Effects and long-term follow-up of using umbilical cord blood-derived mesenchymal stromal cells in pediatric patients with severe BK virus-associated late-onset hemorrhagic cystitis after unrelated cord blood transplantation

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Abstract
This is a retrospective study to evaluate the efficacy and safety of umbilical cord blood-derived mesenchymal stromal cells (MSCs) for the treatment of pediatric patients with severe BK virus-associated late-onset hemorrhagic cystitis (BKV-HC) after unrelated cord blood transplantation (UCBT). Thirteen pediatric patients with severe BKV-HC from December 2013 to December 2015 were treated with MSCs. The number of MSCs transfused in each session was $1 \times 10^6/kg$ once a week until the symptoms improved. The median follow-up time was 1432 (89-2080) days. The median frequency of MSC infusion was 2 (1-3), with eight cured cases and five effective cases; the total efficacy rate was 100%. The copy number of urine BKV DNA was $4.43 \times 10^8/mL$ before MSC infusion and $2.67 \times 10^9/mL$ after MSC infusion; the difference was not significant ($P = .219$). There were no significant differences in the overall survival, disease-free survival, and the incidence of relapse and acute and chronic graft-versus-host disease between the MSC infusion group and non-MSC infusion group. There was also no significant difference in the cytomegalovirus, Epstein-Barr virus (EBV), and fungal and bacterial infection rates between the two groups. Although umbilical cord blood-derived MSCs do not reduce the number of BKV DNA copies in the urine, the cells have a high efficacy rate and minimal side effects in treating severe BKV-HC after UCBT among pediatric patients. MSCs do not affect the rates of relapse, long-term infection, or survival of patients with leukemia.

KEYWORDS
BK virus, hemorrhagic cystitis, mesenchymal stromal cells, unrelated cord blood transplantation

Abbreviations: aGVHD, acute graft-versus-host disease; BCNU, carmustine; BKV, BK virus; BKV-HC, BK virus–associated late-onset hemorrhagic cystitis; cGVHD, chronic graft-versus-host disease; CMV, cytomegalovirus; DFS, disease-free survival; EBV, Epstein-Barr virus; GVHD, graft-versus-host disease; HC, hemorrhagic cystitis; HSCT, hematopoietic stem cell transplantation; JCV, John Cunningham virus; MSCs, mesenchymal stromal cells; OS, overall survival; PTLD, post-transplant lymphoproliferative disorder; UCBT, unrelated cord blood transplantation.
1 | BACKGROUND

Unrelated cord blood is currently one of the important alternative donors for hematopoietic stem cell transplantation (HSCT) in patients with malignant hematological diseases without compatible sibling donors. Hemorrhagic cystitis (HC) is a common and serious complication after unrelated cord blood transplantation (UCBT). The main manifestations of HC are severe hematuria, urinary frequency, urinary urgency, and dysuria. Severe HC can cause urinary tract obstruction, renal failure, hemorrhagic anemia, and death, which seriously affects the quality of life of the patients. At present, HC can be classified according to the time of occurrence as early-onset HC and late-onset HC. Early-onset HC is generally related to the use of cyclophosphamide, isocyclophosphamide, busulfan, and etoposide. Late-onset HC is generally related to post-transplantation viral infections, including those with the BK virus (BKV), cytomegalovirus (CMV), adenovirus, and John Cunningham virus (JCV). Among these, the most common is BK virus–associated late-onset hemorrhagic cystitis (BKV-HC). The incidence of rapid HC has been markedly reduced owing to prophylactic measures, such as hyperhydration, alkalinization, diuresis, and mesna administration. To date, late-onset HC has been the most widely studied HC type. Current studies have confirmed that 70%-100% of late-onset HC cases are related to BKV infection. However, antiviral drugs in China, including ganciclovir, valganciclovir, penciclovir, ribavirin, and foscarnet, have no apparent effect on BKV infection. There is no listing of cidofovir for treating BKV infections in China, and the treatments for BK virus–related HC are limited. Oral leflunomide and oral/intravenous ciprofloxacin are effective in some adult patients but not in children; thus, the treatment options for the affected children are additionally limited. Mesenchymal stromal cells (MSCs) have been used for treating HCs both internationally and locally. However, the average number of cases was relatively small, and the follow-up time was relatively short; further, there are no reports on MSCs from umbilical cord blood for the treatment of pediatric BK virus–associated HC. In this study, we investigated the treatment of severe pediatric BK virus–associated HC after HSCT with MSCs from umbilical cord blood in our transplantation center for the first time.

| Patients | Diseases | Age | Conditioning regimen | HC grade | aGVHD |
|---|---|---|---|---|---|
| 1 | ALL | 14 | Flu + BU + CY | III | II |
| 2 | AML | 11 | Ara-C + BU + CY + BCNU | III | I |
| 3 | ALL | 9 | Flu + BU + CY + BCNU | IV | IV |
| 4 | ALL | 11 | Flu + BU + CY + BCNU | III | NA |
| 5 | ALL | 7 | Flu + BU + CY + BCNU | III | NA |
| 6 | ALL | 11 | Flu + BU + CY | III | NA |
| 7 | ALL | 8 | Flu + BU + CY | III | I |
| 8 | ALL | 10 | Flu + BU + CY + BCNU | III | I |
| 9 | AML | 7 | Flu + BU + CY | III | I |
| 10 | AML | 3 | Flu + BU + CY + BCNU | IV | III |
| 11 | AML | 8 | Flu + BU + CY + BCNU | III | NA |
| 12 | ALL | 11 | Flu + BU + CY + BCNU | IV | II |
| 13 | ALL | 10 | Flu + BU + CY + BCNU | IV | I |

**TABLE 1** Patient characteristics of III–IV HC patients who treated with MSC

Abbreviations: aGVHD, acute graft-versus-host disease; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; Ara-C, cytarabine; BCNU, comoxetine; BU, busulfan; CY, cyclophosphamide; FLU, fludarabine; HC, hemorrhagic cystitis.
conditioning regimen. A patient with acute myeloid leukemia who was not in complete remission before transplantation was treated with a conditioning regimen including high-dose cytarabine (2 g/m² q12h for 1-2 days) + busulfan (12.8-19.2 mg/kg, adjusted according to the patient body weight) + cyclophosphamide (120 mg/kg). All patients were treated with cyclosporine A (Novartis) and mycophenolate mofetil (Roche) for graft-versus-host disease (GVHD) prophylaxis.5

2.3 | Prevention and supportive treatment of infection

Infection prophylaxis consisted of the administration of oral fluconazole, cefprozil, and SMZco. Voriconazole or posaconazole was used to prevent fungal formation in patients with previous fungal infections. Intravenous acyclovir was added to prevent viral infections >1 day after transfusion of the cord blood. Ganciclovir or foscarnet was administered as the preemptive CMV therapy. Heparin sodium and prostaglandin E1 were administered to prevent hepatic venous occlusion disease during the conditioning regimen. Heparin sodium was discontinued when the platelet count was below 20 × 10⁹/L. Granulocyte colony-stimulating factor was added at >6 days after transplantation until the leukocyte count reached >4×10⁹/L and became stable for 3 days. Red blood cells were transfused when the hemoglobin level was <60 g/L, and platelets were transfused when the platelet counts were <20×10⁹/L.

2.4 | Prevention of HC

From the beginning of the conditioning regimen, all patients underwent hydration and alkalization, and the daily fluid infusion volume was 3 L/m², of which alkaline solution accounted for one-third of the volume, maintaining a urine pH of >8.0. From forced diuresis, the urine volume reached 100-150 mL/h. The daily statistics maintained the balance between the incoming and outgoing volumes. Furthermore, mesna was administered at the same time as cyclophosphamide and after 3, 6, and 9 hours. The total dose of cyclophosphamide administered was 100%-120%.

2.5 | Diagnosis and grading of BKV- HC

BKV- HC was diagnosed mainly on the basis of the following two aspects: (a) symptoms of late-onset HC, including urinary frequency, urinary urgency, dysuria, and hematuria (except for urinary tract hemorrhage caused by thrombocytopenia and symptoms of bacterial HC, intravascular hemolysis, and septicemia) and negative bacterial and fungal culture findings in the urine (excluding the presence of other viruses in the urine, such as adenovirus, CMV, and JCV (John Cunningham virus)) and (b) evidence of BKV replication: Late-onset HC is associated with the BKV when more than 10⁶ copies of BKV DNA are found in the urine. The grading of BKV-related HC is the same as that of general HC: Only microscopic hematuria is categorized under degree I; naked hematuria under degree II; naked hematuria with blood clot under degree III; and hemorrhage causing blood transfusion dependence, urethral obstruction, or renal function damage under degree IV. Grade III to IV late-onset HC is considered severe.6

2.6 | Other definitions

Acute GVHD (aGVHD) was diagnosed and graded according to previously published criteria.7 Chronic GVHD (cGVHD) was classified as mild, moderate, or severe according to the 2014 National Institutes of Health consensus criteria.8 Relapse was defined by the morphological evidence of the disease in the peripheral blood, bone marrow, or extramedullary sites; the time to relapse was defined as the number of days from transplantation to the first diagnosis of relapse.9 The overall survival (OS) was defined as the number of days from transplantation to death of any cause. Disease-free survival (DFS) was defined as the number of days from transplantation to the first diagnosis of relapse or death.9

For infections occurring 100 days after transplantation, we compared the following aspects: Severe bacterial infections were defined as sepsisemia or those requiring hospitalization; bacterial agents identified or responding to antibiotics. Severe fungal infections were defined as fungemia or invasive fungal disease, including suspected, clinical, and definitive diagnoses of such infections. The ratio of the CMV-DNA copies (>10¹) and the incidence of CMV infection were compared between the two groups. The proportion of Epstein-Barr virus (EBV)-DNA (>10¹) and the incidence of post-transplant lymphoproliferative disorder (PTLD) were also compared between the groups. The causes of death included infections after the resolution of GVHD and in the absence of leukemia relapse.

2.7 | Preparation of umbilical cord blood-derived MSCs

The umbilical cord blood-derived MSCs (UCB-derived MSCs) were purchased from Shandong Cord Blood Bank. The umbilical cord was obtained under sterile conditions and immersed in serum-free DMEM/F12 medium, and the composition of the culture medium is the core technical information of the bank. The patent number of the MSC is ZL 2009 1 0260704.1. The remaining blood was removed by washing with PBS solution containing penicillin and streptomycin sulfate. The umbilical cord was cut into 1 × 1 × 1 mm pieces by removing the arteries, veins, and envelopes. The adherent cell culture method and enzymatic digestion method (digested by 0.1% collagenase II and 0.25% trypsin, respectively) were used. The PBS solution was washed and inoculated in a 25-cm² culture flask (1.0 × 10⁶/cm²). A cell
culture box was cultured at 37°C, 5% CO₂, and saturated humidity. The culture medium was replaced after approximately 1 week. When the monolayer adherent cells were nearly 80% confluent at 10-14 days, they were digested with 0.25% trypsin and passaged at 1:2. Thereafter, the cells were continuously subcultured in vitro at 1:2 when they reached ~80% confluency with cell expansion. When the cells were cultured to the third generation and were nearly 80% confluent, they were digested with 0.25% trypsin and collected. We use fresh MSCs to treat hemorrhagic cystitis. MSCs were harvested and used at passages 3, and all cell culture steps were performed in good manufacturing practices facility. Approximately 1.0 × 10⁶ cells were suspended in 100 mL PBS solution and analyzed using a flow cytometer. The phenotype of UCB-MSCs is positive for CD73, CD90, and CD105 but negative for CD11b, CD14, CD34, CD45, CD79a, and HLA-DR surface markers according to the International Society for Cellular Therapy (ISCT) released criteria.

2.8 | Treatment of grade III to IV HC with umbilical cord blood–derived MSCs

Four patients had grade III to IV HC at onset. Nine patients had grade I to II HC at onset but advanced to grade III to IV HC after routine hydration, alkalization, antiviral therapy, and bladder irrigation. Once diagnosed with severe HC, the patients were infused with umbilical cord blood–derived MSCs once a week until their symptoms were relieved. The number of MSCs infused in each session was 1 × 10⁶/kg. Dexamethasone (2.5 mg) was administered to prevent anaphylaxis before infusion for approximately 30 minutes.

2.9 | Criteria for the observation and judgment of the therapeutic effects

Follow-up was conducted until August 31, 2019. All patients with HC were routinely tested for BKV DNA, CMV-DNA, JCV-DNA, and adenovirus DNA in the blood and urine twice a week. After treatment, the urine color and presence of urinary frequency, urinary urgency, and dysuria were observed daily; the routine urinary and blood test results were also monitored. The treatment efficacy was categorized as follows¹: (a) cure: resolved symptoms and signs and no gross hematuria and microscopic hematuria (routine urinary examination without red blood cells); (b) effective: improved clinical symptoms and signs (recovered at least 50%) and laboratory results (required routine urinary examination with red blood cells; decreased by at least 50%); and (c) invalidity: persisted or worsened HC-related symptoms and signs and non-reduction in the red blood cell count by more than 50% in the routine urinary examination. The total efficacy rate was calculated as follows: (number of cured cases + number of effective cases)/total number of cases ×100%.
2.10 | Statistical analysis

The two independent samples t test and Fisher’s exact test were used to compare the continuous variables between the two groups. The paired samples t test was used to compare the continuous variables before and after treatment. The chi-square test was used to compare the data on the classifications. The Kaplan-Meier method was used to compare the survival curves and the log-rank test to compare the survival rates between them. SPSS 22.0 was used for the statistical analysis. The rates of relapse and aGVHD were calculated using R software (version 2.11.1), with death as a competitive risk factor.

3 | RESULTS

3.1 | Effect of MSCs

The median follow-up time in the 13 pediatric patients was 1432 (89-2080) days. The onset time of HC was 33 (17-42) days and that of III-IV HC was 38 (25-49) days. The median frequency of MSC infusion was 2 (1-3); there were eight cured cases and five effective cases, and the total efficacy rate was 100%. The disappearance time of gross hematuria was 10 (1-25) days after MSC infusion and that of microscopic hematuria in the eight cured cases was 13 (1-27) days after MSC infusion. All patients had positive BKV DNA findings in their urine. The number of BKV DNA copies in the urine was 4.43 (0.36-56.9) × 10^8/mL before MSC infusion. Negative urine BKV DNA findings were observed in one patient after MSC infusion. After hematuria disappeared under microscope in the eight cured cases, gross hematuria disappeared in the five effective cases. The number of BKV DNA copies was 2.67 (0-56.3) × 10^8/mL. There was no significant difference before and after MSC infusion (P = .219). Two patients had increased creatinine levels; their creatinine levels returned to normal after MSC infusion (Table 2).

3.2 | Safety of the MSC therapy

3.2.1 | Rapid side effects

No fever, rash, or other allergies occurred in all patients during MSC infusion; there were also no cardiovascular or cerebrovascular events.

3.2.2 | Recent and long-term infections

The recent infection rate after MSC infusion was compared with that before MSC infusion and within 3 months after MSC infusion. No patients had worsened bacterial or fungal infections or had new bacterial or fungal infections within 3 months after MSC infusion. After MSC infusion, there was no significant difference in the number of CMV-DNA copies in the blood before and after MSC infusion [3.4 (0-16.2) × 10^2/mL vs 2.2 (0-13.4) × 10^2/mL, P = .243]. There was no EBV infection or other viremia before and after MSC infusion. Because many late infections were defined as those occurring 100 days after transplantation and MSC infusion was conducted within 100 days, the long-term viral, bacterial, and fungal infection rates will be compared between the MSC infusion group and the non-MSC infusion group after 100 days (Table 3). Fungal infections included suspected, clinically diagnosed, and confirmed infections. The diagnostic criteria used were in accordance with the literature.11-13

3.2.3 | aGVHD and cGVHD

Two patients had grade III to IV aGVHD after transplantation in the MSC group; the incidence was 15.4%. Both patients had aGVHD before MSC infusion. No new aGVHD developed after MSC infusion, and the original aGVHD cases were not aggravated. The incidence of aGVHD in the non-MSC infusion group was 10.8%. There was no significant difference between the two groups (P = .76). cGVHD occurred in two patients in the MSC group after transplantation, with an incidence of 19.2%. The incidence of cGVHD in the non-MSC infusion group was 21.0%. There was no significant difference between the two groups (P = .68) (Figures 1 and 2).

3.2.4 | Relapse

In the MSC infusion group, one patient relapsed; thus, the 3-year relapse rate was 10%. In the non-MSC infusion group, the 3-year relapse rate was 26.0%. There was no significant difference between them (P = .24; Figure 3).
3.3 | Survival

In the MSC infusion group, two patients died of grade III to IV aGVHD, one patient died of relapse, and another patient died of infection. All other patients survived disease-free. The OS was 69.2%. The 3-year OS in the non-MSC infusion group was 59.0%. There was no significant difference between the two groups ($P = .517$). The 3-year DFS was 68.1% in the MSC infusion group and 58.8% in the non-MSC infusion group. There was no significant difference between the groups ($P = .681$) (Figures 4 and 5).

4 | DISCUSSION

Hemorrhagic cystitis is one of the major complications after allogeneic HSCT. This condition can develop from microscopic hematuria and gross hematuria to renal failure, directly affecting the success rate of transplantation and the survival rate of patients. HC lacks specific prevention and treatment measures. The common treatment methods are hydration, alkalization, diuresis, bladder irrigation, and drug perfusion. Surgical intervention is often used for severe HC. All of these treatments have unsatisfactory curative effects, and some of them are invasive procedures. Furthermore, affected patients have poor compliance and high risks of bleeding and infection.

To date, the etiology of HC remains unclear. Late-onset HC usually occurs after hematopoietic reconstitution, mainly with BKV infection, followed by CMV, JCV, and EBV infections. In the present study, 13 pediatric patients had severe HC with positive BKV DNA findings in the urine; no other viral infection was found. The human BKV is a subtype of the papillomavirus family. BKV was first found in the urine of a patient with kidney transplantation-related nephropathy. The virus was named BKV\(^1^4\) considering the initials of the patient’s name. The pathological processes of BKV-related HC include the following: (a) damage in the urinary tract mucosa caused by chemotherapy and irradiation; (b) activation of the BKV; and (c) excess inflammation owing to the reconstructed recipient immune system as a response to the BKV.\(^1^5\)
In addition to the traditional treatments for BKV-related HC, the following treatments are also currently used: (a) intravenous cidofovir. The general standard dose is 5 mg/kg once a week, which is changed to twice a week after 2 weeks. Philippe et al.\textsuperscript{17} reported the efficacy of cidofovir in the treatment of BK virus-associated hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation. A total of 27 patients with BK-HC received CV treatment, and the complete response (CR) was achieved in 22 patients (81.5%), partial response (PR) in 2 patients, and 2 treatments failed; response was non-assessable in 1 patient, who received only a single dose of cidofovir. Because cidofovir has a great influence on renal function, it is necessary to take probenecid orally at the same time. A small dose of cidofovir can be used (5 mg/kg) 2-3 times a week, which has minimal effects on renal function and does not require the use of oral probenecid\textsuperscript{18}; (b) oral leflunomide: not reported in children\textsuperscript{19}; (c) oral/intravenous ciprofloxacin: limited use in children; (d) oral estrogen: inexact efficacy, only case reports\textsuperscript{20}, and (e) hyperbaric oxygen: It is difficult to use special instruments to protect and isolate patients in transplantation wards. In this study, all 13 subjects were pediatric patients; thus, oral leflunomide and oral/intravenous ciprofloxacin were not used.

Mesenchymal stromal cells were first found in the bone marrow; however, the preparation of MSCs from damaged bone marrow of donors and the fact that a large amount of bone marrow could not be extracted limited the clinical application of bone marrow-derived MSCs. In 2006, MSCs were isolated from the placenta and umbilical cord tissues in China, which have stronger proliferation and differentiation abilities, convenient collection, and high purity. Since then, umbilical cord blood-derived MSCs have become an ideal substitute for bone marrow-derived MSCs and have a greater potential for application.\textsuperscript{22} MSCs have a strong self-renewal ability and multidirectional differentiation potential. After intravenous infusion, MSCs are scattered in various tissues and organs. If there is inflammation or injury, then these cells respond to the expressed cytokines and then quickly return to the inflammation or injury site for immune regulation and tissue repair.\textsuperscript{23} In addition to hydration, alkalization, and antiviral therapy, this study performed umbilical cord blood-derived MSC intravenous infusion to treat 13 pediatric patients with severe HC after UCBT. The clinical symptoms in each patient disappeared; the efficacy rate reached 100%, and the effect was rapid, which reduced the pain reported by the patients. Considering the relationship between MSCs and the repair function of the urinary tract mucosa, the first step of the pathological process of BKV-related HC was blocked. In addition to tissue repair function, MSCs also have a strong immune regulation function; thus, they can also inhibit excess inflammation owing to the BKV in the immune system of reconstructed recipients, thereby blocking the third step of the pathological process of BKV-related HC. However, the number of BKV DNA copies in the urine of the patients before and after treatment showed no significant change, indicating that MSCs did not inhibit the replication of the BKV and had no anti-BKV effect. At present, the number of BKV DNA copies in the blood and urine...
can be detected simultaneously in patients with HC. The sensitivity of the blood BKV DNA is poor and was not detected in many patients with HC. Most patients with HC had negative findings; however, once BKV DNA is detected, it may be associated with a poor prognosis of the patients. The sensitivity of urine BKV DNA is good; it is often used for the early diagnosis of BKV-related HC. However, the BKV can be found in some patients after transplantation or even in normal human urine; the copy number is low under normal conditions. The BKV copy number is usually more than $10^6$ in the urine, which is considered to be associated with HC.

Due to the immune regulation function of MSCs, they can be used in the treatment of aGVHD, cGVHD, engraftment failure or delay, and bronchiolitis obliterans, and many products are used in other diseases besides hematological diseases. In our study, there was no significant difference in acute and chronic GVHD between the MSC group and the non-MSC group. Firstly, it may be related to the less infusion times of MSC [the median frequency of MSC infusion was only 2 (range, 1-3)]. Secondly, it may be related to few cases in MSC group, so we can increase the number of cases for further study. Third, without MSC infusion, the incidence of GVHD in HC group may be higher than that in the control group. After MSC infusion, the incidence of GVHD in HC group was reduced, so there was no difference between the two groups. But this conclusion needs further study. However, because of their immune regulation function, it is unclear whether MSCs can cause relapse of malignant hematological diseases and increase the infection rate after treatment. Von Bahr and colleagues investigated 31 recipients who underwent HSCT and were treated with MSC infusion for aGVHD (23 recipients) and HC (eight recipients). Each patient was followed up for more than 5 years. aGVHD and HC were controlled via MSC infusion. However, 54% of the patients died of viral, bacterial, and fungal infections within 4 months to 2 years after MSC infusion. Thus, long-term infections after MSC infusion must be managed appropriately. The authors also compared patients with aGVHD and HC and found that the incidence of bacterial and fungal infections was similar. However, the incidence of CMV infection was 32% in the patients with aGVHD treated with MSCs and 0% in the patients with HC; the possibility that aGVHD could increase the incidence of CMV infection could not be disregarded. Two recent studies by Chinese researchers presented with different conclusions. These authors reported that MSCs did not increase the incidence of relapse of primary diseases or the infection rate in patients with acute leukemia after HSCT. In our study, we compared the changes in the VMH copy number before and after MSC infusion. Most patients had a decreased CMV copy number after MSC infusion. There was no significant difference in the blood CMV copy number before and after MSC infusion (P = .243); further, no CMV infection occurred. No patients had worsened bacterial or fungal infections or had new bacterial or fungal infections after MSC infusion. Therefore, we did not find any aggravation of infection in our study in the short term.

Mesenchymal stromal cells have a rapid effect in the treatment of BKV-related HC, greatly reducing the pain of patients. We found that these cells do not have an anti-BKV effect, as there was no significant difference in the urine BKV DNA before and after MSC treatment. To assess whether there is an increase in the incidence of long-term infections after MSC infusion, 13 patients who received MSC infusion were classified into the MSC infusion group and those who received UCBT at the same time into the non-MSC infusion (control) group. The median follow-up time of the patients was >3 years. The infection rates of bacteria, fungi, and viruses were compared between the two groups, and no significant difference was found. These results indicate that MSCs have the function of tissue repair and immune regulation and have no obvious immunosuppressive effect. Thus, we also did not find any aggravation of infection in our study in the long term.

The increased proportion of patients with HC complicated with aGVHD is attributed to the increased viral activation owing to enhanced immunosuppressive therapy in the patients with aGVHD. However, MSCs also have a good therapeutic effect on GVHD. MSC infusion can enhance the repair of pathological tissues and organs in patients with GVHD and can also prevent the occurrence of GVHD in patients who underwent HSCT. At present, MSC infusion is conducted in transplantation centers before HSCT as a preventive strategy for GVHD. Thus, there was no difference in the incidence of aGVHD and cGVHD between the MSC group and control group after neutralization of the two factors. There was also no significant difference in the OS and DFS in the survival analysis between the two groups, indicating that MSC infusion had no significant effect on survival.

## 5 | CONCLUSIONS

In conclusion, although umbilical cord blood–derived MSCs cannot reduce BKV replication, these cells are effective in treating BKV-related HC because of their good tissue repair and immune regulation functions. The treatment efficiency is high, and the symptoms of HC can be rapidly improved. MSCs did not increase the incidence of infection, recurrence rates of aGVHD and leukemia, or rate of survival.

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