Original Article

THE IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME: A CAUSE OF DEATH IN PERSONS ON ANTIRETROVIRAL THERAPY?

Huis in 't Veld D[1, 2], Lukande R[3], Lammens M[4], Conesa-Botella A[1, 5], Vlieghe E[5, 6], Van Marck E[4], Pauwels P[4], Colebunders R[1, 5]

[1]Department of Epidemiology and Social Medicine, University of Antwerp, Antwerpen, Belgium, [2]Fonds Wetenschappelijk Onderzoek-Vlaanderen. Aspirant Mandate holder. FWO, Brussels, Belgium, [3]Department of Pathology, Mulago Hill Road, College of Health Sciences, Makerere University, Kampala, Uganda, [4]Department of Pathology, Antwerp University Hospital, Edegem, Belgium, [5]Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium, [6]Department of Tropical Medicine, Antwerp University Hospital, Edegem, Belgium

Correspondence and offprint requests to: Diana Huis in 't Veld, E-mail: dhuisintveld@itg.be

ABSTRACT

The availability of antiretroviral therapy (ART) has significantly improved the quality of life of persons with HIV infection. However, new problems have arisen as a consequence of this treatment. An immune reconstitution inflammatory syndrome (IRIS) in which patients experience a paradoxical worsening of their clinical condition may occur during recovery of the immunity. Thus far, there is no laboratory test available to diagnose IRIS. The diagnosis therefore remains clinical and by exclusion. In this paper, we describe the autopsy findings of three HIV-infected patients who died at the Antwerp University hospital directly or indirectly related to IRIS. One patient died following a disseminated cryptococcocal and *Mycobacterium avium complex* (MAC) infection. Two other patients died with a disseminated aspergillosis infection after receiving corticosteroids to decrease IRIS induced inflammatory signs. These three patients show the difficulties faced by clinicians in diagnosing IRIS and the importance of performing autopsies in persons with HIV infection who die despite receiving ART.

Key words: HIV, immune reconstitution inflammatory syndrome, mortality, autopsy

INTRODUCTION

Since the discovery of the first antiretroviral drugs much progress has been made in treating persons with HIV infection. However, 11-45% of patients infected with HIV who start antiretroviral therapy (ART) while being treated for an opportunistic infection, experience a syndrome characterized by an excessive inflammatory response and a paradoxical deterioration of their clinical condition (1, 2). This syndrome, called paradoxical immune reconstitution inflammatory syndrome (IRIS), is thought to be an inflammatory response towards circulating antigens during immune restoration (1). Patients without a symptomatic opportunistic infection may also develop inflammatory signs and symptoms caused by an untreated active opportunistic infection shortly after starting ART. This has been termed the "unmasking" type of IRIS (1).

IRIS can occur in response to antigens of pathogens, autoantigens or cancer antigens (1). IRIS remains a condition that is neither well defined nor understood. So far there is no known laboratory test for its diagnosis. Therefore IRIS is a diagnosis of exclusion. Currently there is no internationally agreed case definition for all types of IRIS, but case definitions for tuberculosis (TB) (3) and cryptococcal meningitis-IRIS have been proposed (4). Moreover, it is unclear how to prevent and manage IRIS. Known risk factors for the development of IRIS include a low CD4 T-cell count when starting ART, a disseminated opportunistic infection and a short time interval between opportunistic infection treatment and the start of

ART (1). Some, but not all, studies show that a rapid improvement of immunity and an excellent virological response are also associated with the development of IRIS (5). A meta-analysis by Muller and colleagues (6) showed an overall 4.5% mortality in IRIS patients. Mortality from central nervous system-IRIS is higher (7). Cryptococcal meningitis is the main killer with 20.8% mortality.

To illustrate the difficulties in diagnosis and managing patients suspected of IRIS we describe three patients who died at the Antwerp University hospital, directly or indirectly because of IRIS.

CLINICAL CASES

Case 1

A 37 year old Caucasian man was found to be HIV infected in November 2007 when he presented with a *Pneumocystis jirovecii* pneumonia (PJP). He was treated for the PJP but refused to start ART. In July 2008 he was referred by a general practitioner because of weight loss, weakness and headache since 10 days. Initially he refused to be hospitalized. A serum cryptococcal antigen test (CrAG) was requested but because of a CD4+cell count of 34/µL ART (lamivudine, tenofovir and lopinavir/ritonavir) was started without waiting for the result. Five days after the start of ART he had to be hospitalized because of fever, weight loss and increasing headache. Physical examination revealed mollusca-like skin lesions. The serum CrAG test was positive and a skin biopsy, blood cultures and cerebrospinal fluid (CSF) examination confirmed a Cryptococcus neoformans infection. Cultures did not reveal any other pathogen. Laboratory tests revealed the following abnormal values: alkaline phosphatase 197 U/L (n: 53-128), LDH 1006 U/L (n: 84-246), CK 181 (n: 34-145), AST 40 (n: <31). ART was interrupted and liposomal Amphotericin B was started. Twenty-four hours later, he developed high fever and epilepsy. He was admitted in the intensive care unit but did not recover despite the administration of valproic acid, diphantoin, clonazepam and midazolam with intubation and ventilation and the placement of an intraventricular drainage tube. Corticosteroids were administered, but 2 days later he died with high intracranial pressure refractory to drainage. A postmortem examination revealed a disseminated cryptococcocal and *Mycobacterium avium complex* (MAC) infection. The cause of death at autopsy was considered to be "status epilepticus and refractory cerebral oedema".

Case 2

A 44 year old HIV seropositive Caucasian man who had stopped his ART 4 years earlier was hospitalized in December 2006 because of a left hemiparesis. A magnetic resonance imaging (MRI) of the brain showed a right fronto-temporal brain lesion also involving the brain stem. A PCR-test for *polyomavirus* (JC-virus) on CSF was positive. ART was started 15 days later (zidovudine, lamivudine, abacavir, saquinavir and ritonavir) at a $CD4^+$ cell count of 10/µL. One week after the start of ART, he developed a submandibular abscess and the brain lesion increased. An aspiration of the abscess revealed acid fast bacilli. The abscess was drained and the patient was treated with rifampicin, isoniazide, pyrazinamide, ethambutol, claritromycine and methylprednisolone (48 mg a day for a month, reduced to 32 mg a day after that, until death). Culture of the pus revealed a MAC-infection and no other pathogens. His clinical condition improved, but 6 weeks later he was re-hospitalized because of fever, dyspnea, loss of appetite and fatigue. A polymerase chain reaction (PCR) test on broncho-alveolar lavage fluid was positive for *Cytomegalovirus* (CMV) but negative for PJP. A repeat brain MRI revealed further increase of the brain lesions. He died 2.5 months after the start of ART due to pulmonary and cerebral edema. Postmortem examination revealed a necrotizing bronchopneumonia due to invasive aspergillosis, CMV-infection of the lungs and presence of gram positive cocci in the kidneys and a progressive multifocal leukoencephalopathy (PML) infection of the brain. The most likely immediate cause of death was considered to be the bronchopneumonia.

Case 3

In June 2007, a 67 year old HIV positive Caucasian man was hospitalized because of progressive weight loss, PJP, oesophageal candidiasis and an oral *Herpes simplex virus* infection. A neurological examination revealed loss of memory, disorientation and bradypsychia. A cerebral MRI in T2-weighted setting showed a zone of enhancement compatible with PML or HIV meningo-encephalitis. In addition, there were clinical and biological signs of a syndrome of inappropriate antidiuretic hormone secretion.

One month later, at a CD4 T-cell count of 4/µL, ART was started (lopinavir/ritonavir, emtricitabine and tenofovir). Ethambutol and claritromycine were added at the same moment, because blood cultures revealed a MAC infection. Two months after starting ART, he developed acute renal failure and a pericardial effusion. A renal biopsy showed signs of acute tubular necrosis and acute interstitial nephritis but no signs of focal segmental glomerulosclerosis. Renal dialysis was started. ART was interrupted and methylprednisolone 48 mg/day was given because a nephropathy caused by a MAC-infection-associated IRIS of the kidneys was suspected. He developed an *Enterococcus fecalis* blood stream infection which was treated with vancomycin. He improved clinically and the ART (abacavir, lamivudine and lopinavir/ritonavir) was restarted while the methylprednisolone was continued (48 mg/day).

Three months later, the renal failure had resolved but he was readmitted because of a pulmonary infection with multiple pathogens (*Enterobacter*, *Pseudomonas aeruginosa*, CMV and *Aspergillus fumigatus)* and a possible CMV colitis. He died a few days later with multi-organ failure. Postmortem examination of the lungs showed multiple abscesses with an *Aspergillus* sp. infection in the lung parenchyma, and large areas of hemorrhagic infarction following angio-invasion by *Aspergillus*. Sections of the myocardium showed multiple abscesses with *Aspergillus*, and scarring. A section of the thyroid gland showed multiple abscesses with *Aspergillus*. Examination of the kidney showed features of acute tubular necrosis (granular casts in the tubules) and interstitial abscesses with *Aspergillus*. A section through an adrenal revealed cortical atrophy. Sections of colon, esophagus, liver and pancreas were normal.

DISCUSSION

The three patients who died at the Antwerp University Hospital illustrate the difficulties of diagnosing IRIS and the problems in managing patients suspected of IRIS. Our first

patient's history is compatible with the clinical course of an unmasking cryptococcal associated IRIS following the initiation of ART. Postmortem examination confirmed a disseminated cryptococcal infection, but also a previously unrecognized MAC infection. The cause of death was probably the cryptococcal central nervous system (CNS) infection and the increased intracranial pressure and cerebral oedema caused by inflammation induced by the ART. The result of the CrAG test only became available after the start of ART. Today we certainly would have done this test before the start of ART because his CD4 count was below 100 cells/µL. With a positive CrAG test the cryptococcal meningitis would have been diagnosed earlier and we would have been able to start antifungal treatment before the start of ART. A recent study showed that in HIV infected patients with cryptococcal meningitis the start of ART should be delayed up to 6 weeks after the start of the antifungal treatment (8). This information however was not available at the moment we treated the patient. The ideal time to start ART in HIV infected patients with an opportunistic infection is not very well known but depends on the degree of immune deficiency and the type of opportunistic infection. Indeed in a person with a CD4 count < 50 cells/µL with a TB co-infection in contrast with cryptococcal meningitis ART should be started early, within 2 weeks of the start of the TB treatment. The latter strategy may increase the incidence of IRIS but will decrease mortality. (9, 10)

Our second patient developed MAC-IRIS which was treated with corticosteroids. His clinical situation improved at first, but eventually he developed several infectious problems, including a CMV- and an invasive pulmonary aspergillosis confirmed by postmortem examination.

Patient 3 developed an acute tubular necrosis with acute interstitial nephritis and a pericardial effusion. Whether these manifestations were caused by IRIS or because of tenofovir toxicity is unclear. His renal function improved during corticosteroid therapy but he finally died because of a generalized aspergillus infection probably related to the prolonged corticosteroid use.

The clinical picture of IRIS can be very diverse and there are no diagnostic tests for IRIS. The general approach to the treatment of IRIS is to continue ART and to provide antimicrobial therapy for the provoking infection. Corticosteroids are often used in CNS-IRIS to dampen the effects of inflammation (11). However, it increases the risk of exacerbating other asymptomatic opportunistic infections if screening for these infections has not been thoroughly done (7). In South Africa a placebo-controlled randomized trial evaluated the use of corticosteroids to reduce morbidity in tuberculosis-IRIS (TB-IRIS). Prednisone reduced the need for hospitalization and additional procedures, and resulted in symptom improvement without an excess of corticosteroid related side effects or severe infections (12). Therefore corticosteroids can be considered in the treatment of TB-IRIS, but drug resistant TB should be excluded first.

Whether corticosteroids can reduce IRIS related mortality remains unknown. In patients 2 and 3 corticosteroids were started because IRIS was suspected but this likely contributed to the acquisition of an invasive aspergillus infection (not suspected pre-mortem) and subsequently the death of the patients. It is well known that prolonged corticosteroid treatment particularly in immune deficient patients is a risk factor for a generalized aspergillus infection (13).

Non-steroidal anti-inflammatory drugs (NSAID), tumour necrosis factor (TNF) inhibitors (14, 15), CCR5 inhibitors (Maraviroc), thalidomide (16) and leukotriene receptor antagonists (17) have also been suggested for the treatment of IRIS (11, 18), but sufficient evidence for their clinical use is lacking. Maraviroc, in addition to being an anti-HIV drug, also displays immune modulatory properties – possibly through blocking of CCR5 and interfering with leukotriene recruitment to the site of infection (14). Two on-going clinical trials are currently evaluating Maraviroc and NSAID in the prevention of IRIS.

The most important way to prevent IRIS is to diagnose and treat HIV infection early before opportunistic infections develop. In case of delayed HIV diagnosis screening for opportunistic infections and timely treating such infections before starting ART will reduce the incidence of IRIS and potentially the mortality related to IRIS (19) A placebo controlled randomized trial will evaluate the use of prednisolone to prevent TB IRIS in patients in South Africa (trial coordinated by G. Meintjes (University of Cape Town) in collaboration with the Institute of Tropical Medicine, Antwerp).

CONCLUSION

IRIS directly or indirectly can be a cause of mortality in patients with HIV. Patients with a CD4 lymphocyte count < 100 cell/µL should be carefully screened for the presence of opportunistic infections and this should include serum CrAG testing even in asymptomatic patients before the start of ART. Before starting corticosteroid treatment in a patient suspected of IRIS it is important to investigate whether the patient does not have concomitant infections and during corticosteroid treatment patients should be monitored for the development of co-infections such as a generalized aspergillus infection. More trials are needed to evaluate options to prevent and treat IRIS. The three patients described in this paper demonstrate the importance of performing autopsies to determine causes of death in persons who die after starting ART.

STATEMENT

All authors have made substantial contributions to the manuscript's conception, design and performance.

CONFLICT OF INTEREST: None.

REFERENCES

1. Dhasmana DJ, Dheda K, Ravn *p,* Wilkinson RJ, Meintjes G. Immune reconstitution inflammatory syndrome in HIV-infected patients receiving antiretroviral therapy: pathogenesis, clinical manifestations and management. *Drugs* 2008; 68: 191-208.
2. Lorent N, Conesa-Botella A, Colebunders R. The immune reconstitution inflammatory syndrome and antiretroviral therapy. *Br J Hosp Med (Lond)* 2010; 71: 691-697.
3. Meintjes G, Lawn SD, Scano F, Maartens G, French MA, Worodria W, Elliott JH, Murdoch D, Wilkinson RJ, Seyler C, John L, van der Loeff MS, Reiss *p,* Lynen L, Janoff EN, Gilks C, Colebunders R. Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings. *Lancet Infect Dis* 2008; 8: 516-523.

4. Haddow LJ, Colebunders R, Meintjes G, Lawn SD, Elliott JH, Manabe YC,et al. Cryptococcal immune reconstitution inflammatory syndrome in HIV-1-infected individuals: proposed clinical case definitions. *Lancet Infect Dis* 2010; 10(11): 791-802.
5. Grant PM, Komarow L, Andersen J, Sereti I, Pahwa S, Lederman MM et al. Risk factor analyses for immune reconstitution inflammatory syndrome in a randomized study of early vs. deferred ART during an opportunistic infection. *PLoS One* 2010; 5(7): e11416.
6. Muller M, Wandel S, Colebunders R, Attia S, Furrer H, Egger M. Immune reconstitution inflammatory syndrome in patients starting antiretroviral therapy for HIV infection: a systematic review and meta-analysis. *Lancet Infect Dis* 2010; 10(4): 251-261.
7. Mateen FJ, Nath A. Central nervous system-immune reconstitution inflammatory syndrome in resource-limited settings: current burden and future needs. *AIDS* 2012; 26: 1851-1855.
8. National Institute of Allergy and Infectious Diseases/National Institutes of Health. HIV treatment study in pateints with cryptococcal meningitis ends enrollment early. *NIAID/NIH Bulletin* 2012; accesible on: http://www.niaid.nih.gov/news/newsreleases/2012/Pages/COAT.aspx
9. Abdool Karim SS, Naidoo K, Grobler A, et al. Integration of antiretroviral therapy with tuberculosis treatment. *N Engl J Med.* 2011; 365(16): 1492-1501.
10. Blanc FX, Sok T, Laureillard D, et al. Earlier versus later start of antiretroviral therapy in HIV-infected adults with tuberculosis. *N Engl J Med* 2011; 365(16): 1471-1481.
11. Marais S, Wilkinson RJ, Pepper DJ, Meintjes G. Management of patients with the immune reconstitution inflammatory syndrome. *Curr HIV /AIDS Rep* 2009; 6(3): 162-171.
12. Meintjes G, Wilkinson RJ, Morroni C, et al. Randomized placebo-controlled trial of prednisone for paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome. *AIDS* 2010; 24(15): 2381-2390.
13. Mylonakis E, Barlam TF, Flanigan T, Rich JD. Pulmonary aspergillosis and invasive disease in AIDS: review of 342 cases. *Chest* 1998; 114(1): 251-262.
14. Lawn SD, Meintjes G. Pathogenesis and prevention of immune reconstitution disease during antiretroviral therapy. *Expert Rev Anti Infect Ther* 2011; 9: 415-430.
15. Blackmore TK, Manning L, Taylor WJ, Wallis RS. Therapeutic use of infliximab in tuberculosis to control severe paradoxical reaction of the brain and lymph nodes. *CID* 2008: 47: e83-e85.
16. Brunel A, REynes J, Tuaillon E, et al. Thalidomide for steroid-dependent immune reconstitution inflammatory syndromes during AIDS. *AIDS* 2012; 26(16): 2110-2112.
17. Hardwick C, White D, Morris E, Monteiro EF, Breen RA, Lipman M. Montelukast in the treatment of HIV associated immune reconstitution disease. *Sex Transm Infect* 2006; 82: 513-514.
18. Meintjes G, Scriven J, Marais S. Management of the Immune Reconstitution Inflammatory Syndrome. *Curr HIV/AIDS Rep* 2012; 9: 238-250.
19. Meya DB, Manabe YC, Castelnuovo B, et al. Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+cell count <or=100 cells/microL who start HIV therapy in resource-limited settings. *Clin Infect Dis* 2010 15; 51: 448-455.