

Syphilis in a high-density urban area in the North of Italy

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SUMMARY

Although far less common now than in the past, syphilis continues to pose a danger to public health and should not be overlooked. In this study, we evaluated the presence and characteristics of syphilis in a group of patients attending an STI Clinic in the North of Italy.

A retrospective study was carried out, analysing the data from the 5609 subjects who attended the STI Clinic of St. Orsola-Malpighi Hospital (Bologna) for syphilis screening from January 2016 to December 2017.

Globally, 692 patients (12.3%) were found positive for treponemal tests, with a significant difference between males and females (16.6% vs 4.1%; $P < 0.0001$). Moreover, positive women were more likely foreign (63.3%), in contrast to men, who were more likely Italian (86.1%; $P < 0.0001$).

A total of 306 patients (44.2%), mainly males (47% vs 25%; $P = 0.0003$), received a diagnosis of early syphilis. These cases peaked among patients 35-44 years (31%) and 25-34 years (26.8%).

Overall, 32.9% of the women found positive for treponemal tests were pregnant. Among them, 84.6% were foreign (mainly from Eastern Europe) and 38.4% received a diagnosis of early syphilis. No cases of mother-to-child syphilis were found.

The presence of an HIV-syphilis co-infection was found in 21.5% of patients with early syphilis, with a significant association with the male sex ($P < 0.009$).

In-depth knowledge of the characteristics of syphilis could help set up effective strategies for its control.

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INTRODUCTION

Syphilis is a chronic sexually transmitted infection (STI) caused by the spirochete *Treponema pallidum* subsp. *pallidum* and remains a common worldwide health problem (WHO, 2016; Peeling *et al.*, 2017).

Clinical manifestations and transmissibility to others vary over the natural history of infection. (Workowski and Bolan, 2015; Hook, 2017). It is also important to emphasise that mother-to-child transmission of *T. pallidum* can lead to stillbirth, early foetal death, low birth weight, preterm delivery, neonatal death, or congenital syphilis in new-borns (Marangoni *et al.*, 2016), and that due to their ulcerative nature, syphilis lesions increase the risk of HIV infection transmission and acquisition (Newman *et al.*, 2015).

Despite the availability of effective antibiotic treatment since the mid-twentieth century, syphilis has been re-emerging globally in the last few decades, particularly among men having sex with men (MSM), with a relevant

clinical and economic impact (ECDC, 2018). If left untreated, *T. pallidum* infection can progress over the years, leading to irreversible neurological and/or cardiovascular complications (Gjestland, 1955; Hook and Marra, 1992). Since the natural course of the infection is characterized by periods without clinical signs or by the presence of non-specific symptoms, syphilis diagnosis is primarily based on serological testing. These tests, playing a pivotal role in the epidemiological and diagnostic evaluation of the disease, are divided into non-treponemal and treponemal assays (Marangoni *et al.*, 2013).

In developed countries most laboratories have adopted the 'reverse algorithm.' In this approach, a reactive treponemal screening assay is followed by a quantitative non-treponemal assay to diagnose active disease and to monitor response to treatment (Binnicker, 2012).

The global re-emergence of syphilis as a common STI is forcing healthcare providers to search for the new elements driving this epidemic (ECDC, 2018). In this context, in-depth knowledge of the prevalence of *T. pallidum* infection could help set up effective strategies to prevent the spread of syphilis.

The aim of the study was to evaluate the presence and characteristics of syphilis in a cohort of patients attending an STI Clinic in the North of Italy during a two-year period.

Key words:

Treponema pallidum; syphilis; STIs; sexual health; HIV.

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MATERIALS AND METHODS

Study population

A retrospective study was carried out, analysing the data from all the subjects who attended the STI Outpatients Clinic of St. Orsola-Malpighi Hospital (Bologna) for syphilis screening from January 2016 to December 2017. All the patients, after a preliminary interview and a clinical examination, underwent serological testing for syphilis.

Subjects seeking care from the STI Outpatient Clinic met one or more of the following criteria: having STI related symptoms, having sexual contacts with an infected partner or unsafe intercourse with new or multiple sexual partners.

In the Microbiology Laboratory of St. Orsola-Malpighi Hospital, syphilis diagnosis has been routinely performed by following a 'reverse algorithm' since the beginning of 2000. During the study period, Architect Syphilis TP chemiluminescent (CMIA) immunoassay (Abbott GmbH & Co. KG, Wiesbaden, Germany) was used as a screening test, and positive results were confirmed by both a treponemal (TPHA; Randox Laboratories Ltd., Crumlin, UK) and a non-treponemal test (RPR; Randox), and, eventually, by a Western Blot assay (Mikrogen, Neuried, Germany).

As shown in *Figure 1*, on the basis of clinical, anamnestic and laboratory data (Larsen *et al.*, 1995), patients positive

for treponemal tests were classified in different syphilis stages: early syphilis (primary, secondary or early latent infection), late latent syphilis or tertiary syphilis. A case of past infection was considered when a history of a previous antimicrobial treatment was associated with a significant reduction of the RPR titre or a sero-reversion of this non-treponemal test.

For all the patients with treponemal positive serology, data regarding age, sex and nationality were collected. Moreover, when available, information about the presence of other STIs (HIV, chlamydia and gonorrhoea) was registered as well. The diagnosis of genital and/or extra-genital chlamydial/gonococcal infections was performed by a commercial real-time PCR test simultaneously detecting the presence of *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* DNA (Versant CT/GC DNA 1.0 Assay; Siemens Healthcare Diagnostics, Terrytown, USA) (Marangoni *et al.*, 2015).

HIV serology was performed with Architect HIV Ag/Ab Combo assay (Abbott) as a screening test, whereas VIDAS® HIV DUO Quick assay (bioMérieux, Marcy l'Etoile, France) and INNO-LIA™ HIV I/II Score (Innogenetics, Gent, Belgium) were used as confirmatory tests.

This study was conducted according to the regulations of the St. Orsola-Malpighi University Hospital Ethical Committee and to the 1964 Helsinki declaration and its later amendments.

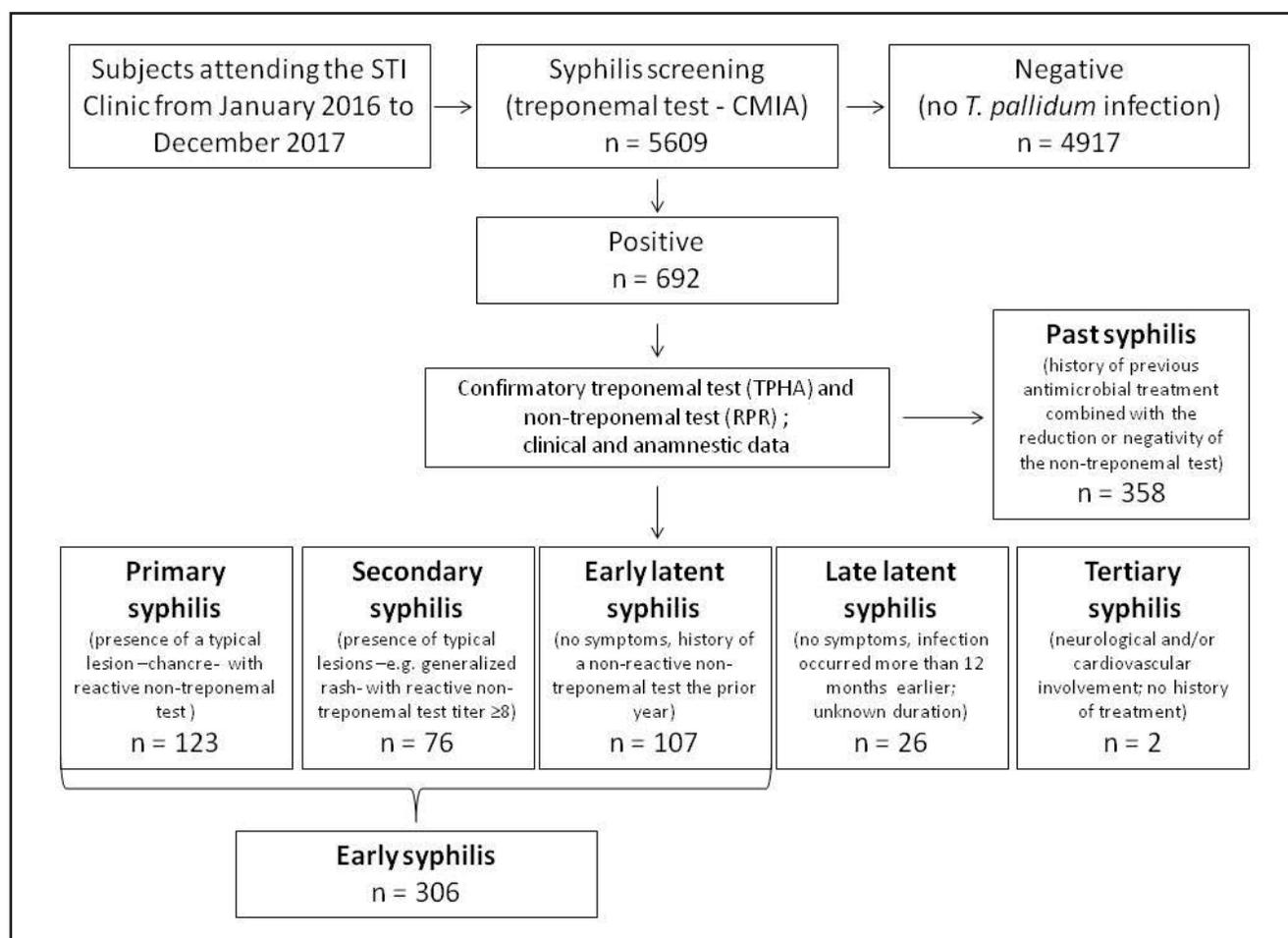


Figure 1 - Flow chart used to categorize the enrolled patients in the different stages of syphilis infection. *T. pallidum* infections were classified in accordance with the clinical and laboratory criteria proposed by Larsen (Larsen *et al.*, 1995).

Table 1 - Demographic characteristics of the subjects enrolled during the study period.

Demographic characteristics of the whole population (n = 5609)	Patients positive for syphilis screening (n = 692)	Patients negative for syphilis screening (n = 4917)	P value
Sex			< 0.0001
Males: 3689 (65.8%)	613/692 (88.5%)	3076/4917 (62.5%)	
Females: 1920 (34.2%)	79/692 (11.5%)	1841/4917 (37.5%)	
Mean age (years ± SD): 33.5 ± 11.0	41.8 ± 12.3	32.3 ± 10.3	< 0.0001
Males: 35.2 ± 11.7			
Females: 30.3 ± 8.7			

Statistical analysis

First, a descriptive analysis was performed to assess the main characteristics of all the subjects enrolled in the study (number of subjects, proportion of males and females, mean age). Second, an evaluation of *T. pallidum* infection stratified by the different available data (e.g. sex, age, nationality, presence of other STIs) was conducted.

To evaluate statistically significant differences, t-test was used to compare quantitative data and categorical data were analysed with Chi-square test or Fisher's exact test. Data were analysed with GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). A $P < 0.05$ was considered statistically significant.

RESULTS

Study population

During the study period, a total of 5609 subjects were screened for syphilis. In particular, the patients included in the study were 3689 males (65.8%) and 1920 females (34.2%), with a mean age of 33.5 ± 11 (\pm standard deviation, SD). Females were significantly younger than males (30.3 years ± 8.7 vs 35.2 ± 11.7 ; $P < 0.0001$) (Table 1).

Globally, in the two-year period, 692 patients (12.3%) showed positive results for treponemal tests. As shown in Table 1, a statistically significant difference was observed between the proportion of positive males and females (male-to-female ratio M:F=7.7:1; $P < 0.0001$). Moreover, positive patients were significantly older than negative ones (41.8 ± 12.3 vs. 32.3 ± 10.3 years; $P < 0.0001$).

In the group of patients positive for treponemal tests, a significant association between nationality and sex was observed: the positive women were more likely foreign (50; 63.3%), in contrast to men, being more likely Italian (528; 86.1%; $P < 0.0001$). Foreign women were mainly from Eastern Europe (36/50; 72%).

Syphilis infection

On the basis of clinical, anamnestic and laboratory results, subjects positive for *T. pallidum* tests (n=692) were categorized as follows (Figure 1). A total of 306 patients (44.2%) received a diagnosis of early syphilis: in particular, 123 (17.7% of the total) cases of primary syphilis, 76 of secondary syphilis (11%) and 107 (15.4%) of early latent infection were found. Thus, primary, secondary and early latent infection accounted, respectively, for 40.2%, 24.8% and 35% of all the cases of early syphilis.

The remaining 386 cases were classified as late latent syphilis (26; 3.8%), tertiary syphilis (2; 0.3%) and past

(previously treated) *T. pallidum* infections (358; 51.7%). Globally, excluding patients with a past syphilis, the frequency of confirmed *T. pallidum* infections was 5.9% (334/5609). Syphilis cases stratified by stage of infection are shown in Figure 2.

Patients with early syphilis were mainly males (286/306; 93.5%), born in Italy (246/286; 86%). Conversely, most of the females with early syphilis were foreign (12/20; 60%), mainly from Eastern Europe (9/12; 75%) ($P < 0.0001$). The cases of early syphilis peaked among subjects age 35-44 years (31%) and 25-34 years (26.8%), followed by 45-54 years (22.5%).

Out of the 79 women with positive treponemal tests, 26 (32.9%) were pregnant. Of them, 22 (84.6%) were foreign (86.3% from Eastern Europe) and 10 (38.4%) received a diagnosis of early syphilis. All these women were correctly managed at the beginning of the pregnancy and no cases of mother-to-child transmission of infection were found.

Analysing the presence of other STIs, we observed that 25.2% (161/640) of the subjects with reactive treponemal tests were HIV-positive and that 16.5% of them showed a concomitant infection due to *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae*. Focusing only on patients with early *T. pallidum* infection, the presence of an HIV-syphilis co-infection was found in 21.5% (66/306) of cases, with a significant association with the male sex (66/268 males vs. 0/20 females; $P < 0.009$). Again, among early syphilis patients, chlamydial/gonococcal co-infec-

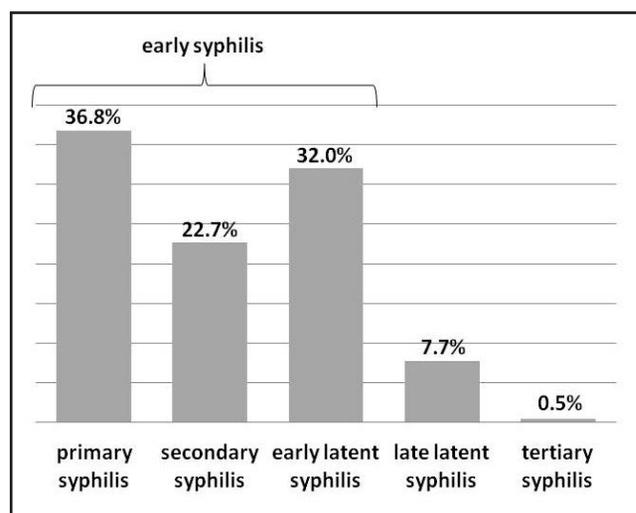


Figure 2 - Distribution of patients on the basis of the stage of *T. pallidum* infection. Patients with past (previously treated) *T. pallidum* infection (n=360) were excluded.

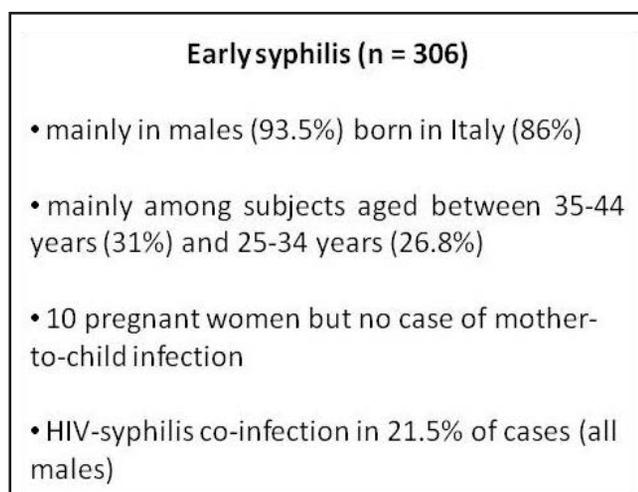


Figure 3 - Main characteristics of early syphilis cases found during the study.

tions were detected only in men (13/286 males vs 0/20 females). The main characteristics of early syphilis cases are summarized in *Figure 3*.

DISCUSSION

Specific prevention and surveillance programs are advisable to face the STIs challenge. In this context, thorough knowledge of the characteristics of *T. pallidum* infection within a population could help set up 'tailored' approaches to reach specific high-risk groups and control the re-emergence of syphilis.

Therefore, in this study, we assessed the presence of syphilis in a cohort of patients attending an STI Clinic in the North of Italy during a two-year period.

At first, in agreement with recent reports, we found that most of the confirmed syphilis cases were detected in men (Zorzi *et al.*, 2017; Pala *et al.*, 2018; ECDC, 2018). Indeed, in Europe in 2016, reported syphilis rates were 8 times higher in men than in women: in particular the mean male-to-female ratio was 7.9 with rates of 10.8 per 100,000 population in men and 1.3 cases per 100,000 population in women (ECDC, 2018). The high proportion of *T. pallidum* infections detected in MSM explains these findings: indeed, this group accounts for the majority of syphilis cases (>60%) in various countries, worldwide (Down *et al.*, 2012; Newman *et al.*, 2015). Thus, the continuous increase in the rate of new cases of syphilis, specifically among MSM, reaffirms the need for screening programs targeted to this high-risk group (Willeford and Bachmann, 2016).

Second, we noticed that, for both men and women, the largest proportion of syphilis cases were reported in the age groups 35-44, followed by 25-34 and 45-54. These data are in agreement with recent European and American reports about the distribution of syphilis cases stratified by age (ECDC, 2018; CDC 2015). Indeed, in the annual epidemiological report for 2016, the ECDC data showed that most of the syphilis cases were registered in the age group above 45 (31%), with almost equally large proportions of cases in the age groups 25-34 (30%) and 35-44 (26%) (ECDC, 2018).

Other interesting aspects emerged from the distribution of syphilis cases by nationality of patients. A significant

association between geographical origin and sex was observed: the positive women were more likely foreign (in particular from Eastern Europe), in contrast to men, being more likely Italian. It has previously been shown that, in our area, immigrant women from Eastern Europe represented a 'core group' for the presence of syphilis during pregnancy (Tridapalli *et al.*, 2007; Marangoni *et al.*, 2008). Indeed, in the recent past (2000-2007), a sizable part of immigrant women did not receive correct prenatal care: thus, they were found positive only at delivery, leading to preterm births and congenital syphilis cases (Tridapalli *et al.*, 2007; Marangoni *et al.*, 2008). Conversely, no cases of mother-to-child transmission have been found since 2014, due to the increased awareness of immigrant women that has allowed them to receive free adequate prenatal care and antenatal screening. In any case, special and constant attention should be paid to foreign women to effectively reduce *T. pallidum* infection incidence in our setting.

When looking at the clinical stage of *T. pallidum* infection, we noticed that a significant proportion of patients (44.2%), mainly males, received a diagnosis of early syphilis, in line with European epidemiological data (ECDC, 2018). Patients with early syphilis, harbouring several mucocutaneous lesions rich in treponemas, can be highly infectious to others through sexual intercourse (Hook, 2017). Therefore, in order to limit syphilis transmission and spread, public health control efforts should focus particularly on primary, secondary, and early latent stages of the infection.

Finally, we observed that 25.2% of the subjects with reactive treponemal tests were HIV-positive and, in particular, that the presence of an HIV-syphilis co-infection was found in 21.5% of cases of early *T. pallidum* infection. In this context, the close association between syphilis and HIV has been frequently described, mainly in MSM, with rate of co-infection exceeding 30-35% (Darrow *et al.*, 1987; Hook, 1989; Newman *et al.*, 2015; ECDC, 2017). The strong relationship between *T. pallidum* and HIV is linked not only to the creation of high-risk sexual networks, but also to the peculiar nature of syphilitic lesions. Indeed, genital ulcers are densely infiltrated with lymphocytes (the primary target cells for HIV infection), providing a portal of entry for HIV acquisition, as well as a focus for STIs transmission to others (Stamm *et al.*, 1988).

In addition to HIV, the association between syphilis and chlamydial/gonococcal infection underlines the existence of an alarming 'STIs burden', mainly among the male population. In this context, specific prevention and surveillance programs are urgently needed to face the STIs challenge (Unemo *et al.*, 2017).

We are fully aware that some limitations have affected our results. The lack of information about the sexual orientation and behavioural factors of positive patients (e.g., sex work, number of sexual partners in recent months...) resulted in the exclusion of significant epidemiological data. Moreover, the absence of detailed data on *T. pallidum*-negative subjects (origins, HIV-positivity, other STIs...) led to an incomplete assessment of syphilis predictors and risk factors.

In conclusion, the major findings of this study are the followings:

- 1) in our area, the characteristics of syphilis infection (i.e., distribution of cases by sex, age and clinical stage; association with HIV) are in agreement with European and American epidemiological reports;

- 2) to limit the spread of syphilis, specific intervention programs should focus on groups at higher risk of infection, such as Italian men and women from Eastern Europe;
- 3) the medical community should be aware that a significant number of syphilis cases are associated with other diseases (HIV, *C. trachomatis*, *N. gonorrhoeae*), with the creation of alarming and peculiar landscapes of STIs.

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Conflict of interest

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