

THE VALUE OF THE WIDAL TEST IN THE DIAGNOSIS OF PROLONGED SEPTICEMIC SALMONELLOSIS (1)

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S U M M A R Y

Twenty patients with prolonged septicemic salmonellosis (Group 1) and 20 with schistosomiasis mansoni (Group 2) were selected for this study. In both groups, the Widal test was done using antigens of the sample Ty 901 (*S. typhi*). The test was also applied in 6 group 1 patients with antigens prepared from *salmonellae* isolated from these patients (autoantigens). Titres over 1:200 were considered significant. Ten group 1 patients (50%) were positive for antigen "H" and 5 (25%) were positive for antigen "O". Three patients with negative "H" and "O" reactions became positive with high titres when using autoantigens. Two other cases maintained the same positive titres and one case showed a fourfold increase in titres when the test was done with antigens of the *Salmonella* isolated. The Widal test was positive in most patients infected with group D *Salmonellae*. Considering titres above 1:200, all cases were negative in Group 2. The authors conclude that the Widal test has low positivity in prolonged septicemic salmonellosis. The test may be valuable in the diagnosis of this disease when using *S. paratyphi* "A" and "B" antigens and a mixture of *Salmonella* antigens taken from other groups.

I N T R O D U C T I O N

The value of the Widal test in the diagnosis of Prolonged Septicemic Salmonellosis (PSS) is not adequately defined. A few studies^{6,7,9,10} have shown wide variations in agglutinin titres, with a significant number of patients presenting negative or inconclusive results. TEIXEIRA¹¹ states that the Widal test is positive in many cases when *S. typhi* and *S. paratyphi* are the causal agents. NEVES & MARTINS⁷ report many negative results, even when *S. typhi* is identified in blood cultures. Various suggestions to explain negative test results were made: a) anti-O agglutinin titres might fall with duration of disease⁹; b) a possible "O" blocking effect may occur in cases with Vi aggluti-

nin⁹; c) patients with disease caused by *Salmonellae* belonging to other groups (not *S. typhi* or *S. paratyphi*, on which the Widal test is based) might not produce antibodies capable of agglutinating classic Widal test antigens⁶; and d) some patients might not produce anti-*salmonella* antibodies due to immunologic failure of the B lymphocyte system⁴.

This paper presents the results of a prospective study on the positivity of the Widal test with classic antigens and with autoantigens (prepared from *Salmonellae* isolated from each patient) in PSS.

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MATERIAL AND METHODS

Identification of patients

Group 1 — twenty patients with PSS whom were admitted to the Carlos Chagas Hospital (Medical School of Federal University of Minas Gerais, Brazil) between 1972 to 1982 were selected. The diagnosis of the disease was based on 3 criteria⁵: a) fever with over 3 weeks duration; b) the presence of *Salmonella* sp in at least two blood cultures; c) viable *S. mansoni* ova in stool examinations. The patients were 5 to 20 years of age (mean 12.7), 11 male, 9 white and 11 non-white.

Group 2 — twenty patients with schistosomiasis mansoni were selected as controls. Fifteen cases were diagnosed by clinical and epidemiologic histories and positive stool examinations for *S. mansoni* ova. One case was diagnosed by a positive rectal biopsy and the remaining 4 by liver biopsies. All cases had the hepatoesplenic form of the disease. Patients were 9 to 46 years of age (mean: 21.7), 13 male, 13 white and 7 non-white.

Patients in both groups had not been vaccinated against typhoid fever and also denied previous history of typhoid infection.

Isolation and identification of Salmonellae

The following procedure was used for the isolation of salmonellae in the Laboratories of the School of Medicine: a) 10 ml of blood were added to 100 ml of BHI (Brain Heart Infusion) broth and kept at 37°C, according to BIER¹; b) the broth was observed up to 30 days, and when turbidity developed, transfer was carried out to blood agar and Teague media. After 24 hours in the former, a smear was stained by Gram's technique, and whenever Gram negative rods were found, 5 colonies were removed to TSI agar media. The same procedure was applied to Teague cultures; c) if suspected salmonellae were observed, the usual biochemical tests were applied; d) organisms with biochemical results characteristic of salmonellae were submitted to agglutination tests with polyvalent somatic and flagellar anti-salmonella serum. After this procedure, the cultures were transferred to Ligineres media and sent to the Oswaldo Cruz Institute (Rio de Janeiro) for serological typing².

Blood cultures were made for all group 1 and 15 group 2 patients.

Widal test with classic and autoantigens

The Widal test with classic antigens was performed for all group 1 and 2 patients, and the reaction with autoantigens was applied in 6 group 1 patients.

Antigens and dilutions

Somatic (O) and flagellar (H) antigens were prepared with Ty 901 samples² for detection of anti-salmonella typhi antibodies. The same method was used for producing antigens with samples taken from patients with PSS (autoantigens) to check antibody titres related to serotypes isolated from each patient.

Agglutinin titres were determined with serum serial dilutions (1:10 to 1:1280), with antigens added as described in the classical method used by WIDAL¹. Titres over 1:200 were considered significant^{10,11}.

Statistical analysis

The chi-square (X^2) test with Yates' correction was used to 5% significance level.

RESULTS

The Widal test with "H" and "O" antigens was negative in 7 (35%) group 1 patients. In 13 cases, the test was positive for antigen "H" and in 12, it was positive for antigen "O". Considering titres over 1:200 as significant, 10 patients (50%) were positive for antigen "H" and 5 (25%) for antigen "O". The Widal test was positive in all patients from which *S. typhi* was isolated (Table I).

The Widal test was negative in 12 group 2 (control) patients (60%). This difference is not significant when compared to group 1 ($P > 0.05$). Considering titres over 1:200 as positive, the reaction was negative in all cases.

Six group 1 patients were tested with autoantigens (cases 2, 6, 7, 9, 12 and 13). Sera of three patients initially non-reacting with "H" and "O" antigens were positive with high titres when using autoantigens. The remaining 3 cases (cases 9, 12 and 13) had positive Widal tests, and two of them maintained titres after

T A B L E I

The Widal test with antigens prepared with Ty 901 (*S. typhi*) sample and with autoantigens, in 20 patients with Prolonged Septicemic Salmonellosis

Cases	Initials	Age (years)	Duration of illness (days)	Isolated <i>Salmonella</i> in (blood cultures)	Classification of <i>Salmonellae</i> Groups	Widal test			
						Classic antigens		Autoantigens	
						H	O	H	O
1.	ERV	13	90	<i>S. paratyphi A</i>	A	neg.	neg.	—(*)	—
2.	JES	15	60	<i>S. typhimurium</i>	B	neg.	neg.	1:1280	neg.
3.	JPS	14	90	<i>S. typhimurium</i>	B	1:160	neg.	—	—
4.	MAR	14	90	<i>S. typhimurium</i>	B	neg.	neg.	—	—
5.	NVC	17	45	<i>S. typhimurium</i>	B	neg.	neg.	—	—
6.	AID	12	60	<i>S. asteca</i>	B	neg.	neg.	1:640	1:1280
7.	MLNP	18	75	<i>S. concordia</i>	C	1:160	1:40	—	—
8.	LNF	9	30	<i>S. concordia</i>	C	neg.	neg.	1:640	1:1280
9.	FAS	7	40	<i>S. kentucky</i>	C	1:160	1:20	1:640	1:80
10.	EAH	18	28	<i>S. typhi</i>	D	1:1280	1:160	—	—
11.	LJS	14	30	<i>S. typhi</i>	D	1:1280	1:160	—	—
12.	LNS	7	105	<i>S. typhi</i>	D	1:320	1:640	—	—
13.	IAC	9	240	<i>S. typhi</i>	D	1:1280	1:160	1:1280	1:160
14.	DBS	12	120	<i>S. dublin</i>	D	1:1280	1:1280	1:1280	1:1280
15.	RFM	5	30	<i>S. dublin</i>	D	1:320	1:320	—	—
16.	RM	20	120	<i>S. dublin</i>	D	1:1280	1:640	—	—
17.	LP	5	150	<i>S. enteritidis</i>	D	1:1280	1:40	—	—
18.	JQS	15	365	<i>S. enteritidis</i>	D	1:1280	1:1280	—	—
19.	MFP	16	210	<i>S. pensacola</i>	D	1:320	1:80	—	—
20.	MMS	15	90	<i>S. berta</i>	D	neg.	neg.	—	—

(*) test not done

autoantigen testing. Case 9 (*S. kentucky*) presented a fourfold increase in agglutinin titres when tested with *Salmonella* autoantigens.

From 11 patients infected with Group D *Salmonellae* only one had a negative Widal test. On the other hand, the same test was negative or presented low titres in patients infected with group A, B and C *Salmonellae* (Table I).

DISCUSSION

We conclude that the Widal test is of little value in the diagnosis of PSS. Significant diagnostic agglutinin titres were found in 25% for "O" and in 50% for "H" antigens. As antigen "H" is less important due to anamnestic reactions with other infections, the serologic diagnosis of PSS becomes restricted. The duration of the disease does not seem to influence positivity, as pointed out by TEIXEIRA⁹. We found cases with high agglutinin titres at both extremes (Table I).

Negative tests converting to positive when using autoantigens are in agreement with predictions made by NEVES & MARTINS⁶. The positivity of the Widal test in patients infected with Group D *Salmonellae* is explained by the

presence of common antigens in *Salmonellae* of the same group of *S. typhi*. The disease caused by *Salmonellae* of other groups shows negative Widal tests. Positive results in the four *S. typhi* cases confirm previous observations¹¹; it must be noted that 3 of these patients had anti-O agglutinin titres under 1:200. In only one case was *S. paratyphi A* isolated and the Widal test was negative. Using *S. paratyphi A* antigens, the test would probably become positive. In case 20, Group D *S. berta* was isolated, and the test was negative. Further explanations are needed in these cases⁴, as both *S. typhi* and *S. berta* have common determinant antigens (antigens 9 and 12).

The Widal test was positive in 8 group 2 (control) patients (40%), with titres under 1:200. Similar results were obtained by SHIKANAI-YASUDA et al.⁸ in patients with hepato-splenic schistosomiasis in the same age groups.

It may be concluded that the Widal test with "H" and "O" antigens yields few positive results in patients with PSS. Using *S. paratyphi A* and B antigens and a selection of antigens of *Salmonellae* belonging to various groups (determined by the local prevalence of *Salmone-*

lae), positivity may be increased, enabling the Widal test to become a useful tool in the diagnosis of this disease.

RESUMO

Valor da reação de Widal no diagnóstico da Salmonelose Septicêmica Prolongada (SSP)

Vinte pacientes com SSP (Grupo 1) e 20 com esquistossomose mansoni (Grupo 2) foram selecionados para o estudo. Realizou-se a reação de Widal, com antígenos preparados da amostra Ty 901 (*S. typhi*), nos pacientes dos Grupos 1 e 2. Em seis pacientes do Grupo 1, a reação de Widal foi feita com antígenos preparados a partir de salmonelas isoladas destes pacientes (autoantígenos). Foram considerados significativos os títulos superiores a 1:200. Dez pacientes do Grupo 1 (50%) tiveram a reação positiva para o antígeno "H" e cinco (25%) para o "O". Em quatro casos em que se isolou a *S. typhi*, a reação de Widal foi positiva. Em três pacientes com reação negativa para "H" e "O", houve viragem da reação com títulos elevados quando se utilizou autoantígenos; outros dois casos positivos mantiveram os mesmos títulos e em um caso houve elevação de quatro vezes nos títulos quando a reação foi feita com antígenos da *Salmonella* isolada. A reação de Widal foi positiva na maioria dos casos quando as salmonelas isoladas eram do Grupo D. Para o grupo 2, caso se aceitem os títulos superiores a 1:200 todos os exames foram negativos. Os Autores concluem que a reação de Widal apresenta baixa positividade na SSP. Com o uso de antígenos de *S. paratyphi* A e B e de uma mistura de antígenos de salmonelas de grupos distintos, a reação provavelmente será de valor no diagnóstico da SSP.

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