HOSPITAL DE CLÍNICAS DE PORTO ALEGRE PROGRAMA DE RESIDÊNCIA MÉDICA EM HEMATOLOGIA E HEMOTERAPIA – ÁREA DE ATUAÇÃO: TRANSPLANTE DE MEDULA ÓSSEA

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TRANSPLANTE SEQUENCIAL DE FÍGADO E MEDULA ÓSSEA: A SOLUÇÃO PARA PROTOPORFIRIA ERITROPOIÉTICA? RELATO DE CASO E REVISÃO DE LITERATURA

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SUMÁRIO

RESUMO

Introdução: A protoporfiria eritropoiética (EPP) é uma doença hereditária rara da biossíntese do heme que resulta no acúmulo de protoporfirinas (PP), caracterizando-se por fotossensibilidade e, em uma minoria de casos, insuficiência hepática. A principal estratégia terapêutica para a doença hepática avançada é o transplante hepático (TH). Entranto, tal estratégia não corrige o defeito primário, culminando na persistência dos sintomas e na recidiva da doença no enxerto hepático. Nesse cenário, o transplante de células-tronco hematopoiéticas (TCTH) posterior ao TH é uma abordagem para EPP. Métodos: objetivamos descrever o primeiro transplante sequencial de fígado e medula óssea realizados no Brasil em uma paciente com EPP, além de revisar a literatura atual. Resultados: paciente do sexo feminino, 13 anos, com histórico de fotossensibilidade, que apresentou síndrome colestática e hepatopulmonar e foi diagnosticada com PPE. A biópsia hepática evidenciou cirrose avançada. Foi submetida a TH com sucesso, com melhoria dos sintomas respiratórios. No entanto, apresentou recorrência da doença no enxerto hepático. Realizou TCTH mieloablativo com doador não aparentado compatível, condicionamento com BuCy e profilaxia DECH (doença enxerto contra o hospedeiro) com ATG, tacrolimus e metotrexato. A pega neutrofílica ocorreu no D+18. Como complicações agudas, apresentou neutropenia febril, reativação de CMV (citomegalovírus) e cistite hemorrágica. Evoluiu com quimerismo misto, mas com normalização dos níveis de PP, estando atualmente 300 dias após TCTH clinicamente bem e com funcionamento normal dos enxertos. Conclusões: TH e TCTH consecutivos para EPP foram descritos em 11 pacientes na literatura, sendo uma população altamente variável, mas com resultados favoráveis. Este conceito de tratamento deve ser considerado em pacientes com doença hepática estabelecida. Novos modelos são necessários para identificar pacientes de alto risco de desenvolver doença hepática os quais poderiam, portanto, se beneficiar de estratégias terapêuticas mais precoces.

Palavras-chave: Protoporfiria eritropoiética; Transplante de fígado; Transplante células-tronco hematopoiéticas;

1 INTRODUCTION

The metabolic disorders characterized by a deficiency in the activity of different enzymes involved in heme biosynthesis are defined as porphyries. Erythropoietic protoporphyria (EPP) is pictured by a failure of the mitochondrial enzyme ferrochelatase (FECH), resulting in the accumulation of protoporphyrin[1]. In EPP, the increased levels of protoporphyrin (PP) occur in tissues (as skin and liver) and blood (erythrocytes and plasma)[2]. Therefore, the disease is expressed as painful photosensitivity, usually starting in early childhood[2]. Hepatic manifestations in EPP are highly variable, occurring in 5 to 20% of patients[3]. The saturation of PP seems to cause direct hepatocellular and biliary damage[4]. Consequently, cholelithiasis is frequent in patients with EPP. Moreover, the parenchymal disease can emerge, with increased levels of aminotransferases and cholestatic enzymes. In about 5% of patients, there is a progressive hepatocellular disease that can culminate in hepatic insufficiency[3]. Liver failure leads to splenomegaly, sequestration of erythrocytes, and hemolysis, increasing erythropoiesis and, therefore, PP formation, creating a vicious cycle[2]. Remarkable, patients who have FECH mutations on both alleles have an increased risk of liver disease[2].

Management of cutaneous disease is mainly based on sunlight protection and oral supplements, such as beta-carotene. Acute liver failure presenting with coagulopathy and cholestasis can be managed with hematin, but it's ineffective to prevent the ultimate liver failure[5,6].

Liver transplantation (LT) is the treatment of choice for end-stage hepatic disease. The first transplant on a patient with EPP was made in 1980, and the patient died from disseminated candidiasis within 4 weeks[7]. Nonetheless, liver transplantation does not alter the consequences of FECH deficiency and PP overproduction. Recurrence of EPP`s liver disease does occur (in about 65% of patients), and the necessity of re-transplantation was already described[8].

In this setting, hematopoietic stem cell transplantation (HSCT) after LT is a curative therapy for patients with EPP and could be capable of preventing recurrent disease on the allograft. The first HSCT in a patient with EPP was performed in 2002[9]. Since then, other centers successfully accomplished HSCT for EPP[7,10].

2 MATHERIALS AND METHODS

We aim to describe the first case of sequential liver and bone marrow transplantation for EPP in Brazil, also providing a review on the previous experiences, to contribute to future clinical decisions regarding those patients. The patient and family gave informed consent for publication.

3 RESULTS – CASE REPORT

A 13 years old teenager was admitted to Hospital de Clínicas de Porto Alegre, a tertiary teaching hospital in southern Brazil, with abdominal pain and jaundice. She had a previous history of painful photosensitivity since early childhood, with a compensatory behavior of avoiding sun exposure. Initial image investigation revealed signs of chronic liver disease and portal hypertension. The Ehrlich test was positive and the patient was put into photo isolation. Hepatic biopsy revealed ductular reaction with marked deposition of dense dark brown pigment in bile canaliculi. First evaluation of total plasma porphyrins was 213µg/dL (reference value < 1µg/dl) and protoporphyrin 207,8µg/dl (reference value < 1µg/dl). Testing for 24-hour urine porphobilinogen and aminolaevulinic acid was normal. The patient was submitted to genetic investigation, with molecular confirmation of a diagnosis of EEP (p.Gln122Arg fs*23 and IVS3-48c variants in the FECH gene, in heterozygosis). Three months after the first hepatic biopsy, she repeated the procedure with evidence of fibrosis progression and the presence of septal cirrhosis. Given her quick hepatic deterioration, she was referred for evaluation with the liver and bone marrow transplant teams. During the assessment, she started with dyspnea during moderate efforts. Laboratory showed markedly hypoxemia and elevation of the alveolar-arterial oxygen gradient. An echocardiogram with microbubbles showed severe pulmonary shunt, and she was diagnosed with hepatopulmonary syndrome, starting therapy with oxygen replacement and garlic supplementation. She evolved with progressive hepatic failure, with a MELD score of 28 points. Subsequently laboratory tests revealed total plasma porphyrins of 125,6 µg/dL, protoporphyrin of 124µg/dL and free erythrocyte protoporphyrin 970 µg/dL (reference value < 55).

She was submitted to orthotopic whole liver transplantation (OLT) from a deceased donor, with an ischemic time of 18 minutes. Immunosuppression was realized with methylprednisolone and tacrolimus. She was submitted to 3 sessions of plasmapheresis for the prevention of post-transplant liver damage. The hepatic explant was enlarged (1.3kg), dark brown color, with a macroscopic puzzle pattern, histology showing biliary pattern cirrhosis.

The graft evolved with prompt function, with normalization of clotting parameters and bilirubin levels. She was discharged from ICU care 11 days after the procedure.

After OLT, she had mild cardiac septal hypertrophy probably secondary to tacrolimus toxicity, being handled with serum level control. She had a progressive improvement in her hepatopulmonary syndrome, getting oxygen-free 90 days after OLT.

She was listed for an HSCT with a matched unrelated donor (MURD). While waiting on the HSCT queue (changed due to the pandemic), nine months after the OLT, she had a worsening of transaminases and canalicular enzymes, repeated hepatic biopsy with evidence of portal enlargement with ductular reduction, inflammatory infiltrate with hepatocyte involvement, and thick bile plugs, possibly corresponding to porphyrins. She had a mild improvement with ursodeoxycholic acid.

She was submitted to MURD HSCT, HLA matched 10x10, masculine donor, ABO isogroup, RhD incompatibility, bone marrow source, both donor and patient CMV (cytomegalovirus) positive. Conditioning was made with busulfan (pharmacokinetic-guided concentration-vs-time curve of 4000 µMol min for 4 days) and cyclophosphamide (120mg/kg) (BuCy), and graft versus host disease (GvHD) prophylaxis with thymoglobulin, methotrexate, and tacrolimus maintenance. Before infusion, she completed 3 sessions of plasmapheresis. She infused 3.5 x 10^6 of CD34+/Kg and 3.7 x 10^8 of TNC/Kg. Neutrophil engraftment occurred in D+18 and platelet engraftment in D+24.

As acute complications, she had febrile neutropenia of oropharyngeal focus, with the identification of Carbapenem-resistant *Klebsiella pneumoniae* in a swab, treated with ceftazidime-avibactam. She had a herpes-simplex cutaneous infection, treated with intravenous acyclovir. Cytomegalovirus reactivation (CMV) was properly managed with ganciclovir. Her D+30 and +60 peripheral blood chimerism was 100% donor. On D+46 after BMT, her free erythrocyte protoporphyrin was 64ug/dL. She was discharged after 54 days of inpatient stay.

On D+60, she had thrombotic microangiopathy related to tacrolimus, with acute renal failure, and the immunosuppression was replaced with sirolimus. She evolved with hemorrhagic cystitis secondary to JC and BK viruses, treated with ciprofloxacin and 10 days of urinary catheter and irrigation, with resolution.

On D+90 she started to present mixed chimerism (80%), which remained stable after increasing sirolimus. She had a favorable evolution since then, being currently 300 days after HSCT, maintaining mixed chimerism (83%), with proper functioning of bone marrow and

hepatic grafts, and normal PP levels. Also, with a satisfactory pulmonary function, being free of oxygen and capable of exercising. Her laboratory evolution can be seen in Table 1.

4 DISCUSSION

EPP is a very rare inherited disease further associated with photosensitivity, and lack of quality of life. A percentage of patients can demonstrate liver complications, including hepatic failure. It is essential to recognize clinical predictors that are associated with liver failure. Among them, erythrocyte protoporphyrin values higher than 1124 μg/dL were associated with severe intrahepatic cholestasis and, above 1517 μg/dL, with cirrhosis[11].

Unfortunately, there is restricted data concerning the efficacy of PP lowering to prevent liver damage. Some authors suggest the use of cholestyramine and ursodeoxycholic acid, in addition to parenteral measures such as hematin infusion, RBC (red blood cells) transfusion, or hypertransfusion and red cell/plasma exchange[12].

Liver transplantation is defined as an approach for end-stage liver disease in patients with EPP. In a case series of 20 LT, pediatric and adult survival rates were 100% and 85% at 1 year, 75% and 69% at 5 years and 50% and 47% at 10 years[13]. A review published in 2014 pointed that 62 liver transplants had been performed in EPP patients, the majority in Europe (35), followed by the United States (23) and Asia (4). Patients were predominantly male (60%), with a MELD (model for end-stage liver disease) score of 21[8].

There are some remarkable issues during the surgery and follow-up of these transplanted patients. It's crucial to decrease PP levels to reduce light damage to the skin and tissues during surgery. These could be achieved through hemin infusion, removing protoporphyrins by plasma or RBC exchange, or increasing its excretion using ursodeoxycholic acid[8], usually measures that are already in place for patients with severe liver disease. Moreover, there is the indication to use specific filters to block the light below 470-nm wavelength during the transplant procedure[8]. In addition, neurological complications can occur, similarly to acute porphyria, with hypertension, tachycardia, abdominal pain, and respiratory paralysis[14]. Among the US series, 6 patients had this kind of complication[8]. Besides, biliary issues were prevalent in this population: they occurred in about 45% of patients[8].

The first HSCT realized on the background of EPP was performed in a 47-year-old female with a history of acute myeloid leukemia (AML). The primary indication for HSCT was, therefore, the AML itself. Nevertheless, the patient had a history of cutaneous

photosensitivity for 25 years, and she was found to have a FECH mutation, being diagnosed with EPP. The HSCT resulted in AML remission and normalization of PP levels[9].

Thus, both the recurrence of liver damage on the hepatic allograft and the anecdotal AML case opened the discussion of the allogeneic HSCT experience in EPP. Particular scenarios are possible indications for HSCT in EPP patients: after LT in older patients with recurrent disease on the hepatic graft; after LT in young patients without liver disease, to prevent it; in patients with progressive liver disease, still without indication for LT[15].

We have found, so far, 11 descriptions of HSCT in patients with EPP, including our patient. Characteristics of this cohort can be seen in Table 2. There are some brief narratives of another two cases, without enough details to portray[15].

From these 11 cases, 9 (81%) had a sequential liver and bone marrow transplant. Seven (63%) were males, with a mean age of 24.6 years old on HSCT realization. Four patients were less than 18 years old. Interestingly, EPP seems to affect both males and females equally[21].

Six patients (54%) underwent a MURD (matched unrelated donor) transplant. This could be related to the concern of a relative also being a mutation carrier. Haploidentical bone marrow transplantation was made in one single case[17], possibly because of worrying about the inherited risks associated with this kind of procedure. Indeed, the only death in the described cohort occurred on this patient.

Concerning graft source, 4 patients only received HSCT from bone marrow (BM) source, including our own. We consider this a suitable practice regarding the benignity of the disease and the reduced GvHD (graft versus host disease) risk. However, one patient who initially received BM source had primary graft failure (GF) and needed a second HSCT using PBSC (peripheral blood stem cells). BM is consistently associated with delayed neutrophil and platelet engraftment across all types of transplant, but the impact on GF depends on donor type and intensity of conditioning[6,22].

The conditioning regimen (CR) was quite variable between the cases. Six (54%) had a RIC (reduced-intensity conditioning), 3 (27.5%) MA (myeloablative), one patient had first a RIC and them a MA, and in 1 case it was not possible to define it. Five patients used thymoglobulin on the regimen. Choosing the CR is certainly one of the biggest challenges in this population, primarily because of the paucity of literature data. As the majority of patients were young people, we can expect a better tolerance of MA regimens. However, as these patients are frequently already transplanted, there is a concern of liver toxicity, especially with drugs such as busulfan. Our patient, however, had a good tolerance using BuCy

(busulfan and cyclophosphamide). There is an explanation for adopting RIC owing to the non-malignant nature of EPP. However, it is known that non-neoplastic disorders are more liable to GF in comparison to acute leukemias. Usually, GF does not carry such an unfavorable outcome in benign diseases as it does in neoplasms. Still, one of the described patients who initially was submitted to a RIC regimen and had secondary GF, developed a therapy-related AML, because recipient hematopoietic stem cells were exposed to potentially mutagenic drugs and irradiation[18]. Therefore, the CR decision should be envisaged with caution.

GvHD prophylaxis was also very contrasting between the subjects. In 3 cases (including ours), the previously IST (immunosuppression) for LT was maintained in HSCT. We and others had fortuitously used MTX (methotrexate) without liver toxicity. The incidence of acute and chronic GvHD was surprisingly low. Acute hepatic GvHD occurred in two patients (18%), and only one had undergone LT. Besides, in this case, the liver biopsy demonstrated a lymphoid infiltrate that could represent either acute cellular rejection or GvHD, and he was treated with methylprednisone 2mg/kg, with a rapid improvement[16]. Only one patient had a description of cGvHD, based on generalized dry skin and impaired hepatic ductal enzymes, which was controlled by mycophenolate mofetil[18]. Our patient had thrombotic microangiopathy related to tacrolimus, which was substituted for sirolimus with success. This resolution was made between both liver and bone marrow transplant teams, additionally based on a protective effect of sirolimus in CMV reactivation, after she had her first reactivation[23].

Hepatic sinusoidal obstruction syndrome (SOS) is a systemic endothelial disease, for which a pre-existing liver disease is one of the major risk factors. Notably, none of the described patients had SOS. Our patient had three additional factors for SOS development: lung disease with reduced diffusion capacity (hepatopulmonary syndrome), use of CR with BuCy, and GvHD prophylaxis with MTX, but she did not develop this intercurrence. The absence of this complication may be related to the frequent and previous use of ursodeoxycholic acid, which is effective prophylaxis for SOS[24,25].

Another concern regarding the pre and post-HSCT settings is the iron overload. It is related to higher rates of infections, VOD, mucositis, liver dysfunction, and acute GvHD, besides decreased survival rates[26]. Our patient did not present hyperferritinemia pre-HSCT, although she had a moderate elevation on recent post-HSTC, which was spontaneously solved after being transfusion-free. Iron damage is even more harmful to the liver graft and should be

assertively managed. Even so, patients who are on an RBC exchange program are at increased risk, and ferritin levels should be carefully monitored.

The incidence of primary or secondary GF was particularly high in these patients, occurring in 4 (36%). Two of them were saved with a second successful HSCT. Usually, GF general incidence ranges from 3.8 to 5.6%. The experience in HSCT for chemotherapy-naïve patients with benign diseases suggests that MA CR may be required to facilitate donor engraftment[27]. The presence of HLA (human leukocyte antigen) antibodies is associated with GF in cord blood and haploidentical HSCT, and donor-specific anti-HLA antibody in MURD HSCT is also a predictor of GT. Our patient had positive anti-HLA testing for DQ2 before transplant, however, it was not specific for the donor and had a low mean fluorescent intensity. Only another case described the absence of anti-HLA antibodies[12]. This evaluation is essential when choosing any donor with a mismatch.

Infections are a major cause of morbidity (and also mortality in the unique case) in this population. This is compatible with an already immune-suppressed community that undergoes HSCT. We can observe a predominance of viral infection among the reported cases. Our patient was the unique note case of CMV reactivation, although this is a very prevalent complication among HSCT receptors, and she was successfully treated with ganciclovir[23].

Among patients who had successful engraftment, complete donor chimerism (> 95%) was present in all cases, except for ours. Our patient had initially complete chimerism that fell after the change in immune suppression for sirolimus. Dose adjustments were made to maintain stable chimerism. There is no evidence in the literature that complete chimerism is required for patients with EPP. Being a benign disease, we hypothesize that the amount of donor chimerism should be sufficient to maintain a normal PP level, and, therefore, to prevent liver damage and symptoms such as photosensitivity.

Hepatopulmonary syndrome is a severe complication in patients with end-stage liver disease and portal hypertension. Our patient had a relevant improvement after LT, becoming oxygen-free. Yet, reduced pretransplant pulmonary function tests, myeloablative busulfan-based conditioning regimens, use of methotrexate for GVHD prophylaxis, and MURD were already described as risk factors for bronchiolitis obliterans syndrome (BOS). Busulfan may induce direct toxicity to the epithelial lining cells of the lungs, contributing to the higher risk. On other hand, the use of ATG as part of the conditioning regimen appears to confer a protective effect against the development of cGVHD and BOS[28].

Decreasing levels of PP is imperative before LT to avoid phototoxic abdominal burns, acute protoporphyrin-mediated damage to the liver allograft and acute neuropathy. One way to achieve this is through therapeutic plasma exchange, especially as a bridge approach. The patient was submitted to this procedure before LT and HSCT, using plasma and albumin. Although this is a possible path, hepatic injury in EPP is a complex complication and may be not be evaded by lowering PP levels.

Despite the defined etiology of the disease - caused by the FECH mutation and heme synthesis defect - there is a hesitation whether EPP is strictly an erythropoietic porphyria. There is possibly a hepatic role contributing to the accumulation of PP, pointed by the loss of photosensitivity in patients submitted to LT[15]. The severity of the hepatic disease is also affected by polymorphism of genes regulation porphyrin homeostasis. In addition, the PP efflux transporter ABCG2 has been associated with photo and hepatotoxicity in patients with EPP. ABCG2 deficiency decreases PP distribution to the skin, preventing damage. In the liver, this deficiency causes a modulation of PP distribution, metabolism, and excretion[29]. We hypothesize that evaluation of the patient`s ABCG2 status could be a way to foresee severe hepatic impairment, and, as a consequence, to plan therapeutic strategies such as HSCT. Besides, it`s a potential target for new EPP approaches.

Sequential LT and HSCT is a very complex treatment that demands mutual cooperation between the hepatic and bone marrow transplant teams. We can observe favorable results with this approach for patients with EPP and end-stage liver disease. There is no standardized literature regarding donor, graft source, CR or GvHD prophylaxis. This decision should be always individualized and based on the local experience and possibilities.

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APÊNDICE

Table 1: Patient`s laboratory tests before and after HSCT and OLT

Days from HSCT OLT	**Total plasma porphyrin (µg/dL) RV < 1**	**Free erythrocyte protoporphyrin (µg/dL)**	**Hb (g/dL) RV>11.7**	**Platelets/µL RV>150000**	**Serum total bilirrubin (mg/dL) RV<1.2**	**Serum direct bilirrubin (mg/dL) RV>0.5**	**ALT (U/L) RV<55**	**AST (U/L) RV<34**	**Albumin (g/dL) RV>3.5**	**Peripheal blood chimerism (% donor)**
D-1003 D-538	213	-	9.6	133000	4.1	2.7	328	404	3.	-
D-496 D-30	125.6	970 (RV<20)	11.9	99000	1.4	0.8	96	100	3.9	-
D-447 D+18	-	169 (RV<55)	11	146000	0.5	0.2	15	14	3.	-
D+46 D+511	-	64 (RV<55)	8.5	111000	0.4	0.1	15	36	3.3	100
D+130 D+595	-	37 (RV<55)	11.5	72000	0.3	0.1	33	30	4.1	75
D+175 D+640	-	50 (RV<55)	11.3	93000	0.3	0.1	57	43	3.9	82

Abbreviations: HSCT – hematopoietic stem cell transplant; OLT – orthotopic liver transplant; RV – reference value; Hb – hemoglobin; ALT – alanine-aminotransferase; AST – aspartate-aminotransferase;

Table 2: Characteristics of patients with EPP

Reference	PohFitzpatrick[9]	Rand[6]	Wahlin[10]	Windon[16]	Smiers[17]	Cheung[18]	Hashmi[19]	Wang[20]	Ardalan[12]	Ardalan[12]	Our patient
Origin	USA	USA	Sweden	USA	Netherlands	Hong-Kong	USA	USA	Australia	Australia	Brazil
Gender	Female	Male	Male	Male	Male	Male	-	Male	Female	Female	Female
Baseline EP	29463nmol/L (RV< 177)	2683µg/dL (RV<100)	170mol/L (RV <1.2)	3235µg/dL (RV< 35)	85000 nmol/L (RV < 560)	140µmol/L (RV <1.5)	3482µg/dL (RV<35)	-	10112 µg/dL	3596 µg/dL	970 µg/dL (RV < 55)
Baseline PP	176 nmol/L (RV< 16)	-	-	61.5 µg/dL (RV < 1)	-	-	-	-	-	-	213µg/dL (RV < 1)
FECH mutation	-	-	Null allele (930G > A)	Heterozygous 315-348 T	Heterozygote missense	Allele IVS3-48C	S264X and IVS3-48T>C intron	Yes, NE	Yes, NE	Yes, NE	p.Gln122Arg fs*23/IVS3-48c
LT	No	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes
MELD Score	-	-	-	30	-	-	-	-	-	20	28
IST for LT	-	-	-	TAC	BSX, TAC, PRED	-	-	-	-	-	MET, TAC
HSCT	Yes	Yes, 2	Yes, 2	Yes	Yes	Yes, 2	Yes	Yes	Yes, 2	Yes, 2	Yes
Age at HSCT	47*	12*	62*	26*	8*	26*	12*	18.1*	25*	22*	13*
Donor type	MRD	MRD	MURD	MURD	HAPLO	MURD	MURD	MURD	MURD	MURD	MURD
Donor match	6x6	6x6	6x6 / 12x12	10x10	5x10	10x10	10x10	12x12	10x10	10x10	10x10
Graft source	-	BM/PBSC	BM	PBSC	BM plus PBSC	-	BM	PBSC	BM	PBSC	BM
Conditioning	BuCy Etoposide	1º Cy-TBI 2ºBuFluCy	1ºFluCyATG 2ºFluCyATG TBI 6 Gy	FluBu TBI 2 Gy	FluCy Treosulfan MEL Alemtuzumab	1º FluCy TBI 6 Gy ATG	FluMEL Thiotepa ATG	FluMEL Alemtuzumab	1º Cy-ATG 2º FluCy Alemtuzumab	1º FluCy ATG 2ºFluMelATG	BuCy ATG
Intensity	MA	-	RIC	RIC	MA	RIC	RIC	RIC	RIC	RIC / MA	MA
GvHD prophylaxis	CsA, PRED	ATG	1ºSiro,TAC 2ºCsA,MTX	TAC, MTX, AZA	TAC, MMF,PRED	-	TAC, MTX	T-cell depletion	TAC,MMF	1º CsA, MTX 2ºTAC,MMF	TAC, MTX
aGvHD	Hepatic	No	No	Hepatic	No	No	No	No	No	No	No
cGvHD	No	No	No	No	No	Skin, hepatic	No	No	No	No	No
VOD	-	-	-	-	No	No	-	No	No	-	No
NE	Yes	No / Yes	Yes	Yes	Yes	Yes	Yes	Yes	No, for both	No / Yes	Yes
Days to NE	-	-	-	17	10	-	14	25	-	-	18
Infections	-	EBV infection	-	-	Scalded skin syndrome, HboV enteritis, VZV encephalitis	-	*Clostridium difficile* colitis, MRSA septic thrombophlebitis	-	-	PTLD-EBV	KPC tonsillitis, HSV, CMV, JC and BK
EP post	3,359 nmol/L	80 g/dL	-	54 µg/dL	< 500 nmol/L	-	Normalized	-	280 µg/dL	34 µg/dL	50 µg/dL (RV < 55)
PP post	14.2 nmol/L	-	-	1 µg/dL	-	-	-	-	-	-	-
Chimerism	-	100%	100%	98% on D+70	100%	33 / 18 / 10%	100% on 18 months	97%	0%	100%	83% on D+300
Alive	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Follow-up	5 years	10 months	30 months	8 months	Died 8 months after BMT	5 months after second HSCT	2 years	-	8 years	1 year	D+310
Comments	AML diagnosis	Splenectomy before the 2º HSCT	Engraftment occurred but lost chimerism until autologous		Slow immune recovery; Defibrotide phophylaxys	TR-AML treated with a 2º MURD HSCT		Diffuse axonal polyneuropathy after LT	Used hematin, RBC exchange, plasmapheresis	PTLD treated with RITUX	Thrombotic microangiopathy with TAC, changed for SIRO

Abbreviations: EP – erythrocyte protoporphyrin; PP – plasma protoporphyrin; FECH – mitochondrial enzyme ferrochelatase; LT – liver transplant; MELD - model for End-stage Liver Disease; IST – immunosuppression; HSCT – hematopoietic stem cell transplantation; GvHD – grfat versus host disease; aGvHD – acute graft versus host disease; cGvHD – chronic graft versus host disease; VOD - veno-occlusive disease; NE – neutrophil engraftment; RV – reference value; MRD – matched related donor; MURD – matched unrelated donor; BuCy – busulfan and cyclophosphamide; CsA – cyclosporine; PRED – prednisone; AML – acute myeloid leukemia; BM – bone marrow; PBSC – peripheral blood stem cell; TBI – total body irradiation; BuFluCy – busulfan, fludarabine and cyclophosphamide; ATG – thymoglobulin; SIRO – sirolimus; TAC –

tacrolimus; MTX – methotrexate; AZA – azathioprine; BSX– basiliximab; HAPLO – haploidentical; MMF – mycophenolate; FluMEL – fludarabine and melphalan; MRSA - methicillin-resistant *Staphylococcus aureus*; MET – methylprednisone; KPC - *Klebsiella pneumoniae* carbapenemase producing; CMV – cytomegalovirus; JC - John Cunningham virus; BK - polyomavirus; NE – not specified; RBC – red blood cells; TR – therapy-related; PTLD – post-transplant lymphoproliferative disease; EBV – Epstein-barr virus; RITUX – rituximab; VZV – varicella zoster virus; HSV – herpes simplex virus; HbOV – human bocavirus; USA –United states of America; *In years;