CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Jan. 1999, p. 137–139 Vol. 6, No. 1
1071-412X/99/$04.00+0

Comparison of Three Different Methods for Measuring Classical Pathway Complement Activity

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Received 30 March 1998/Returned for modification 8 May 1998/Accepted 25 September 1998

The complement system plays an important role in host defense against infection and in most inflammatory processes. The standard 50% hemolytic complement (CH_{50}) assay is the most commonly used method of screening patient sera for functional activity of the classical complement pathway. Our objective in this study was to compare two newer methods (the enzyme immunoassay and the liposome immunoassay) to a commercial CH_{50} assay for measuring total classical complement activity. We conclude that both newer methods compare well with a CH_{50} assay and are equally sensitive in screening routine clinical sera.

Assessing the functional integrity of the complement system (classical pathway) has been accomplished in the clinical laboratory by traditional 50% hemolytic complement (CH_{50}) assays for many years. Hemolytic assays based on the Mayer method (6) require the interaction of titered complement components in patient sera with antibody-sensitized sheep erythrocytes in solution. The titer at which 50% hemolysis occurs (CH_{50} unit) is proportional to the functional activity of the classical pathway in the serum. Only recently have different methods been developed for measuring total classical complement activity. The objective of our study was to compare two newer methods (the enzyme immunoassay [EIA] and the liposome immunoassay [LIA]) to a commercial CH_{50} assay for measuring total classical complement activity.

The levels of complement activity in sera from cord blood and neonates are approximately 50% or less of that in normal adults (1, 3, 8, 11, 12). Moreover, using the CH_{50} method, sera that are deficient (homozygous) in a single classical pathway component show very low or no hemolytic activity, whereas sera with low levels of a single component (heterozygous) have hemolytic activity approximately 50% of the normal level (2, 10). Inherited deficiencies of early complement components are frequently associated with rheumatic disorders, recurrent infection, and various immune abnormalities (2, 4, 9, 10). The most common component deficiency is that of C2 (heterozygous), which has a frequency of 1% in the general population (4).

Three hundred and thirty-one patient sera sent to our laboratory for CH_{50} testing were used for comparison in this study. In addition, sera from cord blood (n = 19 samples), newborns (n = 31), and patients with known complement abnormalities (homozygous, n = 14; heterozygous, n = 3) were included in the study. Sera were donated by Patricia C. Giclas, National Jewish Medical Center, Denver, Colorado; Hajime Kitamura, The Center for Adult Diseases, Osaka, Japan; and by our C2-deficient family at the University of Utah Medical Center, Salt Lake City, Utah. All sera were stored at −70°C until tested. Sera giving discrepant results between assays were retested for result verification.

The CH_{50} assay (Diamedix, Miami, Fla.) utilizes sensitized sheep erythrocytes in solution and is a simplified variation of the Mayer method (6). The degree of cell lysis is proportional to the total classical complement activity present in the serum. Interpretation of CH_{50} units is as follows: <100, low; 100 to 300, normal; and >300, high.

The LIA (Waco Chemicals USA, Richmond, Va.) utilizes dinitrophenyl (DNP)-coated liposomes that contain the enzyme glucose-6-phosphate dehydrogenase. When serum is mixed with the liposomes and a substrate containing anti-DNP antibody, glucose-6-phosphate, and nicotinamide adenine dinucleotide, activated liposomes lyse, and an enzymatic colorimetric reaction occurs which is proportional to total classical complement activity. Interpretation of LIA units is as follows: <23, low; 23 to 60, normal and >60, high. LIA testing was performed with a Hitachi 717 automated analyzer per the manufacturer's protocol.

The EIA combines the principles of the hemolytic assay with the use of a monoclonal antibody specific for neoantigen (C5b-9 complex) produced as a result of complement activation (Incstar, Stillwater, Minn.). The amount of polymerized C5b-9 (final product) is proportional to the functional activity of C1 through C9. Interpretation of EIA units is as follows: <60, low; 60 to 140, normal; and >140, high.

Other than the kits to measure classical complement activity, no funds were derived from the manufacturers for these studies.

Statistical analysis showed the LIA (Waco) to have an agreement of 94.6%, a sensitivity of 93.2%, and a specificity of 95.0% compared to the CH_{50} method (Diamedix) using the 331 patient sera (Table 1). Compared to the CH_{50} method, the EIA (Incstar) showed 94.0% agreement, 95.9% sensitivity, and 93.4% specificity (Table 1). The EIA showed 98.2% agreement, 100.0% sensitivity, and 97.6% specificity compared to the LIA (Table 1).

In the sera from patients known to have a complement deficiency (homozygous, n = 14), all assays gave results far below their cutoff values for normal classical complement activity (Table 2). In contrast, when using sera from patients with known low levels of a complement component (heterozygous), the EIA gave values at or below the cutoff, whereas the CH_{50} assay and LIA indicated these sera had normal levels of classical complement activity (Table 3).

When measuring total complement activity in the 19 cord

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TABLE 1. Comparison of the LIA, EIA, and CH_{50} assay of 331 patient sera for total classical complement activity

Test and level of complement activity	No. of sera per level of complement activity			
	CH_{50}		LIA	
	Low	Normal	Low	Normal
LIA				
Low	68	13		
Normal	5	245		
EIA				
Low	70	17	81	6
Normal	3	241	0	244

TABLE 3. Complement activity units obtained from the CH_{50} (Diamedix), LIA (Waco), and EIA (Incstar) for total classical complement activity in heterozygous C2 sera

Heterozygous sera	Results (complement activity units) for:		
	CH_{50} assay[a]	LIA[b]	EIA[c]
C2	102	31	57
C2	112	30	62
C2	112	31	50

[a] Complement activity units for the CH_{50} assay are as follows: <100, low; 100 to 300, normal; >300, high.
[b] Complement activity units for the LIA are as follows: <23, low; 23 to 60, normal; >60, high.
[c] Complement activity units for the EIA are as follows: <60, low; 60 to 140, normal; >140, high.

and 31 newborn sera, the EIA showed all sera to have classical complement activity less than that of normal adults (Table 4). In contrast, the CH_{50} assay and LIA detected normal adult levels of classical complement activity in 28% and 22%, respectively, of the cord and newborn sera combined (Table 4).

Statistical analysis showed good correlation for both the LIA and EIA compared to the CH_{50} in screening sera for total classical complement activity in the clinical laboratory (Table 1). Moreover, the EIA and LIA showed 100.0% sensitivity compared to each other (Table 1), indicating that these assays may be more comparable than the Diamedix CH_{50} assay when screening patient sera for total classical complement activity.

Using sera with various complement deficiencies (homozygous), all methods showed classical complement activity to be very low or undetectable in all 14 sera (Table 2). The low but detectable levels demonstrated in some sera using these methods may be due to activation of the alternative pathway or to a very low level of the component that is often present even in some homozygous deficient sera (one of our C2-deficient patients had 0.2 mg/dl of C2 while the other had an undetectable concentration). When using sera with low levels of a complement component (heterozygous), the values obtained using the EIA depicted more accurately what one would expect to find as far as functional activity in patients with heterozygous abnormalities (Table 3).

In sera from newborns and cord blood, only the EIA found all sera to have classical complement activity levels less than normal adults (Table 4). Since the EIA is dependent on the amount of neoantigen (C5b-9 complex) generated, one might expect this method to be more sensitive to the low levels of C9 found in neonatal sera (5).

The protocol for the CH_{50} method (Diamedix) is very laborious, especially with a large number of samples. Dilutions and spectrophotometric readings are performed manually for each patient sample in this CH_{50} assay. The LIA (Waco) has been adapted to many common automated analyzers and was the least labor intensive of the three methods. Using a Hitachi 717, LIA results for 60 sera (plus controls) are generated approximately 30 min after test initiation. The EIA (Incstar) requires serum specimens and serum diluent to be kept at 2 to 8°C while dilutions are being made. This method also requires a 37°C incubator and a 2-h total incubation time.

We conclude that the LIA and EIA showed good correlation compared to CH_{50} (Table 1), but that both may be more sensitive than the Diamedix CH_{50} assay when screening patient sera for total complement activity in the clinical laboratory (Table 1). It has been suggested that solid-phase assays such as EIA that use monoclonal antibodies for the detection of neoantigens may be of more value in assessing complement in patient sera (7, 13). Given the results found in the heterozygous, cord, and newborn sera, it is our opinion that the EIA has higher accuracy in detecting low to moderate depressions of complement activity, especially in the case of C9. One should remember that assays such as these measure only the functional activity of the complement components of the classical pathway and are strictly qualitative. When screening for the functional integrity of classic complement components as a whole, the sensitivity of the assay to detect slight depressions is of utmost importance. Since complement activity over time may reflect disease activity, which has been assessed in the past

TABLE 2. Complement activity units obtained from the CH_{50} assay (Diamedix), LIA (Waco), and EIA (Incstar) for total classical complement activity in deficient sera

Homozygous deficiency	CH_{50} assay[a]	LIA[b]	EIA[c]
C1	0	0	0
C1	0	4	0
C2	3	11	0
C2	3	11	0
C2	0	0	0
C2	0	5	0
C6	0	5	11
C6	0	6	1
C7	2	10	10
C8	0	4	23
C8	0	5	12
C9	0	8	4
C9	0	11	0
C9	0	9	0

[a] Complement activity units for the CH_{50} assay are as follows: <100, low; 100 to 300, normal; >300, high.
[b] Complement activity units for the LIA are as follows: <23, low; 23 to 60, normal; >60, high.
[c] Complement activity units for the EIA are as follows: <60, low; 60 to 140, normal; >140, high.

TABLE 4. Results obtained by three different methods for measuring total classical pathway complement activity in cord and newborn sera

Serum type (no. of samples)	No. of sera					
	CH_{50} assay		LIA		EIA	
	Low	Normal	Low	Normal	Low	Normal
Cord (19)	15	4	16	3	19	0
Newborn (31)	21	10	23	8	31	0

using CH_{50} assay values, it will be necessary to validate the LIA and EIA against a CH_{50} assay longitudinally in such disease states.

We thank Tonya Mallory, Waco Chemicals USA, Richmond, Va., for supplying the LIA kits and Patricia C. Giclas, National Jewish Medical Center, Denver, Colo., and Hajime Kitamura, The Center for Adult Diseases, Osaka, Japan, for the complement-deficient sera. We also thank Jennifer Baumgartner and Stacey Avondet, ARUP Laboratories, Salt Lake City, Utah, for technical assistance.

Harry R. Hill was supported by Public Health Service grant AI13150.

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