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International Journal of Infectious Diseases 17 (2013) e892–e896

Contents lists available at SciVerse ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

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

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ARTICLE INFO

Article history:
Received 20 January 2013
Received in revised form 10 March 2013
Accepted 16 March 2013

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
HIV
Toxoplasma gondii
Primary infection
Incidence
Seroconversion

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.
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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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http://dx.doi.org/10.1016/j.ijid.2013.03.017

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 $\geq$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 ($\pm$3 years), age at HIV diagnosis ($\pm$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.

Table 1
HIV-positive patients with Toxoplasma seroconversion. Gender, presumed date of seroconversion, time elapsed from diagnosis of HIV to the date of seroconversion, maximum CFT titers of anti-Toxoplasma antibodies after seroconversion, maximum anti-Toxoplasma IgM values, mean immunological and virological parameters in the 1-year periods before and after seroconversion, and information on antiretroviral treatment at the time of seroconversion are listed. Immunological data were not available for patient No. 8

No.	Gender	Age at HIV diagnosis (years)	Time from HIV diagnosis to Toxo seroconversion (years)	Max CFT titer	Anti-Toxo IgM[a]	Viral load (copies/ml), mean		CD4+T cells/μl, mean		CD8+T cells/μl, mean		NK cells/μl, mean		beta2-micro-globulin (mg/l), mean		CRP (mg/l), mean		IgA total (g/l), mean		IgG total (g/l), mean		IgM total (g/l) mean		cART yes/no
						Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	
1	M	19	3.2	1:16	N	10 584	32 308	479	632	1057	605	33	27	3.2	3.2	2.4	6.0	3.5	3.3	28.8	27.5	4.9	5.0	No
2	M	27	6.2	1:8	N	3460	5125	445	494	445	627	359	628	2.1	2.3	1.5	1.0	3.6	6.4	15.8	21.7	2.0	2.8	No
3	M	38	10.9	1:128	P	181	47	843	1216	4105	4587	810	934	2.9	4.2	3.5	1.2	10.0	8.5	19.9	19.5	1.3	1.0	Yes
4	M	58	6.2	1:16	N	0	0	593	591	1713	2006	183	161	2.2	2.5	1.7	1.3	4.5	5.0	12.8	14.2	0.5	0.7	Yes
5	F	25	0	1:1024	E		7962	663	454	526	1867		417	1.7	2.1	0.0	8.0	2.9	5.3	29.6	32.0	3.3	3.8	No
6	M	27	9.2	1:4096	P	1454	20 392	452	530	533	977	229	238	2.0	2.8	2.7	5.6	5.0	4.2	12.8	23.7	1.2	0.7	No
7	F	23	6.7	1:32	N	8006	25 013	390	621	1973	2104	225	241	2.8	3.1	4.6	0.0	2.8	3.2	26.1	27.1	5.2	4.8	No
8	M	33	6.3	1:16	N																			
9	M	50	2.5	1:8	N	0	<20	272	303	1980	1443	238	163	2.5	2.1	5.8	5.0	4.4	4.5	11.1	9.3	1.0	0.9	Yes
10	F	31	2.6	1:256	N	1150	<20	576	769	1710	1801	155	158	2.0	1.7	4.5	2.8	2.1	2.7	29.1	17.9	2.0	0.9	Yes
11	M	44	5.3	1:512	P	55	<20	251	305	2149	1244	248	195	2.0	2.4	1.1	2.4	4.0	3.7	15.9	12.8	0.7	0.8	Yes
12	M	32	0.8	1:1024	N	913	<20	93	132	1097	1346	85	82	1.1	2.7	0.2	1.0	1.8	2.2	12.6	32.8	0.8	1.9	Yes
13	F	23	1.8	1:64	E	2030	39 900	507	411	614	859	255	165	2.1	1.9	0.2	0.4	1.8	1.9	14.3	18.5	1.3	0.9	No
14	M	55	1.2	1:16	N	33	26	1197	1301	961	881	601	708	3.2		3.5	3.7	3.7		11.9		0.7		Yes

cART, combination antiretroviral therapy; CFT, complement fixation tests; CRP, C-reactive protein; F, female; M, male; NK, natural killer; Toxo, Toxoplasma.
[a] N = negative; E = equivocal; P = positive.

4. Discussion

Primary *T. gondii* infection is usually asymptomatic and often occurs before adulthood. Detection of its incidence therefore requires large longitudinal cohort studies on anti-Toxoplasma-negative individuals, and hence only limited data about the incidence of primary *T. gondii* infection in HIV-infected patients are available from literature sources. In our cohort, the majority of patients (97.5% males and 95.1% females) acquired toxoplasmosis before the diagnosis of HIV infection.

A recent primary *T. gondii* infection was observed in only 1.2% of monitored individuals and the incidence was 0.0046 cases per person-year at risk. These values are consistent with the annual rise in the infection rate (0.0041) observed in German HIV-infected patients, but are significantly lower than the rate of 0.0163 per person-year determined in the SEROCO–HEMOCO study from France.[2,8] Two other French studies also showed higher incidence rates. Derouin et al.[9] observed an annual rate of seroconversion of 0.01, and in the study of Candolfi et al. the annual rate was 0.023.[10] This higher incidence in France apparently reflects a different epidemiological situation and can also be illustrated by the higher incidence of toxoplasmosis in pregnant French women. In a study by Ancelle et al.,[11] a toxoplasmosis incidence of 0.0148 per susceptible pregnancy was found, whereas in Central European countries it was markedly lower: in the Czech Republic 0.0023 cases per pregnancy and in Austria and Germany analogous rates were 0.005 and 0.007, respectively.[12–14] In Poland, the estimated incidence of acquired infection during pregnancy was 0.005 per pregnancy.[15]

CFT seroconversion was detected simultaneously with positive IgM levels in only four out of 14 cases of primary *T. gondii* infection; these IgM levels are considered as determinative markers of recent *T. gondii* infection. Our data do not reveal any direct association between maximum CFT titers and mean CD4, CD8, or NK cell counts in patients. The possible explanation for the weakened serological response is likely to be found in residual immune dysregulation affecting T and B cell quantities and functions, immune activation, or immunosenescence.[16,17] Likewise, the elevated levels of immunoglobulin IgA, IgG, and IgM against controls, which we detected in patients prior to seroconversion, may have a similar root cause. One study on HIV-infected subjects correlated production of specific antibodies with the increase in interleukin (IL)-21 levels and IL-21-R-expressing B cells.[18] Unfortunately, neither in our study, nor in other published studies, were these markers tested.

In some studies, increased serum beta2-microglobulin is cited as a reliable marker of fetal *T. gondii* infection.[19,20] In contrast, our findings did not confirm elevated levels of serum beta2-microglobulin in HIV-infected adult individuals recently infected with *T. gondii*. Likewise, we did not confirm the statistically significant association between *T. gondii* infection and elevated CRP found in the study of Birgisdóttir et al.[21]

Clinical follow-up of the patients showed that in all but one case (patient 5 with transient cervical lymphadenopathy) seroconversion was not accompanied by clinical symptoms. This is similar to the situation in normal immunocompetent individuals, in whom more than 90% of primary *T. gondii* infections are asymptomatic and cervical lymphadenopathy is the most common manifestation of a symptomatic course.[22] None of the cases of cerebral toxoplasmosis in our cohort recorded since 1988 occurred in patients who acquired the disease during the course of monitoring. Similarly, none of the six HIV-infected patients with toxoplasmosis seroconversion described by Reiter-Owona et al.[2] had confirmed cerebral toxoplasmosis. Likewise, in the SEROCO and HEMOCO cohorts, none of the 14 cases with seroconversion were accompanied by clinical manifestations.[8]

Table 2
Immunological and virological parameters of HIV-infected patients before and after Toxoplasma seroconversion in comparison with a control group of matched HIV-positive Toxoplasma-negative individuals. Geometric means for cases with seroconversion are calculated for the period of 1 year before and 1 year after seroconversion. For the controls, we used an equivalent time period as that found in matched patients before seroconversion

Parameter	Cases before seroconversion		Cases after seroconversion			Controls		
	Geometric mean	95% CI	Geometric mean	95% CI	*p*-Value[a]	Geometric mean	95% CI	*p*-Value[b]
CD4+T cells/μl	418	(303, 579)	501	(363, 691)	0.004	479	(418, 549)	0.282
CD8+T cells/μl	1217	(900, 1646)	1336	(991, 1803)	0.262	1106	(1011, 1210)	0.062
NK cells/μl	226	(142, 359)	217	(137, 343)	0.598	260	(228, 297)	0.212
HIV RNA (copies/ml)	336	(47, 2392)	167	(24, 1155)	0.365	103	(36, 292)	0.317
beta2-microglobulin (mg/l)	2.2	(1.9, 2.5)	2.5	(2.1, 2.9)	0.101	2.2	(1.9, 2.5)	0.654
CRP (mg/l)	2.1	(1.4, 3.1)	2.4	(1.4, 4.0)	0.787	2.2	(1.7, 2.7)	0.117
IgA total	3.5	(2.8, 4.5)	3.8	(3.0, 4.8)	0.245	2.6	(2.3, 2.9)	<0.001
IgG total	17.4	(14.5, 20.9)	19.1	(15.9, 23.0)	0.153	14.4	(13.3, 15.7)	<0.001
IgM total	1.4	(1.0, 2.1)	1.4	(0.9, 2.0)	0.632	1.1	(1.0, 1.3)	0.056

CI, confidence interval; NK, natural killer; CRP, C-reactive protein.
[a] Paired comparison of cases before and after seroconversion.
[b] Two-sample comparison of cases before seroconversion to controls.

The clinical picture of primary *T. gondii* infection in our patients was not associated with the state of T-cell-mediated immunity. For reactivation of latent *T. gondii* infection a CD4 count of <100 cells/μl is generally considered as critical.[23,24] The mean CD4 count of 479 cells/μl in our patients was significantly higher, but even in the only case with a CD4 count below 100 cells/μl (patient 12 with a CD4 count of 93 cells/μl) no clinical signs of primary *T. gondii* infection were observed. More than half of our patients had an NK count below the lower limit, but none of them had any symptoms of primary *T. gondii* infection.

It is known that for host resistance to *T. gondii*, the production of interferon-gamma by innate-type NK cells, which are responsible for the initial control of parasite growth, and later by adaptive CD4 and CD8, is crucial.[25–27] It might therefore be expected that individuals with deficits in these key immune cells could be more susceptible to *T. gondii* infection and thus prevail among the persons who acquire the infection. However, our findings do not confirm this hypothesis. The CD4 and NK counts in the HIV-infected patients before primary *T. gondii* infection did not significantly differ from the counts in the control group patients, who remained Toxoplasma-negative. An unexpected finding, which is difficult to explain, is the higher CD8 count before Toxoplasma seroconversion. This increase, similar to the elevation of IgA, IgG, and IgM levels against the control values that we recorded in patients prior to seroconversion, can hardly be associated with higher susceptibility to *T. gondii* infection and it may be a manifestation of an imbalance in the cellular response caused by the HIV infection. On the other hand, treatment, which can significantly improve the patient's immune status, did not prevent infection – both the treated and untreated patients became infected with Toxoplasma.[28] Evidently, other factors such as contact with Toxoplasma oocysts or tissue cysts seem to play a decisive role in the process of infection.

Another hypothesis anticipated a significant increase or decrease in crucial parameters of the immune response after seroconversion as a reaction to Toxoplasma infection. We found a significant increase in CD4 stimulated by Toxoplasma infection. This change was not related to the introduction of cART or to a change of treatment regimen before seroconversion. Thus, the reason for this mild CD4 count increase remains unclear. A possible explanation might be a partial restoration of the immune system after development of the immune response against Toxoplasma and the establishment of a latent infection. On the other hand, the incidence of Toxoplasma seropositivity was previously found to be higher in HIV-infected persons with CD4 counts of 200–499 cells/μl compared to counts of >500 cells/μl.[29] Unlike the CD4 count, other parameters remained unchanged after seroconversion.

Only thanks to years of monitoring very sizeable cohorts of HIV-infected patients did we manage to detect 14 cases of seroconversion and acquire unique data allowing an insight into the dynamics of the immunological and clinical parameters prior to and following Toxoplasma infection. However, the conclusions of our study are limited by the low incidence of seroconversions and the irregular intervals and number of examinations with great fluctuations in values.

Primary *T. gondii* infection is a rare event in Czech adult HIV-infected patients and its incidence is similar to the incidence of primary *T. gondii* infection in pregnant women in the Czech Republic and other Central European countries. Of course, the incidence of *T. gondii* infection can be influenced by preventive education of individuals at risk, but we assume that this factor did not play a major role in our cohort, because our patients were not specifically instructed on how to prevent *T. gondii* infection (e.g., sufficient cooking of meat, washing vegetables, caution when handling cat litter or soil).

The course of primary *T. gondii* infection is usually asymptomatic in HIV-infected individuals, regardless of their immune status, and can only be detected by regular serological screening. The primary *T. gondii* infection itself does obviously not present a significant risk to the health of HIV-infected patients. It is of course always valuable to recommend measures to patients to reduce the risk of exposure to *T. gondii*, but on the other hand it does not necessarily ensure prevention of primary *T. gondii* infection by chemoprophylaxis. Primary *T. gondii* infection, however, also represents the beginning of a process which in individuals with profound immunodeficiency may result in a life-threatening reactivation of latent infection; it is therefore advisable to perform periodic serological screening for *T. gondii* to detect this change in the patient's medical condition in a timely manner.

Acknowledgements

This study was supported by the Grant Agency of the Ministry of Health of the Czech Republic (IGA MZ ČR, No. NT/11429-5). The authors are grateful to Mrs Blanka Širocká and Mrs Jarmila Sedláková for excellent technical assistance.

Conflict of interest: All authors disclose no financial or personal relationships with other people or organizations that could inappropriately influence their work.

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