

EVALUATION OF CARDIOLIPIN ANTIGEN IN THE TESTS FOR SYPHILIS IN LEPROSY

CAPT. JOHN A. SHIVELY, M. C.¹
AND COL. DWIGHT M. KUHNS, M. C.
*Fourth Army Area Medical Laboratory
Brooke Army Medical Center
Fort Sam Houston, Texas*

Pangborn (14) has shown that an antigen for the serologic diagnosis of syphilis can be prepared by the addition of lecithin and cholesterol to a phospholipid obtained from beef heart. Tests for syphilis using these so-called cardiolipin antigens have given few false positive readings in at least one disease, namely, malaria (7, 8, 15, 16). In leprosy, on the other hand, Rein and his coworkers (15, 16) have reported but little difference in the incidence of biologic false positive reactions between the tests made with the cardiolipin antigens and those made with some of the older antigens.

Our own study, here reported, indicates that in the Kolmer complement-fixation test using a cardiolipin antigen the possibility of biological false positive reactions in leprosy is decreased, but that a microfloculation test with a similar antigen is no more specific than the older tests for syphilis.

The percentages of nonsyphilitic leprosy patients who have given positive serologic reactions for syphilis, when the crude beef heart extract antigens were used, have varied not only with the various tests but also with the various investigators. In 1923 Kolmer and Denny (10) reported no false positives in 123 such patients when the Kolmer modification of the Wassermann test was used. Examination of sera from 50 presumably nonsyphilitic patients with leprosy in the 1934 evaluation study of serologic tests for syphilis (2) showed an incidence of false positive reactions ranging from 40 to 72 per cent. Kolmer (9) combined the incidence of doubtful and positive reactions in sera of presumably nonsyphilitic leprosy patients in the 1935 and 1941 Washington serology surveys and found that those varied from 38 to 68 per cent among the various tests. Faget and Ross (4) have reported that 47.3 per cent of the patients admitted to

¹ Present address—South Bend Medical Foundation, 531 North Main Street, South Bend 1, Indiana.

the National Leprosarium at Carville, Louisiana, with all types of leprosy, gave positive reactions to either the Kolmer or Kahn test, or both. Rein and Elsberg (17) reported that of the sera from 80 nonsyphilitic patients with leprosy 85 per cent gave positive reactions with two or more of their battery of tests for the serodiagnosis of syphilis. Using the Rein-Bossack cardiolipin slide test, Rein and his coworkers (15, 16) obtained 51.5 per cent doubtful or positive reactions in leprosy, although by clinical or anamnestic evidence they were able to establish the diagnosis of syphilis in only about 15 per cent of these cases. Since we were unable to find any reference to the incidence of false positive readings in leprosy patients using some of the other cardiolipin tests for syphilis, the present study was undertaken.

MATERIALS AND METHODS

A total of 150 serum specimens from 142 patients at the U. S. Marine Hospital at Carville, Louisiana, were examined. None of these patients gave a history or showed clinical evidence of syphilis, but they had all at some time given positive or doubtful reactions when tested at Carville by the Kahn standard, Kolmer standard, or Mazzini flocculation slide test for syphilis. Most of these patients had been under sulfone treatment for varying lengths of time, and many had shown considerable degrees of clinical improvement. From 8 of them second serum specimens were submitted to us after an interval of about three months.

The sera were forwarded to this laboratory in lots of 10 to 20, at intervals of about every two to three weeks. All of them were subjected to (a) the Kahn standard flocculation test, (b) the Kolmer complement-fixation test using the Kolmer antigen, (c) the Kolmer complement-fixation test using an antigen containing 0.0175 per cent of cardiolipin, 0.0875 per cent of purified lecithin, and 0.3 per cent of cholesterol in ethyl alcohol (6, 11, 12), and (d) a cardiolipin microfloculation technique which is the Army Medical Department Research and Graduate School's adaptation of the Venereal Diseases Research Laboratory (VDRL) microfloculation test (5, 13). The complement-fixation tests were performed simultaneously, using one serum control and identical reagents with the exception that the Kolmer antigen was used in one test-tube and the cardiolipin complement-fixation antigen was used in a second tube.

RESULTS

In the complement-fixation tests 22 of the 142 sera were anti-complementary, but readings with all four tests were obtained

in sera from a total of 120 patients. The results are shown in Table 1.

TABLE 1.—*Results of tests of sera of 120 presumably nonsyphilitic leprosy patients, with percentages of doubtful reactions consolidated.*

Result	Kahn	Kolmer	C.L.C.F. <i>a</i>	C.L.M.F. <i>b</i>
Positive	63	59	31	31
Doubtful	27	5	13	39
Number	90	64	44	70
Per cent	75.0	53.3	36.6	58.3
Negative	30	56	76	50

a Cardiolipin complement fixation test.

b Cardiolipin microflocculation test.

Only 13 sera gave negative results with all four tests. On the other hand, the sera of only 14 patients were positive with all of the four tests. The presumption is that the wholly negative sera represent loss of serologic activity with regression of the clinical picture of leprosy, as pointed out by both Faget and Ross (4) and Badger (1). Comparison of the readings obtained from the paired sera of 8 patients revealed varying readings with these four tests and no definite pattern.

The complement-fixation test using the cardiolipin antigen gave the lowest percentage of positive and doubtful results. While the cardiolipin microflocculation test gave the same number of definitely positive readings as the cardiolipin complement-fixation test, the former gave a larger number of doubtful readings, thus reducing the specificity of this test to approximately that of the Kolmer test. However, the other flocculation test used, the Kahn standard, gave twice as many positive readings and only a few less doubtful ones.

Examination of the various readings (Table 2) shows that the majority of the doubtful ones obtained with the microflocculation test were in sera of patients with a positive Kolmer or Kahn test. The Kahn test gave the largest number of reactions, with 75 per cent either doubtful or positive. It is noteworthy that the Kolmer test gave the fewest doubtful readings.

Another interesting fact is that 22 out of the 150 specimens received were anticomplementary in the complement-fixation tests. While a few of these sera were cloudy and may also have been contaminated, most of them were clear. This incidence of

TABLE 2.—Correlation of the results with the different tests.

	Cases	Kahn			Kolmer			C.L.C.F. <i>a</i>			C.L.M.F. <i>b</i>		
		P	D	N	P	D	N	P	D	N	P	D	N
Kahn													
Positive	63	--	--	--	41	2	20	29	9	25	26	26	11
Doubtful	27	--	--	--	7	2	18	1	1	25	3	4	20
Negative	30	--	--	--	11	1	18	1	3	26	2	9	19
Kolmer													
Positive	59	41	7	11	--	--	--	30	12	17	20	23	16
Doubtful	5	2	2	1	--	--	--	1	0	4	0	2	3
Negative	56	20	18	18	--	--	--	1	1	54	11	12	33
C.L.C.F. <i>a</i>													
Positive	31	29	1	1	29	1	1	--	--	--	15	10	6
Doubtful	13	9	1	3	12	0	1	--	--	--	5	7	1
Negative	76	25	25	26	17	4	55	--	--	--	11	21	44
C.L.M.F. <i>b</i>													
Positive	31	26	3	2	20	0	11	15	5	11	--	--	--
Doubtful	39	26	4	9	23	2	14	11	7	21	--	--	--
Negative	50	11	20	19	16	3	31	6	1	43	--	--	--

a Cardiolipin complement fixation test.*b* Cardiolipin microfloculation test.

anticomplementary sera is much higher than one usually encounters in the serologic tests for syphilis.

Examination of the readings of Table 2 also reveals that of 56 patients who were negative with the Kolmer test, 54 also gave a negative cardiolipin complement-fixation test, while 33 gave a negative microfloculation test and only 18 gave a negative Kahn test. On the other hand, out of 76 patients giving a negative cardiolipin complement-fixation test, 55 had a negative Kolmer test, 44 a negative microfloculation test and 26 a negative Kahn test. This again would tend to indicate that the complement-fixation test using the cardiolipin antigen was more specific than any of the other tests, including the Kolmer complement-fixation test using the Kolmer antigen.

DISCUSSION

From the data presented, it appears that the complement-fixation test for syphilis in which is used the cardiolipin antigen as prepared by the Army Medical Department Research and Graduate School gives the smallest number of false positive readings in sera of presumably nonsyphilitic leprosy patients when compared with the Kahn standard test, the Kolmer complement-fixation test, and a cardiolipin microfloculation test. This finding is in contrast with reports of tests in which a cardiolipin microfloculation slide test, using a different cardiolipin antigen, showed no greater specificity in this disease than did the tests using the crude beef heart extract antigens (15, 16).

The percentages of positive and doubtful readings obtained with the tests employed are not to be taken as truly representing the incidence of false positive results in nonsyphilitic leprosy patients, since only sera which had exhibited some serologic activity on a previous occasion were tested. Of course, there is the possibility that some of the sera from patients who were always negative with the Kahn, Kolmer, and Mazzini tests, and so were not included in our series, would have had positive readings with this cardiolipin complement-fixation test. Another unknown factor is the incidence in this group of patients of asymptomatic latent syphilis, which is frequently discovered in apparently normal individuals when there is no history or clinical evidence of that disease. However, only 14 of the 120 sera examined exhibited positive readings with all of the four tests used, and this would tend to indicate that true syphilitics, if any, were few in this group.

While only 0.1 per cent of the world's leprosy cases are in the United States (3), examination of the records of the City Health Department of San Antonio, Texas, has revealed that a total of 17 cases has been reported in the ten-year period from 1938 to 1947, inclusive. Consequently, this disease may be considered as a rare possible cause for biologic false positive reactions to the tests for syphilis in this area and the Gulf Coast area.

SUMMARY AND CONCLUSIONS

1. Tests were made on 120 sera from presumably nonsyphilitic patients with leprosy, employing the Kahn standard test, the Kolmer complement-fixation test, a cardiolipin complement-fixation test, and a cardiolipin microfloculation technique, an adaption of the Venereal Disease Research Laboratory microfloculation test of the U. S. Public Health Service. All of the

sera examined had exhibited some serologic activity when tested at an earlier date at Carville.

2. The percentages of positive and doubtful readings obtained with these tests were: Kahn, 75.0; Kolmer, 53.3; cardiolipin complement-fixation, 36.6; and cardiolipin microfloculation, 58.3.

3. The Kolmer complement-fixation test using a cardiolipin antigen consisting of 0.0175 per cent cardiolipin, 0.0875 per cent purified lecithin, and 0.3 per cent cholesterol was found to give the smallest number of false positive readings.

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