Serum Globulins and Bronchogenic Carcinoma

ANDERSON NETTLESHPH

(Laboratory Service, Veterans Administration Hospital, Fayetteville, Arkansas)

SUMMARY

In a statistical and controlled analysis, 57 cases of bronchogenic cancer were accurately analyzed from a clinico-pathological and globulin electrophoresis standpoint. There was a significant increase of gamma, beta, alpha 1, and alpha 2 globulins in this disease. A significant increase in these proteins was also found in 102 cases of non-bronchogenic cancer. A marked degree of change was found in all bronchogenic cancer cases, but in some cases, 1 month prior to death, there was an erratic change in these values.

Although the mechanism of the increase in globulins is not understood, it is suggested that the proteins derive either from the neoplasm, as such, or from autogenous neoplastic antibodies. The latter seems a particularly attractive hypothesis at this time.

The present work fits into the emerging pattern of general body immunological responses in neoplastic disease.

It has been clear since the early days of blood protein studies that abnormalities occur in plasma globulins, but the mechanisms responsible for these changes have remained somewhat obscure. A recent statement in the literature concerning this problem is: “In isolated cases of almost all types of neoplasia, in addition to myeloma, there is a striking increase in gamma globulin” (4). To prove whether a specific relationship exists between hyperglobulinemia and cancer there is need for a correlation study between neoplasia thoroughly evaluated by morphologic means and globulin alterations. If this could be shown it would be of immediate practical diagnostic usefulness, as well as contributing to the delineation of criteria concerning neoplastic diseases.

The present study is published in hopes of further defining the problem and indicating to those interested in cancer that the study of the globulin fractions, particularly gamma, beta, and alpha 2, is a useful biological test in clinical diagnosis of the disease. This is most clearly indicated in hypergammaglobulinemia. In the present stage of development, characterization of plasma proteins is not, as such, a test for cancer. There are indications, however, that we may find in the globulin fraction material suitable for a specific test.

The complexity of the problem was recognized in early publications (12, 13) and brought to more thorough definition in 1950 (10) and 1953 (7, 20), when it was generally conceded that the mechanism for hyperglobulinemia had not been found. Since then many contributions to the subject have increased the certainty that there is some connection between hyperglobulinemia and neoplasia, even though the ideal, for an increase in globulins with advance of the disease, needs further study (7). The generally unsatisfactory nature of this particular correlation in human neoplasia will be discussed.

In 1956-57 we observed high gamma-globulin fractions in blood sera of 26 patients with a clinical diagnosis of bronchogenic carcinoma; twelve of these cases were correlated with histopathological findings. During the past 26 months, 57 additional cases, proved by biopsy or autopsy, were studied. The problem was to determine whether or not individual alterations in value of alpha 1, alpha 2, beta, and gamma have a statistically valid relation to bronchogenic carcinoma. After blood serum values of these fractions were obtained, the findings were tested with $\chi^2$ values and standard deviations to determine whether the blood serum globulins in bronchogenic carcinoma actually show significant variations from median normal values. The possibility of a statistical validation of abnormalities in certain globulin fractions will be shown during the development of the subject, as well as other interesting features.
Some years ago it was discovered that serum albumin and globulin alterations occur in diverse diseases. Later, it was shown electrophoretically, by the ultracentrifuge and more refined chemical methods, that globulin is nonhomogeneous and composed of various fractions. With the use of paper electrophoresis numerous diseases have been studied, and the significance of some of these changes in the globulin fractions is becoming recognized.

The present report is a statistical analysis of a series of human globulins, from normal controls and histopathologically proved bronchogenic and other carcinomas. The study includes sera from normal people as well as patients with other neoplasms, infectious diseases, and a variety of degenerative disorders.

MATERIALS AND METHODS

The control blood sera for globulin determinations were from 100 consecutive blood bank pilot tubes. Test sera were from patients in the hospital. Except for four post-mortem sera, all other sera were obtained during life. The selective criteria used were neoplastic histopathologically positive proof for bronchogenic and other carcinomas. Patients were studied and listed for tabulation in order of chronologic occurrence. Twenty-six months after beginning the study we were able to evaluate 51 noninfectious degenerative disease patients, 38 infectious cases, 102 nonbronchogenic cancers, and 57 proved bronchogenic cancer cases. All neoplasms in this study were examined microscopically either during life or at post mortem, and only cases proved by usually acceptable morphologic criteria are included. The infectious group were sub-acute or chronic infections, usually pyogenic. The degenerative diseases ran the gamut from rheumatoid arthritis and arteriosclerosis to disseminated lupus (two cases) and scleroderma (one case).

Globulin fractions were quantitated by paper electrophoresis. This technic has today become standardized (14, 19). Blood bank sera controls were from donors 16-65 years of age. The patient's blood was obtained by vena puncture or by opening the heart at post mortem. No blood was used later than 16.5 hours after withdrawal, the average time being 4.7 hours. Grossly hemolyzed or contaminated sera were discarded. The nature of the histological evidence in the cancer cases was proved in 48 cases at autopsy and nine by biopsy. Histopathological preparations were made with routine hematoxylin and eosin stains; when necessary, special stains were used to differentiate type of neoplasm. Conditions associated with the bronchogenic group showed six proved bronchopneumonias, and the usual chronic pulmonary emphysema, anthracosis, and bronchiectasis were present in a high percentage of all patients.

The equipment used in these experiments was that of paper electrophoresis, with Spinco Durum-type cell with Duostat power control.

Following separation from clot, .006 ml. serum was placed on S & S 2048 A paper strips. The system was buffered with veronal buffer at pH 8.6 with ionic strength of 0.075 and run at 2.5 constant MA voltage of approximately 75 for 16 hours. The strips were fixed with heat and methyl alcohol and stained with bromphenol blue; the resultant color changes were translated into curves by Model RB Analytrol. The biuret method was used to determine total proteins. Early in our analysis it was determined that ethanol precipitate fractions II, III, and IV of Cohn (5) followed the same alpha, beta, and gamma globulin electrophoretic patterns, respectively, as those of unprecipitated serum; but the relative proportions of these fractions were not duplicated. As would be expected, the relative proportions were not the same, since the methods are different; nevertheless it does indicate that we are dealing with similar molecular groups.

Statistical analyses were calculated with a non-parametric rank-order analysis used for variance (6, 11, 17).

RESULTS

The results of globulin determinations are given in Table 1. The statistical analyses of gamma values for bronchogenic carcinoma show x^2 values of 37.60 for alpha 1, 50.00 alpha 2, 27.00 beta, and 49.54 gamma.

The basic control group values in Table 1 agree with results published in the literature (14, 19). The majority of these people were normal; they passed a routine physical and blood check for donor blood and came from a healthy population. As can be seen from the table their gamma values are lower than for all other groups. The noninfectious degenerative group shows slight increment in values; in infectious diseases this is increased, and finally with nonbronchogenic cancers and bronchogenic carcinomas it is strikingly increased.

Fifty-six of the bronchogenic carcinomas were epidermoid, oat-cell, or squamous type; one was an adenocarcinoma. The other cancers studied were carcinomas of the colon, stomach, pancreas, liver, kidney, and various other sites; also included was one osteogenic sarcoma and one mesothelioma. No cancers of the skin are included.

Most of the infectious cases were acute or sub-
acute pyogenic infections and included furuncles, pneumonias, infectious hepatitis, one pneumococ- cic pericarditis.

We were able to study thirteen cases of bronchogenic carcinoma electrophoretically during a period of 6 months. Of these nine showed an increase in percentage of gamma globulin with progression of disease, and six showed, with detailed analyses during the period, an increment from 20.4 to 33.3 per cent. Two cases studied 4 months and 1 month, respectively, had high gammas which showed no change, and three cases showed erratic elevations 1 month prior to death and a minimum point, 1 week before death, which approached the previous 1-month point. What is significant here is the change, with its marked increase, and that some cases drop off somewhat directly before death.

Another unexpected finding was the heterogeneity of the gamma peak in bronchogenic car-
cinoma; this was often doubled or even tripled. Variability and heterogeneity in beta and alpha 1 and 2 are also common. No exact pattern emerges, but long familiarity with the curves increases predictability as regards whether or not the patient has neoplastic disease and, particularly, bronchogenic carcinoma.

DISCUSSION

The statistical analyses leave no doubt about correlation of hypergammaglobulin, beta, and alpha 1 and 2 states with neoplastic diseases. This confirms earlier reports (8, 10, 12). The most pronounced hyper states are found in bronchogenic carcinomas. Thus, by studying paper electrophoresis curves of serum globulins, it is possible to obtain information that is clinically useful in helping to differentiate neoplastic diseases, particularly bronchogenic carcinomas (7, 8, 12). To be of clinical significance it is evident that these values

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Globulin</th>
<th>Average in per cent</th>
<th>Gram per cent</th>
<th>Range in per cent</th>
<th>Statistical Calculations</th>
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<tbody>
<tr>
<td>Normals</td>
<td>100</td>
<td>Alpha 1</td>
<td>3.40±0.85</td>
<td>0.24</td>
<td>2.3-5.3</td>
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<td>Alpha 2</td>
<td>7.86±1.4</td>
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<td>3.5-14.4</td>
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<td>Beta</td>
<td>9.89±1.4</td>
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<td>5.5-15.6</td>
<td>(to be read: more than)</td>
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<tr>
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<td>Gamma</td>
<td>12.32±3.55</td>
<td>0.88</td>
<td>7.0-20.0</td>
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<td>Degenerative diseases</td>
<td>51</td>
<td>Alpha 1</td>
<td>3.96</td>
<td>0.37</td>
<td>1.6-9.5</td>
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<td>0.61</td>
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<td>Beta</td>
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<td>0.80</td>
<td>5.8-17.0</td>
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<td>1.03</td>
<td>7.0-25.1</td>
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<td></td>
<td>Tot. prot.</td>
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<td>6.80</td>
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<tr>
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<td>Tot. prot.</td>
<td>100</td>
<td>6.65</td>
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</table>

* A statistical median was determined for each group of diseases separately, incorporating the values for 100 normals in each, and 2 X 2 contingency tables were set up. The statistical significance was calculated by means of the nonparametric $\chi^2$ formula:

$$\chi^2 = \frac{N\left(AD - BC\right) - N_1^2}{(A + B)(C + D)(A + C)(B + D)}$$

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must be related to the clinical disease picture—a necessary condition in any clinical laboratory test. In a dramatic way, these findings again illustrate the dynamic state of body proteins (3, 16). What they do not answer is why a neoplasm should bring about such constant globulin increase. It has been stated that hypergammaglobulinemia is merely related to the chronic state in cancer or complications such as fever, infection, or related liver disease (2, 13). In our studies a deliberate effort was made to evaluate these factors as thoroughly as possible. We noted six cases in which terminal bronchopneumonia was recorded at autopsy. Careful gross and microscopic examinations ruled this out as a major contributing factor in the other cases. No single complicating factor could be found in enough cases to weight the statistics.

It has been generally accepted since 1950 (10), as a general principle, that plasma protein changes should increase as the extent of the disease increases. Although such a value may be valid in experimentally transmissible tumors, this value cannot be accurately predicted in human cancer at this time; and, indeed, from our studies it appears that it does not necessarily increase. The reasons for this are: (a) Clinical progress of cancer does not correlate with the extent or rate of growth, since a small cancer may press on a vital structure to the cancer growing large; (b) it is impossible to evaluate the amount of necrosis present in advanced human cancer; (c) the extreme complexity of the interrelationship of neoplastic proteins to the body’s store of proteins may be a changing value as the disease progresses, and finally if antibodies against the neoplastic disease are the cause of the hypergammaglobulinemia this would vary according to antigen release (tumor necrosis) plus the body’s ability to build antibodies.

None of the explanations offered seem sufficient to explain the hyperglobulin states in neoplasia. In multiple myeloma the abundant M-globulin is thought to be a product of myeloma cells, a kind of secretory product. In a different light, neoplasia (when they necrose) may readily release globulins into the blood stream. These in turn may produce autogenous globulin antibodies; both occurrences would then show up in the blood serum as hyperglobulin states. There has been considerable discussion concerning the lack of a typical serum protein response pattern for all malignancies (2, 15, 18). Hyper state of any one, two, or three globulins may be found in any particular patient’s serum. The neoplastic curves for the gamma state are often heterogeneous, the single most consistent finding being a double peak and the second peak being close to the beta curve; the beta curve itself may be broad or double-peak.

Straight-line increment of globulin values in the series of diseases studied is an expected finding, since it is well known that globulin is involved in antibody production. Yet unaccounted for are the hypogammaglobulinemic states occasionally found with neoplasm, particularly in some of the chronic lymphatic leukemias. These may be understood as neoplasms which destroy antibody production sites or occur through some other, as yet, unexplained mechanism.

In addition to hyper states reported in the literature, dysgammaglobulinemia is recognized (2). However, there are exceptions to this (1, 9, 13, 18). One of the most productive thoughts which has come from these data is that when a neoplasm arises at a particular site it probably reflects fairly early in the blood stream. That such reactions are found in the globulins is not an isolated phenomenon but is one of the general biologic reactions to focal neoplastic disease; further, this may be a part of the body’s general defense to neoplasm. It is true that more data are needed to clarify the earlier stages in the process; but if the mechanism is present early it may be of profound significance. Certainly in those cases of proved spontaneous cure of human cancer this may be the major role of such occurrences. The nature of the final solution to this complex and exciting problem can only be surmised at this time.

ACKNOWLEDGMENTS

The excellent technical assistance of J. A. Strother and W. G. Champlin is acknowledged, as well as that of Dr. Charles V. Lair for aid on statistical analysis and H. Siemsen Smith on manuscript.

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