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Cryptococcal meningitis in an HIV-1-infected person: relapses or IRIS? Case report and review of the literature

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Abstract. – After starting highly active antiretroviral therapy (HAART), HIV-infected patients may experience what is termed immune reconstitution inflammatory syndrome (IRIS). IRIS is characterized by a paradoxical inflammatory response to either previously or recently treated infections or unmasked subclinical infections, when the patient regains the ability to mount a suitable immune response against specific antigens or pathogens. Cryptococcal IRIS (C-IRIS) is thought to be mediated by recovery of Cryptococcus-specific immune responses, resulting in exaggerated host inflammatory responses. In HIV-positive subjects, two distinct modes of presentation of C-IRIS are recognized, "paradoxical" and "unmasking" C-IRIS. "Paradoxical" C-IRIS presents as worsening or recurrence of treated cryptococcal disease following HAART initiation, despite microbiological treatment success. In the "unmasking" form, patients with no prior diagnosis may develop acute symptoms of cryptococcosis, such as meningitis or necrotizing lymphadenopathy, after starting HAART.

Here, we present the case of an HIV-positive man, who developed cryptococcal meningitis two months after having started HAART and experienced several meningeal relapses and a "paradoxical" C-IRIS during the following year.

Key Words:
Antiretroviral therapy, Cryptococcal meningitis, HIV.

Introduction

Since its introduction, highly active antiretroviral therapy (HAART) has led to reduced mortality and morbidity and immunological reconstitution, with a significant decrease in the appearance of AIDS-related opportunistic illnesses (OIs) and the emergence of non-AIDS related events and malignancies, such as pancreatic, lung, colon and liver cancer[1-14]. Nonetheless, viral persistence in viral reservoirs and residual viremia still represent major obstacles to HIV eradication[15-24].

HAART-induced immunological recovery may be associated with the reactivation of latent infections, such as those due to *Mycobacterium tuberculosis, Mycobacterium avium, Cytomegalovirus*, *Pneumocystis jiroveci* and *Cryptococcus neoformans*[23]. Immune reconstitution inflammatory syndrome (IRIS) is characterized by a paradoxical inflammatory response to either previously or recently treated infections or unmasked subclinical infections, when the patient regains the ability to mount a suitable immune response against specific antigens or pathogens[25]. IRIS has been reported to have an overall prevalence at least as high as 10-25%[24]. This syndrome often occurs during the first two weeks of antiretroviral treatment among patients beginning HAART early after an OI, with low baseline CD4 T-cell count (< 100/μl) and an excellent virological response to therapy[27-30].

Cryptococcal IRIS (C-IRIS) is thought to be mediated by recovery of *Cryptococcus*-specific immune responses, resulting in exaggerated host inflammatory responses. In HIV infection, this reversal is driven by HAART, but C-IRIS has also been described as occurring after solid organ transplantation or during pregnancy[31-33]. In HIV-positive subjects, two distinct modes of presentation of C-IRIS are recognized, "paradoxical" and "unmasking" C-IRIS[34]. "Paradoxical" C-IRIS presents as worsening or recurrence of treated cryptococcal disease following HAART initiation, despite microbiological treatment success. In the "unmasking" form, patients with no prior diagnosis may develop acute symptoms of cryptococcosis, such as meningitis or necrotizing lymphadenopathy, after starting HAART.

Here, we present the case of an HIV-positive man, who developed cryptococcal meningitis two months after having started HAART and ex-

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perienced several meningeal relapses and a "paradoxical" C-IRIS during the following year.

Case Report

In May 2007, M. M., a 42-year-old man having sex with men, was admitted to our Department, due to sharp headache, with neither fever nor neurological signs.

Since January 2007, he had mucocutaneous lesions highly suggestive for Kaposi's sarcoma in his penis, arms and legs; in March 2007 he was diagnosed with HIV infection in another Outpatient Unit, his CD4 T-cell count was 56 cells/μl and HIV RNA viral load was 52,298 copies/mL. HAART was started with Emtricitabine-Tenofovir-Lopinavir/r (FTC-TDF-LPV/r).

On admission, his CD4 T-cell count was 76 cells/μl and HIV RNA viral load was 650 copies/mL. Cerebral computerized tomography (CT) did not show any pathological alteration. A lumbar puncture (LP) was performed and cerebrospinal fluid (CSF) culture was positive for *C. neoformans*. On the basis of these findings, a 18-day course of intravenous Amphotericin B (AmpB) was started (total dose 900 mg), followed by maintenance therapy with Fluconazole (Flu) (400 mg orally every day), while on HAART.

Although maintenance therapy with Flu was never interrupted, during the following 10 months the patient had some meningeal relapses (Table I). A LP was performed in 4 of these episodes (1, 8, 9 and 10 months after the first one) and each time CSF had the same characteristics: it was clear, with low glucose and high protein levels; cultures always tested positive for *C. neoformans* and negative for common bacteria and M. *tuberculosis*. During each relapse, the patient was treated with AmpB (50 mg/die), followed by maintenance therapy with Flu (400 mg/die). During the last episode, antibiogram showed sensitivity to all the most common antifungal drugs; a cerebral CT scan described the presence of "hypoxia of the right subcortical precentral rostral paramedian parenchyma", while encephalic magnetic resonance imaging (MRI) showed "areas of aberrant signal in the right caudate nucleus, with strong enhancement after gadolinium infusion, highly suggestive for cryptococcal relapse".

In July 2008, while still receiving prophylaxis with Flu 400 mg/die, the patient was admitted again to our Department, due to severe headache and vomiting. LP was performed and showed a slightly torbid CSF, with 22 white blood cells/μl,

Table I. Viro-immunological, clinical and microbiological aspects of relapsing cryptococcal meningitis, as found in our patient.

Period	May 2007	June 2007	January 2008	February 2008	March 2008	July 2008
CD4 T-cell count (cells/μl)	76	99	211	104	105	362
Viral load) (copies/mL	650	< 50	< 50	< 50	< 50	< 20
HAART	FTV-TDF-LPV/r	FTV-TDF-LPV/r	FTV-TDF-LPV/r	FTV-TDF-LPV/r	FTV-TDF-LPV/r	FTV-TDF-LPV/r
Symptoms	Headache	Headache	Headache	Headache	Headache	Headache
CSF parameters: aspect	Clear	Clear	–	Clear	Clear	Slightly torbid
pH	9	8	–	8	9	8.5
Glucose (mg/dL)	37	39		32	30	39
Proteins (mg/dL)	79	61		87	80	133
LDH (mg/dL)	44	20		24	22	18
Cells/μl		4		80	1	22
Cultures	Positive for C. *neoformans*	Positive for C. *neoformans*		Positive for C. *neoformans*	Positive for C. *neoformans*	Negative for common bacteria and fungi
Other data						India Ink stain for Cryptococcus negative; cryptococcal antigen positive (not quantified)

CSF: Cerebrospinal fluid; FTC-TDF-LPV/r: Emtricitabine-Tenofovir-Lopinavir/Ritonavir; HAART: Highly active antiretroviral therapy.

low glucose and high protein levels. India Ink stain was negative, whereas CSF cryptococcal antigen detection was positive. In addition to old lesions, encephalic MRI described new signs of leptomeningeal and cortical inflammation and a right midbrain active inflammatory lesion. While waiting for culture results, empiric antibiotic and antiviral therapy was started with high-dose Cefotaxime sodium and Acyclovir plus Dexamethasone for two weeks, followed by liposomal AmpB 250 mg/die for two weeks and Dexamethasone for a week, without achieving complete clinical resolution. The patient continued to receive HAART, his CD4 T-cell count was 362 cells/μl and HIV RNA was < 20 copies/ml. When finally fungal cultures were reported as negative, symptoms were assumed to be caused by C-IRIS. The patient was treated with Dexamethasone 3 mg/die for 3 months, obtaining a fast and complete resolution of headache. After this period, he began anti-inflammatory therapy with Indomethacin 50 mg/die and then Diclofenac 100 mg/die, for total 6 months.

The patient is currently on HAART and he is in good conditions, just complaining of sporadic episodes of short-term headache.

Discussion

Even though CNS disease (i.e., meningitis, meningoencephalitis, space occupying brain lesions) represents the most common presentation for C-IRIS, accounting for around 70% of cases, the clinical spectrum of disease also includes necrotic limphadenopathy, suppurating skin or soft tissue disease and lung disease[34].

The reported time of onset of paradoxical C-IRIS after the initiation of HAART varied from 1 to 10 months in cohort studies; on the other hand, the median time on HAART was 4 weeks in HAART-associated cryptococcosis[34]. In our case report, the first episode of meningitis occurred two months after starting HAART with FTC-TDF-LPV/r; several relapses occurred during the following 10 months.

Several retrospective studies indicated higher pre-HAART HIV viral load, earlier initiation of antiretroviral therapy and greater CD4 T-cell increase in the first 6 months of HAART as risk factors for paradoxical C-IRIS[29,35,36]. However, in prospective studies HIV viral load, time to start HAART and baseline CD4 T-cell count were not risk factors for C-IRIS[36-38]. Analogously, it is not clear if markers of fungal burden, i.e. higher serum cryptococcal antigen (CrAg) titer, may predict the risk for C-IRIS[36-38]. In a prospective cohort, the paucity of CSF inflammation (CSF protein < 50 mg/dL and WBC < 25 cells/μl) prior to HAART was associated with a seven-fold increase in IRIS risk[37]. As for HAART-associated cryptococcosis, the incidence may be as high as 33% in individuals with subclinical cryptococcal antigenemia, without fluconazole preemptive therapy[39,40]. Although HAART-associated cryptococcosis has been described even among subjects who were serum CrAg negative before HAART initiation, pre-HAART screening for cryptococcal antigenemia may be a useful strategy for identifying and treating subclinical infection and reducing the incidence of HAART-associated cryptococcosis, especially in high prevalence regions[34].

Clinical presentation of meningeal C-IRIS is indistinguishable from relapses, so culture results may help discriminating between them[34]. In our case, CSF cultures were always positive for *C. neoformans*, with the exception of the last episode, when only CSF cryptococcal antigen tested positive. Therefore, it may be hypothesized that the last episode was due to C-IRIS, whereas the previous clinical events were due to relapsing cryptococcal meningitis. However, it should be taken into account that a negative cryptococcal culture is not an absolute requirement for the diagnosis of IRIS, given the variable timing of CSF culture sterility in patients treated with AmphB or high-dose fluconazole. In fact, if patients commence HAART shortly after antifungal therapy, they may still have positive CSF culture when they present with paradoxical C-IRIS. Nevertheless, a positive fungal culture after 3 months of antifungal therapy is considered as therapeutic failure, excluding the diagnosis of C-IRIS[32].

Without secondary prophylaxis or effective immune reconstitution, HIV-positive patients with cryptococcal infection are at high risk for relapses. Until recently, life-long maintenance therapy to prevent disease relapse was recommended for all patients with HIV after successful completion of primary induction therapy for cryptococcal meningoencephalitis. However, on the basis of recent evidences, the Infectious Diseases Society of America (IDSA) guidelines suggest considering discontinuation of suppressive therapy during HAART in patients having received at least 12 months of antifungal therapy, with a CD4 T-cell count > 100 cells/μl and an undetectable or very low HIV RNA level sustained for > 3 months[41].

Conclusions

C-IRIS therapeutic management may require the use of anti-inflammatory drugs, in addition to HAART and antifungal therapy[41]. In case of severe CNS complications, i.e. elevated intracranial pressure, oral prednisolone (0.5-1 mg/kg/die) and possibly dexamethasone at higher doses may be used. In our case report, the patient experienced a rapid response to corticosteroids. Time of response to anti-inflammatory therapy is not predictable and a strict follow-up is required. Considering that prolonged steroid therapy is not free of side effects, Nonsteroidal Anti-Inflammatory drugs (NSAIDs) may be effective substitutes. In our case, steroid therapy was discontinued, because of the appearance of peripheral edema, moon facies and exacerbation of Kaposi's skin lesions. Switch to NSAIDs, which were administered for further seven months, was associated with a complete and prolonged remission of symptoms.

Conflict of Interest

None to declare.

References

1) Palella FJ, Baker RK, Moorman AC, Chmiel JS, Wood Kc, Brooks JT, Holmberg SD; HIV Outpatient Study Investigators. Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. J Acquir Immune Defic Syndr 2006; 43: 27-34.
2) Zanet E, Berretta M, Di Benedetto F, Talamini R, Ballarin R, Nunnari G, Berretta S, Ridolfo A, Lleshi A, Zanghì A, Cappellani A, Tirelli U. Pancreatic cancer in HIV-positive patients: a clinical case-control study. Pancreas 2012; 41: 1331-1335.
3) Berretta M, Garlassi E, Cacopardo B, Cappellani A, Guaraldi G, Cocchi S, De Paoli P, Lleshi A, Izzi I, Torresin A, Di Gangi P, Pietrangelo A, Ferrari M, Bearz A, Berretta S, Nasti G, Di Benedetto F, Balestreri L, Tirelli U, Ventura P. Hepatocellular carcinoma in HIV-infected patients: check early, treat hard. Oncologist 2011; 16: 1258-1269.
4) Berretta M, Lleshi A, Cappellani A, Bearz A, Spina M, Talamini R, Cacopardo B, Nunnari G, Montesarchio V, Izzi I, Lanzafame M, Nasti G, Basile F, Berretta S, Fisichella R, Schiantarelli C, Garlassi E, Ridolfo A, Guella L, Tirelli U. Oxaliplatin based chemotherapy and concomitant highly active antiretroviral therapy in the treatment of 24 patients with colorectal cancer and HIV infection. Curr HIV Res 2010; 8: 218-222.
5) Berretta M, Cappellani A, Di Benedetto F, Lleshi A, Talamini R, Canzonieri V, Zanet E, Bearz A, Nasti G, Lacchin T, Berretta S, Fisichella R, Balestreri L, Torresin A, Izzi I, Ortolani P, Tirelli U. Clinical presentation and outcome of colorectal cancer in hiv-positive patients: a clinical case-control study. Onkologie 2009; 32: 319-324.
6) Berretta M, Zanet E, Basile F, Ridolfo Al, Di Benedetto F, Bearz A, Berretta S, Nasti G, Tirelli U. HIV-positive patients with liver metastases from colorectal cancer deserve the same therapeutic approach as the general population. Onkologie 2010; 33: 203-204.
7) Zanet E, Berretta M, Martellotta F, Cacopardo B, Fisichella R, Tavio M, Berretta S, Tirelli U. Anal cancer: focus on HIV-positive patients in the HAART era. Curr HIV Res 2011; 9: 70-81.
8) Nunnari G, Xu Y, Acheampong Ea, Fang J, Daniel R, Zhang C, Zhang H, Mukhtar M, Pomerantz RJ. Exogenous IL-7 induces Fas-mediated human neuronal apoptosis: potential effects during human immunodeficiency virus type 1 infection. J Neurovirol 2005; 11: 319-328.
9) Nunnari G, Pomerantz RJ. IL-7 as a potential therapy for HIV-1-infected individuals. Expert Opin Biol Ther 2005; 5: 1421-1426.
10) Nunnari G, Berretta M, Pinzone MR, Di Rosa M, Cappellani A, Berretta S, Tirelli U, Malaguarnera M, Schnell JM, Cacopardo B. Hepatocellular carcinoma in HIV positive patients. Eur Rev Med Pharmacol Sci 2012; 16: 1257-1270.
11) Martellotta F, Berretta M, Cacopardo B, Fisichella R, Schioppa O, Zanghì A, Spartà D, Cappellani A, Talamini R, Izzi I, Ridolfo A, Torresin A, Fiorica F, Tirelli U. Clinical presentation and outcome of squamous cell carcinoma of the anus in HIV-infected patients in the HAART-era: a GICAT experience. Eur Rev Med Pharmacol Sci 2012; 16: 1283-1291.
12) Berretta M, Di Benedetto F, Dal Maso L, Cacopardo B, Nasti G, Facchini G, Bearz A, Spina M, Garlassi E, De Re V, Fiorica F, Lleshi A, Tirelli U. Sorafenib for the treatment of unresectable hepatocellular carcinoma in HIV-positive patients. Anticancer Drugs 2013; 24: 212-218.
13) Martellotta F, Berretta M, Vaccher E, Schioppa O, Zanet E, Tirelli U. AIDS-related Kaposi's sarcoma: state of the art and therapeutic strategies. Curr HIV Res 2009; 7: 634-638.
14) Berretta M, Cinelli R, Martellotta F, Spina M, Vaccher E, Tirelli U. Therapeutic approaches to AIDS-related malignancies. Oncogene 2003; 22: 6646-6659.
15) Pinzone MR, Di Rosa M, Cacopardo B, Nunnari G. HIV RNA suppression and immune restoration: can we do better? Clin Dev Immunol 2012; 2012: 515962.
16) Nunnari G, Coco C, Pinzone MR, Pavone P, Berretta M, Di Rosa M, Schnell M, Calabrese G, Cacopardo B. The role of micronutrients in the diet of HIV-1-infected individuals. Front Biosci (Elite Ed) 2012; 4: 2442-2456.
17) Pinzone MR, Cacopardo B, Condorelli F, Di Rosa M, Nunnari G. Sirtuin-1 and HIV-1: An Overview. Curr Drug Targets 2013; 14: 648-652.
18) Pinzone MR, Fiorica F, Di Rosa M, Malaguarnera G, Malaguarnera L, Cacopardo B, Zanghì G, Nunnari G. Non-AIDS-defining cancers among HIV-infected people. Eur Rev Med Pharmacol Sci 2012; 16: 1377-1388.

19) Nunnari G, Smith JA, Daniel R. HIV-1 Tat and AIDS-associated cancer: targeting the cellular anti-cancer barrier. J Exp Clin Cancer Res 2008; 27: 3.
20) Nunnari G, Otero M, Dornadula G, Vanella M, Zhang H, Frank I, Pomerantz RJ. Residual HIV-1 disease in seminal cells of HIV-1-infected men on suppressive HAART: latency without on-going cellular infections. AIDS 2002; 16: 39-45.
21) Dornadula G, Nunnari G, Vanella M, Roman J, Babinchak T, DeSimone J, Stern J, Braffman M, Zhang H, Pomerantz RJ. Human immunodeficiency virus type 1-infected persons with residual disease and virus reservoirs on suppressive highly active antiretroviral therapy can be stratified into relevant virologic and immunologic subgroups. J Infect Dis 2001; 183: 1682-1687.
22) Otero M, Nunnari G, Leto D, Sullivan J, Wang FX, Frank I, Xu Y, Patel C, Dornadula G, Kulkosky J, Pomerantz RJ. Peripheral blood Dendritic cells are not a major reservoir for HIV type 1 in infected individuals on virally suppressive HAART. AIDS Res Hum Retroviruses 2003; 19: 1097-1103.
23) Nunnari G, Argyris E, Fang J, Mehlman KE, Pomerantz RJ, Daniel R. Inhibition of HIV-1 replication by caffeine and caffeine-related methylxanthines. Virology 2005; 335: 177-184.
24) Smith JA, Nunnari G, Preuss M, Pomerantz RJ, Daniel R. Pentoxifylline suppresses transduction by HIV-1-based vectors. Intervirology 2007; 50: 377-386.
25) Pinzone MR, Di Rosa M, Malaguarnera M, Madeddu G, Focà E, Ceccarelli G, d'Ettorre G, Vullo V, Fisichella R, Cacopardo B, Nunnari G. Vitamin D deficiency in HIV infection: an underestimated and undertreated epidemic. Eur Rev Med Pharmacol Sci 2013; 17: 1218-1232.
26) Hirsch HH, Kaufmann G, Sendi P, Battegay M. Immune Reconstitution in HIV-Infected Patients. Clin Infect Dis 2004; 38: 1159-1166.
27) Ratnam I, Chiu C, Kandala NB, Easterbrook PJ. Incidence and risk factors for immune reconstitution inflammatory syndrome in an ethnically diverse HIV type 1-infected cohort. Clin Infect Dis 2006; 42: 418-427.
28) Bonham S, Meya DB, Bohjanen PR, Boulware DR. Biomarkers of HIV Immune Reconstitution Inflammatory Syndrome. Biomark Med 2008; 2: 349-361.
29) Lortholary O, Fontanet A, Mémain N, Martin A, Sitbon K, Dromer F; French Cryptococcosis Study Group. Incidence and risk factors of immune reconstitution inflammatory syndrome complicating HIV-associated cryptococcosis in France. AIDS 2005; 19: 1043-1049.
30) Putignani L, Antonucci G, Paglia MG, Vincenzi L, Festa A, De Mori P, Loiacono L, Visca P. Cryptococcal lymphadenitis as a manifestation of immune reconstitution inflammatory syndrome in an HIV-positive patient: a case report and review of the literature. Int J Immunopathol Pharmacol 2008; 21: 751-756.
31) Singh N, Perfect JR. Immune reconstitution syndrome associated with opportunistic mycoses. Lancet Infect Dis 2007; 7: 395-401.
32) Singh N, Lortholary O, Alexander BD, Gupta KL, John GT, Pursell K, Munoz P, Klintmalm GB, Stosor V, del Busto R, Limaye AP, Somani J, Lyon M, Houston S, House AA, Pruett TL, Orloff S, Humar A, Dowdy L, Garcia-Diaz J, Kalil AC, Fisher RA, Husain S; Cryptococcal Collaborative Transplant Study Group. An immune reconstitution syndrome-like illness associated with Cryptococcus neoformans infection in organ transplant recipients. Clin Infect Dis 2005; 40: 1756-1761.
33) Singh N, Perfect JR. Immune reconstitution syndrome and exacerbation of infections after pregnancy. Clin Infect Dis 2007; 45: 1192-1199.
34) Haddow LJ, Colebunders R, Meintjes G, Lawn SD, Elliott JH, Manabe YC, Bohjanen PR, Sungkanuparph S, Easterbrook PJ, French MA, Boulware DR, on behalf of the International Network for the Study of HIV associated IRIS (INSHI). Cryptococcal immune reconstitution inflammatory syndrome in hiv-1-infected individuals: literature review and proposed clinical case definitions. Lancet Infect Dis 2010; 10: 791-802.
35) Shelburne SA, Darcourt J, White WA, Greenberg SB, Hamill RJ, Atmar RL, Visnegarwala F. The role of immune reconstitution inflammatory syndrome in AIDS-related Cryptococcus neoformans disease in the era of highly active antiretroviral therapy. Clin Infect Dis 2005; 40: 1049-1052.
36) Bicanic T, Meintjes G, Rebe K, Williams A, Loyse A, Wood R, Hayes M, Jaffar S, Harrison T. Immune reconstitution inflammatory syndrome in HIV-associated cryptococcal meningitis: a prospective study. J Acquir Immune Defic Syndr 2009; 51: 130-134.
37) Boulware DR, Bonham SC, Meya DB, Wiesner DL, Park GS, Kambugu A, Janoff EN, Bohjanen PR. Paucity of initial cerebrospinal fluid inflammation in cryptococcal meningitis is associated with subsequent immune reconstitution inflammatory syndrome. J Infect Dis 2010; 202: 962-970.
38) Sungkanuparph S, Filler SG, Chetchotisakd P, Pappas PG, Nolen TL, Manosuthi W, Anekthananon T, Morris MI, Supparatpinyo K, Kopetskie H, Kendrick AS, Johnson PC, Sobel JD, Larsen RA. Cryptococcal immune reconstitution inflammatory syndrome after antiretroviral therapy in AIDS patients with cryptococcal meningitis: a prospective multicenter study. Clin Infect Dis 2009; 49: 931-934.
39) Jarvis JN, Lawn SD, Vogt M, Bangani N, Wood R, Harrison TS. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. Clin Infect Dis 2009; 48: 856-862.
40) Pongsai P, Atamasirikul K, Sungkanuparph S. The role of serum cryptococcal antigen screening for the early diagnosis of cryptococcosis in HIV-infected patients with different ranges of CD4 cell counts. J Infect 2010; 60: 474-477.
41) Perfect JR, Dismukes WE, Dromer F, Goldman DL, Graybill JR, Hamill RJ, Harrison TS, Larsen RA, Lortholary O, Nguyen MH, Pappas PG, Powderly WG, Singh N, Sobel JD, Sorrell TC. Clinical practice guidelines for the management of cryptococcal disease: 2010 Update by the Infectious Diseases Society of America. Clin Infect Dis 2010; 50: 291-322.