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SUCCESSFUL AUXILIARY LIVER TRANSPLANT FOLLOWED BY HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN A BOY WITH X-LINKED LYMPHOPROLIFERATIVE DISEASE TYPE 1

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Abbreviations: ALF – Acute liver failure, ALT - Alanine aminotransferase, AST - Aspartate aminotransferase, ATG - Anti-thymoglobulin, CMV – Cytomegalovirus, EBV – Epstein-Barr Virus, GGT - Gamma-glutamyl transferase, HLH - Haemophagocytic lymphohistiocytosis, HSCT - Haematopoietic stem cell transplant, INR – International Normalised Ratio, LT - Liver transplantation, PID - Primary immunodeficiency, PTLD – Post-transplant lymphoproliferative disease, SAP - SLAM-Associated Protein, SLAM - Signalling Lymphocyte Activation Molecule, SRR - steroid-resistance rejection, XLP1 - X-linked lymphoproliferative disease type 1

Contributors' Statements:

Dr. Chartier wrote the manuscript, and approved the final manuscript as submitted.

Dr Hadzic conceptualized the project and was the main supervisor.

Dr. Deheragoda provided the histology slides and associated comments as well as reviewed and revised the manuscript and approved the final manuscript as submitted.

Dr. Deheragoda, Dr. Gattens, Dr. Dhawan, Dr. Heaton, Dr Booth and Dr. Hadzic reviewed and revised the manuscript and approved the final manuscript as submitted.

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ABSTRACT

We described a five-year-old boy who presented with acute liver failure of indeterminate aetiology, requiring urgent liver transplant. Post-operative course was complicated by pancytopaenia, hypogammaglobulinaemia and cerebral lesions, histologically confirmed as EBV-driven post-transplant lymphoproliferative disease. Genetic testing showed XLP1 mutation, prompting matched-unrelated haematopoietic stem cell transplant to cure his primary immunodeficiency.

INTRODUCTION

X-linked lymphoproliferative disease type 1 (XLP1) is a rare, potentially life-threatening primary immunodeficiency (PID) with a range of clinical manifestations, including haemophagocytic lymphohistiocytosis (HLH), lymphoma, hypogammaglobulinaemia and autoimmune phenomena. It is caused by mutations in the SH2D1A gene, which encodes for the signalling lymphocyte activation molecule (SLAM)-associated protein (SAP)[1]. The absence of SAP in XLP1 patients leads to multiple immunological defects including NK- and T-cell cytotoxicity[2], the lack of NKT-cell development and defective CD4+ T-follicular cell (T_{FH}) help[3], leading to abnormal humoral function. The clinical disease phenotype is characterised by severe immune dysregulation including development of lymphoma, T-cell activation defects leading to HLH (or dysregulated immune response to infection), abnormalities in immunoglobulin production and T-dependent humoral immune responses. SAP acts as a 'natural blocker' of other SH2-domain containing inhibitory molecules from binding to SLAM-family receptors. In the absence of SAP, SLAM-family receptors switch function and mediate inhibitory signals[4].

XLP1 is often regarded as an EBV-driven disease and although susceptible to overwhelming EBV infection, up to 35% of patients develop symptoms in its absence, including HLH, lymphoma, lymphoid vasculitis, hepatitis and cytopaenias[5-6]. Historically, outcome for XLP1 patients was extremely poor with survival as low as 4% when associated with HLH[7]. However, with the advent of improved HLH and lymphoma protocols, anti-CD20 monoclonal antibodies and immunoglobulin replacement the mortality has fallen. Haematopoietic stem cell transplant (HSCT) offers a curative treatment with survival reaching 80% with a well-matched donor[5]. Survival is reduced to ~50% in

non-transplanted patients and those requiring HSCT with a poorly-matched donor and active disease at the time[5].

CASE REPORT

A five-year-old Caucasian boy was admitted in acute liver failure (ALF) after a two-week history of jaundice, abdominal pain and lethargy. His past medical history included an episode of aplastic anaemia (AA) at the age of 3 years. Diagnosis was confirmed by a markedly hypocellular bone marrow aspirate (<10% cellularity) without evidence of haemophagocytosis or infiltrative disease and he received supportive treatment with blood products and intravenous immunoglobulin. He was negative for EBV, parvovirus B19, HIV but positive for CMV IgG. HSCT was considered, but he recovered spontaneously.

At presentation with ALF, he had grade I encephalopathy, jaundice (total bilirubin 227 μmol/L, conjugated 171 μmol/L), elevated transaminases (ALT 1006 IU/L, AST 1374 IU/L), and abnormal synthetic function (INR 1.83, ammonia 70 μmol/L, albumin 35 g/L). He was initially started on intravenous vitamin K, broad-spectrum antibiotics and antifungals. Ultrasound showed no splenomegaly or portal hypertension, but the liver parenchyma appeared abnormal. Aetiologic work-up was negative for hepatitis A, B, C and E, CMV, adenovirus, enterovirus, parvovirus B19 and HHV-6. He had positive EBV IgG, but EBV IgM and PCR RNA were negative. Two weeks earlier, EBV capsid IgG and IgM as well as EBV nuclear antigen antibodies were negative. He had mildly positive serum anti-nuclear antibodies (titre 1:40), while serum IgG levels were slightly decreased at 4.72 g/L (normal, 4.9-16.1 g/L), with normal IgM and IgA levels. The remainder of his autoantibody panel was negative for anti-smooth muscle, anti-mitochondrial, anti-gastric parietal cell and anti-liver kidney microsome antibodies. His lymphocyte count varied between 6-13 $x10^9$/L (normal, 2-9 $x10^9$/L). Serum ferritin was raised to 2088 ng/ml (normal, 20-300 ng/ml) with borderline low fibrinogen of 1.3 g/L (normal, 1.5-4.5 g/L), but normal triglyceride of 1.1 mmol/L (normal, 0.5-1.7 mmol/L). Given his background of AA and weakly positive autoantibodies, he was empirically started on methylprednisolone (2 mg/kg/d) for possible autoimmune hepatitis. Three days later, as INR increased to 2.5, a transjugular liver biopsy was performed. Histopathology showed a porto-lobular

hepatitis with interface activity and early portal fibrosis that was compatible with partially treated autoimmune hepatitis (figure 1A-B). Given the poor biochemical response to steroids, he received one dose of anti-CD20 monoclonal antibody (rituximab 400 mg/m^2) 12 days later.

On day 18 after admission, he developed grade II/III encephalopathy, with ammonia of 170 μmol/L, INR 3.15 and profound hypoglycaemia. He was intubated, transferred to the intensive care unit and listed for emergency liver transplant (LT). Two days later, he received an auxiliary partial orthotopic LT with a left lateral segment from a cadaveric adult EBV-positive/CMV-negative donor. Auxiliary grafting was performed based on the absence of macroscopic evidence of chronic liver disease and presumed "indetermined" aetiology of ALF, according to the standard practice in our unit[8].

The immediate post-operative course was complicated by severe T-cell mediated rejection requiring high-dose methylprednisolone (10 mg/kg/d x3) followed by rabbit anti-thymoglobulin (ATG) (1.5 mg/kg/d x10 consecutive days), as repeated biopsy, one week later, showed persistent T-cell mediated rejection with bile duct damage. As his liver enzymes improved with ATG, no liver biopsy was repeated at the end of treatment. During the rejection episode, he was found to have significant ascites with high abdominal drain output, hypoalbuminaemia (22 g/L) and hypogammaglobulinaemia (IgG 1.07 g/L). Observed pancytopaenia prompted a bone marrow aspirate showing hypocellularity, but no haemophagocytosis, interpreted as likely secondary to mycophenolate mofetil (MMF), which had been added as second immunosuppressant to tacrolimus. The pancytopaenia improved after converting MMF to azathioprine within 3 days.

Two months after LT he was discharged home, but continued having fluctuating transaminases (ALT 70-445 IU/L, AST 48-484 IU/L). One month post-LT, elective hepatobiliary scintigraphy (HIDA) showed that only 18% of the liver function and biliary excretion was performed by the native liver, while three months later this increased to 90% (figure 2A-B). Six months post-LT, liver biopsy demonstrated good regeneration and minimal lobular inflammation of the native liver (figure 1E), whereas the left transplanted liver showed moderate rejection. CT volumetry confirmed the native liver was increasing in size while the graft was getting smaller (figure 2D). With the evidence of a

good radiological and functional recovery of the native liver, we initiated gradual reduction in immunosuppression with target tacrolimus levels between 2-4 ug/L.

Seven months post-LT, the boy re-presented with prolonged generalised tonic-clonic seizure, fever and maculopapular rash. Brain MRI revealed two focal lesions in the right and left temporal regions. HSV PCR and bacterial cultures were negative, but he was highly EBV viraemic (EBV DNA 4,100,000 copies/ml). A brain biopsy revealed heavy mononuclear CD20+ infiltrates and EBER expressing B-lymphoid infiltrate, supporting the diagnosis of PTLD (figure 1F). Immunosuppression was discontinued and a course of anti-CD20 monoclonal antibody (rituximab 400 mg/m^2/week x4) was given. However, the brain lesions progressed radiologically, prompting treatment escalation with chemotherapy (cytarabine, methotrexate, and etoposide). Due to the rarity of cerebral PTLD post-LT, his background of AA and ALF of indeterminate aetiology, he underwent genetic testing which identified a pathogenic c.245 duplication (Asn82Lysfs*22) mutation of SH2D1A gene, confirming the diagnosis of XLP1.

Following clinical remission of PTLD, the boy underwent evaluation for HSCT. By then, his native liver had fully recovered, as documented on repeated HIDA scan, MRI volumetry (figure 2B/2E). and liver biopsy, which showed only minimal lobular inflammation and mild porto-septal fibrosis (figure 1E). Therefore, his liver function was deemed sufficiently recovered for the challenges of the next transplant. He was conditioned with fludarabine, treosulfan and alemtuzumab and underwent a 10/10 matched unrelated donor peripheral stem cell transplant 18 months after his LT. Twenty days later, 100% donor cell engraftment was documented. He remained EBV viraemic during and after the HSCT, but without further complications. Post-HSCT immunosuppression was maintained with cyclosporin and MMF as he required no medications for the liver graft. His liver function tests remained normal. With excellent engraftment, immune recovery and no evidence of graft-versus-host disease, MMF was stopped after 4 months, followed by cyclosporin tapering 8 months post-HSCT. Thirty months after LT and one-year post-HSCT the boy remains completely well, off immune suppression and cured from life-threatening ALF, cerebral lymphoma and, most importantly, underlying XLP1 (See Figure 3 for complete timeline of critical events).

DISCUSSION

We describe a highly unusual presentation of XLP1, which required combined interventions to treat ALF, cerebral PTLD and immune dysregulation. Our patient became immunosuppression independent after "bridging" his ALF with auxiliary LT and subsequent successful HSCT engraftment.

According to a recent multicentre study of 91 XLP1 patients[5], HLH was the most common presenting feature (31.9%), with further 22% having dysgammaglobulinaemia and 14.3% lymphoma. Less than 10% presented with other symptoms such as anaemia, gastritis, vasculitis or hepatitis. It appears that EBV-negative XLP1 patients are less likely to have HLH as the first presentation[5]. Our patient initially presented with AA and negative EBV serology. This emphasizes that a comprehensive immunological assessment, and possibility of XLP, should be considered in all AA patients with negative initial investigations.

The clinical management of our patient was symptom-driven. After he developed progressive ALF with encephalopathy and failed to respond to empirical course of steroids for presumed autoimmune aetiology, he received emergency LT. Intraoperatively, it was decided to perform auxiliary partial LT, as potential for regeneration of the native liver was anticipated.

It is well established that patients with XLP1 can present with fulminant hepatitis. Purtilo described 61 patients who died from EBV infection (30 with sporadic infectious mononucleosis and 31 with XLP1)[6]. Fifty-eight percent of XLP1 patients died of fulminant hepatitis, at a mean of 7 weeks after onset of symptoms. On necropsy, their liver biopsy typically showed prominent portal infiltrate composed of lymphocytes, plasma cells, immunoblasts and histiocytes[6]. The inflammatory component was similar to that reported in our patient transjugular biopsy, misleading us to suspect autoimmune hepatitis, given the previous history of AA and low-titre seropositivity for anti-nuclear antibodies. Therefore, differential diagnosis between ALF secondary to autoimmune hepatitis or XLP1 may not be straightforward. PIDs, including XLP, should be excluded in all indeterminate

ALFs of childhood, particularly in presence of pancytopaenia, dysgammaglobulinaemia, and supportive history.

The immediate post-LT course of our patient was complicated by episodes of T-cell mediated rejection, which could lead to increased abdominal drain losses due to the graft stiffness. Thus, he received standard treatment with high-dose steroids followed by ATG for steroid-resistant rejection (SRR), as ATG by depleting T-cells can be effective in treating solid organ SRR[9] and late cholestatic rejection in pediatric liver transplant recipients[10]. This would have made him even more susceptible to EBV-related injury during primary exposure to the virus, likely acquired from the adult graft donor. The hypogammaglobulinaemia observed post-LT was initially interpreted as secondary to immunoglobulin losses from the significant abdominal drain output, but in retrospect it is more likely to have been related to XLP1.

The most unusual complication in our patient was the development of cerebral PTLD seven months after LT. PTLD ranges from non-destructive lesions (follicular hyperplasia), to malignant forms (lymphoma-like lesions), as a result of chronic immunosuppression in transplant recipients. About 70% of paediatric cases are EBV-driven, especially early post-transplant. One study reported a mean onset latency for brain PTLD of 31 months (range, 3-131 months) after transplantation[11]. With our experience, it is mandatory to exclude PIDs in all children developing cerebral PTLD after LT, as their ultimate treatment could require additional management, including HSCT.

PTLD remission was achieved after chemotherapy, providing a window for potentially curative treatment. HSCT can be associated with a number of hepatic complications such as graft-versus-host disease, sinusoidal obstructive syndrome, drug-induced liver injury or sepsis. Our patient underwent elective assessment of the native liver function, including ultrasound studies, MRI volumetry, HIDA and histology, all indicating that the liver regeneration was likely to be adequate for the challenges of HSCT. He indeed had no hepatic complications and remains in good health, after his minimal immunosuppression was completely withdrawn one year after HSCT.

In conclusion, a diagnosis of XLP1 links all previous symptoms including cytopaenias, ALF and EBV-driven brain PTLD. Our unusual experience demonstrates that LT can be life-saving in patients with XLP1 who present with ALF, but vigorous diagnostic attempts should be made in all indeterminate cases to rule out PIDs. Had the diagnosis been reached earlier, HSCT could have been performed sooner to avoid additional complications associated with PIDs.

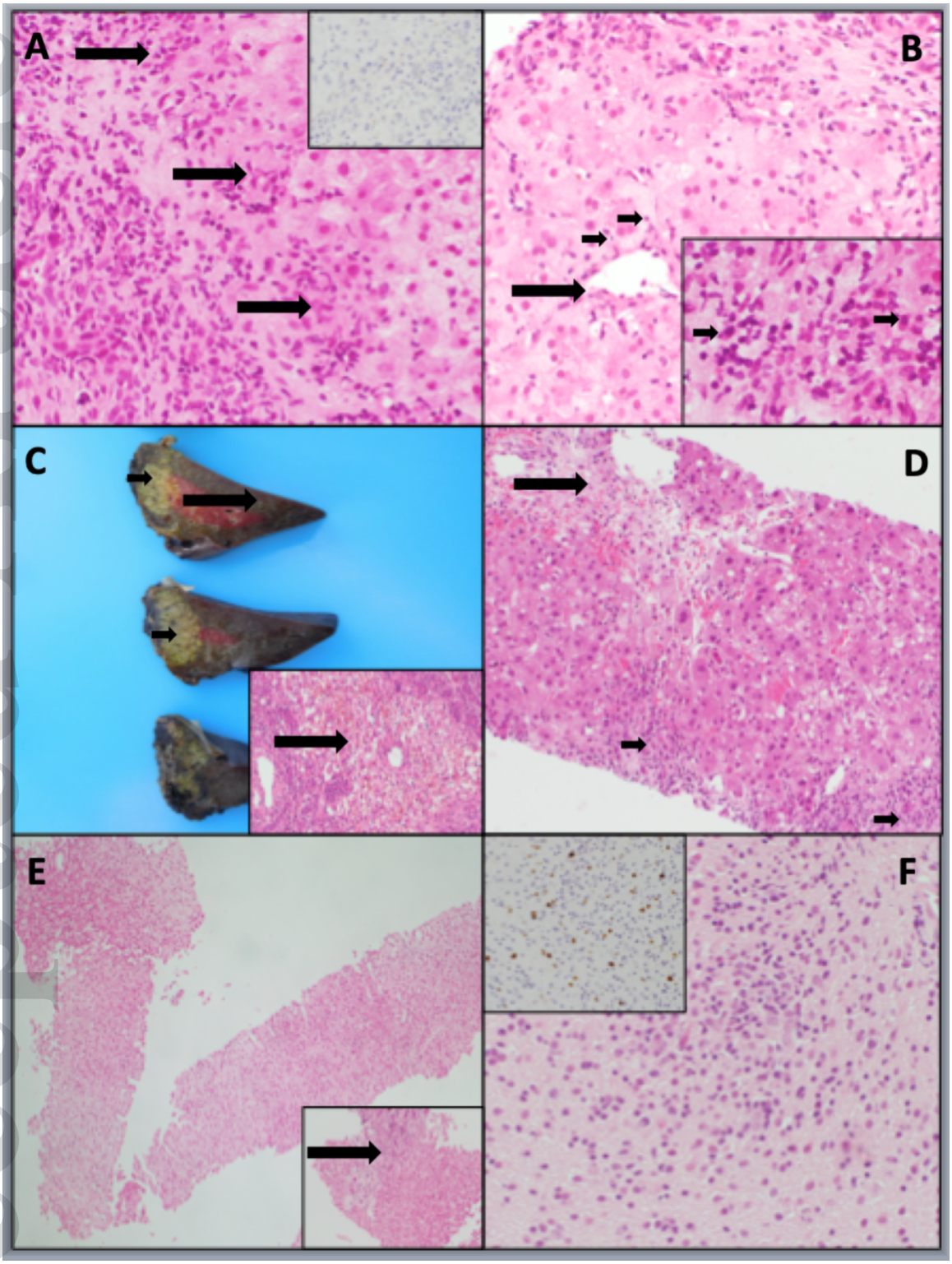

Figure 1: Histopathology findings pre- and post-liver transplant

(A-B) Transjugular liver biopsy performed following three days of steroid treatment, demonstrated a porto-lobular hepatitis with interface activity (A, Main image, H&E x 200 magnification, black arrows indicating the interface activity). Evidence for EBV infection was not found (inset, EBER in-situ hybridisation x200 magnification). The interface activity was predominantly lymphocyte-rich, with very occasional plasma cells (B, inset image, H&E x200 magnification, short arrows indicating the plasma cells). Other features observed in autoimmune hepatitis such as hepatocellular lymphocyte emperipolesis (B, Main image H&E x 200 magnification, short arrows) and perivenular inflammation (B, Main image, long arrow) were present. Haematopathology review of the biopsy found no evidence of lymphoproliferative disease.

(C) The explanted left liver lobe at LT demonstrated widespread collapse (Main image and inset, long arrows indicating collapsed areas, with inset arrow demonstrating panacinar confluent hepatocyte necrosis) with foci of residual viable cholestatic lobules (Main image, short arrows indicate residual viable parenchyma).

(D) Allograft biopsies undertaken at 10-, 13- and 23-days post-LT demonstrated severe T-cell mediated rejection (main image, H&E x 100 magnification, short arrows indicate portal inflammation and long arrows show inflammation affecting the terminal hepatic venules with associated hepatocyte necrosis, 10 days post-LT).

(E) Subsequent biopsies of native liver at 6 months post-LT demonstrate regeneration with only mild residual portal inflammation, but no significant interface or lobular inflammation (main image, H&E x 100 magnification and inset showing portal tract and interface H&E x 100 magnification).

(F) Cerebral cortex biopsy undertaken 8 months post-LT demonstrate a high grade B-cell PTLD (Main image H&E x 200 magnification, image courtesy of Dr Andrew King, Clinical Neuropathology, King's College Hospital) which demonstrate nuclear EBER expression (inset image, EBER in situ hybridisation x 200 magnification, image courtesy of Dr Hadil Abu Arqoub, King's College Hospital).

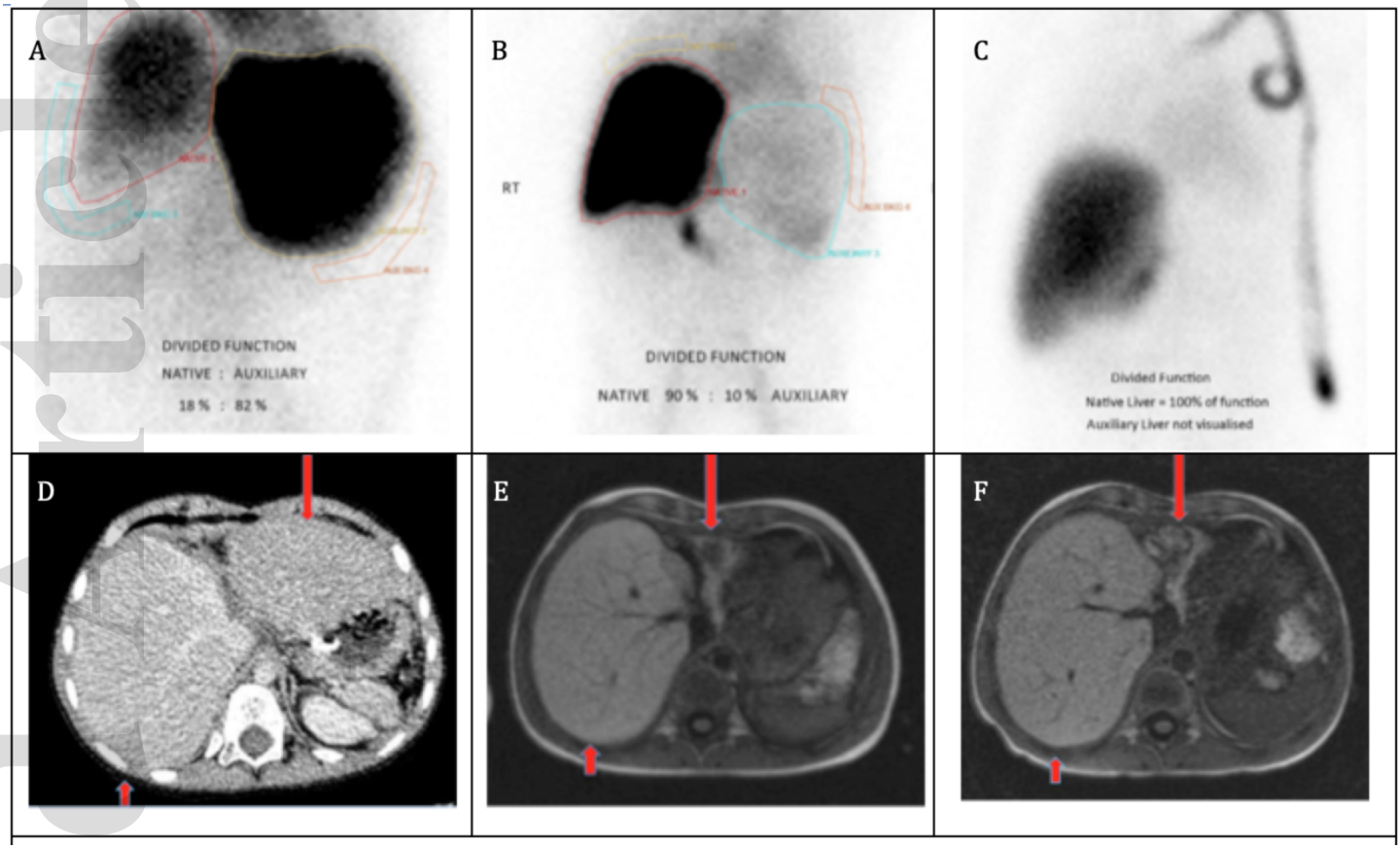

Figure 2: Evolving changes in differential liver function and liver volumes (right native vs. left graft liver) after the auxiliary partial orthotopic LT.

Panel A-C shows hepatobiliary scintigraphy at 1, 4 and 24 months post-LT. There has been a progressive increase in the function of the native liver (from 18% in Panel A, to 90% in Panel B to 100% in Panel C) with complete inactivity of the grafted liver after stopping immunosuppression. Panel A and B are pre-HSCT while Panel C is post HSCT.

Panel D-F shows the progressive change in native (small arrow) and graft liver (long arrow) volume. Panel D is a CT scan done 4 months post-LT, whereas E and F are MRI done at 15 and 24 months, respectively. Estimated liver volumes on MRI done at 15 months (Panel E), as part of investigation pre-HSCT, is 819 ccm for native liver and 14 ccm for liver graft.

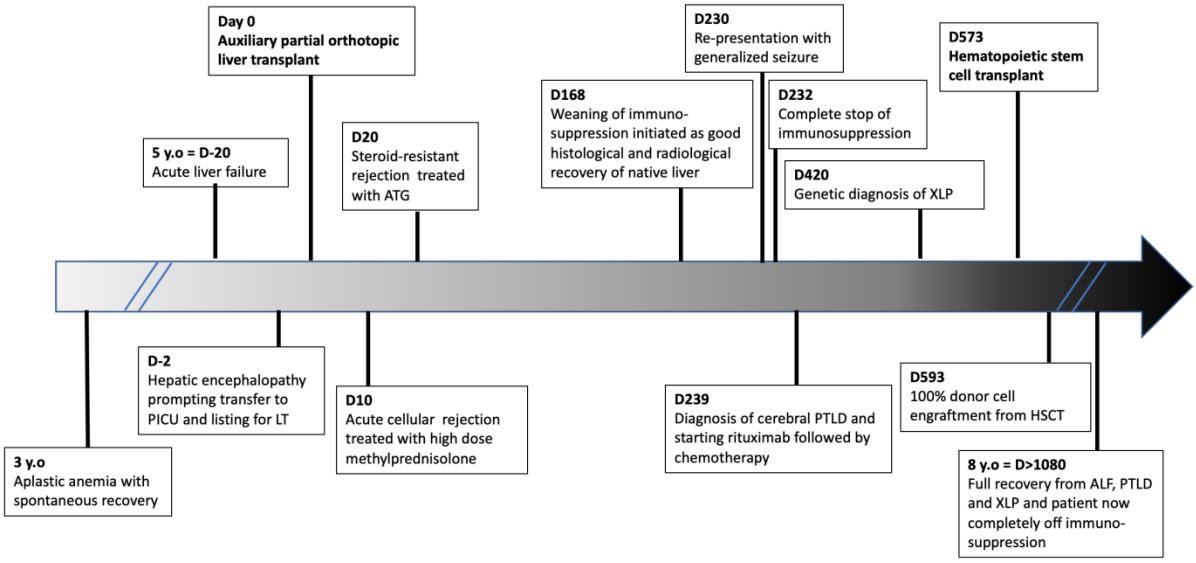

Figure 3: Timeline of events according to age and days after liver transplant

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