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**Management of patients with EPP related progressive liver disease**

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**Kew words**

1. Protoporphyrin IX
2. XLP
3. Red cell exchange
4. Liver transplantation
5. Allogeneic stem cell transplant



Footnote page

| **Abbreviation** | **Definition** |
|---|---|

| | |
|---|---|
| ALAS2 | 5-aminolevulinic acid synthase 2 |
| AlloSCT | Allogeneic stem cell transplantation |
| ALP | Alkaline phosphatase |
| AST | Aspartate aminotransferase |
| EPP | Erythropoietic protoporphyria |
| Erc-PPIX | Erythrocyte protoporphyrin |
| FECH | Ferrochelatase |
| GVHD | Graft versus host disease |
| Hb | Hemoglobin |
| IDA | Iron deficiency anemia |
| INR | International normalised ratio |
| LFT | Liver function test |
| MELD | Model for End Stage Liver Disease |
| MUD | Matched unrelated donor |
| OLT | Orthotopic liver transplant |
| P-PPIX | Plasma Protoporphyrin |
| PBSCT | Peripheral blood stem cell transplant |
| PPIX | Protoporphyrin IX |
| RBC | Red blood cell |
| RBCs | Red blood cells |
| RCE | Red cell exchange |
| XLP | X-Linked EPP |

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**Abstract**

Erythropoietic protoporphyria (EPP) is an inherited metabolic disorder of heme synthesis resulting from overproduction of protoporphyrin IX (PPIX) which can lead to progressive liver disease characterized by recurrent EPP crisis and end stage liver disease. We utilized the Australian Transplant Registry to identify five patients referred for liver transplantation between 2008 and 2017. Four patients had EPP secondary to ferrochelatase (FECH) deficiency and one had X-Linked EPP (XLP). No patient had specialist follow-up prior to the diagnosis of progressive liver disease. Three patients underwent orthotopic liver transplant (OLT), while two died while on the transplant waiting list. Parenteral PPIX-lowering therapy was utilized in four patients and was effective in three patients although two of these had rebound porphyria and worsening liver function following decreasing the intensity of therapy. Early disease recurrence in the allograft following transplantation occurred in two patients requiring red cell exchange (RCE) to successfully attain and maintain low PPIX levels, but RCE was associated with hemosiderosis in one case. Allogeneic stem cell transplantation (AlloSCT) was performed in two patients. One failed engraftment twice while the second rejected their first graft but achieved full donor chimerism with a second graft and increased immunosuppression. Conclusion: Our observations suggest that progressive liver disease needs parenteral PPIX-lowering treatment with

the intensity adjusted to achieve a target Erc-PPIX level. Since recurrent EPP liver disease is the rule, AlloSCT should be considered in all patients with adequate immunosuppression to facilitate engraftment. RCE appears effective for recurrent EPP liver disease but is associated with an increased risk of iron overload.

## Introduction

Erythropoietic protoporphyria (EPP) is an inherited metabolic disorder of heme synthesis resulting in overproduction of protoporphyrin IX (PPIX) which accumulates in erythrocytes, skin and liver. It is most commonly an autosomal recessive disorder, characterized by reduced production of ferrochelatase (FECH), required to convert protoporphyrin to heme.(1, 2) Less commonly, EPP may result from an X-linked gain of function mutation in the 5-aminolevulinic acid synthase 2 (ALAS2) gene, required for the synthesis of 5-ALA, the rate limiting enzyme in heme synthesis;(3) this is known as X-Linked Protoporphyria(XLP).

Excess PPIX production occurs primarily in the bone marrow and diffuses across red blood cell (RBC) membranes into the plasma, where it is albumin bound and taken up by the liver for secretion into bile. Liver damage, characterised by cholestasis, inflammation, fibrosis and cirrhosis,(4) has been attributed to precipitation of toxic insoluble PPIX in bile canaliculi and to protoporphyrin-induced oxidative damage to hepatic membrane-bound enzymes.(1, 5)

Progressive liver disease manifests in only 1-5% of patients with EPP and impaired hepatobiliary clearance can result in further increases in erythrocyte protoporphyrin (Erc-PPIX) leading to rapid progression of liver disease.(6) Rising Erc-PPIX levels can also precipitate EPP crises in patients with pre-existing liver disease, characterized by acute deterioration in liver function, severe abdominal pain, jaundice, and worsening photosensitivity.(7)

Various treatment strategies are used for the management of EPP related liver disease, although evidence for their efficacy is limited. (10-13). (Table 1)

Orthotopic liver transplantation (OLT) is the only effective treatment for EPP-related end stage liver disease.(6, 8, 9) However, OLT does not correct defective heme synthesis, so disease recurrence in the allograft is common and ongoing PPIX-lowering therapy is required in the post-transplant setting. The only definitive cure for EPP remains an AlloSCT to correct the defect in heme synthesis.(8, 10)

We report five cases of EPP-related progressive liver disease referred for OLT in Australia between 2008-2017.

## METHODS AND RESULTS

Five patients referred with EPP-related progressive liver disease for OLT between the years 2008 and 2017 were identified using the Australian Liver Transplant Registry which reported 2276 liver transplants over this time period. Tables 2 and 3 summarise the key features of the five cases.

### CASE 1

A 41-year-old female was referred for OLT with EPP-crisis. EPP, secondary to FECH deficiency, was diagnosed at the age of 22 years and managed with strict sun avoidance and beta-carotene. Liver biopsy three years prior to referral confirmed EPP-related liver disease with minimal periportal fibrosis.

EPP crisis manifested with jaundice, abdominal pain and vomiting with an aspartate aminotransferase (AST) 248 IU/L (reference range <31 IU/L), an alkaline phosphatase (ALP) of 167 IU/L (reference range 30-120 IU/L), a bilirubin of 68 μmol/L (reference range 0-20 μmol/L) and an international normalised ratio (INR) of 1.2. Erc-PPIX was 149 μmol/L (8371 μg/dL) (reference range <1.8 μmol/L; <101 μg/dL). Liver biopsy confirmed cirrhosis with abundant protoporphyrin pigment consistent with EPP-related cirrhosis. PPIX-lowering treatment was instituted with daily infusions of heme arginate 3mg/kg for three days and 500 mg of ursodeoxycholic acid twice daily. Whilst symptoms resolved and bilirubin decreased to 38 μmol/L there was no change in Erc-PPIX.

Two weeks later there was a recurrence of EPP crisis in the setting of an elevated Erc-PPIX of 331 μmol/L (18596 μg/dL). PPIX-lowering treatments were reinstituted with heme arginate, plasma exchange and RBC hypertransfusions (Hemoglobin (Hb) target of ≥ 120 g/L). The Erc-PPIX reduced to 120 μmol/L by week 4 of admission and plasma exchange was performed weekly thereafter. A month later, she developed progressive neuropathy in the context of rising Erc-PPIX (401 μmol/L or 22528 μg/dL) and a dropping Hb (90 g/L). Daily plasma exchange and heme arginate were recommenced. Despite Erc-PPIX levels falling to 105 μmol/L (5899 μg/dL), neuropathy worsened and she developed type 2 respiratory failure and hepatorenal syndrome leading to death.

**Case 2**

A 20-year-old female was referred for OLT with complications of end stage liver disease. EPP, secondary to FECH deficiency, was diagnosed at the age of four years and managed with strict sun avoidance and beta-carotene. Liver involvement was noted two years prior to referral on liver biopsy with features of cholestasis and bridging fibrosis for which cholestyramine was commenced. She was admitted with decompensated cirrhosis with a Model for End Stage Liver Disease (MELD) score of 24 and an Erc-PPIX of 172 μmol/L (9663 μg/dL). Heme arginate 3mg/kg four days per week for two weeks failed to produce clinical improvement despite a falling Erc-PPIX level to 105 μmol/L (5899 μg/dL). While on the transplant waiting list, she developed multi-organ failure and sepsis leading to her death.

**Case 3**

A 25-year-old female was referred to the liver transplant and hematology units for consideration of sequential OLT and AlloSCT for EPP-related end stage liver disease. X-Linked Protoporphyria (XLP) was diagnosed at the age of 18 months and managed with strict sun avoidance and beta-carotene. At the age of 15 years she developed recurrent episodes of EPP crisis. A year later, decompensated cirrhosis due to XLP was confirmed on liver biopsy. At the time Erc-PPIX was 180 μmol/L (10112 μg/dL) and she had iron deficiency anemia (IDA) with a Hb of 95 g/L and a serum ferritin of 13 μg/L. PPIX-lowering treatment included 7 days of IV hematin (3mg/kg), ursodeoxycholic acid 250 mg three times daily (12mg/kg/d) and oral iron replacement. OLT was considered, but refused by the patient. Liver function tests (LFTs) remained normal for 2.5 years on this treatment and the mean Erc-PPIX was

19.7 µmol/L (1107 µg/dL). At the age of 20, splenic embolization was performed for hypersplenism associated with severe neutropenia, recurrent infections and thrombocytopenia. For the following 5 years she remained stable with a mean Erc-PPIX of 11 µmol/L (618 µg/dL) on ursodeoxycholic acid.

At the age of 25, she developed end stage liver disease. Erc-PPIX was 210 µmol/L (11798 µg/dL) with concomitant IDA. She was worked up for sequential OLT and AlloSCT. While awaiting liver transplantation she had two admissions for EPP crisis in the setting of rising Erc-PPIX with a mean of 74 µmol/L (4157 µg/dL) and iron deficiency. Both crises were managed with hematin and plasmapheresis. The second crisis was followed by eight infusions of iron fortnightly, and subsequently daily 15ml of liquid iron (30 mg/ml) until OLT four months later when Erc-PPIX was 16 µmol/L (899 µg/dL). (Figure 1).

OLT was performed with the use of light filters omitting wavelength <420nm. The explant showed cirrhosis with birefringent protoporphyrin pigment and moderate hepatocyte iron accumulation (grade 2 siderosis). The early post-transplant course was complicated by acute cellular rejection and an anastomotic biliary stricture requiring stenting. On day 20, a liver biopsy performed for ongoing derangement in LFTs and rising Erc-PPIX (55 µmol/L or 3090 µg/dL), demonstrated recurrent EPP liver disease, despite being on ursodeoxycholic acid 750mg bd and cholestyramine 4gm twice daily post-operatively. This failed to respond to five days of hematin, intravenous iron, RBC hypertransfusion and a total of 13 sessions of plasma exchange over 3 weeks, but responded rapidly to red cell exchange, initially twice weekly then weekly for nearly 7 months successfully attaining and maintaining Erc-PPIX of <5 µmol/L (<280 µg/dL). Subsequently, the frequency of red cell exchange was reduced to monthly.

Two AlloSCTs from unrelated donors were performed. The first occurred four months post-OLT using marrow from a 38-year-old, nulliparous female, 10/10 HLA-matched unrelated donor (MUD). Conditioning was with cyclophosphamide (50mg/kg/day -5 to -2) and anti-thymocyte globulin 30mg/kg/day (days -5 to -3). Graft versus host disease (GVHD) prophylaxis was with tacrolimus and mycophenolate. Engraftment failure was documented by day 22 and 28 chimerism studies of 10% and 0% donor total mononuclear cells, respectively. A second AlloSCT was performed 4 months later using a different donor; a 44-year-old male, 10/10-HLA matched MUD, with no donor HLA antibodies and with more immunosuppressive conditioning regimen consisting of fludarabine 25mg/m2/day (day -6 to -2), cyclophosphamide 500mg/m2/day (day -6 to -3), Alemtuzumab 20 mg/day (Days -5 to -1), and similar GVHD prophylaxis. Despite these measures the donor failed to engraft.

Eight years post OLT, the patient remains on ursodeoxycholic acid 750mg bd, cholestyramine 4gm bd and regular red cell exchange every 4-5 weeks with a mean Erc-PPIX of 5 µmol/L (280µg/dL), normal LFTs, a mean serum ferritin of 312 µg/L and transferrin saturation of 35% without any iron chelating agents.

**CASE 4**

A 21-year-old female was referred for OLT with EPP crisis. She was diagnosed with EPP, secondary to FECH deficiency, at the age of 2 years managed with strict sun avoidance. Blood test two years prior to referral, showed normal liver function but an Erc-PPIX of 64 µmol/L (3596 µg/dL). A liver biopsy confirmed EPP-related cirrhosis with severe cholestasis and protoporphyrin pigment. MELD score was 20, Erc-PPIX was 157µmol/L (8820 µg/dL) and Hb was 95 g/L with a serum ferritin of 14 µg/L. PPIX-lowering therapy was commenced, consisting of cholestyramine 8 gm twice daily, ursodeoxycholic 15 mg/kg/day, RBC hypertransfusion, 3mg/kg/d of heme arginate infusion and four days of daily plasmapheresis, reducing the Erc-PPIX to 87 µmol/L (4888 µg/dL). In the setting of line-related sepsis, no further parenteral PPIX-lowering treatment was given. She was readmitted six weeks later with EPP crisis with an Erc-PPIX of 142 µmol/L (7978 µg/dL). PPIX-lowering therapy was re-instituted with heme arginate, plasma exchange and RBC hypertransfusion. This resulted in reduction of Erc-PPIX to 42 µmol/L (2360 µg/dL). Upon discharge, heme arginate and plasma exchange were maintained at once a week up until OLT two months later when Erc-PPIX was 101 µmol/L (5674 µg/dL). (Figure 2)

At liver transplantation, yellow filters, omitting wavelengths <470 nm, were placed in theatre. A single plasma exchange was performed on day 3. She was discharged with an Erc-PPIX of 59 µmol/L (3314 µg/dL) on ursodeoxycholic acid 500 mg twice daily and ferrous sulphate (105 mg of elemental iron) daily. Cotrimoxazole (160/800 mg) three times a week was used for pneumocystis jiroveci pneumonia (PJP) prophylaxis for 6 months. A biliary anastomotic stricture was diagnosed, dilated and stented at day 39 post-OLT. On day 60 post-OLT, a liver biopsy, performed for abnormal LFTs and a rising Erc-PPIX (118 µmol/L or 6629 µg/dL), confirmed recurrent EPP liver disease. Reinstitution of plasma exchange and heme arginate was ineffective. Consequently, plasma exchange was replaced with weekly red cell exchange, using erythrocytapheresis. This involved removing 6 units of red blood cells (RBCs) from the patient and transfusing equivalent units of matched RBCs. This successfully reduced Erc-PPIX to 36 µmol/L (2023 µg/dL) over 6 weeks. Red cell exchange was gradually reduced to third weekly maintaining a mean Erc-PPIX of 79 µmol/L (4438 µg/dL). Approximately 1 year after EPP recurrence, a liver biopsy revealed cirrhosis with minimal protoporphyrin pigment and significant hepatocyte iron accumulation (grade 3 siderosis); serum ferritin was 1600 µg/L with transferrin saturation of 86%. Haemochromatosis gene testing showed heterozygosity for the C282Y mutation. Ferrous sulphate was stopped and iron chelation with deferasirox commenced at a dose of 500 mg daily (7.5mg/kg/day) but discontinued a month later due to intolerance. Third weekly red cell exchange for four years maintained a mean Erc-PPIX of 63 µmol/L (3539 µg/dL), a mean ALT of 45 IU/L, and a normal bilirubin. Despite this, decompensated cirrhosis developed. A repeat liver biopsy 15 months post-OLT showed cirrhosis with minimal protoporphyrin pigment and grade 4 hemosiderosis with deposition of hemosiderin in hepatocytes, portal stroma and bile duct epithelium. The mean serum ferritin over the preceding 4 years was 2174 µg/L and mean transferrin saturation was 90%. Deferasirox was recommenced at a small tolerated dose of 125 mg bd and the patient was worked up for sequential OLT and a peripheral blood stem cell transplant (PBSCT). (Figure 3)

Due to massive splenomegaly, a splenectomy was performed at the time of second OLT with the intention to improve stem cell engraftment. While awaiting PBSCT, PPIX-lowering measures included cholestyramine, ursodeoxycholic acid and red cell exchange (thrice weekly initially then twice weekly) along with 20mg/kg/d of deferasirox, maintaining Erc-PPIX levels around 25 μmol/L (1405 μg/dL). Pentamidine was used instead of cotrimoxazole for PJP prophylaxis. The first PBSCT, five months post-OLT, was from a 29-year-old female HLA-MUD, matched at HLA-A, B, C, -DRB1, DRB4/5 and DQB1 and mismatched at 2 HLA-DPB1 alleles. Conditioning regimen was fludarabine 25mg/m2 for 5 days, cyclophosphamide (60mg/kg for 2 days)) and thymoglobulin (4.5mg/kg in total over 3 days), and GVHD prophylaxis with cyclosporine and methotrexate (day 1,3,6 and 11). It resulted in partial donor chimerism (donor CD3 <30%) with eventual graft loss by day 100, concomitant with a mild rise in Erc-PPIX to a mean of 33 μmol/L (1854 μg/dL). The allograft was complicated at day 56 by an EBV-positive posttransplant lymphoproliferative disease presenting with a transverse myelitis-like sensory neuropathy and intensely PET avid tonsillar, cervical and paratracheal nodes with EBV DNA viremia (peaking at 2645 copies/ml of plasma). This responded to a single 375mg/m$^2$ dose of rituximab and withdrawal of immunosuppression with resolution of viremia, lymphadenopathy and steady improvement of sensory neuropathy.

A second PBSCT was performed six months after the first, with an unchanged fludarabine schedule but replacement of cyclophosphamide with melphalan 100mg/m$^2$ followed by infusion of cryopreserved peripheral blood stem cells from original donor. The increased conditioning intensity and omission of T-cell depletion were due to previous concerns of early graft rejection.(11, 12) Successful donor chimerism were confirmed at day 20+ and maintained at 1year post second allograft without active GVHD while on immunosuppression regimen of tacrolimus and mycophenolate substituting for cyclosporine due to intolerance. Normal Erc-PPIX of 0.6 μmol/L (34 μg/dL) was also achieved at day 20+ post second allograft and maintained, off all invasive PPIX-lowering measures, at the latest follow-up at 1 year, with only minimally abnormal LFTs without any veno-occlusive disease and able to tolerate sunlight without cutaneous photosensitivity.

**CASE 5**

A 64-year-old man with EPP, secondary to FECH deficiency; diagnosed at the age of 39 years and managed with strict sun avoidance, was referred for OLT with a 1-year history of end stage liver disease. This occurred on a background of known EPP-related cirrhosis diagnosed 12 years previously on no PPIX-lowering treatment.

At the time of the referral, he had a MELD of 23 with a bilirubin of 467 µmol/L, and an Erc-PPIX of 210 µmol/L (11798 µg/dL). He was considered for liver transplantation but spontaneously recovered with normalisation of his bilirubin and INR. Six months later, he developed worsening ascites and jaundice with a bilirubin of 530 µmol/L, an INR of 1.8 and creatinine of 140 µmol/L yielding a MELD of 30. This developed five days following an iron infusion for IDA with a Hb of 117g/L, and a serum ferritin of 11µg/L. Erc-PPIX was 100 µmol/L (5618 µg/dL). He was worked up for OLT and responded to supportive measures but no PPIX-lowering treatment was initiated until OLT three weeks later at which time his Erc-PPIX was 150 µmol/L (8427 µg/dL). At transplantation flexible yellow filters, omitting wavelengths <470 nm, were placed in theatre, and plasma exchange was performed perioperatively. Explant liver had features consistent with EPP-cirrhosis, severe cholestasis and grade I siderosis, with iron deposits in the hepatocytes.

Post OLT, plasma exchange was performed day 1 and 3, and heme arginate at a dose of 3mg/kg was infused daily for 4 days. Erc-PPIX was 75 µmol/L (4214 µg/dL) upon discharge 3 weeks later. Subsequently, cholestyramine and ursodeoxycholic acid were used as the only PPIX-lowering treatment. At 2 years post OLT, he remains well with normal LFTs and no evidence of recurrent EPP liver disease with a mean Erc-PPIX of 73 µmol/L (4101 µg/dL).

## DISCUSSION

EPP-related progressive liver disease is rare. Therefore, there is much variation in management across centres as demonstrated in our case series.

We present five cases with EPP progressive liver disease and focus on lessons learned in each case combined with a literature review to recommend some strategies to help better manage this unique group of patients.

As up to 5% of patients with EPP develop progressive irreversible liver disease, it is important to be able to identify patients with early liver disease at risk of progression so they can be closely monitored and hepatoprotective therapy initiated. Whilst there are no predictors for the development of progressive liver disease, mild LFT derangement, with values up to three-fold the upper limit of normal, is an abnormality suggestive of liver involvement with greater elevation correlating with more severe liver injury. (13-15) However, LFTs may be normal in patients with EPP-related fibrosis and cholestasis. (13, 15-17) Since PPIX excretion decreases with progressive liver injury with a consequent rise in Erc-PPIX, measuring Erc-PPIX may identify patients with early liver involvement at risk of progression. A 20-year study on hepatobiliary complications of EPP found that those with EPP liver disease had higher Erc-PPIX levels than those without liver involvement( $38 \pm 8$ μmol/L vs $12 \pm 3$ μmol/L ) or (1573 ± 449 μg/dL vs 674 ± 169 μg/dL), and that Erc-PPIX levels of >20μmol/L (1124 μg/dL) were associated with severe intrahepatic cholestasis and levels >27μmol/L (1517 μg/dL) were associated with cirrhosis.(5) An increase in urinary coproporphyrin excretion to >0.2 μmol/24 hours (normal <0.1 μmol/24hours) and an isomer I/III ratio of up to 40-80%(normal <31%) has also been considered sensitive and specific for liver involvement and cholestasis.(5) In our cohort, one patient (case 3) had developed transient transaminitis at childhood and another (case 4) had normal LFTs, but an Erc-PPIX of 64 μmol/L (3596 μg/dL) two years prior. In the absence of any published guidelines on monitoring patients with EPP for liver involvement, we recommend routine LFTs, Erc-PPIX and 24-hour urine for coproporphyrin excretion and isomer I/III ratio annually or when photosensitivity worsens in all patients with EPP and to proceed to a liver biopsy when there is any degree of LFT derangement, an Erc-PPIX level of > 20 μmol/L (1124 μg/dL) or an increase of urinary coproporphyrin to >0.2 μmol/24 hours with an isomer I/III ratio of >40%. In those whose Erc-PPIX and urinary coproporphyrin levels are above the aforementioned values and in those with deranged LFTs and evidence of PPIX-associated portal intrahepatic cholestasis or fibrosis in the liver biopsy, we recommend PPIX-lowering treatment and close monitoring. (Figure 4)

There are limited data on the efficacy of PPIX-lowering treatment in halting or slowing the progression of EPP liver disease. Oral medications which increase PPIX excretion, such as cholestyramine and ursodeoxycholic acid, have been utilised. There are three case reports supporting the efficacy of cholestyramine at a dose of 12 g daily in normalizing LFTs in those with early liver disease and, in two of the reports, reducing Erc-PPIX levels and improving liver fibrosis a year after therapy. (18-20) Similarly, two case reports supported the efficacy of 15 mg/kg/d of ursodeoxycholic acid in improving LFTs and slowing fibrosis progression. (13, 21) In our cohort, cases 1 and 2 were diagnosed with hepatic EPP with periportal and bridging fibrosis respectively but only the latter was started on cholestyramine. We recommend using both cholestyramine,12g daily, and ursodeoxycholic 10-15mg/kg/d as PPIX-lowering treatment in patients with early EPP liver disease, with close monitoring of LFTs and Erc-PPIX, aiming to normalise LFTS and to reduce Erc-PPIX to <20 μmol/L (1124 μg/dL). We recommend escalating treatment to parenteral PPIX-lowering treatment if this strategy fails. (Figure 4)

In patients presenting with progressive liver disease, manifesting with cirrhosis, and/or recurrent EPP crisis, ursodeoxycholic acid and cholestyramine should be started, but parenteral therapy to rapidly and profoundly reduce PPIX levels is needed along with referral for OLT and AlloSCT. This can prevent further liver damage and is essential in preventing perioperative complications of transplantation and early post-OLT EPP recurrence.(5, 8, 22, 23) Parenteral therapy includes measures to inhibit PPIX production such as heme (hematin) infusion, RBC transfusion and/or RBC hyper transfusion combined with measures to remove circulating PPIX with red cell exchange  or plasma exchange.  There is no consensus on when plasma exchange should be used over red cell exchange. Since hepatic uptake of PPIX is mainly from the plasma compartment, plasma exchange offers the most rapid reduction of plasma protoporphyrin (P-PPIX) accessible by the liver and would be theoretically ideal for EPP crises. Conversely, red cell exchange causes a more substantial reduction in total PPIX, reducing both Erc-PPIX and P-PPIX, making it theoretically more effective at durably lowering and maintaining lower total PPIX levels.(24) The literature suggests that the frequency at which combination therapy is administered is as important as the treatment type.(25, 26) A recurring reported phenomenon, also observed in cases 1 and 4, is the rapid rebound rise in PPIX levels associated with episodes of crisis, axonal neuropathy and rapid deterioration of liver disease when the frequency of parenteral treatment is reduced following clinical and biochemical improvement. This can be fatal despite reinstituting PPIX-lowering measures. (22, 25) Hence, aiming for a target Erc-PPIX and adjusting the frequency of combination therapy accordingly may be a rational approach. There is currently no accepted target Erc-PPIX level to aim for, but maintaining Erc-PPIX level below the 20 μmol/L (1124 μg/dL)-cut-off associated with liver involvement-seems reasonable but may prove difficult to achieve in established cirrhosis due to impaired PPIX excretion. Crises have been reported to occur with Erc-PPIX levels of ≥82μmol/L (4607 μg/dL). (8) and neuropathy has been reported to occur with levels as low as 41 μmol/L (2304 μg/dL),(27) although the majority of cases have been reported with higher levels.(9) Therefore, we recommend reducing Erc-PPIX levels in those with progressive liver disease to < 40 μmol/L (2247 μg/dL), preferably to <20 μmol/L (1124 μg/dL), and to regularly monitor Erc-PPIX levels on a weekly basis allowing early adjustment of parenteral treatment to a frequency which maintains these levels. (Figure 4)

Iron deficiency and IDA are common in patients with EPP.(28, 29) In patients with X-linked protoporphyria(XLP) with iron deficiency, there is sufficient evidence that iron repletion improves photosensitivity, reduces Erc-PPIX and even liver damage;(29, 30) We therefore, recommend that all patients with XLP should have iron studies done regularly to ensure iron stores remain replete. In EPP secondary to FECH deficiency with concomitant iron deficiency, the benefits of iron supplementation are less. In a few case reports, it reduces PPIX and photosensitivity. (13, 31) However, in most published reports, iron supplementation has been associated with increasing PPIX levels, worsening photosensitivity and liver function. (29, 31, 32) Iron deficiency may in fact be protective in these patients, as aminolevulinic acid synthase 2 (ALAS2) expression is positively regulated by iron.(28), The increased ALAS2 function following iron administration explains the increase in PPIX levels. In our fifth case, iron infusion, given for a serum ferritin of 11 µg/L and a Hb of 119g/L was associated with a rapid deterioration of liver function, expediting OLT. We therefore think it prudent to follow the suggestion that iron supplementation in EPP due to FECH deficiency should be reserved to those with symptomatic IDA with Hb of <100 g/L at small doses with close monitoring of PPIX.(30)

Post liver transplantation, recurrent EPP liver disease has been reported in 65%-80% of patients.(8, 9, 26) There are limited published data on which PPIX-lowering treatment can effectively prevent or delay recurrent liver disease. Cholestyramine and ursodeoxycholic acid have been used with variable success. (6, 26) In our cohort, early recurrent EPP liver disease was diagnosed in two of three patients despite ursodeoxycholic acid in both and cholestyramine in one. Recurrent EPP liver disease usually requires parenteral PPIX-lowering treatment. RBC hypertransfusion, hematin infusion and plasmapheresis have been used with variable success. (8, 33), Red cell exchange has been successfully used but its long-term use is associated with risk of iron overload. (26) In our cohort, red cell exchange was successfully used for recurrence in two cases (3 and 4). Iron overload occurred in one of these and potentially contributed to the progression of liver disease as evident by the high serum ferritin and high grade siderosis that can't be explained by EPP or cirrhosis in the absence of iron overload. (34, 35) Hemosiderosis post long-term red cell exchange is reported(36) despite adjustments to achieve minimal iron loading by modulating the post-exchange haematocrit; when performed monthly, there is still a net iron load of 0.05mg/kg/day (37), that needs at least 20mg/kg/day of deferasirox to prevent iron overload.(36) , Factors predisposing to iron overload in our case include third weekly exchange, concomitant oral iron supplementation, inability to tolerate adequate doses of deferasirox and possibly C282Y HFE gene heterozygosity state. Of note, even before cirrhosis was diagnosed, frequent red cell exchange was only able to achieve Erc-PPIX levels of 60-80 µmol/L (3371-4494 µg/dL); it is conceivable that increased iron load was part of a vicious cycle by increasing expression of ALAS2, increasing the PPIX and leading to the need for more frequent red cell exchange. Since recurrent EPP liver disease is the rule, and PPIX-lowering treatments are invasive with variable efficacy with potential complications, AlloSCT early after OLT, to cure the defect in heme synthesis should be the goal. (9, 26, 38) Until AlloSCT is carried out, some form of PPIX-lowering treatment is needed, with treatment choice, dose and frequency adjusted to achieved low Erc-PPIX. We suggest aiming for levels <20µmol/L (1124 µg/dL), as higher levels are associated with intrahepatic cholestasis and progressive liver disease in native livers, if regular red cell exchange is needed, serum ferritin levels should be monitored and, if rising, oral iron chelation should be considered. (Figure 4)

The results of only a handful of AlloSCT for EPP have been published, 2 in the pediatric population and 2 in the adult population (2, 38-41). In the pediatric literature, there are two reported cases of sequential OLT and AlloSCT. The first, from an HLA-matched sister, resulted in graft rejection but using a more aggressive myeloablative conditioning regimen in the second attempt resulted in successful engraftment.(2) The latter achieved engraftment using a myeloablative conditioning regimen but was complicated with delayed immune recovery and fatal disseminated varicella zoster attributed to T-cell depletion.(38)

In the adult population, two cases have been described in detail, both from matched-unrelated donors. The first was complicated with graft failure overcome in the second attempt with more aggressive myeloablation. (41) The second, which was a sequential OLT and AlloSCT, was successful using a reduced-intensity conditioning regimen consisting of fludarabine, busulfan and total body irradiation without T cell depletion.(40) In our cases, both allograft attempts resulted in initial graft failure which was overcome successfully in one by more aggressive conditioning with melphalan and omission of T cell depletion; in the other the addition of fludarabine was unsuccessful. These observations are consistent with the emerging experience in AlloSCT for chemotherapy naïve patients with non-malignant disease which suggests that aggressive myelo-lymphoablative conditioning may be needed to facilitate donor engraftment. Limited experience in EPP patients suggest that, as in some allograft series for leukaemia, profound depletion of donor T cells may increase the risk of engraftment failure.(11, 12)

In conclusion, our observations and literature review suggest that patients with EPP with Erc-PPIX levels >20 μmol/L (>1124 μg/dL), increase in urinary coproporphyrin excretion and/or with deranged LFTS should undergo a liver biopsy and considered for oral PPIX-lowering medications as cholestyramine and ursodeoxycholic acid with an aim to normalise LFTS and reduce Erc-PPIX to less than 20 μmol/L (1124 μg/dL). Iron deficiency should be treated in all XLP patients, but only used in EPP FECH deficient patients if it causes severe anaemia. Those with more serious liver disease, should have parenteral PPIX-lowering therapy and be considered for sequential OLT and AlloSCT. Following aggressive PPIX-lowering, ongoing maintenance therapy is required to maintain low levels of Erc-PPIX and prevent recurrence post-OLT. Regular red cell exchange appears to be effective in maintaining low PPIX levels but may result in iron overload without other interventions such as iron chelation. The definitive cure remains an AlloSCT.

SUMMARY

As up to 5% of patients with EPP develop progressive irreversible liver disease, it is critical to have a clinical strategy for managing this unique group of patients. The EPP management algorithm presented in figure 4 offers a practical approach to the management of EPP related liver disease. Cutaneous manifestations of EPP, characterised by immediate painful photosensitivity occurring in infancy or early childhood, always precedes hepatic manifestations. (42) The biochemical diagnosis of EPP is established by the detection of significantly elevated total Erc-PPIX levels. It is important to differentiate EPP secondary to FECH deficiency from XLP as their management differs in several aspects. EPP secondary to FECH deficiency is suggested by decreased FECH levels to < 50% of normal and metal-free protoporphyrin accounting for (85%–100%) total Erc-PPIX, and confirmed by identification of biallelic mutations by sequencing the FECH gene.(43, 44) X-Linked protoporphyria is suggested by normal FECH levels and a lower fraction of metal-free protoporphyrin (50%–85% of the total Erc-PPIX), and confirmed by ALAS2 sequencing. (45, 46) All patients with EPP should be assessed for overt liver involvement. In those with no overt liver involvement, LFTs, Erc-PPIX and 24-hour urine for coproporphyrin excretion and isomer I/III ratio should be done annually or whenever photosensitivity worsens. When Erc-PPIX levels >20 μmol/L, there is an increase in urinary coproporphyrin excretion and/or LFTS become deranged, a liver biopsy is needed and patients should be considered for oral PPIX-lowering medications as cholestyramine and ursodeoxycholic acid with an aim to normalize LFTS and reduce Erc-PPIX to less than 20 μmol/L (1124 μg/dL). Iron deficiency should be treated in all XLP patients, but only used in EPP FECH deficient patients if it causes severe anemia. Features of overt liver involvement are not specific and include mild to moderate liver enzymes elevation, jaundice, recurrent episodes of EPP crisis, cirrhosis and end stage liver disease. Other common liver diseases need to be ruled out with the appropriate blood tests and imaging modalities. A liver biopsy is essential to confirm EPP related liver disease and to stage fibrosis. Those with early EPP liver disease should initially be managed with oral PPIX-lowering treatment as detailed above. Patients failing oral PPIX-lowering treatment and patients with more serious liver disease should have parenteral PPIX-lowering therapy and be considered for sequential OLT and AlloSCT. Following aggressive PPIX-lowering, ongoing maintenance therapy is required and adjusted to maintain low levels of Erc-PPIX aiming for a target of <40 μmol/L (2248 μg/dL) or preferably of <20 μmol/L (1124 μg/dL). Post-OLT, PPIX-lowering treatment should be resumed aiming for a target Erc-PPIX of <20 μmol/L (1124 μg/dL). Regular red cell exchange appears to be effective in maintaining low PPIX levels but may result in iron overload without other interventions such as iron chelation. The definitive cure remains an AlloSCT. Myelo-lymphoablative conditioning and omitting T-cell depletion may be needed to facilitate donor engraftment.

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| Table 1: Protoporphyrin IX (PPIX) -Lowering Therapies | |
|---|---|
| Mechanism | Example |
| Increasing PPIX* excretion | Ursodeoxycholic acid<br>Cholestyramine |
| Suppressing erythropoiesis and PPIX production | Iron<br>Hematin or heme arginate infusions<br>Red cell hypertransfusion (target Hb >120g/l) |
| Removing circulating PPIX | Plasmapheresis<br>Plasma exchange<br>Red cell exchange |
| PPIX: Protoporphyrin IX<br>Hb: Hemoglobin | |

**Table 2. A summary of the five cases with EPP-progressive liver disease referred for consideration of Orthotopic liver transplantation (OLT)**

| | Age at EPP liver disease (years) | Genetic testing | Available Erc-PPIX levels Y/N (value) | PPIX-lowering treatment before cirrhosis or EPP crisis Y/N (medication) | Age at EPP crisis or cirrhosis | Erc-PPIX μmol/L (μg/dL) at EPP crisis or Cirrhosis | Serum Ferritin (μg/L) at EPP crisis or cirrhosis | Age at OLT referral | Pre-OLT PPIX-lowering treatment | OLT Y/N (cause if no OLT) |
|---|---|---|---|---|---|---|---|---|---|---|
| Case 1 | 38 | NA | N | N | 41 | 149 (8371 μg/dL) | 57 | 41 | Ursodeoxycholic acid<br>Heme arginate<br>RBC hypertransfusion<br>Plasma exchange | N (Died from ESLD and sensorimotor neuropathy on the OLT list) |
| Case 2 | 18 | NA | N | Y Cholestyramine | 20 | 172 (9663 μg/dL) | NA | 20 | Heme arginate<br>plasmapheresis | N (Died from ESLD and HRS-1[+] while on the waiting list) |
| Case 3 (XLP) | 1.5* | XLP mutation (exon 11) | N | N | 15 | 180 (10112 μg/dL) | 13 | 15 And 25** | A) Initial referral<br>Ursodeoxycholic acid<br>Oral iron<br>B) Second referral<br>Plasmapheresis, IV hematin, IV iron and oral iron | Y |
| Case 4 | 19 | NA | Y (65 μmol/L) | N | 21 | 157 (8820 μg/dL) | 14 | 21 | Ursodeoxycholic acid<br>Cholestyramine<br>Heme arginate<br>RBC hypertransfusion | Y |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Plasma exchange | |
| Case 5 | 52 | Heterozygous c.616+1G>T c.617-3C>T variants in the FECH gene | N | N | 52 | 210[#] (11798 µg/dL) | 11 | 64 | None | Y |

+ HRS: Hepatorenal syndrome

*Transient transaminitis noticed at 1.5 years. No regular Liver function tests from then till EPP crisis and cirrhosis.

** Initial referral at the age of 15, but liver transplantation was refused by the patient. Second referral at the age of 25

**#** This Erc-PPIX level is at the time of ESLD and not the diagnosis of cirrhosis 12 years prior.

Reference ranges: Erc-PPIX <1.8 µmol/L.  Serum ferritin 20-150 µg/L.

**Table 3. A summary of the three cases that had successful orthotopic liver transplantation (OLT)**

| | Post OLT preventative PPIX-lowering treatment Y/N (medications) | Recurrent EPP liver disease Y/N (Days post OLT | Erc-PPIX (µmol/L) at recurrence | Effective PPIX-lowering treatment | Mean Erc-PPIX µmol/L (µg/dL) | Mean serum ferritin µg/L | Iron chelating agent Y/N (Medication and dose) | Second OLT | AlloSCT Y/N (No.) | Type of AlloSCT | Successful grafting | Need for PPIX lowering treatment Y/N (medication |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Case 3 | Ursodeoxycholic acid Cholestyramine | Y(20) | 55 | RCE+ | 5 (280µg/dL) | 312 | N | N | Y (2) | 1-MUD BMSCT* 2-MUD PBSCT ** | 1-N (graft loss day 28 2-N (Failed to graft) | Y (Cholestyramine, ursodeoxycholic acid and RCE 4-5 weekly) |
| Case 4 | Ursodeoxycholic acid Ferrous sulphate Plasma exchange day 3 | Y(39) | 118 | RCE | 63 (3539µg/dL) | 2174 | Y (Deferasirox) # | N | Y (2) | 1-MUD PBSCT 2-MUD PBSCT ** | 1-N (graft loss day100) 2-Y (100% donor chimerism day 20 | N |
| Case 5 | Ursodeoxycholic acid Cholestyramine Plasma exchange day 1 | N | | | 73 (4101 µg/dL) | NA | N | N | N | NA | | Y (Cholestyramine ursodeoxycholic acid) |

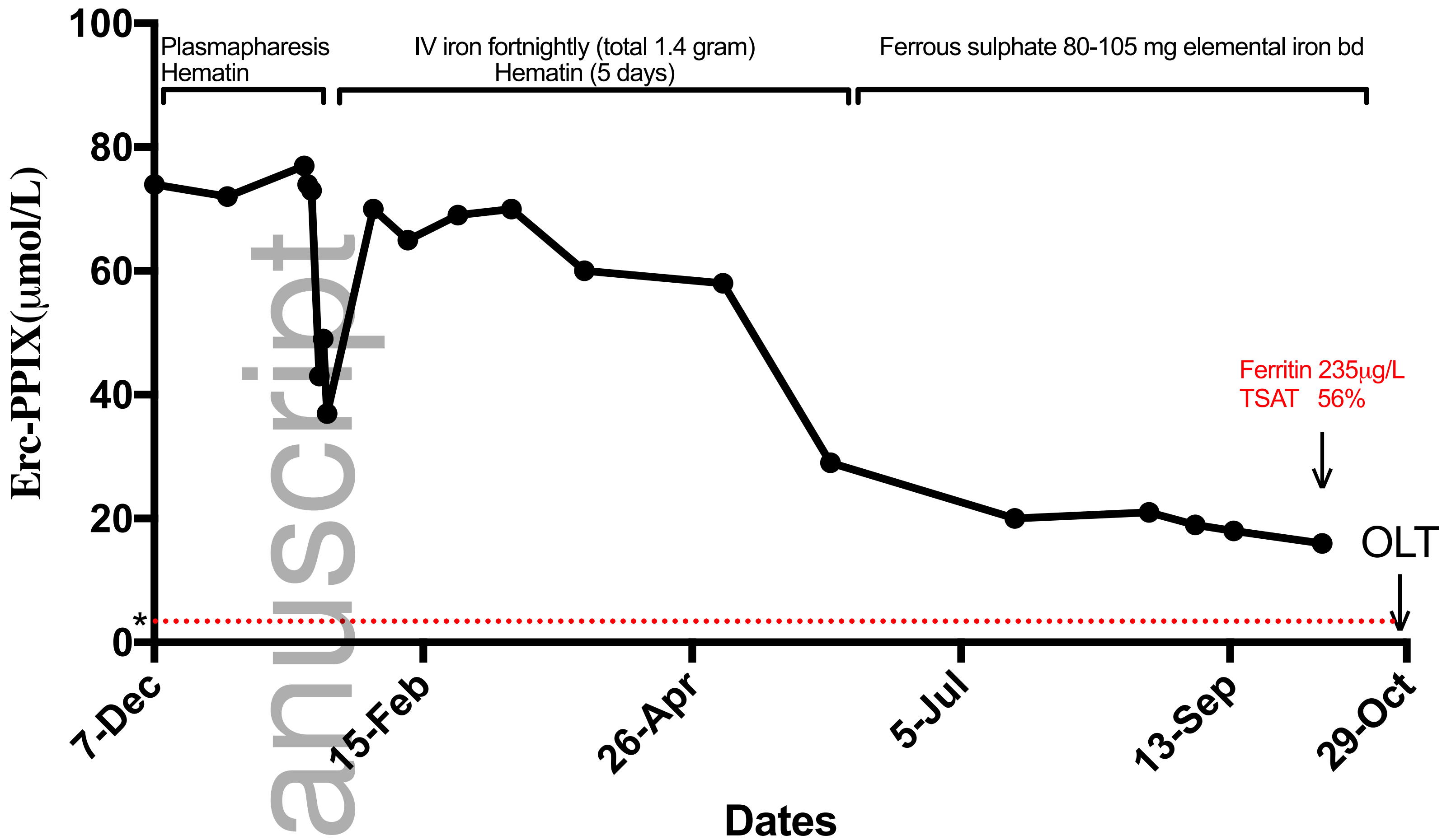
| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | and 3 | | | | | | | | | | | |

+ RCE: Red cell exchange

* MUD BMSCT: Matched unrelated donor bone marrow stem cell transplant

**MUD PBSCT: Matched unrelated donor peripheral blood stem cell transplant

# Deferasirox: A dose of 7.5 mg/kg/d tolerated only for 3 weeks. After cirrhosis and grade 4 siderosis was diagnosed with a mean serum ferritin of 2174 µg/L. Deferasirox 250 mg daily was commenced and tolerated.

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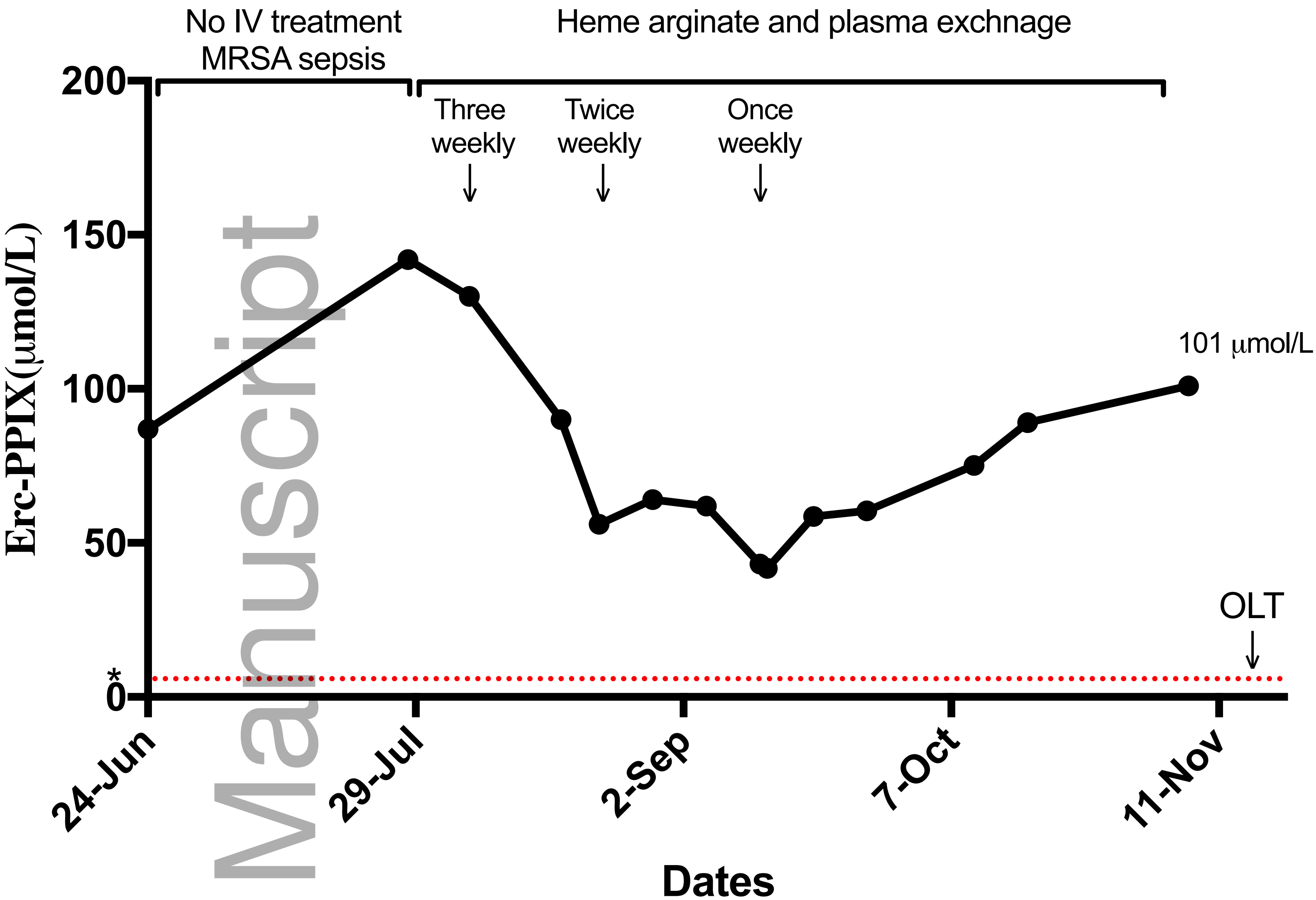


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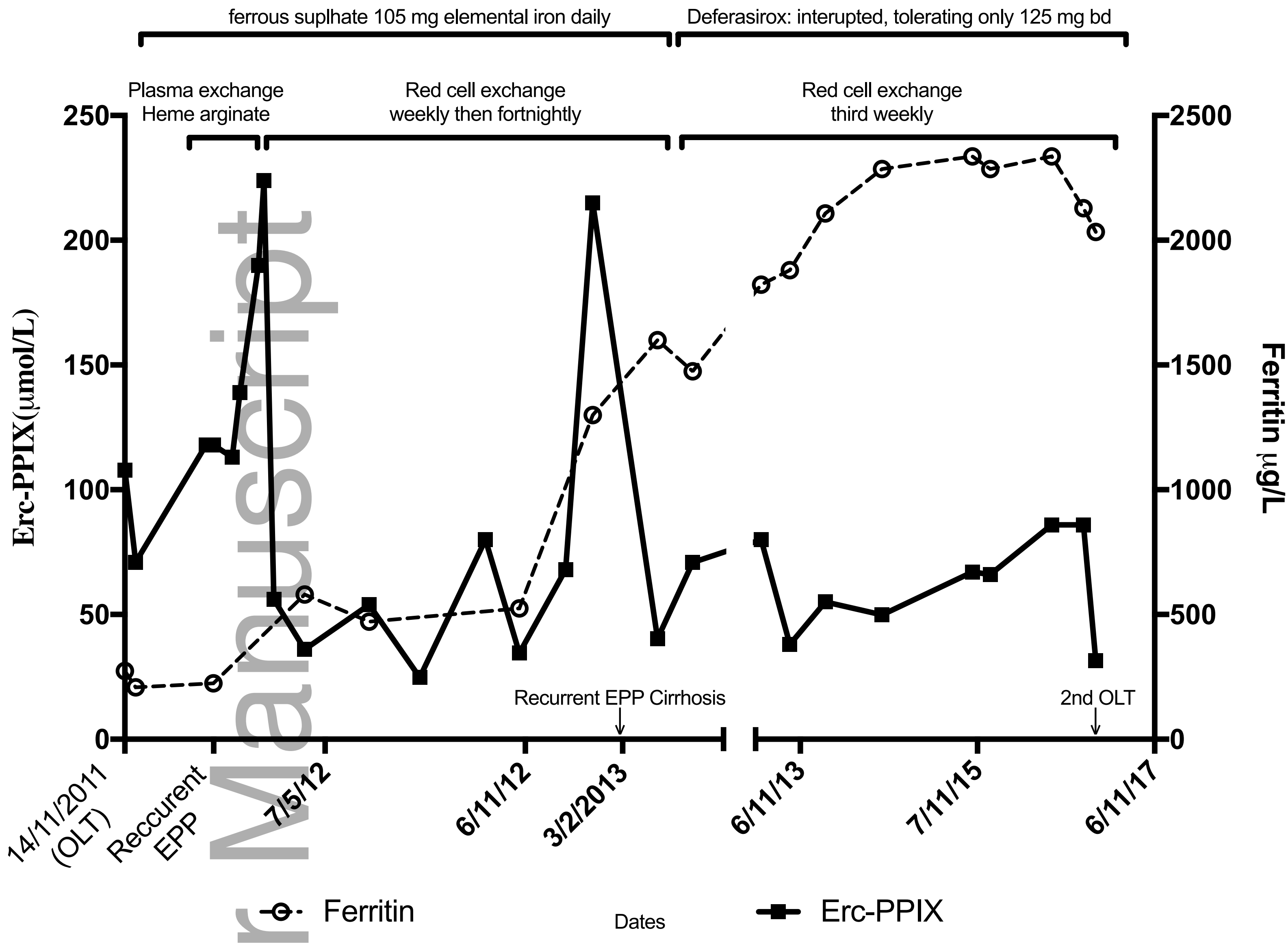
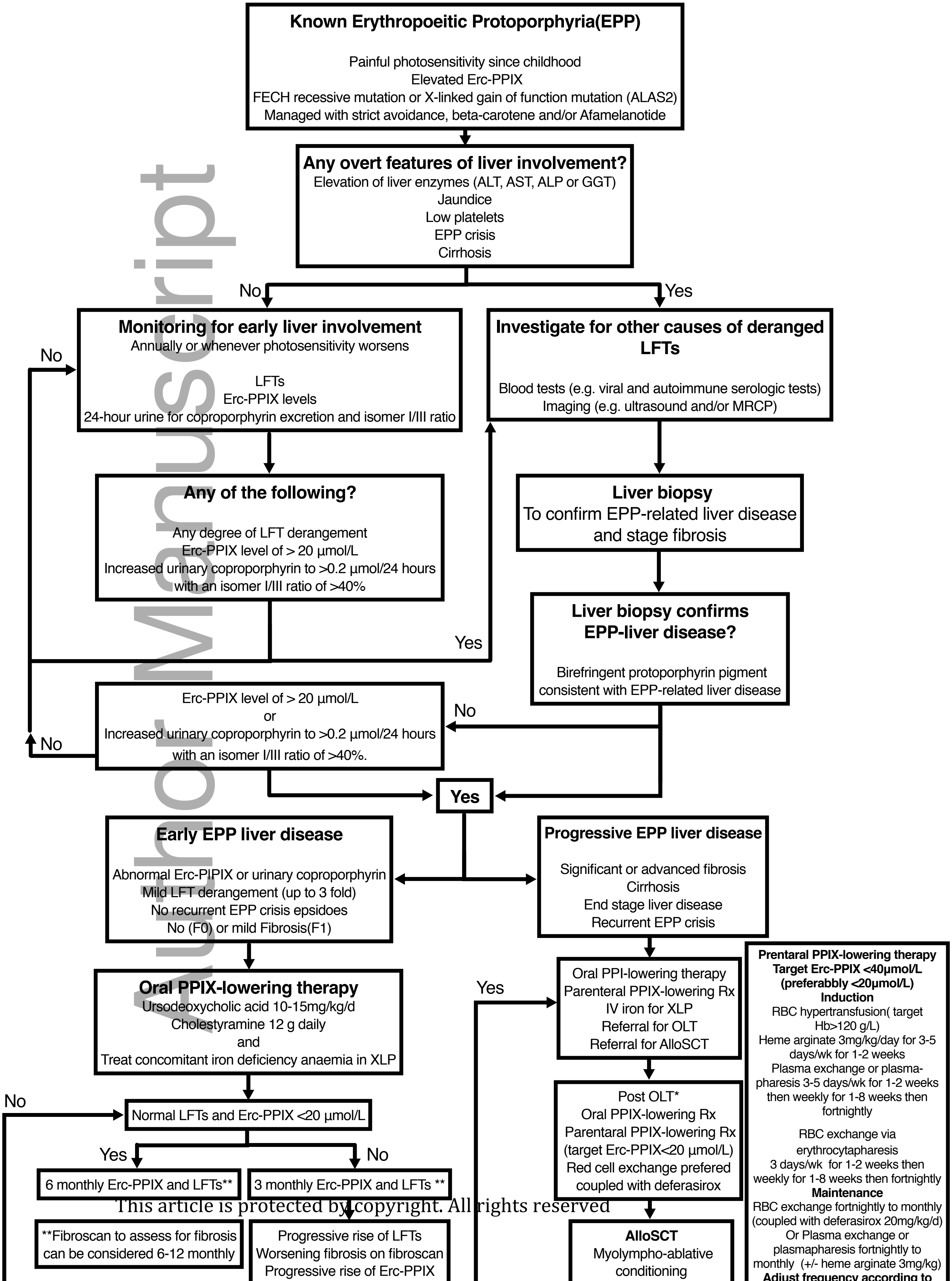



lt_25632_f3.eps


Known Erythropoeitic Protoporphyria(EPP)
Painful photosensitivity since childhood
Elevated Erc-PPIX
FECH recessive mutation or X-linked gain of function mutation (ALAS2)
Managed with strict avoidance, beta-carotene and/or Afamelanotide
Any overt features of liver involvement?
Elevation of liver enzymes (ALT, AST, ALP or GGT)
Jaundice
Low platelets
EPP crisis
Cirrhosis
No
Yes
Monitoring for early liver involvement
Annually or whenever photosensitivity worsens
LFTs
Erc-PPIX levels
24-hour urine for coproporphyrin excretion and isomer I/III ratio
Investigate for other causes of deranged LFTs
Blood tests (e.g. viral and autoimmune serologic tests)
Imaging (e.g. ultrasound and/or MRCP)
No
Any of the following?
Any degree of LFT derangement
Erc-PPIX level of > 20 µmol/L
Increased urinary coproporphyrin to >0.2 µmol/24 hours with an isomer I/III ratio of >40%
Yes
Liver biopsy
To confirm EPP-related liver disease and stage fibrosis
Liver biopsy confirms EPP-liver disease?
Birefringent protoporphyrin pigment consistent with EPP-related liver disease
Erc-PPIX level of > 20 µmol/L
or
Increased urinary coproporphyrin to >0.2 µmol/24 hours with an isomer I/III ratio of >40%.
No
No
Yes
Early EPP liver disease
Abnormal Erc-PIPIX or urinary coproporphyrin
Mild LFT derangement (up to 3 fold)
No recurrent EPP crisis epsidoes
No (F0) or mild Fibrosis(F1)
Progressive EPP liver disease
Significant or advanced fibrosis
Cirrhosis
End stage liver disease
Recurrent EPP crisis
Oral PPIX-lowering therapy
Ursodeoxycholic acid 10-15mg/kg/d
Cholestyramine 12 g daily
and
Treat concomitant iron deficiency anaemia in XLP
Yes
Oral PPI-lowering therapy
Parentaral PPIX-lowering Rx
IV iron for XLP
Referral for OLT
Referral for AlloSCT
Prentaral PPIX-lowering therapy
Target Erc-PPIX <40µmol/L (preferabbly <20µmol/L)
Induction
RBC hypertransfusion( target Hb>120 g/L)
Heme arginate 3mg/kg/day for 3-5 days/wk for 1-2 weeks
Plasma exchange or plasma-pharesis 3-5 days/wk for 1-2 weeks then weekly for 1-8 weeks then fortnightly
RBC exchange via erythrocytapharesis
3 days/wk for 1-2 weeks then weekly for 1-8 weeks then fortnightly
Maintenance
RBC exchange fortnightly to monthly (coupled with deferasirox 20mg/kg/d)
Or Plasma exchange or plasmapharesis fortnightly to monthly (+/- heme arginate 3mg/kg)
Adjust frequency according to
No
Normal LFTs and Erc-PPIX <20 µmol/L
Yes
No
6 monthly Erc-PPIX and LFTs**
3 monthly Erc-PPIX and LFTs **
Post OLT*
Oral PPIX-lowering Rx
Parentaral PPIX-lowering Rx
(target Erc-PPIX<20 µmol/L)
Red cell exchange prefered coupled with deferasirox
**Fibroscan to assess for fibrosis can be considered 6-12 monthly
Progressive rise of LFTs
Worsening fibrosis on fibroscan
Progressive rise of Erc-PPIX
AlloSCT
Myolympho-ablative conditioning