Clinicopathological Conference Report—PM 25535

Disseminated Nonreactive Miliary Tuberculosis, Dual Infection with *Mycobacterium tuberculosis/avium* with Hemophagocytic Syndrome in a HIV-Positive Individual

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This case (PM 25535) was discussed on 16th September 2013 as a student clinicopathological exercise at PGIMER, Chandigarh, India.

Clinical details and case analysis—Dr Umesh Kumar Chandel, Junior Resident, Department of Internal Medicine, PGIMER, Chandigarh, India.

CLINICAL DETAILS

A 40 years old male presented on 12th July 2013 with complaints of fever, cough, loose stools, headache for 3 months and altered sensorium for 1 week. Fever was low grade (38.5°F), on and off and not associated with chills and rigor. Cough was productive, yellowish, 20 to 30 ml, not foul smelling without any hemoptysis. Loose stools were intermittent, watery, 4 to 5 times/day, without any blood. The headache was intermittent, holocranial, dull aching, associated with nausea but no vomiting. There was altered sensorium in the form of decreased responsiveness to verbal commands, not recognising relatives, irrelevant talk but without any history of seizures or loss of consciousness. There was history of loss of weight and appetite. He was a chronic alcoholic for last 15 years with a history of high risk sexual behavior. He was admitted in Sector 16 hospital Chandigarh, 2 months back with complaints of fever and cough, was diagnosed as a case of pneumonia and was managed conservatively with antibiotics. On general physical examination he was found to have generalized wasting, was disoriented, drowsy, (E4V3M5), febrile, there was tachycardia (PR-122/min), BP-110/70 mm Hg, tachypnea (RR-20/min) with room air oxygen saturation 95%, pallor +, icterus +, and no lymphadenopathy. Respiratory system revealed b/l coarse crepitations. Per abdomen examination revealed hepatosplenomegaly (both liver and spleen palpable one finger below the respective costal margins). CNS examination showed neck rigidity with normal deep tendon reflexes and plantar reflexes were flexor.

Investigations revealed-HIV + by ELISA, pancytopenia (Hb-7.8 gm%, TLC-1800 × 103/ul, platelets-74,000/ul), deranged liver function tests-hyperbilirubinemia (total 2.4 mg%, direct 1.2 mg%), hypoalbuminemia (albumin-2.6 gm), transaminitis (SGOT-260/ul, SGPT-270/ul, ALP-631/ul), LDH 6092/ul, respiratory alkalosis (pH/PO₂/PCO₂/HCO₃/SO₂-7.58/113/18.3/17.3/99.1% on room air), X-ray showed right upper lobe opacity and USG abdomen revealed hepatosplenomegaly. CSF examination-TLC-40 cells, predominantly polymorphs, with protein 106, sugar 06 (CBS-106), ADA-10, crypto LA and India ink negative and gram stain did not show any organism. CECT head did not show any parenchymal lesion/meningeal enhancement/basal exudates or hydrocephalous. HRCT+ CECT chest (Fig. 1) and abdomen revealed multiple b/l centrilobular nodules with tree in bud appearance and patchy consolidation in right upper lobe/superior segment of right lower lobe. Etiology likely tuberculosis. Units diagnosis was disseminated tuberculosis with tubercular meningitis and? granulomatous hepatitis. He was managed with I/V antibiotics (ceftriaxone/vancomycin), modified ATT in view of deranged liver function and steroids. However, he did not improve and his sensorium/condition worsened and he died on 17th July 2013. Unit's Final diagnosis was AIDS, clinical stage 4 with disseminated tuberculosis and sepsis.

CASE ANALYSIS

In the above clinical scenario, we have a 40 years male who was symptomatic for last 3 months. He had fever, cough,

loose stools, headache for 3 months and altered sensorium for 1 week. On general physical examination he was disoriented, drowsy, (E4V3M5), febrile, there was tachycardia (PR- 122/min), tachypnea (RR-20/min), pallor +, icterus +, b/l coarse crepitations, hepatosplenomegaly and neck rigidity. Investigations revealed HIV + status, pancytopenia, hyperbilirubinemia, hypoalbuminemia, transaminitis, raised LDH, CSF revealed a high protein, low sugar and high ADA, on USG there was hepatosplenomegaly, CECT- chest revealed multiple b/l centrilobular nodules with tree in bud appearance and patchy consolidation in right upper lobe/superior segment of right lower lobe.

Considering the data base the following differential diagnosis are kept:

- Disseminated tuberculosis
- Disseminated fungal infection (?cryptococcosis)
- Sepsis
- CMV infection
- Hemophagocytic lymphohistiocytic syndrome (HLH)
- *Mycobacterium avium* complex infection
- Lymphoma.

Disseminated Tuberculosis

In HIV patients tuberculosis is the most common opportunistic infection. Our patient has a long duration of h/o fever and cough with h/o loss of weight/appetite and CECT chest findings are s/o tuberculosis. Headache/alterd sensorium of more than 1 week with CSF having high protein and ADA of 10 are in favor of tubercular meningitis. In view of the hepatosplenomegaly with deranged liver functions with ALP more than SGOT and SGPT, a possibility of granulomatous hepatitis is kept. Pancytopenia was thought to be due to bone marrow involvement with tuberculosis. So considering all these findings a diagnosis of disseminated tuberculosis is most likely.

Fig. 1: CECT chest-black arrow showing patchy consolidation and the red arrows show multiple centrilobular nodules with 'tree in bud' appearance

Disseminated Fungal Infection

In HIV patients fungal infections are also very common. To diagnose disseminated fungal infection we need to fulfill some criterias.¹ Our patient had host criteris and 2 or more minor clinical criterias but no microbiological criteria (blood/body fluid microscopy/cytology/culture positive for fungus or fungal antigen positivity) and major clinical criterias (no e/o fungal infection in CECT head). So, fungal infection seems less likely.

Sepsis

This patient fulfilled most of the criterias for sepsis (fever/tachycardia/tachypnea/hypoxemia/hypotension/alterd mental state/pancytopenia/deranged liver function tests/CECT chest showing focus of infection). Irrespective of the sterile blood culture a possibility of sepsis is kept because culture positivity is seen in only 19% patients with sepsis.²

CMV Infection

CMV infection is common in HIV patients to the tune of 45 to 65%. Patients with CMV infection can present like our index case (prolonged fever/cough/diarrhoea/alterd sensorium/pancytopenia/deranged liver enzymes). CMV infection is more common in HIV patients with CD4 count <50 cu mm, but in our case CD4 count is not available and CECT head/chest did not show any e/o CMV infection. Moreover CMV serology/PP65 antigen test were not done. So in view of this, CMV infection cannot be ruled out.

Hemophagocytic Lymphohistiocytic Syndrome (HLH Syndrome)

Though HLH syndrome is not common (but can occur) in HIV patients but there are studies where it is associated in patients with tuberculosis. It needs 5 out of 8 criterias to diagnose HLH.³ Our patient fulfilled 3/8 criterias (fever/hepatosplenomegaly/cytopenia ≥ 2 cell lines), TG level were in normal range and patient was not investigated for other remaining criterias (histopathologic evidence of hemophagocytosis in spleen/bone marrow/lymph node, low or absent NK cell activity, serum ferritin > 500 $\mu\text{g/l}$). So to rule out HLH syndrome, we need a more comprehensive work up. Hence HLH is a likely possibility in this case.

Mycobacterium avium Complex Infection

MAC infection is another common opportunistic infection in HIV patients and is seen in 35 to 45% patients. These patients present with prolonged fever/cough/diarrhea/hepatosplenomegaly/pancytopenia/deranged liver function like in our patient. CECT chest findings shows tree in bud

appearance as seen here. So a possibility of MAC infection is high on the cards in our patient.

Lymphoma

Lymphomas are most common among all malignancies seen in HIV patients. Our patient has clinical findings consistent with lymphoma patients like prolonged fever, cough, loss of weight/appetite, hepatosplenomegaly, pancytopenia, deranged liver enzymes, raised LDH with CSF showing raised TLC, high protein and low sugar. There was no seizure/lymphadenopathy and CECT chest + abdomen findings were not consistent with lymphoma. So lymphoma is kept as unlikely possibility in this patient.

So, the most likely clinical diagnosis in this patient is HIV clinical stage 4 with disseminated tuberculosis.

OPEN HOUSE DISCUSSION

- *Senior resident treating unit:* This patient was treated on the lines of tuberculosis and modified ATT was given as liver function tests were deranged. He was also started on fluconazole to cover for fungal infection. But despite everything he went into shock which never recovered despite fluids and vasopressors.
- *Dr A Chakrabarty:* In cases with HIV, opportunistic infection with aspergillous and candida is rare. More common is cryptococcus and histoplasma. These infections occur when the count falls below 50.

Autopsy Protocol

PM 25535—Dr Abin Koshy, Junior Resident, Department of Pathology, PGIMER, Chandigarh.

A complete autopsy was performed on this 40 years old, HIV positive male. On external examination the patient was moderately built and emaciated. The serous cavities were within normal limits.

- *Lungs:* They were heavy and weighed 2000 gm. The pleural surface was dull with many fibrinous tags. The left upper lobe was adherent to the chest wall. The cut surface showed many tiny miliary tubercles present diffusely along with few larger white nodules measuring 0.1 to 0.3 cm in diameter. The right upper lobe revealed a confluent greyish white lesion (Fig. 2). On microscopic examination there were multiple foci of acute necrosis with nuclear debris (Figs 3A and B); however, no epithelioid cell granuloma or Langhans giant cells were seen. Adjacent lung parenchyma showed pulmonary edema and macrophage collections. No fungal profile was seen even with PAS and Grocott's stain. Ziehl Neelson stain was strongly positive (5+) for acid fast bacilli in the areas of acute necrosis (Fig. 3C). Hilar and carinal lymph nodes also showed extensive areas of acute

necrosis (Fig. 3D) with no granulomas/Langhans giant cells. The Ziehl Neelson stain for AFB was as strongly positive as in the lungs in the areas of acute necrosis. Liver weighed 1650 gm (mildly enlarged). The capsular and cut surface (Figs 4A and B) showed miliary tubercles and small white nodules measuring 0.1 to 0.3 cm in diameter scattered all over. Microscopy revealed multiple foci of acute necrosis, with no epithelioid cells/giant cells. The Ziehl Neelson Stain was teaming with AFB (Fig. 4D). Spleen was enlarged weighing 340 gm and was studded with miliary tubercles (Fig. 5A) and small white nodules measuring 0.1 to 0.4 cm in diameter. Representative sections revealed similar areas of acute necrosis (Fig. 5B), replete with AFB (Fig. 5C). Kidneys also showed miliary tubercles on the capsular and cut section which revealed a similar pathology as seen above.

Fig. 2: The cut surface of the lungs shows tiny miliary tubercles present diffusely. The right upper lobe shows a confluent white lesion

Figs 3A to D: (A and B) Microphotograph from the lung showing areas of acute bland necrosis with absence of epithelioid cells and Langhans giant cells, (C) Ziehl Neelson stain teaming with acid fast bacilli, (D) Microphotograph from the hilar lymph nodes showing similar areas of acute necrosis which showed similar florid presence of acid fast bacilli

The bone marrow was hypercellular for age, with many foci of acute necrosis (Fig. 6A) with a florid AFB positivity (Fig. 6B). There were increased number of histiocytes, many showing hemophagocytosis (Fig. 6C). The brain weighed 1300 gm. The leptomeninges was dull with prominent venous channels. No basal meningitis was however noted grossly. On microscopy, the meninges revealed multiple necrotic foci surrounded by a sparse lymphohistiocytic infiltrate, with minimal inflammatory change in vessels without any evidence of vessel wall necrosis or obliterative endarteritis (Fig. 7A). Choroid plexus also showed areas of acute necrosis (Fig. 7B). The parenchyma of hippocampus and pons adjacent to meningeal investment showed small foci of necrosis amounting to borderzone encephalitis (Fig. 7C). All these necrotic foci were strongly positive for AFB (Fig. 7D). The esophagus at its lower showed small superficial hemorrhagic ulcers with pseudohyphae and spores (which are better seen in PAS stain) of candida. The mesentric lymph nodes microscopically revealed lymphoid involution with only a few preserved germinal centers in the periphery amounting to intermediate involution stage of HIV lymphadenopathy. Rest of the organs were within normal limits.

- **PCR:** Dr Kusum Sharma, Department of Microbiology—Postmortem lung was subjected to multiplex PCR by using primers specific for *Mycobacterium tuberculosis* and *avium*. It was positive for both (Fig. 8).

FINAL AUTOPSY DIAGNOSIS- 40 M, HIV POSITIVE

- Disseminated nonreactive miliary tuberculosis involving the lungs, liver, spleen, kidneys and bone marrow with tubercular meningitis, borderzone encephalitis and choroid plexitis. AFB strongly positive (5+). MPCR positive for *Mycobacterium tuberculosis* and *avium*.
- Secondary hemophagocytic lymphohistiocytosis in bone marrow.
- Esophageal candidiasis.

OPEN HOUSE DISCUSSION

Prof BD Radotra: On gross examination of the brain there were no exudates but the meninges was dull. On microscopic examination there is no arteritis and the meningeal exudate is very sparse with no granulomas. All that is seen are areas of acute necrosis teeming with acid fast bacilli. This is the classical picture of tubercular meningitis in HIV patients. On morphology it is not possible to differentiate between *Mycobacterium tuberculosis* and *avium*. This can only be done by PCR.

Figs 4A to D: (A and B) Capsular and cut section of the liver showing miliary tubercles, (C) microscopic examination of the liver showing the tubercles to be composed of acute necrosis with nuclear debris (arrows), (D) Ziehl Neelson stain in the liver strongly positive for AFB

Figs 5A to C: (A) Slice of the spleen studded with tiny miliary tubercles, (B) Microphotograph from the spleen showing the tubercles to be composed of acute necrosis (arrows) which were teeming with (C) AFB

Figs 6A to C: Microphotograph from the bone marrow showing areas of bland necrosis (arrow) (A) teeming with acid fast bacilli (B). Bone marrow showing evidence of hemophagocytosis (C)

Figs 7A to D: Microphotograph from the brain showing a sparse meningeal exudate with areas of bland necrosis (arrows) (A) which were also seen in the choroid plexus (B), in the superficial cortex of the brain (C) and were teeming with acid fast bacilli (D)

Fig. 8: Postmortem lung was subjected to multiplex PCR by using primers specific for *Mycobacterium tuberculosis* and *avium*. It was positive for both (L1: MM; L2: positive control; L3: NC; L4 and L5: DNA from lung tissue)

COMMENTARY

The important issues in this case are immunocompromised state due to HIV positivity, disseminated nonreactive miliary tuberculosis, florid bacillary load, a coexistence of *Mycobacterium tuberculosis* and *avium* infection and pancytopenia with hemophagocytic lymphohistiocytosis (HLH).

Nonreactive tuberculosis shows a bland acute necrosis composed of nuclear debris without the presence of any epithelioid cells and Langhans giant cells and these areas show a very heavy bacillary load just like what was seen in the index case in the lungs, liver, spleen, bone marrow, kidneys and the brain. In the brain, no exudates were appreciated on gross examination but the microscopic examination revealed areas of bland necrosis in the meninges, choroid plexus and superficial cortex replete with

AFB. This is the classical picture of tubercular meningitis in an immunocompromised individual. The nonreaction occurs because the individual cannot mount an inflammatory response due to the defective immune system.

Hemophagocytic lymphohistiocytic syndrome⁴ is a disorder characterized by fevers, lymphadenopathy, hepatosplenomegaly, cytopenias, and hyperferritinemia due to dysregulated activation and proliferation of macrophages, leading to uncontrolled phagocytosis of hematopoietic precursors throughout the reticuloendothelial system. Primary or familial hemophagocytic syndrome has a genetic etiology, whereas secondary hemophagocytic syndrome is associated with malignancy, autoimmune disease, or infections. Epstein-Barr virus is the commonest infection implicated in hemophagocytic syndrome, but the syndrome has been associated with a variety of other viral, bacterial and parasitic pathogens. The hemophagocytic syndrome (HS) occurs more frequently but not exclusively associated with infections in individuals with pre-existing immunologic abnormalities. Tuberculosis associated hemophagocytic syndromes are reported in literature.⁵ High-dose methylprednisolone and intravenous immunoglobulin are at times added and this contributes to the favorable outcome of these patients. The similarities in HLA phenotypes in these patient reported in the literature provide evidence for an underlying immune dysregulation in some cases of infection-associated HS.⁵ Grateau et al⁶ reported nine cases of hemophagocytic lymphohistiocytic syndrome (HLH) in patients with human immunodeficiency virus infection. HLH developed during an advanced stage of immunodeficiency. Clinical and hematological signs are not specific in this setting, and the diagnosis relies on histological features, mainly bone marrow examination. An opportunistic infection was associated in three cases and a lymphoid malignancy in two of their cases. The role of the human immunodeficiency virus in the occurrence of the HLH remains to be defined but the overall prognosis is poor. In the index case 3/8 criteria for HLH syndrome were present and the rest were not looked for. Post mortem bone marrow confirmed the presence of HLH. The underlying etiology for this secondary HLH points to the florid tubercular infection and to the underlying HIV infection both of which can be the causative factors.

Most mycobacterial infections are still caused by *Mycobacterium tuberculosis* complex (MTC) strains; however, infections by nontuberculous mycobacteria (NTM) are increasing, particularly among the immunocompromised patients. Dual infections with *Mycobacterium tuberculosis* and *avium* are well-known. Polymerase chain reaction (PCR) is the most rapid and sensitive method for diagnosing

mycobacterial infections and identifying the etiological Mycobacterial species in order to administer the appropriate and timely therapy for better patient management.⁷

REFERENCES

1. Ascioğlu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients: an International Consensus. *Clinical Infectious Diseases* 2002; 34:7-14.
2. Previsdomini M, Gini M, Cerutti B, Dolina M, Perren A. Predictors of positive blood cultures in critically ill patients: A retrospective evaluation. *Croat Med J* 2012;53:30-39.
3. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al. 2004, Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* 2007;48:124-131.
4. Brastianos PK, Swanson JW, Torbenson M, Sperati J, Karakousis PC. Tuberculosis-associated haemophagocytic syndrome. *Lancet Infect Dis* 2006 Jul;6(7):447-454.
5. Undar L, Karpuzoğlu G, Karadoğan I, Gelen T, Artvinli M. Tuberculosis-associated haemophagocytic syndrome: a report of two cases and a review of the literature. *Acta Haematol* 1996; 96(2):73-78.
6. Grateau G, Bachmeyer C, Blanche P, Jouanne M, Tulliez M, Galland C, Sicard D, Séréni D. Haemophagocytic syndrome in patients infected with the human immunodeficiency virus: nine cases and a review. *J Infect* 1997 May;34(3):219-225.
7. Gopinath K, Singh S. Multiplex PCR assay for simultaneous detection and differentiation of *Mycobacterium tuberculosis*, *Mycobacterium avium* complexes and other *Mycobacterial* species directly from clinical specimens. *Asian Pac J Trop Med* 2012 Jan;5(1):71-74.

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