# Incidence, Clinical Presentation and Outcome of Cerebral Toxoplasmosis in HIV-infected patients during the Highly Active Antiretroviral Therapy Era: A Nationwide Cohort Study

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## Introduction

Cerebral toxoplasmosis (CTX) is the most common of the opportunistic infections (OI) in the central nervous system (CNS) of HIV-infected patients. Since the introduction of cART, the overall IR of OI and the post-OI mortality rate (MR) have declined dramatically (1-4). Still, incidence and mortality of CTX vary considerably between studies probably due to differences in sociodemographic characteristics, access to care and percentage of late presentation of HIV-infection of the different study populations. As CTX still remains an important cause of morbidity and mortality in HIV-infected patients (1,5-6), it seems important to further investigate the incidence, presenting symptoms, risk factors and prognosis of HIV-associated CTX as of today.

## Objectives

We conducted a cohort study to assess the risk of CTX and associated mortality in HIV-infected patients during the pre-cART (1995-1996) and cART-era (1997-2014), and assessed the associated predictive and prognostic factors. We further described the presenting characteristics and the clinical course of patients with CTX.

## Materials & Methods

From the Danish HIV Cohort Study (DHCS), we identified 6,325 Danish HIV-infected individuals aged ≥16 years (study period: 1995-2014). Data on CTX were obtained through medical files review. We assessed incidence rate (IR), mortality rate (MR), predictive factors, clinical presentation and prognosis of CTX during the pre-combination antiretroviral therapy (pre-cART: 1995-1996) and cART-era (1997-2014). We used Poisson regression analysis to assess adjusted incidence rate ratios (aIRR), mortality rate ratios (aMRR) and 95% confidence intervals (CI).

## Results

72 patients were diagnosed with CTX (IR: 1.17; 95% CI 0.93-1.47), of whom the majority (56.9%) were diagnosed with HIV before 1995. All CTX patients had advanced HIV-disease (table 1).

| | HIV-infected individuals (n=6,325) | HIV-infected individuals with CTX diagnosis (n=72) |
|---|---|---|
| Male, n (%) | 4,817 (76.1) | 53 (73.6) |
| Age at baseline, median years (IQR) | 37 (31-45) | 36 (32-47) |
| Caucasian, n (%) | 5,001 (79.0) | 55 (76.4) |
| Infection mode: | | |
| MSM, n (%) | 2,936 (46.8) | 30 (41.7) |
| Heterosexually infected, n (%) | 2,353 (37.4) | 31 (43.1) |
| IDU, n (%) | 612 (9.7) | 7 (9.7) |
| HCV, n (%) | 921 (14.6) | 7 (9.7) |
| HIV diagnosis before 1995, n (%) | 2,023 (32.0) | 41 (56.9) |
| AIDS before inclusion, n (%) | 533 (8.4) | 17 (23.6) |
| On cART before inclusion, n (%) | 276 (4.4) | 0 |
| CD4+ cell count at study inclusion, median cells/µL (IQR) | 300(117-497) | 61 (20-194) |
| VL at study inclusion, median $\log_{10}$ copies/mL (IQR) | 4.6 (3.7-5.3) | 5.3 (4.5-5.9) |
| PYR, n (%) | 61,698 | 160 |
| Median observation time (IQR) | 8.9 (3.7-15.7) | 0.9 (0.2-3.4) |
| Emigration during the study period, n (%) | 307 (4.9) | 0 |
| Loss to follow, n (%) | 29 (0.5) | 0 |
| Death, n (%) | 1,556 (24.6) | 42 (58.3) |
| CTX, n (%) | 72 (1.0) | 72 (100) |
| Age at CTX diagnosis, n (%) | - | 40 (33-48) |
| Median time from HIV to CTX diagnosis, years (IQR) | . | 4.4 (0.5-9.6) |
| AIDS before CTX diagnosis (%) | - | 32 (44.4) |
| CD4+ cell count <200 cells/µL at CTX diagnosis, n (%) | . | 65 (90.3) |
| On cART at CTX diagnosis, n (%) | . | 22 (31.0) |

**Table 1. Demographics and HIV-related characteristics of the study patients and the patients who developed cerebral toxoplasmosis (CTX) diagnosed during the study period**

**Incidence and predictive factors associated with CTX**: From the pre-cART to the cART-era we observed an unchanged risk of CTX during the first year after study inclusion (i.e. HIV diagnosis) (aIRR: 0.81; 95%CI: 0.42-1.56). In contrast, a substantial reduction in risk of CTX was observed in the subsequent years during the cART-era (aIRR: 0.06; 0.03-0.11. Higher risk of CTX was significantly associated with a low CD4+ cell count (< 200 cells/µL) and high VL (VL ≥100.000 c/mL) (table 2).

| Category | CTX events | PYR | IR per 1,000 PYR (95% CI) | IRR (95% CI) | aIRR (95% CI) |
|---|---|---|---|---|---|
| Total | 72 | 61,698 | 1.17 (0.93-1.47) | - | - |
| Calendar time (time-updated) | | | | | |
| Calendar time 1995-1996: | | | | | |
| -Year 0-1 after inclusion | 20 | 2,156 | 9.28 (5.98-14.38) | Ref (1) | Ref (1) |
| -Year > 1 after inclusion | 12 | 1,708 | 7.03 (3.99-12.37) | 0.76 (0.37-1.55) | 0.82 (0.40-1.67) [1] |
| Calendar time 1997-2014: | | | | | |
| -Year 0-1 after inclusion | 19 | 3,838 | 4.95 (3.16-7.76) | 0.53 (0.28-1.00) | 0.75 (0.47-1.54) [1] |
| -Year > 1 after inclusion | 21 | 53,996 | 0.39 (0.25-0.60) | 0.04 (0.02-0.08) | 0.05 (0.03-0.10) [1] |
| Gender | | | | | |
| Female | 19 | 15,945 | 1.19 (0.76-1.87) | Ref (1) | Ref (1) |
| Male | 53 | 45,752 | 1.16 (0.89-1.52) | 0.97 (0.58-1.64) | 0.85 (0.47-1.54) [1] |
| Age at baseline (per 1 year) | | | | 1.02 (1.00-1.04) | 1.01 (0.98-1.03) [1] |
| Race | | | | | |
| Caucasian | 55 | 48,715 | 1.13 (0.87-1.47) | Ref (1) | Ref (1) |
| Non-Caucasian | 17 | 12,982 | 1.31 (0.81-2.11) | 1.16 (0.67-2.00) | 1.46 (0.77-2.79) [1] |
| Injection drug use | | | | | |
| No injection drug use | 65 | 56,104 | 1.16 (0.91-1.48) | Ref (1) | Ref (1) |
| Injection drug use | 7 | 5,593 | 1.25 (0.60-2.63) | 1.08 (0.50-2.36) | 1.06 (0.48-2.38) [1] |
| CD4+ cell count at baseline | | | | | |
| CD4+cell count ≥ 200 cells / µL | 26 | 45,027 | 0.58 (0.39-0.85) | Ref (1) | Ref (1) |
| CD4+ cell count <200 cells/µL | 46 | 16,671 | 2.76 (2.07-3.68) | 4.78 (2.95-7.73) | 3.91 (2.39-6.39) [1] |
| VL at baseline | | | | | |
| VL < 100.000 c/mL | 9 | 22,154.2 | 0.41 (0.21-0.78) | Ref (1) | Ref (1) |
| VL >=100.000 c/mL | 63 | 39,543.3 | 1.59 (1.24-2.04) | 3.92 (1.95-7.89) | 3.03 (1.44-6.38) [1] |
| Sub analysis: | | | | | |
| Calendar time 1997-2014 and year >1 after inclusion stratified by: | | | | | |
| Inclusion < 1997 | 15 | 26,642 | 0.56 (0.34-0.93) | Ref (1) | Ref (1) |
| Inclusion >= 1997 | 6 | 27,354 | 0.22 (0.10-0.49) | 0.39 (0.15-1.00) | 0.51 (0.16-1.61) [2] |

Abbreviations: CTX, cerebral toxoplasmosis; PYR, person-years at risk; IR, incidence rates; IRR, incidence rate ratio; aIRR, adjusted incidence rate ratio; CI, confidence interval; VL, viral load; IDU, injection drug use; AIDS, Acquired immunodeficiency syndrome.
[1]Adjusted for all other variables in the model than the one observed.
[2] Adjusted for the following baseline variables: gender, age (continuous variable, per 1 year), race, injection drug use, calendar time, CD4+ cell count and viral load.

**Table 2. Predictive factors for cerebral toxoplasmosis in HIV-infected individuals**

**Mortality**: Forty-two patients (58.3%) diagnosed with CTX died during the study period of whom thirty (71%) died within the first year and nine (25%) within 30 days after the CTX diagnosis (data not shown).

We observed a substantial reduction in post CTX mortality with later calendar periods (1995-1996 vs. 1997-2014) during both the first (aMRR: 0.13; 0.06-0.30) and subsequent years (aMMR: 0.02; 0.01-0.04). And, during 1997-2014, no statistically significant difference in risk was observed between patients who had survived the first year after CTX diagnosis and in whom CTX was diagnosed before and after year 1996 (aMRR: 0.36; 0.08-1.74).

When evaluating prognostic factors, age at CTX diagnosis, injection drug use, and no exposure to cART before CTX diagnosis were associated with a statistically significant higher risk of mortality in univariate models (table 3).

| Prognostic factors | Death | PYR | MR (95% CI) per 1000 PYR | MRR (95% CI) | aMRR (95% CI) |
|---|---|---|---|---|---|
| Mortality overall | 42 | 388 | 108 (80-146) | - | - |
| Calendar time (time-updated) | | | | | |
| Calendar time 1995-1996: | | | | | |
| -Year 0-1 after CTX | 22 | 12 | 1,867 (1,229-2,835) | Ref (1) | Ref (1) |
| -Year > 1 after CTX | 2 | 1 | 3,336 (834-13,337) | 1.79 (0.42-7.60) | 1.51 (0.32-7.02) [1] |
| Calendar time 1997-2014 | | | | | |
| -Year 0-1 after CTX | 8 | 37 | 216 (108-433) | 0.12 (0.05-0.26) | 0.15 (0.06-0.38) [1] |
| -Year > 1 after CTX | 10 | 339 | 29 (16-55) | 0.02 (0.01-0.03) | 0.02 (0.01-0.05) [1] |
| Gender | | | | | |
| Female | 8 | 100 | 80 (40-160) | Ref (1) | Ref (1) |
| Male | 34 | 289 | 118 (84-165) | 1.47 (0.68-3.17) | 0.90 (0.38-2.11) [1] |
| Age at CTX (per 1 year) | | | | 1.04 (1.01-1.07) | 1.02 (0.99-1.06) [1] |
| Race | | | | | |
| Caucasian | 37 | 306 | 121 (88-167) | Ref (1) | Ref (1) |
| Non-Caucasian | 5 | 83 | 60 (25-145) | 0.50 (0.20-1.27) | 0.61 (0.21-1.77) [1] |
| IDU/no IDU | | | | | |
| No IDU | 35 | 374 | 94 (67-130) | Ref (1) | Ref (1) |
| IDU | 7 | 14 | 494 (235-1036) | 5.28 (2.34-11.88) | 1.38 (0.54-3.55) [1] |
| CD4+ count at CTX ≥< 50 | | | | | |
| CD4+ cell count ≥ 50 cells/µL | 9 | 126 | 72 (37-138) | Ref (1) | Ref (1) |
| CD4+ cell count < 50 cells/µL | 33 | 263 | 125 (89-177) | 1.75 (0.84-3.66) | 1.81 (0.81-4.01) [1] |
| cART before CTX | | | | | |
| No | 34 | 219 | 155 (111-218) | Ref (1) | Ref (1) |
| Yes | 8 | 170 | 47 (24-94) | 0.30 (0.14-0.66) | 0.83 (0.33-2.07) [1] |
| Sub analysis | | | | | |
| Calendar time 1997-2014 and year >1 after CTX stratified by: | | | | | |
| CTX < 1997 | 7 | 170 | 41 (20-86) | Ref (1) | Ref (1) |
| CTX >= 1997 | 3 | 169 | 18 (6-55) | 0.41 (0.11-1.66) | 0.28 (0.04-1.90) [2] |

Abbreviations: PYR, person-years at risk; MR: Mortality Rate, MRR Crude mortality rate ratio, aMRR: adjusted Mortality Rate Ratio; cART, combination antiretroviral therapy; IDU, injection drug use; AIDS, Acquired immunodeficiency syndrome.
[1]Adjusted for all other variables in the model than the one observed.
[2]Adjusted for the following baseline variables: gender, age (continuous variable, per 1 year), race, injection drug use, CD4 </>= 50cells/µL and cART before CTX.

**Table 3. Prognostic factors for death in HIV-infected individuals diagnosed with cerebral toxoplasmosis.**

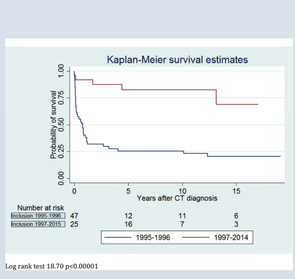


**Figure 1. Kaplan-Meier curves for overall survival of HIV-infected individuals with cerebral toxoplasmosis (CTX) by calendar time of CTX diagnosis (1995-1996) (blue), 1997-2014 (red).**

**Presenting symptoms and and neurological outcome in patients diagnosed with CTX** (table 3,4)

Headache (37.5%), cognitive deficit (41.7%), limb paresis (36.1%) and fever (75.9% > 37.5°C and 31% > 38.5°C) were the most common symptoms at presentation (table 4).

Four months after CTX, 61.2% of the patients experienced an improvement of their neurological symptoms and 18.4% experienced a complete resolution of their deficits. Three years after CTX, further improvement was observed, with 45.5% experiencing additional improvement of the neurological symptoms and 30% reporting a complete resolution of symptoms (table 5).

| | Disease presentation N=72 n (%) | Follow-up 4 months (N=49) n (%) | Follow-up 3 years (N=33) n (%) |
|---|---|---|---|
| Neurological symptoms | | | |
| Headache | 27 (38) | 3 (6) | 0 (0) |
| Nausea and vomiting | 14 (19) | 2 (4) | 1 (3) |
| Cognitive deficit | 30 (42) | 8 (16) | 3 (9) |
| Coordination disturbance | 21 (29) | 4 (8) | 0 (0) |
| Speech disturbance | 22 (31) | 6 (12) | 1 (3) |
| Visual impairment | 14 (19) | 3 (6) | 0 (0) |
| Facial palsy | 19 (26) | 3 (6) | 0 (0) |
| Limb paresis | 26 (36) | 13 (27) | 3 (9) |
| Sensory affection | 5 (7) | 2 (4) | 1 (3) |
| Seizures | 22 (31) | 5 (10) | 3 (9) |
| Need help in everyday life | 6 (8) | 6 (12) | 1 (3) |
| Status at follow-up | | | |
| Progression of neurological symptoms | | 6 (12) | 1 (3) |
| Unchanged neurological symptoms | | 2 (4) | 3 (9) |
| Improvement of neurological symptoms | | 30 (61) | 15 (46) |
| Resolution of neurological symptoms | | 9 (18) | 10 (30) |

**Table 4. Neurological symptoms of cerebral toxoplasmosis in HIV-infected individuals at primary presentation, at first follow-up visit after 4 months, and after 3 years**

| Category, parameter | Proportion (%) |
|---|---|
| Clinical and paraclinical signs at presentation | |
| Severely impaired GCS | 5/72 (7) |
| Moderately impaired GCS | 8/72 (11) |
| Mildly impaired GCS | 59/72 (82) |
| Fever > 37.5°C | 22/29 (76) |
| Fever > 38.5°C | 9/29 (31) |
| Elevated CRP or sedimentation rate[1] | 32/51 (63) |
| Leukocytosis in peripheral blood (>9 x $10^9$ cells/L) | 10/66 (15) |
| CSF pleocytosis (>10 cells/µL) | 10/45 (22) |
| Elevated CSF protein level (>0.5 g/L) | 33/44 (75) |
| Abnormal glucose level in CSF (< 2 mmol/L) | 2/44 (5) |
| Results of diagnostic test for CTX | |
| Positive IgG titers for toxoplasma | 59/66 (89) |
| Positive IgM titers for toxoplasma | 5/56 (9) |
| CTX diagnosed by suggestive MRI/CT-scans and clinical symptoms | 66/72 (92) |
| CTX diagnosed by PCR in CSF | 4/23 (17) |
| CTX diagnosed by brain biopsy | 3/5 (60) |
| CTX diagnosed at autopsy | 1/1 (100)[1] |
| Diagnosis | |
| Definitive diagnosis[2] | 8/72 (11) |
| Presumptive diagnosis with positive toxoplasma serology[3] | 53/72 (74) |
| Presumptive diagnosis with negative toxoplasma serology[3] | 6/72 (8) |
| Presumptive diagnosis with unknown toxoplasma serology[4] | 5/72 (7) |

Abbreviations: CRP: c reactive protein; CSF cerebrospinal fluid; CTX cerebral toxoplasmosis
[1] Autopsy might have been performed in more patients; however, the results of the autopsy is generally not presented in the patient files
[2] Definitive diagnosis was based on clinical and neuroradiological findings compatible with CTX and detection of *Toxoplasma gondii* by neuropathology or by polymerase chain reaction (PCR).
[3] Presumptive diagnosis was based on clinical and neuroradiological findings compatible with CTX, positive toxoplasma serology and favourable response to CTX.

**Table 5. Clinical data and laboratory results at presentation of HIV-infected individuals with cerebral toxoplasmosis.**

## Conclusions

In conclusion, CT still remains an important cause of morbidity and mortality among HIV-infected patients with advanced immunosuppression. However, the incidence of CT and post CT mortality has declined substantially during the cART-era, especially when surviving the first year of HIV-infection and CT, respectively. As a result, individuals diagnosed with HIV or CT during the pre-cART-era can be assured a low risk of CT or post CT mortality when compliant to cART. Hence, early diagnosis of HIV and cART initiation remains paramount.

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