Safety of Mesenchymal Stem Cells Therapy in Patients with Inflammatory Bowel Diseases - 5 Year Follow-up

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ABSTRACT

Aim: To compare safety profile of therapy in patients with ulcerative colitis (UC) and Crohn’s disease (CD), receiving anti-inflammatory therapy, using bone marrow-derived mesenchymal stromal cells (MSC) and standard therapy with 5-aminosalicylic acid (5-ASA), glucocorticosteroids (GCS) and immunosuppressive agents (IS).

Materials and Methods: Adverse events were analyzed in 103 patients with inflammatory bowel disease (IBD) after administration MSCs (56 patients UC and 47 patients CD). The findings were compared with data obtained in 208 patients with UC and CD, receiving standard anti-inflammatory therapy. All analyzed patients were similar in demographic characteristics, the duration of disease, the extent of disease, course of disease, phenotype and degree of disease.
The analysed groups did not include patients, treated with anti-TNF therapy. The safety of therapy was evaluated by presence of complications, developed during follow-up period.

**Results:** We conducted analysis of side effects in 103 IBD patients, treated with mesenchymal stem cells, comparing with 208 UC and CD patients, treated with standard anti-inflammatory therapy and finally we did not reveal any differences in developing acute posttransfusional toxicity, infectious complications, exacerbation of chronic inflammatory diseases, serious infectious complications, malignancy and death in UC and CD patients, besides transitory febrile.

**Conclusion:** Results of our study show that innovative method of cell therapy is safe in clinical practice.

**Keywords:** safety of cell therapy, mesenchymal stromal cells, Crohn disease, ulcerative colitis, inflammatory bowel diseases

**INTRODUCTION**

Inflammatory bowel diseases- is the umbrella term for group of chronic diseases of gastrointestinal tract with unknown etiology, that is characterized by nonspecific destructive immune inflammation of intestine, resulted in local and systemic complications [1].

Inflammatory bowel diseases include ulcerative colitis, Crohn’s disease and undifferentiated colitis. Ulcerative colitis and Crohn’s diseases share many similarities, such as histological pattern of inflammation, pathogenesis and clinical manifestations. But there are some differences in ulcerative colitis, the large intestine (colon) is typically the only site that is affected, whereas in Crohn’s disease, the location of the inflammation may occur anywhere along the digestive tract from the mouth to the anus. The clinical manifestations of Crohn’s disease are much more variable than those of ulcerative colitis. These two types of inflammatory bowel diseases differ also in its various complications, prognosis and response to treatment (Table 1). Crohn’s disease is characterized by unpredictable course of diseases, variability and difficult-to-control response to treatment. Crohn disease is devious, because there is a well-known disconnect between its clinical manifestations (general state, stool frequency, body weight, abdominal pain) and degree of mucosal lesions, course and prognosis of disease. Accumulating evidence indicates that we need to look beyond clinical symptoms of CD while determination degree and extent of remission.
Table 1: Clinical and epidemiological features of the two major inflammatory bowel disease subtypes, Crohn’s disease and ulcerative colitis.

|                      | Crohn’s disease | Ulcerative colitis |
|----------------------|----------------|-------------------|
| Terminal ileum involvement | Commonly       | Seldom            |
| Colon involvement    | Usually        | Always            |
| Rectum involvement  | Seldom         | Usually           |
| Perianal involvement | Common         | Seldom            |
| Endoscopy            | Discrete deep ulcers | Continuous diffuse ulcers |
| Depth of inflammation| May be transmural, deep into tissues | Shallow mucosal |
| Fistulae             | Common         | Seldom            |
| Smoking              | Higher risk for smokers | Lower risk for smokers |

Mucosal healing is now considered as one of major goals of treatment in clinical trials for patients with inflammatory bowel disease, and achieving the mucosal healing in clinical practice might be the best way to modify IBD course and maintain of intestine functions. In recent studies was reported, that mucosal healing in UC and CD is reliable associated with longer duration of clinical remission, lower rate of recurrence, substantively lower frequency of surgical procedures, more lower risk of colorectal cancer and higher quality of life [2]. So, for example, in population-based cohort study K.F. Frosile et al. [3] reported significant decrease of surgical management (colectomy, intestine resection) in IBD patients with complete mucosal healing. J.F. Colombel, et al [4] determined that there is strong correlation between one-year reduced rate of hospitalizations frequency and achievement of deep remission at 12 week of adalimumab treatment. It was revealed that complete mucosal healing also is one of the predictors of sustained clinical remission. Now there is no validated definition of deep remission in CD. However, in the near future the concept of deep remission might include histologic and immunobiologic components. Deep remission in CD is defined as absence of clinical, immunobiological and histologic signs of inflammation - remission beyond symptoms [5]. It means also normalization of serum and fecal biomarkers of active inflammation.

Randomized clinical trials suggest that mucosal healing in CD can be achieved by anticytokine treatment. Anti-TNF therapy has been shown to change options in CD treatment. The strategy in CD treatment in the pre-cytokine era was only achievement of clinical remission and reduction in the use of glucocorticosteroids. Current goals of CD treatment include induction and maintenance nonsteroidal remission, achievement and maintenance of mucosal healing, prevention of complications, reduction of hospitalization and surgery rate, improvement quality of life in CD patients.

Bone-marrow derived mesenchymal stem cells therapy is one of the innovative methods of CD treatment [6]. Mesenchymal stromal cells (mesenchymal stem cells; MSC) area heterogeneous group of cells, that can be isolated from many tissues (bone marrow, adipose tissue, dental
pulpe). First described in 1960-years of XX century [7], MSC have recently received attention in a number of different clinical fields for their potential therapeutic effects. Although often described as «adult stem cells», MSC’s have limited cellular differentiation ability. Instead, pre-clinical evidence suggests that MSCs exert their beneficial effects largely through immunomodulatory and paracrine mechanisms. MSCs home to sites of inflammation and secrete bioactive molecules, and thus may be especially effective in different proinflammatory diseases [8].

There is a growing body of literature demonstrating the efficacy of MSC therapy in a variety of pre-clinical models, including acute lung injury [9,10], septic shock [11], acute myocardial infarction [12]. Several small clinical trials have investigated efficacy and safety of MSCs in diseases including chronic heart failure, acute myocardial infarction, hematological malignancies, Crohn’s disease [13] and graft-versus-host disease.

However, safety concerns represent a significant barrier to the successful translation of MSCs into an acceptable clinical therapeutic. Potential risk is associated with its proliferative capacity, susceptibility to infectious complications given their immunosuppressive effects, embolism of the cells, zoonoses associated with cell culture reagents, and acute or chronic immunogenicity of the cells themselves [14].

Therefore, we conducted a systematic review of randomized and non-randomized controlled trials as well as uncontrolled clinical trials in foreign literature, that examined the safety and efficacy of intravascularly delivered MSCs, and revealed their most frequent adverse events [15]. Adverse events were grouped according to the immediacy of the event-acute infusional toxicity, fever, the occurrence of organ system complications (neurological, pulmonary, cardiovascular, gastrointestinal and renal, and hematologic systems), infection, and the occurrence of longer term adverse effects (death, malignancy).

Included studies were conducted in 14 different countries from Asia, the Middle East, Europe, and North America. There were eight RCTs (n = 369 patients) [16-23], 10 non-RCTs (n = 466 patients) [24-33] and and 18 uncontrolled clinical trials (n = 252 patients)[34-51]. Six of 36 studies were multi-centre [12,13,20,23,32,33]. One non-controlled study had a mixed adult-pediatric population [39], all other studies included only adult participants. The follow-up period was reported in all studies and the duration ranged from 0.5 to 60 months.

There were following diseases analyzed: eight randomized controlled studies included patient populations with cardiovascular diseases-acute myocardial infarction [11,12], chronic heart failure [10,16], with neurological disease either ischemic stroke [13], spinal cord injury [17], following stem cell transplantation for hematological malignancies [15]. The 10 non-RCTs included patient populations with old myocardial infarctions [25], stem-cell transplant post renal transplant[27], stem cell transplant for hematological malignancy [18,19,23], graft-versus-host disease [20,26], or healthy volunteers [24].
Sixteen studies used autologous MSC [10,11,13,14,16,17,22,24,25,27,29,31,32,37,43,45], eight used allogenic MSC [12,18,20,34,35,39,40,41]. Nine of the 36 studies cryopreserved MSCs prior to administration [12,18,20,21,23,29,31,32,44], and one study used both fresh and cryopreserved MSC[33], while the remainder of studies used only fresh MSCs. A meta-analysis revealed no significant differences in the occurrence of acute infusional toxicity, infectious complications, recurrence of chronic inflammatory diseases, serious infectious complications, malignancy and death between patients treated with MSC and control group. Significant association was demonstrated between MSC injection and transient fever.

Further we demonstrate our data for safety profile of allogenic mesenchymal stromal bone marrow cells in patients with inflammatory bowel diseases over a 5-year follow-up period.

Aim of study: to compare safety profile of therapy in patients with ulcerative colitis (UC) and Crohn disease (CD), received combined anti-inflammatory therapy including MSC and standard therapy, including 5-ASA, Glucocorticosteroids (GCS) and immunosuppressive therapy.

Materials and methods: Systemic transplantation of allogenic bone marrow MSC was performed in 74 UC and 64 CD patients ranging from 2008 to 2014 years.

First analysed group included 56 UC patients, follow-up period comprised in median 62±4 months. This group consists of 29 (51.78%) man and 27 (48.22%) women (Table 2). Mean age was 35.4±1.42 years. The second, control group included 84 UC patients, receiving standard anti-inflammatory therapy with 5-ASA and GCS. This group consists of 46 (54.8%) man and 38 (45.2%) women. Mean age - 34.98±1.23 years.

Third group included 47 CD patients, mean follow-up period was 64±4 months. Nineteen (40.4%) man and twenty-eight (59.26%) women were included in the third group. Mean age was 30.4±1.2 years. Fourth control group consisted of 124 CD patients, receiving standard anti-inflammatory therapy including 5-ASA, GCS and IS. In this group were 56 (45.2%) man and 68 (54.8%) women. Mean age was- 36.8±1.5 years.

We did not include patients, receiving anti-TNF therapy, in analyzed groups.

Technique of receiving and cultivation MSC in appropriate for systemic transplantation amount (150-200 millions of cells) was published [46]. This method is validated by Federal Supervisory Agency for Health Care and Social Development Ministry of Healthcare and Social Development of the Russian Federation (License 2006/206).
Table 2: Demographic characteristics of patients with CD and UC.

| Group | Male-to-female ratio (%) | Mean Age, years |
|-------|--------------------------|-----------------|
| 1     | 29:27 (51:48)            | 35.4±1.42       |
| 2     | 46:38 (54:45)            | 34.98±1.23      |
| 3     | 19:28 (40:59)            | 30.4±1.2        |
| 4     | 56:68 (45:54)            | 36.8±1.5        |

Bone marrow cells were isolated by means of flushing the sternum or iliac crest of healthy donor under local anesthesia and aseptic conditions. All donors signed informed consent for using bone marrow samples for scientific purposes. MSC culture was injected intravenous drip-feed in dosage 1.5-2 mln by 1 kg body weight. For systemic transplantation 130-160mln allogenic MSC, cultivated, were suspended in 200 ml steril isotonic solution, consisting of heparin in concentration of 50 U/ml. MSC culture was injected during 40-60 minutes by means of intravenous drip-feed infusion. Mathematical modeling of MSC treatment was performed to assess maximal efficacy and minimal side effects of MSCs. We analyzed several trials, in which regimen of MSC administration, frequency and the rationale for the cell dose were examined [13,14,44,45]. All patients signed inform consent for participating in study before MSC injection. Thus, procedure of MSC cultivation was performed according to GMP.

Safety of therapy was assessed by presence of complications, occurred during follow-up period, for example acute infusional toxicity, fever; complications (neurological, pulmonary, cardiovascular (arrhythmias etc.), urinary, gastrointestinal tract and blood system), infection complications, exacerbation of chronic inflammatory diseases, serious infectious complications (pneumonia, sepsis, abscess), malignancy, death. All persons, monitoring the complications were blinded with the treatment.

**Results and discussion:** In the first group 3/56 UC patients (5.4%) have acute infusional toxicity–looks like hives immediately or after MSC injection, in the second group allergic reaction like papulearurticaria was noticed in 1/84(1.2%) patient, treated with sulfasalazin (Table 3).

Table 3: Summary analysis of side effects in UC patients, receiving MSC and in control group.

| Side effects                          | Frequency in 1 group UC patients | Frequency in 2 group UC patients | 95% CI       | p           |
|--------------------------------------|----------------------------------|----------------------------------|--------------|-------------|
| Acute infusion reaction              | 3/56                             | 1/84                             | 0.48-42.18   | 0.87        |
| Fever                                | 16/56                            | 1/84                             | 3.27 - 175.89| 0.0000043  |
| Serious infectious complications      | 1/56                             | 5/84                             | 0.04-2.5     | 0.44        |
| Non-serious infectious complications | 7/56                             | 14/84                            | 1.5-23.58    | 0.66        |
| Malignancy                           | 1/56                             | 4/84                             | 0.05-4.96    | 0.97        |
| Lethal cases                         | 1/56                             | 1/56                             | 0.1-23.49    | 0.66        |
Allergic reaction like hives in first group patients had no statistically significance in compare with second group of patients ($x^2=0.35; p=0.87$). In 16/56 (28.6%) patients of first group were noted increasing of temperature around 37.2-37.4 °C during 12 hours after MSC injection or fever around 38.0 °C, in 1/84 (1.2%) patients of second group was reported increasing temperature above 37.7 °C, caused by intravenous injection of prednisolone. Fever and temperature increasing after MSC injection were statistically significant compared to control group-relative risk (RR) was 24.0 (95% CI 3.27 - 175.89); $x^2=21.12; p=0.0000043$. In the first UC group non-serious infectious complications and exacerbation of chronic inflammatory diseases were revealed in 7/56 (12.5%) patients, in second group - in 14/84 (16.7%) patients.

There was no significant difference in risk of infectious complications and exacerbation of chronic inflammatory diseases between two groups of UC patient, receiving standard antiinflammatory therapy and MSC (RR=0.75; 95% CI 1.5-23.58; $x^2=0.16; p=0.66$). In the first group serious infectious complications (pneumonia, pleurisy, activation of latent tuberculosis) were detected in 1/56 patients (1.8%), in the second -in 5/84 (5.9%). There was no difference in these complications between two groups (RR=0.3; 95%CI 0.04-2.5; $x^2=0.59; p=0.44$). Colorectal cancer was documented only in 1/56 (1.8%) patient in the first group. Diagnosis of colon cancer was established in 10 days after MSC injection.

During five-year follow-up period malignancy was found in 4/84 (4.8%) in the second group (RR=0.5, 95% CI 0.05-4.96; $x^2=0.01; p=0.97$). In the first and in the second groups during five-year follow-up one lethal case from each group was documented and it was 1.8% vs 1.2%, respectively (RR=1.5; 95% CI 0.1-23.49; $x^2=0.19; p=0.66$).

In the third group of CD patients acute infusional toxicity like hives and Quincke’s edema were detected in 2/47 patients (4.25%) immediately after MSC injection, in the fourth group there were no complications during antiinflammatory therapy, but these manifestations have no statistically significance between groups ($x^2=2.3, p=0.07$) (Table 4). Increase in body temperature up to 37.2-37.40 °C during 12 hours after MSC injection or fever up to 38.00 °C was noticed in 22 patients of third group (46.8%), in the fourth group of patients there was no fever, associated with intravenous interventions (medication injection) or per se administration was found in 0/124 (0%). Fever and mild increase of temperature after MSC injection were statistically significant compare to control group - RR - 58.5 (95% CI 8.1 - 422.0), x2=58.5, p<0.001. Non-serious infectious complications and exacerbation of chronic inflammatory diseases during therapy observed in 12 patients of 47 in the third group, that accounts 25.5%, in the fourth group-in 48 (38.7%) patients of 124, that had no significant difference: RR - 0.67 (95% CI 0.39 - 1.15), x2=1.86, p=0.17.

There were no differences between third and fourth groups in risk of serious infectious complications (pneumonia, pleurisy, activation of latent tuberculosis) during standard anti-inflammatory CD therapy and therapy with MSC. In the third group one patient developed pneumonia 1/47 (2.1%), in the fourth group two cases of pneumonia and one case of latent tuberculosis activation were detected - 3/124 (2.4%) (RR=0.88, 95% CI 0.09-1.85; x2=0.21; p=0.7).
In the third group of CD patients no cases of colorectal cancer were found. In the third group during five-year follow-up period no lethal outcomes were documented, in the fourth group one lethal case (0.8%), unlinked to underlying disease was found (x²=0.26; p=0.61). In the fourth group malignant transformation was noted in 2 patients (1.6%) from 124 (x²=0.01; p=0.93).

In patients with UC and CD, receiving MSC treatment, no cardiovascular, pulmonary, neurological, renal, and hematologic systems complications were detected.

**Table 4:** Summary analysis of side effects, occurred after MSC injection and in control group of patients with CD.

| Side effects in all analysed clinical trials | Frequency in 3 group CD patients | Frequency in 4 group CD patients | 95% CI     | p     |
|-------------------------------------------|---------------------------------|---------------------------------|------------|-------|
| Acute infusion reaction                   | 2/47                            | 0                               | -          | 0.07  |
| Fever                                     | 22/47                           | 0                               | 8.1 - 422.0| <0.001|
| Serious infectious complications           | 1/47                            | 3/124                           | 0.09-1.85  | 0.7   |
| Non-serious infectious complications      | 12/47                           | 48/124                          | 1.5-23.58  | 0.66  |
| Malignancy                                | 0                               | 2/124                           | -          | 0.93  |
| Lethal cases                              | 0                               | 1                               | -          | 0.61  |

**CONCLUSION**

Our study includes comparative analysis of adverse events, associated with MSC treatment and standard anti-inflammatory therapy in UC and CD patients. We analyzed advanced outcomes in 103 IBD patients, receiving MSC therapy and compared this data with 208 UC and CD patients, who had the same demographic characteristics, disease duration, extent of disease, course of disease, phenotype of disease, type of severity. Thus, we did not observe any significant differences in MSC safety, aside from transient fever.

This analysis did not reveal any differences in acute post transfisional toxicity, infectious complications, exacerbation of chronic inflammatory diseases, serious infectious complications, malignancy and lethal cases in UC and CD patients, treated with standard anti-inflammatory therapy.

We have detected significant association between MSC injection and fever. However, fever was transient and not associated with long term sequelae. The mechanisms for fever are not clear but could be related to acute inflammatory reactions by a subset of patients to particular preparations of MSCs, not unlike similar reactions occasionally observed with red blood cell and fresh frozen plasma administration [47].

Although malignant transformation is a theoretical risk, our own experience and literature analysis, presented in this chapter found no association between MSCs and tumour formation. Concerns related to tumourgenicity of MSCs were raised by preclinical studies demonstrating increased tumour burden in vivo [48]. Although recent position papers have suggested low
probability of malignant transformation and tumour formation with MSCs [8]. Malignancy occurred only in studies involving participants with ongoing or previous malignancies; no de novo malignancies were observed.

Although MSC immunomodulatory effects may be beneficial in pro-inflammatory diseases, these same effects may leave a patient susceptible to infection.[49]. The question arised-whether immunosuppressive therapy could increase risk of infections? This review did not demonstrate any evidence of increased susceptibility to infections with MSC administration.

In our chapter, infections were common in already immunosuppressed patients (e.g. following hematopoietic stem cell transplant), however the infection rates were similar to those in control group of patients [47,48].

Currently obtained data show that despite of strong immunosuppressive effect due to autoimmune agression, MSC did not hinder the activity of immunocompetent cells, directed against infectious agents [50-56].

Absence of posttransfusional reaction may be explained by low MSC immunogenicity, due to absence HLA class II and low level of expression HLA I class at their surface [56]. The use of fetal bovine serum for culturing MSCs could be one of the reasons for above mentioned posttransfusional toxicity, and another potential concern with MSC therapy application is the use of dimethylsulfoxide as cryopreservative, which has toxic side effects and could cause hypersensitivity reactions [57]. Thus, greater vigilance may be needed in future studies for reporting cellular viability and monitoring for potential dimethylsulfoxide related adverse events. Results from our study should provide some assurance to investigators and health regulators that, with the present evidence, this innovative therapy appears safe.

References
1. Vorobyev GI, Khalif IL. Non-specific inflammatory bowel disease. Miklos. 2008.
2. Pineton de Chambrun G, Peyrin-Biroulet L, Lémann M, Colombel JF. Clinical implications of mucosal healing for the management of IBD // Nat. Rev. Gastroenterol. Hepatol. 2010; 1: 15-29.
3. Froslie KF, Jahnsen J, Moun BA. Mucosal healing in inflammatory bowel disease: results from a Norwegian population-based cohort /Gastroenterology. 2007; 2: 412-422.
4. Colombel J, Rutgeerts P, Sandborn WJ. Deep remission for adalimumab-treated patients with moderate to severe ileocolonic Crohn’s disease: results from EXTEND // J. Crohn’s Colitis. 2010; 4: S11.
5. Zallot C, Peyrin-Biroulet L. Deep remission in inflammatory bowel disease: looking beyond symptoms. Curr Gastroenterol Rep. 2013; 3: 315.
6. OV Knyazev, Al Parfenov, PL Shcherbakov, IN Ruchkina, AG Konoplyanikov. Refractory forms of Crohn’s disease cell therapy. Cellular technologies in biology and medicine. 2013; 3: 145-152.
7. Friedenstein AJ, Piatetzky S, Petrakova KV. Osteogenesis in transplants of bone marrow cells. J Embryol Exp Morphol. 1966; 16: 381-90.
8. Francois S, Bensidhoum M, Mouiseddine M, Mazurier C, Allenet B, et al. Local irradiation not only induces homing of human mesenchymal stem cells at exposed sites but promotes their widespread engraftment to multiple organs: a study of their quantitative distribution after irradiation damage. Stem Cells. 2006; 4: 1020-1029.
9. Matthay MA, Goolarats A, Howard JP, Lee JW. Mesenchymal stem cells for acute lung injury: preclinical evidence. Crit Care Med. 2010; 38: S569-573.
10. Mei SH, McCarter SD, Deng Y, Parker CH, Liles WC, et al. Prevention of LPS-induced acute lung injury in mice by mesenchymal stem cells overexpressing angiopoietin. PLoS Med. 2007; 4: e269.

11. Mei SHJ, Haitsma JJ, Dos Santos CC, Deng Y, Lai PFH, et al. Mesenchymal stem cells reduce inflammation while enhancing bacterial clearance and improving survival in sepsis. American Journal of Respiratory and Critical Care Medicine. 2010; 182: 1047-1057.

12. Boyle AJ, McNiece IK, Hare JM. Mesenchymal stem cell therapy for cardiac repair. Methods Mol Biol. 2010; 660: 65-84.

13. Knyazev OV, Parfenov AI, Sherbakov PL, Homeriki SG, Ruchkina IN, et al. Efficacy and safety of mesenchymal stem cells in patients with refractory Crohn disease. Cell transplantation and tissue engineering. 2013; 1: 76-84.

14. Prockop DJ, Brenner M, Fibbe WE, Horwitz E, Le Blanc K, et al. Defining the risks of mesenchymal stromal cell therapy. Cytotherapy. 2010; 12: 576-578.

15. Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, et al. Safety of Cell Therapy with Mesenchymal Stromal Cells (SafeCell): A Systematic Review and Meta-Analysis of Clinical Trials. PLoS One. 2012; 7: e47559.

16. Chen S, Liu Z, Tian N, Zhang J, Ye F, et al. Intracoronary transplantation of autologous bone marrow mesenchymal stem cells for ischemic cardiomyopathy due to isolated chronic occluded left anterior descending artery. J Invasive Cardiol. 2006; 18: 552-556.

17. Chen SL, Fang WW, Qian J, Ye F, Liu YH, et al. Improvement of cardiac function after transplantation of autologous bone marrow mesenchymal stem cells in patients with acute myocardial infarction. Chin Med J (Engl). 2004; 117: 1443-1448.

18. Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, et al. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. J Am Coll Cardiol. 2009; 54: 2277-2286.

19. Lee JS, Hong JM, Moon GJ, Lee PH, Ahn YH, et al. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. Stem Cells. 2010; 28: 1099-1106.

20. Lee PH, Kim JW, Bang OY, Ahn YH, Joo IS, et al. Autologous mesenchymal stem cell therapy delays the progression of neurological deficits in patients with multiple system atrophy. Clin Pharmacol Ther. 2010; 83: 723-730.

21. Ning H, Yang F, Jiang M, Hu L, Feng K, et al. The correlation between cotransplantation of mesenchymal stem cells and higher recurrence rate in hematologic malignancy patients: outcome of a pilot clinical study. Leukemia. 2008; 22: 593-599.

22. Wang JA, Xie XJ, He H, Sun Y, Jiang J, et al. A prospective, randomized, controlled trial of autologous mesenchymal stem cells transplantation for dilated cardiomyopathy. Zhonghua Xin Xue Guan Bing Za Zhi. 2006; 34: 107-10.

23. Xie ZW, Cui GX, Li YZ, Li BW, Zhu SW, et al. Cursive effect of autologous mesenchymal stem cell transplantation on spinal cord injury. Journal of Clinical Rehabilitative Tissue Engineering Research. 2007; 11: 1277-1279.

24. Baron F, Lechanteur C, Willems E, Bruck F, Baudoux E, et al. Cotransplantation of mesenchymal stem cells might prevent death from graft-versus-host disease (GVHD) without abrogating graft-versus-tumor effects after HLA-mismatched allogeneic transplantation following nonmyeloablative conditioning. Biol Blood Marrow Transplant. 2010; 16: 838-847.

25. Gonzalo-Daganzo R, Regidor C, Martin-Donaire T, Rico MA, Bautista G, et al. Results of a pilot study on the use of third-party donor mesenchymal stromal cells in cord blood transplantation in adults. Cytotherapy. 2009; 11: 278-288.

26. Kebrhai P, Isola L, Bahceci E, Holland K, Rowley S, et al. Adult human mesenchymal stem cells added to corticosteroid therapy for the treatment of acute graft-versus-host disease. Biol Blood Marrow Transplant. 2009; 15: 804-811.

27. Koc ON, Day J, Nieder M, Gerson SL, Lazarus HM, et al. Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH). Bone Marrow Transplant. 2008; 30: 215-222.

28. Lazarus HM, Haynesworth SE, Gerson SL, Rosenthal NS. Caplan AI Ex vivo expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): implications for therapeutic use. Bone Marrow Transplant. 1995; 16: 557-564.

29. Lazarus HM, Koc ON, Devine SM, Curtin P, Maziarz RT, et al. Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. Biol Blood Marrow Transplant. 2005; 11: 389-398.

30. Liu L, Sun Z, Chen B, Han Q, Liao L, et al. Ex vivo expansion and in vivo infusion of bone marrow-derived Flk-1+CD31-CD34-mesenchymal stem cells: feasibility and safety from monkey to human. Stem Cells Dev. 2006; 15: 349-357.

31. Mohyeddin-Bonab M, Mohamad-Hassani MR, Alimoghaddam K, Sanatkari M, Gasemi M, et al. Autologous in vitro expanded mesenchymal stem cell therapy for human old myocardial infarction. Arch Iran Med. 2007; 10: 467-473.

32. Ringden O, Uzunel M, Rasmusson I, Remberger M, Sundberg B, et al. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. Transplantation. 2006; 81: 1390-1397.
33. Vanikar AV, Trivedi HL, Feroze A, Kanodia KV, Dave SD, et al. Effect of co-transplantation of mesenchymal stem cells and hematopoietic stem cells as compared to hematopoietic stem cell transplantation alone in renal transplantation to achieve donor hypo-responsiveness. Int Urol Nephrol. 2011; 43: 225-232.

34. Arima N, Nakamura F, Fukunaga A, Hirata H, Machida H, et al. Single intra-arterial injection of mesenchymal stromal cells for treatment of steroid-refractory acute graft-versus-host disease: a pilot study. Cytotherapy. 2010; 12: 265-268.

35. Duijvestein M, Vos AC, Roelofs H, Wildenberg ME, Wendrich BB, et al. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn’s disease: results of a phase I study. Gut. 2010; 59: 1662-669.

36. Fang B, Song Y, Liao L, Zhang Y, Zhao RC. Favorable response to human adipose tissue-derived mesenchymal stem cells in steroid-refractory acute graft-versus-host disease. Transplant Proc. 2007; 39: 3358-3362.

37. Honmou O, Houkin K, Matsunaga T, Niitsu Y, Ishiai S, et al. Intravenous administration of auto serum-expanded autologous mesenchymal stem cells in stroke. Brain. 2011; 134: 1790-1807.

38. Karussis D, Karageorgiou C, Vaknin-Dembinsky A, Gowda-Kurkalli B, Gomori JM, et al. Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis. Arch Neurol. 2010; 67: 1187-1194.

39. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host-disease: a phase II study. Lancet. 2008; 371: 1579-1586.

40. Liang J, Zhang H, Hua B, Wang H, Lu L, et al. Allogeneic mesenchymal stem cells transplantation in refractory systemic lupus erythematosus: a pilot clinical study. Ann Rheum Dis. 2010; 69: 1423-1429.

41. Liang J, Zhang H, Wang D, Feng X, Wang H, et al. Allogeneic mesenchymal stem cell transplantation in seven patients with refractory inflammatory bowel disease. Gut. 2012; 61: 468-469.

42. Meuleman N, Tondreau T, Ahmad I, Kwan J, Crokaert F, et al. Infusion of mesenchymal stromal cells can aid hematopoietic recovery following allogeneic hematopoietic stem cell myeloablative transplant: a pilot study. Stem Cells Dev. 2009; 18: 1247-1252.

43. Mohammadnejad M, Alimoghaddam K, Mohyeddin-Bonab M, Bagheri M, Bashtar M, et al. Phase 1 trial of autologous bone marrow mesenchymal stem cell transplantation in patients with decompensated liver cirrhosis. Arch Iran Med. 2007; 10: 459-466.

44. Ringden O, Uzunel M, Sundberg B, Lonnies L, Nava S, et al. Tissue repair using allogeneic mesenchymal stem cells for hemorrhagic cystitis, pneumomediastinum and perforated colon. Leukemia. 2007; 21: 2271-2276.

45. Sun L, Akiyama K, Zhang H, Yamaza T, Hou Y, et al. Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans. Stem Cells. 2009; 27: 1421-1432.

46. Sun L, Wang D, Liang J, Zhang H, Feng X, et al. Umbilical cord mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus. Arthritis Rheum. 2010; 62: 2467-2475.

47. Wang D, Zhang H, Cao M, Tang Y, Liang J, et al. Efficacy of allogeneic mesenchymal stem cell transplantation in patients with drug-resistant polymyositis and dermatomyositis. Ann Rheum Dis. 2011; 70: 1285-1288.

48. Weng JY, Du X, Geng SX, Peng YW, Wang Z, et al. Mesenchymal stem cell as salvage treatment for refractory chronic GVHD. Bone Marrow Transplant. 2010; 45: 1732-1740.

49. Yang Z, Zhang F, Ma W, Chen B, Zhou F, et al. A novel approach to transplanting bone marrow stem cells to repair human myocardial infarction: delivery via a noninfarct-relate artery. Cardiovasc Ther. 2010; 28: 380-385.

50. Zhang X, Li JY, Cao K, Lu H, Hong M, et al. Cotransplantation of HLA-identical mesenchymal stem cells and hematopoietic stem cells in Chinese patients with hematologic diseases. Int J Lab Hematol. 2010; 32: 256-264.

51. Zhang ZX, Guan LX, Zhang K, Zhang Q, Dai LJ. A combined procedure to deliver autologous mesenchymal stem cells to patients with traumatic brain injury. Cytotherapy. 2008; 10: 134-139.

52. Zib AF, Konoplyannikov AG, Kolesnikova AI, PavlovVV. Application of cell cultures in medicine from mesenchymal stem cells of human bone marrow. News of Russian Academy of science. 2004; 59: 71-76.

53. Hendrickson JE, Hillyer CD. Noninfectious serious hazards of transfusion. Anesth Analg. 2009; 108: 759-769.

54. Djouad F, Plence P, Bony C, Tropel P, Apparailly F, et al. Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. Blood. 2003; 102: 3837-3844.

55. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunol. 2008; 8: 726-736.

56. Karlsson H, Samarasinghe S, Ball LM, Sundberg B, Lankester AC, et al. Mesenchymal stem cells exert differential effects on alloantigen and virus-specific T-cell responses. Blood. 2008; 112: 532-541.

57. Majumdar MX, Keane-Moore M, Buyaner D. Characterization and functionality of cells surface molecules on human mesenchymal stem cell. Journal of Biomedical Science. 2003; 2: 228-241.