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Modified Kasai operation combined with autologous bone marrow mononuclear cell infusion for biliary atresia

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Abstract

Aim To evaluate the safety and outcomes of modified Kasai operation combined with autologous bone marrow mononuclear cell (BMMNC) infusion for biliary atresia (BA).

Methods A matched control study was conducted between January 2015 and December 2021. Ten consecutive children with biliary atresia (BA) who underwent the modified Kasai operation combined with autologous BMMNC infusion (cell therapy group) and ten children who had only the modified Kasai operation (control group) were included in the study. The Kasai operation was performed with two modifications: partial exteriorization of the liver, and encirclement with lateral retraction of two hepatic pedicles to facilitate the removal of fibrotic tissue.

Bone marrow was harvested through anterior iliac crest under general anesthesia then a modified Kasai operation was performed. After processing, bone marrow mononuclear cells were infused through the umbilical vein at the end of the operation.

Serum bilirubin, albumin, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, and prothrombin time were monitored at baseline, six months, twelve months, and the last follow-up (4.5 years) after the operation. In addition, esophagoscopy and liver biopsies were performed on patients whose parents agreed. Mixed-effects analysis was used to evaluate the changes in Pediatric End-Stage Liver Disease (PELD) scores.

Results There were no intraoperative or postoperative complications related to the operation or cell infusion. The average infused BMMNC and CD34 + cell counts per kg bodyweight were $85.5 \pm 56.0 \times 10^6$/kg and $10.0 \pm 3.6 \times 10^6$ for the injection, respectively. Following the intervention, all ten patients in the cell therapy group survived, with a mean follow-up duration of 4.5 ± 0.9 years. Meanwhile, three patients in the control group died due to end-stage liver failure, with a mean follow-up time of 4.3 ± 0.9 years. Liver function of the cell therapy group was maintained or improved after the operation and cell infusion, as assessed by biochemical tests. The disease severity reduced markedly in the CT group compared to the control group, with a significant reduction in PELD scores ($p < 0.05$).

Conclusion Autologous BMMNC administration combined with Kasai operation for BA is safe and may maintain or improve liver function in the studied patients.

Trial registration ClinicalTrials.gov Identifier: NCT05517317 on August 26th, 2022.

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Keywords Biliary atresia, Kasai operation, Bone marrow mononuclear cell infusion

Introduction

BA is a progressive obliterative cholangiopathy, rare in Europe and North America, but common in some countries in Asia [1–3].

BA is divided into two groups: the curable group and the incurable group. Before 1958, there was no effective treatment for the incurable group. In 1959, Morio Kasai introduced the portoenterostomy technique for incurable BA [4]. Since then, many patients have been saved [3, 5]. However, the long-term prognosis for patients after Kasai surgery is still far from satisfactory. According to many studies, the 5-year survival rate of patients with their native liver is about 40% [6–8]. This rate decreases further over time, dropping to 17.8%−30% after 20 years [6, 8]. Patients who can live with their native liver also feature many severe complications. A study conducted by Lee et al. with 52 patients whose mean age was 7.4 years old demonstrated that portal hypertension was observed in 40%, cholangitis was 36%, and bleeding varices were 25% [9]. The goal to improve the survival rate for patients with native livers triggered many modifications in surgical techniques that have been applied with varying success [10–12]. Furthermore, various adjuvant treatments have also been carried out [13]. Steroids have been used as postoperative adjuvant therapy to alleviate the inflammatory reaction and improve postoperative bile. However, this treatment may improve bile flow in the short term but could not sustain long-term results [14–16].

Given the constraints of existing treatment in BA, there is an urgent need to develop a new and effective treatment. In recent years, cell-based therapy, including bone marrow mononuclear cells (BMMNCs), has demonstrated encouraging outcomes in addressing liver fibrosis. BMMNCs encompass a varied array of cells, such as hematopoietic stem cells, mesenchymal stromal/stem cells, and endothelial progenitor cells [17]. In animals with hepatic fibrosis, BMMNC transplantation induced apoptosis of fibrogenic cells and stimulated hepatocyte proliferation during liver generation leading to improvement of liver function [18]. BMMNCs also reduced liver fibrosis associated with downregulation of inflammatory and the extracellular matrix in CCl_4-indcued liver fibrosis mice [19].

In human, the safety and efficacy of BMMNC transplantation have been demonstrated by many clinical trial [20–24]. In 2015, Pankaj P et al. reported the outcomes of BMMNC transplantation in patients with cirrhosis across eight clinical trials. The findings indicated that BMMNC transplantation elevated serum albumin levels, reduced total bilirubin levels, and temporarily decreased levels of alanine transaminase and aspartate transaminase [21]. In 2007, Gupta and colleagues initiated the injection of BMMNC into eight children with BA, including five of them underwent simultaneous BMMNC injection and the Kasai procedure. Researchers administered the cells to the liver through one of three different routes: the hepatic artery route, portal vein route, and direct injection into the hepatobiliary radicals. However, determining the results post-intervention is challenging because BA, choledochal cysts, and cholestasis were combined into a single group [25]. In 2011, Sharma S et al. reported the results of BMMNC infusion through the hepatic artery and/or portal vein for BA patients at the time of Kasai or after Kasai surgery. Although the group of patients receiving cell transfusion had better early outcome than the control group, the long-term results were not as expected. Remarked improvement of biochemical parameters and scintigraphy was recorded, but the median survival time of treated group was only 181 days [26].

In 2022, we published the results of BMMNC infusion via the arterial route in 19 BA patients with cirrhosis after Kasai surgery. Follow-up after 12 months showed that only one patient died five months after intervention, while 18 patients were still alive and with normal liver functions [27].

Nevertheless, we hypothesize administering cells concurrently with Kasai surgery might yield superior results and cell delivery through the umbilical vein is a feasible and safe approach, offering a less invasive alternative than the arterial route and potentially delivering a higher cell quantity to the liver than the peripheral route.

The aim of this study was to evaluate the safety and preliminary outcomes of modified Kasai operation combined with autologous BMMNC infusion via the umbilical cord vein for patients with BA.

Patients and methods

Study design

A prospective study was performed from January 2015 to December 2021.

Patients

Twenty patients were enrolled in this study, including 10 patients in the cell therapy (CT) group who underwent a modified Kasai operation combined with autologous BMMNC administration at Vinmec Times City International Hospital, Hanoi, Vietnam, and 10 patients in the

control group who underwent the same Kasai technique at Vietnam National Children's Hospital, Hanoi. The matching criteria between the two groups included age at the time of surgery (with a difference of no more than 14 days), the same sex, and the use of a similar surgical technique.

Inclusion criteria Children of both sexes aged ≤ 4 months old with BA at the intervention were included.

Exclusion criteria Patients with coagulation disorders, allergies to anesthetic agents, or hepatic coma were excluded.

Methods

Bone marrow aspiration and isolation of BMMNCs

Bone marrow was collected via anterior iliac crest puncture under general anesthesia in the operating room just prior to the Kasai procedure. Based on improved safety data from our previous studies, the collected volume of bone marrow was set at 8 ml/kg [22–24]. BMMNCs were isolated using Ficoll-Paque density gradient centrifugation (GE Healthcare, Sweden), following the method described in our previous research (35,287,722). Briefly, aspirated bone marrow was carefully layered onto Ficoll-Paque at a 1:1 volume ratio and centrifuged at 1,400 × g for 18 min at 20 °C without braking. After centrifugation, the plasma layer was retained for resuspending the final cell product. The mononuclear cell layer was collected and washed with 1X phosphate-buffered saline (PBS). Finally, the cell product was resuspended in 10 mL of autologous plasma for further injection.

The final cell product was subjected to a quality control process to ensure cell viability, absence of mycoplasma, endotoxin safety, and sterility. The release criteria required for the cell product included: ≥ 80% cell viability as measured by the Trypan blue exclusion method, a negative result for mycoplasma using the MycoAlert™ Mycoplasma Detection Kit (Lonza, Switzerland), endotoxin levels below 5.0 EU/kg/infusion hour using the Endosafe-PTS Kit (Charles River, USA), and a negative microbiology test verified by the BacT/Alert® 3D microbial detection system (Biomerieux, USA). Additionally, total blood components before and after Ficoll separation were analyzed with a Beckman Coulter LH780 hematology analyzer (USA). The CD34 + hematopoietic stem cell count was determined using the Stem-Kit™ Reagent (Beckman Coulter, USA) on a Navios flow cytometer (Beckman Coulter, USA).

Kasai operation

An incison below the right flank extended to the left was made. The umbilical vein was identified and divided. The #6 catheter was inserted into the proximal umbilical vein until blood came out.

The liver was mobilized by dividing the falciform ligament, the left and the right triangular ligaments, and a part of the coronary ligaments and was then partially exteriorized (Fig. 1A). The left and right hepatic pedicles were isolated, encircled by vessel loops, and retracted laterally (Fig. 1B). The gallbladder was detached from its bed, then the dissection was continued along the cholecystic duct to the fibrotic mass of the liver hilar. A stay suture was placed on the fibrotic tissue to hold it during its removal. The fibrotic mass was maximally removed. Any small vessel from the fibrotic mass running to the portal veins was coagulated by bipolar diathermy.

Portoenterostomy was carried out with separate sutures. The length of the Y limb from the duodenal angle to the entero-enterostomy anastomosis was 30 cm while the Y loop from the portoenterostomy anastomosis to the entero-enterostomy anastomosis was 20 cm in length. Ten ml autologous plasma containing bone marrow mononuclear cells were infused

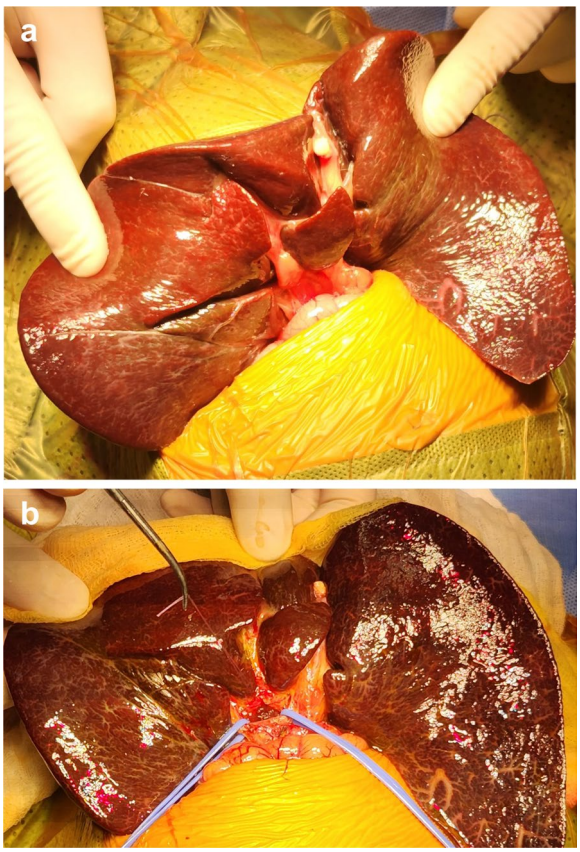

Fig. 1 **A** Exteriorization of the liver. **B** Isolation and retraction of the hepatic pedicles

through the umbilical vein catheter within a period of 10 min, then the abdomen was closed.

Histopathological analysis

In the CT group, histopathological examination was executed by pathologists at Vinmec Times City International Hospital, using eosin staining and hematoxylin. The first liver biopsy was performed intraoperatively, with staining. An ultrasonography-guided fine-needle core liver biopsy was performed for the second liver biopsy. We used 18-gauge core biopsy needles, and the biopsy target site was the right lobe. Three core biopsies were immediately placed in formalin. This was followed by tissue block staining with hematoxylin and eosin and analysis under microscopy.

Four histopathological findings including fibrosis, bile duct proliferation, cholestasis and portal inflammation were graded as follows:

Fibrosis was graded from mild to severe [20, 28].

- Grade I: Mild fibrosis with fibrous expansion affecting less than 50% of portal tracts.
- Grade II: Moderate fibrosis with Porto-portal bridging affecting more than 50% of portal tracts, without liver nodules.
- Grade III: Severe fibrosis with nodular liver structure and bridging fibrosis in more than 50% of portal tracts.

Bile Duct Proliferation was scored semi-quantitatively [29].

- Mild: 5–9 bile ducts per portal tract.
- Moderate: 10 or more bile ducts per tract.
- Severe: 10 or more ducts per tract, with ducts elongated, attenuated, and angulated

Cholestasis was classified by bile accumulation [30].

- Absent.
- Mild: Bile in centrilobular hepatocytes.
- Moderate: Bile in both centrilobular and periportal hepatocytes
- Severe: Presence of bile infarcts

Portal and periportal inflammation were assessed based on cell density [31].

- Mild: Cells in less than one-third of portal tracts.
- Moderate: Cells in one-third to two-thirds of tracts.
- Severe: Dense cell presence in more than two-thirds of tracts. Duct llate malformation is identified by unusual bile duct formations around a fibrous or vascular core, and giant cell transformation is noted as either present or absent.

In the control group, liver biopsies were performed by a single pathologist at the National Hospital of Pediatrics, utilizing eosin and hematoxylin staining. Liver fibrosis was staged according to the METAVIR scoring system, which includes five stages: F0 (normal, no fibrosis), F1 (portal fibrosis with mild fibrosis and pericellular collagen deposits), F2 (fibrosis with few septa, moderate fibrosis, and early bridging), F3 (numerous septa, severe fibrosis, and bridging between fibrotic areas), and F4 (cirrhosis, indicating advanced scarring) [32].

Outcome measures

+ *Monitoring procedure-related adverse events*

Safety evaluation: Procedure-related adverse events (AEs) and serious adverse events (SAEs), such as fever, pain, intraabdominal bleeding, bile leakage, cholangitis, and rupture of esophageal varices were recorded during a 48-h post-infusion period as well as after discharge.

+ *Monitoring the changes in liver function and the severity of the disease after the intervention*

Biochemical indicators, including serum bilirubin, albumin, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase (GGT), international normalized ratio (INR), and prothrombin time, were evaluated at baseline and six months, 12 months, and the last follow-up visit after transplantation. In addition, liver biopsies were performed at baseline and twelve months after the BMMNC infusion for patients whose parents agreed to it.

The Pediatric End-Stage Liver Disease (PELD) score, which consists of age, albumin, total bilirubin, prothrombin time, INR, and growth failure, was calculated at baseline and six months, 12 months, and the last follow-up after transplantation to estimate the relative disease severity. PELD scores in which higher scores indicate a poorer condition or worse outcomes, were calculated as follows [33]:

$$\mathbf{PELDScore} = 10\mathbf{x}(0.480\mathbf{xln}(\mathbf{bilirubin}) + 1.857\mathbf{xln}(\mathbf{INR}) - 0.687\mathbf{xln}(\mathbf{albumin}) + 0.436(\text{if the patient is under 12 months}) + 0.667(\text{if the history of growth failure is positive}).$$

Table 1 Baseline characteristics of the patients

Characteristics		CT group *N* = 10 (100%)	Control group *N* = 10 (100%)	*P* value
Age at Kasai operation (month)	**Mean ± SD [Min, Max]**	**2.6 ± 0.8 [1.7, 3.8]**	**2.7 ± 0.8 [1.9, 3.9]**	**0.859**
Height (cm)	Mean ± SD [Min, Max]	59.3 ± 3.1 [54.0, 62.0]	59.9 ± 2.1 [57.0, 63.0]	0.621
Weight (kg)	Mean ± SD [Min, Max]	5.3 ± 0.7 [4.5, 7.0]	5.4 ± 0.8 [4.4, 7.0]	0.656
Sex	**Male** (n, %)	5 (50%)	5 (50%)	1.000
	Female (n, %)	5 (50%)	5 (50%)	

Mixed-effect analysis was applied to evaluate the changes in PELD scores at each visit.

Ethics

The study protocol was reviewed and approved by the Ethics Committee of Vinmec Times City International Hospital with approval number 150115/2015/QD-VMEC. The study was registered on ClinicalTrials.gov on August 26th, 2022, with identity number NCT05517317. The written informed consent was obtained from all patient's parents.

Statistical analysis

Each individual was a unit of analysis. The Wilcoxon signed-rank test was used to compare the total PELD scores at six months, 12 months, and the last follow-up with those at baseline. Mixed-effects analysis was applied to evaluate the changes in PELD scores. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using R software version 3.6.1.

Results

The CT group consisted of five girls and five boys, with ages at the time of the Kasai operation and BMMNC infusion ranging from 1.7 to 3.8 months (mean: 2.6 ± 0.8 months) and an average body weight of 5.3 ± 0.7 kg. The control group also included five girls and five boys, with ages at the time of the Kasai operation ranging from 1.9 to 3.9 months (mean: 2.7 ± 0.8 months) and an average body weight of 5.4 ± 0.8 kg. There were no significant differences between CT and control groups in terms of age at Kasai operation, height, weight, and sex. The basline parameters of the patients in the two groups are shown in Table 1.

Before intervention, the mean PELD scores for the CT and control groups were 9.8 ± 2.7 and 8.4 ± 5.2, respectively. In the CT group, 8 (80%) patients had hepatomegaly, 4 (40%) had splenomegaly, and one presented with collateral circulation at baseline. In the control group, 5 (50%) patients had hepatomegaly and 4 (40%) had splenomegaly. In addition, all patients had elevated bilirubinemia, elevated transaminase, and elevated GGT before the intervention.

The liver function indicators an PELD scores at baseline are presented in Table 2.

Table 2 Liver function and PELD scores of the patients at baseline

Indicator	Baseline Mean ± SD [Min, Max]		
	CT group (*N* = 10)	**Control group (*N* = 10)**	***P* value**
Albumin (*μmol/L*)	38.7 ± 3.1 [34.3; 42.7]	37.8 ± 4.3 [30.0; 44.6]	0.594
Serum Bilirubine *(mg/dL)*	9.98 ± 3.4 [6.1; 15.4]	5.30 ± 4.0 [0.9, 13.2]	0.012
Aspartate Aminotransferase (AST) *(U/L)*	340.0 ± 132 [193; 581]	290.0 ± 96.0 [99.6, 409]	0.071
Alanine Aminotransferase (ALT) *(U/L)*	283.0 ± 145 [122; 593]	166.0 ± 117 [59.3, 456]	0.068
Gamma–Glutamyl Transferase (GGT)	496.0 ± 287 [200; 1090]	504.0 ± 269 [257.0, 1180.0]	0.956
Prothrombin Time (PT) *(second)*	13.2 ± 2.57 [10.4; 18.0]	12.1 ± 1.63 [10.1; 14.9]	0.276
International Normalized Ratio (INR)	1.08 ± 0.19 [0.9; 1.5]	1.07 ± 0.09 [0.9, 1.2]	0.885
PELD score	9.8 ± 2.7 [5.00; 13.0]	8.4 ± 5.2 [2.00, 18.0]	0.495

Table 3 Histopathological features of CT patients at Kasai operation and cell infusion

	Number (*N*)	Percentage (%)
Fibrosis (*N*=10)		
Grade I (mild)	0	0
Grade II (moderate)	3	30%
Grade III (severe)	7	70%
Bile duct proliferation (*N*=10)		
Mild: 5–9 bile ducts	3	30%
Moderate: ≥10 bile ducts	2	20%
Severe: ≥10 bile ducts	5	50%
Cholestasis (*N*=10)		
Absent	4	40%
Mild	3	30%
Moderate	1	10%
Severe	2	20%
Portal and periportal inflammation (*N*=10)		
Mild	6	60%
Moderate	2	20%
Severe	2	20%

Liver biopsies were performed on ten CT patients at baseline; among them two patients' parents agreed to the second liver biopsy. The degree of fibrosis, bile duct proliferation, cholestasis, and portal inflammation on liver biopsies was assessed according to Gunadi et al. [30] and were presented in Table 3.

In the control group, liver biopsies were performed on nine patients at baseline, with most classified as having moderate to advanced liver fibrosis: 22.2% had mild fibrosis (F1), 33.3% moderate fibrosis (F2), 33.3% advanced portal fibrosis (F3), and 11.2% severe liver scarring (F4) (Table 4).

Each CT patient underwent one BMMNC administration, with an average of 44.3 ml of bone marrow collected. The average mononuclear cell and $CD34^+$ cell counts per kg body weight were $85.5 \pm 56.0 \times 10^6$/kg and $10.0 \pm 3.6 \times 10^6$. The average cell viabilities before the infusion were 96.7%.

Table 4 Histopathological features of control patients at Kasai operation

Fibrosis (*N* =9)	Number (*N*)	Percentage (%)
F0 (no fibrosis)	0	0
F1 (portal fibrosis without septa)	2	22.2%
F2 (portal fibrosis with rare septa)	3	33.3%
F3 (mumerous septa without cirrhosis)	3	33.3%
F4 (cirrhosis)	1	11.2%

There were no severe complications during bone marrow collection, Kasai operation, or cell infusion. The average follow-up duration for children who received BMMNC infusion after surgery was 4.5 ± 0.9 years (ranging from 2.8 to 5.7 years), while the control group had an average follow-up time of 4.08 ± 1.3 years (ranging from 2.2 to 6.1 years).

Liver function of CT children improved markedly over the follow-up period. The mean of ALT and AST decreased from 283 UI/L and 340 UI/L, respectively, to 106 UI/L and 104 UI/L, at six months and 99.8 UI/L and 92.8 UI/L at last visit. While in the control group, the mean of ALT and AST decreased from 166 UI/L and 290 UI/L, respectively, to 121 UI/L and 198 UI/L six month and 121 UI/L and 146 UI/L at the last visit.

The GGT mean of children in CT group significantly reduced after 6 and 12 months, compared to the baseline and compared to the control group ($p = 0.033$ and $p = 0.016$).

After 12 months, albumin level of CT patients showed significant improvement compared to the control group ($p = 0.001$).

All CT patients had bilirubinemia at baseline over 2 mg/dL; six months after surgery, five patients had serum bilirubin lower than 2 mg/dL. This number of CT patients increased to seven cases after 12 months. The mean of bilirubinemia decreased from 9.98 mg/dL, to 4.21 mg/dL at six months and 4.92 mg/dL at the last visit. While in the control group, the mean of bilirubinemia increased from 5.3 mg/dL, to 10.6 mg/dL at six months and 9.66 mg/dL at the last visit. In CT group, normal coagulation function manifested by INR and PT was maintained in normal ranges after six months, 12 months, and the last visit. In addition, albumin was maintained at a normal value in all postoperative visits among CT children.

The liver functions before and after the surgery are in Table 5.

Only two of ten CT patients had their parents' consent to a second liver biopsy 12 months after surgery. The first liver biopsy showed that both patients have grade III fibrosis, severe cholestasis in the bile duct, severe bile duct hyperplasia, severe cholestasis, and moderate inflammation in the portal and periportal.

The results showed that grade III fibrosis and severe biliary hyperplasia are still observed in both patients, but cholestasis has improved (cholestasis was no longer observed in the biliary canaliculus) and only mild inflammation in the portal and periportal. The degree of liver cirrhosis is not exacerbated when compared to the liver biopsy conducted during the Kasai procedure; it remained at degree 3 (Fig. 2).

Table 5 Liver function tests after Kasai operation and cell infusion

Indicator	6 months Mean ± SD [Min; Max]			12 months Mean ± SD [Min; Max]			At the final follow - up timeMean ± SD [Min; Max]		
	CT group (*N* =9)	Control group (*N*=10)	*P* value (compared 2 groups)	CTgroup (*N* =9)	Control group (*N*=10)	*P* value (compared 2 groups)	CT group (*N*=9)	Control group (*N*=7)	*P* value (compared 2 groups)
Albumin *(µmol/L)*	40.1 ± 6.59 [28.6; 48.7] *P**= 0.400	36.8 ± 4.48 [29.7; 41.8] *P**= 0.630	0.227	40.6 ± 5.20 [32.0; 46.8] *P**= 0.277	29.2 ± 7.26 [14.9; 37.0] *P**= 0.003	0.001	40.9 ± 6.04 [31.9; 49.8] *P**= 0.279	42.7 ± 3.46 [38.3; 46.6] *P**= 0.528	0.450
Serum Bilirubin *(mg/dL)*	4.21 ± 4.78 [0.3; 12.3] *P**= 0.022	5.08 ± 5.40 [0.4; 15.0] *P**= 0.529	0.714	3.41 ± 1.70 [0.1; 4.8] *P**< 0.001	10.6 ± 10.5 [1.7; 30.5] *P**= 0.353	0.023	4.92 ± 3.42 [0.3; 9.9] *P**= 0.001	9.66 ± 13.1 [1.0; 34.9] *P**= 0.523	0.146
Aspartate Aminotransferase (AST) *(U/L)*	104 ± 70.9 [36.4; 219] *P**< 0.001	198 ± 84.1 [88.6; 363] *P**= 0.904	0.017	93.3 ± 56.4 [36.2; 202] *P**< 0.001	147 ± 62.1 [53.2; 217] *P**= 0.278	0.062	92.8 ± 75.3 [25.6; 279] *P**< 0.001	146 ± 94.9 [77.7; 343] *P**= 0.095	0.182
Alanine Aminotransferase (ALT) *(U/L)*	106 ± 102 [12.6; 322] *P**= 0.010	121 ± 46.7 [69.6; 225] *P**= 0.549	0.697	94.5 ± 68.5 [20.2; 211] *P**= 0.002	92.9 ± 58.1 [31.4; 231] *P**= 0.065	0.956	99.8 ± 97.5 [13.3; 291] *P**= 0.0007	121 ± 59.7 [50.6; 247] *P**= 0.315	0.567
Gamma–Glutamyl Transferase (GGT)	282 ± 271 [9.10; 883] *P**= 0.094	376 ± 247 [290.0; 740] *P**= 0.603	0.033	133 ± 122 [9.40; 375] *P**= 0.0009	257 ± 239 [95.3; 1150] *P**= 0.278	0.016	188 ± 201 [14.0; 557] *P**=0.011	262 ± 197 [122; 1070] *P**= 0.065	0.146
Prothrombin Time (PT) *(second)*	12.4 ± 1.42 [11.1; 15.3] *P**= 0.719	13.9 ± 3.30 [9.9; 21.4] *P**= 0.211	0.197	12.7 ± 1.56 [10.9; 15.4] *P**= 0.780	13.3 ± 2.99 [10.2; 20.1] *P**= 0.447	0.597	12.4 ± 1.13 [10.9; 14.0] *P**= 0.795	13.9 ± 3.03 [11.1; 21.4] *P**= 0.156	0.175
International Normalized Ratio (INR)	1.08 ± 0.16 [0.9; 1.3] *P**= 0.780	1.16 ± 0.30 [0.9; 1.9] *P**= 0.529	0.448	1.01 ± 0.1 [0.95; 1.04] *P**= 0.842	1.18 ± 0.24 [0.9; 1.7] *P**= 0.579	0.043	1.04 ± 0.08 [0.97; 1.20] *P**= 0.853	1.20 ± 0.22 [0.8; 1.6] *P**= 0.075	0.055

*P** *P* - value compare with baseline

The mean PELD scores of CT patients decreased from 9.8 points (range: 5.0 to 13.0) at baseline to −2.6 points (range: −10.0 to 7.0) at the last follow-up, with an average follow-up time of 4.5 ± 0.9 years ($p < 0.05$). The mean PELD scores of control patients decreased from 8.4 points (range: 2.0 to 18.0) at baseline to 0.1 points (range: −3.0 to 7.0) at the last follow-up, with an average follow-up time of 4.1 ± 1.3 years. The progress of PELD scores for both groups is presented in Fig. 3.

At the baseline, the two groups had similar PELD scores (9.8 and 8.4, $p = 0.495$). The results of a mixed-effects analyses suggest that each visit was associated with a decrease in the PELD score of 3.1 points in the PELD score of CT group compared to the control group, and this change was statistically significant (Table 6).

A total of 16 episodes of cholangitis in six CT patients were observed during the follow-up period. All cholangitis incidences responded to antibiotic treatment. In contrast, the control group experienced 49 episodes of cholangitis across ten patients, averaging 5 episodes per patient. One patient had hematemesis and one patient had melena. At the last visit, all CT children defecated with colored stools, whereas three children in the control group presented with acholic stools.

At the last visit, all CT patients were alive with their native liver, while three patients in the control group had died due to end-stage liver failure. The survival times for both groups are shown in Fig. 4.

Discussion

Kasai portoenterostomy is a standard surgical operation for BA. However, this procedure is associated with technical challenges in removing the fibrotic mass and performing the portoenterostomy.

In our surgical operation technique, the liver was exteriorized, and the left and the right hepatic pedicles were encircled and retracted laterally. With these techniques, the fibrotic mass in the hilar liver is well exposed and easy to remove completely. Moreover, portoenterostomy is also performed more easily in comparision with the standard procedure.

The rate of CT patients with biliary drainage in our study was high. After the operation, all CT patients had bile drainage with color stools.

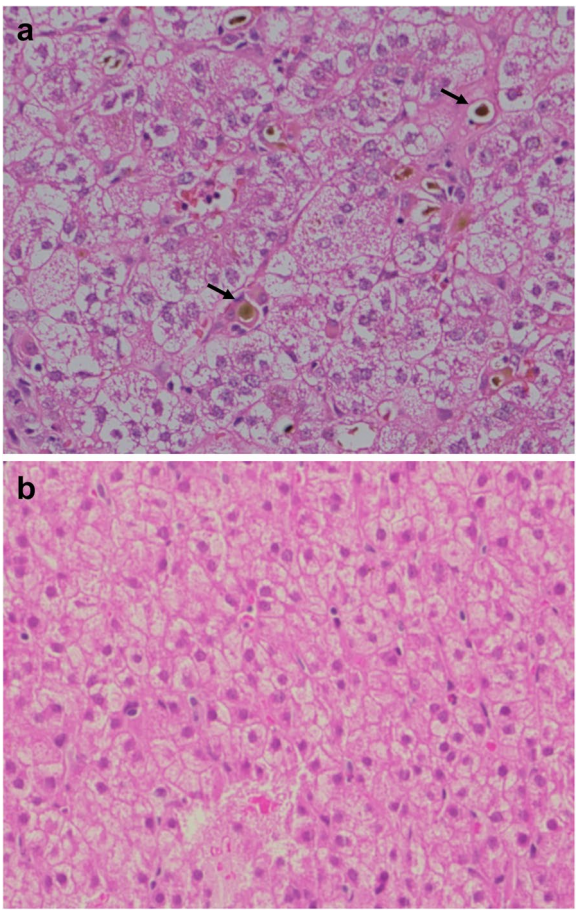

Fig. 2 Portal and periportal inflammation has disappeared at 12 months after BMMNC administration. **A** Moderate portal and periportal inflammation with dense infiltration of lymphocytes and plasma cells. B Inflammatory cells can no longer be observed in the portal and periportal areas

Our results demonstrate that modified Kasai operation combined with BMMNC infusion is safe for a small, young infant. We did not observe any surgical complications during or after the procedure. There were no complications related to bone marrow harvest or cell infusion. BMMNCs were effortlessly infused through the umbilical vein. We believe that this route is more accessible than through the hepatic artery route or via the portal vein trunk [25, 26].

The insertion of an umbilical venous catheter is a routine procedure in neonatology, providing rapid and secure access for medication delivery. Despite its advantages, this procedure carries a potential risk of portal vein thrombosis. In 2021, a systematic study, encompassing 16 reports, was conducted to assess the incidence of umbilical venous catheterization-related portal vein thrombosis in infants with postnatal age up to three months. The results revealed a mean pooled incidence of 12%, with variations ranging from 0 to 49% across different studies [34].

In our study, the catheter was only placed in the umbilical vein for a very short time, potentially reducing the risk of portal vein thrombosis. Nevertheless, this complication requires vigilant monitoring and early detection through doppler ultrasound in the post-surgical period.

Our results revealed that the modified Kasai operation combined with BMMNC infusion was more effective for BA than the Kasai operation alone. Bilirubinemia decreased significantly more in the combined treatment (CT) group compared to the control group. Albumin levels were maintained within the normal range during the postoperative follow-up. Notably, the albumin levels in the CT group were significantly higher than those in the control group at twelve months.

In the CT group, liver enzyme levels, including ALT and AST, showed a decline following the intervention. Each follow-up visit revealed that this drop was statistically significant. By the six-month mark, AST levels in the CT group were notably lower than those in the control group, showing the effectiveness of the combined treatment.

Although GGT decreased more slowly than ALT and AST, the reductions were still statistically significant between visits among CT patients. At six and twelve months, GGT levels in the infusion group were significantly lower than those in the control group.

Improvements in liver histology were noted in two CT patients who underwent the second liver biopsie. The degree of the portal and periportal inflammation was reduced from moderate at base line to mild in both patients after 12 months.

The severity of disease based on Pediatric End-Stage Liver Disease (PELD) was significantly reduced in the CT group compared to the control group, with a 3.1-point decrease in PELD scores per visit. In the CT group, PELD scores dropped from 9.8 (range: 5.0 to 13.0) at baseline to −2.6 (range: −10.0 to 7.0), with all children remaining alive after an average follow-up of 4.5 ± 0.9 years. In contrast, the control group's PELD scores decreased from 8.4 (range: 2.0 to 18.0) to 0.1 (range: −3.0 to 7.0), with 3 patients dying after an average follow-up of 4.1 ± 1.3 years.

Our results are consistent with the results of other publications using cell therapy for BA. In 2007, Gupta et al. injected autologous bone marrow mononuclear cells in eight patients with biliary atresia. The procedure was assessed as safe but the results were not clearly defined [25]. In 2011, Sharma et al. reported the results of injection of bone marrow mononuclear cells in 11 patients with biliary atresia. Although the authors showed that bone marrow mononuclear cell infusion could improve

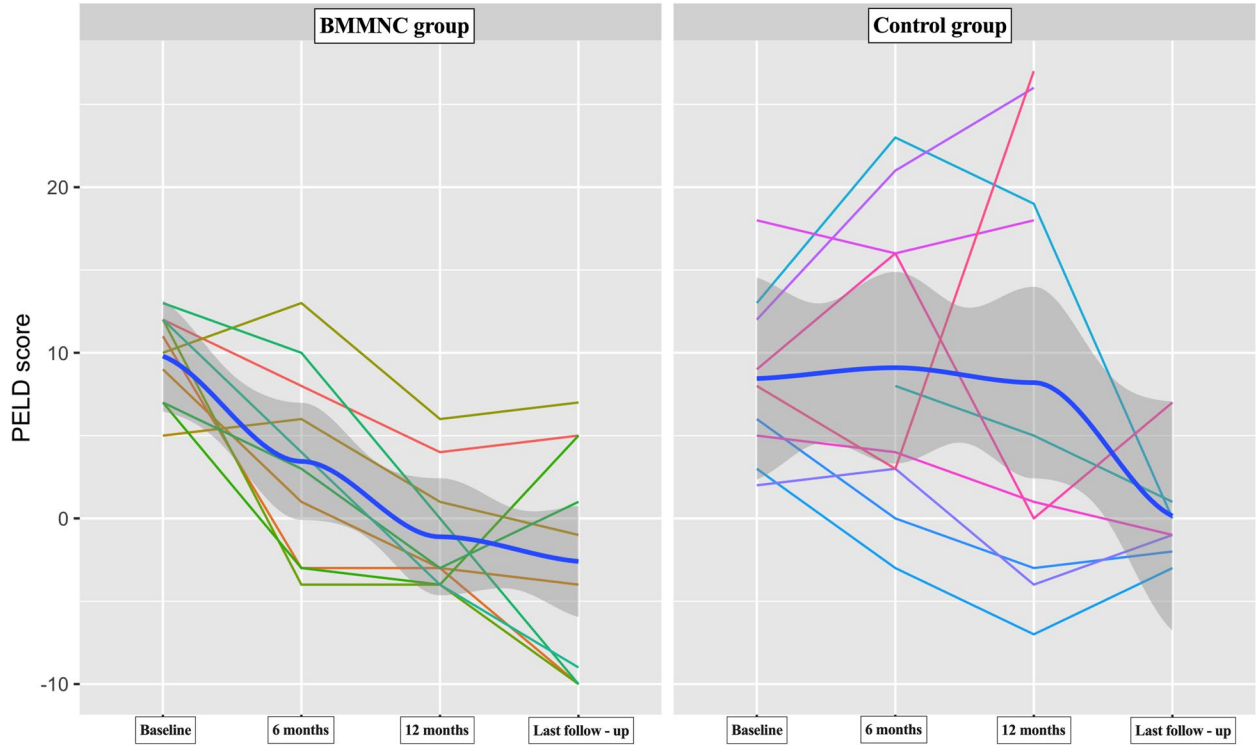

Fig. 3 PELD scores of the two groups before and after BMMNC administration

Table 6 Results from the mixed-effects analysis of PELD scores

Fixed effects	Estimate ± SE*	t value	*p* value
(Intercept)	15.1 ± 4.6	3.28	0.002
Group	-2.4 ± 2.9	-0.79	0.431
Time	-7.2 ± 1.9	-3.76	0.001
Group: time	3.1 ± 1.3	2.45	0.022
Correlation of fixed effects:			
	(Intr)	**Group**	**time**
Group	-0.95		
Time	-0.616	0.594	
Group: time	0.588	-0.635	-0.946

*Standard Error

biochemical parameters and scintigraphy, the survival rate at 12 months after surgery was only 27.3% [26]. In 2021, we reported the results of BMMNC infusion for patients with biliary atresia after Kasai surgery in 19 children. Liver function was maintained or had improved after infusion and the severity of the disease reduced markedly, with a significant reduction in PELD scores. Only one patient died after 5 months post cell infusion while the remaining 18 patients were still alive after 12 months [27].

The mechanism of action of combined cell therapy for BA still needs to be fully understood.

Experimental studies suggest that BMMNCs may have anti-cirrhotic effects through different mechanisms. For example, de Carvalho revealed that BMMNC transplantation could increase metalloproteinase-9 and 13, as well as decreasing tissue inhibitors of metalloproteinase-1 and 2 expressions. In addition, they are also associated with fibrogenic cell apoptosis during hepatic regeneration in cholestatic rats [18, 35]. Furthermore, the expression of anti-fibrotic cytokines (IL-10, IL-13, and hepatocyte growth factor, HGF) and the reduction in pro-inflammatory cytokines (IL-17A and IL-6) were observed after BMMNC transplantation [36, 37]. These factors play an essential role in the quiescence and apoptosis of hepatic stellate cells (HSCs) and contribute to the regeneration of liver function [38]. Transfused BMMNCs can migrate and localize in the liver and may transdifferentiate into albumin-secreting hepatocytes [39–42]. Besides, BMMNCs can also help improve the condition of local hepatocytes by beneficial paracrine effects on hepatic mitochondrial bioenergetics, increasing oxidative capacity, and reducing oxidative stress [43]. The small sample size and absence of randomization are limitations of this study. Larger randomized clinical trials with an expanded control group are necessary to better assess the efficacy of combining the Kasai operation with cell therapy for BA.

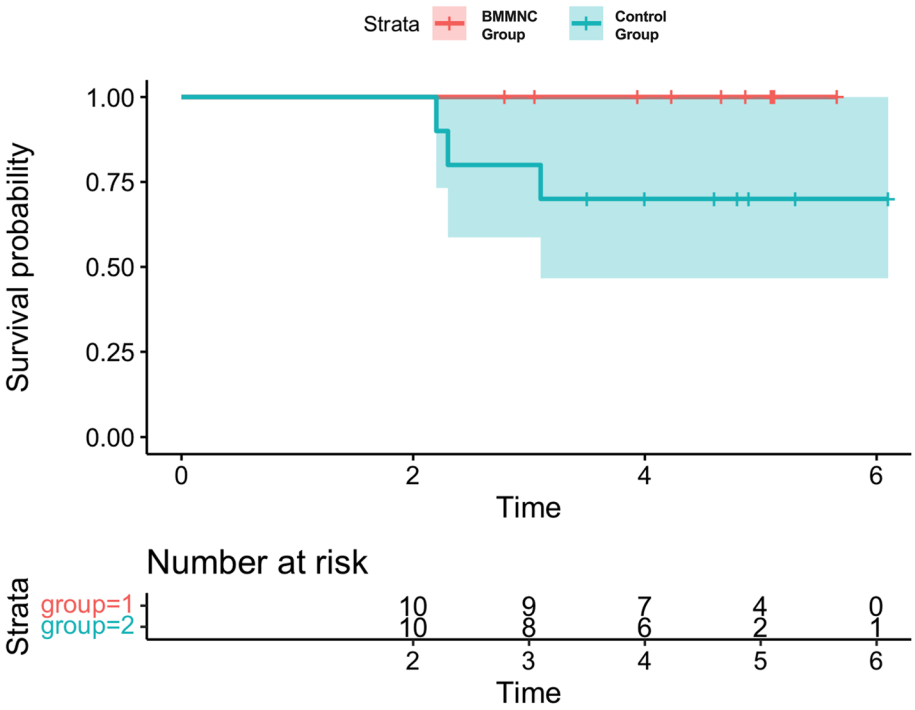

Fig. 4 Survival time of patients enrolled in this study

Conclusion

The results of this study indicate that autologous BMMNC administration combined with Kasai operation is safe. Furthermore, liver functions were maintained or improved during the mean follow-up duration of 4.5 years in our study patients. This combination may be an alternative approach for the effective treatment of BA. However, a larger randomized study is required to yield a more accurate conclusion.

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Authors' contributions
LNT, PNH, TPTK, MND, and QNT participated in the study conceptualization, experimental design, and data collection. PNH performed the data analysis. LNT, PNH, TPTK, MND, HHTT, HBT, QNT, PNV, DHP and DTQ participated in the acquisition and interpretation of the data and drafted the manuscript. LNT and QNT have accessed and verified the underlying data. All authors have read and approved the final version of the manuscript.

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Data availability
The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Autologous BMMNC infusion for children with BA was approved by the Ministry of Health with approval number: 612/QD-BYT on February 21st, 2014. The Institutional Review Board of Vinmec International Hospital evaluated the ethical aspects of the study in accordance with the World Medical Association's Declaration of Helsinki. The study was explained in detail to the parents of the participants.

Consent to participate
This manuscript does not include personal information of participants. We confirm that informed consent to receive the cell therapy/interventions, containing explanation of benefits and risks, was obtained from the parents of all patients.
Autologous BMMNC infusion for children with BA was approved by the Ministry of Health (Approval number: 612/QD-BYT). Participants in this study did pay for treatment.

Competing interests
The authors declare no competing interests.

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References

1. Liu MB, et al. Biliary atresia in Vietnam: Management and the burden of disease. Surgery. 2017;161(2):533–7.
2. Lakshminarayanan B, Davenport M. Biliary atresia: A comprehensive review. J Autoimmun. 2016;73:1–9.
3. Nio M, et al. Five- and 10-year survival rates after surgery for biliary atresia: a report from the Japanese Biliary Atresia Registry. J Pediatr Surg. 2003;38(7):997–1000.

4. Kasai M, et al. Surgical treatment of biliary atresia. J Pediatr Surg. 1968;3(6):665–75.
5. Chardot C, et al. Prognosis of biliary atresia in the era of liver transplantation: French national study from 1986 to 1996. Hepatology (Baltimore, Md). 1999;30(3):606–11.
6. Chardot C, et al. Improving outcomes of biliary atresia: French national series 1986–2009. J Hepatol. 2013;58(6):1209–17.
7. Davenport M, et al. Biliary atresia in England and Wales: results of centralization and new benchmark. J Pediatr Surg. 2011;46(9):1689–94.
8. Parolini F, et al. Biliary atresia: 20–40-year follow-up with native liver in an Italian centre. J Pediatr Surg. 2019;54(7):1440–4.
9. Lee WS, et al. Chronic liver disease is universal in children with biliary atresia living with native liver. World J Gastroenterol. 2017;23(43):7776–84.
10. Suzuki T, et al. Evaluating patients' outcome post-Kasai operation: a 19-year experience with modification of the hepatic portoenterostomy and applying a novel steroid therapy regimen. Pediatr Surg Int. 2010;26(8):825–30.
11. Wada M, et al. Experience of treating biliary atresia with three types of portoenterostomy at a single institution: extended, modified Kasai, and laparoscopic modified Kasai. Pediatr Surg Int. 2014;30(9):863–70.
12. Park CJ, et al. Surgical modifications of the Kasai hepatoportoenterostomy minimize invasiveness without compromising short- and medium-term outcomes. J Pediatr Surg. 2019;54(3):537–42.
13. Sumida W, et al. Oral administration of eicosapentaenoic acid suppresses liver fibrosis in postoperative patients with biliary atresia. Pediatr Surg Int. 2018;34(10):1059–63.
14. Zhang M-Z, et al. Adjuvant steroid treatment following Kasai portoenterostomy and clinical outcomes of biliary atresia patients: an updated meta-analysis. World J Pediatr. 2017;13(1):20–6.
15. Yang C-Z, et al. Effects of postoperative adjuvant steroid therapy on the outcomes of biliary atresia: a systematic review and updated meta-analysis. Front Pharmacol. 2022;13:956093.
16. Davenport M, et al. Steroids in biliary atresia: single surgeon, single centre, prospective study. J Hepatol. 2013;59(5):1054–8.
17. Nguyen QT, et al. Bone Marrow-Derived Mononuclear Cells in the Treatment of Neurological Diseases: Knowns and Unknowns. Cell Mol Neurobiol. 2023;43(7):3211–50.
18. de Carvalho SN, et al. Bone marrow cell transplantation is associated with fibrogenic cells apoptosis during hepatic regeneration in cholestatic rats. Biochem Cell Biol. 2013;91(2):88–94.
19. Sakaida I, et al. Transplantation of bone marrow cells reduces CCl4-induced liver fibrosis in mice. Hepatology. 2004;40(6):1304–11.
20. Weerasooriya VS, White FV, Shepherd RW. Hepatic fibrosis and survival in biliary atresia. J Pediatr. 2004;144(1):123–5.
21. Pankaj P, et al. Autologous bone marrow transplantation in decompensated liver: Systematic review and meta-analysis. World J Gastroenterol. 2015;21(28):8697–710.
22. Nguyen TL, Nguyen HP, Nguyen TK. The effects of bone marrow mononuclear cell transplantation on the quality of life of children with cerebral palsy. Health Qual Life Outcomes. 2018;16(1):164.
23. Thanh LN, et al. Improvement in gross motor function and muscle tone in children with cerebral palsy related to neonatal icterus: an open-label, uncontrolled clinical trial. BMC Pediatr. 2019;19(1):290.
24. Nguyen LT, et al. Outcomes of autologous bone marrow mononuclear cells for cerebral palsy: an open label uncontrolled clinical trial. BMC Pediatr. 2017;17(1):104.
25. Gupta DK, et al. Stem cells as a therapeutic modality in pediatric malformations. Transplant Procs. 2007;39(3):700–2.
26. Sharma S, et al. Bone marrow mononuclear stem cell infusion improves biochemical parameters and scintigraphy in infants with biliary atresia. Pediatr Surg Int. 2011;27(1):81–9.
27. Nguyen TL, et al. Autologous bone marrow mononuclear cell infusion for liver cirrhosis after the Kasai operation in children with biliary atresia. Stem Cell Res Ther. 2022;13(1):108.
28. Mukhopadhyay SG, et al. A histopathological study of liver and biliary remnants in the long-term survivors (>10 years) of cases of biliary atresia. Indian J Pathol Microbiol. 2014;57(3):380–5.
29. Lee WS, Looi LM. Usefulness of a scoring system in the interpretation of histology in neonatal cholestasis. World J Gastroenterol. 2009;15(42):5326–33.
30. Gunadi A, et al. Histopathological findings for prediction of liver cirrhosis and survival in biliary atresia patients after Kasai procedure. Diagn Pathol. 2020;15(1):79–79.
31. Roy P, et al. A histopathological study of liver and biliary remnants with clinical outcome in cases of extrahepatic biliary atresia. Indian J Pathol Microbiol. 2010;53(1):101–5.
32. The French METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. J Hepatol. 1994;20:15–20.
33. Wiesner RH, et al. MELD and PELD: application of survival models to liver allocation. Liver Transplant. 2001;7(7):567–80.
34. Bersani I, et al. Incidence of umbilical vein catheter-associated thrombosis of the portal system: a systematic review and meta-analysis. World J Hepatol. 2021;13(11):1802–15.
35. de Carvalho SN, et al. Bone marrow mononuclear cell transplantation increases metalloproteinase-9 and 13 and decreases tissue inhibitors of metalloproteinase-1 and 2 expression in the liver of cholestatic rats. Cells Tissues Organs. 2013;198(2):139–48.
36. de Souza VCA, et al. Bone marrow-derived monocyte infusion improves hepatic fibrosis by decreasing osteopontin, TGF-β1, IL-13 and oxidative stress. World J Gastroenterol. 2017;23(28):5146–57.
37. Pinheiro D, et al. Cytokines, hepatic cell profiling and cell interactions during bone marrow cell therapy for liver fibrosis in cholestatic mice. PloS One. 2017;12(11):e0187970.
38. Pinheiro D, et al. Mechanisms Underlying Cell Therapy in Liver Fibrosis: An Overview. Cells. 2019;8(11):1339.
39. Schwartz RE, et al. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. J Clin Invest. 2002;109(10):1291–302.
40. Terai S, et al. An in vivo model for monitoring trans-differentiation of bone marrow cells into functional hepatocytes. J Biochem. 2003;134(4):551–8.
41. Chen Y, et al. In vitro differentiation of mouse bone marrow stromal stem cells into hepatocytes induced by conditioned culture medium of hepatocytes. J Cell Biochem. 2007;102(1):52–63.
42. Stock P, et al. Human bone marrow mesenchymal stem cell-derived hepatocytes improve the mouse liver after acute acetaminophen intoxication by preventing progress of injury. Int J Mol Sci. 2014;15(4):7004–28.
43. de Andrade DC, et al. Bone marrow mononuclear cell transplantation improves mitochondrial bioenergetics in the liver of cholestatic rats. Exp Cell Res. 2015;336(1):15–22.

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