

IMMUNOGLOBULIN DETERMINATION

EVALUATION OF THE RESULTS OF IMMUNOELECTROPHORETIC ANALYSIS AND THE RADIAL DIFFUSION METHOD

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SUMMARY

A comparative study was made of the results of immunoglobulin determination in sera using immunoelectrophoresis and the radial diffusion method of Mancini. The results indicated that, except for certain ranges of immunoglobulin concentration (low, normal or slightly increased) the data obtained by immunoelectrophoresis are unreliable and do not correlate with the values obtained by the Mancini method.

The immunoelectrophoretic analysis of human serum proteins is widely used in clinical studies¹. Apart from qualitative aspects, estimations on levels of various serum proteins can be made^{2,3}. The semi-quantitative data obtained from the immunoelectrophoretic patterns, however, have to be interpreted carefully, since many factors may influence the results. As, at present, more accurate quantitative methods are available⁴⁻⁶, a comparative study was undertaken to evaluate the results of the estimation on serum immunoglobulin levels in the immunoelectrophoretic pattern compared with the data obtained by the radial diffusion method⁵. The results are presented in this paper and might be of value in choosing the appropriate method in studies on serum proteins.

MATERIAL AND METHODS

Immunoelectrophoresis was carried out on micro-scale⁷ using the LKB-apparatus*, following the manufacturers' instructions. The antisera used were anti-human antiserum of the horse (Central Laboratory of the Blood Transfusion Service, Amsterdam) and antisera specific for IgM, IgG and IgA, prepared in rabbits (full details in ref. 8). The immunoglobulin levels were estimated using the stained immunoelectrophoretic patterns. On each slide the "unknown" — or test serum was evaluated against

* Immunophor—LKB Produkter AB, Stockholm.

a reference serum (see below), made of serum samples from forty healthy adults. In some cases where evaluation was difficult, the analysis was repeated at various dilutions. The results were expressed as normal, slight increase (or slight decrease), moderate increase (or moderate decrease), considerable increase (or considerable decrease), respectively.

The radial diffusion method of Mancini *et al.*⁵ was used for the quantitative analysis of the various immunoglobulins. The determinations were carried out as described by van Munster and Stoelinga⁹. On each immuno-plate (containing 25 antigen reservoirs) five dilutions of a reference serum (the same as used for the immunoelectrophoretic experiments) were included, to plot a standard curve. The test sera were applied to the plate in two dilutions. The plates were allowed to diffuse at room temperature. The diffusion was complete after 48–72 hours. The area of the precipitate was determined by measuring (with a magnifying glass) the diameter (*d*) of the ring. Dilutions of the reference serum were plotted against the diameters squared to obtain the standard curve. The immunoglobulin concentrations of the test sera were expressed in percentage of the reference serum. Absolute IgG, IgA and IgM values were obtained by comparison of the reference serum with several standards, *viz.* a standard serum from Behringwerke, the standard sera supplied with Hyland's immuno-plates and, so far as IgA was concerned, a standard serum of known IgA concentration made available by Dr. P. J. J. van Munster. Results with these different standards were fairly similar. As, for the moment, no international standard is available it was tentatively decided to accept as absolute values of the reference serum: 1002 mg IgG/100 ml, 180 mg IgA/100 ml, 83 mg IgM/100 ml.

RESULTS

The correlation between the estimated serum levels of the various immunoglobulins in immunoelectrophoresis and the data obtained by the Mancini radial diffusion method is presented in Figs. 1–3. Fairly good agreement was obtained for IgA and IgM in the ranges scored as normal, slight, or moderate decrease and in the range designated as slight increase. A distinction between moderate and considerable increase could not be made by immunoelectrophoresis. For IgG, only the estimations scored as considerable and excessive decrease showed reasonable agreement. Values estimated as slight decrease, normal, slight increase, considerable increase and excessive increase varied widely compared with the quantitative data.

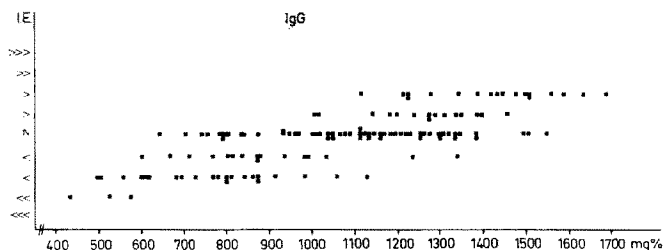


Fig. 1. Correlation of immunoglobulin-G serum levels, estimated by immunoelectrophoresis (ordinate) and determined by radial diffusion (abscissa). The arbitrary units on the ordinate are explained under MATERIAL AND METHODS. The radial diffusion values on the abscissa are expressed in mg/100 ml.

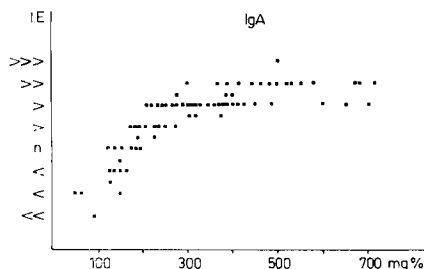


Fig. 2. Correlation of immunoglobulin-A serum levels, estimated by immunoelectrophoresis (ordinate) and determined by radial diffusion (abscissa).

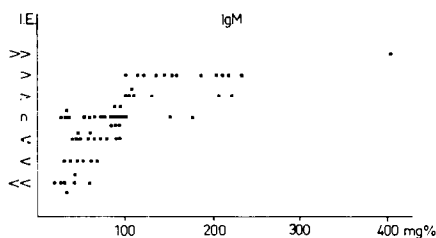


Fig. 3. Correlation of immunoglobulin-M serum levels, estimated by immunoelectrophoresis (ordinate) and determined by radial diffusion (abscissa).

DISCUSSION

From the data presented in this study, it is clear that the estimation of immunoglobulins from the immunoelectrophoretic patterns is unreliable in certain concentration ranges. In particular, high levels of IgG, IgA and IgM are difficult to identify. Concerning IgG, this also holds for levels between "normal" and "moderate increase". In contrast, low serum levels of the various immunoglobulins can be scored correctly.

Three critical remarks should be made in this context, however. First, very low levels of immunoglobulins are not revealed by immunoelectrophoresis and will be interpreted as total lack of immunoglobulins of a certain class. Since the radial diffusion method is more sensitive, low but significant levels can be measured in these cases. This may have clinical significance in studies on various types of immunological defects. Second, estimations of serum immunoglobulin levels by immunoelectrophoreses are usually made using a reference serum obtained from healthy adults. Since it is known that serum immunoglobulin concentrations in children are lower than in adults, a correct evaluation of immunoglobulin levels in young children can only be obtained by comparison with normal age-mates⁸. In all cases, however, the interpretation of the values obtained is difficult due to the great variation in immunoglobulin concentrations in a normal healthy population. This, of course, holds also for the radial diffusion method. Finally, it must be emphasized that using the radial diffusion method, important qualitative aberrations in the immunoglobulin pattern may remain unrecognized. This is particularly true for cases of restricted heterogeneity of IgG in certain hypogammaglobulinaemic patients¹⁰ and for the presence of monoclonal immunoglobulins and Bence Jones proteins.

In conclusion, it can be stated that the estimation of immunoglobulin levels by immunoelectrophoresis gives inaccurate results, except in certain concentration ranges. Nevertheless, the technique is most helpful for studying the qualitative aspects of the immunoglobulin pattern, and in our opinion, it is indispensable for studies on serum proteins.

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