Study results indicate a need for careful and complete evaluation of each patient with an unexplained low titer reaction to the VDRL serologic test for syphilis.

An Evaluation of the Kolmer Reiter Protein and Fluorescent Treponemal Antibody Tests

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WHEN quantitative serologic tests for syphilis came into general use in the early 1940's, clinicians were immediately confronted with the problem of interpreting serologic reports which indicated low titer results. Some physicians are prone to consider the low titer results of Venereal Disease Research Laboratory (VDRL) quantitative tests as biologic false positive reactions when the patient has no clinical or historical evidence of syphilis infection. Because it has been our impression that many of the so-called false positive reactions are in fact evidence of infection, we decided to interpret, by reference tests, a series of specimens which yielded low titer results.

Kolmer Reiter protein (KRP) and fluorescent treponemal antibody (FTA) tests were performed on 1,000 consecutive blood specimens which gave reactions in the range of 1 to 4 dilutions with the VDRL tube test. The physicians and clinicians who had submitted the specimens were asked to complete a form including information as to previous history of disease and treatment, present evidence of syphilis, spinal fluid findings, if performed, and their diagnosis of the case. They were not informed of the results of the KRP and FTA tests, and the information they submitted was not given to the laboratory personnel.

Because it was not possible to obtain clinical information for some of the persons whose blood specimens were submitted and because of duplicate specimens from a few persons in the original 1,000, the final study group consisted of 842 persons. Besides the KRP and FTA tests, *Treponema pallidum* immobilization (TPI) tests were performed on second blood specimens from 96 selected persons. Also, as a control, KRP and FTA tests were conducted on 100 consecutive blood specimens which had previously shown nonreactive results with the VDRL test.

In April 1951 the division of laboratories of the Tennessee Department of Public Health replaced the Kahn standard quantitative test for routine diagnosis with the VDRL tube quantitative test, which is performed according to the recommended technique (*1*). We have found that the VDRL test has consistently shown good reproducibility, and it has agreed well with control performance on individual serums, as demonstrated in the annual Public Health Service standard serologic evaluation study. The VDRL tube test is used rather than the VDRL slide test because it has better reproducibility. This usage is particularly true

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for private laboratories which must meet certain standards in the performance of serologic tests under the Tennessee premarital law. Also, proper performance of the VDRL tube technique is easier to teach technicians than the slide technique.

The KRP test (*2*) was used experimentally in our laboratory in 1958, and from January 1, 1959, to December 31, 1960, it was used routinely as a reference for problem cases. The FTA test (*3,4*) was used experimentally in our laboratory in 1959, and since January 1, 1961, it has replaced the KRP test as a reference. Two of us have had special training in the performance of the VDRL and KRP tests, and another has also had this training in FTA tests at the Venereal Disease Research Laboratory. The antigens used in this study for the KRP and FTA tests were supplied by the Venereal Disease Research Laboratory, Communicable Disease Center, Atlanta, Ga., and the TPI tests were performed there.

FTA tests were performed on the 842 blood specimens using a serum dilution of 1:5 on the first 177 specimens and a serum dilution of 1:200 on the remainder as well as on the controls. Use of the higher dilution was recommended by the Venereal Disease Research Laboratory.

Results and Discussion

The results of the FTA and KRP tests on 842 blood specimens with low titer VDRL test reactions for the 2 dilutions of serum with the FTA test are shown in table 1. The reactivity rate of the FTA test using the two dilutions was about the same. Therefore, for reactivity

Table 1. Results of FTA and KRP tests on 842 blood specimens with low titer VDRL reactions for 2 dilutions of serum with the FTA test, Tennessee, 1961

VDRL reaction	Number of specimens	FTA reactive		KRP reactive	
		Number	Percent	Number	Percent
Total	842	697	82.8	469	55.7
1 dil	312	247	79.2	163	52.2
2 dils	319	269	84.3	180	56.4
4 dils	211	181	85.8	126	59.7
FTA dilution 1:5	177	153	86.4	99	55.9
1 dil	65	58	89.2	39	60.0
2 dils	75	65	86.7	42	56.0
4 dils	37	30	81.1	18	48.6
FTA dilution 1:200	665	544	81.8	370	55.6
1 dil	247	189	76.5	124	50.2
2 dils	244	204	83.6	138	56.6
4 dils	174	151	86.8	108	62.1

Table 2. Results of TPI, FTA, and KRP tests on 96 blood specimens with low titer VDRL reactions, according to clinical diagnosis, Tennessee, 1961

Clinical diagnosis	Number of specimens	TPI reactive		FTA reactive		KRP reactive	
		Number	Percent	Number	Percent	Number	Percent
Total	96	53	55.2	31	32.3	18	18.8
Syphilitic	36	21	58.3	10	27.8	6	16.7
Biologic false positive	60	32	53.3	21	35.0	12	20.0

rates with the FTA test in this study, the figures are combined rather than separated by serum dilution. For uniformity, weakly reactive KRP and TPI test results are considered reactive.

A marked difference occurred in the reactivity rates of the FTA and KRP tests, 82.8 percent for the FTA and 55.7 percent for the KRP (table 1). Thus, the FTA test yielded 49 percent more reactors than the KRP test. Variations in the strength of the VDRL reactions in the range of 1 to 4 dilutions had little effect on the reactivity rates of either test.

The results of TPI, FTA, and KRP tests on 96 blood specimens with low titer VDRL reactions, according to clinical diagnosis, are given in table 2. This small sample was selected and is heavily weighted with biologic false positive diagnoses as made by the attending physician. Also, the TPI tests were not performed on the same blood specimens as the FTA and KRP tests. Second blood specimens were obtained for the TPI tests, since it was necessary to submit sterile specimens in vials with paraffin-coated corks. These blood specimens were obtained at varying intervals after the first specimen. Table 2 also shows a difference in reactivity rates of the TPI, FTA, and KRP tests. The FTA test appears less sensitive than the TPI, and the KRP less sensitive than either of the other tests. Of 60 blood specimens from persons diagnosed as biologic false positive, 53.3 percent were reactive by the TPI test, 35.0 percent by the FTA test, and 20.0 percent by the KRP test.

A breakdown of the general clinical status of the 96 selected patients is shown in table 3. A reversion to a nonreactive status apparently had occurred with the reference tests for the two treated primary and secondary cases. A disagreement appears between the TPI and FTA tests for one untreated dark-field positive primary case which was reactive by the FTA test and nonreactive by the KRP test before treatment. The blood specimen for this TPI test was obtained 7 months after treatment. A portion of each specimen sent for the performance of the TPI test was refrigerated. For this particular untreated primary case only, an FTA test was performed on the refrigerated portion. The portion yielded a negative result, indicating a reversion to a nonreactive status after treatment.

Seven of these patients were diagnosed as having early latent syphilis, two treated and five untreated (table 3). Of the treated patients, one was reactive by the TPI test but nonreactive by the FTA and KRP tests. For

Table 3. Results of TPI, FTA, and KRP tests on 96 blood specimens with low titer VDRL reactions, according to clinical diagnosis and previous treatment, Tennessee, 1961

Diagnosis and treatment	Number of specimens	TPI		FTA		KRP	
		Reactive	Nonreactive	Reactive	Nonreactive	Reactive	Nonreactive
Total	96	53	43	31	65	18	78
Primary	3	0	3	1	2	0	3
Treated	1	0	1	0	1	0	1
Untreated	2	0	2	1	1	0	2
Secondary	1	0	1	0	1	0	1
Treated	1	0	1	0	1	0	1
Untreated	0	0	0	0	0	0	0
Early latent	7	3	4	2	5	1	6
Treated	2	1	1	0	2	0	2
Untreated	5	2	3	2	3	1	4
Late latent	21	15	6	7	14	4	17
Treated	13	9	4	3	10	2	11
Untreated	8	6	2	4	4	2	6
Congenital	4	3	1	0	4	1	3
Treated	2	2	0	0	2	1	1
Untreated	2	1	1	0	2	0	2
Biologic false positive	60	32	28	21	39	12	48

the untreated early latent cases there was agreement between the TPI and FTA tests, but the KRP test showed a lack of sensitivity for one case. Three cases diagnosed as early latent syphilis which were nonreactive by the TPI and FTA tests were probably biologic false positives.

Twenty-one patients were diagnosed as having late latent syphilis, 13 with and 8 without previous treatment (table 3). The FTA test was less sensitive than the TPI, and KRP less sensitive than either of the others for these patients. Two of the untreated late latent patients were probably biologic false positive reactors, based on the TPI test. Four patients had been diagnosed as having congenital syphilis, two treated and two untreated. For the two treated cases, a disagreement appeared between the TPI and FTA tests. Of 60 biologic false positive reactors, 32 were positive by the TPI test, 21 by the FTA test, and 12 by the KRP test. One patient with nonreactive TPI and FTA tests was reactive by the KRP test. Does this represent a technical false positive KRP test?

Among the control group of 100 consecutive blood specimens, which were nonreactive by the VDRL test, 2 were reactive by the KRP test but none was reactive by the FTA test. TPI tests were performed on second blood specimens from the two persons with reactive KRP tests, one of whom was a 44-year-old nonwhite male applicant for a foodhandler's certificate who had no history or evidence of syphilis infection. His specimen was nonreactive by the TPI test. The other second blood specimen was drawn from a 63-year-old nonwhite male, hospitalized for tuberculosis at the time, who also had no history or evidence of syphilis infection. His condition was diagnosed as active tuberculosis and arteriosclerotic heart disease. His specimen was reactive by the TPI test.

The comparative results of FTA and KRP tests on 842 blood specimens with low titer VDRL reactions are shown in table 4. For 70.3 percent of these specimens there was agreement between the FTA and KRP tests, 54.4 percent where both tests were reactive and 15.9 percent where both tests were nonreactive. A disagreement appeared between the two tests for 29.7 percent of the specimens. There was disagreement for 28.4 percent of the specimens in which the FTA test was reactive and the KRP test nonreactive. Specimens from 11 patients, 1.3 percent, were nonreactive by the FTA test and reactive by the KRP test. Three of these patients had been diagnosed as having treated late latent syphilis, one as untreated late latent, one as treated congenital, and six as biologic false positive. Two of the six biologic false positive reactors are included in table 3, one reactive and one nonreactive by the TPI test.

The results of FTA and KRP tests on the 842 blood specimens according to clinical diagnosis and previous treatment are given in table 5. In this tabulation the various diagnoses under late syphilis are combined, as the numbers were too few to make comparisons. Cases of neurosyphilis, cardiovascular syphilis, and late cutaneous syphilis are included. The results shown in the table indicate again the difference in sensitivity between the FTA and KRP tests. Considering only the untreated latent and biologic false positive classifications, 79, or 11.3 percent of the 697 persons reactive by the FTA test, would have been missed if only the KRP test had been used as a guide to infection.

Of the 149 persons diagnosed as biologic false positive reactors, 78, or 52.3 percent, had a syphilitic infection according to the results of the FTA test. Conversely, using the FTA test as a gauge and including untreated latent, untreated congenital, and biologic false positive classifications where a nonreactive result was obtained by the FTA test, 87, or 10.3 percent, of the 842 low titer VDRL blood specimens may

Table 4. Comparative results of FTA and KRP tests on 842 blood specimens with low titer VDRL reactions, Tennessee, 1961

Comparative results	Number	Percent
Total specimens	842	100.0
Agreement	592	70.3
Both reactive	458	54.4
Both nonreactive	134	15.9
Disagreement	250	29.7
FTA reactive, KRP nonreactive	239	28.4
FTA nonreactive, KRP reactive	11	1.3

Table 5. Results of FTA and KRP tests on 842 blood specimens with low titer VDRL reactions, according to clinical diagnosis and previous treatment, Tennessee, 1961

Diagnosis and treatment	Number of specimens	FTA reactive		KRP reactive	
		Number	Percent	Number	Percent
Total	842	697	82. 8	469	55. 7
Primary	39	29	74. 4	16	41. 0
Treated	16	8	50. 0	3	18. 8
Untreated	23	21	91. 3	13	56. 5
Secondary	20	15	75. 0	9	45. 0
Treated	16	11	68. 8	7	43. 8
Untreated	4	4	[1]	2	[1]
Early latent	71	61	85. 9	41	57. 7
Treated	42	37	88. 1	24	57. 1
Untreated	29	24	82. 8	17	58. 6
Late latent	488	450	92. 2	321	65. 8
Treated	344	314	91. 3	224	65. 1
Untreated	144	136	94. 4	97	67. 4
Late	32	31	96. 9	22	68. 8
Treated	18	17	94. 4	12	66. 7
Untreated	14	14	100. 0	10	71. 4
Congenital	43	33	76. 7	15	34. 9
Treated	33	26	78. 8	9	27. 3
Untreated	10	7	70. 0	6	60. 0
Biologic false positive	149	78	52. 3	45	30. 2

[1] Percentage not computed for less than 10 specimens.

be evidence of true biologic false positive reactions.

Comments

This study, although not as complete as desirable, suggests answers to certain questions relative to the comparative values of the FTA and KRP tests and to the significance of the low titer VDRL test. Ideally, a study should include a complete and careful clinical evaluation of each patient in the sample and FTA, KRP, and TPI tests performed on the same blood specimen from each patient. Such procedures were impossible in our study. A study of this type would best be performed over a relatively long period of time with a clinic population. The cost of the TPI test would be the greatest hindrance.

The findings of this study indicate that the KRP test is low in sensitivity. It has been stated (*5*) that the pattern of reactive serologic tests for syphilis and nonreactive KRP tests can be interpreted clinically in three ways: as recently acquired untreated syphilis, adequately treated early or late syphilis, or nonsyphilitic. Based on the TPI and FTA tests in our study, a negative KRP can occur in the presence of syphilis other than recently acquired untreated syphilis and adequately treated early or late syphilis. For these reasons, our laboratory no longer uses the KRP test as a reference.

We agree with others (*6,7*) that the FTA test is a valuable reference. Its specificity is of high value. This can be expected, since *T. pallidum* antigen is used in its performance. Theoretically, the FTA test should be less subject to technical error than the TPI since extraneous factors could possibly influence the motility of *T. pallidum* in the TPI test, which would not be a factor in the performance of the FTA test. Although the sensitivity of the FTA test was not as high in our sample as in the experience of its authors, it was considerably more sensitive than the KRP test. Because of the unavailability of the TPI test as a routine reference in most State laboratories, due to its cost, we feel that with satisfactory antigens and goat antihuman serum globulin, the FTA test, properly performed, is the reference of choice.

Since the completion of this study, commercial FTA antigens have been used in our laboratory. Unfortunately, we have encountered difficulties with all the lots of commercial anti-

gen we have checked. When the test has been performed with the commercial antigen, using antigen produced at the Venereal Disease Research Laboratory as a control, we have found the commercial antigen is either low in sensitivity or it loses its sensitivity shortly after being reconstituted. More thorough controls on each lot of commercial antigen prior to its release are indicated. (Since this paper was prepared, we have obtained an excellent commercial antigen for the FTA test.)

The findings of this study stress the need for clinical evaluation of all patients with an unexplained low titer VDRL test. They also reveal that only about 10 percent of low titer VDRL tests represented biologic false positive reactions. If a clinical study of the sample had been possible, we feel the results would have been most interesting.

More frequent use of a reference test to evaluate patients with unexplained low titer diagnostic tests is indicated. In the absence of historical and clinical evidence of syphilis infection, reference tests should also be used for clinical evaluation of patients whose VDRL tests show high titers since biologic false positive reactions can be encountered with such results, as we have noted in Tennessee.

Conclusions

1. The Kolmer Reiter protein test is of limited value as a reference test because of its low sensitivity.

2. The fluorescent treponemal antibody test is high in specificity and is relatively high in sensitivity as measured by the *Treponema pallidum* immobilization test.

3. When the *T. pallidum* immobilization test is unavailable, the fluorescent treponemal antibody test appears to be a reference test of choice.

4. In this study, only about 10 percent of blood specimens giving low titer VDRL tests represented biologic false positive reactions when based on the fluorescent treponemal antibody test.

5. The need for careful and complete clinical evaluation of each patient with an unexplained low VDRL test is indicated.

REFERENCES

(*1*) Harris, A., Rosenberg, A. A., and Del Vecchio, E. R.: A macroflocculation test for syphilis using cardiolipin-lecithin antigen. J. Ven. Dis. Inform. 29: 313–316, October 1948.

(*2*) Bossak, H. N., Falcone, V. H., Duncan, W. P., and Harris, A.: Kolmer test with Reiter protein antigen. Pub. Health Lab. 16: 39–48, March 1958.

(*3*) Deacon, W. E., Falcone, V. H., and Harris, A.: A fluorescent test for treponemal antibodies. Proc. Soc. Exper. Biol. & Med. 96: 477–480 (1957).

(*4*) Deacon, W. E., Freeman, E. M., and Harris, A.: Fluorescent treponemal antibody test. A modification based on quantitation (FTA 200). Proc. Soc. Exper. Biol. & Med. 103: 827–829 (1960).

(*5*) Pariser, H.: Sensitivity of Reiter protein complement fixation and standard serologic tests. South. M.J. 53: 1543 (1960).

(*6*) Olansky, S., and McCormick, G. E., Jr.: Experiences with fluorescent treponemal antibody for syphilis (FTA). A.M.A. Arch. Dermat. 81: 59 (1960).

(*7*) Montgomery, C. H., Suhrland, S., and Knox, J. M.: Behavior of the fluorescent treponemal antibody test in early syphilis. Pub. Health Lab. 18: 44, May 1960.