

Successful treatment of an enterovesical fistula due to Crohn's disease with stem cell transplantation: a case report

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Crohn's disease (CD) is a chronic, progressive inflammatory disease affecting the digestive tract. Up to 30% of patients develop a fistulising disease during their lifetime, leading to the development of a variety of fistulas between the gastrointestinal tract and nearby tissues, such as enterocutaneous, enterovesical or enterovaginal fistulas. Even in the era of biological agents, including anti-TNF drugs, approximately one-third of patients will suffer from fistulas that are chronic and resistant to standard treatments [1], which may lead to dangerous and life-threatening complications.

Recently, mesenchymal stem cells (MSC) were tested for the treatment of fistulising disease in phase II and phase III clinical trials [2–5]. Thanks to their high anti-inflammatory and regenerative potential, MSC exerted strong paracrine effects in perianal fistulas, producing positive effects [5]. However, in these trials, the regenerative cells were obtained from healthy donors. Among the various methods used to obtain stromal cells, the isolation of autologous adipose tissue-derived regenerative cells (ADRC) using the Celution system is one of the most novel and clinically applicable. Herein, we provide the first report of complete enterovesical fistula resolution following treatment with autologous ADRC.

A 52-year-old female who had been suffering from CD for 12 years was admitted to our clinic with a severe

bacterial urinary tract infection. She had never attained clinical remission using steroids, immunosuppressants, anti-TNF agents with a short dosing interval, or alternative treatments such as cyclosporine, tacrolimus, or thalidomide. Consequently, she suffered from numerous complications, which led to a significant number of laparotomies. She had undergone multiple segmental resections of the small intestine and colectomy with ileostomy. Only maintenance therapy with adalimumab once a week with concomitant azathioprine resulted in a relative clinical remission. However, she still suffered from frequent perianal and peristomal fistulas, which led to several surgeries to reposition the stoma.

At the time of admission, the patient was suffering from pyrexia (up to 39°C) and dysuria; she also noted the passage of air and faeces during urination. The general state of the patient was critical because of severe undernutrition (BMI 15) and progressive urinary tract infection. A urine culture revealed severe polybacterial infection with *Klebsiella pneumoniae*, *Escherichia coli* ESBL (+), and *Pseudomonas fluorescens*, which was only susceptible to carbapenems. Despite the administration of targeted antibiotic therapy, the state of the patient continued to worsen. A cystoscopy performed after the oral application of methylene blue showed the presence of an enterovesical fistula, approximately 3 cm long and

1 cm wide, with active suppuration of intestinal contents into the urinary bladder.

Surgery was considered a high-risk treatment for several reasons. First, the multiple past interventions had led to the formation of multiple adhesions in the abdomen cavity, leading to a so-called 'frozen abdomen', which made surgical intervention impossible. Second, the healing process would have been heavily impaired because of the critical state of the patient, including the high CD activity and cachexia. Finally, even with maintenance therapy using adalimumab once a week with concomitant thiopurines, the patient suffered from the formation of new and re-opening fistulas. Peristomal and perianal fistulas were both active at the time of admission.

In this case, conservative treatment with biologics was not expected to be effective, and broad-spectrum antibiotics did not stop the progression of sepsis. Because of the critical state of the patient, we decided to use an alternative treatment as a 'rescue therapy'.

Based on promising previous reports that show the effectiveness of the local injection of adipose-de-

rived regenerative cells (ADRC) into enterocutaneous [2], enterovaginal [3], and perianal fistulas [4, 5], we decided to apply the novel technique in this particular case. After approval was obtained from an independent Ethics Committee, the local injection of regenerative cells into the enterovesical fistula was performed during cystoscopy.

Manual liposuction was performed under general anaesthesia. A 260-ml aliquot of lipoaspirate was immediately transferred to the CellCelution 800 system (Cytori Therapeutics Inc.). After 2 h of tissue processing, 5 ml of an ADRC suspension was obtained. During cystoscopy, the suspension was injected around the fistula through an 18 G needle, the size routinely used for botulin injections, in 0.5-ml aliquots into the detrusor muscle (Figure 1).

Over the next few days, the signs of severe infection gradually resolved. Treatment with adalimumab and azathioprine was continued. Cystoscopies performed 12 and 22 days after the treatment revealed no active leakage of the methylene blue colorant into the urinary bladder, and to our surprise, the enterove-

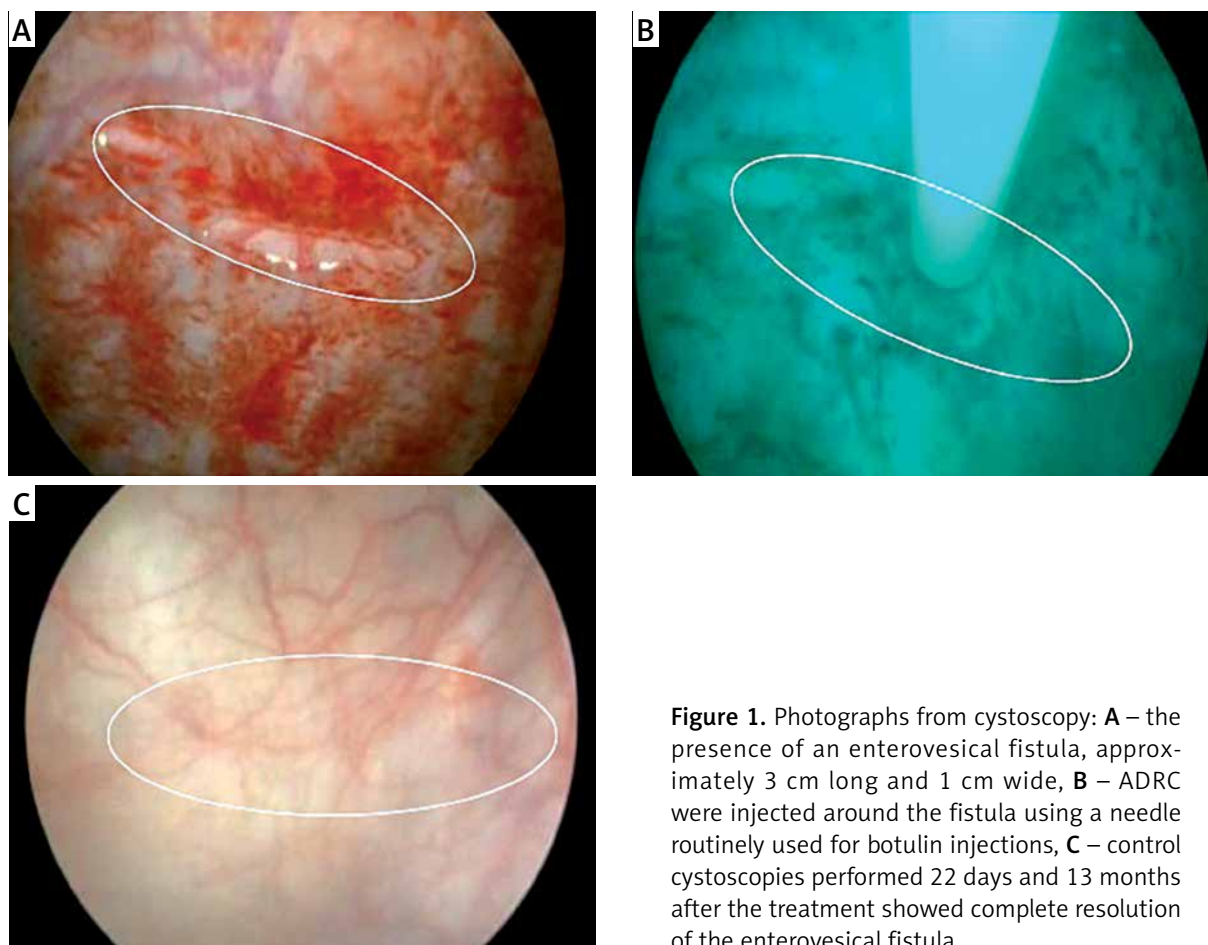


Figure 1. Photographs from cystoscopy: **A** – the presence of an enterovesical fistula, approximately 3 cm long and 1 cm wide, **B** – ADRC were injected around the fistula using a needle routinely used for botulin injections, **C** – control cystoscopies performed 22 days and 13 months after the treatment showed complete resolution of the enterovesical fistula

sical fistula had completely resolved. Urine culture did not reveal any evidence of pathogenic bacteria. No adverse events occurred during hospitalisation or during one year of clinical observation. Follow-up cystoscopy performed 13 months after the treatment demonstrated complete fistula healing. The clinical effect was maintained after 36 months of follow-up, despite subsequent exacerbations of CD leading to escalation of biological treatment.

Using a 1-ml suspension of ADRC, the phenotype and expression of molecular markers were evaluated using microscopy and fluorescence-activated cell-sort-

ing (FACS). Freshly isolated cells were counted ($27 \times 10^6/5$ ml ADRC), and cells with 95% viability were obtained. The cell suspension was cultured in a standard humidified incubator under a 21%-O₂ and 5%-CO₂ atmosphere at 37°C. After several days, the ADRC became spindle-shaped and fibre-like. The obtained ADRC expressed CD73 (99.3% of cells), CD90 (99.6%), and CD105 (89.1%) as well as CD34, CD19, CD11b, and HLA-DR (all in 1.7% of cells) as surface markers (Figure 2).

The evolution of lesions in CD has a significant inflammation-induced component. Unfortunately, the currently used immunosuppressive therapy is burdensome

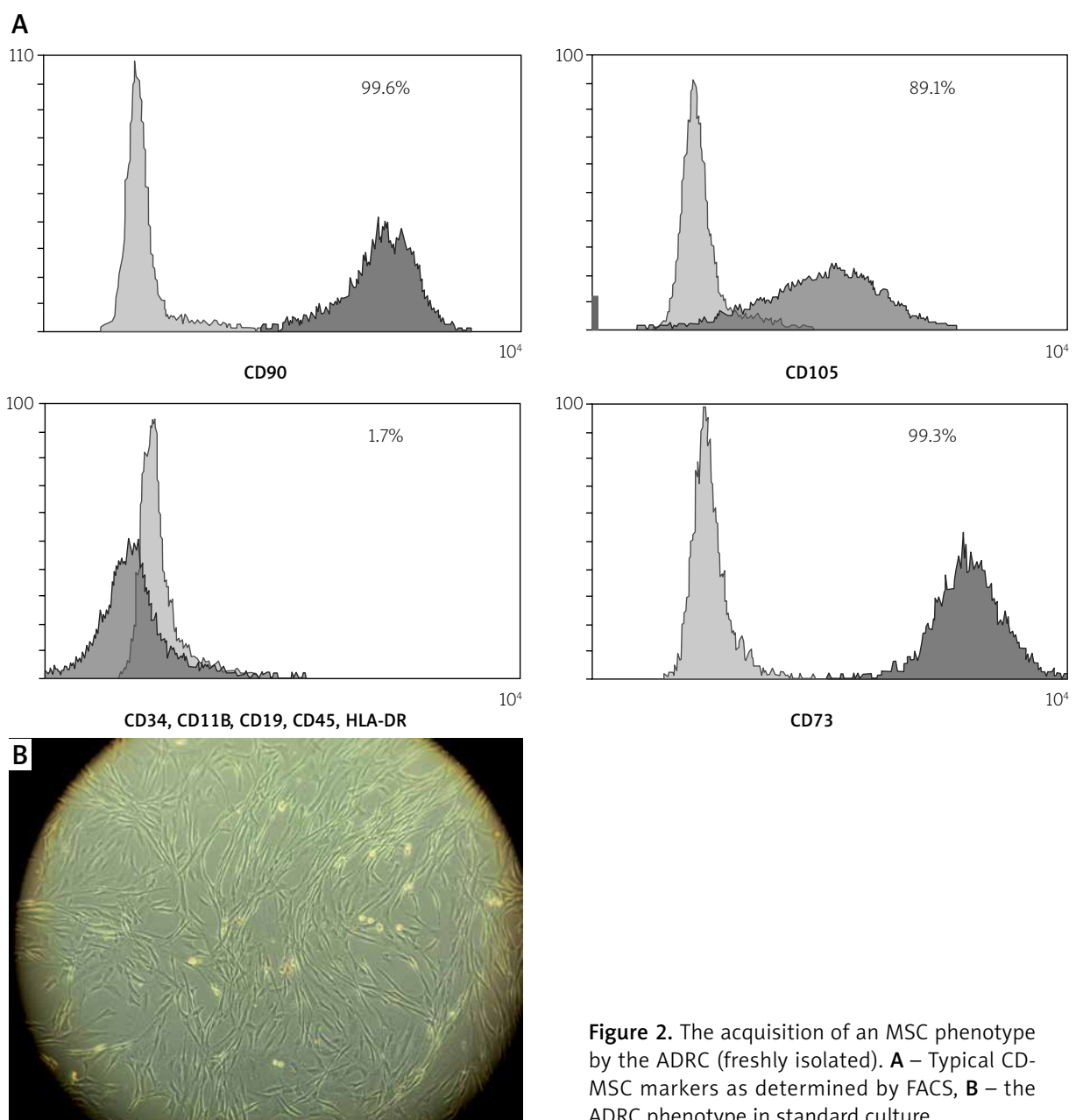


Figure 2. The acquisition of an MSC phenotype by the ADRC (freshly isolated). **A** – Typical CD-MSc markers as determined by FACS, **B** – the ADRC phenotype in standard culture

for the patient and not always effective [1]. Thus, the application of ADRC is thought to be a promising therapy. ADRCs have been described to modulate and reduce the inflammatory response through paracrine effects [6]. So far, the application of these cells in CD has been described in only a few papers and has been mainly used in the treatment of enterovaginal and enterocutaneous fistulas [7, 8]. However, only their effectiveness in healing perianal fistulas has been assessed in large randomised clinical trials, mainly using allogenic adipose-derived MSC. There have been no previous case reports concerning the use of ADRC to treat enterovesical fistulas.

In the phase I/IIa clinical trial concerning persistent fistulas in CD conducted by de la Portilla *et al.* [5], the authors demonstrated a significant positive effect of allogenic adipose-derived stem cell-based therapy for complex perianal fistulas. More than half (56.3%) of the patients achieved complete closure of the treated fistula. However, the treatment involved long laboratory procedures, e.g. digestion of the healthy donors' adipose tissue, culture and incubation, followed by expansion *in vitro* and long cryopreservation until use. In a South Korean phase II study [9], 78.8% of the CD patients suffering from perianal fistulas showed complete closure after the first injection, which was notably superior compared to other studies, possibly due to the higher number of cells used per fistula tract.

In our case, we used freshly isolated autologous cells, not expanded ADRC, and injected them 2 h after isolation. For the first time, we observed successful closure of an enterovesical fistula in a critically ill patient suffering from CD with a high risk of complications and failure of standard surgical treatment. Adapting the Cytori[®] system reduced the time between the isolation of the adipose tissue and the injection of cells in the operating room. The number of cells obtained and injected around the fistula was comparable to that used in the described clinical trials. We successfully demonstrated the therapeutic effects of ADRC in uroepithelial regeneration and observed no adverse effects.

The mechanisms involved in the closure of an enterovesical fistula might be associated with the immunomodulatory effects of the injected ADRC or with their regenerative potential. Increased regeneration of epithelial integrity modulated by adipose-derived MSC has been shown in an animal model by Chang *et al.* [10]. The regeneration of the intestinal epithelium, together with a reduction of the inflammatory response, led to increased survival in rats that underwent high-dose abdominal irradiation. Moreover, ADRCs were previously described to have a high potential for differentiation into connective tissue cells [11], which may have con-

tributed to the successful closure of the fistula in our patient.

Furthermore, Shi *et al.* [12] recently documented the differentiation of human adipose-derived stem cells into urothelial cells. In an earlier paper by Jack *et al.* [13], it was shown that human processed lipoaspirate cells injected into the urinary tract exhibited morphological and phenotypic evidence of smooth muscle incorporation and differentiation over time. In addition, the cells were maintained in the muscle for at least 12 weeks in mice.

As described above, in pre-clinical and clinical studies, different populations of mesenchymal cells derived from adipose tissue were applied. Here, the cells were not characterised prior to administration to the patient. An aliquot of the obtained mesenchymal cells was sent to the research laboratory for a full phenotypic and survival analysis.

In conclusion, it appears that using freshly isolated, autologous ADRC-based therapy could be used as a safe, effective and non-invasive alternative for the treatment of CD patients with enterovesical fistulas, with potential long-term clinical effect. Further research is needed to evaluate the effectiveness and safety of this novel method in IBD patients.

Acknowledgments

The study was reviewed and approved by the Ethical Committee of the Central Clinical Hospital of the Ministry of the Interior and Administration, Warsaw, Poland.

Conflict of interest

The authors declare no conflict of interest.

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