COLD HAEMAGGLUTINATION IN PRIMARY ATYPICAL PNEUMONIA

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Cold haemagglutination has been the subject of investigation by many workers, and has been shown to occur in healthy human beings as well as in many animals. It has also been demonstrated in association with many diseases, usually to a much higher titre than in normal, healthy people. Li-Chen-Pien[1] described it in a patient suffering from hypertrophic cirrhosis of the liver. It has been found in association with leukaemia by Alexander and Thompson,[2] Boxwell and Bigger,[3] and Manheims and Brunner.[4] Dameshek[5] mentioned its association with infectious mononucleosis, and Stats and Wasserman[6] and Muschel[7] described its occurrence in pregnancy. Increased titres of cold haemagglutinins, however, have been found consistently only in trypanosomiasis (York[8]) and in primary atypical pneumonia.

Clough and Richter,[9] in 1918, were the first to describe a raised cold haemagglutinin titre, in association with a condition which would now be diagnosed as primary atypical pneumonia. They did not associate this serological abnormality with the respiratory condition, and thought it to be coincidental. Since 1943, however, numerous references have been made in the literature to this association between raised cold-haemagglutinin titres and primary atypical pneumonia. Turner *et al.*[10] investigated 83 cases of primary atypical pneumonia. Of these, 44 (53%) had titres of at least 32, and 23 patients had titres of 128 or higher. In their control series, consisting of 132 patients suffering from various other diseases, in only 5 (3·7%) did the titre exceed 16. Horstmann and Tatlock[11] investigated 43 cases and found an incidence of 67·5% of significantly raised cold-haemagglutinin titres. Peterson *et al.*[12] mention that the great majority of the patients with primary atypical pneumonia which they had encountered showed cold haemagglutinins in dilutions of serum ranging from 1/10 to 1/10,000. Young,[13] in studying a large series of cases, reported an incidence of over 51% in which the titre was 128 or higher, whilst his normal controls and other respiratory diseases showed an incidence of only 0·7%. Finland and Barnes[14] investigated 676 patients suffering from a wide variety of acute respiratory infections. They came to the following conclusions: (1) The great majority of uncomplicated cases of primary atypical pneumonia develop significantly raised cold-haemagglutinin titres; (2) the demonstration of high-titre cold haemagglutinins is almost always associated with primary atypical pneumonia, although the finding of this disease was sometimes obscured by complicating conditions; (3) patients with acute respiratory infections other than primary atypical pneumonia fail to develop high-titre cold haemagglutinins; (4) the demonstration of high-titre cold haemagglutinins is of considerable value in the diagnosis of primary atypical pneumonia.

The spate of articles describing the occurrence of high-titre cold haemagglutinins in primary atypical pneumonia appearing in the medical press in 1943 and at sporadic intervals afterwards originated mainly from the USA. In virtually every article the significant incidence of high-titre cold haemagglutination was stressed. It has, in fact, become customary for cold-agglutinin investigations to be carried out as an aid to diagnosis in cases of atypical respiratory infections.

The demonstration of high-titre cold haemagglutinins in primary atypical pneumonia, or any respiratory infection for that matter, is a rare occurrence in the experience of our laboratories during the past 11 years, and it is probable that physicians in this country have been struck by the monotonous regularity with which such investigations have given negative results. The investigations described in this paper were carried out in order to confirm these findings.

The differentiation between 'normal' cold haemagglutinins present in many normal, healthy people, and 'abnormal' cold haemagglutinins associated with disease, depends to a large extent on the concentration in which the cold haemagglutinins are present in the serum. The exact dividing line between

'normal' and 'abnormal' cold haemagglutinins is debatable, although a titre of 16 is the most generally accepted upper limit of the normal range. This figure is questionable; many workers consider the upper limit of normal to be 128, and titres as high as 500 have been described in apparently normal, healthy people (Kyuhei Nakadate[15]). The confusion is increased by variations in technique. It is probable that before definite pathological significance can be attached to cold haemagglutinins the titre would have to be well in excess of 16, but for purposes of this investigation the latter figure was accepted as the upper limit of the normal range.

Cold-haemagglutinin technique:

A clotted specimen of the patient's blood is incubated at 37°C for 2 hours to allow elution of cold haemagglutinins to take place. The serum is separated immediately after the specimen has been removed from the incubator. Twofold serial dilutions of the serum in saline are prepared, and a drop of each dilution is added to each of two tubes. One drop of a washed 2% suspension of the patient's cells in isotonic saline is added to each tube in one row of serum dilutions, and one drop of freshly prepared, washed 2% suspension of group O cells in isotonic saline is added to each tube in the other row. The cells and diluted serum are mixed, and then refrigerated at 4°C for 12-18 hours. The results are read macroscopically, and scored from ++++(solid agglutinate) to +(visible granularity). The highest titre at which visible granularity can be detected is taken as the end-point of the titration.

The investigation consisted of a 160 patients tested for cold haemagglutinins (Table I). Of these patients 27 had been diagnosed clinically as virus or primary atypical pneumonia, 33 as pulmonary tuberculosis, 12 as some other pulmonary infection (lobar pneumonia or bronchopneumonia), and 88 as suffering from various non-respiratory conditions. It is apparent from an analysis of these figures that the incidence of high-titre cold haemagglutinins in primary atypical pneumonia (7.6%) is insignificant when compared with figures quoted by American authors, in which the incidence ranged from 51% to 67.5%. The incidence compares more

TABLE I. 160 PATIENTS TESTED FOR COLD HAEMAGGLUTININS

	Primary atypical pneumonia	*Pulmonary tuberculosis*	*Other respiratory conditions*	*Various non-respiratory diseases*
No. of cases investigated ..	27	33	12	88
No. in which cold haemagglutinin titre exceeded 16	2	1	0	2
Incidence	7·6%	3%	0%	2·3%

favourably with that of their control series, consisting of various respiratory infections other than primary atypical pneumonia. The records of cold-haemagglutinin investigations carried out at the Blood-Group Research Laboratories from 1951 to 1954 were also analysed (Table II). These results are in close agreement with those of Table I. The final analysis is therefore based on 590 investigations comprising 79 cases of primary atypical pneumonia, 33 cases of pulmonary tuberculosis, 33 cases of other types of respiratory infections, and 445 patients suffering from various non-respiratory diseases (Table III). The most important point which emerges from an analysis of these findings is that the incidence of

TABLE II. ROUTINE INVESTIGATIONS CARRIED OUT AT THE BLOOD-GROUP RESEARCH LABORATORIES 1951–54

	Primary atypical pneumonia	*Pulmonary tuberculosis*	*Other respiratory conditions*	*Various non-respiratory diseases*
No. of cases investigated ..	52	0	21	357
No. in which cold haemagglutinin titre exceeded 16	0	—	2	1
Incidence	0%	—	8%	0·28%

high-titre cold haemagglutinins in primary atypical pneumonia (2.6%) is low, being similar to the incidence in other types of respiratory infections (4.5%), in contrast to the highly significant incidence described by American workers. It would appear therefore, that the presence or absence of high-

TABLE III. FINAL ANALYSIS—588 INVESTIGATIONS

	Primary atypical pneumonia	*Pulmonary tuberculosis*	*Other respiratory conditions*	*Various non-respiratory diseases*
No. of cases investigated ..	79	33	33	445
No. in which cold haemagglutinin titre exceeded 16	2	1	2	3
Incidence	2·6%	3%	6%	0·67%

titre cold haemagglutinins is of no value in diagnosing the nature of a respiratory infection. These remarks are certainly applicable to the Witwatersrand area, from which most of the specimens used in the investigation originated, and presumably also to the rest of Southern Africa.

It should be pointed out that the specimens used in this investigation were submitted to our laboratory for routine investigation, and originated from many different sources. It is certain that the diagnostic criteria used in the diagnosis of primary atypical pneumonia, being the enigma that it is, were not uniform, and that in some instances the condition did not conform to the clinical entity described by American workers. Some of the patients probably suffered from Q fever or one of the lesser known diseases which may simulate virus pneumonia. As the analysis, however, included all specimens investigated by our laboratory since 1949, one cannot but come to the conclusion that either the incidence of virus pneumonia is not significant in this country or, if it is, then the disease process must in some way differ from that occurring in the United States.

Macaulay[16] considers cold haemagglutination to be a non-specific response to lung-tissue destruction, occurring in all types of lung disease, although most commonly in primary atypical pneumonia. In the light of the above findings it seems much more likely that the development of high-titre cold haemagglutinins can be correlated with the aetiological agent, presumably a virus[17] different strains of which would

be found in various geographical regions, and only some of which are capable of stimulating the formation of cold haemagglutinins.

SUMMARY

An analysis of cold-haemagglutinin investigations carried out in 79 patients suffering from primary atypical pneumonia, 66 patients suffering from other respiratory conditions, and 445 patients suffering from various non-respiratory diseases, show that there is no significant difference in the incidence of high-titre cold-haemagglutinins in primary atypical pneumonia and other respiratory infections and that, in South Africa, the demonstration of the presence or absence of high-titre cold haemagglutination is of no value in diagnosing the nature of a respiratory infection.

The mechanism of cold haemagglutinin formation is briefly discussed.

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REFERENCES

1. Li-Chen-Pien (1926): J. Immunol., **11,** 297.
2. Alexander, H. L. and Thompson, L. D. (1925): J. Amer. Med. Assoc., **85,** 1707.
3. Boxwell, W. and Bigger, J. W. (1931): J. Path. Bact., **34,** 407.
4. Manheims, P. J. and Brunner, E. K. (1933): J. Amer. Med. Assoc., **101,** 207.
5. Dameshek, W. (1943): *Ibid.*, **123,** 77.
6. Stats, D. and Wasserman, L. R. (1943): Medicine, **22,** 363.
7. Muschel, L. H., Nolte, L. B. and Keiderling, L. J. (1957): Amer. J. Clin. Path., **27,** 42.
8. York, W. (1910): Ann. Trop. Med. Parasit., **4,** 529.
9. Clough, M. C. and Richter, I. M. (1918): Johns Hopk. Hosp. Bull., **29,** 86.
10. Turner, J. C., Jackson, E. B., Nisnewitz, S. and Berney, R. (1943): Lancet, **1,** 765.
11. Horstmann, D. M. and Tatlock, H. (1943): J. Amer. Med. Assoc., **122,** 369.
12. Peterson, O. L., Ham, T. H. and Finland, M. (1943): Science, **97,** 167.
13. Young, L. E. (1946): Amer. J. Med. Sci., **211,** 23.
14. Finland, M. and Barnes, M. W. (1951): *Ibid.*, **221,** 152.
15. Kyuhei Nakadate (1956): Keio J. Med., **5,** 51.
16. Macaulay, D. (1951): Arch. Dis. Childh., **26,** 601.
17. Editorial (1956): Lancet, **2,** 180.