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Alteration of paraluic seroreactions in Leprosy

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Introduction

Seroreactions play an important rôle in the diagnosis of syphilis, but it should be always kept in mind that seroreactions are only one of the evidences required for diagnosis.

All seroreactions for syphilis but one (Nelson's immobilisation test, which is still in development) (1) are not specific antigen-antibody reactions in the sense of a Gruber-Widal or a reaction between toxin and antitoxin, but are reactions between lipoids extracted from human or animal organs and antilipidal antibodies produced by the infected organism. Hence it is clear that not only syphilis, but many other diseases may cause the production of such antibodies by the patient. Many diseases and conditions, from the common cold to tuberculosis and leprosy and even the physiological changes of the female organism during pregnancy, are known to give paraluic (false positive) readings of the seroreactions for syphilis.

Other treponemal diseases, such as yaws, relapsing fever, etc. also interfere, but this interference must be considered from another point of view, as there are common characters in this group.

Therefore the diagnosis of syphilis should not be based on positive seroreactions only. The clinical evidence and physical signs of the patient should always be considered before a final diagnosis is arrived at.

Another source of error in interpreting seroreactions may be due to the fact that some syphilitic sera are unable to react with the antigens immediately after the withdrawal of the blood. It takes some time for these sera to reach their final colloidal status before representative results are obtained. This phenomenon is called alteration (2).

A modern syphilis survey involves mass field testings with a quick seroreaction for screening purposes. Such results, if not confirmed, may lead to wrong interpretations on the positive as well as on the negative side. Interfering diseases may escape observation as well.

In the exchange of samples between distant laboratories for inter-laboratory comparisons, the comparability of the results may be affected by alteration, since the tests may be done at different time-intervals after collection. Thus the serologist may be mislead regarding the specifity and reliability of the reactions performed.

In the tropics leprosy may interfere seriously with the interpretation of seroreactions for syphilis (3). This interference could be ruled out if a correct diagnosis of leprosy were made. But in mass surveys it so happens that cases of leprosy

pass undiagnosed and this could contribute to a misinterpretation of the seroreactions.

A comparative testing of 400 blood samples (150 from pregnancy, 250 from leprosy) done at the WHO laboratory, Simla (India), and at the Medical Research Institute, Colombo (Ceylon) in 1949 yielded interesting results. Two samples were collected from each patient, one was taken as whole blood to Simla by air, the other was tested in Colombo 24 hours after bleeding, thus resulting in a difference of about 70 hours between the times the tests were performed at the respective laboratories.

In pregnancy the results were nearly uniform in both laboratories (only 5 per cent. of cases showing slight differences), whereas in leprosy, disparity was observed in 30 per cent. of cases, the readings being mostly negative or weaker positive in the tests performed later at Simla.

These results strongly suggest that the alteration which occurs in syphilitic sera may occur as well in sera giving paraluic reactions.

Methods

To find out whether the alteration of syphilitic sera may apply to sera giving paraluic reactions, 479 patients of the Leprosy Hospital at Hendala (Ceylon) were tested serologically and clinically. It is realised the number of cases is small, but no more were available for this investigation.

The patients were bled in the forenoon, 10 ml of blood being taken. The serum was separated from the clot immediately after coagulation, and was divided into 3 parts, namely a small part for testing on the average 2 hours after bleeding, and 2 larger parts which were stored without inactivation at room temperature, and at 4° C in a refrigerator, respectively. All specimens were handled under sterile conditions. The amounts required for testing were inactivated at 60° C for 3 minutes (4, 5) shortly before the tests were performed. Tests were done on both fractions (room temperature and 4° C) 24, 48, 96 and 144 hours after drawing the blood.

The tests performed were complement fixation reaction (WR) (Wyler's technique) (6), Standard Kahn reaction (KR) (7), and Cardiolipin microscopic slide flocculation test (CL) (Kline's modification) (8, 9). On sera with positive readings the Kahn verification test (V) (10) was performed additionally.

Results

Table 1 shows the distribution of the results by sex and type of leprosy. The distribution of sex and type of leprosy among the patients tested agrees with previously reported results (3).

TABLE 1

TYPE / Sex		L Cases	L %	N Cases	N %	LN Cases	LN %	TOTAL Cases	TOTAL %
Male	–	97	20·3	112	23·4	6	1·3	215	45·0
	+	71	14·7	32	6·8	5	1·0	108	22·5
	Total	168	35·0	144	30·2	11	2·3	323	67·5
Female	–	43	9·0	66	13·7	1	0·2	110	22·9
	+	38	7·9	8	1·7	—	—	46	9·6
	Total	81	16·9	74	15·4	1	0·2	156	32·5
Grand Total	–	140	29·3	178	37·1	7	1·5	325	67·9
	+	109	22·6	40	8·5	5	1·0	154	32·1
	Total	249	51·9	218	45·6	12	2·5	479	100·0

Key :— Seronegative, + at least one positive reading, L lepromatous, N neural, LN mixed leprosy (Cairo nomenclature) (3).

Three hundred and twenty-five (67·9 per cent.) of the 479 patients tested gave a negative response to all reactions, and 154 (32·1) of them had a positive reading and showed all strengths of reactions (± to + + + +), including even a single positive reading of a single reaction at any time.

The frequency with which a single reaction or a combination of reactions gave positive readings is shown in Table 2. Sub-division into sexes as in Table 1 was not done for this and all further tables, as the respective figures were too small.

From Table 2 it may be seen that the WR gave the highest number of patients with a positive result in a single reaction. Hence in selecting a single screening test for mass surveys, priority should be given to the KR or CL reaction.

TABLE 2

TYPE Reaction	L Cases	%	N Cases	%	LN Cases	%	TOTAL Cases	%
WR + KR, CL–	28	5·9	10	2·1	4	0·8	42	8·8
KR + WR, CL–	10	2·1	2	0·4	—	—	12	2·5
CL + WR, KR–	13	2·6	7	1·5	—	—	20	4·1
WR, KR + CL–	15	3·1	5	1·0	—	—	20	4·1
WR, CL + KR–	6	1·2	2	0·5	—	—	8	1·7
KR, CL + WR–	6	1·2	1	0·3	—	—	7	1·5
WR, KR, CL +	31	6·5	13	2·7	1	0·2	45	9·4
Total	109	22·6	40	8·5	5	1·0	154	32·1

% calculated on the total of 479 patients.

Any threshold in biological work above or below which a result is to be taken as positive or negative respectively, must be considered an arbitrary one. For statistical purposes, however, a threshold is necessary. Therefore the interpretation of Wyler (6), Kahn (7), and Kline (8) were given an arbitrary value as shown in Table 3.

TABLE 3

WR	KR	CL	Interpretation	Value
–	–	–	negative	0
±	+	+	doubtful	1
+	+ +	+ +	weak positive	2
+ ±	+ + +	+ + +	positive	3
+ +	+ + + +	+ + + +	positive	4

For each set of reactions the values of the single reactions were totalled. The total value of 6 was accepted as minimum threshold below which results were negative or doubtful, and above which results were positive. The respective minimum values for 24, 48, 96, 144 hours were therefore 18, 30, 42 and 54 (Table 4).

TABLE 4

No._____ Date_____

Name_____ Age_____ Sex_____

Duration of leprosy_____

WR	KR	CL	2h
+(2)	+ +(2)	+ +(2)	6

TABLE 4 (*Contd.*)

Room temperature				Ice box		
WR	KR	CL		WR	KR	CL
+(2)	++(2)	++(2) $\overset{6}{\underline{18}}$	24h	$\overset{6}{\underline{30}}$ +(2)	++(2)	++(2)
+(2)	++(2)	++(2) $\overset{6}{\underline{42}}$	48h	$\overset{6}{\underline{54}}$ +(2)	++(2)	++(2)
+(2)	++(2)	++(2) $\overset{6}{\underline{}}$	96h	$\overset{6}{\underline{}}$ +(2)	++(2)	++(2)
+(2)	++(2)	++(2)	144h	+(2)	++(2)	++(2)

All cases were valued, totalled accordingly (Table 5), and tabulated in a graph (Fig. 1), and seropositivity was decided from the distribution of values in relation to the above thresholds.

TABLE 5

No. 191 Date : 3-10-50
Name : Charlet Age : 20 Sex : Female
Duration of leprosy : 15 years

WR	KR	CL	2h
++(4)	+(1)	++(2)	$\overset{7}{\underline{}}$

Room temperature				Ice box		
WR	KR	CL		WR	KR	CL
++(4)	++(2)	+(1) $\overset{7}{\underline{21}}$	24h	$\overset{7}{\underline{}}$ ++(4)	++(2)	+(1)
++(4)	+(1)	−(0) $\overset{5}{\underline{33}}$	48h	$\overset{7}{\underline{}}$ ++(4)	++(2)	+(1)
+(2)	−(0)	−(0) $\overset{2}{\underline{38}}$	96h	$\overset{3}{\underline{}}$ +(2)	+(1)	−(0)
±(1)	−(0)	−(0) $\overset{1}{\underline{41}}$	144h	$\overset{2}{\underline{}}$ ±(1)	+(1)	−(0)

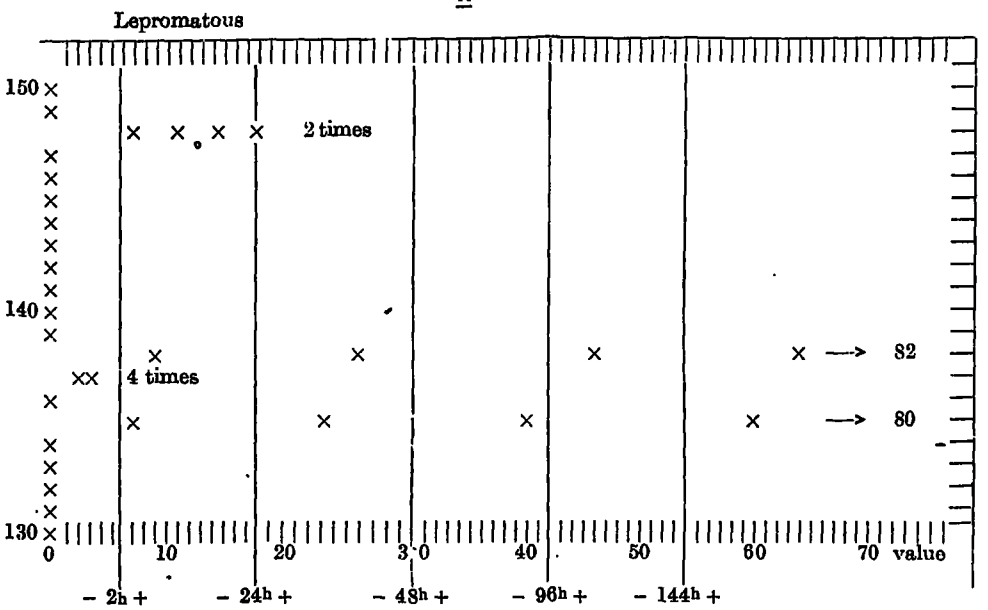

Figure 1 (Selected part).

The results obtained by this way, including clinical evidences, and Kahn verification tests are enlisted in Table 6.

TABLE 6

Type / Result	L						N						LN						Total						Grand Total
		Pos. hours after bleeding						Pos. hours after bleeding						Pos. hours after bleeding						Pos. hours after bleeding					
	±	2	2–24	2–48	2–96	2–144	±	2	2–24	2–48	2–96	2–144	±	2	2–24	2–48	2–96	2–144	±	2	2–24	2–48	2–96	2–144	
S+C+V+	—	—	1	—	—	2	—	—	—	—	—	3	—	—	—	—	—	—	—	—	1	—	—	5	6
S+C–V+	—	—	—	1	—	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1	—	2	3
S+C–V–	—	5	1	4	—	5	—	1	—	—	1	5	—	—	—	—	1	—	—	6	1	4	2	10	23
S±C–V+	2	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	3
S±C–V–	87	—	—	—	—	—	28	—	—	—	—	—	4	—	—	—	—	—	119	—	—	—	—	—	119
Total ..	89	5	2	5	—	8	29	1	—	—	1	9	4	—	—	—	1	—	122	6	2	5	2	17	154

Key : S seroreactions, C clinical evidence, V Kahn verification test – negative, ± doubtful, + positive.

From Table 6 it will be seen that six patients proved to be syphilitic, and one of them showed an alteration towards seronegativity. In all other patients, clinical evidence of syphilis was missing, and the Kahn verification test was negative too, except in 6 cases which had to be left undecided, as these patients were not willing to have a lumbar puncture done on them for the cerebrospinal fluid examination.

All other readings proved to be paraluic ones, 13 of them showing an alteration towards seronegativity in the later tests (Table 7).

TABLE 7

Type / Results	L	N	LN	Total	%
Syphilitic (Alterations)	3 (1)	3 (–)	– (–)	6 (1)	1·2
Undecided (Alterations)	4 (1)	2 (–)	– (–)	6 (1)	1·2
Paraluic (Alterations)	102 (10)	35 (2)	5 (1)	142 (13)	29·7
Negative	140	178	7	325	67·9
Total	249	218	12	479	100·0

Conclusions and Summary

This investigation shows that paraluic seroreactions due to leprosy undergo an alteration similar to that observed in cases of true syphilitic sera.

Six patients were proved to be syphilitic, six other patients could not be clarified, as lumbar puncture was refused.

Of 142 patients with paraluic seroreactions 13 showed an alteration towards seronegativity.

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