CASE REPORT

Long-term follow-up of hepatitis-associated aplastic anaemia

Cristina Gonçalves, Sandra Ferreira, Susana Nobre, Isabel Gonçalves

Unidade de Transplantação
Hepática - PA, Centro Hospitalar
e Universitário de Coimbra EPE,
Coimbra, Portugal

Correspondence to

Dr Cristina Gonçalves,
cristina.campos.goncalves@
gmail.com

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SUMMARY

Prognosis of hepatitis-associated aplastic anaemia (HAAA) was improved with haematopoietic stem cell transplantation (HSCT) and immunosuppression, but the long-term outcome remains undefined. Case 1: a girl aged 3 years with acute liver failure (ALF) submitted to orthotopic liver transplantation (OLT) subsequently developed aplastic anaemia and HSCT from a compatible sibling was performed. Post-HSCT, the patient developed post-transplant lymphoproliferative disorder and rituximab was administered with good response. Fifteen years later, both grafts show good outcome. Case 2: a girl aged 10 years submitted to OLT due to ALF, developed pancytopenia 2 months later. Due to the absence of a human leucocyte antigen compatible donor, she was treated with ciclosporin and antithymocyte globulin with very good long-term outcome. These clinical cases suggest that, for patients with HAAA that underwent OLT, aggressive therapy with HSCT or immunosuppression may provide a benign long-term outcome.

BACKGROUND

Hepatitis-associated aplastic anaemia (HAAA) is a subtype of aplastic anaemia (AA) that emerges after acute hepatitis, mainly affecting young males. However, the severity of AA does not correlate with hepatitis severity, and the onset of AA is usually life threatening if left untreated.¹

HAAA occurs in 28% of young adults after orthotopic liver transplantation (OLT) for acute liver failure (ALF) of indeterminate cause.² Therapeutic options include haematopoietic stem cell transplant (HSCT) or intensive immunosuppression. Although the outcome had initially been reported as very poor, more recent studies suggest that HSCT or immunosuppression may lead to 10-year survival rate of up to 70%.³

We report two cases of HAAA in girls submitted to OLT following ALF of unknown cause. We highlight the long-term follow-up without major complications, despite different therapeutic approaches were chosen. These cases emphasise that medical therapy may be a valuable alternative to HSCT when there is no compatible donor.

CASE PRESENTATION**Case 1**

A girl aged 3 years underwent OLT due to ALF with no identifiable aetiology, after exclusion of common causes (virus, metabolic conditions, Wilson's

disease, alpha-1-antitrypsin deficiency) and tacrolimus-based immunosuppression was started. No dysmorphic features were noted. Two weeks after OLT, pancytopenia was noticed (minimum haemoglobin (Hb): 4.2 g/dL; leucocytes: $0.09 \times 10^9/L$; neutrophils: $0 \times 10^9/L$; platelets: $0 \times 10^9/L$) and CD4/CD8 ratio was low (0.3). Bone marrow biopsy was hypocellular, affecting all blood cell lines equally and a small number of histiocytes were noted. Diepoxybutane chromosomal breakage test and telomere length analysis were normal. Diagnosis of AA was established and antithymocyte globulin (ATG) was administered without response. A high dependency on platelet and red blood cell transfusions and to granulocyte colony-stimulating factor administration was noticed. A human leucocyte antigen (HLA)—haploidentical bone marrow donor (sister) was found and HSCT was performed 5 months after OLT.

After HSCT, a left lung lower lobe mass was recognised on chest X-ray. Although sputum cultures (bacteria, mycobacteria and fungus) were negative, empiric itraconazole therapy was started without improvement. Lung biopsy disclosed a polymorphic mononuclear cell infiltrate with small lymphocytes and mature plasmacytes CD20+ and EBV+ (Epstein Barr Virus), compatible with post-transplant lymphoproliferative disorder (PTLD) with CD20+ cells (plasmocyte hyperplasia). EBV was also identified in serum using PCR test. Tacrolimus therapy was stopped and prednisolone (in monotherapy) was maintained. Additionally, acyclovir 30 mg/kg/day was administered. The patient received four treatment cycles with rituximab (375 mg/m^2) and there was progressive mass shrinking. Also, EBV genome became undetectable soon after starting therapy. No further signs of systemic disease were observed and she had normal growth. Liver biopsy 10 years post-OLT disclosed only minimal fibrosis. Currently, the patient is aged 19 years and the liver graft remains stable with normal enzymes and no other haematological changes occurred (table 1).

Case 2

A girl aged 10 years, with a history of congenital toxoplasmosis and familial history of autoimmune disorders (grandmother with inflammatory bowel disease and colangiopathy; aunt with rheumatoid arthritis and severe uveitis), presented with ALF associated with lymphopenia (minimum: $0.2 \times 10^9/L$) after flu-like prodromes. There was no identifiable cause (exclusion of virus, toxics,

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Table 1 Clinical and laboratorial data of patients 1 and 2 before liver transplantation, at aplastic anaemia (immediately before starting treatment for aplastic anaemia) and 5 and 10 years after liver transplantation

	Case 1				Case 2		
	Before LT	AA	5Y	10Y	Before LT	AA	5Y
Hb (g/dL)	8.3	4.5	14.9	15.7	14.0	7.2	12.8
WBC ($\times 10^9/L$)	5.25	0.09	4.88	4.96	4.6	0.44	4.49
Neut ($\times 10^9/L$)	1.27	0	1.6	2.5	3.7	0.2	2.29
Lym ($\times 10^9/L$)	0.31	0	2.9	2.0	0.2	0.2	1.74
Plat ($\times 10^9/L$)	111	0	161	167	103	8	123
CD4/CD8	NA	0,3	NA	NA	0.05	0.21	NA
INR	6.4	1.0	1.0	1.0	3.5	0.9	0.9
AST (U/L)	416	10	30	22	374	35	20
AST (U/L)	839	12	24	21	452	35	15
Bone marrow	NA	Hypocellular	NA	NA	Lymphopenia; remaining blood lines NL	Hypocellular	NA
Liver biopsy	NA	NA	Hemosiderosis. No fibrosis (F0)	Minimal fibrosis (F1)	NA	Normal liver graft	No fibrosis
Treatment modality for AA	HSCT from compatible donor				ATG and CyA		

LT, liver transplantation; AA, aplastic anaemia; Hb, haemoglobin; WBC, white blood cells; Neut, neutrophils; Lym, lymphocytes; Plat, platelets; NA, non-available; 5Y, 5 years after liver transplantation; 10Y, 10 years after liver transplantation.

autoimmunity, Wilson's disease, alpha-1-antitrypsin deficiency) and no dysmorphic features were noted. OLT was performed and immunosuppression (tacrolimus and prednisolone) was started. Two months later, pancytopenia developed with progressive worsening. Fanconi anaemia and diskieratosis congenita were excluded. PCR for EBV, B19 Parvovirus, HSV 1/2/6/7 and enterovirus were negative, as well as serologies for echovirus, toxoplasma, CMV, HVB, HVA, HVC, HIV, *Leishmania* and *Leptospira*. Bone marrow at biopsy was hypocellular with 20% haematopoietic cells affecting all three cell lines. No fibrosis was observed and hemophagocytosis was excluded.

Peripheral blood mononuclear cells were examined by flow cytometry and an inversion of CD4/CD8 was noticed. Primary immunodeficiency was also excluded by conventional tests. Due to progressive worsening reaching very severe AA criteria (Hb 7.2 g/dL; $0.44 \times 10^9/L$ leucocytes; $0.2 \times 10^9/L$ neutrophils; $8 \times 10^9/L$ platelets; bone marrow persisted hypocellular with CD34 counts <1%), and in the absence of a related bone marrow donor, treatment with ATG (2 administrations of 10 mg/kg) and ciclosporin (through levels 80–100 ng/mL) was started 14 months after OLT with a very good haematological response. The outcome of the patient and liver graft was good. Liver biopsy performed 5 years post-OLT was normal. Currently, the patient is aged 16 years and has normal red blood cell count and aminotransferases (table 1).

DISCUSSION

HAAA was first reported in 1955 by Lorenz and Quaiser⁴ and is characterised by pancytopenia following an episode of hepatitis, with at least two of the following criteria: an absolute neutrophil count of $<500/mL^3$, a platelet count of $<20\,000/mL^3$ and a reticulocyte count of $<60\,000/mL^3$, in association with a bone marrow cellularity of $<30\%$.¹ For this purpose, hepatitis is defined as an increase in serum aminotransferase levels to at least three times the upper limit of the normal range.¹ The median time from the diagnosis of hepatitis until the development of AA is 3 weeks.¹ HAAA is more common in young males and the hepatitis can be mild and transient, fulminant or become chronic. We report two cases of HAAA occurring in girls after ALF leading to OLT. Although it has been associated with some

viral infections,^{5,6} none had been proved and the aetiology of HAAA remains unknown. An immune-mediated pathogenesis was suggested because of the evidence of peripheral blood-activated CD8 T lymphocytes and inverted CD4/CD8 ratio,^{17,8} as observed in both cases. The familial history of autoimmunity in patient 2, also supports the immunological disturbance as a contribution to pathogenesis. Additionally, in patient 2 lymphopenia was noted before the OLT. It was previously reported by Honkamen *et al* in three of six children at the onset of hepatitis⁹ and HAAA was also associated with lower lymphocyte counts when compared with other forms of AA.⁷ Concerning treatment, there are two main options: immunosuppressive therapy and HSCT. Although in the first case of HAAA the prognosis was very poor, more recent series reported better treatment response rates.^{10–13} In the cases we are describing, two different treatment modalities were applied with success.

Patient 1 was treated with HSCT from a compatible sibling. When using HSCT, the prognosis is equivalent in HAAA to AA from other causes¹ and if a compatible donor is available, precocious HSCT is recommended, avoiding complications.¹ The subsequent management of PTLD including rituximab, may have contributed for haematological improvement. Moreover, rituximab was previously used to treat severe AA, although not associated with hepatitis.^{14,15}

On the other hand, although response to immunosuppressive therapy was reported to be lower than in other causes of AA,¹⁶ there is growing evidence of a good response to immunosuppression in HAAA¹⁷ (as occurred with patient 2), with outcome and survival at 5 years approaching those of HSCT from a compatible donor.¹¹ If patients are unresponsive to initial treatment, high-dose cyclophosphamide can be offered¹² or, alternatively, HSCT from an unrelated donor, with poorer prognosis when compared with HSCT from compatible donors.¹⁰

We report the long-term follow-up (16 and 6 years) of two cases of HAAA after OLT. In fact, there are few reports of long-term follow-up of this entity. Our patients have been well without major complications either from ALF and OLT or from AA, although different treatment choices were taken. Additionally, although the prognosis is reported to be poorer when the interval between pancytopenia and immunosuppression is longer than 26 days,³

patient 2 (treated within 12 months) had a good response. On the other hand, although one of our patients developed PTLT, no other major complications were found, in contrast with previous reports of high early infection rate and infection-related mortality in the first years after diagnosis of HAAA.¹⁶

Most series report no recurrence after recovering from AA,¹¹ but Al-Nadhi *et al* described a case of an episode of HAAA occurring in adulthood, similar to the episode occurring in infancy.¹⁸ An association with acute lymphoblastic leukaemia was also reported.¹⁹ In the cases we are reporting, during the follow-up, no haematological abnormalities were found after resolution of AA.

In summary, although the follow-up of HAAA was described as very poor, current therapy either with HSCT or immunosuppression with ATG and ciclosporin successfully treat about 70% of patients. We highlight the long-term follow-up of two patients treated differently, without haematological complications and also with stable liver graft.

Learning points

- ▶ Aetiology of hepatitis-associated aplastic anaemia remains unclear.
- ▶ Therapeutic options for hepatitis-associated aplastic anaemia include haematopoietic stem cell transplant or intensive immunosuppression, when a compatible donor is not available.
- ▶ Current treatment strategies for hepatitis-associated aplastic anaemia successfully treat about 70% of patients disclosing a good long-term prognosis without major complications.

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Contributors All authors contributed to this paper. CG was involved in patient follow-up, acquisition of data and draft the paper; SF was involved in acquisition of data, patient follow-up and critical review of the paper; SN was involved in patient follow-up and critical review of the paper; IG was involved in patient follow-up, critical review of the paper and approved the final version.

Competing interests None declared.

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