The research of human umbilical cord mesenchymal stem cells therapy in decompensated liver cirrhosis associated with hepatitis virus B: a long-term follow up analysis of a single-center, on-limits and stepped research.

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Research Article

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Abstract

Background: There is a large number of decompensated liver cirrhosis patients in China, caused by infection of hepatitis B virus(HBV) mainly. These patient suffered a process of irreversible liver cirrhosis. Umbilical cord-derived mesenchymal stem cells (UC-MSCs) has the potential of self-renewal and multidirectional differentiation, which makes it possible for curing decompensated liver cirrhosis.

Methods: 24 participants was recruited and divided into 3 groups. hUC-MSCs would be infused via peripheral vein 3 times. A 24 weeks follow up visit would be proceeded, including detecting liver function, coagulation function, general condition, immune system etc. Adverse event also would be recorded. A 1.5 year visit of survival would be proceeded subsequently.

Results: After infusion, liver function was improved in a short time. ALB increased in 57^{th} and 85^{th} day, but descended to baseline level in 169^{th} day. PTTA was significantly improved since 29^{th} day till 157^{th} day. IL-8 was decreased during the whole visit. AE of class 1 and 2 were over 70%, while SAE occurred only 3 times. The 6-month survival rate is 75%, 75%, 100% in low, medium, high dose group. The interaction between dosage and efficacy is weak.

Conclusion: hUC-MSCs has great potential for treating patients of decompensated liver cirrhosis associated with HBV, with satisfied safety. The large sample size and RCT trial is required to prove its therapeutic effect.

Trial registration: This trial was registered in clinicaltrials.gov and the registration ID is NCT05442437. The name of registry is “Clinical Study of hUC-MSCs Treating Decompensated Liver Cirrhosis With HBV”. The registry published in 1^{st} of July,2022.

Introduction

Stem cells is immature cells with self-differentiation, self-renewal and self-replication. Stem cells are usually divided into embryonic stem cells and adult stem cells (1). Mesenchymal stem cells (MSCs) are a common family of stem cells. MSCs origins from mesoderm and ectoderm in the early stage of embryonic development (2). MSC is mainly found in fibrous connective tissue and periorgan stroma. MSC was originally discovered in bone marrow, and has attracted increasing attention of researchers because of its multi-directional differentiation potential, supporting effect on hematopoietic system, promoting stem cell implantation, immune regulation and self-renewal and replication (3). In recent years, studies around the world have shown that MSC can differentiate into bone (4), muscle (5), fat, cartilage, tendon, liver, nervous system, myocardium (6), endothelium and other tissues (7) and participate in the repair and regeneration process under the corresponding induction conditions in vivo or in vitro. After continuous subculture and cryopreservation, MSC still has the potential of multidirectional differentiation and can be used as an ideal seed cell for repairing organ or tissue damage caused by aging and disease (8).

Umbilical Cord-derived Mesenchymal Stem Cells (UC-MSCs) is one kind of adult stem cells with self-renewal and multidirectional differentiation potential. In addition, UC-MSCs also have the advantages of convenient sampling, stable biological performance, ethical controversy free, low immunogenicity and so on. These characteristics drive the wide application and research of UC-MSCs in the field of tissue engineering.

The liver is one of the most important and complex organs in the human body. Drugs, alcohol, viruses and other factors may damage the liver, resulting in hepatitis, fatty liver disease, cirrhosis and other liver diseases which are most-common liver diseases in China. Most of the patients with decompensated hepatitis B cirrhosis are not satisfied with conventional therapy. As the disease develop, patients might got hepatic encephalopathy, esophageal variceal bleeding, liver failure, hepatorenal syndrome and other severe disease which threaten their lives. Because of multi-directional differentiation potential and good ability of proliferation, mesenchymal stem cells (MSCs) can produce a variety of cytokines and growth factors, also has capability of hematopoiesis, immune regulation and anti-inflammatory function, that confirmed in animal experiments and clinical studies, we believe that MSCs for treatment of hepatitis B viral hepatitis cirrhosis of the liver decompensation has great potential, and it is suitable for cell transplantation.

Therefore, we design this clinical trial for exploring a new therapy method of decompensated liver cirrhosis associated with HBV. The main object of this clinical trial is investigating the survival rate, promotion of the liver function, improvement of health, and also the safety and the tolerability of patients after infusing hUC-MSCs. We expected this trial would bring new hope for deconpenstaed liver cirrhosis patients.

Patients and methods

Study design

Our study is a open-labelled, stepped-up and single center research. We plan to recruit 24 voluntary patients of decompensate liver cirrhosis with HBV, dividing them into 3 group:1.low-dose group, accepting infusion of 100mL with 2.5×10^7 cells; 2.medium-dose group, accepting infusion of 100mL with 5.0×10^7 cells; 3.high-dose group, accepting infusion of 100mL with 1.0×10^8 cells. Each group contains 8 patients.

We treat the subjects with human umbilical cord mesenchymal stem cells via venous transfusion. First investigators arrange a whole test for participants, such as vital sign examination, laboratory test, ECG, CT, MRI, ultrasound etc. Investigators screen these patients with a complete eligibility criteria. Then investigators proceed the therapy in the 1st, 8th and 15th day. There are 8 times of follow-up visit for these patients. The first 4 times of those are proceeded during the hospitalization, while last 4 times happens after the discharge which should be proceeded in our clinics for necessary examination.

The 4 times follow-up visit includes vital sign examination, laboratory test, ECG, CT, MRI, ultrasound, Child-Pugh grade, MELD grade and SF-36 test. These follow-up visit last 24 weeks (probably 6 months) since the first treatment. After that, investigators also arrange a survival visit through telephone or clinic each 6 months, lasting another 1.5 years.

Participants recruitement

Decompensated liver cirrhosis with HBV is defined as patients who has liver cirrhosis caused by HBV when occurs complication such as stubborn ascite, upper gastrointestinal hemorrhage, hypersplenism or even hepatic encephalopathy.

We recruited participants according to a strict inclusion and exclusion criteria.

1.Inclusion criteria included:

• aged between 18 and 65 (including 18 and 65), regardless of gender;

• decompensated stage of viral hepatitis B cirrhosis;

• the conventional medical treatment failed, and the health condition got worse;

• serum albumin < 35g/L, total bilirubin < 170μmol/L, prothrombin activity > 30%;

• Child-pugh score ≥7;

• MELD score ≤15;

• hemoglobin > 70g/L, blood platelet > 3×10^9/L;

• patients who cannot accept liver transplantation for now or forever;

• patients participating in the clinical study voluntarily, willing to sign informed consent.

2.Exclusion criteria included:

• patients with spontaneous peritonitis or other serious infection;

• patients with hepatorenal syndrome;

• severe hepatic encephalopathy, massive hemorrhage of varicose in upper digestive tract in recent 1 month;

• portal vein thrombosis;

• complicated with serious diseases of heart, lung, kidney, blood and endocrine system;

• HIV positive;

• positive auto-antibodies related to autoimmune liver disease;

• presence of liver or any type of malignant tumor;

• pregnant women, breast-feeding women or those with recent plan of pregnancy;

• patients have a history of alcohol and drug abuse and failed to quit it;

• participated in other clinical trials within 3 months prior to enrollment;

• participated in clinical research on stem cells;

• unwillingness to sign informed consent;

• other situation that investigator considers inappropriate for patients to participate in this study.

Treatment procedure

The infusion follows the procedure below: 1) Examination: checking the appearance of the hUC-MSCs bag package to ensure no leakage or damage, and the label is complete and clear. 2) Blending: shaking the hUC-MSCs bag 10 times with wrist force to fully mix the cells (because the cell suspension contains human blood albumin, it is normal for the cell suspension to increase foam after weak shaking). 3) Establish venous access; establishing a fluent venous access and flush the tube fully with 0.9% sodium chloride solution. 4) Intravenous drip: in the low-dose group, human umbilical cord mesenchymal stem cells (100mL), containing 2.5×10^7 stem cells, were injected intravenously for 3 times (day 1, day 8 and day 15, respectively) at an interval of 7 days; medium dose group: human umbilical cord mesenchymal stem cell preparation 100mL, containing 5.0×10^7 stem cells, intravenous infusion interval of 7 days, respectively on day 1^{st}, day 8^{th}, day 15^{th}, a total of 3 times; high-dose group: human umbilical cord mesenchymal stem cells (100mL), containing 1.0×10^8 stem cells, were injected intravenously for 3 times (1, 8 and 15 days, respectively) at an interval of 7 days.

There are some notification for the infusion procedure:1) Human umbilical cord mesenchymal stem cell preparation diluted with 0.9% sodium chloride solution should be used within 6 hours, and the diluted human umbilical cord mesenchymal stem cell preparation should be stored at room temperature (15-30 ℃), away from light; 2) Infusion speed control: The infusion rate is about 30 drops /min in the first 15 minutes, and it can be increased to about 45 drops /min in 15-30 minutes. If the subject has no discomfort, the infusion rate can be increased to a maximum of 70 drops /min. Gently shake the infusion bag every 5-10 minutes to avoid cell sedimentation and accumulation during the infusion. The total infusion time should be limited in 1 hour; 3) At the end of infusion, rinsing the pipeline with 0.9% sodium chloride solution to avoid cell waste; press the puncture point for more than 10 minutes after needle withdrawal after finishing the infusion

Outcome mearsures

1. Survival: We evaluate the survival rate of participant with 6-month and 2-year survival rate, which excludes the participant dead, loss to follow-up, quiting the clinical trials.
2. Liver function: We test the following index in first day (baseline), 29th day, 57th day, 85th day and 169th day, such as, serum albumin (ALB, g/L), alanine aminotransferase (ALT, U/L), aspartate aminotransferase (AST, U/L), cholinesterase (CHE,U/L), total bilirubin (T-Bil, μmol/L), direct bilirubin (D-Bil, μmol/L), serum total cholesterol (TC, mmol/L), then figure out their tendency, for describing the liver function variation after therapy of hUC-MSCs.
3. Coagulation function: We test the following index in first day (baseline), 29th day, 57th day, 85th day and 169th day, such as, prothrombin activity (PTTA, %), and antithrombin-III (AT-III, mg/L), then figure out their tendency, for describing the coagulation function variation after therapy of hUC-MSCs.
4. Clinical feature: Including Symptoms and general condition evaluation.

Symptoms we focus including ascites, edema of legs, haematemesis, jaundice and poor appetite. These symptoms are recorded in the first day, 29th day, 57th day, 85th day and 169th day. It is performed as “condition in baseline/condition in visit”, and symbol “-” represents negative of this symptoms, meanwhile symbol “+” represents positive of this symptoms.

We measure the weight and calculate Child-Pugh and MELD score for participants in first day, 29th day, 57th day, 85th day and 169th day, and also the medical outcomes study 36-item Short Form Health Survay scale (SF-36 scale) in 85th day and 169th day. Child-Pugh score is the most commonly used tool for liver reserve function, which is measured according to hepatic encephalopathy, ascites, serum albumin, total bilirubin, prothrombin time. Score 5-6 is grade A. Score 7-9 is grade B. Score 10-15 is grade C. MELD score is a efficient tool for evaluating the severity of terminal liver disease. The caculation formula is MELD=3.78*ln(total bilirubin mg/dL) +11.2*ln(INR)+ 9.57*ln(serum creatine mg/dL)+ 6.43 (for HBV patient). Higher score in both Child-Pugh and MELD declares worse liver function and general condition. The SF-36 scale is a measurement for patients' living quality. The scores contain 8 parts, including physical functioning, role-physica, bodily pain, general health, vitality, social functioning, role-emotional and mental health. Conversion score=(actual score-minimum score in this part)/(maximum score in this part-minimum score in this part)*100. Score of each part range from 0 to 100. The higher score means more healthy.

5. Immune system: We test the following index in first day (baseline), 29th day, 57th day, 85th day and 169th day, such as CD4+ T cell, CD8+ T cell, Th1 cells proportion, Th2 cells proportion, Naural Killer cells, Natural Killer-T cells(NK-T cells) proportion, Interleukin-1β (IL-1β, pg/mL), Interleukin-4 (IL-4 , pg/mL), Interleukin-6 (IL-6, pg/mL), Interleukin-8 (IL-8, pg/mL), Interleukin-12 (IL-12, pg/mL), Interleukin-15 (IL-15, pg/mL), and Interleukin-17A (IL-17A, pg/mL), to performing the effect of hUC-MSCs to immunity system.
6. Adverse event: Adverse event (AE) including infusion reaction, anaphylaxis, hemolysis, acute liver failure, acute kidney failure and other obvious variations of examination. We observe and record

whether participants shows these adverse event during the first whole 2 weeks and 29th day, 57th day, 85th day and 169th day. All records of adverse event must include name of AE, beginning and terminal time of AE, management of AE, association with hUC-MSCs, prognosis of AE, whether serious adverse event (SAE) or not.

serious adverse event (SAE) is defined as the AE caused the following situation, including death, threat to life, admission to hospital or delay of discharge from hospital, loss of function(eternal or severely). When SAE occurs, it must be recorded and reported to the department of health of government in our electronic data capture systems in 24 hours.

Statistical analysis

For continuous variables, descriptive statistics will include number of subjects (N), number of Missing visitors (Missing), Mean (Mean), standard deviation (SD), quartile (Q1, Q3). We adopt Shapro-Wilk test for normality test of samples. Signed-rank test was proceeded for comparison within group (any visit compared with baseline). As the comparison among low, medium and high-dose group, we use Fisher test for normal distribution and Kruskal-Wallis test for inparameter test.

For categorical variables, frequency and frequency or component ratio (%) were used for statistical description. The frequency and percentage are reserved as 1 decimal place. Chi-square test is used for analyzing the statistic difference.

Survival analysis is proceeded by Kaplan-Meier curves and statistical analysis use logrank analysis.

For all analysis, we define p value <0.05 as statistical significance.

Results

Enrollment and Survival

In our study we enrolled all 39 patients and 15 isn't suitable for our criteria. All of 24 enrolled participants had received whole 3 infusion of hUC-MSCs. However, 1 in low-dose group and 2 in medium-dose group haven't finished the follow-up visit.

For the low-dose group, the 6-month survival rate is 75%(6 of 8). One participant withdraw the follow-up because the newly diagnosed liver cancer in 7th visit (day 85th). Another participant missed the follow-up after 7th visit and he/she cannot be connected till now. The 2-year survival rate is 50%(4 of 8). Besides the withdraw one and drop out one above, 2 participants were dead. One is dead because of cerebral hemorrhage caused by trama (23 months after first infusion), another is dead due to hepatic encephalopathy(11 months after first infusion). Our conclusion is that these 2 deaths are irrelevant to the hUC-MSCs preparation.

For the medium-dose group, the 6-month survival rate is 75%(6 of 8). One participant missed all the follow-up visit and we couldn't connect to him/her. There is another participant couldn't arrive hospital for visit due to the epidemic of COVID-19 (which led the curfew in his/her city for a longtime), but we are able to connect to him/her and confirm his/her alive. Yet this case was still defined as drop out. 2-year survival rate is also 75% (6 of 8)

For the high-dose group, the 6-month survival rate is 100%(8 of 8). 2-year follow-up hasn't finished yet while the last participant accepted the first infusion in December 2021, who will finished the survival visit till December 2023. However, the current survival rate is 87.5%(7 of 8) according to our investigation. One participant was dead nearly 7 months after first infusion, because of hepatic failure and hepatic encephalopathy. The whole study flow is shown in Fig. 1.

Kaplan-Meier survival analysis was applied for researching the survival of three groups. As the Fig. 2 displays, there is no significant difference in survival ratio among low, medium and high-dose group (logrank = 0.1741, P = 0.9166), inferring infusing higher concentration of hUC-MSCs might not raise the risk of life.

Liver function

For the patients of decompensated liver cirrhosis, most of them cannot maintain the liver function as good as healthy person. Thus the evaluation of liver function is one of the most essential test. In our trials we test serum albumin, alanine aminotransferase, aspartate aminotransferase, cholinesterase, total bilirubin, direct bilirubin, and serum total cholesterol for description of liver function. As we figured out, in day 169th, all these indexes were not significantly different from the baseline level (**S-Table 1**). But during the follow-up visit some indexes have been obviously changed which will be decribed below. The viariation was without statistically different among these 3 groups. This might suggested dosage didn't effect well on liver function.

The serum albumin level was significantly raised in low and high-dose groups in day 29th (P = 0.0156, 0.0156) and day 57th (P = 0.0156, 0.0078), and raised in all 3 groups in day 85th (P = 0.0156, P = 0.0313, P = 0.0313)**(**Fig. 3A**)**. However, Alb level of all 3 groups dropped down in day 169th and didn't show statistical significance. Besides, during all visit time, there was no statistic difference of variation of Alb (which compared with baseline level) among these 3 groups. These might suggest infusion of different dose of hUC-MSCs doesn't effect the serum albumin level.

ALT level only raised in day 85th of high-dose group **(P = 0.0469)**, and it didn't beyond the ULN (45U/L). The difference among 3 groups shows no significant statistical difference. The variation of ALT level hasn't performed significant difference in each visit **(**Fig. 3B**)**. Variation of AST among groups also shows no statistical significance **(**Fig. 3C**)**. In day 29th, CHE level raised remarkably in high-dose group (), and in day 85th it increased in low-dose group, but both max level still less than ULN (12500U/L) **(S-Table 1)**. As the time went by, CHE level return as no difference compared to baseline. Likewise, variation of CHE

among groups has no statistical significance. These indexes infer the hUC-MSCs might not damage the hepatocyte.

Total bilirubin was discovered that in day 29th, the medium-dose group performed a significant increasing of T-Bil (P = 0.0156), however it returned to normal level since day 57th (Fig. 3D). The variation of T-Bil among groups shows statistic difference in day 29th (P = 0.0108), but it did not have a dose-effect relationship between dosage of hUC-MSCs and T-Bil. As Fig. 3E shown variation of direct bilirubin expressed neither significant difference in each visit compared to baseline, nor among these 3 groups.

Figure 3F performed the variation of total cholesterol level. We found that in day 29th, TC level raised in low-dose group (P = 0.0391), and recovered as no difference since day 57th. Moreover, high-dose group showed significant augmentation in day 57th (P = 0.0078). The variation of TC of all 3 groups remains no significantly different.

Coagulation function

Most of the decompensated liver cirrhosis patients doesn't have normal coagulation function that might result in upper gastrointestinal hemorrhage, and it could be very dangerous. Therefore we test prothrombin activity and antithrombin-III level to judge coagulation function of participants.

Tabel 2 showed in the last visit PTTA of high-dose group was remarkably increased comparing the baseline (P = 0.00156), meanwhile this variation wasn't obviously different comparing the variation of low and medium-dose group. It is obviously that high-dose group enhanced the PTTA since day 29th and lasted till the final visit (day 29th P = 0.0078, day 57th P = 0.0391, day 85th P = 0.0156, day 169th P = 0.0156)(Fig. 4A). While the comparison of among 3 groups has no statistical significance. Figure 4B illustrates the AT-III variation in all visit. Day 29th we could be told that AT-III raised remarkably in low (P = 0.0156) and high-dose group (P = 0.0156), besides AT-III level of high-dose group still remained higher in day 57th(P = 0.0156). The last visit it doesn't varied significantly in all three groups and no difference of variation among 3 groups **(S-Table 2)**. Unfortunately the medium-dose group loss some data. The reason will be described in discussion part.

Clinical feature

Patients of decompensated liver cirrhosis might represent various Clinical feature. For estimating the whole body situation, many methods was created. In this trials we applied most common methods, the Child-Pugh score, the MELD score and SF-36 scale. As recorded, in the last visit the weight of participant didn't change significantly, and Child-Pugh and MELD score remains similar compared with baseline **(S-Table 3)**.

We could be told that weight of participants remains in a stable level, and 3 different groups show similar variation (Fig. 5A). The low-dose group performed a significant lower Child-Pugh score in day 57th (P = 0.0313)(Fig. 5B). The variation among groups has no statistic difference. Besides could find that MELD

score has unconspicuously changed in each visit, and 3 groups all have no significant alteration of MELD(Fig. 5C). Our data also shows that all three groups perform a healthy life and maintain well in the last visit **(S-Table 3)**. However, only 2 participants of low-dose group filled this scale, and 5 participants of medium-dose group finished this. Fortunately, high-dose group was highly completed of SF-36 scale investigation **(S-Table 3)**.

We also records symptoms of all participants in baseline and all follow-up visit. These symptoms include ascites, edema of legs, haematemesis, jaundice and poor appetite (from **S-Table 4** to **S-Table 8**).

From these records, we discover that in low-dose group, most of the participants had ascites in baseline. After therapy of hUC-MSCs, 5 of 8 (62.5%) participants became ascites free in day 29th. As times went by, the number decreased, and finally only 1 of 4 (25%) participant got improvement on ascites. In medium-dose group, the situation was better that 3 of 5 (60%) participant get rid of ascites. As to high-dose group, 2 of 8 (25%) participant became ascites free, whereas there are 2 of 8 (25%) participant developed the ascites, who were negative of ascites at the beginning.

Majority of low-dose groups participants had edema in legs in baseline. In day 57th and day 85th, 5 of 7 (71.4%) participants improve their symptoms of edema, but at last only 1 of 3 (33.3%) maintained this improvement. There is 1 of 5 (20%) participant got cure of edema in legs of medium-dose group. 1 of 8 (12.5%) participant of high-dose group became better in edema, meanwhile 1 of 8 (12.5%) get worse.

Only 1 participant in low-dose group occurred haematemesis in day 29. No one had haematemesis in the first day. We also find that 1 of 7 (14.3%) in low-dose group develop jaundice in day 29th, but after that got better. 1 of 8 (12.5%) in high-dose group ameliorate his/her jaundice. It was reported that only 1 of 7 (14.3%) in low-dose group develop poor appetite in day 29th but got improvement in following visit. No one has poor appetite in baseline visit.

Immune function

Patients of decompensated liver cirrhosis are susceptible to infection, due to hyperplenism and the establishment of collateral circulation between portal veins. So it is easy to occur bronchitis, pneumonia, peritonitis or biliary tract infection in these patients. As Fig. 6 demonstrated, only IL-8 was significant decreased comparing the baseline in the last visit ($P = 0.0156$), besides the variation of IL-8 in 3 groups showed statistical significant ($P = 0.0491$).

We ran a detailed test on immunity function. Over 10 indexes were examined for all participants. After infusion of hUC-MCSs, CD4 + T cell and CD8 + T cell didn't show a significant decreasing in follow-up visit (Fig. 6A, Fig. 6B). Similarly, Th1 cell and Th2 cell neither descended remarkably in all 4 times visit. Natural Killer increased obviously in day 29th of low-dose group ($P = 0.0469$), and also ascended in day 57th of high-dose group ($P = 0.0391$), but these variation represent no statistical significance among the 3 groups (Fig. 6C). Natural Killer T cell didn't alter evidently through the whole follow-up visit. There are 7 kinds of interleukin were tested during the visit, and it is easy to find that IL-8 was evidently decreased in day 29th both in low ($P = 0.0156$) and high-dose group ($P = 0.0234$), and the decreasing lasted till day 169th in

high-dose group (day 57th and day 169th both P = 0.0156) (Fig. 6D). Moreover, In day 169th the variation was significantly different among 3 groups (P = 0.0491), while IL-8 of high-dose group descend more remarkably. IL-17α obvious raised in day 57th of low-dose group than high-dose group (P = 0.0425, medium-dose group missed all visit in day 57th), while IL-17α in high-dose group kept on 0 in day 57th (**S-Table 9**) .

Adverse event

During the whole trial, we monitored the adverse event carefully and recorded as quickly as possible, then handled it appropriately. If serious adverse event occurred, there is a emergency report system which will be activated and all information will be sent to the health department of our province.

As recorded, there are 63, 34 and 54 times of AE happened in low-dose, medium-dose and high dose group (Table 1). Most of these AE was classified as class 1 or 2 grade, whose proportion together was 90%, 82% and 74% in low, medium and high-dose group. The high proportion of class 1 and 2 AE means the AE effect is mild. Class 4 AE only occurred 0, 1 and 1 times in three groups. ANNOVA test shows the difference among three group is not significant (P = 0.2101). It inferred that the dosage won't raise the hazard of AE.

Table 1
Summary of adverse event

		Low-dose group (N = 8)	Medium-dose group (N = 8)	High-dose group (N = 8)	F value	P value
Total adverse event		63	34	54		
Severity of AE	Class 1	33	18	24	2.341	0.2101
	Class 2	24	10	16		
	Class 3	6	5	13		
	Class 4	0	1	1		
Association with hUC-MSCs	absolutely relevant	0	0	0	1.262	0.3435
	possibly relevant	4	0	0		
	possibly irrelevant	1	1	0		
	absolutely irrelevant	58	33	54		
AE led to reduce dosage		0	0	0		
AE led to terminate therapy		0	0	0		
AE led to death		0	0	0		
Annotation:This table excludes the severe adverse event						

All these AE will be judged whether they are relevant to the infusion of hUC-MSCs, but only 4 in low-dose group were possibly relevant to the therapy. Most of AE are absolutely irrelevant, and ANNOVA test show no statistical significance among three groups (P = 0.3435).

When analyzing these AE we figure out that there isn't any AE led to reduce the dosage of hUC-MSCs therapy, nor terminate the therapy. Besides, the AEs didn't cause any death in our trial.

Most common AE is alteration of laboratory examination (71 times). Neutropenia took place 9 times totally. For low-dose group, 1 time is class 2. For medium-dose group, 2 times are class 2. For high-dose group, there are 6 times, defined 1of class1, 3 of class 2 and 2 of class 3. It occurs 8 times of hyperbilirubinemia during all visit. There are 4 times in low-dose group (2 of class 1, 2 of class 2), 1 time in medium-dose group (class 1), 3 times in high-dose group (2 of class 2, 1 of class 4). Ascend of interleukin also took place 8 times, which all classified as class 1 (5 times in low-dose group and 3 times in high dose group). Thrombocytopenia happens 6 times, including 2 in low-dose group (1 of class 1, 1 of

class 3), and 4 in high-dose group (2 of class 2, 2 of class 3). Moreover, there are other AEs such as leukopenia(5 times), lymphopenia(4 times), ascend of urobilinogen (3 times) and so on.

The second most common is gastroentergic AE (20 times). Ascites is the most among the gastroentergic AE, which occurred 5 in low-dose group (1 of class 1, 3 of class 2, 1 of class 3), 1 in medium-dose group (class 2) and 4 in high-dose group (2 of class 1, 1 of class 2 and 1 of class 1). Ventosity also took place commonly, which include 4 in low-dose group (2 of class 1, 2 of class 2) and 2 in high-dose group (class 3).

The metabolic and nutritional AE ranks the third (18 times). There are 9 in low-dose group, containing hyperlipemia (1 of class 1), hypoglycemia (1 of class 2), hypokalemia (1 of class 1), hypocalcemia (1 of class 1, 2 of class 2) and hypoalbuminemia (1 of class 2, 2 of class 3). 4 happened in medium-dose group, containing electrolyte imbalance (1 of class 2), hypocalcemia (1 of class 2) and hypoalbuminemia (1 of class 2, 1 of class 3). For high-dose group, there are 5 times, containing poor appetite (2 of class 3), hyperlipemia (1 of class 1) and hypoalbuminemia (2 of class 2).

Change of general condition is also common, which is 7 times. Four times in low-dose group contain 3 times edema of limbs (2 of class1, 1 of class 2), 1time fever (class 1). Two times in medium-dose group contain 1 time edema of whole body (class 4) and 1time fever (class 1). There is only 1 time edema of limbs in high-dose group, defined as class 3.

Other common AE includes proteinuria (3, 2, 1 times in low, medium, high-dose group, class 2), sinus arrhythmia (2 in low-dose group, 1 in medium-dose group, all class 1), cholecystitis (3 times of class 1, 1 time for each group), urinary tract infection (1 in low-dose group, class 2; 1 in medium-dose group, class 1), peritonitis (1 time in low-dose group, class 1), portal thrombosis (1 time in low-dose group, class 1) and hepatic encephalopathy (1 time in high-dose group, class 3) and so on. The most common AE was listed in Fig. 7.

Serious adverse event(SAE) was reported 3 times.1 participant in low-dose group was admission into our hospital because of a de novo liver cancer, which happened 3 months after inclusion into study. Participant accepted percutaneous ethanol injection(PEI) and radiofrequency ablation(RFA) for treating the tumor. The curative effect was good and this participant is alive without HCC recurrence. For 2nd SAE, participant was in medium-dose group who suffered upper gastrointestinal hemorrhage and was admission into hospital of his/her city, then received endoscopy therapy and drug therapy.The therapy stopped the bleeding. This happens 5 months after our infusion. Third participant has SAE is in high-dose group, who suffered hepatic encephalopathy nearly 5 months after first infusion of hUC-MSCs. This patient accepted therapy in his/her local hospital. Unfortunately the condition got worse and he/she died in over 6 months after first infusion, which is later than the last follow-up visit.

Discussion

Hepatitis B virus (HBV) is one of the most common virus which cause complicated disease in China. The major hazards of HBV are liver cirrhosis and hepatocellular carcinoma (9). Since the cirrhosis process is irreversible, liver transplantation is the only method for patients of decompensated liver cirrhosis to get cured (10). However, great cost of hospitalization, high risk of transplantation surgery and continously post-operative immunosuppressive therapy result in that minority of decompensated liver cirrhosis patients can receive a new liver for a better living. As a department of hepatic surgery, we have receive a large mount of decompensated liver cirrhosis patients every year. The therapeutic effect was unsatisfied. Most of them died of liver failure, hepatic encephalopathy, esophagogastric variceal hemorrhage, hepatorenal syndrome and other complications (11, 12). Therefore, we try hard to seek for a new, safe and effective method for patients who cannot receive liver transplantation, or who will wait longtime for liver transplantation, in order to improve their living quality and lengthen their survival.

Our earlier non-clinical trials in rats showed hUC-MSCs is safe enough for the treatment. It doesn't increase the risk of de novo tumor, nor poison the subject. Trial of Shi Ming (13) discover the HCC-free survival ratio of patients accepted UC-MSCs treatment and control group has no significant difference after 75-month investigation. Bartolucci (6) proceeded a clinical trial of hUC-MSCs for treating heart failure, and there was only one patient developed malignant melanoma, among 30 participants. This trial also reported 2 death in the trial, but one death because of cardiovascular disease took place in placebo group. Some researchers recorded one mortality caused by liver tumor, in a trial of treatment for COVID-19 with hUC-MSCs (14). Thus they monitored the tumor marker of patients with, figuring out there was no difference of these tumor marker between patient receiving placebo and hUC-MSCs. In our trial, there is only one participant who discovered de novo HCC after the infusion. We considered the development of HCC was irrelevant with hUC-MSCs since it had been passed 3 months from the first infusion. In addition, hUC-MSCs actually improve his general situation. This participant first came to our hospital with a large amount of ascites and 9 score in Child-Pugh. When he was diagnosed HCC, the ascites disappeared and Child-Pugh descended to 6, which allowed him to accepted the RFA by laparoscopy. Nevertheless, it still remains unclear whether the mesenchymal stemm cell promotes or inhibits tumor, since it is able to differentiate into a wide variety of cell kind (15). Interaction between MSCs and tumor are complicated. A meta-analysis concluded that MSCs will either suppress or promote tumor, according to its derivation, whether modified or not, and the type of tumor (16). This affect was determined by different signaling pathway that MSCs mediating as well. For example, MSCs play a tumor suppressor by inhibiting REC2 via PI3K/AKT pathway, but also act as tumor promoter by upregulated cyclin D through WNT pathway (17). In any way, we should notice the tumorigenesis of MSCs when proceeding the clinical trial.

Most of adverse event in our trial are classified as class 1 or 2 (total proportion over 70%), which inferred the toxicity and other side effect of hUC-MSCs are weak. A phase I/II clinical trial of MSCs for treating knee osteoarthritis demonstrated no SAE, permanent disability, neoplasia, or septic arthritis was recorded during trial (18). Another trial of hUC-MSCs for treating relapsing remitting multiple sclerosis (RRMS) claimed that no developed tumor and organ disorder was observed throughout 10-year follow up, although partial demyelination lesions was detected (19). As to our trial, the most common AEs are ascites, neutropenia, hyperbilirubin, ascend of interleukin, hypoalbuminemia. Most of them are irrelevant

to hUC-MSCs, we just applied common diuretics or hepatinica for a few days, or even without interference. A few are probably resulted by hUC-MSCs, but the mechanism requires deeper study.

Summarily, the safety of mesencheymal stem cell is reliable, providing essential theoretical basis for clinical application.

Not only the safety, but also the efficacy of hUC-MSCs is crucial for treating patients. Before the trial started, we've explored lots of relevant references and discovered theoretical basis of the treatment of hUC-MSCs on decompensated liver cirrhosis.. Liu (15) summarized several probably mechanism how MSCs repairing the liver injury, such as direct differentiation into hepatocytes, fuse with hepatocytes to repair, paracrine of plenty of cytokine and growth factors for hepatocyte regeneration, modulation on immune system (CD4 + T cells, CD8 + T cells, NK cells, etc.) for curing the immune injury in liver, and inhibiting hepatocellular apoptosis by MSC-conditioned medium. Yin (20) has similar theory with Liu, but also mentioned that the exosome derived from hUC-MSCs might alleviate liver fibrosis. This theory was proved by many trials. One trial demonstrated after injection of exosome from hUC-MSCs into CCl_4-induced injured mice liver, the liver texture became soft, by repressing the expression of TGF-β1, collagen types I and III, and the phosphorylation of Smad2, at the meantime overexpressing E-Cadherin, a epithelial-mesenchymal transition-associated marker (21). Some other researchers discovered hUC-MSCs inhibited proliferation hepatic stellate cells via TGF-β/Smad signaling pathway, when hepatic stellate cells are responsible for liver fibrosis (22). A novel marker in TGF-β/Smad signaling pathway, named the milk fat globule-EGF factor 8 (MFGE8) has been proved one of the critical anti-fibrosis factors (23). Moreover, Chen (24) also figure out that MSCs not only suppressed the activation of hepatic stellate cells but also attenuated collagen deposition, by secret monocyte chemoattractant protein-1, interleukin-6, hepatocyte growth factor and so on, in order to repress liver fibrosis.

Based on these large amount of theory, we believed hUC-MSCs is highly-possible to improve the liver function of decompensated liver cirrhosis. From the data collected we concluded that hUC-MSCs are able to improve the liver function in perhaps 2–3 months since infusion, but the efficacy weaken as time went by. Thus we discovered only PTTA got better, and IL-8 decreased remarkably in the last visit (169th day). One RCT research in Iran illustrated that at the 12-month follow-up, Child scores, MELD scores, serum albumin, INR, serum transaminases and liver volumes haven't got improvement in patients received MSCs compared to placebo (25). The author suggested instead of peripheral vein, infusing MSCs via hepatic artery or portal vein might have potential for better treating the liver cirrhotic patients. A meta-analysis referred, intra-arterial injection exhibited greater efficacy than peripheral vein, improving MELD score and ALB and T-BiL levels (26). It was reported that infusion via artery significantly make MSCs locate in the injury tissue more precisely (27). Furthermore, when infusing via peripheral vein, MSCs initially arrived lung with the most concentration and the second destination was liver (28). Therefore, it was a new idea that we might infuse the hUC-MSCs via hepatic artery or portal vein, in order that as more cells as possible could act on liver injury. However, the difficulty, high consumption and high risk of performing new infusion path are still important issue to be solved.

There are still several limitation of our trial. First, it was not a randomized clinical trial, but a opened labelled one, which has weaker proof on efficacy than former one. We expected we could apply the next-stage trial of hUC-MSCs for treating decompensated liver cirrhosis, on the basis of this one, that would be RCT. Second, the sample size is still small. We also expected a large size of sample could be recruited in the next-stage trial. Third, the infusion frequency and path requires more researching. In this trial we just figure out the interaction of cellular concentration and the efficacy. In a word, there is still a long way to explorer the application of hUC-MSCs for treating decompensated liver cirrhosis patients associated with HBV, for a better therapeutic effect.

Conclusion

hUC-MSCs have great potential in treating decompensated liver cirrhosis patients associated with HBV, but it requires a larger sample size, a RCT trial to be identified. In our trial we demonstrate that hUC-MSCs could improve the liver function in a short time, especially the effect on coagulation function. It is also safe enough for clinical application with low risk of serious adverse event and tumor development. hUC-MSCs effect hardly on immune system. It improve the patient's general condition in some aspect.

Abbreviations

hUC-MSCs: human umbilical cord mesenchymal stem cells

HBV: hepatitis B virus

Alb: albumine

AST: alanine aminotransferase

ALT: aspartate aminotransferase

CHE: cholinesterase

Bil: total bilirubin

TC: cholesterol

PTTA: prothrombin activity

AT-III: antithrombin

NK: natural killer

IL: interleukin

AE: adverse event

SAE: serious adverse event

RCT: randomized controlled trial

Declarations

Ethics approval and consent to participate

Ethics approval was granted by ethics comittee of Zhongnan Hospital of Wuhan University, with number [2018]001, in 30th August, 2018.

All UCs were obtained from healthy adult donors after a written and informed consent, following the Helsinki's Declaration and Administration of Clinical Research on Stem Cells (National Health Commission of China, 2015).

Author contribution

Xian Qin the first author of this manuscript, is the sub-investigator of this clinical trial, in charge of the recruitment, signature of inform consent, proceeding the infusion of UC-MSCs, recording and analyzing the index and adverse event of participant, follow-up visit and the manuscript writing.

Jing Chen has contributed the statistical analysis, figure manufacture, table editing and type composing of the manuscript, as the co-first author.

Li Du, Yan Ma, Yi Li, Yu Lu, Yating Wang, Liufang Wu, Zihui Yu, Mengjing Hu1and Lijun Li are nurses in this clinical trial, who manipulating the infusion of UC-MSCs, observing and recording all the event of participant. Moreover they draw blood and exam the vital sign in each visit.

Bo Liao and Zhen Li are responsible for the observation of participants after infusion and record the vital sign and clinical feature.

Zhiyong Yang is the chief of our department, who is in charge of the communication with other department for the exam arrangement, to ensure the well operation of the clinical trial, as co-correspondence author.

Kun Li is responsible for supervising the correct process of the clinical trial, inspecting the medical record and collating the statistical data, as co-correspondence author.

Yufeng Yuan is the principle investigator of this clinical trial, who designed the whole research plan, and supervising the whole clinical trial, correcting the manuscript and auditing the statistical analysis, as the main correspondence author.

All authors read and approved the final manuscript.

Patient consent

All participants were detailedly notified the procedure, the profit and the risk of this clinical trial and all participants signed the informed consent voluntarily.

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Consent for publication

All authors consent to publication of the present manuscript

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

All the authors declare no competing interests

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Figures

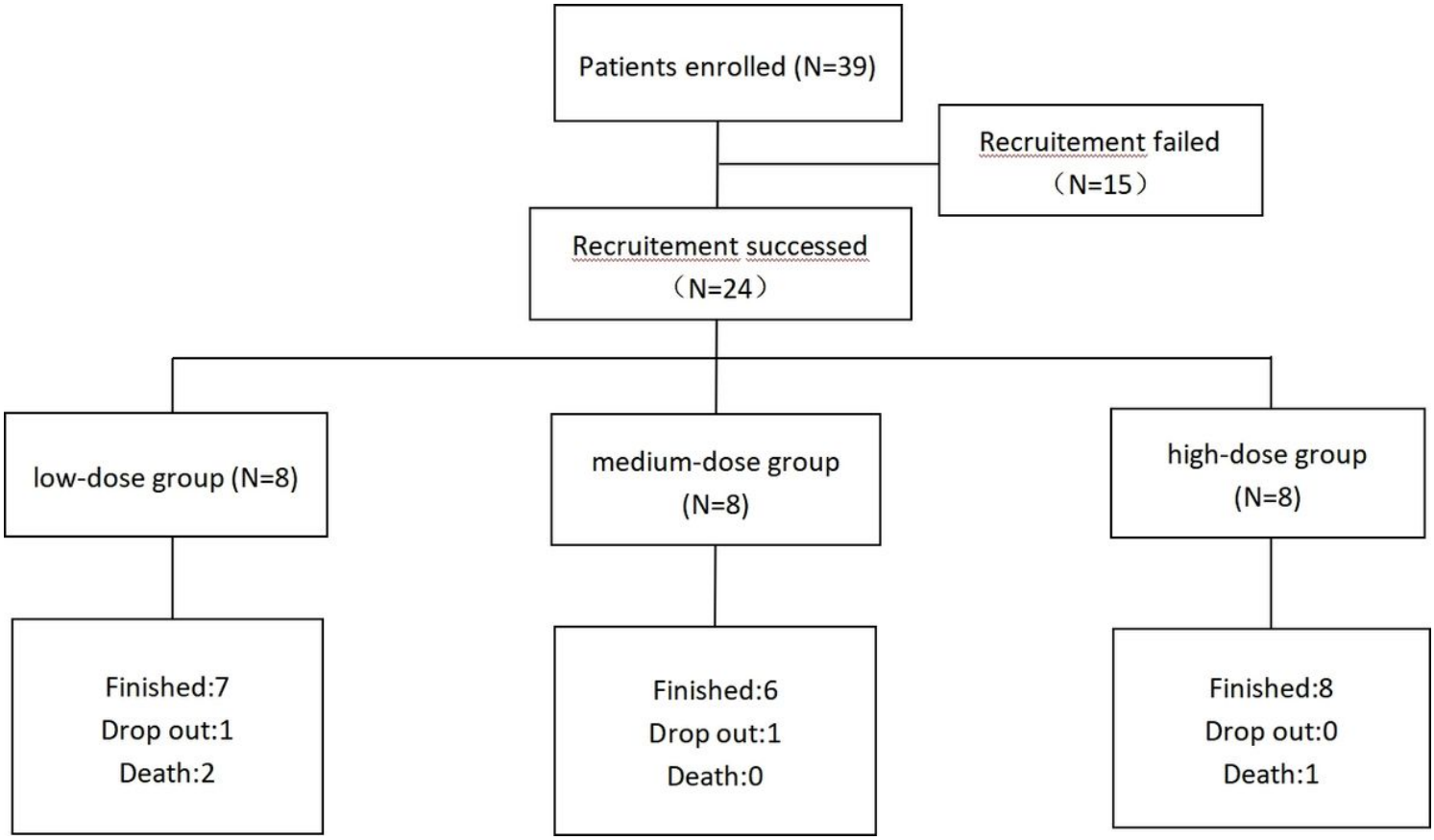

Figure 1

Study flow chart: 39 patients were enrolled and 24 were recruited. Finally 21 of them have finished the whole follow-up visit.

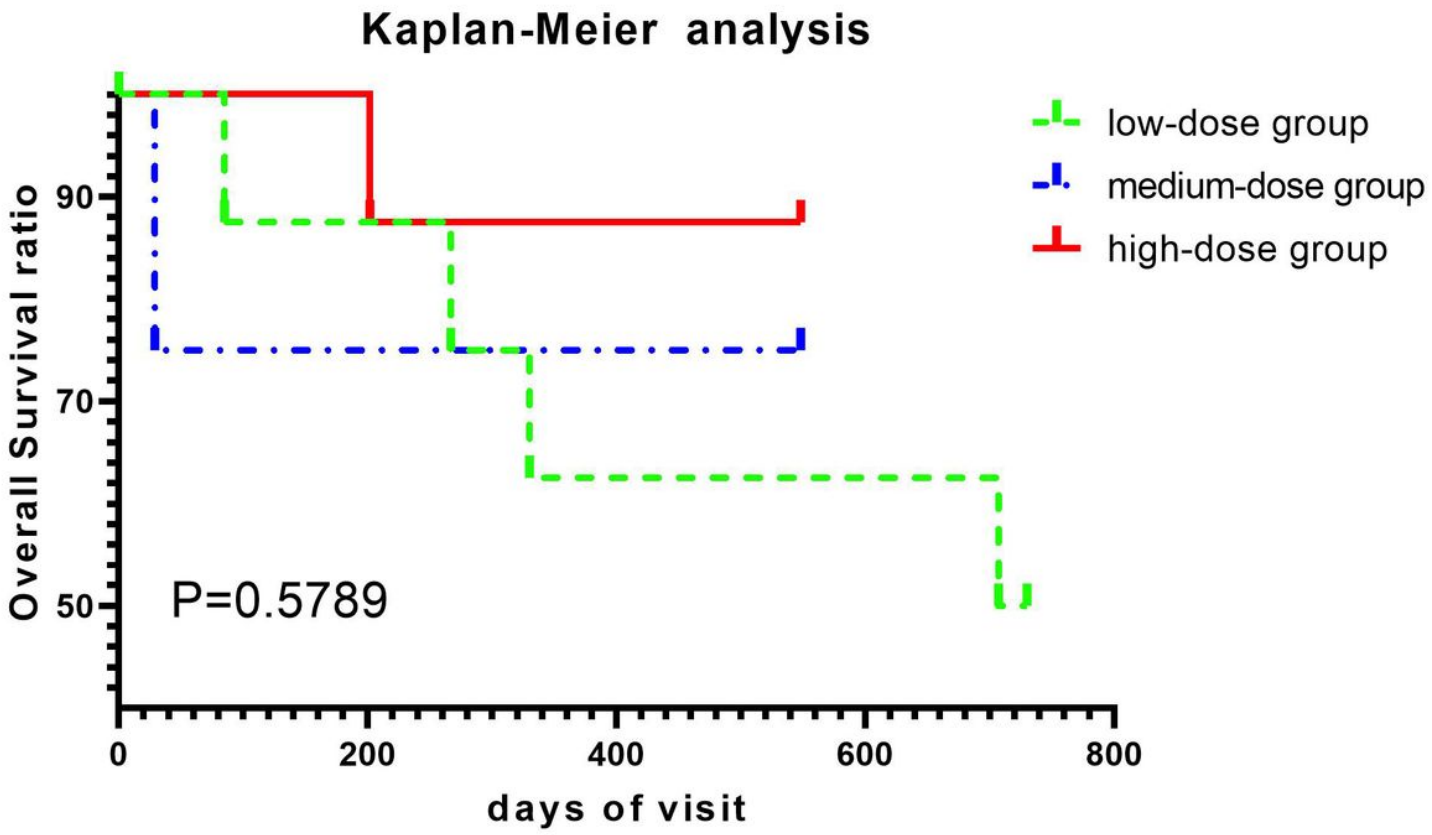

Figure 2

Kaplan-Meier analysis of 3 groups There is no statistical difference among 3 groups (P=0.9166)

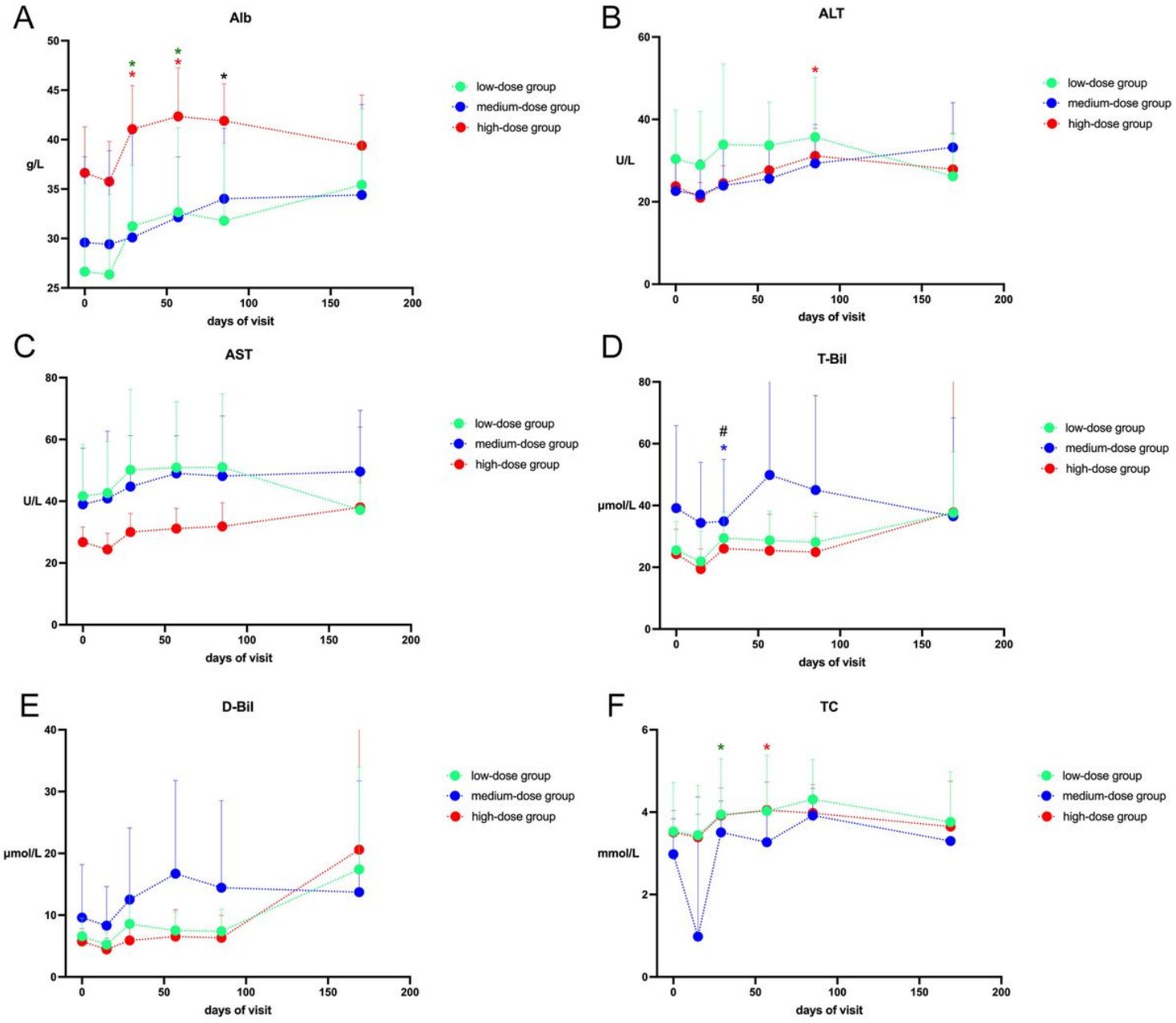

Figure 3

Variation of liver function: (A) serum albumin(B) alanine aminotransferase (C) aspartate aminotransferase (D) serum total bilirubin (E) serum direct bilirubin (F) total cholesterol.

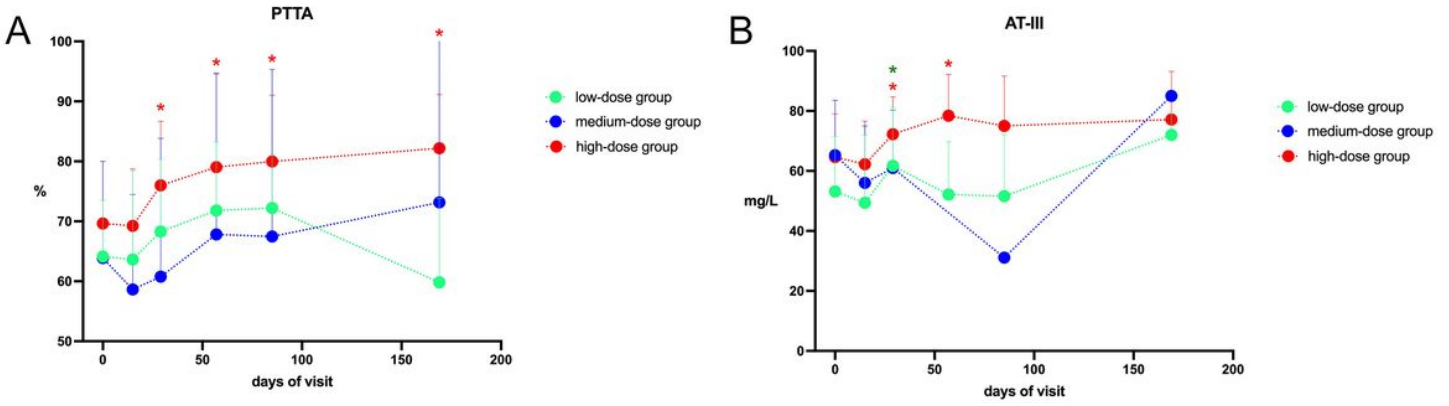

Figure 4

Variation of coagulation function: (A) prothrombin time activity (B) antithrombin-III.

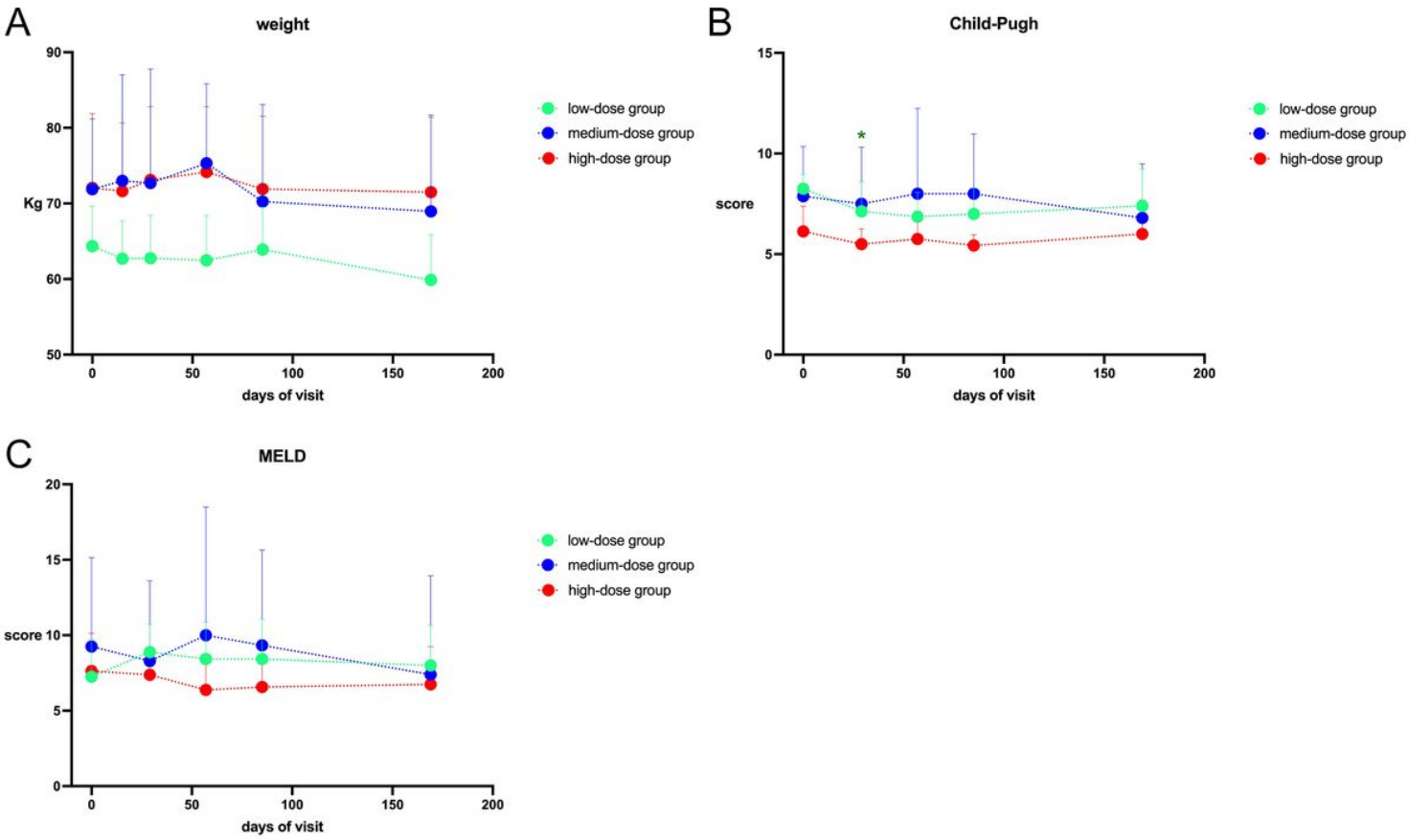

Figure 5

Variation of general condition: (A) Weight of participant (B) Child-Pugh score (C) MELD score

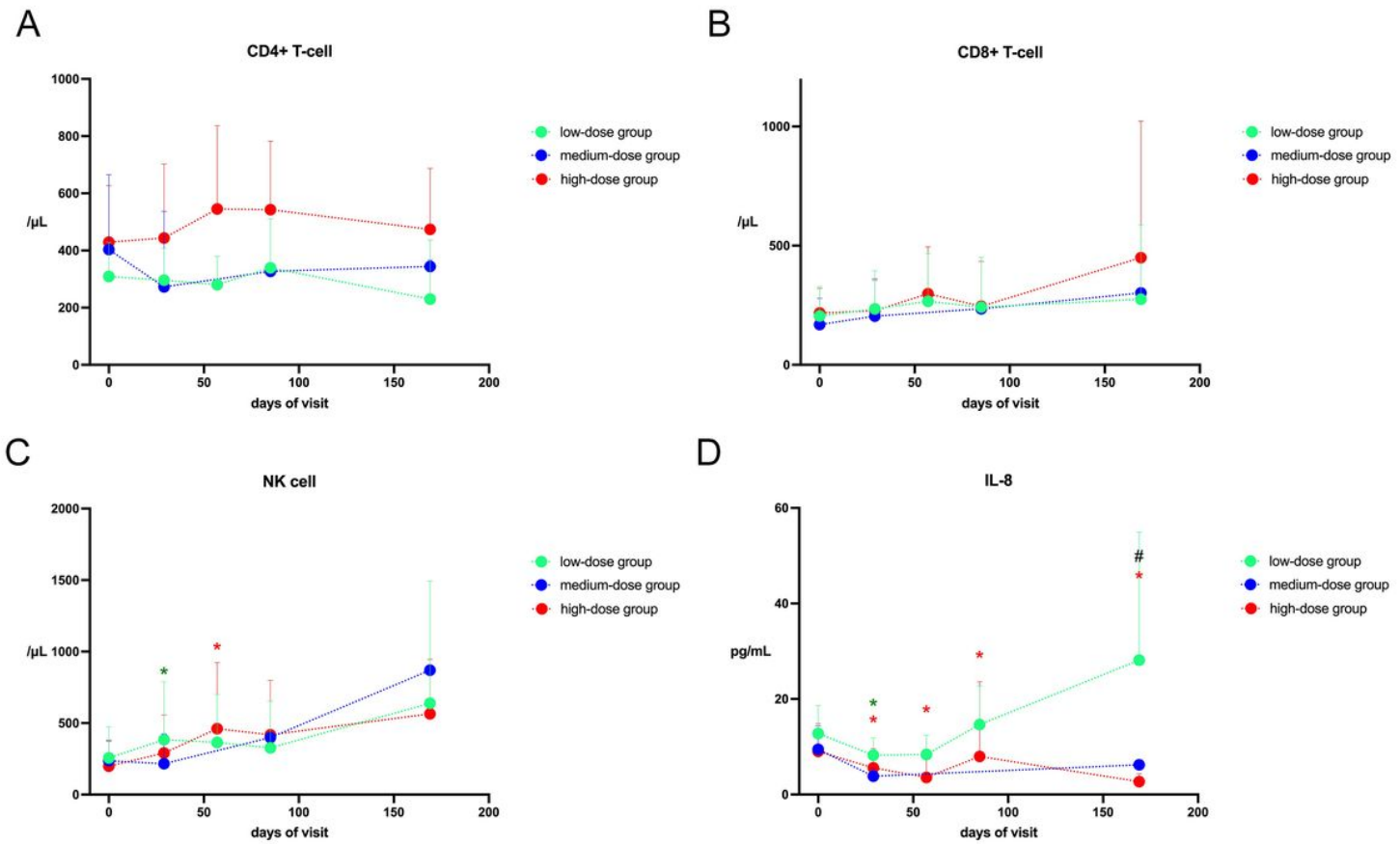

Figure 6

Variation of immune system: (A) $CD4^+$ T cell (B) $CD8^+$ cell (C) Natural killer cell (D) interleukin-8

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Figure 7

Most common adverse event: the most common 15 adverse event were displayed and the horizontal axis represents person-time, which display the frequency in each group.

Supplementary Files

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

- hUCMSCsSupplimentarydata.docx