirely comparable. Higher incidence of autoantibodies in the previously examined patients cannot be attributed to a changed selection, since all patients were included in the same randomized trial. It is possible that some unregistered change in sensitivity or specificity of Fluorescein isothiocyanate anti-Ig (FITC) conjugates for different immunoglobulin classes or minimal changes in grading of fluorescence could account for the observed difference. In order to elucidate this discrepancy, further investigations are planned. Furthermore, the observation of PAPATESTAS et coll. (1976) was not confirmed. In a retrospective analysis of 453 patients with malignant breast tumours he found that low pretreatment lymphocyte counts were associated with a poorer prognosis. In contrast, in the present material only a weakly positive correlation was found between lymphocyte counts and the presence of axillary node involvement, a well recognized unfavourable prognostic sign. It should be pointed out that the few correlations observed between the immunologic variables on the one hand and clinical and follow-up variables on the other were significant only at the 5 per cent level.

Since the number of patients was limited, the present analysis does not definitely rule out the possibility that the immune status of the patients, as this is reflected by the tests used, may be of importance for the prognosis of the disease. On the other hand, the number of patients was sufficiently high to allow the detection of the well-known and expected correlations between the size of the primary tumour, involvement of axillary nodes, the histological malignancy grade and the prognosis of the disease (BLOOM & RICHARDSON 1957, FISHER et coll. 1969, HAAGENSEN 1971).

In conclusion, the results of this analysis indicate that the initial peripheral lymphocyte counts, their PHA and PPD reactivities and the occurrence of certain autoantibodies are not correlated with the clinical features of the disease in operable carcinomas of the breast, nor do they seem to give any basis for a more certain prognosis of the disease.

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## SUMMARY

A series of 203 consecutive patients with operable carcinoma of the breast was analysed with regard to correlations between a set of immunologic and clinical variables existing at the time of the diagnosis. No major correlations were revealed between immunologic

variables on the one hand and clinical features or the course of the disease on the other. The well-known prognostic relevance of tumour size, involvement of the axilla and the histological grade of malignancy was evident.

## ZUSAMMENFASSUNG

Eine Serie von 203 konsekutiven Patienten mit einem operablen Karzinom der Brust wunde hinsichtlich der Korrelationen zwischen einer Anzahl immunologischer und klinischer Variablen zur Zeit der Diagnose analysiert. Keine Korrelation wurde zwischen den immunologischen Variablen auf der einen Seite und dem klinischen Verlauf oder dem Erscheinungsbild der Erkrankung auf der anderen Seite gefunden. Die wohl bekannte prognostische Relevanz der Tumorgrösse, der Beteiligung der Axilla und der histologischen Gradierung der Malignität war offenbar.

## RÉSUMÉ

Une série de 203 malades consécutives ayant un cancer du sein opérable a été étudiée en ce qui concerne les corrélations entre un groupe de variables immunologiques et de variables cliniques au moment du diagnostic. Les auteurs n'ont pas trouvé de corrélations importantes entre les variables immunologiques d'une part et les caractères cliniques ou l'évolution de la maladie d'autre part. La signification pronostique bien connue du volume tumoral, de l'atteinte de l'aisselle et du type histologique de malignité a été évidente.

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