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Research Article

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Abstract

Background: Acute graft-versus-host disease (aGVHD) is a life-threatening complication after allogeneic hematopoietic stem cell transplantation. First-line treatment of aGVHD is corticosteroid. Second-line therapy mainly comprises immunosuppressants. However, ~ 25% of the patients have a steroid-resistant and therapy-refractory disease, which is associated with a very poor prognosis. An alternative therapy option for steroid-refractory and therapy-refractory aGVHD is the use of mesenchymal stromal cells (MSCs). Here, we report the results of 88 patients with grade III-IV aGVHD treated with human umbilical cord derived mesenchymal stromal cells (UC-MSC).

Methods: There were 18 children and 70 adults with grade III/ IV aGVHD (82% grade IV). These patients were either resistant to steroids or refractory to 1–5 additional immunosuppressants. UC-MSCs were transfused at a median dose of $1 \times 10^6$ cells/kg with a median of 4 times (range, 1 to 16).

Results: Median time between the onset of aGVHD and the first infusion of UC-MSC was 7 days (range, 3–88). The day 28 overall response rate was 51.14%, of these, 24 patients (27.27%) showed complete remission (CR), 21 (23.86%) showed partial remission (PR). The estimated survival probability at 100 days was 43.3%. And after a median follow-up of 66 months (26–122 months), the survival rate was approximately 33% (29/88). Patients developed acute gastrointestinal (GI) tract and liver GVHD showed worse overall response in day 28 than patients only with acute GI GVHD (21% vs. 58%; p= 0.037). No patient had severe side effects.

Conclusions: These results suggest that UC-MSC treatment was safe and effective in children and adults, and should be considered for treating steroid-refractory aGVHD.


Background

Acute graft-versus-host disease (aGVHD) is a frequent complication after allogeneic hematopoietic stem cell transplantation (HSCT), mainly affecting skin, liver and gastrointestinal (GI) tract. In clinical practice, acute GI GVHD needs more attention since the grade III to IV acute GI GVHD usually lead to poor prognosis. Despite of GVHD prophylaxis, approximately 40%~60% transplantation recipients develop aGVHD(1). The accepted standard first-line therapy for aGVHD is corticosteroid, to which about 40–50% grades II to IV acute GVHD patients respond(2). The prognosis of patients who had grade III to IV is dismal with a 2-year survival of 20% and 5 year survival 8%(3). And the survival of patients who are refractory to steroids is even worse (10%). Currently, there is no clear option for second line treatment to improve the outcomes of these patients, partly because of drug-specific side effects and increased risk for infectious complications(2, 4).
Mesenchymal stem cells (MSCs) are a heterogeneous population cells possess multi-lineage differentiation potential, nonspecific immunosuppressive activities and immunomodulatory effects, and can be isolated from bone marrow, adipose tissue, placental tissues and umbilical cord\(^{(5, 6)}\). In 2004, LeBlanc et al firstly reported the effectiveness of haplo-identical BM-derived MSC therapy in a pediatric patient with severe treatment-resistant grade IV gut and liver aGVHD\(^{(7)}\). In 2008, a first phase II, multicenter clinical trial in patients with steroid-resistant severe aGVHD was conducted, 39 of 55 patients responded to MSCs treatment with no side-effect infusions observed\(^{(8)}\). Since then, MSCs have been considered as a candidate treatment option for patients suffering from steroid-resistant and therapy-refractory aGVHD.

Discussion is ongoing on the optimal source of MSCs even though most studies have been conducted using bone marrow-derived MSCs \(^{(7, 9–22)}\). However, to harvest bone-marrow MSC is an invasive procedure which may cause pain, infection or hemorrhage. And the quality of BM-MSCs is associated with donor age\(^{(23)}\). Compare to BM, human umbilical cord (UC) is an abundant source of MSCs \(^{(24, 25)}\) since it is a kind of “waste” after delivery. Besides, the harvesting procedure of UC-MSC is non-invasive, and has no ethical issues. However, there are not many of clinical trails to treat aGVHD with UC-MSCs\(^{(26, 27)}\). Our group has been long studied how to obtain abundant MSCs from UC efficiently\(^{(28, 29)}\). In this study, we report the outcomes of 88 patients with steroid-resistant and treatment-refractory acute GI GVHD treated with UC-MSCs as a salvage therapy. As far as we know, this is the largest cohort of acute GI GVHD patients receiving UC-MSCs reported so far.

**Subjects And Methods**

**Patients**

We conducted an open-label, single-center, self-control and phase I/II study between November 2009 and April 2018. Patients of all ages with grade III to IV steroid-resistant and treatment-refractory acute GI GVHD were eligible for this study. In total, 88 patients were enrolled in this study, 84 developed acute GI GVHD after allogeneic HSCT and four after donor lymphocyte infusion.

**GVHD**

Grading and staging of acute GI GVHD was performed using the Seattle-Glucksberg modified criteria. Steroid-resistance was defined as GVHD symptoms does not improve for 5 days or worsen over 3 days of treatment with 2mg/kg per day steroids\(^{(4)}\). Treatment-refractoriness was defined as progression or no improvement in aGVHD despite 5 days of two or more second-line therapy (CD25 monoclonal antibody, tacrolimus, mycophenolate mofetil, CsA, azathioprine, sirolimus, maraviroc, ATG, methotrexate and CTX).

**MSC Manufacture**

MSCs were derived from the umbilical cord of unrelated HLA-mismatched donors. UC-MSCs culture and expansion were undertaken by modifying methods published previously\(^{(28, 29)}\). Briefly, umbilical cords
are washed with phosphate buffered saline (PBS; Gibco, Grand Island, NY, USA) repeatedly and shredded into small pieces. The tissues are digested with 0.05% type II collagenase (Sigma, St Louis, USA) at 37°C for 2–3 h, then cell suspension is collected by filtering through a stainless steel mesh. After washing with PBS twice, cells are resuspended in serum-free MSC culture media and seeded in 75cm² culture bottles. After culture at 37°C in 5% CO₂ atmosphere with over 95% humidity for 72h, non-adherent cells are discarded. Media are changed every 3 to 4 days until cells can be observed growing to 80–90% confluence. The adherent cells were detached with 0.05% Trypsin and 0.01% EDTA (Gibco, Grand Island, NY, USA). Cells are passaged at 4000–6000 cell/cm². Fifth-passage cells are frozen at 5×10⁶/ml, 2ml per tube for following infusions. Each batch of UC-MSCs were identified for their phenotype by flow cytometry and in some cases tested for their ability to differentiate into adipocytes, osteoblasts and chondrocytes. The UC-MSCs suspensions were culture negative for bacteria and mycoplasma before infusion.

**MSC Administration**

MSC treatment was started as early as possible after occurrence of steroid-resistant and therapy-refractory grade III-IV acute GI GVHD. Patients were given 1×10⁶/kg UC-MSCs infusion once or twice a week according to their severity of symptoms, concomitantly with second line therapy (Table 1) until aGVHD showed a response.

**Evaluation Points**

Primary end point in this study was response (complete, partial, overall) rate. Complete response (CR) was defined as loss of all symptoms of aGVHD, partial response (PR) was defined as clinical improvement of at least one GVHD grade. Overall response (OR) included CR and PR. Response to MSC treatment was evaluated on day 28, day 56 and day 100 after the first MSC infusion or on the date of death if it occurred before 28 days. No response (NR) was defined as patients with stable disease (SD) who showed no change or patients with progressive disease (PD) who showed worsening of symptoms.

**Statistical analysis**

The response rates per categories were compared using Fisher’s exact test. We estimated the probability of survival with the Kaplan–Meier method and compared using log-rank test. A P-value of < 0.05 was considered statistically significant. Statistical analyses were performed using the statistical software R.

**Results**

**Patient Characteristics**

We report 88 patients with steroid-resistant and therapy-refractory grade III-IV acute GI GVHD who were treated with UC-MSC from November 2009 to April 2018 at the Fifth Medical Center of PLA General
Hospital. Patient characteristics are summarized in Table 1.

Median age of patients was 27.5 years old (range, 11–54 years). In 38 patients, acute myeloid leukemia (AML) was the indication for allogeneic HSCT. Other patients received allogeneic transplantation for acute lymphoblastic leukemia (ALL) (n = 27), myelodysplastic syndrome (n = 8), chronic myeloid leukemia (CML) (n = 5), AA (n = 3), NHL (n = 2) and other diseases (n = 5). HSCT was performed with granulocyte colony-stimulating factor mobilized peripheral blood stem cells (n = 86) or bone marrow (n = 1) or both (n = 1) of HLA-compatible (n = 19) or partially HLA matched donors (n = 69) after myeloablative regimen. Forty four patients received ATG as a part of their conditioning regimen. GVHD prophylaxis mostly comprised CsA + methotrexate + MMF (n = 45). All aGVHD occurred while receiving prophylactic immunosuppressive drugs.

Median time from HSCT or DLI to the onset of aGVHD was 37 days (range, 7–216 days). Eighty-four patients had de novo aGVHD and 4 had secondary aGVHD (occurred after donor lymphocyte infusion). Majority of patients (n = 72; 82%) had grade IV acute GI GVHD, 19 (21.6%) patients developed acute GI and liver GVHD.

**Treatments Of Agyvd Before Mscs Infusions**

All patients received steroids as first-line treatment for aGVHD and did not respond. 31 (35.2%) patients received one or two second-line immunosuppressive drug, while 57 (64.8%) patients did not respond to three or additional immunosuppressive therapies.

Median time from diagnosis of aGVHD to the first UC-MSC infusion was 7 days (range, 3–88 days). Cell dose per infusion was $1 \times 10^6$/kg bodyweight. Each patient received a median of 4 infusions (range 1–16). Uc-MSC infusions were not associated with any acute or late side effects during or after infusion.

**Response To Msc Treatment**

At day 28, over half the patients achieved an overall response (OR) (45/88, 51.14%), including CR in 24 patients (27.27%), PR in 21 (23.86%); 43 patients were classified as nonresponders because of NR (n = 23, 26.14%) or PD (n = 14, 15.91%) or died (n = 6, 6.82%)(Table 2). Patients developed acute GI and liver GVHD showed significantly worse overall response in day 28 than patients only with acute GI GVHD (21% vs. 58%; p = 0.037)( Table 3, Fig. 1).

Clinical responsiveness did not differ between different age groups: Of children under 18 years old, 6 (35.29%) reached CR and 2 (11.76%) PR by day 28. Among those 20 patients at 18–25 years of age, 5 (25%) achieved CR, 6 (30%) PR; Among those 51 patients above 25 years of age, 13 (25.49%) achieved CR, 13 (25.49%) PR (Table 4). No significant differences were noted between patients receiving once or twice UC-MSC infusions per week (OR: 47% vs. 56%; p = 0.78) (Table 5). No differences were seen in patients who was in CR1 status or not before transplantation (OS 49% vs. 52%, p = 0.917) (Table 6).
Survival

The overall survival (OS) at 100 days was 43% for the entire cohort of patients, and 56% for children (P = 0.442). The OS for the group of only with acute GI GVHD was 53% and in the liver involved group was 11% (P = 0.001). 29 (33%) patients were alive at last follow-up (July 2020), with a median follow-up of 66 months (26–122 months) from the first infusion of UC-MSCs. All the survivors did not have recurrence of the original disease or secondary tumors. Reasons of death for the treated patients were related to aGVHD (29), infection (7), original disease relapse (5), diffuse alveolar hemorrhage (5), multi-organ failure (3) and others (8).

Discussion

Steroid-resistant and therapy-refractory aGVHD is still the main cause of allogeneic HSCT related mortality. The 2-year nonrelapse mortalities of these patients were up to 84% (30). Since the first report of MSC administration to treat a child with refractory steroid-resistant aGVHD (7), numerous studies confirmed the potential benefit of this therapy to steroid-refractory aGVHD. However, there are not many of clinical trails to treat aGVHD with UC-MSCs (26, 27). Compare to BM, UC is an abundant source of MSCs (24, 25) since it is a kind of “waste” after delivery. Besides, the harvesting procedure of UC-MSC is non-invasive, and has no ethical issues. What’s more, UC-MSCs revealed higher potential of immunomodulatory capacity than BM-MSCs (31). To the best of our knowledge, this is the results of so far the largest single-center cohorts of 88 patients receiving UC-MSCs to treat steroid-resistant and therapy-refractory acute GI GVHD. Though the findings have some limitations as it is a single-center retrospective study and there is no control group, it can be viewed as a real-world scenario to assess the therapeutic effect of UC-MSCs. In our study, no patients showed any adverse effects due to UC-MSC infusions, the application of UC-MSCs was confirmed to be safe.

Our results showed that UC-MSC infusion to treat steroid-resistant and therapy-refractory acute GI GVHD has led to an overall response rate (OR) of 51.14% (45 of 88 patients) by day 28. Le Blanc et al. reported an overall response rate of 71% (39 of 55 patients), 84% in children and 60% in adults (8). However, the response of the treatment was assessed at a median of 18 days (3–63 days) after the first MSC infusion. In our study, we used day 28 response as an outcome parameter, according to updated recommendations (32). Kaipe et al. (33) summarized the data of 190 patients with aGVHD treated with MSCs of published studies and found a CR of 52% and PR of 23% (75% OR) after 4 weeks of MSCs infusion. A possible explanation for the inferior results we reported could be the disease severity of our patients: in our study, most of our patients (82%) had grade IV aGVHD. This is a more severely disease cohort than in most published series. Besides, the only prospective, randomized, double-blind phase III placebo-controlled trial of an industrial third-party BMSC product (Prochymal) for treating steroid-refractory GVHD reported no significant differences in effects between BMSC and placebo-treated groups. This indicates the complex of this disease and the difficulty to value a single treatment in supplement with other second-line immunosuppressive therapies — most of our patients had received more than two additional treatments before UC-MSC infusion. Still, these results we obtained with such a highly
challenging patient cohort (18% grade III and 82% IV) suggests the advantage of the treatment of aGVHD with UC-MSC.

Liver involvement along with GI aGVHD is associated with a worse response in our study. This probably cannot be regarded as a MSC-specific predictor since it has been frequently identified in non-MSC approaches.

We did not observe any difference in response rates between children and adults (P = 0.76) and this disagrees with most of the studies demonstrated a trend towards a better clinical response in children(11, 12). Our pediatric cohort demonstrated an OR of 46%, which in inferior to that reported by Introna et al(10) of 66.7%. However, in their patient cohort, only 25% of the patients exhibited aGVHD over grade III, whereas in our cohort, 23.5% of the patients exhibited grade III aGVHD and 76.5% grade IV, this may be a possible explanation for our finding.

In addition, MSC infusion frequency, once a week or twice a week, has not been shown to affect response rates. Among the final survivors, 14 patients received UC-MSCs once a week, 13 patients twice a week, and 2 patients only received UC-MSCs for one time. And from our experience, the UC-MSCs will be announced invalid after 4-week treatment.

The survival at the last follow-up in July 2020 in our cohort, with a median follow-up of 66 months (26–122 months) from the onset of aGVHD, was approximately 33% (29/88), better than that of a previous report with an OS of 22% for their MSC-treated patients with GVHD after a median follow-up of 767 days (range 74–1270 days)(34). To be more specific, the survival rate was 25.7% in our adult cohort, which was similar with the result of von Dalowski et al.(35), while 61.1% in the child cohort. In our study, children have significant better survival rate than adults.

Conclusion

In conclusion, UC-derived MSC infusion is safe in this large cohort of patients with steroid-resistant and therapy-refractory III-IV acute GI GVHD and is effective in 51% of patients. This therapeutic modality could be considered as an important option. And we recommend MSCs could be used as early as along with other second-line treatment, rather than after the failure of second-line treatment. Further insights into MSCs biology and preclinical handling are still needed to optimize in randomized, placebo-controlled, double-blind studies.

Abbreviations

acute graft-versus-host disease (aGVHD), mesenchymal stromal cells (MSCs), umbilical cord (UC), umbilical cord derived mesenchymal stromal cells (UC-MSC), hematopoietic stem cell transplantation (HSCT), gastrointestinal (GI)

Declarations
Ethics approval and consent to participate

The study was approved by the ethics committees of the Fifth Medical Center of PLA General Hospital. The study was registered at www.clinicaltrials.gov (#NCT01754454). Patients or their legal guardians provided written informed consent.

Consent for publication

Patients or their legal guardians provided written informed consent.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing Interests

The authors declare that they have no competing interests.

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Authors’ contributions

JWN managed the patients, collected, analyzed and interpreted the patient data, and was a major contributor in writing the manuscript. YHL managed the patients, collected the data and set up the database. CX managed the patients and collected the data. HXS and CT provided the UC-MSCs. HMN, JWH, JLC, JW, XL, NL, YFS, YS, ZQQ, LW, YZ, SCL, JX, JR managed the patients. HC designed the clinical trial and supervised the Implementation. BZ designed the clinical trial and supervised the UC-MSC preparation. Liangding Hu designed the clinical trial, supervised the implementation and took part in writing. All authors read and approved the final manuscript.

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References


Tables

Tables 1 to 6 are available in the Supplementary Files section.

Figures
Figure 1

One-year survival estimates for patients with steroid-resistant and therapy-refractory III-IV acute GI GVHD from time of first MSC infusion. (A) according to MSC infusion frequency; (B) according to age groups; (C) according to disease status before HSCT; (D) according to whether liver aGVHD involved.

Abbreviations: aGVHD, acute graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; CR, complete response; MSC, mesenchymal stem cell.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1CharacteristicsOfPatients.xlsx
- Table2GVHDResponseAndOutcome.xlsx
- Table3GVHDResponseAndOutcomeAccordingToLiver.xlsx
- Table4GVHDResponseAndOutcomeAccordingToAgeGroups.xlsx
• Table5GVHDresponseandoutcomeaccordingtofrequency.xlsx
• Table6GVHDresponseandoutcomeaccordingtostatus.xlsx