

Influence of Treatment of Biologic False Positive Syphilis Tests in Leprosy¹

H. G. S. Ruge²

Leprosy is one of the diseases in which many of the so-called biologic false positive (BFP) reactions occur. Reports dealing with this phenomenon present figures fluctuating from zero to 100 per cent (⁹). The differences probably will be explained by the fact that, besides the clinical status, the eventual effect of the treatment applied and the time of the disease have been often neglected.

In the following paper data are compiled on 210 patients suffering from lepromatous leprosy, which generally exhibits the greatest number of BFP reactions. These patients could be followed serologically with a battery of syphilis tests, in three consecutive series 24 weeks apart in each case, designed to determine the behavior and frequency of BFP reactions.

For the study five standard syphilis tests (STS) were used, viz., three complement fixation tests (CFR), including (1) the original Wassermann test (OWaR), (2) the cardiolipin Wassermann test (CWaR), and (3) the Reiter complement fixation test,³ (RCF), and two flocculation tests, viz., (1) the Meinicke clarification test II (MKR), and (2) the Venereal Disease Research Laboratory test (VDRL). As standard tests the treponema immobilization test (TPI) was chosen, assuming its reliability to be 100 per cent.

The sera came from the Cebu Skin Dispensary, Leonard Wood Memorial, Cebu City, Cebu, Philippines. These were derived from a group composed, originally, of 420 patients, including 293 males and 127 females (Table 1).

As can be seen in Table 1, 42 cases of burnt-out yaws and two cases of latent

syphilis were detected, made up of 31 males and 13 females (10.6% of the males in the group and 10.4% of the females). These cases had to be excluded from the present study. Since 166 cases could not be followed regularly for the three series of tests, only 210 patients could be examined completely.

For obvious reasons the results of the TPI tests are not considered here in detail, since the TPI test was used only to make sure that eventual doubtful STS reactions could be regarded as specific if backed by the results obtained with TPI. In this connection it may be mentioned that even a small number of TPI tests were to be regarded as false-positive or inconclusive (¹¹). These cases are, of course, not included in this study.

The treatment given the patients consisted of Ciba 1906, Camoquine, and DDS in doses of 4 mgm./kgm. bodyweight daily, except Sundays, or 2.5 mgm./kgm. bodyweight daily. The different treatment groups and the corresponding BFP reactions observed are summarized in Table 2, which includes the anticomplementary (ac) reactions, which, besides BFR reactions, are not infrequently encountered (⁹, ¹¹). In addition, the table includes the amounts of DDS that had been applied eventually before the beginning of this investigation.

Ninety BFP and 40 ac reactions were observed among 68 patients. It can be concluded from Table 2 that the number of BFP and ac reactions has been reduced considerably by the therapy employed. When the results are calculated by chi squares, the reduction of BFP and ac reactions is not significant in the case of patients treated with Ciba 1906 or Camoquine ($\chi^2 = 0.024$ and 0.41 respectively). The significance is much higher in

¹ Received for publication 1 February 1968.

² H. G. S. Ruge, M.D., D.T.M. & H., Kiel University, Ravensberg 3, D 23 Kiel, Germany.

³ Pallida-Antigen, Promonta, Hamburg, Germany.

TABLE 1. Cases of yaws and syphilis, by age and sex.

Age (yrs.)	Male	Female
15	31 (5)	16 (1)
16-25	124 (10 + 1) ^a	52 (4)
26-35	77 (5 + 1) ^a	27 (3)
36-45	45 (9)	19 (3)
>45	16 (2)	13 (2)
Total	293 (31 + 2)	127 (13)
Per cent	10.6	10.4

^a Figures within parens = cases of burnt-out yaws; + 1 = case of latent syphilis

the case of DDS in a dosage of 2.5 mgm./kgm. daily, where $\chi^2 = 4.41$. A truly statistical significance is reached only with DDS in a dosage of 4 mgm./kgm., where $\chi^2 = 13.61$. In the grand total $\chi^2 = 11.34$ (significance $\chi^2 = > 9$).

Altogether 3,150 tests have been performed, dealing with five reactions in three series in 210 sera. Table 3 summarizes the number and kinds of tests performed in which the 90 BFP reactions and 40 ac reactions occurred in the course of 630 (3 x 210) serum examinations. The table records also the percentages of tests in which these reactions occurred. Altogether, reaction occurred in 20.62 per cent (630/130) of the sera tested. Clearly the percentages calculated depend greatly on the kind of calculation employed.

The percentage of 4.12 for 130 reactions in 3,150 tests appears rather low in the light of the number of tests made. The number of BFP reactions in flocculation reaction tests is 2.3 times as high as that of the complement reaction tests and twice as high as the percentage of ac reactions. It is worth noting that ac reactions take second place. This means that ac reactions in leprosy indicate the presence of a high concentration of γ globulins in the serum. Their presence has been proven already by a series of investigators (^{4, 6, 7}) and studies by other authors have shown an increased tendency toward self-aggregation of the γ globulins that cause ac reactions

(^{12, 13}). Also, besides syphilis, the so-called collagen diseases (e.g., scleroderma and lupus erythematosus) and certain autoimmune liver diseases are known to produce ac reactions (^{1, 2, 3, 7}).

Table 4 presents the details of the BFP and ac reactions observed.

The distribution of BFP and ac reactions among the various groups of cases of lepromatous leprosy; i.e., L1, L1-2, L2, L2-3, and L3, shows a preponderance of reactions among the more serious forms. If, in the first series, the 58 patients suffering from L1 and L1-2 leprosy are compared with the 152 patients suffering from L2, L2-3, and L3 disease respectively (97% of all of them had previously had no treatment or only a little) 7/58 and 27/152 cases are found to have BFP and ac reactions, i.e., 12.1 per cent and 17.8 per cent respectively. Yet this difference is not significant. The same holds true for the percentages of the BFP and ac reactions observed in these two groups; i.e., 13/340 : 40/760 = 3.8 per cent : 5.3 per cent.

A general influence of erythema nodosum leprosum (ENL) on the number or character of BFP and ac reactions encountered could not be established, although in several cases a certain mutual dependence could not be excluded.

Concerning the two different types of reactions, i.e., OWaR and CWaR, on the one hand, and flocculation reactions on the other, it can be said that OWaR and CWaR disappear more quickly and are not likely to reoccur during the treatment. The downhill course of these two complement fixation reactions is rather steady; on the other hand, additional flocculation reactions are observed more often during treatment, and the general tendency to fade is less pronounced. A possible explanation is that at least partly different reagins are bound in these two types of reactions. It should be added that the RCF antigen, which consists of ultrasonically disrupted treponemata Reiter and contains a group antigen against *Treponema pallidum* has given a doubtful result only twice. This means that in lepromatous leprosy generally the nonspecific antibodies; i.e., reagins, are concerned, and that group-specific anti-

TABLE 2. Treatment groups.

DDS treatment before (gm.)	Ciba 1906			Camoquine			DDS 4 mgm./kgm.			DDS 2.5 mgm./kgm.			Grand total		
	No. cases			No. cases			No. cases			No. cases			All cases		
	Neg	p/df	Total	Neg.	p/df	Total	Neg.	p/df	Total	Neg.	p/df	Total	Neg.	p/df	Total
0-10	19	12	31	14	10	24	25	9	34	24	11	35	82	42	124
10-100	10	5	15	15	3	18	16	11	27	15	5	20	56	24	80
>100	1	—	1	2	1	3	1	—	1	—	1	1	4	2	6
Total	30	17	47	31	14	45	42	20	62	39	17	56	142	68	210

DDS treatment before (gm.)	Reactions																			
	Series																			
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III					
0-10	1/4	0/4	1/3	1/6	3/2	1/2	1/3	3/5	—	1/4	1/5	1/3	4/17	7/16	3/8					
10-100	0/5	1/2	0/1	2/1	0/1	1/0	0/3	0/8	0/1	5 ac	2 ac	11 ac	11 ac	5 ac	9 ac					
>100	—	—	—	3 ac	3 ac	—	3 ac	3 ac	—	—	3 ac	—	6 ac	9 ac	2/2					
Total	1/9	1/6	1/4	3/7	4/3	2/2	1/6	3/13	0/1	2/7	1/8	2/3	7/29	9/30	5/10					
Ser. I : Ser. III	$\chi^2 = 0.024$					$\chi^2 = 0.41$					$\chi^2 = 4.41$					$\chi^2 = 11.34$				

Symbols: Neg. = negative; p = positive; df = doubtful. Numerator = positive reaction. Denominator = doubtful reaction. ac = anticomplementary. Grand total: 21/69 = 90 BFP and 40 ac reactions.

TABLE 3. *Number and kinds of tests performed.*

No.	Tests	Reactions		Total	%
		BFP	ac		
1,890	Complement fixation	35	40	75	3.97
1,260	Flocculation	55	—	55	4.37
3,150	All tests	90	40	130	4.12

TABLE 4. *Distribution of BFP and ac reactions in Series 1, 2 and 3.*

Test	Series 1		Series 2		Series 3		Total	
	Pos.	Dbtfl.	Pos.	Dbtfl.	Pos.	Dbtfl.	Pos.	Dbtfl.
OWaR	3	2	2	8	2	—	7	10
CWaR	1	5	2	8	—	—	3	13
RCF	—	1	—	1	—	—	—	2
MKR	2	11	1	4	1	6	4	21
VDRL	1	10	4	9	2	4	7	23
ac	17		14		9		40	

bodies do not play an important role, if any at all^(5, 8).

In the author's opinion this is the first time in which the sera of three consecutive series of lepromatous leprosy patients could be investigated serologically. From the results observed here it can be said that the behavior of OWaR, CWaR and ac reactions could give certain indications regarding the beginning normalization of the sera in question, which would probably correspond with the gradual regression of other serologic changes, such as increased cold agglutinins, altered albumin/globulin quotients, and increased γ globulins.

SUMMARY

In three consecutive series of tests, made at intervals of 24 weeks each on sera from 210 patients suffering with lepromatous leprosy, it could be demonstrated that the number of biologic false positive syphilis tests decreased considerably, especially during DDS treatment. This therapy proved to be more effective than treatment with Ciba 1906 and Camoquine, if the fading of reactions can be taken as an

indication of the beginning normalization of the sera in question. Complement fixation reactions disappeared more quickly than the flocculation reactions, and the Reiter treponema antigen presented two doubtful tests only. Anticomplementary sera were considerably increased—probably as a consequence of a great increase in γ globulins, which is generally found in lepromatous leprosy.

RESUMEN

En tres series consecutivas de pruebas, hechas con intervalos de 24 semanas cada una, en sueros de 210 enfermos con lepra lepromatosa, se demostró que el número de reacciones biológicas falso-positivas en las pruebas para sífilis disminuyeron considerablemente, especialmente durante el tratamiento con DDS. Este tratamiento probó ser más efectivo que el tratamiento con Ciba 1906 y Camoquina si el debilitamiento de las reacciones puede tomarse como una indicación del comienzo de la normalización de los sueros en cuestión. Los sueros anticomplementarios aumentaron considerablemente, probablemente como consecuencia de un gran aumento de los gama-globulinas, lo que corrientemente ocurre en lepra lepromatosa.

RÉSUMÉ

Au cours de trois séries consécutives d'épreuves, effectuées à des intervalles de 24 semaines chacune, sur le sérum de 210 malades atteints de lèpre lépromateuse, on a pu montrer que le nombre d'épreuves biologiques faussement positives pour la syphilis diminuait considérablement, surtout au cours du traitement par la DDS. Cette thérapeutique est apparue plus efficace que le traitement par le Ciba 1906 et par la Camoquine, du moins si l'affaiblissement des réactions peut être considéré comme une indication du retour à la normale du sérum considéré. Les réactions de fixation du complément ont disparu plus rapidement que les réactions de flocculation; l'antigène au tréponème de Reiter n'a présenté que deux résultats douteux. Le sérum anti-complément était considérablement augmenté, probablement par suite de l'augmentation prononcée des γ globulines qui est généralement observée dans la lèpre lépromateuse.

REFERENCES

1. BERNDT, J. and HIPPIUS, H. Über Eigenhemmungen in den klassischen Luesreaktionen bei neurologisch-psychiatrischen Kranken. *Arch. Psych.* **210** (1967) 198-210.
2. CASTANEDO, J. P. and WILLIAMS, R. C. Anticomplementary activity of sera from patients with connective tissue disease and normal subjects. *J. Lab. Clin. Med.* **69** (1967) 217-228.
3. LIGHTER, A. G. Observations on anti-complementary reactions. *Arch. Derm. Syph.* **67** (1953) 362-368.
4. MATTHEWS, L. J. and TRAUTMAN, J. R. Clinical and serological profiles in leprosy. *Lancet* **2** (1965) 915-917.
5. MILLER, J. N., DE BRUIJN, J. H., BEKKER, J. H. and ONVLEE, P. C. Antigenic structure of *Treponema pallidum*, Nichols strain. I. The demonstration, nature and location of specific and shared antigens. *J. Immunol.* **96** (1966) 450-458.
6. NØRGAARD, O. Investigations into anti-complementary human sera. *Acta Path. Microbiol. Scandinavica* **37** (1955) 329-339.
7. OLHAGEN, B. Studies on thermostable anticomplementary human sera. *Acta Med. Scandinavica Suppl.* **162**, 1945.
8. PILLOT, J., BETZ, A., COLOMBANI, J. and RIPAUT, J. Problem of positive complement-fixation reactions with the Reiter treponeme in the absence of syphilis. *British J. Vener. Dis.* **41** (1965) 170-176.
9. RUGE, H. G. S. Serological findings in leprosy and tuberculosis with the Wassermann, Meinicke and VDRL tests. *Bull. WHO* **13** (1955) 861-886.
10. RUGE, H. G. S. Luesreaktionen an Lepra-seren. (Reihenuntersuchungen) *Med. Welt* **17** N.F. (1966) 2820-2827.
11. RUGE, H. G. S. Treponemal immobilization tests in leprosy. *British J. Vener. Dis.* **43** (1967) 191-196.
12. SCHIERZ, G. Zur antikomplementären Wirkung von Serum und Gammaglobulinen. *Z. Immunforsch.* **127** (1964) 417-427.
13. VARGUES, R., MORAUD, B. and GONTHIER, F. Etude cinétique de l'inactivation du complément immunologique C' par les γ globulines humaines aggrégées. *Compt. Rend. Soc. Biol. (Paris)* **158** (1964) 1736-1739.