ARCHIVES OF ACOUSTICS
9, 1-2, 117-120 (1984)

THE PASSIVE HAEMAGGLUTINATION REACTION WITH SONICATED *T. PALLIDUM* (NICHOLS STRAIN) IN THE SERODIAGNOSIS OF SYPHILIS (TPHA)

VOICHITZA LAZAR, FLORICA KEVORKIAN,
IULIU CĂPUŞAN, DEZIDERIU AUSLANDER, EVA VERESS

Clinic of Dermatology, Institute of Medicine and Pharmacy

Babes-Bolyai University, Ultrasonic Laboratory, Cluj-Napoca, Romania

The preparation of the antigen for TPHA sonicated suspension of *T. pallidum* (Nichols strain) being so far a "secret" of the procedures, we have attempted to reproduce it by our own means of ultrasonic irradiation. The best results in agreement with the standard reactions for syphilis were obtained by sonicating *T. pallidum* suspensions for a duration of 20 min. by the following parameters of the ultrasonic field: frequency 1 MHz, intensity 0.9 W/cm^2. Using this method we obtained microfragments of 1-1.3 microns. The most reliable dilution for the antigen was 1/1000. The haemagglutination was performed in 30 patients with different forms of late syphilis after treatment.

The results were in agreement with the clinical diagnosis, being more sensitive and consistent compared with the classical and standard reactions.

TPHA is highly useful in mass screenings, as an indicator of the therapeutical efficiency and in the evaluation of the persistent positive perological reactions or in discordant serology.

TPHA was introduced in 1965 by RATHLEV [6, 7] for syphilis serodiagnosis, and it was completed in a standardized reaction by TOMIZAWA and KASAMATSU in 1966 [8], a laboratory standardized technique being realized by COX *et al.* in 1968 [2].

The first attempt to introduce this technique in Romania, using treponemic antigens or standardized Japanese antigen (Fujikoki Pharm. Ltd.) was made by BĂDĂNOIU *et al.* in 1976 [1] and GEORGESCU and IONESCU in 1979 [3].

The advantages of TPHA include: simplicity, specificity by use of pathogen Treponema strains, high sensitivity, fidelity in excess of 95 per cent over the standard reaction, including the most specific reaction as FTA and TPI, precocity and persistence, permitting also a retrospective serological diagnosis [1]

The field in which TPHA is recommended is: mass syphilis screening together with a lipoidic reaction, serological diagnosis in the late syphilis replacing other treponemic reactions as FTA-Abs and TPI. TPHA can be used with a higher specificity than FTA-Abs and TPI as a control serological test after the treatment.

There are some geographical variations of TPHA, the test being used with antigens from Japan (Fujikoki — 5), England and BRD (Cellagnost — 4).

The preparation of the antigen (sonicated suspension of *T. pallidum* (Nichols strain)) being a "secret" of the above procedures, we have attempted to reproduce it by our own means of ultrasonic irradiation.

Using 1 ml of *T. pallidum* (Nichols strain) liofilized antigen diluted in distilled water, exposed to ultrasonic irradiation in an ultrasonic TESLA type apparatus with a 40 mm diameter quartz, we checked the fragmentation by an ultramicroscopic procedure in dark field.

The best results agreeing with the standard reactions for syphilis were obtained by sonicating *T. pallidum* suspensions for a period of 20 min., in several intervals (30 s, 5, 4 and 10 min.), by the following parameters of the ultrasonic field; frequency 1 MHz, intensity 0.9 W/cm². Using this method we obtained microfragments of 1-1.3 microns (Fig. 1).

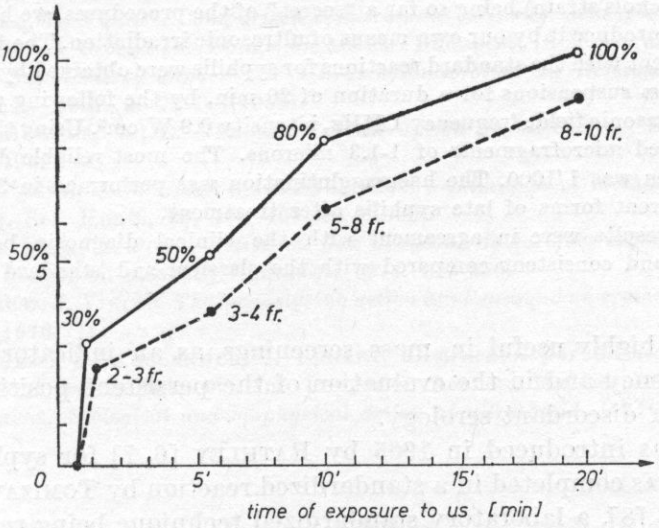

Fig. 1. T. pallidum fregmentation by ultrasound related to the time parameter. — fragmentation at % elements, — — number of fragments

A graduate disintegration of *T. pallidum* after ultrasonic exposure, using the same frequency and intensity of US and changing the time parameter, is due to the quality of treponemic suspension. This is a mixture of different susceptibilities of the microorganism varying with the length, age and biological stage.

The haemagglutination was performed with progressive dilutions of the sonicated *T. pallidum* antigen, the most reliable dilution being 1/100. The dilutions of patient serum ranged from 1/2 to 1/256.

We used the serum of 30 patients with different forms of late (secondary, latent) and congenital syphilis, the cases being after the treatment. The reference case was a patient with psoriasis and no clinical nor serological signs of syphilis (Table 1).

Table 1. The results of serological reactions for syphilis using TPHA with sonicated *T. pallidum*

Clinical diagnosis of syphilis	Number of cases	Type of serological reaction									
		RFC		Floculation		Reaction		Treponemic			
		RBW		Citochol		KAHN		VDRL		TPHA	
		+	−	+	−	+	−	+	−	+	−
Secondary syph.	12	7	5	7	5	7	5	7	5	10	2
Latent pyph.	11	2	9	10	1	10	1	10	1	6	5
Seroresistent syph.	2	2	—	2	—	2	—	2	—	2	—
Congenital syph.	1	1	—	1	—	1	—	1	—	1	—
Discordant serology	4	—	4	2	2	2	2	2	2	2	2
Total cases	30	12	18	22	8	22	8	22	8	21	9
[%]	100	40	60	73.3	26.6	73.3	26.6	73.3	26.6	70	30

The results show the following aspects:

1. The TPHA test was in agreement with clinical diagnosis, being more sensitive and consistent compared with the classical and standard reactions.

2. TPHA is more sensitive than RBW and with almost the same sensitivity than floculation reactions.

3. Cases with positive floculation reactions and negative RBW and TPHA were considered recovered, the floculation reactions being false because of liver dysfunctions (4 cases).

4. After the treatment TPHA is the only test that indicates a real recovery. The treatment will be continued when the TPHA test remains positive, even in cases with negative lipoidic reactions (7 cases).

5. TPHA can be erroneously positive in patients with severe disglobulinemia (1 case).

6. In condition of discordant serological reactions TPHA will coroborate with the floculation reactions, even when RBW gives different results (2 cases).

7. TPHA is highly useful when it is used together with VDRL in mass screenings, as an indicator of the therapeutical efficiency, and in the evaluation

of the persistent positive serological reactions or in discordant serology. Being much simpler than other treponemic reactions (FTA – Abs, TPI), this test must be introduced in all laboratories as a routine test for the diagnosis in syphilis.

8. Our method for antigen preparation by sonicating *T. pallidum* (Nichols strain), changing the time parameter, is the first attempt in Romania to obtained sonicated suspensions for TPHA.

References

[1] Al. Bădănoiu, G. Nicolau, Florica Brăilescu, *Observaţii privind comportarea testului de hemaglutinare pasivă cu antigene treponemice in cursul sifilisului,* Derm. Ven. (Buc.), **21**, 161-165 (1976).

[2] P. U. Cox, L. C. Logan, L. C. Norins, *Automated quantitative microchemagglutination assay for Tr. pallidum antibodies,* J. Appl. Microbiol., **18**, 485-489 (1968).

[3] M. Georgescu, D. Ionescu, Silvia Dumitru, *Reacţia de hemaglutinare pasivă in serodiagnosticul sifilisului,* Microbiologia (Buc.), **24**, 109-114 (1979).

[4] Jaj Barbare, Salker Rajas, Lalji Fatima, T. D. Davies, J. D. Harris, *An economical, simplified haemagglutination test for mass syphilis screening,* J. of Clinical Pathol., **33**, 1216-1221 (1980).

[5] J. Lesinski, J. B. Dudzisz, E. Kadziewicz, WHO/VDT/Res., 317-319 (1974).

[6] T. Rathlev, WHO/VDT/Res, 77-79 (1965).

[7] T. Rathlev, *Haemagglutination test utilizing pathogenic Tr. pallidum for the serodiagnosis of syphilis,* Brit. J. Vener. Dis., **43**, 142-148 (1967).

[8] T. Tomizawa, S. Kasamatsu, *Haemagglutination test for diagnosis of syphilis,* Japan, J. Med. Sci. Biol., **19**, 305-311 (1966).