

## Investigation of syphilis coinfection and performance of the Architect Syphilis Tp ELISA screening test in HIV positive patients

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**Background/aim:** Limited data on syphilis coinfection in human immunodeficiency virus (HIV) positive cases exist in Turkey. Our aim is to investigate syphilis coinfection and to evaluate the compatibility of the screening Architect Syphilis Tp ELISA with the fluorescent treponemal antibody absorption (FTA-abs) confirmation test in HIV positive cases.

**Materials and methods:** Totally 519 HIV positive patients were included in the study. Enzyme linked fluorescent assay (ELFA) was used as a screening test and positive samples were confirmed by line immunassay (LIA). In order to discriminate acute HIV infection and false ELISA positivity, HIV-1 RNA PCR was performed in ELFA positive and LIA negative samples. Architect Syphilis TP ELISA was used for the detection of total antibodies against *Treponema pallidum* in HIV positive patients. Positive results were confirmed by the FTA-abs test.

**Results:** Out of 519 HIV-1 positive patients, IgG and IgM positivity, and only IgG positivity was detected as 1.9% and 11.4% in all the samples, respectively. A total of 79 (15.2%) sera were positive with Architect Syphilis Tp ELISA test and 69 (13.3%) were positive with FTA-abs test. Statistically significant, almost perfect agreement was found between Architect Syphilis Tp ELISA and FTA-abs tests ( $\kappa = 0.921$  and  $P < 0.001$ ).

**Conclusion:** Implementation of syphilis and HIV screening tests together among risk groups is considered to be appropriate.

**Key words:** Syphilis, HIV/AIDS, coinfection

### 1. Introduction

Sexually transmitted infections (STIs) remain a major public health problem all over the world. Each year around three-fourths of the estimated 340 million new cases are reported from developing countries and STIs account for 17% of all economic losses due to treatment costs (1). Nowadays, AIDS and syphilis are the most commonly reported sexually transmitted diseases (2). Although the incidence of syphilis has declined since the 1940s with the widespread use of penicillin, this infection reemerged due to the dramatic increase in HIV positive patients in the 1980s. In addition, syphilis is still one of the most important causes of death in developing countries where HIV infection is frequently detected (3). The incidence of HIV/STI coinfection is more common among individuals who are newly diagnosed with HIV (4). Coinfections between HIV and *Treponema pallidum*, the causative agent of syphilis, are common due to shared routes of sexual transmission and epidemiological similarities (3,5). HIV may affect syphilis transmission,

clinical course, and response to treatment and can also alter its serological diagnosis (6). On the other hand, syphilis can mimic various clinical aspects and cause cardiovascular and neurological problems among HIV positive patients (3).

In recent years, different control programs have been carried out throughout the world to control STIs. Syphilis and HIV screening have been implemented together among risk groups in some countries (1). Given the increased rates of syphilis in HIV infected patients, routine periodic screening (two to four times in a year, at least once) is highly recommended (7). Recently, syphilis coinfection in HIV infected individuals has been reported from several countries, mostly among African Americans (6). In Turkey, there are limited data on syphilis coinfection in HIV positive cases. The aim of the present study was to determine syphilis coinfection and to evaluate the compatibility of the Architect Syphilis Tp ELISA screening test with the confirmatory fluorescent treponemal antibody absorption (FTA-abs) test in HIV positive cases.

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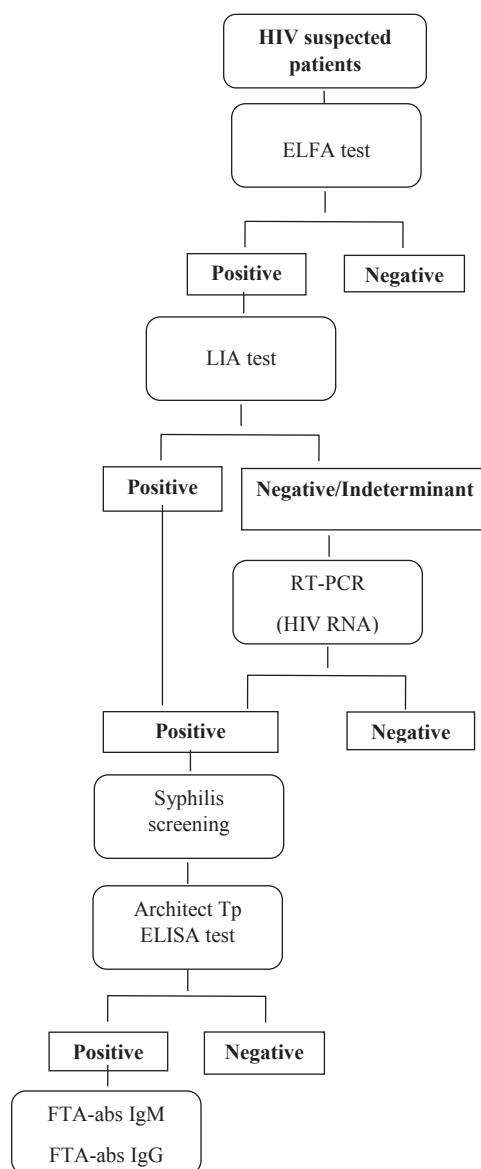
## 2. Materials and Methods

During the period of May 2016 to April 2017, a total of 519 confirmed HIV-1 positive serum samples of the patients aged  $\geq 15$  years that were sent for HIV confirmation to the Ministry of Health National HIV-AIDS and Viral Hepatitis Reference Laboratory in Ankara, from all provinces in Turkey, except İzmir, İstanbul, and the Thrace Region, were included in the study. Samples with sufficient sera for syphilis screening and FTA-abs confirmation tests were included in the study. All samples were checked and repeated samples were excluded. Only the first samples of the patients were evaluated. A two-step HIV diagnostic algorithm was performed as recommended by the Centers for Disease Control and Prevention (CDC) (8). The VIDAS HIV DUO Ultra (BioMérieux, France) enzyme linked fluorescent assay was used as a screening method for HIV suspected patients. Positive test results with ELFA were confirmed by testing for antibody presence with line immunoassay (LIA; Inno-LIA HIV-1/2 Score, Fujirebio, Belgium). ELFA positive and LIA negative or indeterminate samples were tested by reverse transcription polymerase chain reaction (RT-PCR; Artus HIVirus-1 RT-PCR, QIAGEN, Germany) to identify acute HIV infection. HIV RNA positive specimens were evaluated as HIV positive. The positivity of ELFA method was interpreted as a false positive reaction in the samples without viral load.

In HIV positive patients, syphilis serostatus screening was determined by total antibody (IgM+IgG) Architect Syphilis TP ELISA (Abbot Diagnostics, USA) test. Samples, which were positive by screening test, were confirmed by the FTA-abs IgM and FTA-abs IgG (Euroimmun, Germany) tests (Figure 1). HIV positive patients who were positive with Architect Tp ELISA, FTA-abs IgM and FTA-abs IgG tests were evaluated as having acute coinfection and rapid plasma reagin (RPR) test (Omega, UK) was performed for all these sera. IBM SPSS version 23 statistical program was used for statistical evaluations and descriptive information was shown by number and percentage distributions. The agreement between ELISA and FTA-abs tests was assessed by the kappa test. A P-value below 0.05 was considered as statistically significant.

## 3. Results

Out of 519 HIV-1 positive patients, 69 (13.3%) were positive for syphilis. IgG positivity was detected in 85.5% (59/69) of positive patients, and IgM positivity together with IgG positivity was detected with a rate of 14.5% (10/69). IgG and IgM positivity was detected in 1.9% (10/519) and only IgG positivity was detected in 11.4% (59/519) (Table 1) of all samples. All samples, which were IgM and IgG positive by the confirmatory test, were positive by RPR test with  $\geq 1/32$  titer.



**Figure 1.** Algorithm of the laboratory diagnosis of HIV and syphilis coinfection.

The mean age was  $40.9 \pm 13.4$  and syphilis positivity was most frequently detected in the 35–44 age group with a percentage of 30.4% (21/69). This was followed by the age groups 25–34 and 45–54 with the rates of 27.5% (19/69) and 17.4% (12/69), respectively (Figure 2). When all the age groups were evaluated separately, IgG positivity was higher than the acute infection (IgM and IgG positivity) rates (Figure 3).

HIV positivity was detected among patients who were resident from a total of 50 different provinces for HIV confirmation in our study. IgG positivity was determined in patients from 20 provinces. In addition, IgM and IgG

**Table 1.** Antibody positivity rates by FTA-abs test in HIV-positive patients (n = 519).

Antibody positivity	Number (n)	Percent (%)
Only IgG positivity	59	11.4
IgM and IgG positivity	10	1.9
Negative	450	86.7
Total	519	100.0

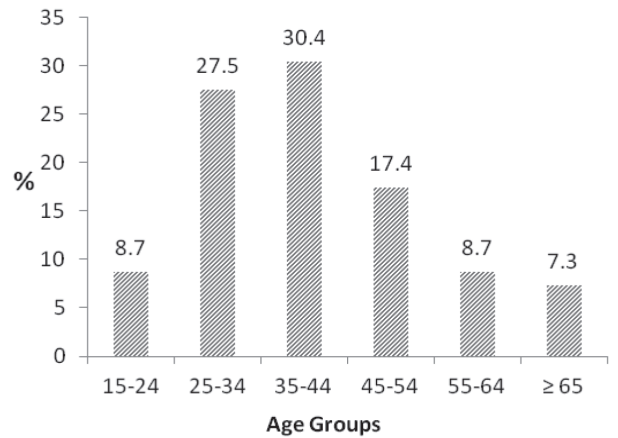
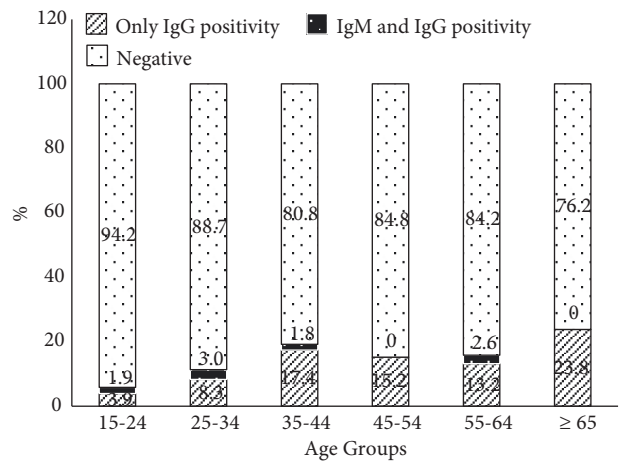
positivity were detected in samples sent from Düzce, Bursa, Isparta, Antalya, Adana, Ordu, Diyarbakır, and Mardin (Figure 4).

Out of 519 HIV-1 positive patients, 79 (15.2%) were positive with Architect Syphilis Tp ELISA test. Of the 79 ELISA reactive samples 69 (87.3%) were positive by FTA-abs test. When the FTA-abs test was considered as the confirmation method, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Architect Syphilis Tp ELISA test was 100% (69/69), 97.8% (440/450), 87.3% (69/79), and 100% (440/440), respectively. Statistically significant, almost perfect agreement was found between Architect Syphilis Tp ELISA and FTA-abs tests ( $\kappa = 0.921$  and  $P < 0.001$ ) (Table 2).

#### 4. Discussion

*Treponema pallidum* and HIV are both sexually transmitted agents with similar epidemiological characteristics (5). HIV and syphilis coinfection is complex and diagnosis of syphilis is more difficult in HIV positive patients (9). False positive and false negative reactions have been frequently reported in nontreponemal RPR/VDRL tests in HIV patients. False positive reactions may occur due to viral infections such as HIV and HCV, malignancy, drug addiction, autoimmune diseases, and pregnancy (10). Moreover, false negative results can be detected in all stages of syphilis and can result from prezonal phenomenon in the secondary stage where the antibody levels are the highest in most cases (11). For these reasons, it is recommended to perform treponemal tests such as ELISA in HIV positive patients. (12). Hence a FTA-abs positive/ELISA positive result implies that patient has treponemal infection. Our study was planned to determine the syphilis coinfection in HIV positive patients and also to evaluate the compatibility of the ELISA test, which is now being used as a screening test for syphilis diagnosis, with the FTA-abs test.

In our study, as suggested by the European Center for Disease Prevention and Control (ECDC) algorithm, positive treponemal screening test results were confirmed

**Figure 2.** Distribution of syphilis positivity according to age groups in HIV positive patients (n = 69).**Figure 3.** Antibody positivity rates by FTA-abs test in each age group of HIV positive patients (n = 519).

by a second different treponemal test (13). Nowadays, contrary to the nontreponemal (RPR and VDRL test) tests in routine use, ELISA tests using recombinant *T. pallidum* antigens that are used successfully especially in blood banking are highly sensitive and specific automated tests (5). A total antibody ELISA test based on chemiluminescent enzyme immunoassay technology using treponemal antigens with high sensitivity ( $\geq 99\%$ ) and specificity ( $\geq 99\%$ ) was used as a screening test in the present study. Samples, which were positive by ELISA test, were tested by a second treponemal test, the FTA-abs test, which is accepted as a confirmation test for syphilis.

Evaluation of our data revealed that the ELISA test determined 15.2% and the FTA-abs test 13.3% syphilis antibody positivity in HIV positive cases. False positivity with the ELISA test was 1.9%. The positivity detected by the

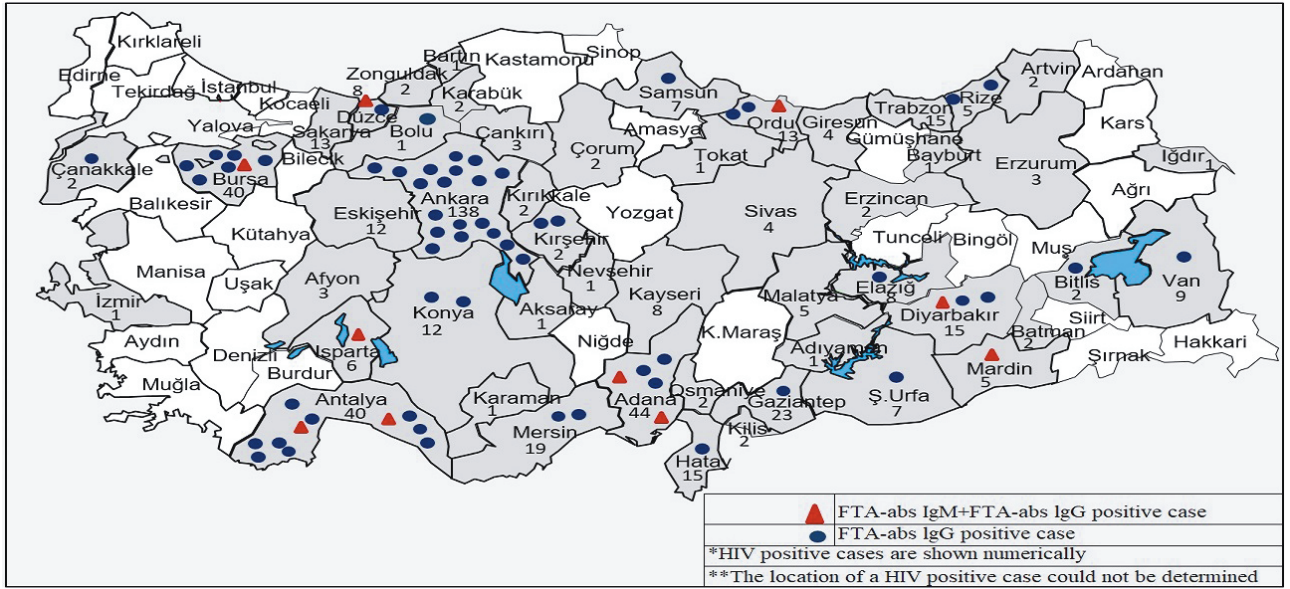


Figure 4. Distribution of HIV and syphilis positive patients attending from different provinces.

Table 2. Evaluation of Architect Syphilis Tp ELISA in comparison with the FTA-abs test (n = 519).

	Number (n)	Percent (%)	Cohen's kappa test		
			Kappa coefficient	P-value	Interpretation
Sensitivity	69/69	100.0	0.921	P < 0.001	Perfect agreement
Specificity	440/450	97.8			
PPV*	69/79	87.3			
NPV**	440/440	100.0			

\* Positive predictive value.  
\*\* Negative predictive value.

FTA-abs test was considered to include syphilis infection prior to HIV infection, syphilis infection concurrently with HIV infection, or syphilis infection after acquiring HIV infection. When FTA-abs IgM and FTA-abs IgG test results were evaluated, 11.4% of 13.3% cases were determined as past syphilis infection, and 1.9% of them were favorably interpreted as acute syphilis infection. The incidence of syphilis coinfection in HIV-infected patients ranges from 0.5% to 24.4% in different studies, consistent with the frequency of 13.3% detected in our study (14). On the other hand, the frequency of acute syphilis infection is reported as 2%–4% in Europe and 1%–21% in North America (15). In our study the reported 1.9% acute syphilis infection rate in HIV positive patients was lower than the ones reported from Rio de Janeiro (2.7%), Recife (8.8%), Londrina (24.4%), and Porto Alegre but somewhat higher than the rates detected in HIV infected patients

in Eastern Nigeria (0.3%), South Nigeria (1.5%), and Northeast Nigeria (1.7%) (14,16). The difference in the rates of syphilis among studies may be due to sampling, study design, different algorithms and diagnostic tests used, and epidemiological characteristics.

Most HIV positive cases are thought to be concentrated in İstanbul and other big cities, mainly Ankara and İzmir, where access to information and screening tests used can facilitate to diagnose HIV infection. Additionally, due to the stigmatism followed by a positive HIV test, most patients prefer not to be tested and treated in their hometown. This could be the reason for clustering of the cases generally in the bigger cities. Therefore, it was determined that IgG positivity was mostly detected in the patients attending from Ankara and IgM positivity together with IgG positivity was detected only from patients attending from Düzce, Antalya, Adana, Ordu, Diyarbakır, Mardin, Bursa, and Isparta among

our study patient group. These findings indicate that the rate of coinfection is not limited to patients from specific provinces. In this respect, the necessary actions for control measures need to be planned at the national level rather than the regional level. It should also be noted that patients who are suspected to be HIV or syphilis positive by the clinician may have a coinfection risk. According to our results when age groups were investigated, it was determined that the most common syphilis positivity was detected in the 35–44 years, followed by 25–34 and 45–54 years age groups. These age groups constitute the sexually active part of societies (9). Consistent with our findings, similar studies have reported that syphilis positivity was mostly detected among people 20–40 years of age, suggesting that the highest positivity for syphilis coinfection is generally detected in this age group (9,17).

Syphilis IgG positivity remains lifelong, even after the treatment. Past infection (IgG positivity only) rate in our study was higher than the acute infection (IgM and IgG positivity) rate in all age groups. The high percentage of past syphilis infections in HIV positive cases compared to acute infections suggests that either syphilis coinfection is being treated in HIV positive patients or syphilis infection has been acquired in the pre-HIV-infection period. Due to the effects on the immune system and common transmission routes, HIV predisposes to other STIs and vice versa (4). STIs facilitate HIV transmission by breaching protective mucosal barriers and causing immune cells to migrate to the infection site (18). Thus, the risk of HIV transmission increases with syphilis ulcers (3). In addition, several studies have corroborated the negative synergistic effect of syphilis on HIV infection; during syphilis infection viral load in blood plasma increases and CD4 cell counts decrease transiently (19). For this reason, testing all syphilis diagnosed patients for HIV infection is critical (20). Likewise, patients who have been diagnosed with HIV need to be screened for syphilis; therefore, rapid diagnosis in syphilis is very important (21,22).

As syphilis clinical findings and serological response may change in HIV positive patients, delayed type atypical responses may develop in the treponemal and nontreponemal tests; hence, the results of the diagnostic tests may be difficult to interpret. In HIV infection, anticardiolipin, antilecithin antibodies, and polyclonal gammopathies are formed. It is

therefore suggested that these results are biologically false positive and do not show syphilis infection (23). Although there are some reports of unexpected serological responses in HIV infected patients (7), syphilis serological diagnostic tests are evaluated among HIV positive patients in the same way as in the normal population, in the present study.

Nowadays, ELISA tests seem to be advantageous as they can be automated and use multiple samples at the same time for screening purposes (13). In our study, when the Architect Tp screening ELISA test results were evaluated by the FTA-abs test, the interrater reliability of the ELISA test was almost perfect with a sensitivity and specificity of 100% and 97.8% respectively. Compatible with our results, Marangoni et al. also reported the sensitivity and specificity of the ELISA test as 99.2% and 98.4% respectively, when compared to a second treponemal test (24). However, considering that false positive reactions can be obtained by ELISA tests, syphilis should not be determined with only one treponemal test, as suggested in the ECDC algorithm, and therefore positive results should be confirmed with a second different treponemal test for proper diagnosis of syphilis (13).

It is thought that the presence of HIV and syphilis coinfection in our study will raise awareness among clinicians. In this context, timely demand for diagnostic tests will lead to early diagnosis of the patients and prevent the development of complications. One limitation of this study is that as personal data (coded data) are not always comprehensive, risk factors other than HIV and syphilis including sociodemographic characteristics were not assessed in this study. In addition, as the study population does not cover all HIV positive patients of the provinces, the rate of syphilis-HIV coinfection could not be detected at the provincial and national levels.

In conclusion, as syphilis coinfection is detected among HIV positive patients, implementation of syphilis and HIV screening tests together is considered to be appropriate among risk groups.

It is suggested that the data within this study will provide information for public health actions, as well as development of the target strategies in order to monitor the frequency of syphilis coinfection and control of the transmission. Thus, detection and treatment of undetected cases will contribute to reducing HIV and syphilis infections in our country.

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