

# 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 presense 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 transitive 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.

	<b>Crohn's disease</b>	<b>Ulcerative colitis</b>
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, etal [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 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 standart 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  $62 \pm 4$  months. This group consists of 29 (51.78%) man and 27 (48.22%) women (Table 2). Mean age was  $35.4 \pm 1.42$  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 -  $34.98 \pm 1.23$  years.

Third group included 47 CD patients, mean follow-up period was  $64 \pm 4$  months. Nineteen (40.4%) man and twenty-eight (59.26%) women were included in the third group. Mean age was  $30.4 \pm 1.2$  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-  $36.8 \pm 1.5$  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.

	Male-to-female ratio (%)	MeanAge, years
1 group (n=56)	29:27 (51:48)	35.4±1.42
2 group (n=84)	46:38 (54:45)	34.98±1.23
3 group (n=47)	19:28 (40:59)	30.4±1.2
4 group (n=124)	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 papularurticaria 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 in all analyzed clinical trials	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 ( $\chi^2$ -0.35;  $p=0.87$ ). In 16/56 (28.6%) patients of first group were noted increasing of temperature around 37.2-37.4 0 C during 12 hours after MSC injection or fever around 38.0 0 C, in 1/84 (1.2%) patients of second group was reported increasing temperature above 37.7 0 C, caused by intravenous injection of prednisolon. 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);  $\chi^2$ -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;  $\chi^2$ -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;  $\chi^2$ -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;  $\chi^2$ -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;  $\chi^2$ -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 ( $\chi^2$ -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),  $\chi^2$ -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),  $\chi^2$ -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;  $\chi^2$ -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 ( $x^2=0.26$ ;  $p=0.61$ ). In the fourth group malignant transformation was noted in 2 patients (1.6%) from 124 ( $x^2=0.01$ ;  $p=0.93$ ).

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 transfusional 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.

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