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Analysis of the serological diagnosis of syphilis: A proposal for improvement

Análisis del diagnóstico serológico de la sífilis: una propuesta de mejora

Serological techniques are the gold standard for the diagnosis of syphilis, with the reverse algorithm being the most widely used. This consists of an automated treponemal screening test, followed by a second confirmatory treponemal test and a third non-treponemal test. However, since it was published in the early 2000s, this algorithm has barely been modified.[1,2] Numerous studies now point to the utility of the index provided by screening platforms for predicting infected patients who do not require additional confirmatory tests, and for predicting potential false positives.[3] However, for application in routine clinical practice, this index has to be validated for each individual diagnostic screening platform.

In view of the above, we set out to determine the relationship between the index value, expressed as a signal-to-*cut off* (S/CO) ratio, of samples reactive for the Alinity Syphilis TP assay (Abbott Diagnostics, Abbott Park, IL) based on chemiluminescent microparticle enzyme immunoassay (CMIA), and the result of a second confirmatory treponemal test. To our knowledge, this is the first evaluation of these characteristics performed with the Alinity Syphilis TP assay.

We studied serum samples positive for the Syphilis TP assay processed in two third-level hospitals, corresponding to the two health areas of Valladolid, over a two-year period (January 2022–January 2024). A sample with an index value ≥1 S/CO was considered positive. Depending on the processing centre, confirmatory treponemal techniques available during the study period were INNO-LIA Syphilis Score (Fujirebio, Gent, Belgium) or TPHA (Master Labor SL, Madrid, Spain). We consider a true positive (TP) to be a patient with a positive Syphilis TP and positive confirmatory test, and a false positive (FP) to be a patient with a positive Syphilis TP and a negative or indeterminate confirmatory test, confirmed with a second serum sample. We excluded patients with positive Syphilis TP and negative confirmatory test, in whom a second serum sample was not analysed. Continuous quantitative variables were expressed as median and interquartile range (IQR). The relationship between the S/CO of the Syphilis TP assay and the outcome of confirmatory screening tests was assessed using ROC curves. Statistical differences were evaluated using the Mann–Whitney U test ($p < 0.05$).

We included 1676 Syphilis TP assay-positive samples, corresponding to 832 patients. The median age was 43 (IQR: 33–56) and 76% were male. The confirmatory techniques used were INNO-LIA-Syphilis Score in 42.9% of cases and TPHA in 57.1%. A total of 776 patients (93.3%) were diagnosed as true positives and 56 (6.7%) as false positives. The median for true positives was 17 S/CO (IQR: 11–20.6) while for the false positives it was 2.4 S/CO (IQR: 1.6–2.9) ($p < 0.001$). ROC analysis revealed that 100% of patients with a screening test score greater than 10.37 S/CO were true positives, so it would not have been necessary to perform confirmatory tests and, taking into account the price of these techniques (20.57 euros for the INNO-LIA Syphilis Score and 2.05 euros for the TPHA), this would have meant a saving of 11,993.70 euros in our study period (Fig. 1). In terms of reducing the percentage of false positives, the ROC analysis showed an optimal cut-off point of 5.88 S/CO

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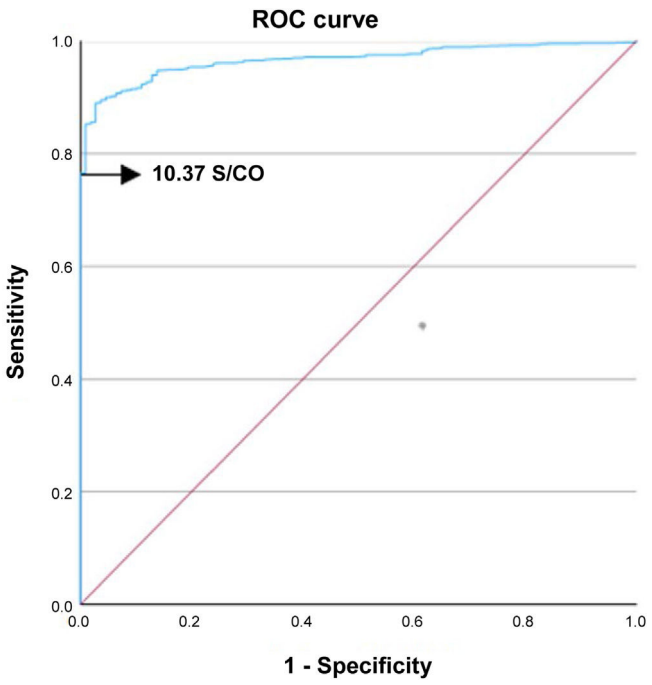

Fig. 1. ROC curve for the S/CO of samples positive for the Alinity Syphilis TP assay and confirmatory test results. The value 10.37 S/CO is marked. The area under the ROC curve is 0.967 (95% CI: 0.957–0.976; $p < 0.001$).

(Youden Index = 0.863), which would reduce the false positives by 97.2% while maintaining 89% sensitivity.

Our results are consistent with those obtained by Demir Çuha et al.[4] and Dai et al.[5] who, in separate studies using the Architect platform (Abbott Diagnostic), also based on CMIA, obtained an index value of 10.47 and 9.9 S/CO respectively, above which 100% of the samples were true positives, highlighting the reproducibility and robustness of the value obtained in our study. In contrast to these results, in a multicentre study conducted in Canada using the Architect platform, Serhir et al.[6] a result was obtained of 16.4 S/CO, consequently, a confirmatory test would not be necessary.

Based on all the results, we can conclude that increasing the cut-off point of the Syphilis TP assay would lead to a decrease in sensitivity inappropriate for a screening test. It would be of interest to establish defined cut-off points (S/CO) above which confirmatory testing would not be necessary, as this would enable cost savings and optimisation of diagnostic tests. However, we currently do not have universal cut-off points that allow us to standardise these techniques. To do this, it would first be necessary to validate these *cut-offs* for each of the different diagnostic screening platforms. Secondly, each case would have to be individualised, taking into account the particular characteristics of each patient that might interfere with this type of technique,[7] as well as the prevalence of infection in different populations. Lastly, until more conclusive results than those available to date are obtained, it is essential to be cautious when interpreting and reporting these values.

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