Hematopoietic Stem Cell Transplantation (HSCT) for Mitochondrial Neurogastrointestinal Encephalopathy (MNGIE): a Single Center Experience Underscoring the Multiple Factors Involved in the Prognosis.

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Abstract

Background: Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE) is a progressive autosomal recessive disorder characterized by cachexia, gastrointestinal (GI) dysmotility, ptosis, peripheral neuropathy and brain MRI white matter changes. Bi-allelic TYMP mutations lead to deficient thymidine phosphorylase (TP) activity, toxic accumulation of plasma nucleosides (thymidine and deoxyuridine), nucleotide pool imbalances and mtDNA instability. Death is mainly due to GI complications: intestinal perforation, peritonitis, and/or liver failure. Based on our previous observations in 3 patients with MNGIE, that platelet infusions resulted in a transient 40% reduction of plasma nucleoside levels, in 2005 we performed the first HSCT worldwide as a life-long source of TP in a patient with MNGIE. Procedure: HSCT was performed in a total of six patients with MNGIE. The multiple factors involved in the prognosis of this cohort were analyzed and compared to the literature experience. Results: Cell source was bone marrow in five patients and peripheral stem cells in one, all from fully HLA-matched related donors, including four who were TYMP mutation carriers. Four of six (66%) survived compared to the 37% survival rate in the literature. Reduced intensity conditioning regimen contributed to secondary graft failure in 2 patients. 15 years post-HSCT the first transplanted patient is seemingly cured. Severe GI symptoms pre-transplantation were mostly irreversible and a poor prognostic factor. Conclusions: Allogenic HSCT could constitute a curative therapeutic option for carefully selected, young, pre-symptomatic or mildly affected patients. Timing, donor selection and optimal conditioning protocol are major determinants of outcome. HSCT is inadvisable in patients with advanced MNGIE disease.

Introduction

Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE) is an ultra-rare, progressive and fatal autosomal recessive disorder. It is a multi-organ disease characterized by cachexia, gastrointestinal dysmotility, ptosis, peripheral neuropathy, sensorineural deafness and leukoencephalopathy with intact cognition in most studied patients. MNGIE is caused by loss of thymidine phosphorylase (TP) activity due to *TYMP* mutations which lead to toxic increase in levels of thymidine and deoxyuridine in plasma and tissues. The accumulation of nucleosides results in unbalanced pools of intramitochondrial deoxynucleotides causing point mutations and deletions, thus impairing mitochondrial DNA (mtDNA) replication and leading to mtDNA depletion.

As a consequence, patients develop progressive mitochondrial respiratory chain oxidative phosphorylation dysfunction resulting in a multisystem mitochondrial disorder. The disease is progressive and death is primarily due to severe malnutrition and gastrointestinal complications such as perforations and peritonitis. Other infections originate frequently from central venous catheters used in patients with MNGIE for chronic total parenteral nutrition (TPN) as the main, sometimes even the only source of nutritional intake. Most patients die between the 3rd and 4th decade.

Over the years, attempts to reduce toxic nucleoside accumulation by hemodialysis failed because of rapid re-accumulation of thymidine. Enzyme replacement therapy provided as erythrocyte-encapsulated TP showed some efficacy in a very few patients but needs to be repeatedly infused. A trial of repeated transfusions of platelets, which are rich in TP, produced transient reduction of nucleoside levels. This therapeutic modality could not constitute a long-term therapy but raised the concept of performing HSCT as a continuous lifelong replacement therapy of TP by the donor-derived cells which are rich in TP Since nucleosides diffuse between extra-and intracellular compartments, TP from normal donor cells has been shown to clear thymidine and deoxyuridine from plasma and presumably also from tissues of patients with MNGIE. We performed repeated platelet infusions in three patients, which resulted in ˜40% transient reduction of the nucleoside levels and led us to perform the first HSCT in a patient with MNGIE in 2005. Since 2005 to 2016 we have performed allogeneic HSCT in 6 patients with MNGIE disease from 3 consanguineous families. Here we describe our single center experience with long-term follow up.

Methods and study design

Data Collection

We conducted a retrospective analysis of patients undergoing allogeneic HSCT for MNGIE disease with long-term follow up at a single center. From July 2005 to June 2016, six patients from 3 consanguineous families with MNGIE disease were referred to Rambam Health Care Center and all of them were treated with HSCT. Partial details of patients 1 and 2 have been previously published. The study was approved by the Institutional Review Board.

The collected data included age and clinical symptoms at diagnosis, TP activity, nucleoside serum level, *TYMP* genotype in the patient and the HSCT donor, age and clinical status at HSCT, conditioning regimen and the clinical, biochemical and genetic (chimerism) consequences post-transplantation, as well as survival and outcome.

MNGIE diagnosis

The diagnosis was based on the patients' clinical, biochemical and genetic features (as depicted in Table 1). Serum deoxyuridine and thymidine were measured using high-performance liquid chromatography. Thymidine phosphorylase activity in buffy coat leukocytes was performed as previously reported in patients 1 and 2.

The mutation causing MNGIE in each family was determined by sequencing the *TYMP* gene as previously reported.

All studied patients underwent thorough gastrointestinal and neurological evaluation including abdominal X-ray and/or computerized tomography (CT), upper endoscopy, brain magnetic resonance imaging (MRI), electromyography and nerve conduction studies prior to and following the HSCT procedure.

Transplantation, outcome and long-term follow-up

Transplant characteristics, including age at transplant, donor type and *TYMP* genotype, graft source, number of cells (total nucleated cells(TNC)/CD34), conditioning protocol, chimerism, biochemical correction (deoxyuridine/thymidine levels) post HSCT and outcome are described in the text in detail and summarized in Table 2. Median follow up of patients alive at discharge from HSCT was 5.5 years, ranging from over 15 years for our first patient to 4 years for the last transplanted patient.

Tables 3 and 4 summarize a literature review of HSCT for MNGIE patients below and above 20 years of age, respectively. The literature review was conducted using the National Library of Medicine's PubMed database through 2019, using the keyword MNGIE with particular interest in HSCT.

Results

Demographics

All patients originated from three highly consanguineous Arab families, two from Northern Israel and one from The Palestine Authority.

The kindred of two families are depicted in Figures 1.A and 1.B.

Pre-Transplant Clinical Characteristics

The salient patients' characteristics pre-transplant are summarized in Table 1. All our patients were diagnosed before the age of 22 years. All had gastrointestinal symptoms at the time of HSCT, which included underweight (patients 1-4) and extreme thinness (patients 5-6). All six patients had abdominal pain as well as recurrent vomiting, which occurred only rarely for patient 4. Only patient 3 had bowel obstruction, necessitating surgical intervention and partial bowel resection. Patients 2 and 3 had a gastrostomy placed for more than a year prior to HSCT to allow decompression of the dilated stomach and symptom relief. Patients 4 and 6 underwent gastrostomy insertion only one month prior to transplantation, for the same indication. Patients 1-3 were on complementary parenteral feeding for nutritional support prior to transplantation. All patients had normal liver transaminases. Pre-transplantation abdominal ultrasound performed in patients 2-6 revealed normal liver size and appearance except for patient 3 who had hepatomegaly with sonographic findings suggestive of fatty liver.

All patients had some degree of clinical signs suggestive of demyelinating polyneuropathy with motor and sensor involvement. Patients 1 and 2 underwent brain MRI before transplant, with abnormal white matter changes (Figures 1.C-E).

All patients had a thorough evaluation for infectious disease risk factors and organ function in relation to HSCT, as per standard practice.

Conditioning Regimen, Transplant, Graft and Donor Characteristics

Transplant characteristics are shown in Table 2. Patients 1 and 2 underwent busulfan-based myeloablative preparative regimens. Patients 3-6 had a treosulfan-based reduced intensity conditioning regimen. All patients were transplanted from a fully 10/10 HLA-matched related donor, including three siblings (patients 2, 5 and 6), one mother (patient 1) and two close family members (patients 3 and 4). The donors for four patients (patients 1, 2, 5 and 6) were heterozygous carriers of the *TYMP* mutation; the donor for patient 4 was wild type and in patient 3, unknown carrier status of donor..

Only patient 1 received peripheral blood stem cells collected from her matched mother, while all other patients were transplanted using bone marrow as the source of stem cells. Median total nucleated cell dose was 7.58 $x10^8$/kg (range 4.2-13.9) and median CD34+ dose was 6.98 $x10^6$/kg (range 3.0-12.4). All had graft versus host disease (GVHD) prophylaxis with cyclosporine and 3 doses of methotrexate.

Engraftment and Chimerism

Neutrophil engraftment with a sustained absolute neutrophil count of greater than 500 (10^9/L) occurred in 5 patients at a median of 15 (range 8-21) days post-transplantation. Platelet engraftment with a sustained platelet count greater than 20,000 (10^9/L) without transfusion occurred at a median of 12 (range 9-16) days post-transplantation. Patient 3 started engraftment with an absolute neutrophil count 400 $x10^9$/L on day +11 with 100% chimerism, however she died before engraftment due to sepsis and multiorgan failure. Patients 5 and 6 engrafted with 90% of donor cells but 3 months post-transplantation started to decrease chimerism; despite early discontinuation of immunosuppression and repeated donor lymphocyte infusions they developed secondary engraftment failure one-year post-transplantation.

Biochemical Correction post-HSCT

Patients 1, 2 and 4, who maintained 100% chimerism, also showed sustained biochemical correction post-HSCT (normal deoxyuridine and thymidine levels in blood). Patient 3, who died 12 days after transplant, showed no biochemical correction. For patients 5 and 6 who had secondary engraftment failure, whereas 23 days post-transplant patient 5 had essentially normal results, nucleoside levels 18 and 14 months post-transplant, respectively, were elevated (all details are summarized in Table 2).

GVHD and Organ Toxicity

Only one patient (patient 1) developed skin symptoms of acute GVHD stage 2-3 during the first 3 months post-transplant. She was treated with steroids and extracorporeal photopheresis with gradual improvement. She had no symptoms of chronic GVHD.

Patient 2 developed multiple complications during the first post-transplantation year: several infections with line sepsis including disseminated candidiasis with infected thrombus in her right atrium, requiring prolonged hospitalization for antifungal and anticoagulant treatment, as well as diabetes mellitus that required insulin treatment. She developed mild to moderate abnormalities of liver function, most probably secondary to prolonged TPN.

Patient 3 developed septic shock and multiorgan failure on day +3, and died on day +12 post-transplantation.

The other patients (4, 5 and 6) had no serious complications related to transplantation (all data is summarized in Table 2).

Survival and Outcome

Four of the six transplanted patients are alive with a median follow-up of 7 (range 4-15) years; only two of the surviving patients (patients 1 and 4) have 100% donor chimerism, whereas patients 5 and 6 developed secondary engraftment failure.

Patient 1 is now 15 years post-transplantation. During the first five years post-transplantation, she complained of mild abdominal pain and poor appetite necessitating the continuation of TPN for 10 years. Over the last 5 years, without requiring TPN, she is progressively, although slowly, gaining weight to her current weight of 43 kg and is seemingly asymptomatic. Her peripheral neuropathy improved clinically and her latest nerve conduction test, performed a year ago, is within normal range. Brain MRI revealed slow progression of the white matter changes over the years (Fig. 1C-E) but despite the MRI changes she has no cognitive deterioration. Over a year ago she had a single episode of loss of consciousness initially suspected to be a generalized seizure. However, a subsequent EEG was normal and there have been no further recurrent episodes. She graduated from high school and college and works in her profession. She has hypergonadotropic hypogonadism, which can be a secondary long-term endocrine complication in a female survivor of HSCT, or a primary abnormal endocrine manifestation of MNGIE.

Patient 2, despite full donor chimerism, continued having recurrent episodes of abdominal pain and vomiting necessitating daily usage of her gastrostomy for stomach drainage was dependent on daily opioids for pain treatment and TPN constantly for 7 years. She remained the same weight as she was prior to transplantation. Six years following HSCT she was admitted frequently with severe abdominal pain, partial bowel obstruction, leukopenia, thrombocytopenia and increased inflammatory markers. An abdominal abscess was found on CT and she was treated conservatively with antibiotics, with temporary improvement. It was not possible to determine if the abscess was a result of intestinal diverticula or micro-perforation of the small intestine. In total, she had 12 hospitalizations during the first 4 years after HSCT and 22 hospitalizations in the next 4 years. She succumbed to her disease seven and a half years post-HSCT form septic shock and multiple organ failure.

Patient 3 died before engraftment on day+12 post-transplantation from sepsis and multi organ failure.

Patient 4 is now 4 years after HSCT, with full donor chimerism and biochemical correction. She still uses her

gastrostomy and is TPN dependent as her main nutritional support. She requires chronic opioid treatment for pain management. During the last 2 years she was admitted several times for central line infection. A recent attempt to stop TPN failed with a subsequent 30% loss of her weight.

Patients 5 and 6 lost their graft during the first year following transplantation. Clinically, patient 5 remained stable during the first year after HSCT with rare and short episodes of abdominal pain and vomiting and stable weight and a second transplant was considered. However, when she was admitted for a thorough assessment before repeating HSCT, is was clear that there was severe gastrointestinal involvement with weight loss, daily vomiting, and distressful abdominal pain. She had electrolytes abnormalities with hypokalemia and contraction alkalosis. A gastrostomy was placed and for a while TPN was given but was stopped due to technical issues and the family's request. Currently, she uses her gastrostomy daily for drainage and attempted feeding via jejunostomy is currently ongoing. At this stage, she is not considered a candidate for HSCT. Her youngest sister (patient 6) also developed secondary engraftment failure and due to severe gastrointestinal involvement, including daily usage of her gastrostomy for stomach drainage and almost daily vomiting, the decision was made not to proceed to a second transplant.

Discussion

In this retrospective study, we present our single center long-term experience of allogeneic HSCT in six patients with MNGIE disease, including the first patient (patient 1) who underwent HSCT. Despite obvious statistical limitations of this relatively small cohort and heterogeneity of pre- and post-HCST characteristics, important lessons can be learned. Detailed assessment of the long-term follow-up of our first patient as compared to the other five as well as to previously reported patients allows us to reinforce previously suggested guidelines and recent position paper on diagnosis, prognosis and treatment of MNGIE, when carefully considering selected patients for this procedure.

The first multicenter experience of allogeneic HSCT for MNGIE published by Halter summarized HSCT in 24 patients with a follow up ranging from 27 months to 8.5 years. The mortality of 15 patients out of a total of 24 was substantial. Overall, they concluded that allogenic HSCT can alter the natural course of MNGIE and therefore should be considered as a therapeutic option for carefully selected patients and that the clinical status of the surviving patients improved significantly over time, even in patients with very severe disease manifestations. This is not inline with our experience showing that patients who exhibited symptoms of progressive disease at the time of transplantation did not improve to any significant extent following HSCT and their GI-related symptoms and complications continued to progress. An important parameter to be followed is weight gain, which occurred very slowly even in patients who resumed oral feeding and were able to ingest high caloric nutrients. In fact, only patient 1, who was transplanted early presumably before the development of nonreversible changes, has started gaining weight years post-transplant.

Age at diagnosis and timing of transplantation are of paramount importance. HSCT should be carried out as early as possible, to maximize the potential for recovery as well as minimizing the risks associated with HCST. In our cohort, the best outcome was achieved in patient 1 who was diagnosed early at age 8 years owing to a family history of two affected cousins (fig. 1A) and had only mild symptoms at the time of HSCT. Patient 3, who was 27 years of age and had severe GI manifestations at the time of transplant, died 12 days post-transplant. This is keeping with the perception that late transplantation is a significant risk factor for HSCT. It is possible, as was previously suggested, and as featured by our patient 1, that longer follow-up is required to evaluate the outcome of these patients. Since patients with MNGIE are born with this progressive mitochondrial disorder, and age of HSCT is of paramount importance, including MNGIE in panels of newborn screening should be considered.

According to the previous consensus proposal for a standardized approach for allogeneic HSCT, careful donor selection is important, with an HLA-identical sibling as the first preferred option, with both non-carriers and heterozygous *TYMP* mutation carriers as potential donors. All our patients were transplanted from a fully 10/10 HLA-matched related donor. The family trees of the two kindred, depicted in Figures 1A and 1B, highlight the role of consanguineous marriages in the causation of fatal, ultra-rare autosomal

recessive disorders such as MNGIE. On the other hand, the detailed plotting of the consanguineous pedigrees enabled tracking and identification of 10/10 HLA-matched donors for all transplanted patients. In general, carrier status of the HSCT donor in inborn errors of metabolism is considered to be a significant factor by influencing post-transplant enzyme levels which appear to be important in determining long-term results, including neurocognitive outcome in some disorders. In our group, four patients were transplanted from heterozygous carriers (patients 1,2,5,6). It is possible that the slow recovery of patient no 1 is related to the fact that she was transplanted from her heterozygous mother, but on the other hand her favorable outcome over time could indicate that carrier status is only one of several factors to be taken into consideration. Therefore, whilst a fully matched related donor who is not a carrier is the preferred donor, our experience suggests that in selected patients, for whom a carrier is the only available fully-matched related donor, it is acceptable to proceed with HSCT from a carrier.

Furthermore, based on published data regarding matched unrelated donors (Tables 3 and 4), 10 out of 12 published patients with MNGIE who underwent matched unrelated donor HSCT engrafted and developed GVHD, 50% of them having grade 3-4 acute GVHD and dying from GVHD or infections. Another known factor for developing GVHD is the source of cells. Only patient 1 received peripheral stem cell collection, which probably contributed to her GVHD, whereas 4/5 patients who received bone marrow as a source of stem cells (excluding patient 3 who died on day +12 post HSCT) did not develop GVHD.

Toxicity of the conditioning intensity regimen needs to be balanced against the risk of secondary engraftment failure while using reduced intensity and taking into consideration the toxic effect of specific medications on the mitochondria. Favorable results can be achieved by careful selection of HLA-matched donors, an adequate number of cells in the graft and strict monitoring of chimerism to detect graft failure early and guide therapeutic approaches to prevent graft loss. In our cohort, four of our patients (patients 3-6) received a reduced to treosulfan-based conditioning regimen instead of a busulfan-based regimen to try and minimize GI toxicity for symptomatic patients. Patient 3 started engraftment with full donor chimerism but died on day 12 post-transplantation from multiple organ failure. Patient 4 had full donor chimerism 3 years post-HSCT and did not have any significant organ toxicity. In contrast, our young patients 5 and 6 engrafted with mixed chimerism and during the first year post-transplant had progressively decreasing donor cells leading to secondary engraftment failure despite repeated infusions of donor lymphocytes. Five out of six published transplanted patients who underwent a non-busulfan-based conditioning regimen or cord blood source had primary or secondary engraftment failure (Tables 3 and 4) in contrast to patients who were conditioned with a busulfan-based regimen. Thus, according to published literature and our own experience, reduced intensity conditioning is not sufficient for these patients with normal bone marrow and a healthy immune system. The conditioning regimen for patients with MNGIE should be busulfan-based with addition of anti-thymoglobulin to achieve a higher percentage of donor chimerism. We conclude that the risk of secondary engraftment failure while using a reduced toxicity conditioning regimen outweighs the risk of organ toxicity associated with a busulfan-based regimen, bearing in mind that a second HSCT might not be possible due to disease progression, as happened with two of our patients (5 and 6).

According to published data, overall survival of transplanted patient with MNGIE is about 37%, but almost all these patients were older than 16 years of ageand had severe manifestations of their disease (Table 3 and 4). Survival in our cohort was 66%.

Overall, our experience reinforces the significance of HSCT timing with regard to HSCT-related risks, as well as a prognostic factor for transplant outcome. We conclude that severe GI symptoms are mostly irreversible and constitute a poor prognostic indicator both short and long-term. HSCT should therefore be carefully considered in those patients.

In conclusion, our present study emphasizes the short and long-term outcome of allogenic bone marrow transplantation as a curative option for patients with MNGIE if the following factors are carefully followed: early diagnosis before irreversible GI symptoms occur, careful selection of HLA-matched related donor and the use of a busulfan-based conditioning regimen. Further studies might lead to other promising therapeutic options and future developments such as newborn screening may contribute to early diagnosis.

Conflict-of-interest disclosure :

All authors declare no conflict of interest.

References:

FIGURE CAPTIONS

Figure 1 **Pedigrees of studied kindred and axial FLAIR brain MRI images of patient 1.**. A, Kindred of patient 1 (patient VII-7). A known family history of MNGIE (patients VII-1, VII-2) led to early diagnosis of patient 1 at eight years of age. The mother (individual VI-4) was a fully matched HLA (10/10) donor for her daughter; B, Kindred of three siblings: patient 2 (patient V-2), patients 3 (patient V-1) and patient 4 (patient V-6). All donors were fully HLA-matched related donors: individual V-7 for patient 2, individual IV-6 for patient 3 and individual V-17 for patient 4; C, Axial FLAIR brain MRI of patient 1 showing pre-HSCT abnormal high signal intensity of the deep and periventricular white matter, as well as foci of high intensity in both frontal lobes with sparing of the subcortical U-fibers. Brainstem, shown here at the level of middle cerebral peduncles, is unaffected; D, Six years post HSCT. Minimal progression visualized in the deep and periventricular white matter. New foci of high signal intensity were recognized in the brainstem; E, Thirteen years post HSCT. Progression of the abnormal signal intensity in the white matter of both hemispheres. Minimal progression in brainstem structures. Of note, it is possible that part of the progression of MRI changes may be related to improvement in image quality over the years.

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`Table 1 Patients characteristics before HSCT.pdf` available at https://authorea.com/users/362729/articles/483800-hematopoietic-stem-cell-transplantation-hsct-for-mitochondrial-neurogastrointestinal-encephalopathy-mngie-a-single-center-experience-underscoring-the-multiple-factors-involved-in-the-prognosis

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