Laboratory and clinical study

LIMITATIONS AND CONSEQUENCES OF BASING LATE SYPHILIS SEROASSESSMENTS ON VDRL TEST

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ABSTRACT

The purpose of this paper is to caution about a recommendation made in 1996 by the 39th Meeting of the European Committee on Health, which operates within the Council of Europe. It prescribes a screening for the agents of major microbial infections, syphilis included, that endanger transplantation. This leaves an alternative in choosing between the VDRL (non-treponemal) and TPHA (treponemal) tests. With a retrospective analysis of the findings of our tests collected in a proficiency testing program, we have attempted to estimate the size of a potential diagnostic miss that could result from such recommendation. The results were obtained on 18,094 subjects (nonvenereal inpatients, psychiatric asylum patients, persons undergoing preemployment examination, persons seeking medical certificates or international health documents, pregnant women screened for syphilis) who were tested with both tests simultaneously and came from two public health laboratories. A false negative VDRL ratio calculated from the 265 infected (i.e., TPHA-reactive) was defined as a diagnostic miss. It amounted to 84.5%. A statistical procedure derived from the Bayes theorem was used to assess the active late syphilis, yielding a value as high as 21.0% per 265 subjects infected, which was significantly more than the percentage suggested by the VDRL reactive test (15.5%). These results suggest that, for the needs of transplantation, organ and tissue donors (and all venerological or nonvenerological subjects) should also undergo the screening for syphilis with the TPHA test as is usual in transfusiology.

KEY WORDS

VDRL, screening disadvantages, late syphilis detection model.

INTRODUCTION

Recommendations on the methods to use in serological screenings for the most prevalent microbial infections (one being syphilis) linked with tissue and organ transplantation came from the 39th Meeting of the European Health Committee (CDSP) held in Strasbourg in June 1996 (1). This committee operates within the Council of Europe. Although the risk of syphilis transmission through transplantations may be small, positive reaction in a syphilis test is indicative of the donor's hazardous sex behavior.

Table 1. The value of the VDRL test's nosological sensitivity (SE_X)[a] based on the TPHA reference test[b], as well as the VDRL false negative (FN) rate[c] were determined for a public health population of 18,094 subjects[d].

Value of the VDRL test (SE_X)[a]	
SE_X	VDRL FN rate
15.5%	84.5%

[a] SE_X = (VDRL+ /TPHA+) • 100

[b] All 265 TPHA reactors were considered to be infected with *T. pallidum,* all nonreactors being uninfected.

[c] In the parallel strategy (both tests done simultaneously) users, the VDRL FN rate represented a hypothetical miss in the detection of those infected with syphilis. Conversely, in serial strategy (VDRL used as a screening test and TPHA as a confirmation test), the VDRL FN rate constituted a genuine diagnostic miss.

[d] Of the 18,094 subjects tested the 265 syphilis infected comprised almost exclusively old syphilis either late treated syphilis or untreated syphilis.

Further, the cost of the test is minimal, it is the most widely used serotest, and one with which there is a long experience. Besides, it unveils the risk of other known sexually and parenterally transmitted diseases and of the still unexplored diseases for which efficient screening techniques have not become routine yet.

There is thus every justification to perform an effective screening for syphilis e.g. at STD clinics, public health dispensaries, in blood donors' centers. Such selection of tests differs from the one undertaken to detect patients suspected to be infected with (early) syphilis.

In screenings for syphilis in addition to the TPHA hemagglutination test (or an alternative test) VDRL (non-treponemal antigen serologic test) should be used. The latter is known for its lower specificity and considerably lower sensitivity at later stages of the disease, a fact which is mostly disregarded or overlooked.

A different practice derived from some older misunderstandings, is found to cause negative public health effects as confirmed by the recent findings in a proficiency testing program carried out by the Croatian National Institute of Public Health from 1993 to 1995 in our clinical, public health and blood transfusion laboratories which perform syphilis serotests (2). As in most European countries, a request for serological test in Croatia assumes that syphilis will be investigated at any stage. A problem may arise under the influence of the above meeting's conclusions (1) or the routine in the USA, where unless accompanied by other indications, a request for syphilis screening will result in the use of algorithm needed to detect the epidemiologically more dangerous and markedly infectious early syphilis (3). This was stressed by one of us (I.V.) at the Strasbourg meeting. According to its conclusion VDRL is the only test or the first in a series with the TPHA test (screening with VDRL and confirming with TPHA only the VDRL positives). If a general practitioner in the US wants to look for latent syphilis or for late active syphilis, he is expected to specially emphasize this on the request form or add

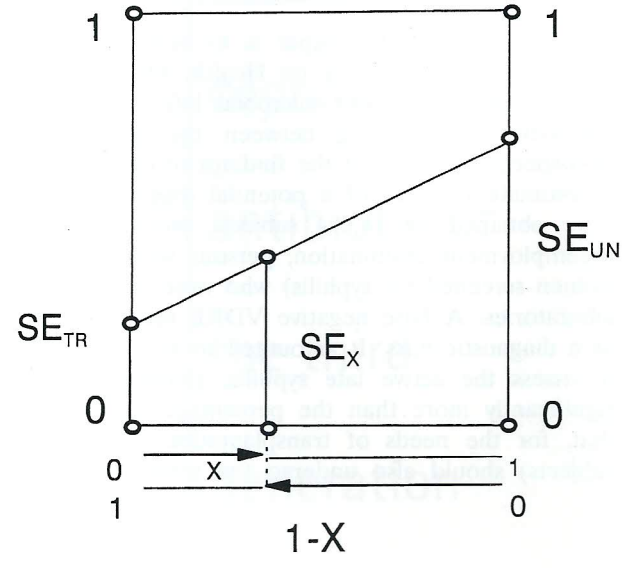

Figure 1. Graphic model of the detection of late active untreated syphilis.

SE_X - VDRL test sensitivity observed in our study
SE_{TR} - VDRL test sensitivity in treated late syphilis
SE_{UN} - VDRL test sensitivity in patients with untreated late syphilis
x - proportion of untreated late active syphilis among the infected found in the study
Note. All terms are expressed in terms of proportion.

Table 2. Nosological sensitivity and specificity of the tests used in testing for syphilis, and some characteristics of the 18,094 population tested.

Nosological sensitivity and specificity				
Syphilis stages with highest presumed prevalence among the study population	Estimated age of VDRL and TPHA reactors	Screening tests used	Nosological sensitivity (SE) of tests (%) Range (in brackets) of literature given values (3,4)	Nosological specificity (SP) of tests (%)
Untreated cases of latent and late syphilis	x=60.1 yrs. Range 37 to 80 yrs N=28	VDRL	SE_{UN}=70[a] (34-94)	98 (96-99)[c]
		TPHA	100 (97-100)[c]	99 (98-100)[c]
Cases of late treated or of self-healed syphilis		VDRL	SE_{TR}=1[b] (0-1)	98 (96-99)[c]
		TPHA	100 (97-100)[c]	99 (98-100)[c]

a SE_{UN} - VDRL test sensitivity in untreated latent and late syphilis.
b SE_{TR} - VDRL test sensitivity in treated late and self-healed syphilis.
c For simplicity it was assumed that the above values equalled 100%. Also ignored was the 0.3% of the cases registered as isolated VDRL reactors (biological false positive reactions or early syphilis).

such a remark to it. After that, the laboratory will employ other tests, i.e. those for antitreponemal antibody demonstration (mainly of the IgG class) such as the TPHA test, and the tests for the demonstration of process activity (19 S IgM tests) - for the individuals whose infection had previously been demonstrated.

As our old population is practically free from early syphilis the combination VDRL+ and TPHA+ primarily indicates late active untreated syphilis.

MATERIALS AND METHODS

Based on our proficiency testing program findings (2), we have attempted to assess the size of two potential misses:

1. The miss due to interlaboratory differences in the effectiveness of detection of individuals infected with *T. pallidum* at any time in their lives. Miss of this kind can be readily detected (and is demonstrable directly or indirectly) by using in addition the parallel testing strategy on the same or similar population. In this case both tests are done simultaneously on the same analytical sample. Mathematically, this is expressed as VDRL test's false negative rate in percentages as determined on a population of the TPHA positive patients (i.e., on the syphilis-infected in the course of their lives regardless of the stage of the disease or process activity) (Table 1).

2. The second type of potential miss occurs when detecting late, and probably active, forms of syphilis. It was assumed that the VDRL test has a maximum specificity, but various sensitivity (ranging from 0.01 to 0.70) which depends on the stage of the disease and its status of treated or untreated (3,4) (Table 2). From this we proceeded to making the assessment using the statistical procedure derived from the Bayes theorem (5), which is based on a ratio (x) of two differences, i.e., the nosological sensitivity (SE_X) observed in our study for VDRL, and the VDRL sensitivity already known from the literature (3) for persons treated for late or latent syphilis (SE_{TR}). All this is shown in the numerator. The second difference is that between the VDRL test's sensitivity to the persons untreated for latent and late syphilis (SE_{UN}) and VDRL's sensitivity to the persons treated for latent and late syphilis (SE_{TR}). Both of these have been previously established by other workers (4). This difference is represented by the denominator.

Table 3. The number of cases infected with T. pallidum who were detectable by means of a parallel (P), respectively serial (S) testing strategy of combining the VDRL and TPHA tests.

Number of cases infected with *T. pallidum*			
Subject total tested	No. of infected detected by strategy P	No. of infected detected by strategy S	The infected total detected by both strategies (S+P)
18,098	265	41	265

x Parallel strategy (P) involves simultaneous use of the VDRL and TPHA tests on a sample.
In the serial strategy (S), only VDRL test reactors are additionally checked with the TPHA test.

The thus calculated quotient (x), is the proportion of presumed late active but untreated syphilis among the infected.

$$x = SE_x - SE_{TR}/SE_{UN} - SE_{TR}$$

RESULTS AND DISCUSSION

Graphically, a solution to "x" is illustrated in Fig. 1. The error first mentioned, or a potential miss, was calculated from a public health laboratory's test findings on 18,094 subjects (without clinical or epidemiological data). Of the 265 infected whose *T. pallidum* infection was demonstrated using the TPHA test, 41 were simultaneously also reactive in the VDRL test. Consequently, we regarded them (because of their average age of 60.1 yrs; range 37 to 80; N= 28) as having late untreated syphilis, or as (not very likely) cases of recently treated but not yet seronegativized syphilis. Had the infected been detected by the often recommended serial strategy, which combines the VDRL and TPHA tests, and not with the parallel strategy, as few as 41 (15.5%) infected individuals would have been found instead of the 265, with the miss rate amounting to 84.5% (Tables 1,3).

The second error or miss was assessed by taking the same material. It consisted in making a statistical assessment of the share of presumably late active syphilis in our sample of the 265 infected. On introducing in the afore-mentioned formula (5,6,7) an VDRL SE_x of 15.5% previously noted in our study (Table 1), that of 70% (3) cited by most reports for the untreated with late syphilis (SE_{UN}), and one of 1% (4) for the treated cases (SE_{TR}) (Table 2), the assessment showed that the 265 infected who had been detected included as many as 56 (21%) people affected by late active syphilis (Table 4). This is 15 persons more (56 instead of 41, i.e. 21% instead of 15.5% of 265 infected) than screening with the VDRL test only, would reveal (although the method does not permit an identification of affected individuals) (6,7). Moreover, the method enables calculations of the confidence

Table 4. The results of a retrospective analysis of a screening for syphilis covering 18,094 public health subjects: different numbers of late active syphilis suspects under standard interpretation and according to the Bayes theorem.

Results of a retrospective analysis of a screening for syphilis			
Subject total tested	No. of infected detected	No. of late active syphilis (VDRL+ and TPHA+)	Estimate of the number of late active syphilis cases based on Bayes theorem
18,094	256	41	56

limits of an assessment, ignored here for the sake of clarity.

Both diagnostic misunderstandings may be avoided by planning the screening with regard to the type of test(s), the strategy of combining them and by the presumed prevalence of individual stages of the syphilitic process in a patient population.

CONCLUSIONS

An attempt has been made to evaluate a diagnostic miss in detecting old (and probably also active) syphilis in the routine use of VDRL and TPHA tests (VDRL test being the first in serial testing). Our data confirm the size of the miss to be specifically dependent on the mode of combining the two tests. By using an original statistical procedure we have uncovered a probably additional diagnostic miss.

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