

Autoantibodies in patients with bronchial carcinoma

MARGARET E. HODSON
and MARGARET TURNER-WARWICK

Brompton Hospital and Cardiothoracic Institute, London SW3

Hodson, Margaret E. and Turner-Warwick, Margaret (1975). *Thorax*, 30, 367-370. **Autoantibodies in patients with bronchial carcinoma.** No overall increase in the incidence of antinuclear, smooth muscle or reticulin antibodies was observed in a group of 105 patients with bronchial carcinoma of various classified histological types. Smooth muscle antibody was, however, demonstrated in 27% of patients with undifferentiated carcinoma compared to 5% of controls ($P < 0.05$). A highly significant increase in antinuclear antibodies in patients with adenocarcinoma was found (31% compared with 5% of controls— $P < 0.01$). There was no apparent correlation between the presence of these antibodies and age, sex or other clinical features studied. A detailed study of the reproducibility of the antibody results, studied by indirect immunofluorescence in patients with bronchial carcinoma, is reported.

Smooth muscle antibody has been reported in 67.5% of 80 patients with malignant disease in various sites (Whitehouse and Holborow, 1971). These workers also found an overall increase in the incidence of antinuclear antibody (24%) and they reported a new antigen presumed to be located in bile canaliculi.

It has been suggested that contractile protein components present on the surface of the malignant cell might provide the stimulus for smooth muscle antibody production (Whitehouse and Holborow, 1971). If this were the case, one might expect that these antibodies would occur with different frequencies in association with tumours of different histological types.

Our aim was to study these antibodies in a larger series of patients with bronchial carcinoma of different histological types and to attempt to relate the antibody prevalence to a number of clinical features.

PATIENTS AND METHODS

The study includes 105 patients with bronchial carcinoma; histological classification of the tumour was made at biopsy in 50 cases and by cytological examination of the sputum in 55. Clinical history, physical examination, and radiographic findings were recorded, and blood was taken for autoantibody studies. Thirty-nine subjects matched for sex and without lung disease

were used as controls. The histology of the tumours was classified by Dr. K. F. W. Hinson as squamous in 50, oat cell in 25, adenocarcinoma in 19, and undifferentiated in 11.

IMMUNOLOGICAL METHODS The antibodies were studied without previous knowledge of the clinical or histological features of the case. Sera diluted 1/10 were examined for antinuclear, smooth muscle, and 'reticulin' antibodies, using a standard indirect double layer immunofluorescent system (Coons and Kaplan, 1950) on 4 μ cryostat sections of rat liver and stomach. Sheep antihuman immunoglobulin (Wellcome) labelled with fluorescein diluted 1 : 8 was used to detect IgG, IgM, and IgA antibodies. The preparations were viewed, using transmitted ultraviolet light, with a Reichert Zetopan microscope.

Titres of antinuclear antibody were measured using the same method with different serial dilutions of serum.

Fluorescence of smooth muscle in the outer layer of the stomach wall and muscularis mucosae, and in the arterial walls, was recorded. A score of the intensity of the smooth muscle fluorescence was recorded as 1-3 for each structure (as suggested to us by Dr. Holborow), making a total possible score of 9. In our study a total score of 3 or more was counted as positive.

The patterns of 'reticulin' antibody observed on

liver tissue were classified following the system suggested by Rizzetto and Doniach (1973): R_1 —coarse or lumpy fluorescence surrounding portal tracts; R_2 —fine reticular fluorescence of vessel walls; R_3 —Kupffer cell fluorescence.

When antibodies are being detected in fairly low titres by immunofluorescent methods, special care over the reproducibility of results is needed. Therefore we studied intra-observer error by requiring the same observer (MTW) to read the results for each serum sample in random order on at least two occasions. If both readings were not in complete agreement for all antibodies studied then the sample was read again on a further two occasions, making four in all. All the laboratory work was done by the same person (MEH) using the same technique on each occasion, but of course it was necessary to use tissue from different rats during the course of the study. For counting purposes, a serum was considered to be positive or negative for a given antibody if it was either consistent on the first two readings or positive or negative on three out of four occasions. Readings on sera which were uncertain after four readings (i.e., two negative and two positive) were counted as negative for purposes of correlation with the clinical and histological features.

RESULTS

Antinuclear antibody was detected in 13 of 105 sera from carcinoma patients (12%) but the prevalence was not significantly different from that in the controls (Table I). The titre of antinuclear antibody found is shown (Table II). A prevalence of smooth muscle (12%) and 'reticulin' (4%) was not greater in the carcinoma group than in the controls. When sera from patients with different histological types of tumour are considered separately (Table III), smooth muscle antibody was significantly increased in undifferentiated tumour ($P < 0.05$) and antinuclear antibody in adenocar-

TABLE I
TISSUE ANTIBODIES IN CONTROL AND CARCINOMA SERA

| Antibody | 39 Control Sera | | 105 Carcinoma Sera | | P Value |
|---------------|-----------------|---|--------------------|----|---------|
| | No. Positive | % | No. Positive | % | |
| Antinuclear | 2 | 5 | 13 | 12 | NS |
| Smooth muscle | 2 | 5 | 13 | 12 | NS |
| Reticulin | 0 | 0 | 4 ¹ | 4 | NS |

NS The sera from the carcinoma patients showed no significant difference from the controls.

¹Reticulin found was R_1 in three cases and R_2 in one case (for terminology see Rizzetto and Doniach (1973)).

TABLE II
DISTRIBUTION OF ANTINUCLEAR ANTIBODY TITRES

| Titre No. of patients | ANA Negative | ANA Positive | | | Total Positive |
|--------------------------|-----------------|--------------|-----------|-----------|-------------------|
| | 1/10 92 | 1/10 6 | 1/20 3 | 1/40 3 | 1/80 1 |

cinoma of the bronchus ($P < 0.01$). Forty-nine of our patients had chronic bronchitis as defined by MRC criteria (Medical Research Council Report, 1965) but this did not correlate with the presence or absence of antinuclear antibody (Table IV).

There was no correlation between the autoantibodies found and various clinical features of the disease. The latter included age, sex, smoking history (both duration and amount), duration of symptoms, nature of the first symptom, presence or absence of haemoptysis, finger clubbing, neuropathy, weight loss, lymph node enlargement, and liver involvement.

REPRODUCIBILITY OF RESULTS Three hundred and five readings for antinuclear antibody were made on the 105 sera (Fig. 1). Only three sera were inconsistent. Thirteen sera were considered to be positive and 89 negative. Six of the sera which were finally regarded as negative gave positive readings on one occasion (false positive rate

TABLE III
FREQUENCY WITH WHICH ANTIBODIES ARE FOUND, ACCORDING TO DIFFERENT HISTOLOGICAL TYPES OF TUMOUR

| Sera | | Antibodies | | | | | |
|------------------|----------------|-------------|-----------------|---------------|-----------------|-------------|---|
| Histology | Number Studied | Antinuclear | | Smooth Muscle | | 'Reticulin' | |
| | | No. | % | No. | % | No. | % |
| | | Carcinoma | 105 | | | | |
| Squamous | 50 | 4 | 8 | 5 | 10 | 4 | 9 |
| Oat | 25 | 2 | 8 | 2 | 8 | 0 | 0 |
| Adenocarcinoma | 19 | 6 | 31 ¹ | 3 | 16 | 0 | 0 |
| Undifferentiated | 11 | 1 | 9 | 3 | 27 ² | 0 | 0 |
| Controls | 39 | 2 | 5 | 2 | 5 | 0 | 0 |

¹ $P < 0.01$ compared with controls.

² $0.05 > P > 0.02$ compared with controls.

TABLE IV

| Carcinoma Patients | | With Positive ANA | |
|----------------------------|------------|-------------------|----|
| | | No. | % |
| With chronic bronchitis | 49 | 6 | 12 |
| Without chronic bronchitis | 56 | 7 | 12 |
| Total | 105 | 13 | |

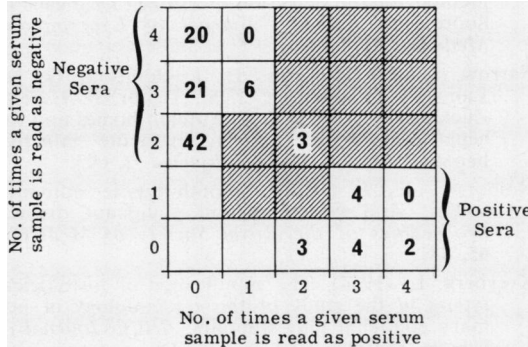


FIG. 1. Chart showing the reproducibility of antinuclear antibody. The numbers in each box indicate the number of sera which have been read a given number of times positive and a given number of times negative, e.g., '6' shows that six sera were read as negative on three occasions and positive on one occasion. Sera falling within the shaded area in Figures 1 and 2 are considered to be inconsistent.

(6/251) 2%). Four of the sera which were finally regarded as positive gave a negative reading on one occasion (false negative rate (4/42) 10%).

Three hundred readings for smooth muscle antibody, using the scoring method detailed above, were made on 105 sera (Fig. 2). Only three sera were inconsistent. Thirteen sera were considered to be positive and 89 negative. Sixteen sera which were finally regarded as negative gave a positive reading on one occasion (false positive rate (16/251) 6%). Six sera which were finally regarded as positive gave negative readings on one occasion (false negative rate (6/41) 15%).

DISCUSSION

Holborow (1972) reported an increased prevalence of antinuclear antibody (24%) and smooth muscle antibody (67.5%) in carcinoma from many different sites. This study included only six patients with carcinoma of the bronchus.

We have been unable to confirm this high overall incidence of antinuclear and smooth muscle antibody in a group of 105 patients with bronchial carcinoma. The reason for our different results

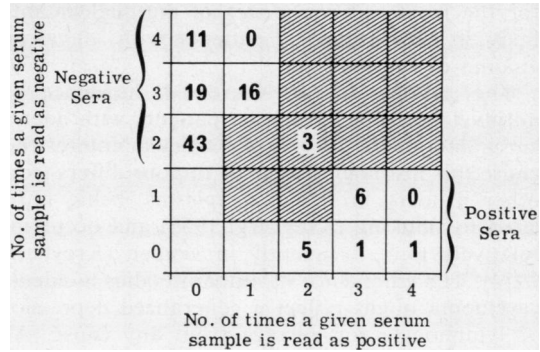


FIG. 2. Shows the reproducibility of smooth muscle antibody.

may be that bronchial carcinoma differs from other forms of carcinoma in this respect, or the explanation may be technical. The fluorescent antibody technique, the screening sera dilution, the tissue species used, and the methods of quantifying smooth muscle antibody were all similar to those used by Holborow but we used transmitted ultraviolet light with a Reichert Zetopan microscope, not incident light. We also included only positive results when sera were found to give reproducible results on two occasions or at least three times out of four repeated observations. Using this method of checking on our intraobserver error, only 3 out of 105 sera gave discordant results for antinuclear antibody and only 3 out of the 105 for smooth muscle antibody.

We have, however, demonstrated a significant increase in certain antibodies in relation to individual histological types of tumour although the results need to be confirmed in a larger group of patients.

Antinuclear antibody was found in six (31%) of our group of 19 patients with adenocarcinoma of the bronchus; four of these six patients were men. This sex distribution is of some importance because Beck (1963) has shown that antinuclear antibody (ANA) occurs more frequently in women, especially over 60 years of age. Among our ANA positive patients with adenocarcinoma there were only two women, aged 49 and 60.

An increased incidence of antinuclear antibody has been found in chronic bronchitis with gross infection but not in simple chronic bronchitis (Hodson and Turner-Warwick, in press). Chronic bronchitis was present in six of the carcinoma patients with antinuclear antibodies but none had gross infection. No patient in the study had more than three infective episodes in a year. Thus coincident chronic bronchitis is unlikely to account

for the increased prevalence of antinuclear antibody in this group of patients with adenocarcinoma of the bronchus.

The finding of an increased incidence of antinuclear antibody among patients with adenocarcinoma of the lung is of particular interest because this histological type of tumour differs from other primary bronchial neoplasms, being unrelated to smoking (Kreyberg, 1955) and occurring relatively more frequently in women (Kreyberg, 1954). The emergence of autoantibodies in adenocarcinoma might reflect a generalized depression of lymphocyte surveillance from any cause. Alternatively, stimulation of lymphocytes by tissue antigens might facilitate the replication of oncogenic virus (Schwartz, 1972). The increased frequency of tissue antibodies in many other types of viral infection is now well established (Farrow *et al.*, 1970; Holborow, 1972). It is also possible that the presence of antinuclear antibody is a marker for some other genetic factor, so far undetermined, which acts as the predisposing factor in the development of adenocarcinoma of the lung in some individuals.

The increased incidence of smooth muscle antibody in undifferentiated carcinoma suggests that in this tumour the malignant cell membranes may be structurally different from other histological variants. We were unable to find any clinical explanation for this association; in particular, liver involvement was not more frequent in this group of cases.

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- Requests for reprints to: Professor Margaret Turner-Warwick, Cardiothoracic Institute, Fulham Road, Brompton, London SW3 6HP.