DIFFUSE INFILTRATIVE LYMPHOCYTOSIS SYNDROME AND ACUTE CYTOMEGALOVIRUS INFECTION IN A HIV INFECTED PATIENT: A ROMANIAN CASE REPORT

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ABSTRACT

Diffuse infiltrative lymphocytosis syndrome (DILS), a particular feature of HIV infection, is defined by the oligoclonal expansion of CD8+ T lymphocytes in response to HIV antigens. This syndrom is often associated with lymphocytic infiltration of various organs, especially the salivary glands, followed by the onset of symptoms of Sjögren's-like syndrome (xerostomia and xerophthalmia). The lung is the most common extra glandular site affected during the evolution of the disease, causing a clinical and imaging pattern of interstitial pneumonia.

We describe a case of a 43-years-old patient with HIV infection who presented, at the time of diagnosis, a clinical Sicca syndrome and interstitial pneumonia highly suggestive for PCP. Initial biological evaluation showed a high CD8+ lymphocytosis (8819/mm^3) which raised the suspicion of DILS. Simultaneously, acute CMV infection with CMV pneumonitis was confirmed. The subsequent investigations ruled out the diagnosis of PCP, autoimmune diseases and malignancies. This case has some peculiarities related to HIV infection (higher viral replication [HIV RNA 115,000,000 cp/mL], acute HIV infection being excluded), and acute CMV infection associated with CD8+ lymphocytosis syndrome with common clinical and biological features.

The clinical and laboratory evolution was favorable under treatment with ganciclovir, corticosteroids and antiretroviral drugs.

Keywords: CD8+ lymphocytosis, interstitial pneumonitis, CMV acute infection, HIV

INTRODUCTION

Infection with human immunodeficiency virus (HIV) causes a progressive decrease of CD4+ T cells and therefore patients are at risk for developing opportunistic diseases. Normally the CD8+ T cell population increases in the initial phase of HIV infection as part of the host immune response against HIV antigens (1). This response is not sustained and is followed by a subsequent decline in the CD8+ T cells during disease evolution. However, persistent expansion of the CD8+ lymphocytes is observed in two related settings: diffuse infiltrative lymphocytosis syndrome (DILS) and HIV associated CD8+ lymphocytosis syndrome (1,2).

DILS is defined as a oligoclonal expansion of CD8+ T lymphocytes and lymphocytic infiltration of various organs. These cells infiltrate multiple organs and the most common sites involved in this process are salivary glands and lungs (2,3). Patients typically present with bilateral parotid gland enlargement (90% to 100% of cases), and often with symptoms of the Sicca syndrome or Sjögren's-like syndrome (xerostomia in 82% and xerophtalmia in

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35%) but without anti-Ro or anti-La antibodies (3,4). Unilateral parotid swelling has been described, although it is a rare finding. Generalized lymphadenopathy was described in 80% to 100% of the patients (3). In a previous large study (5), 31% of patients were diagnosed with lymphocytic interstitial pneumonitis. These patients can be asymptomatic or may present cough (71%), dyspnea (61%), weight loss (16%), fever (10%), pleuritic chest pain (6%), fatigue and arthralgia (2,4). The pneumonitis is clinically and radiologically very similar to *Pneumocystis jiroveci* pneumonia (PCP) and therefore it's important to perform a diagnostic bronchoscopy with broncho-alveolar lavage (BAL) (4). Other less common features can occur in HIV patients with DILS, such as myositis (26%), peripheral neuropathy (20-25%), lymphocytic hepatitis (23%), interstitial nephritis and interstitial gastrointestinal disease (2,3,4).

The exact prevalence of the disease is insufficiently documented to date. Most studies have reported data ranging from 0.8% to 7.8%, and it seems to be more frequent in African populations[4].

In addition to a positive HIV test, the diagnostic criteria of DILS are bilateral salivary gland enlargement or xerostomia for more than six months and histological evidence of CD8+ focal lymphocytic infiltration in the salivary or lacrimal glands in the absence of granulomatous or neoplastic involvement (6). The histological diagnosis of DILS can be performed from a salivary gland biopsy or other tissue biopsies, including bronchoscopy with biopsy and liver biopsy. Also, MRI and CT scans can be useful for diagnosis of DILS, as well as gallium scintigraphy as a substitute for salivary gland biopsy (2,4).

Commonly, the progression of HIV disease in patients with DILS is slower than in patients with HIV alone, presumably as a result of the strong immune response mediated by CD8+ T lymphocytes elicited against the virus (3,4).

The goal of treatment is to minimize damage caused by the lymphocytic infiltration in different organs. Thus, the therapy for DILS includes the use of corticosteroids in order to decrease inflammation and antiretroviral drugs to remove the antigenic burden, the main trigger of the disease (2-4).

CASE REPORT

A 43-years-old male patient from Bucharest presented in October, 2012 at "Matei Bals" National Institute for Infectious Diseases with a clinical Sicca syndrome and interstitial pneumonia. His symptoms started 10-weeks before the admission with worsening asthenia, dry cough and headache. After 4 weeks of sickness a lung CT scan was performed without any abnormalities detected on native scan. These symptoms were followed by thrush and 2 weeks later by fever (39-40°C), chills and sweats. After 10 days of antibiotics (clarithromycin and amoxicillin-clavulanate) the symptomatology was still persisting. The patient accused also dryness of mouth and eyes and started to complain about chest pain and dyspnea on exertion. In early October the patient was admitted to an internal medicine hospital and was treated again with antibiotics (ampicillin, gentamycin and metronidazole) and intravenous steroids (dexamethasone). The fever disappeared but because of the persistence of chest pain, dyspnea and cough a cardiac disease was suspected (myocarditis, endocarditis or pulmonary thromboembolism). The patient was transferred to the cardiology department but a cardiac impairment was excluded. Additionally, an ELISA-HIV blood test was performed with positive result and the patient was sent to our hospital for further investigation and treatment.

The patient has a medical history of hypertension without regular treatment. He was married and had a 1 year-old child (obtained through in vitro fertilization). He denied smoking, alcohol consumption, intravenous drug use or extramarital sexual intercourses. His wife was HIV negative.

On examination, we found an anxious patient with thrush, painless liver enlargement (4 cm below the costal margin) and symmetrical upper limb tremor without any pathological changes at the respiratory and cardiovascular physical exam. His vital signs were normal and there was no lymphadenopathy or parotid gland enlargement.

Extensive laboratory and imaging tests were performed in order to establish the final diagnosis. Two HIV-1 ELISA 4th generation test were positive, subsequently confirmed by Western-Blot HIV-1 with 10 positive bands (excluding the diagnosis of acute HIV infection). The quantitative HIV-1 RNA level was above 10,000,000 copies/mL (HIV-RNA Cobas TaqMan), respectively 115,000,000 copies/mL. Baseline resistance testing showed a wild type virus.

White blood count (WBC) revealed leukocytosis (16,100/mm^3) with lymphocytosis (9900/mm^3) and monocytosis (1,600/mm^3) with a CD4+ T cell count of 316 cells/mm^3 and a marked elevation of CD8+ T cell count -8,819 cells/mm^3 (normal range, 190-1,140 cells/mm^3). Peripheral blood smears did not identify the presence of blasts.

The liver test results were outside the normal range: alanine aminotransferase level was 829 UI/L (normal level, < 72 UI/L), aspartate aminotransferase level 636 UI/L (normal level, < 59 UI/L), gamma glutamyltransferase 403 UI/L (normal level, < 73 UI/L), alkaline phosphatase 191 UI/L (normal level, < 126 UI/L) with a slightly increase in bilirubin level 0.9 mg/dL (normal level, < 0.4 mg/dL). The amylase and lipase levels were increased at 132 UI/L (normal range, 30-110 UI/L), respectively at 497 UI/L (normal range, 23-300 UI/L). Protein electrophoresis showed hypergammaglobulinemia.

IgM anti CMV were intense positive, IgG anti CMV positive and the serum CMV-DNA was 36,832 copies/mL, suggestive for an acute CMV infection. IgG anti EBV antibodies were positive.

All other blood tests (erythrocyte sedimentation rate, coagulation parameters, fibrinogen level, hepatitis A, B, C and E serology, leptospirosis serology, syphilis, toxoplasmosis serology, HTLV-1,2 antibodies, herpes simplex viruses-1,2 antibodies, Quantiferon TB gold, cryptococcal antigen, cryoglobulin, anti-nuclear, anti-Ro and anti-La antibodies) were within normal limits or negative.

Neurological evaluation did not confirm the presence of peripheral neuropathy.

Chest-X ray showed bilateral diffuse micronodular interstitial infiltrates in the lower lung lobes very similar to PCP. Oropharyngeal culture revealed Candida albicans with fluconazole susceptibility. Although the CD4+ count was above 200 cells/mm^3, considering the suspicion of PCP, we promptly started treatment with cotrimoxazole (15 mg/kg of body weight/day for trimethoprim, TID) and steroids (intravenous dexamethasone 16 mg/day as a starting dose, BID). A diagnostic bronchoscopy with BAL was performed. Analysis of the BAL samples revealed frequently CMV-infected cells with no other pathogens (bacteria, fungi, acid fast bacilli or *Pneumocystis jiroveci*) on examined smears. All bacterial and fungal cultures were negative. The polymerase chain reaction (Plex-ID) from BAL specimen was positive for CMV and two bacteria (*Stenotrophomonas maltophilia* and *Streptococcus pneumoniae*). Based on these data we were able to rule out PCP and CMV pneumonitis was confirmed. Subsequently cotrimoxazole treatment was stopped and intravenous treatment with ganciclovir (5 mg/Kg of body weight, twice a day) was started and maintained for a total period of 21 days. Treatment with dexamethasone was stopped after 14 days.

The diagnosis of DILS was established in the presence of CD8+ lymphocytosis and Sicca syndrome in an HIV infected patient. We did not perform any tissue biopsy for histological confirmation.

During treatment with ganciclovir, in the 14th day, we initiated antiretroviral therapy (ART) with tenofovir (TDF), emtricitabine (FTC) and boosted darunavir (DRV) 800 mg once daily. We mention that at the beginning of cART, the CD4+ and CD8+ cell count had dropped to 108 cells/mm^3 and 2093 cells/mm^3, respectively.

One month after starting cART, the outcome was favorable with complete resolution of Sicca syndrome and respiratory symptoms associated with pulmonary infiltrates disappearance and normalization of liver and pancreatic function tests.

The immune response during cART was favorable, the CD4+ cell count was constantly increasing, reaching 625 cells/mm^3 at 6 months. In addition, the level of CD8+ cells had a fluctuating evolution, without getting into the normal range, the level at 6 months being 3903 cells/mm^3. Considering the very high level of HIV RNA, the viral response was initially evaluated after 2 months of cART. At that point the viral load (VL) was 2284 cp/mL and we decided to increase the daily dose of DRV to 1200 mg. The VL at 6 months of cART was still detectable (2761 copies/mL) with a slightly increase compared to the previous value. Genotypic resistance test showed the presence of M184V mutation in the reverse transcriptase gene. cART was changed according to resistance profile, the new antiretroviral regimen containing tenofovir, etravirine and boosted DRV 1200 mg/day. After another six months, the VL became undetectable with stable immune recovery (Figure 1). At two years from diagnosis, the patient continued to do well, without any symptoms and with fully suppressed virus. However, despite viral suppression and immune reconstruction, CD8+ cell count is still elevated to a value of 2787 cells/mm^3 (Figure 2).

DISCUSSIONS

In our opinion this case has some particularities related to HIV infection itself and acute CMV infection in combination with DILS.

DILS may be seen in all stages of HIV infection, but more commonly in early HIV stage with higher CD4+ cell counts (usually above 200 cells/mm^3) and fewer opportunistic infection (3,4). Although our patient was not diagnosed in acute HIV phase, the level of HIV RNA was very high which in-

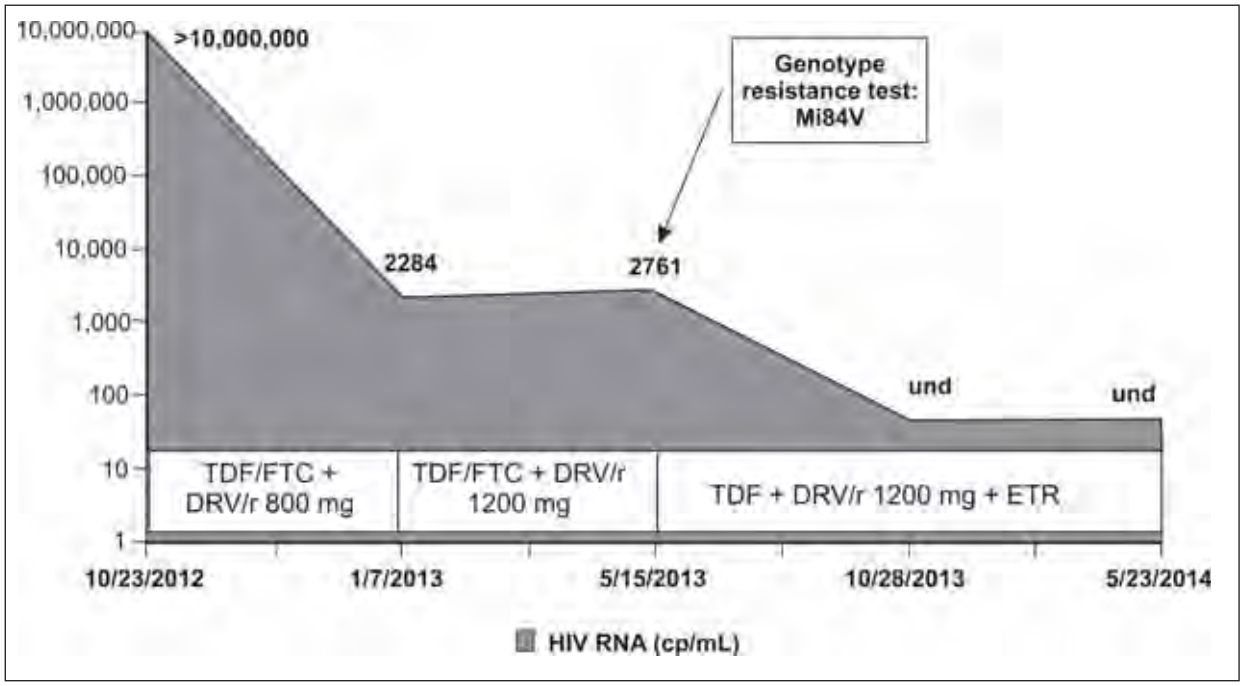

FIGURE 1. *Evolution of HIV-RNA (cp/mL) during antiretroviral treatment with different regimens (TDF – tenofovir; FTC – emtricitabine; DRV – darunavir; ETR – etravirine; und – undetectable VL)*

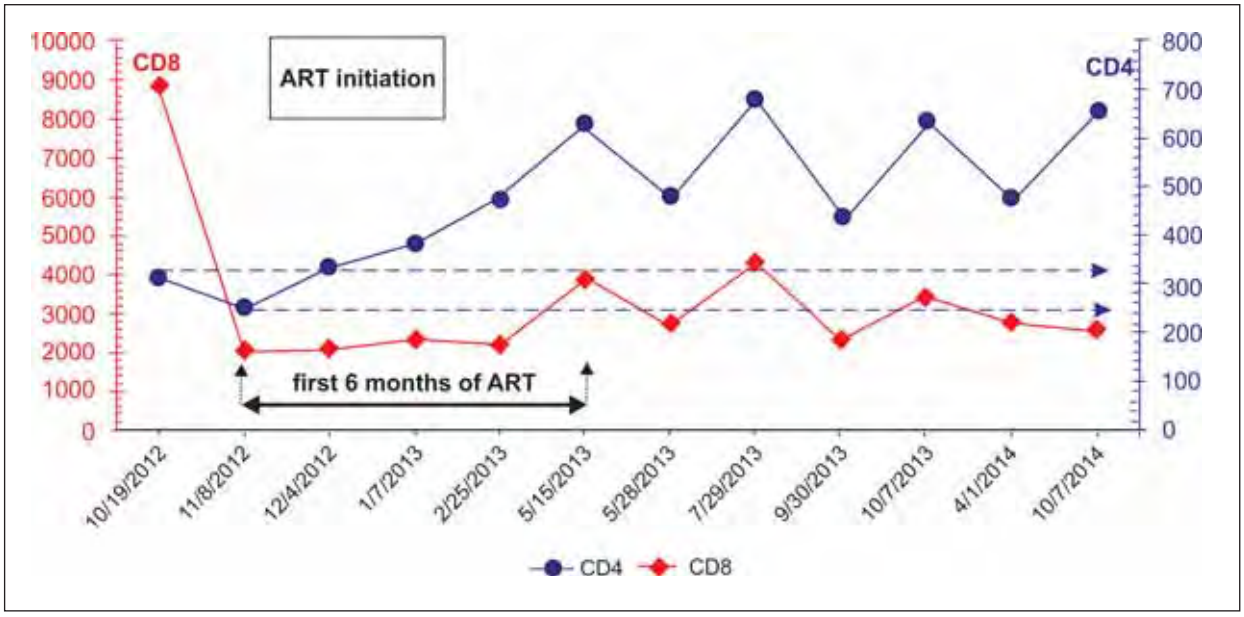

FIGURE 2. *Evolution of CD4+ cell count and CD8+ cell count during antiretroviral treatment with different regimens*

creased the risk of virologic failure and emergence of resistance.

In these particular settings of HIV positive naive patients with very high level of viremia at baseline, we recommend to start from the beginning with a higher dose of boosted DRV (1200 mg/day) to decrease the risk of resistance.

There is no clear data on the CD8+ cell counts in patients with DILS (3). In one study, the mean CD8+ cell count was 1639/mm^3, with a broad range of values (560/mm^3 to almost 5000/mm^3) (5). The CD8+ T cell count in our case was extremely high, to 8819 cells/mm^3, much higher than has been published to date. Our possible explanation for this particular immune response is the combination of two different viruses (HIV and CMV, both with high antigenic load), which acted as a super-antigen with strong booster effect on the immune system. DILS is mostly associated with HIV infection, although isolated case reports of HIV negative pa-

tients with DILS (7) have been described. Another study found EBV markers in histological specimen of salivary glands which may emphasize a role for EBV in pathophysiology of DILS (8). In our case we definitely think about the role of CMV acute infection in triggering the massive CD8+ cells elevation and DILS occurrence.

Another specific feature of our patient was the combination of acute CMV infection and DILS. Both diseases share the same clinical patterns with interstitial pneumonitis and hepatitis. After all laboratory results we were able to confirm CMV pneumonitis but we could not exclude DILS as coexisting condition for respiratory impairment. Also, the liver dysfunction could be related to CMV acute infection or DILS or both conditions. Perhaps in these particular cases the biopsy play a major role.

There are no randomized control studies comparing different treatments for DILS. Several authors reported that cART might be effective for treating sicca symptoms and visceral manifestations. However, little is known about the role of boosted protease inhibitor based regimen (9). Several visceral manifestations such as lymphocytic interstitial pneumonitis require corticosteroids in moderate to high doses (10). In our case we used simultaneously steroids for 14 days, ganciclovir for 21 days and cART containing protease inhibitor with favorable clinical, virological and immune outcomes. However, the CD8+ cell count was still elevated after two years of cART with fully suppressed virus and good immune recovery. We are doing also regular checking of the patient because of the risk of developing lymphoma.

In conclusion, we emphasize that DILS is a particular immune response commonly described in HIV positive patients with large spectrum of clinical features, including salivary glands and respiratory tract involvement which may result in delayed or wrong diagnosis. ART alone or associated with corticosteroids for a short period of time have been shown to be effective for the treatment of DILS.

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