Case presentation

A 70-year-old male Albanian arable farmer was found to have disseminated *Mycobacterium tuberculosis* (fully sensitive, smear positive pulmonary infection and meningitis) soon after arrival in UK. HIV-1 infection (CD4 47 cells/mm^3, HIV viral load 2.3 million copies/mL) was diagnosed at this time. Prophylactic co-trimoxazole was started and rifampicin, isoniazid, pyrazinamide and ethambutol was commenced, reducing to continuation phase rifampicin/isoniazid after 8 weeks. Despite the low CD4 count at presentation starting antiretroviral therapy had been delayed, initially given the CNS tuberculosis, and later because TB treatment was complicated by nausea and vomiting after medication and meals.

Four weeks after starting anti-retroviral therapy (ART) with a fixed-dose combination of tenofovir- disoproxil fumarate/emtricitabine/efavirenz, and five months into tuberculosis treatment the patient developed fever. His family reported he was intermittently confused and experiencing vivid nightmares. The patient was transferred to our hospital.

On arrival he was confused (GCS 14), cachectic, pyrexial (>39^oC) and rigoring. A palpable spleen tip was noted. Full blood count demonstrated a pancytopenia, with iron-deficiency anaemia (Figure 1: baseline investigations). His chest radiograph showed interval improvement in right upper zone consolidation consistent with response to TB treatment. A septic screen (including an atypical pneumonia screen, blood and urine cultures) revealed no focus of infection. He was empirically treated with piperacillin-tazobactam, with no clinical improvement after one week. An ^{18}FDG CT-PET scan showed increased avidity in a mildly enlarged (13cm) spleen and in the right upper zone pulmonary tuberculosis focus. Bone marrow aspirate showed many Giemsa-staining intracellular organisms of leishmaniasis (Figure 2), confirmed with a positive blood recombinant K39 antigen immunochromatographic test and direct agglutination test (DAT) (1:102,400). Infection in the Mediterranean basin suggested *Leishmania infantum*. Emergent visceral leishmaniasis symptoms after one month of anti-retroviral therapy suggests 'unmasking' immune-reconstitution-inflammatory-syndrome (IRIS).

A treatment course of 40mg/kg liposomal amphotericin in 10 divided doses over 38 days was started, following WHO and IDSA guidelines for *L. infantum*/HIV co-infection (Aronson et al, 2016 and Lindoso et al, 2018). The first week of treatment was complicated by acute kidney injury and metabolic acidosis due to Fanconi syndrome, precipitated by the combination of amphotericin and tenofovir-disproxil fumarate (part of the ART combination). Along with supportive care and close monitoring ART was changed to dolutegravir/abacavir/lamivudine (fixed dose combination), with an additional 50mg of dolutegravir (separated by 12h, because of concurrent rifampicin administration).

Fever defervesced after one week's amphotericin treatment. One month into therapy a bone marrow aspirate showed few residual destroyed amastigotes, reduced leishmaniasis DAT (1:12,800), undetectable HIV viral load, and increased CD4 (137 cells/mm^3). To prevent relapse, secondary prophylaxis with three weekly liposomal-amphotericin infusions were maintained for $\geq$6 months, until the CD4 count was >200-350 cells/mm^3 (Aronson et al, 2016).

Discussion:

In a patient with fevers, pancytopenia, weight loss and mild splenomegaly against a background of advanced HIV and tuberculosis, differentials include bone marrow infiltrative processes; malignancy, visceral leishmaniasis, dimorphic fungal infections including *Histoplasmosis,* and *Mycobacterium avium* complex, and bone marrow suppression and failure, caused

by; HIV, Epstein-Barr virus, parvovirus, drug reactions, autoimmune conditions and haemophagocytic lymphohistiocytosis. In this patient investigations revealed the cause to be unmasking IRIS due to leishmaniasis.

Visceral leishmaniasis (VL) is a protozoal infection occurring when prometacyclic *Leishmania donovani* protozoa are transmitted by sandfly bites. The species of sandfly and leishmania depend on geographical location. The 500,000 annual VL cases occur predominately in India and East Africa, but clinicians should be aware of acquisition in Europe particularly among immunocompromised patients. VL presents with irregular fevers, splenomegaly, and, later, pancytopenia, hepatomegaly, hypergammaglobulinaemia, and weight-loss. Asymptomatic infection occurs in 3-30% of populations in endemic areas. Immunocompromised patients can present with subtle, atypical symptoms making diagnosis more challenging.

HIV and leishmaniasis synergistically impair cell-mediated immunity, accelerating progression of both diseases. In HIV/VL coinfected cohorts, mortality and relapse rates are high (Lindoso et al, 2018 and van Griensven et al, 2018). Anti-leishmanial antibodies are diminished or undetectable making serological tests unreliable (Lindoso et al, 2018). Management is more complex because pentavalent antimonials are more toxic when administered with antiretrovirals. Atypical disseminated leishmaniasis is a WHO clinical stage 4 disease (Lindoso et al, 2018).

HIV/VL co-infection is associated with high rates of relapse and poor prognosis. Risk factors for relapse of VL include CD4 <200 cells/mm^3, poor CD4 response to ART, no secondary prophylaxis and history of relapse. HIV viral load response is a marker of antimonial effectiveness (Lindoso et al, 2018). In this case, the viral load was undetectable (<40 copies/ml) by the end of treatment.

The co-occurrence of VL and TB in HIV infected persons is not rare, reflecting the global epidemiology of these infections (van Griensven et al, 2018). This case contrasts with a previous report of 19 cases of VL IRIS which were secondary to 'paradoxical IRIS' (Badaró et al, 2014). In 'paradoxical IRIS' a previously diagnosed and treated opportunistic infection recurs leading to clinical deterioration (Lawn et al, 2006, Walker et al, 2015). Visceral leishmaniasis 'unmasking IRIS' is rare. In 'unmasking IRIS' no underlying opportunistic infection was diagnosed prior to initiation of ART.

Learning points:

- Consider visceral leishmaniasis in patients with persistent fevers from endemic areas, (including from southern Europe).
- Visceral leishmaniasis can present as an unmasking IRIS in patients taking antiretroviral therapy, and should be considered in the differential diagnosis in patients from endemic regions presenting with fever, pancytopenia or splenomegaly post-ART.

References

Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, et al. (2016) Diagnosis and Treatment of Leishmaniasis: Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). *Clin Infect Dis* **63**:e202-e264. (doi:10.1093/cid/ciw670)

Badaró R, Gonçlaves LO, Gois LL, Maia ZP, Benson C, Grassi MF. (2014) Leishmaniasis as a Manifestation of Immune Reconstitution Inflammatory Syndrome (IRIS) in HIV-Infected Patients: A Literature Review. J Int Ass Prov AIDS Care.**14(5)**:402-407. DOI: 10.1177/2325957414555225.

Lawn SD & Wilkinson RJ. (2006) Immune reconstitution disease associated with parasitic infection following antiretroviral treatment. *Parasite Immunol* **28**:625–633. (doi: 10.1111/j.1365-3024.2006.00900.x)

Lindoso JAL, Moreira CH, Cunha MA, Queiroz IT. (2018) Visceral leishmaniasis and HIV coinfection: current perspectives. *HIV/AIDS (Auckl)* **10**:193–201. (doi:10.2147/HIV.S143929)

van Griensven J, Mohammed R, Ritmeijer K, Burza S, Diro E. (2018) Tuberculosis in Visceral Leishmaniasis-Human Immunodeficiency Virus Coinfection: An Evidence Gap in Improving Patient Outcomes. *Open Forum Infectious Diseases* **5**: ofy059. (doi:10.1093/ofid/ofy059)

Walker NF, Scriven J, Meintjes G, Wilkinson RJ. (2015) Immune reconstitution inflammatory syndrome in HIV-infected patients. *HIV/AIDS (Auckl)* ;**7**:49-64. (doi:10.2147/HIV.S42328)