An evaluation of nephelometry and complement-based tests for the detection of circulating immune complexes

L. IZZARD, H. A. VAN WYK, N. POTGIETER, S. BRIGHTON, R. ANDERSON

Summary

Sera from 20 normal adult control subjects and 28 patients suffering from various diseases which may be associated with an immune complex disorder were investigated, using three different techniques for detection of circulating immune complexes (CICs). The sera from the patients were assigned expected positive or negative ratings by the clinicians according to clinical and laboratory criteria. This information as well as the diagnoses was withheld until the results of immune complex determinations were available. The three tests used to detect CICs were laser nephelometry (LN), ^{125}I-C1q binding and measurement of the C3 breakdown product C3c. Serum levels of the complement components C3 and C4 were assessed on the serum specimens from the patients. Results obtained from normal control sera showed that 18 of the 20 and all 20 were negative with the C1q binding technique and LN respectively. Of 16 sera for which a positive result was expected, 5 (31,3%) and 14 (87%) were positive when examined by the C1q binding technique and LN respectively; C3c determination produced no positive results. No false-negative results were obtained with the C1q binding and C3c tests, but 2 out of 16 (13%) results obtained with the LN test were false negative. LN is a rapid, sensitive test for the detection of CICs.

S Afr Med J 1983; **63:** 244-246.

Immune complexes have been implicated in the pathogenesis of a variety of disorders including infections, auto-immune diseases and malignant tumours.[1] The presence of circulating immune complexes (CICs) often correlates with the stage and prognosis of the disease and may be of value in monitoring the course and activity of some diseases. The requirement for accurate, reliable techniques for the detection of CICs is therefore well recognized and has been the subject of much developmental research. However, the techniques for immune complex detection in serum specimens available at present are extremely complex. They are time-consuming and require considerable technical skill, and are therefore unsuitable as routine procedures in most laboratories. However, recent reports have indicated that laser nephelometry (LN) may be a rapid, inexpensive and reproducible technique for the measurement of CICs.[2,3] In this study we compared three different methods, LN, ^{125}I-C1q binding, and measurement of circulating C3c, for the detection of immune complexes in the sera of patients with various diseases which may be associated with an immune complex disorder.

Immunology Section, Department of Medical Microbiology, Institute of Pathology, University of Pretoria
L. IZZARD, DIP. MED. LAB. TECH.
H. A. VAN WYK, B.SC.
R. ANDERSON, PH.D.
Renal and Rheumatology Units, Department of Internal Medicine, H. F. Verwoerd Hospital, Pretoria
N. POTGIETER, M.B. CH.B., M.MED. (INT.)
S. BRIGHTON, M.B. CH.B., M.MED. (PHYS. MED.), F.F. PHYS. MED.

Date received: 19 March 1982.

Material and methods

Patients

Serum specimens were obtained from 28 patients: 11 had renal disease (2 nephrotic syndrome, 2 post-streptococcal glomerulonephritis, 1 minimal-change glomerulonephritis, 1 minimal-change glomerulonephritis with nephrotic syndrome, 1 analgesic nephropathy, 1 acute proliferative glomerulonephritis, 1 glomerulonephritis with vasculitis, 1 glomerulitis and nephrotic syndrome and 1 end-stage renal failure), 8 had systemic lupus erythematosus (SLE), 3 had rheumatoid arthritis, 1 had Sjögren's syndrome, 2 had *Yersinia* arthritis (both HLA B27 positive), 1 had juvenile polyarthritis, 1 had Hashimoto's disease and 1 had allergic granulomatous angiitis. The patients had various degrees of disease activity according to clinical and laboratory criteria (ESR, acute-phase reactant tests, presence of auto-antibodies, immunoglobulin levels, biopsies), and the sera were assigned expected positive or expected negative ratings by the clinicians. Neither this information nor the diagnosis was revealed until the results of the immune complex determinations had become available. These results were then correlated with the clinical findings. Blood specimens were delivered to the laboratory within 30 minutes of being taken. The sera were separated and stored at −70°C before measurement of the immune complexes.

Serum specimens were also taken from 20 normal adult volunteers and used as negative controls.

Immune complex determinations

Laser nephelometry. Sera were diluted 1 in 10 with 0,15M saline and tested for spontaneous light scattering in a helium-neon laser nephelometer (Behring). Light scattering was expressed as volts displayed on a digital meter. Sera, in 0,2 ml volumes, were transferred into cuvettes of low background reading (LN cuvettes, Behring) and measured for turbidity causing scattering of the laser beam. Standard curves were constructed using IgG aggregates. Human IgG was obtained from Pel-Freez Biologicals, Rogers, Ark., USA, and reconstituted to 10 mg IgG per millilitre. Aggregation was accomplished by heating at 63°C for 20 minutes. This preparation was used to construct a standard curve in the range 0,05 - 5 mg aggregated IgG per millilitre. A standard curve was constructed for each series of determinations. Nephelometric readings are expressed in volts.

^{125}I-Clq binding. The binding of ^{125}I-Clq to immune complexes was determined by radio-immunoprecipitation with 3% polyethylene glycol (PEG) as previously described.[4] ^{125}I-Clq was obtained from Lodge Diagnostics, Hampton-on-the-Hill, Warwickshire, UK. Positive controls were constructed using aggregated IgG. Results were considered positive if 10% or more of the added ^{125}I-Clq was present in the PEG precipitate.

Detection of C3c in serum. Blood for these investigations was collected into tubes containing the protease inhibitor Trasylol at 200 Krein inactivator units per millilitre to prevent spontaneous breakdown of C3. C3c was measured by a crossed-immuno-electrophoresis technique as previously described.[5] C3c was separated from C3 by electrophoresis for 2 hours at 20 V in 1% agarose using a tris/glycine/barbitol buffer, pH 8,6. The separated components were then re-electrophoresed into 1% agarose containing 50 μl antisera to human C3 (Behring) under the same conditions of electrophoresis.

Measurement of serum C3 and C4 levels. Serum C3 and C4 concentrations were measured using a rocket immuno-electrophoresis technique as previously described.[6] Results are expressed as milligrams per millilitre of serum.

Results

Expected results. According to the assessments of the clinicians 16 of the 28 serum specimens from the patients were expected to be positive for CICs and 12 negative (Table I).

LN. The mean value ($\pm$ SE) obtained with normal control sera was 0,6 $\pm$ 0,12 V. The upper limit for negative sera was arbitrarily set at double this mean value, i.e. 1,3 V. All control sera gave readings of $<$ 1,3 V. Of 16 serum samples from the patients for which a positive result was expected, 14 were positive for CICs (Table I) and 2 were negative. Of these 2 'false-negative' results 1 was probably a true false negative because the clinical diagnosis of glomerulonephritis with nephrotic syndrome was accompanied by a biopsy report documenting acute exudative proliferative glomerulonephritis with deposition of IgM and IgG. The second false-negative result was also obtained on serum from a patient with acute proliferative glomerulonephritis in whom biopsy had revealed IgM, IgG and C4 deposition. However, the LN reading for this serum specimen was 1,2 V, which is higher than any of the values obtained for control sera. Of the 12 sera for which a negative result was expected, 2 gave positive results. One of the positive results was obtained on serum from a patient with minimally active SLE and was considered a truly false-positive result. However, the other apparent false-positive result obtained on serum from a patient with minimal-change glomerulonephritis with nephrotic syndrome was probably truly positive, because the high LN reading correlated with low serum C3 and C4 values. A typical standard curve obtained with varying concentrations of heat-aggregated IgG is shown in Fig. 1.

^{125}I-Clq binding. Of the 20 control sera, 2 were found to have ^{125}I-Clq binding values of $\geqslant$ 10% (15% and 20%). Only 5 of the 16 samples from the patients for which a positive result was expected were actually positive. In all 5 cases LN also revealed the presence of CICs. No false-positive results for the sera for which a negative result was expected were obtained (Table I).

Serum C3c concentrations. This test produced no positive results, indicating its relative insensitivity.

Serum C3 and C4 levels. One patient with *Yersinia* arthritis had elevated C3 and C4 levels, but as expected no test produced positive results for CICs. Four patients had decreased C3 and C4 levels which correlated with disease activity and positive results on LN (Table I).

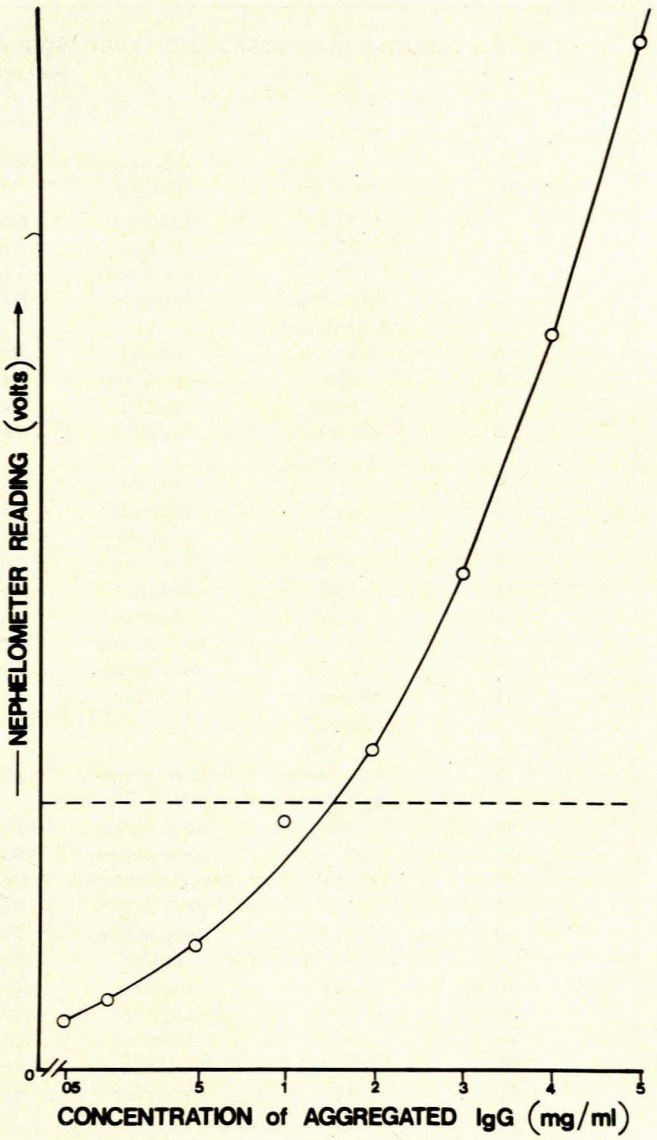

Fig. 1. The effects of increasing concentrations of heat-aggregated IgG on light scattering (measured in volts) using a helium-neon laser nephelometer.

Discussion

The results of this study confirm previous reports that LN is a rapid, useful test for the detection of CICs in serum specimens.[2] Although 2 out of 12 results were classified as falsely positive according to the clinical evaluation, it is likely that the true figure is 1 out of 12 (8%) since the other apparent false-positive result was obtained on serum from a patient with minimal-change glomerulonephritis and nephrotic syndrome; low serum C3 and C4 levels correlated with a positive result on LN. These results indicate systemic activity of the disease, and the LN result can probably be considered truly positive. Of the 16 sera for which a positive result was expected 14 (87%) were found to be positive on LN, leaving 2 results which were apparently falsely negative; 1 of these samples had an LN reading of 1,2 V, which could be considered as bordering on positive.

The ^{125}I-C1q binding technique produced a positive result in only 5 (31,3%) of the 16 cases in which a positive result was expected. These 5 positive sera also gave positive LN readings. Two false-positive results were obtained with the control sera and none with the sera from the patients for which a negative result was expected. This apparent insensitivity of the ^{125}I-Clq binding test is surprising. However, our findings are in agreement with those described in a recent report by Füst *et al.*,[7] who found that tests based on binding of complement components to

TABLE I. CLINICAL DIAGNOSES AND EXPECTED AND OBSERVED RESULTS OF CIC DETERMINATIONS ON PATIENT SERA

Patient No.	Diagnosis	Disease activity	CIC determination: Expected result	CIC determination: Observed result LN* (V)	CIC determination: Observed result ^{125}I-C1q binding† (%)	Serum C3 and C4 levels (mg/ml): C3	Serum C3 and C4 levels (mg/ml): C4
1	SLE	Inactive	Negative	1,2	7	61	16
2	PSG	Active	Positive	*2,5*	9	59	47
3	RA	Very active	Positive	*4,0*	*11*	53	15
4	End-stage renal failure	Inactive	Negative	0,4	5	54	25
5	RA + G	Active	Positive	*1,9*	8	62	18
6	SS	Very active	Positive	*2,8*	*14*	56	19
7	APG	Active	Positive	1,2	3	57	20
8	Minimal-change G	Inactive	Negative	0,5	5	100	31
9	YA	Active	Negative	0,5	7	225	100
10	SLE	Minimal activity	Negative	*5,0*	6	50	32
11	SLE	Very active	Positive	*5,3*	8	110	34
12	PSG	Inactive	Negative	0,5	3	68	36
13	AGA	Active	Positive	*5,0*	9	109	34
14	SLE + V	Very active	Positive	*5,0*	*15*	65	36
15	G + V	Very active	Positive	*4,5*	*31*	61	32
16	Minimal-change G + NS	Inactive	Negative	*3,1*	7	*22*	*11*
17	JPA + wide-spread V	Very active	Positive	*1,5*	4	76	64
18	RA	Very active	Positive	*4,4*	*17*	100	66
19	SLE + G	Very active	Positive	*2,0*	5	*14*	*4*
20	SLE	Minimal activity	Negative	0,3	5	91	25
21	HD	Very active	Positive	*2,4*	5	*13*	*12*
22	SLE	Very active	Positive	*1,7*	5	*21*	19
23	G + NS	Active	Positive	*4,8*	2	—	—
24	AN	Inactive	Negative	0,1	4	40	39
25	YA	Active	Negative	0,2	3	74	24
26	NS	Inactive	Negative	0,9	5	68	33
27	G + NS	Very active	Positive	0,2	5	56	25
28	SLE	Inactive	Negative	0,4	5	105	26

*A result of > 1,3 V is considered positive. Positive results are given in italics.
†A result of ≥ 10% is considered positive. Positive results are given in italics.
SLE = systemic lupus erythematosus; PSG = post-streptococcal glomerulonephritis; RA = rheumatoid arthritis; G = glomerulonephritis; SS = Sjögren's syndrome; APG = acute proliferative glomerulonephritis; YA = *Yersinia* arthritis; AGA = allergic granulomatous angiitis; V = vasculitis; NS = nephrotic syndrome; JPA = juvenile polyarthritis; HD = Hashimoto's disease; AN = analgesic nephropathy.

CICs were much less sensitive than Fc-receptor-dependent assays. It is possible that in the detection of CICs false-negative results may occur if the antigen-antibody complexes are already saturated with Clq or if a weakly complement-activating antibody is involved. The crossed immuno-electrophoresis test, which gave no positive results, is too insensitive for use in the detection of CICs.

In conclusion, LN may provide a relatively rapid, sensitive uncomplicated test for the routine detection of CICs in serum specimens. However, as with all presently available tests for the detection of CICs in serum the inherent assumption that positive results are due to CICs or that negative results exclude CICs may be incorrect in some cases. Results obtained by LN are therefore subject to confirmation by other clinical and laboratory tests.

REFERENCES

1. Editorial. Immune complexes in health and disease. *Lancet* 1977; **i:** 580-581.
2. Höffken K, Bestek U, Sperber U, Schmidt CG. Quantitation of immune complexes by nephelometry. *J Immunol Methods* 1979; **29:** 237-244.
3. Arai K, Shimizu A. A simple quantitative assay of circulating immune complexes by laser nephelometry, using a rabbit IgG antibody against human aggregated IgG. *Clin Exp Immunol* 1981; **46:** 541-546.
4. Zubler RH, Lange G, Lambert PH, Miescher PA. Detection of immune complexes in unheated sera by a modified ^{125}I-Clq binding test. *J Immunol* 1976; **116:** 232-235.
5. Laurell CB. Antigen-antibody crossed electrophoresis. *Anal Biochem* 1965; **10:** 358-361.
6. Laurell CB. Quantitative estimation of proteins by electrophoresis in agarose gell containing antibodies. *Anal Biochem* 1966; **10:** 45-52.
7. Füst G, Fekete B, Angyal I *et al.* Evaluation of different methods for detecting circulating immune complexes: studies in patients with lung cancer. *J Immunol Methods* 1981; **46:** 259-276.