Hepatic Sinusoidal-obstruction Syndrome and Busulfan-induced Lung Injury in a Post-autologous Stem Cell Transplant Recipient

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Veno-occlusive disease of the liver is mostly encountered as a complication of hematopoietic stem cell transplantation with myeloablative regimens with an incidence estimated to be 13.7%. It is clinically characterized by tender hepatomegaly, jaundice, weight gain and ascites. Strong clinical suspicion and an early recognition of clinical signs are essential to establish the diagnosis and institute effective regimen. Another complication of cytotoxic drugs given for cancers, is development of busulfan-induced lung injury. A strong index of suspicion is needed for its diagnosis, especially in setting where opportunistic fungal and viral infections manifest similarly. We illustrate the clinical and autopsy finings in a 2½-year-old boy who received autologous stem-cell transplantation following resection of stage IV neuroblastoma. He subsequently developed both hepatic veno-occlusive disease and busulfan-induced lung injury**.** The autopsy findings are remarkable for their rarity.

Keywords: *Autopsy, Hepatic veno-occlusive disease, Neuroblastoma.*

Clinical Protocol

A 2.5-year-old boy with stage IV neuroblastoma (primary tumor in the left adrenal, with bone marrow and multiple bone metastases) was undergoing therapy at our institute. The patient was treated with 8-cycles of induction chemotherapy, consisting of vincristine, carboplatin, cisplatin, cyclophosphamide and etoposide, as per the SIOP high-risk neuroblastoma protocol. Chemotherapy was administered at 10-day intervals with G-CSF prophylaxis. Induction was uneventful, with no episodes of febrile neutropenia. A re-evaluation bone marrow examination performed post-induction demonstrated a complete response. At this stage, tumor excision along with left nephrectomy was performed with resection of approximately 95% of the tumor. On histology, it was categorized as differentiating neuroblastoma. Post-surgery, patient was taken up for high-dose chemotherapy with autologous stem cell transplantation (auto-SCT). Myeloablation was performed with busulfan and melphalan with dose adjustment for weight (10 kg). Busulfan was administered orally with a cumulative dose of 480 mg/m^2 and melphalan (120 mg/m^2) was administered on day -1. Busulfan level monitoring was not performed due to non-availability. Prophylaxis for sinusoidal obstruction syndrome (SOS) was ensured with ursodiol. The dose of CD-34+ cells infused was 4×10^6/kg. The child had a relatively uneventful post-transplant period with fever and mild mucositis lasting for 2 days. Neutrophil engraftment occurred on day 13; however, platelet engraftment had not occurred by day 35. He was discharged on day 14, on ursodiol prophylaxis. *Pneumocystis jirovecii* (PCJ) prophylaxis had not been initiated on discharge.

On day 35, he presented with abdominal distension, icterus (serum total bilirubin 35mmol/L) and weight gain of 2.5% above the baseline. He had tender hepatomegaly (3 cms below costal margin). He remained afebrile and non-neutropenic. Abdominal sonography and Doppler demonstrated moderate ascites and left pleural effusion. The hepatic and portal venous flow was normal. Liver and kidney functions were unremarkable. A diagnosis of mild sinusoidal obstruction syndrome (SOS) was made as per Seattle criterion [1]. He was managed with supportive care (sodium and fluid restriction, ursodiol and spironolactone) as an in-patient for 2 days, after which he improved and could be discharged. He was well, and was being assessed on an OPD basis at weekly intervals till day +60, when he presented with cough, tachypnea and hypoxia. The illness started with respiratory distress two days prior to presentation. The cough was non-productive and non-paroxysmal. There was absence of fever, rash, neurological involvement and/or bleeding.

Clinical examination

Triage examination at day +60 showed an open and stable airway, tachypnea, (respiratory rate 60/minute) with

increased efforts, hypoxia (saturation on room air: 90%; increasing to 95% on 40% FiO2). Tachycardia was present (heart rate 132/minute); however, circulatory parameters were normal (BP 98/54 mm Hg, normal capillary refill time and pulse pressure, warm extremities). Pallor was present, with no evidence of skin or mucosal bleeding. Systemic examination was consistent with pneumonitis as the patient had tachypnea, bilateral equal air entry, and presence of coarse crepitations in bilateral lung fields. Abdominal examination showed dark brown pigmentation over abdomen with no tenderness, guarding or rigidity. Hepatomegaly was present with liver palpable 3 cm below costal margin (span 8 cm). Spleen was not palpable. There was no free fluid. Cardiac and neurological examinations were essentially normal.

Course and management

The index case was managed as a case of pneumonitis post auto-SCT. Respiratory support was initially provided with nasal prongs-continuous positive airway pressure. Intravenous antibiotics were started cefoperazone-sulbactam, amikacin and azithromycin. Due to rapidly progressive respiratory distress, child was transferred to Pediatric intensive care unit where mechanical ventilation was provided. There was single episode of fever on the day of admission (38.7ºC) with a subsequent afebrile period throughout the hospital stay. Progressive respiratory distress worsened into acute respiratory distress syndrome (ARDS). Ventilation strategy was modified accordingly. On day 63, there was development of hypotensive shock, initially responding to fluid boluses. On day 65, the shock necessitated inotropic support (dopamine, adrenaline, noradrenaline and pre-terminally, and vasopressin). There was development of left sided pneumothorax followed by cardiac arrest on the same day. The patient could be revived and pneumothorax was drained. Multi-organ dysfunction developed with acute kidney injury (onset day 63), requiring peritoneal dialysis. Significant transaminitis with elevated bilirubin levels was documented. Antibiotics were changed to vancomycin and meropenam on day 63; azithromycin was continued. Intravenous co-trimoxazole and gancyclovir were added in therapeutic, renal modified doses. Platelet concentrates were transfused to maintain a platelet count above $20 \times 10^6/mm^3$. There was development of refractory shock on day 67. The child suffered another cardiac arrest on day 68, and could not be revived.

Investigations

The hematological and biochemical investigations are outlined in ***WebTable* I**. Echocardiography on day 62 showed normal cardiac function with no evidence of pulmonary hypertension. Coagulopathy was present (day 68) with elevated PT: 52s (normal 12-14 s); aPTT: 69s (normal: 33-36 seconds); with an INR of 3.67.

Microbiological analysis: Multiple blood cultures (thrice) and urine culture (once) were sterile. Endotracheal aspirate (ETA) on day 63 and day 64 showed presence of yeast. Serum Galactomannan was negative. Both ETA and gastric aspirates were negative for PCJ and acid fast bacilli (AFB) on two occasions. Pleural fluid examination was unremarkable. Qualitative cytomegalovirus (CMV) PCR was positive in ETA but negative in blood.

Radiology findings (Radiologist): Initial few radiographs revealed ill-defined reticulo-nodular opacities in bilateral lung fields (left > right) with relative sparing of bilateral upper lobes and periphery. Subsequently, serial chest radiographs revealed progressive pulmonary lesions on day 63 and day 65. A chest radiograph on day 65 revealed pneumothorax, subtle pneumo-mediastinum and subcutaneous emphysema. Expansion of left side of lung on the radiograph done on day 66 was noted. Radiograph done on the day of demise revealed significant increase in subcutaneous emphysema involving bilateral lower cervical region (***Fig.* 1*A-D***).

With these chest radiographs, possible etiologies considered were infective, including fungal pneumonia and PCJ pneumonia, CMV disease and miliary tuberculosis (TB). Non-infective etiologies under consideration included pulmonary graft-versus host disease (GvHD) and pulmonary veno-occlusive disease (VOD).

Discussion (Clinical Discussant)

This is a case of neuroblastoma, stage IV, day 68 post auto-SCT, presenting with fever, pneumonia, hypoxia, and investigations showing polymorphic leucocytosis with deranged liver function tests (LFT). In a post-transplant patient, complications can be divided according to the duration subsequent to transplant. In the initial 30 days, there is presence of neutropenia; between 30-100 days is the early post-engraftment phase and beyond 100 days is late post-engraftment phase.

The index case developed symptoms in the early post engraftment phase. Common complications seen in early post-engraftment phase can be divided into infective and non-infective. Infective etiologies include CMV which can explain both pneumonia and hepatitis. It is a common pathogen causing disease 3 weeks post SCT. India is an endemic country for CMV. In the index case, ETA demonstrated polymerase chain reaction (PCR) positivity

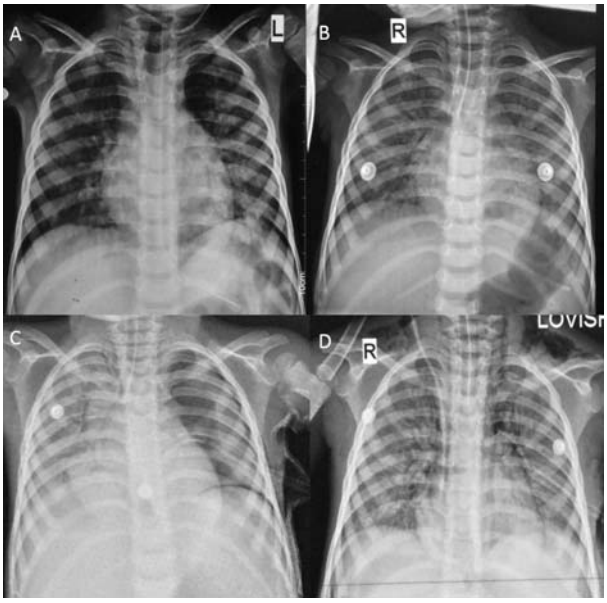

FIG. 1 *(A) Chest X-ray (CXR) showing ill-defined reticulonodular opacities in both lungs, which significantly increased in the CXR done after 48 hours (B); (C) Follow-up CXR depicting pneumothorax on the left side, for which a drainage tube was inserted; (D) Repeat CXR after 48 hours demonstrates expansion of lung on left side with persistent opacities in bilateral lungs. Pneumomediastinum and subcutaneous emphysema can also be seen.*

for CMV along with radiological findings which were supportive of the diagnosis. However, the child deteriorated despite administration of gancyclovir from day 2 onwards, which is unusual. Typically blood PCR is positive in such cases, though not mandatory for diagnosis of CMV pneumonia. Fungal infections are the next possibility, supported by the presence of candida in ETA on two occasions. Presence of normal neutrophil count and a normal serum Galactomannan are odd points. Galactomannan <0.5 has shown a good negative predictive value for Aspergillus infection [2]. Other viral infections that are important in post-SCT scenario include Respiratory syncytial virus (RSV), Para-Influenza virus, Influenza, Metapneumovirus, and Coronavirus. Multiorgan failure and lymphopenia is common in these patients. Patients with RSV often require ventilation. Associated co-infection with fungus, especially aspergillus can be seen. In the absence of investigations directed towards the myriad respiratory viruses, it is difficult to rule in or rule out these infections. Tuberculosis should be considered in an immunocompromised patient in an endemic country; however, the rapid onset of disease, absence of a contact and negative evaluation make it unlikely. In our case other bacterial infections typically seen in an immunocompromised child are also unlikely in view of sterile cultures, complete absence of fever and normal C-reactive protein (CRP).Though this clinical presentation can be caused by infection with PCJ, it is an uncommon infection. Other atypical infections like Nocardia and Cryptococcus are rarer still. The non-infective etiologies causing respiratory symptoms in a post-transplant setting can be pulmonary GvHD, Idiopathic pneumonia syndrome (IPS), Bronchiolitis obliterans syndrome (BOS), Cryptogenic organising pneumonia (COP) and SOS. IPS is a very common disease in this situation, but is typically seen post allo-SCT and hepatitis is not an associated feature. On-going hepatic SOS is unlikely as there was no weight gain or tender hepatomegaly. GvHD and BOS are also typically diseases seen in allo-SCT setting. Pulmonary SOS is very rare and normal echo findings negate this possibility. The clinical presentation is consistent with COP, though it is more common in females undergoing allogenic transplant.

The final diagnosis is neuroblastoma stage IV, day + 68 post auto-SCT (Bu-Mel) with pneumonitis, ARDS and multi-organ failure; likely etiology being fungal pneumonia or CMV pneumonia and hepatitis secondary to ischemia with underlying SOS.

Pediatrician I: This patient presented in 2nd month post auto-SCT. Lymphocytes are still not functional at this stage. Moreover, he had documented lymphopenia. There is a high likelihood of infective causes with CMV and PCJ pneumonia. Other important etiologies are Busulfan-induced lung injury, which typically occurs in 2nd month post-transplant. He had received conditioning regimen of melphalan-busulfan.

Pediatric hemato-oncologist 1: IPS occurs post SCT day+60 to 80. This child had typical bilateral basilar infiltrates and hypoxia. Moreover, IPS has a relationship with use of busulfan and pre-existing SOS. Presence of CMV positivity in ETA is of questionable significance as it is a common organism. Histopathological evidence from lung biopsy is essential to prove CMV pneumonia. Liver dysfunction in the form of transaminitis was likely due to shock and ischemia.

Pediatric hemato-oncologist 2: Bacterial and fungal infections cannot be excluded despite absence of fever, several sterile cultures and continued normal values of CRP, though less likely. However, both CMV and PCJ infections are possible with normal CRP. Absence of adventitious lung sounds at initial presentation, along with presence of hypoxia may be a pointer towards PCJ pneumonia. Immunocompromised state, lymphopenia and the fact that the child was not on PCJ prophylaxis are important here. Moreover, CMV is ubiquitous in our

pediatric population, and in pediatric oncology patients, we have seen a near 100% seropositivity. Reactivation of CMV can occur at any point of time in these patients. Important non-infective possibilities are IPS and cryptogenic pneumonia. Pulmonary SOS is quite unlikely given the normal echo findings.

Adult hematologist: Immune reconstitution post-transplant takes typically 6 to 12 months. This child was immunocompromised. Adenovirus infection can be considered. It can be rarely seen in association with hepato-pulmonary syndrome.

PATHOLOGY PROTOCOL

The excised tumor on histology was categorized as differentiating neuroblastoma (***Fig.* 2*A-C***). Autopsy revealed normal serous cavities. Liver weighed 290 g and revealed irregular areas of sinusoidal congestion, confluent at places with necrosis of adjoining parenchyma involving both right and left lobe (***Fig.* 2*D***). No thrombi were identified in right and left hepatic vein or inferior vena cava. Microscopically, areas of centrizonal congestion were identified (***Fig.* 2*E***). Furthermore, the dominant pathology was seen in the central vein and terminal hepatic venule (THV). There was varying degree of obliterative changes with subendothelial fibrosis and laying down of reticulin fibres and collection of extracellular matrix in subintimal zone. At places, the THV was completely obliterated with wipe out of centrizonal hepatocytes while the periportal hepatocytes were preserved (***Web Fig.* 1*A-E***). In other regions, extravasated RBCs and areas of hemorrhage were noted in centri-zonal regions. Besides acute obliterative changes, subacute changes in form of deposition of collagen around the hepatic venule and collection of hemosiderin laden macrophages were also noted. Loss of hepatic parenchyma resulted in approximation of central veins structures (***Web Fig.* 1*A***).

Both lungs weighed 245g with dull pleura. Microscopy revealed features of busulfan-induced lung injury with marked prominence of Type II pneumocytes; many of them demonstrated nuclear atypia and hyperchromasia. Marked thickening of interstitium with fibrosis was also noted (***Web Fig.* 2*A-B***). Other regions showed patchy acute bronchopneumonia and alveolar haemorrhages. Features of pulmonary arteriopathy were also noted with prominence of intra-acinar arterioles. There were no features of VOD in the pulmonary veins. An occasional focus of septic emboli with Candida infiltration into parenchyma was noted. No CMV inclusions were noted in lungs. PCR carried out on lung tissue for adenovirus, RSV and metapneumovirus were negative.

Acute ulcers with Candida infiltration were noted in stomach and small intestine. Candida had disseminated to heart causing mural endocarditis, myocardial abscess and tiny (2-4 mm) vegetations on left atrial wall (***WebFig.* 2 C-F**). Both tricuspid and mitral valves were normal. Dissemination with formation of fungal abscesses were also detected in psoas muscle and omental fat. Subsequent to septic emboli, infarcts were detected in right kidney (upper pole) with thrombi within the branches of renal vessels and spleen. Right kidney also revealed features of acute tubular necrosis in non-infracted regions. No residual tumor was detected in lymph nodes, thymus and bone marrow.

The autopsy diagnosis is concluded as follows:

In a known case-of neuroblastoma, undifferentiated (adrenal) post-autologous stem-cell transplant:

- Features of busulfan-induced lung injury with organizing bronchopneumonia and pulmonary arterial hypertension;
- Veno-occlusive disease in liver.
- Fungal (Candida) ulcers in GIT with extensive dissemination to heart (mural endocarditis and myocardial abscess), lungs, skeletal muscle and omental fat producing embolic infarcts in right kidney and spleen.
- No residual disease in bone marrow.

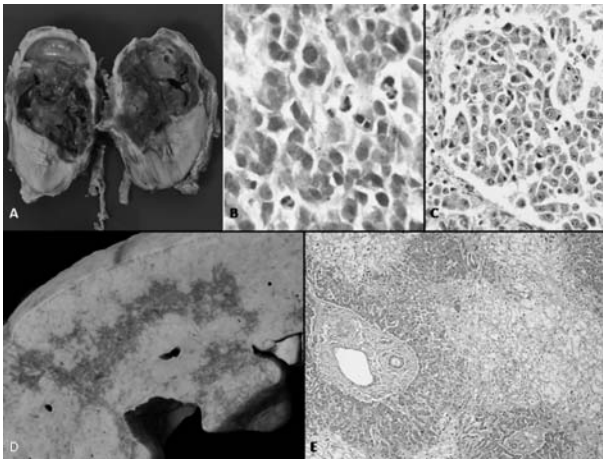

FIG. 2 *(A) Gross of resected tumor at the upper pole of the left kidney. It is largely necrotic; (B) Round to oval cells with high N/C ratio with abundant mitoses and apoptotic debris (H&E × 400); (C) About 40% of cells demonstrated nodular areas of differentiating cells of large size, with nucleolar prominence and moderate cytoplasm (H&E × 200); (D) Gross of live showing haemorrhagic discoloration involving both right and left lobe; (E) Areas of loss of hepatic parenchyma with congestion in the centrizonal areas, only the peri-portal areas are preserved (H&E × 100).*

DISCUSSION

Hepatic sinusoidal obstruction syndrome (SOS) is an obliterative venulitis of THV which occurs as a result of cytoreductive therapy prior to hematopoietic stem cell transplantation (HSCT), ingestion of pyrrolizidine alkaloids, or radiation therapy [3-6]. The primary pathogenetic event is the endothelial injury of sinusoids and small hepatic veins. Following which, there is deposition of fibrin-related aggregates and oedema in the subendothelial zone [3]. Accumulation of these aggregates and entrapment of fluid and cellular debris progressively occlude the hepatic venous flow and leads to post-sinusoidal intrahepatic hypertension. This is accompanied by necrosis of perivenular hepatocytes. Histologically, acute, sub-acute and chronic forms of SOS have been described depending upon collagenization and fibrosis of terminal hepatic venule. Incidence of SOS varies from 0-70%, as it depends on the conditioning regimen used as well as upon the patient's risk factors [4-6]. SOS occurs more often after allo- than after auto-HSCT (8 v/s 3%, respectively), suggesting a role of immune reactions in this disorder [7]. Few independent studies have documented increase in circulating levels of plasminogen activator inhibitor-1 (PAI-1), a molecule released by the endothelial cells, in patients developing SOS [8,9]. Increased PAI-1 levels might be of clinical utility in challenging clinical situations in patients with hyperbilirubinemia occurring after HSCT. It forms one of the therapeutic targets for defibrotide, which reduces circulating PAI-1 levels along with other actions. Other endothelial markers, like intercellular adhesion molecule-1 (ICAM-1), E-selectin, von Willebrand factor (VWF), and thrombomodulin may also be helpful in early identification of patients at risk of SOS who may benefit from early introduction to therapies [10]. Diagnosis of SOS is based on constellation of signs and symptoms and serum bilirubin levels. Hepatic SOS is clinically characterized by jaundice caused mainly by conjugated hyperbilirubinemia, tender hepatomegaly, fluid accumulation manifested as rapid weight gain and ascites [4]. Most commonly used diagnostic criteria for SOS includes the Seattle criteria [11], the modified Seattle criteria [1], and the Baltimore criteria [12]. Because of its high incidence and mortality, prophylaxis for hepatic SOS is widely practiced, using different regimens in different centres. When hepatic SOS is established, specific therapy is usually given in addition to general supportive care, especially in moderate or severe cases. Hepatic SOS is a formidable challenge both for patients undergoing stem cell transplantation and for their physicians.

The second pathology in this child which significantly contributed to his downhill course was busulfan induced lung injury. Intriguingly, in the present clinical setting, busulfan induced lung injury remains an diagnosis of exclusion, particularly with respect to considering usual and atypical infections. Its clinical presentation includes a spectrum ranging from acute, rapidly progressive respiratory distress to chronic, interstitial lung disease with insidious onset [13,14]. The pathophysiology of drug-induced lung injury is not fully understood but direct toxicity of the drug to parenchymal cells, cell-mediated immune reactions and release of cytokines are believed to contribute to the lung injury. The pathologic findings consist of mainly diffuse interstitial pneumonitis, organizing alveolitis and cellular atypia within type II pneumocytes. The injury pattern with busulfan is diffuse alveolar damage (DAD) either in acute exudative phase with alveolar and interstitial oedema and hyaline membranes; or late reparative phase, which is characterized by proliferation of type II pneumocytes and interstitial fibrosis [15]. Marked atypia of the type II pneumocytes is a morphological clue in favour of busulfan induced lung injury in contrast to organizing bacterial pneumonia. Moreover, PCR for CMV is helpful in excluding viral pneumonia.

The prevalence of drug-induced pulmonary toxicity is increasing, and more than 100 drugs are now known to cause lung injury. Because this lung injury can be progressive and fatal, early recognition is important. The diagnosis of pulmonary drug toxicity should be considered in any patient with a history of drug therapy who presents with new or progressive respiratory complaints.

The superadded fungal ulcer which developed preterminally with extensive dissemination to heart causing mural endocarditis and myocardial abscess eventually led to the demise of the child.

Hepatic SOS contributes considerably to transplantation-related morbidity and mortality. Recognition of this disease in the post-transplantation setting remains a challenge in the absence of specific diagnostic features as many other more common conditions can mimic it. A high index of suspicion is needed to identify patients with SOS. While hepatic SOS and busulfan induced lung injury are commonly reported as isolated findings following autologous SCT, the co-existence of these are extremely rare and have not been documented in the literature thus far. The present case adds observational data to the existing literature and highlights the importance of keeping high index of suspicion for these two entities in patients following HSCT, and early institution of effective therapy.

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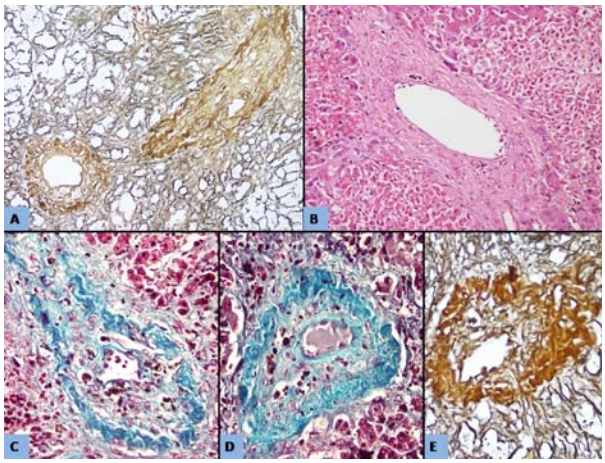
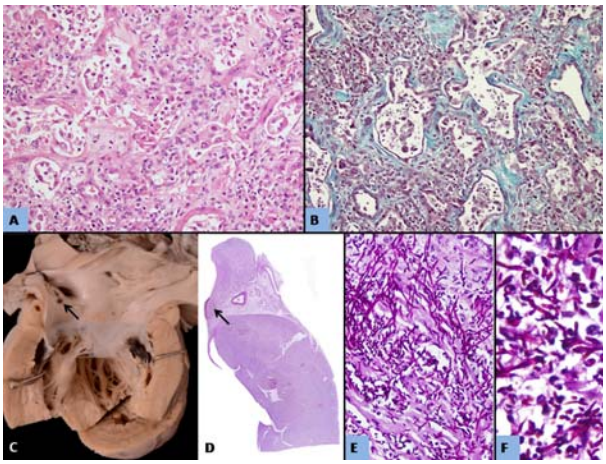
WEBTABLE I HEMATOLOGICAL AND BIOCHEMICAL INVESTIGATIONS IN THE PRESENT CASE

Test	*Days post- transplant*						
	60	61	62	64	65	67	68
Hb(g/dL)	11.7	10.6	7.7	8.0		9.2	7.0
TLC (/mm^3)	9200	19300	6900	6700		39100	25200
DLC (N/L/M/E)	-	91/5/3/1	90/08/1/1	67/26/4/2		80/16/1/1	97/02/01
Platelets (10^6/mm^3)	36	65	25	31		50	90
CRP	26	41	17	11		12	15
Na/ K	131/ 4.1			130 / 5.1	129 /5.5	140 /4.7	1304.2
Urea/ Creatinine	35/0.6			59/1.2	80/2.2	90/2.7	89/3
Bilirubin / conj.	0.7/-			0.7/-	0.7/-	2.3/1.6	3.1/1.5
AST/ALT	92/24			71/24	59/24	794/390	6756/1112
ALP	132			101	108	169	183
Ca/ PO4/ Mg	9.9/3.5/1.3			9.4/4.8/1.4	9.8/4.4/1.5	9.6/5.4/-	10.4/5.6/2.4
Total protein/ Albumin	6.6/2.6			5.5/2.3	5.8/2.6	4.6/2	4.6/2.1

Ca: calcium, CRP: C-Reactive protein, DLC: Differential leukocyte count, E: Eosinophils, Hb: Hemoglobin, K: potassium, L: Lymphocytes, M: Monocytes, Mg: magnesium, N: Neutrophils, Na: sodium, PO4: serum phosphorus, TLC: total leukocyte count



WEB FIG. 1 *(A) Approximated terminal hepatic venue with laying down of reticulin fibers and obliteration of lumen (reticulin x400); (B) Sub-acute changes in the central vein with perivenular fibrosis and collection of hemosiderin laden macrophages (H&E x400); (C&D) Masson's trichrome stain highlights varying degree of obliteration of lumen and collection of extracellular matrix in the subintimal zone (Masson's trichrome x400); (E) High power demonstrates intraluminal deposition of extracellular matrix and reduced luminal diameter (reticulin x400).*



WEB FIG. 2 *(A): High magnification showing marked prominence of Type II pneumocytes and interstitial widening (H&E x400); (B) Masson's trichrome stain highlights interstitial widening with fibrosis (Masson's trichrome x400); (C): Gross of heart with tiny vegetations on left atrial wall (arrow); (D) Scanner view of left atrial and ventricular wall showing vegetation (arrow); (E) Periodic acid-Schiff (PAS) stain demonstrates pseudohyphae of Candida infiltrating the myocardial fibers (PAS x400); (F) Candida yeast and pseudohyphae highlighted by PAS stain (PAS x1000).*