J Clin Pathol 1981;34:396-399

Nephelometry *v* differential agglutination titre in the measurement of rheumatoid factors

MH PRITCHARD AND K JOBBINS

From the Departments of Rheumatology and Haematology, University Hospital of Wales, Cardiff CF4 4XW, UK

SUMMARY IgM rheumatoid factors have been measured in 92 patients by both laser nephelometry and the traditional hospital procedure of a latex slide test followed by differential agglutination titre (DAT). Of the results 86% were in accordance by both methods, but seronegative patients with a high nephelometry score all showed, after reference to their clinical state and previous investigations, that the nephelometry result more accurately represented their clinical state than the DAT, which was shown to be in error in five out of six cases. Six seropositive patients with low nephelometry scores were also studied; three were in complete remission on gold or penicillamine therapy. Nephelometry is both technically simpler and more reliable than the traditional DAT method, and our results suggest that it has some advantages over the DAT in routine use.

The observation that sera from rheumatoid arthritic patients were able to aggregate sensitised sheep cells was made independently by Waaler in 1940[1] and by Rose in 1948.[2] It was only after the latter publication, however, that serious interest was shown in this observation as a possible diagnostic test for patients with rheumatoid arthritis. The test is based on the agglutination reaction between particles coated with gammaglobulin and IgM class autoantibodies (rheumatoid factors) present in the serum of the rheumatoid patient, and as the reaction may take place at high dilution the test is usually carried out in the laboratory by doubly diluting the serum until no further agglutination takes place. This dilution is then reported as the agglutination titre.

Numerous variations have been made to the basic procedure in the last 30 years but this basic principle has remained the same. The main variants of the test involve either sheep red blood cells coated with rabbit IgG (differential agglutination titre or sensitised sheep cell test—DAT or SCAT), tanned sheep red cells coated with human Cohn fraction II gammaglobulin (FII), or latex particles coated with human gammaglobulin. The main differences in the tests in practice are in their sensitivities. Zutshi *et al.*[3] compared the three tests and found that the DAT/SCAT test was the least sensitive but did not produce any false-positive results in normal controls while the FII test gave the most positive results in the rheumatoid arthritis population, but 11% of normals were also positive.

A new approach to the measurement of rheumatoid factors is nephelometry, a technique that has many laboratory applications in the routine measurement of proteins.[4 5] The principle of this process is based on the fact that an antibody-antigen complex in a beam of light will scatter the light in proportion to the concentration of the complex present. The procedure therefore is to establish a baseline for each individual sample by placing the sample in the beam of light and then adding a known amount of specific antibody for the protein in question, allowing it to incubate for 20 minutes. The sample is then placed in the beam of light again, and the difference in light scattering before and after adding the antibody is recorded on the machine in arbitrary units and is proportional to the amount of antibody-antigen complex present. For this study the IgM rheumatoid factor in the patient's serum is reacted with human heat-aggregated IgG as supplied by the manufacturers (LAS-R rheumatoid factor antigen (human)).

We describe a comparative study on 92 patients from the rheumatology clinic at the University Hospital of Wales whose sera were measured for rheumatoid factor by the routine latex screening test plus DAT and, secondly, by nephelometry. The laser nephelometer was kindly loaned by Travenol Laboratories—Model No. PDQ (semi-automated).

Accepted for publication 30 September 1980

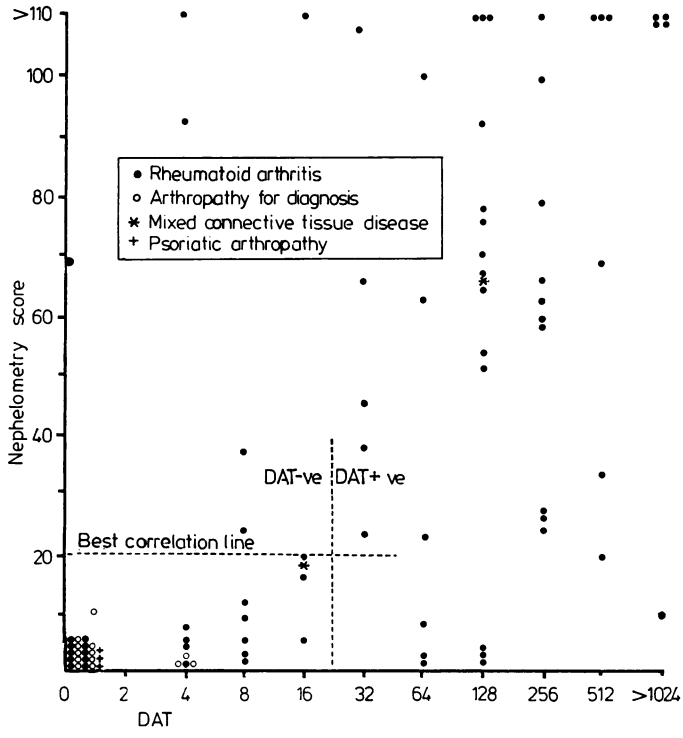
J Clin Pathol: first published as 10.1136/jcp.34.4.396 on 1 April 1981. Downloaded from http://jcp.bmj.com/ on November 30, 2022 by guest. Protected by copyright.

Material and methods

A total of 92 patients attending the rheumatology clinic with a variety of arthritic complaints were selected at random, and venous blood was aspirated in a routine manner into a plastic syringe; 10 ml was then sent to the laboratory in a plastic tube for latex and DAT tests while a further 10 ml was retained in the rheumatology department. This was allowed to clot for 1 hour at room temperature and was then centrifuged. The serum was taken off, decanted into a fresh container, relabelled, and frozen at −20°C. When approximately 35 samples had been collected in this way they were transported without defrosting to the haematology laboratory where they were subsequently tested on the nephelometer. The samples, after being renumbered, were tested under blind conditions, and after each batch had been measured the results were plotted on a graph of DAT titre *v* nephelometry score. In order to test the variability of the nephelometry method, blood was taken from 21 inpatients with rheumatoid arthritis. Each sample was divided into two, and the batch of specimens was sent to the laboratory as described above after randomising and renumbering. The duplicate specimens were tested in a single batch.

Results

The results of the study are shown in the Figure, which plots the DAT measured in dilutions on the horizontal axis and the nephelometer results measured in arbitrary units on the vertical axis. A correlation coefficient between the two has not been calculated because the DAT titre, being measured by double dilutions, is non-linear. The object of the graph, therefore, is to ascertain whether seropositive patients (DAT > 1:32) also had a high nephelometry score and seronegative patients a low score. Seropositive and seronegative results were differentiated on the graph by a vertical line, but as there is no figure in current use dividing significant nephelometry scores from non-significant ones this has been calculated in the first instance to give the best agreement with the DAT results. This line has been drawn at a nephelometry score of 20 units, and patients are therefore considered to be 'nephelometry +ve' or 'nephelometry −ve'. If the graph is considered to be divided into four areas by these two lines it can be seen that 80 out of 92 results are either positive by both methods or negative by both methods. Of the 12 discordant results, six had a high nephelometry score but were seronegative, and six



DAT (1/x) in dilutions against nephelometry score in arbitrary units.

J Clin Pathol: first published as 10.1136/jcp.34.4.396 on 1 April 1981. Downloaded from http://jcp.bmj.com/ on November 30, 2022 by guest. Protected by copyright.

were seropositive but had a low nephelometry score. Thus 86% of the samples tested gave the same results by both methods, an error of 14%.

The discordant results were then investigated in more detail to see if it could be deduced which method was more accurately reflecting the patient's clinical state. The clinical and other biochemical data on the six seronegative patients with a high nephelometry score are shown in Table 1, and the six seropositive patients with a low nephelometry score are shown in Table 2. All patients in both groups had erosive rheumatoid arthritis. In Table 1 it can be seen that five out of the six patients were strongly seropositive on previous measurements. The sixth patient was always seronegative. This, when taken with the raised ESR and the clinical details in the case notes, suggested that every one of the six patients had active rheumatoid disease clinically and biochemically. It is clear, therefore, that in a single sample situation the nephelometry score accurately reflected the patient's clinical status in each case, and that in five out of six of the cases the DAT result was neither in accordance with the patient's clinical state nor with readings taken on previous occasions. In the sixth patient the previous DAT results had always been negative, but clinically the patient had an erosive arthropathy. In the second group of patients, who were seropositive with a low nephelometry score, the same analysis was made. Table 2 shows that three of the six patients were in full remission—both clinically and biochemically—at the time of the test, after treatment with either sodium aurothiomalate (Myocrisin) or penicillamine for at least one year. Two other patients had had rheumatoid arthritis for more than 30 years, and the clinical picture was complicated by widespread secondary degenerative disease and other medical conditions. An accurate clinical assessment was therefore difficult. The last patient had had a recent flare-up in the rheumatoid disease and previously low rheumatoid factor titres, and the nephelometry score of 19 units was only just below the arbitrary calculated cut-off level.

In order to establish the reproducibility of this technique the coefficient of variation was calculated from the differences between 21 duplicate readings measured in one batch. The samples taken covered the whole expected range of results from 0 to 134 units. The results gave a standard deviation of 2·8 and a coefficient of variation of 5·4%. Although repeated batches have not been tested, this coefficient of variation calculated on the basis of a single batch shows that the reproducibility of the method is extremely high.

Discussion

Seropositive rheumatoid arthritic patients who have been successfully treated with gold or penicillamine and who have gone into clinical remission frequently drop their rheumatoid factor titres to a low level or even lose them altogether.[6] Some of the groups of patients who have zero nephelometry and negative DATs on the graph (Figure) are in this group and have become seronegative on this treatment. It is very interesting, therefore, that three of the low nephelometry/seropositive patients should have been in this clinical category, suggesting that the nephelometry score is more sensitive than the DAT in this situation. It could be speculated that this sensitivity is related to the fact that the nephelometry method uses human IgG as a substrate whereas the DAT uses rabbit IgG.

Measurements of rheumatoid factors are an

Table 1 *Seronegative patients with high nephelometry score: clinical and biochemical details*

Patient	*ESR*	*Nephelometry score*	*Present DAT 1/x*	*Previous DATs 1/x*	*Clinical state*
Ha	109	108	32	128, 512, 256	Progressive disease
Hu	44	93	4	128	
Bu	53	66	– ve	– ve	
Ki	28	37	8	1024, 128	
Mu	112	132	4	256, 128	
Da	30	24	8	– ve, 128	Mild activity only

Table 2 *Seropositive patients with low nephelometry score: clinical and biochemical details*

Patient	*ESR*	*Nephelometry score*	*Present DAT 1/x*	*Previous DATs 1/x*	*Clinical state*
Mo	12	0	128	n a	Complete remission
Mi	13	3·3	128	1024, 512, 256	
No	5	8·2	64	128	
Pa	47	2·5	64	n a	Burnt-out R A
Br	40	2·9	128	256	
Po	33	19	512	32, 64	Recent flare-up

J Clin Pathol: first published as 10.1136/jcp.34.4.396 on 1 April 1981. Downloaded from http://jcp.bmj.com/ on November 30, 2022 by guest. Protected by copyright.

essential part of a rheumatology clinic, and it is helpful in clinical practice to be able to do this test on a routine basis. As it is a basic screening test it is important to have the results reasonably quickly and for the tests to be reliable. Another factor to be considered in a routine test is the time taken for it to be carried out. It was calculated during the survey that it took one technician a total of 5 hours spread over two and a half days to set up and read the DAT measurements. The same technician took 2½ hours in one afternoon to set up and calibrate the nephelometer and to read the average weekly load of 35 samples. Thus nephelometry can save 2½ hours of technical time per week. Reading the results of the DAT is subject to substantial intra and inter observer errors,[7] and it is current practice in this laboratory for two observers to read the results and to average their interpretations. There is a high degree of variability with time in DAT readings in any case, as this survey has shown, five results being reported as negative in patients with active rheumatoid disease and previously high DAT readings. The reproducibility of the nephelometry, on the other hand, is extremely high, the largely automated results eliminating one of the major errors of the DAT technique.

Routine rheumatoid factor measurements have been in use now for 30 years and they ought perhaps to be able to provide more information than simply a screening test for rheumatoid disease. This can in any case be carried out as a latex slide test (Hyland, Rheumaton), a result being obtained in less than 2 minutes. The sensitivity of the Rheumaton test is very close to that of the conventional DAT,[7] and common laboratory practice is to use this as a screening test and the more laborious DAT only on those patients who gave a positive result. The slide test, however, gives only a positive or negative result, and no more information is available from it.

Attempts have been made to correlate the DAT titre with prognosis or severity of the rheumatoid disease, but Lloyd *et al.*[7] could not detect any significant correlation with any other parameters in rheumatoid patients. Although the unreliability of the test must be a major factor in this, the cut-off point of 1/32, below which the test is not considered to be significantly positive, results in a number of patients with rheumatoid arthritis by all other criteria being considered seronegative. This often gives the unwary clinician a false sense of security, whereas in practice such patients are frequently as liable to erosive disease as those who are strongly seropositive.

The figure of 1/32 has been reached on epidemiological grounds,[8 9] and the result in practical terms is that there is a significant overlap between the rheumatoid population and the normal population in the low DAT titre range. It is not yet clear whether there is a similar overlap in the nephelometry scores, but the results shown in the Figure show that the nephelometry significance point could be reduced from 20 to 10 units and still discriminate accurately between the rheumatoid and the non-rheumatoid population. Further studies are in progress to establish exactly where this cut-off point should be.

References

1 Waaler E. Occurrence of factors in human serum activating specific agglutination of sheep blood corpuscles. *Acta Path et Microbiol Scand* 1940;**17**:172-88.

2 Rose HM, Ragan C, Pearce E, Lipman MO. Differential agglutination of normal and sensitised sheep erythrocytes by sera of patients with rheumatoid arthritis. *Proc Soc Exp Biol Med* 1948;**68**:1-6.

3 Zutshi DW, Reading CA, Epstein WV, Ansell BM, Holborow EJ. FII haemagglutination test for serum antigammaglobulin in arthritides sero-positive and sero-negative by other tests. *Ann Rheum Dis* 1969;**28**:289-99.

4 Deaton CD, Maxwell KW, Smith RS, Reveling RL. Use of laser nephelometry in the measurement of serum proteins. *Clin Chem* 1976;**22/9**:1465-71.

5 Finley PR, Hicks J, Williams RJ, Hinlicky J, Lichti DA. Rate nephelometric measurement of rheumatoid factor in serum. *Clin Chem* 1979;**25**:1909-14.

6 Pritchard MH, Nuki G. Gold and penicillamine: a proposed mode of action in rheumatoid arthritis based on synovial fluid analysis. *Ann Rheum Dis* 1978;**37**:493-503.

7 Lloyd KN, Williams PI, Williams M. Reproducibility and sensitivity of serological rheumatoid tests. XIV International Congress of Rheumatology, San Francisco. Abstract 16. 1977.

8 Kellgren JH, Ball J. Clinical significance of the rheumatoid serum factor. *Br Med J* 1959;**i**:523-31.

9 Ball J, Lawrence JS. Epidemiology of the sheep cell agglutination test. *Ann Rheum Dis* 1961;**20**:235-43.

Requests for reprints to: Dr MH Pritchard, Department of Rheumatology, University Hospital of Wales, Cardiff CF4 4XW.

J Clin Pathol: first published as 10.1136/jcp.34.4.396 on 1 April 1981. Downloaded from http://jcp.bmj.com/ on November 30, 2022 by guest. Protected by copyright.