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Incidence and clinical and immunological characteristics of primary *Toxoplasma gondii* infection in HIV-infected patients

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SUMMARY

Objectives: To determine the incidence and laboratory characteristics of primary *Toxoplasma gondii* infection in HIV-infected individuals.

Methods: This retrospective study was conducted between 1988 and 2012 on a cohort of 1130 HIV-infected patients at the AIDS Center Prague. Toxoplasma serology, standard laboratory parameters, and health status were evaluated at 3–6-month intervals for all patients.

Results: The total person-time of follow-up of patients at risk of Toxoplasma seroconversion was 3046.3 years; there were 14 primary *T. gondii* infections, yielding an incidence rate of 0.0046 (95% confidence interval 0.0027–0.0078). Most of the subjects were clinically asymptomatic, but in one case seroconversion was accompanied by transient cervical lymphadenopathy. The CD4+ T-lymphocyte count geometric mean increased from 418 (95% confidence interval 303–579) cells/μl before seroconversion to 501 (95% confidence interval 363–691) cells/μl after seroconversion (p = 0.004), while other parameters (CD8+ T-lymphocytes, natural killer cells, viral load, beta2-microglobulin, total immunoglobulins) remained unchanged. As compared to the control group, patients with primary toxoplasmosis had higher initial levels of total immunoglobulins IgA and IgG and a tendency to higher CD8+ T lymphocyte counts.

Conclusions: Neither the incidence nor the course of the primary Toxoplasma infection was influenced by the immune status of the patients. Immune parameters of patients with primary Toxoplasma infection did not differ from those of the controls.

1. Introduction

Toxoplasmosis caused by the protozoon *Toxoplasma gondii* (Apicomplexa) is one of the most common widespread parasitic diseases worldwide and is one of the major opportunistic infections afflicting patients with advanced HIV infection. The primary infection in immunocompetent individuals, which is asymptomatic or accompanied by mild and non-specific symptoms in most cases, is usually followed by a lifelong latent infection. Any subsequent reactivation of latent toxoplasmosis due to severe immunodeficiency is manifested most often as cerebral toxoplasmosis. Since the mid-1980s this disease has been the focus of greatly increased attention and the circumstances of its pathogenesis and clinical and laboratory symptoms are relatively well known, whilst effective therapy and prophylaxis are available.[1] Nonetheless, little is known about the incidence and manifestations of primary *T. gondii* infection in HIV-infected individuals.[2]

For this reason we decided to carry out a retrospective analysis of medical records pertaining to HIV-infected patients at the AIDS Center Prague in order to determine the incidence and laboratory and clinical characteristics of primary *T. gondii* infection. In this health care setting, a total of 1130 HIV-infected patients are followed up, representing approximately 65% of all diagnosed HIV-infected patients in the Czech Republic.[3] This study was possible thanks to many years of close cooperation between the AIDS Center at the Bulovka Hospital in Prague and the National Reference Laboratory for Toxoplasmosis at the National Institute of Public Health in Prague.

2. Methods

All HIV-infected patients attending the AIDS Center at Bulovka Hospital in Prague between November 1988 and April 2012 were included in this retrospective study. Blood samples were collected

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from all confirmed HIV-infected patients at 3–6-month intervals for testing *T. gondii* serology as well as immunological, hematological, and biochemical parameters. Throughout the study, complement fixation tests (CFT), IgG ELISA, and IgM ELISA were used for the detection of anti-Toxoplasma antibodies. According to the manufacturer's information, TestLine Toxoplasma diagnostic kits show the following sensitivity/specificity: CFT 97–99%/95–98%, ELISA IgG 98.9%/99.2%, and ELISA IgM 96.4%/97.9%.[4–7] CFT titers ≥1:8 were considered positive. The Toxoplasma status of patients whose test results fluctuated during follow-up was considered negative when the initial sample was negative and no more than one positive result was detected thereafter; the Toxoplasma status was considered positive when the initial sample was positive and repeated (twice or more) positive samples were detected. Seroconversions were detected by follow-up of patients if the initial (at least two) negative samples were followed by an uninterrupted sequence of samples positive both by CFT and ELISA IgG. The dynamics of the antibody response were monitored. Patients were classified retrospectively into groups according to their *T. gondii* infection status as recorded at serological follow-ups.

Other monitored laboratory markers included the HIV RNA viral load measured by PCR (limit of detection 20 copies/ml) and parameters of both humoral immunity (serum immunoglobulins IgG (normal range 7.51–15.6 g/l), IgM (normal range 0.46–3.04 g/l) and IgA (normal range 0.82–4.53 g/l), and beta2-microglobulin (normal range 0.7–1.8 mg/l)) and cellular immunity (such as numbers of natural killer cells (NK; normal range 300–700/μl), CD4+ T lymphocytes (CD4; normal range 700–1100/μl), and CD8+ T lymphocytes (CD8; normal range 500–900/μl) tested by flow cytometry and also levels of serum C-reactive protein (normal range 0–8 mg/l)).

The clinical status of all patients was examined at the AIDS Center every 3–6 months and it was noted whether these patients were receiving combination antiretroviral therapy (cART) or anti-Toxoplasma prophylaxis. In the case of seroconversion, the medical records were retrospectively reviewed for possible clinical symptoms during the previous 12 weeks and patients were additionally interviewed for the same information.

The study was approved by the local ethics committee of Bulovka Hospital and was conducted in accordance with the ethical standards laid down in the 1975 Declaration of Helsinki. All patients agreed to participate in the study and signed an informed consent.

2.1. Statistical analysis

Model-based geometric means together with corresponding 95% confidence intervals (95% CI) were calculated to characterize the central tendency and variability of the analyzed variables in the groups. Within-group and between-group comparisons were based on a mixed-effects linear regression model with random intercepts fitted via maximum likelihood.

For comparison of the basic characteristics of patients with anti-Toxoplasma seroconversion with patients without toxoplasmosis, a control group of 56 individually matched Toxoplasma-negative patients (four controls per case) was randomly selected from the cohort. Controls were matched to cases on year of HIV diagnosis (±3 years), age at HIV diagnosis (±7 years), gender, and, if possible, also by transmission route of HIV infection. It was required that each control patient had negative Toxoplasma tests on at least four different days. For comparison, the clinical data of cases at 1 year pre-seroconversion and 1 year post-seroconversion were used. For the controls, we used the data covering an equivalent time period as that found in matched patients before seroconversion. All controls during this period were asymptomatic for HIV.

The anticipated time of the seroconversion was determined as the middle of the time interval between the last negative and the first positive serology. The incidence rate of *T. gondii* in HIV-infected patients was calculated from the total follow-up time of Toxoplasma-negative persons and the number of seroconversion cases.

Tests of categorical variables were based on Fisher's exact test and its generalization.

All statistical tests were treated as two-sided, and results with p-values less than 0.05 were considered statistically significant. The data were analyzed with a Stata software package, version 9.2 (Stata Corporation, College Station, TX, USA).

3. Results

A total of 1130 patients – 956 males (mean age at HIV diagnosis 33.7 years) and 174 females (mean age at HIV diagnosis 28.1 years) – were evaluated in the study, representing 5530.8 person-years of follow-up time. The median follow-up period of repeatedly tested patients with 2–49 samples was 3.2 years; the maximum follow-up was 22.4 years.

As evident from positive CFT and ELISA IgG results, 396 (41.4%) males and 78 (44.8%) females were infected with *T. gondii* before diagnosis of the HIV infection. In total, 642 seronegative patients (550 (57.5%) males and 92 (52.9%) females) had no change in their negative status during the whole follow-up time.

Seroconversion indicating a recent *T. gondii* infection was observed in 14 patients (10 (1.0%) males and four (2.3%) females). The total person-time of follow-up of HIV-infected patients at risk of Toxoplasma seroconversion was 3046.3 years. The resulting incidence rate of primary toxoplasmosis in the cohort was therefore 0.0046 with a 95% confidence interval of 0.0027–0.0078. The age of patients with observed seroconversion was 22–63 years (median 44 years) in males and 24–33 years (median 27 years) in females, and the interval of follow-up after diagnosis of HIV was 0–11 years (median 4.2 years).

Among patients with a recent *T. gondii* primary infection, the highest CFT titers did not exceed 1:32 in seven cases; one patient reached a maximum titer of 1:64. Higher CFT titers (1:128–1:4096) occurred in six cases, although only in three of the cases was the positive CFT accompanied by positive anti-Toxoplasma IgM in the ELISA test (Table 1). IgA ELISA antibodies were detected in two cases only (patients 3 and 6).

The mean CD4 count in patients with seroconversion was 479 (range 93–1197) cells/μl and seven patients were on cART consisting of one protease inhibitor boosted with ritonavir and two nucleoside reverse transcriptase inhibitors. In the majority of cases the seroconversion was not accompanied by clinical symptoms with possible relevance to primary *T. gondii* infection. Only one patient (patient 5), a pregnant woman with a CD4 count of 663 cells/μl, had a diagnosis of cervical lymphadenopathy lasting for 3 weeks. None of the patients with observed seroconversion were taking any anti-Toxoplasma prophylactic regimen.

The values of all monitored immunological parameters differed substantially between patients (Table 1). Comparison of mean values of monitored parameters pre-seroconversion and post-seroconversion revealed a significant increase in CD4 counts following infection by Toxoplasma (Table 2). No significant differences were detected for any of the other parameters.

Comparison with the control group (Table 2) showed that Toxoplasma seroconversion was preceded by increased CD8 values ($p = 0.062$) and total IgG, IgA (both $p < 0.001$), and IgM ($p = 0.056$). For the other monitored parameters, no significant differences between groups were observed.

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