Present day practice

Immunoglobulin and intrinsic factor antibody in the sera of patients with pernicious anaemia

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Blocking antibody to intrinsic factor has been found to be present in the serum of 50 to 60% of patients with pernicious anaemia (Ardeman and Chanarin, 1963; Irvine, 1966; Ungar, Whittingham, and Francis, 1967). This antibody has been shown to be a gamma globulin (Ardeman and Chanarin, 1963), possibly IgG (Chanarin, 1969). It has been suggested that the production of this antibody is an autoimmune phenomenon (Chanarin, 1969). To our knowledge, no studies relating the serum immunoglobulin levels and the incidence of intrinsic factor blocking antibody in patients with pernicious anaemia have yet been reported. We report the results of such a study.

Forty-two patients with proven pernicious anaemia were studied. The ages of these patients ranged from 50 to 89 years (mean age = 72 years). All the patients were admitted to hospital for investigation of anaemia. Investigation procedures included routine haematological profile studies, bone marrow aspiration, estimation of serum vitamin B_{12} and folic acid, and Schilling tests without and with hog intrinsic factor preparations. A final diagnosis of pernicious anaemia was based on a consideration of all such data. Before commencing regular vitamin B_{12} therapy, serum samples were collected for the estimation of immunoglobulin levels and intrinsic factor blocking antibody. The serum immunoglobulins were measured by the radial immunodiffusion method[1]. The serum intrinsic factor blocking antibody was assayed by the method of Shum, O'Neill, and Streeter (1971).

Of the 42 patients studied, only 16 had detectable serum intrinsic factor blocking antibody. The titre of antibody ranged from 7 to $\geqslant 80$ ng units/ml. The patients were then divided into two groups, those who did not have serum intrinsic factor blocking antibody and those who did. Results of the serum IgM, IgA, and IgG levels of these two groups of patients are shown in Figure 1. Statistical analyses by Student's t test and Wilcoxon's test (Geigy, 1962) (Table) show that there is no significant difference in the mean serum immunoglobulin levels of these two groups. Furthermore, in the group of patients with detectable serum intrinsic factor blocking antibody, there was no obvious correlation between the level of the serum immunoglobulins and the titre of the antibody (Fig. 2).

Statistical Test	*Serum Immunoglobulin*	P	*Difference at 5% Level*
Student t	IgG	>0·70	Not significant
	IgA	>0·50	Not significant
	IgM	>0·30	Not significant
Wilcoxon's	IgG	>0·10	Not significant
	IgA	>0·10	Not significant
	IgM	>0·05	Not significant

Table *Statistical analysis on serum immunoglobulin levels of pernicious anaemia patients without and with IFAB*[1]

[1]IFAB = serum intrinsic factor blocking antibody.

Our results indicate that the presence of intrinsic factor blocking antibody in the serum of patients with pernicious anaemia is not associated with a measurable rise in serum IgM, IgA, or IgG levels. Presumably this is because the concentration of immunoglobulin which has intrinsic factor blocking antibody activity is small compared with the total immunoglobulin concentration.

Considering the 42 cases of pernicious anaemia as one group, the overall results of their serum immunoglobulin levels are as follows: IgM = 116 ± 78 (mean ± SD), IgA = 381 ± 150, IgG = 1158 ± 380. A control group of 39 normal subjects gave IgM = 106 ± 42 (mean ± SD), IgA = 247 ± 73, IgG = 1212 ± 258. It is of interest to note that, when compared to the normal subjects, the patients with pernicious anaemia showed a significantly elevated IgA level whilst the IgM and IgG levels were not significantly different. Other groups of patients in this hospital have also shown elevated IgA levels. A more detailed and comprehensive study of these observations will be reported.

[1]Hyland immuno-plate.

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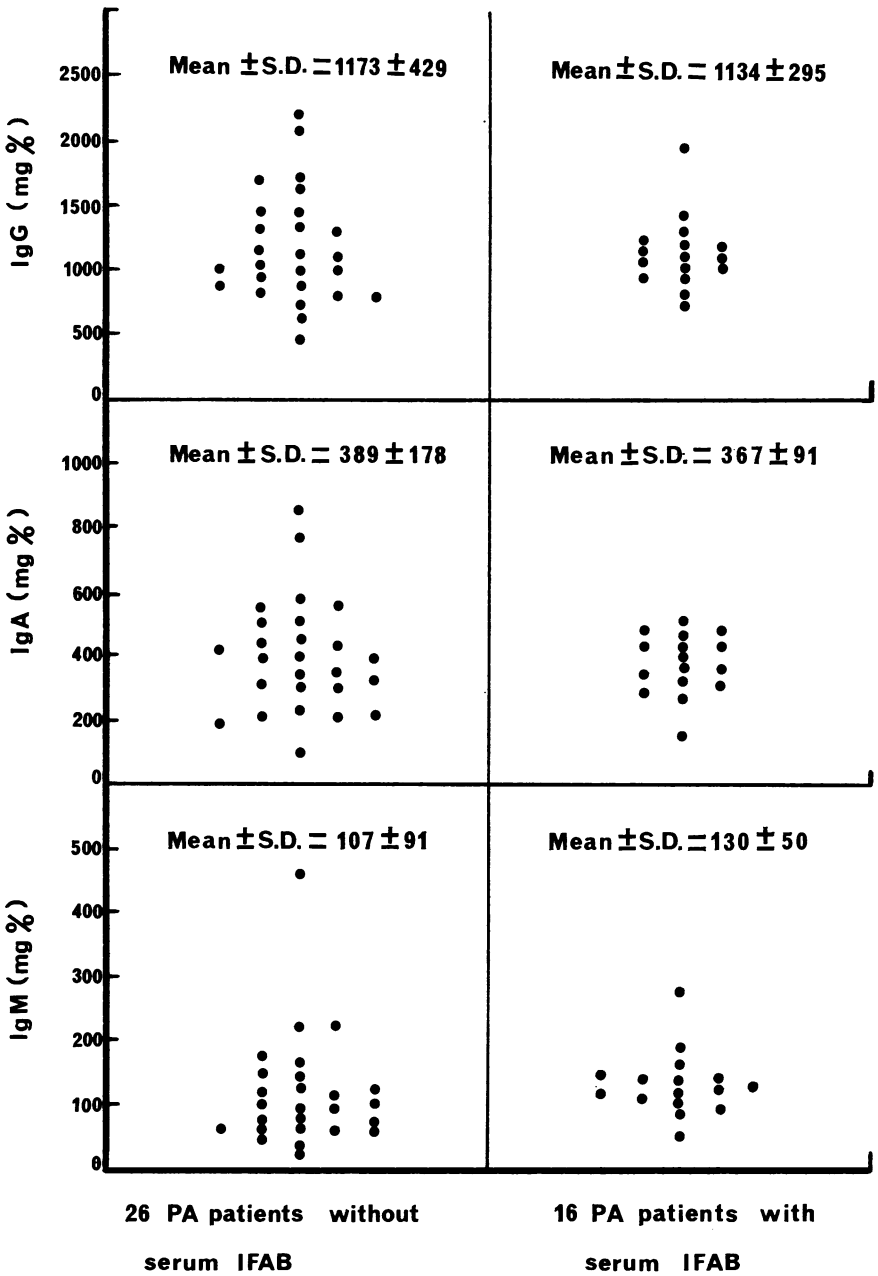

Fig. 1 *Results of the serum immunoglobulin levels from 26 pernicious anaemia (PA) patients without serum intrinsic factor blocking antibody (IFAB) and from 16 PA patients with serum IFAB.*

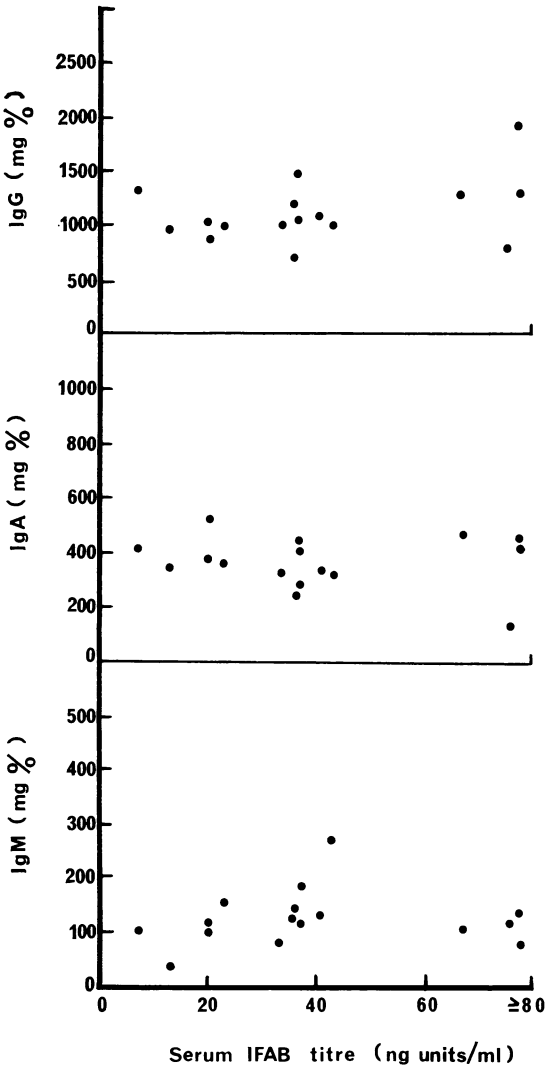

Fig. 2 *Results of the serum immunoglobulin levels and the serum intrinsic factor blocking antibody (IFAB) titre from 16 patients with pernicious anaemia.*

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Technical method

Simultaneous staining of phospholipids, basic proteins, and glycogen on the same slide

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At least three different sections or smears are normally required when staining for glycogen, phospholipids, and basic proteins. The following procedure uses a single section or smear for the identification of the three substances, and demonstrates the site of each of the three components in the same cell. It is also useful when the number of sections or smears is limited.

The procedure is based on our observation that luxol fast blue selectively stains basic proteins in the media used for staining phospholipids according to Lison (1952). This double staining does not interfere with the period acid Schiff (PAS) staining method for polysacharides.

A combined staining procedure for Luxol fast blue and PAS has already been introduced by Shanklin and Nassar (1959).

Materials and Methods

Blood or bone marrow smears, touch preparation (impression smears), and frozen sections.

SOLUTIONS

Sudan black B staining solution

The method is according to Lison (1952).

Luxol fast blue-Sudan black B staining solution

To 100 ml of Sudan black B solution add 0·1 g Luxol fast blue.

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