Appendix S1.

METHODS

Patients

This was a retrospective study of HIV-infected individuals ≥ 18 years of age diagnosed with syphilis between 1 May 2004 and 31 October 2009. All HIV-infected individuals attending the Department of Infectious Diseases at Copenhagen University Hospital, Rigshospitalet, the Department of Infectious Diseases at Copenhagen University Hospital, Hvidovre, and the sexually transmitted disease (STD) clinic at the Department of Dermato-venereology at Copenhagen University Hospital, Bispebjerg, were included. Exclusion criteria were: patients who received intravenous antibiotics, who were diagnosed with neurosyphilis or who lacked information on therapy. An individual could contribute with more than one episode, provided that treatment and appropriate treatment response was documented in the patient files.

Antibiotic treatment

Therapy consisted of doxycycline (100 mg orally twice daily for 14 days for early syphilis, i.e. primary, secondary and early latent stages, and for 30 days for late latent syphilis) or penicillin (a single dose of intramuscular 2.4 million units of benzathine penicillin G for early syphilis and 3 doses each at 1-week intervals for late latent syphilis). At the beginning of the study period 15 patients were treated with intramuscular procaine penicillin (1 dose of 600,000 units once daily for 10 days) and these cases were grouped with the benzathine penicillin G treated cases.

Definition of syphilis stages

Disease stage was based on clinical examination, patient history and result of serological tests. Patients were classified as having primary syphilis (i.e. ulcer), secondary syphilis (i.e. seroreactivity with clinical manifestations such as skin rash or mucocutaneous lesions), early latent syphilis (i.e. seroreactivity, no clinical manifestations and known duration of less than a year), late latent syphilis (i.e. seroreactivity, no clinical manifestations and known duration of more than one year or unknown duration) or tertiary syphilis (i.e. seroreactivity with cardiac or gummatous lesions). CNS involvement can occur during all stages of syphilis, and neurosyphilis was defined as seroreactivity in the cerebrospinal fluid (CSF) or elevated CSF cell count combined with unexplained neurological manifestations consistent with neurosyphilis (1, 2).

Data collection

Sociodemographic information, mode of acquisition (e.g. men who have sex with men (MSM) status), syphilis disease stage, CNS symptoms, treatment of syphilis, country of acquisition, history of previous syphilis infection, HIV RNA, CD4 cell counts, cART, hepatitis B virus status (presence of hepatitis B surface antigen), hepatitis C virus status (presence of hepatitis C antibody) and information on concurrent STDs were extracted from the patient files.

Laboratory tests

Serological test results were obtained from Statens Serum Institut, where all serological testing of syphilis was centralized during the study period. The non-treponemal test rapid plasma reagin (RPR) was determined by agglutination and, furthermore, 3 treponemal tests were used: anti-flagellum IgM (AF-M) was determined by a capture ELISA, anti-flagellum IgG (AF-G) was determined by an indirect ELISA (20–22) and the fluorescent treponemal antibody absorption test (FTA-ABS) was done by immunofluorescence microscopy. Examination of CSF included total protein, number of mononuclear cells, AF-M, AF-G and intrathecal synthesis of immunoglobulins.

Definition of outcomes

Serological cure was defined as a ≥ 4-fold decline in RPR titres following therapy. Serological failure was defined as a lack of a 4-fold decline. Serological test results were allocated to a specific follow-up visit (at 3, 6, 9 or 12 months post-therapy) if the test was performed between 30 days before and 30 days after the relevant time-point. Furthermore, the last-observation-carried-forward principle was used to handle missing values of RPR, e.g. if a patient had no available tests at 12 months, but had reached serological cure at 9 months the patient was classified as serologically cured at 9 months.

Data analysis

Descriptive statistics were used to characterize the study population. Sex, ethnicity, MSM status, country of acquisition, history of syphilis, hepatitis B and C virus status and cART were evaluated as dichotomous variables. RPR titres were evaluated as discrete variables. CD4 cell count was evaluated as a continuous variable and as a categorical variable (CD4 cell count ≤ 200 cells/µl or > 200 cells/µl). HIV RNA was evaluated both as a logarithm base 10-transformed continuous variable and as a categorical variable (≤ 200, > 200 and < 100,000 or ≥ 100,000 copies/ml).

Where appropriate, the χ^2 or Fisher's exact test were used to compare independent proportions. For comparison of continuous variables the *t*-test and the Mann–Whitney test were used for normal distributed and non-normal distributed variables, respectively. The Kruskal–Wallis test was used for comparison of titres between different syphilis stages. Odds ratios (ORs) with 95% confidence intervals (CIs) were computed by logistic regression analysis. Propensity score methods were used, in which the predicted probability of treatment with doxycycline was derived from unconditional logistic regression, utilizing a manual backward-elimination approach. The predicted probability of the model was used as the propensity score for each patient. For the propensity-score-matched case-control study, patients in the doxycycline treatment group were matched with patients in the penicillin treatment group who had the closest propensity scores within a calliper size of one-quarter of the standard deviation of the propensity score. Thus, we excluded cases in which the propensity score difference was more than 0.09. Serological outcome 12 months after treatment was compared between the propensity-score-matched groups. Differences with $p < 0.05$ (2-sided) were considered statistically significant. Data analysis was done using SPSS version 16.0 (SPSS Inc., Chicago, Illinois, USA). Exemption for review by the ethics committee system and for obtaining informed consent was obtained from the Committee on Biomedical Research Ethics for the Capital Region of Denmark.