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A 12-Month Follow-up After a Single Intracavernous Injection of Autologous Adipose-derived Regenerative Cells in Patients with Erectile Dysfunction Following Radical Prostatectomy: An Open-label Phase I Clinical Trial

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Key words (1-5 in) at the bottom of the title page.

Autologous adipose-derived stem cells, erectile dysfunction, radical prostatectomy, stem cell therapy.

Running head: Phase 1: Treatment of erectile dysfunction with stem cells

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Abstract

Objective

To explore safety in adipose-derived regenerative cells (ADRC) therapy, treating ED.

Methods

Twenty-one patients with ED after radical prostatectomy (RP), with no signs of recovery using conventional therapy, received a single intracavernous injection of autologous ADRC and were followed for 1 year. Six men were incontinent, and 15 were continent at inclusion. The primary (safety of ADRC therapy) and secondary endpoints (sexual function) were evaluated at 1, 3, 6, and 12 months after ADRC injection by registration of adverse events and validated questionnaires using the International Index of Erectile Function-5 (IIEF-5) and Erection Hardness Score (EHS).

Results

No serious adverse events occurred, but 8 reversible minor events related to the liposuction were noted. Eight out of 15 (53%) patients in the continent group reported erectile function sufficient for intercourse at 12 months.

Baseline median IIEF-5 scores (6.0; IQR 3) were unchanged 1 month after the treatment, but significantly increased after 6 to 7 (IQR 17). This effect was sustained at 12 months (median 8; IQR 14). We did not see any improvements in erectile function the group of incontinent men or among men with erectile dysfunction prior to radical prostatectomy.

Conclusions

Intracavernous injection of ADRC is safe in this phase 1 study with a 12 month follow-up.

Introduction

Erectile dysfunction (ED) represents a substantial burden in men following radical prostatectomy (RP). Post RP-ED can be viewed as a multifactorial disorder in which the interlinked mechanisms include denervation, endothelial dysfunction, and structural alterations in connective tissue and smooth-muscle cells. Corporeal cell apoptosis has been documented in experimental models of post RP-ED and may contribute to post-RP veno-occlusive dysfunction [1]. Apoptosis affects several cell types including smooth muscle cells, supportive stromal cells, and vascular smooth-muscle and endothelial cell [2]. Preclinical studies have investigated the use of intracavernous stem cell injections as a mean of repairing this complex set of cell damage. There is a lack of evidence of engraftment or even differentiation of the cells in vivo [3] and mounting evidence suggests stem cell mediated repair by paracrine effect rather than stem cell differentiation[4]. In vivo findings suggest a paracrine mechanism being responsible for the regeneration, as treatment with ADRC-lysate alone improves EF in a rat nerve crush injury model [5]. Other potential mechanisms of action seen in animal studies include cell protective and anti-apoptotic effects [6], neurotrophic effects on the cavernous nerves, mitochondrial transfer through nanotube formation, and activation of host progenitor cells [7].

Phosphodiesterase type 5 inhibitor (PDE-5i) has revolutionized the treatment of ED, but is not always effective, and second-line treatment such as penile vacuum devices and injection therapy are associated with patient dissatisfaction and dropout [8]. In the past decade, preclinical studies [9] have shown promising efficacy of various stem cells sources for restoring the complex set of cell injury in erectile function after RP. Translation into clinical use is limited, however, and at present, only 4 clinical

trials using intracavernosal injections of either allogenic umbilical cord blood stem cells [10], allogenic placental matrix-derived mesenchymal cells [11], autologous bone marrow mononuclear cells [12] or freshly isolated autologous adipose tissue-derived regenerative cells (ADRC) have been reported [13].

Nonetheless, many private entities worldwide have been offering questionable stem cell treatments for ED without preceding rigorous clinical data regarding safety and efficacy [14].

We previously demonstrated the 6-month safety and feasibility of a single intracavernous injection of ADRC in 17 patients with ED following RP [13]. The study was designed to include 30 patients with an interim efficacy analysis when approximately half of the patients had been evaluated at their 6-month follow-up. At this point, the study included a total of 21 patients. Herein, we report the 1-year outcome of all 21 patients enrolled in the clinical pilot study.

Methods

Patients and Inclusion

Eligible patients were: men 6 to 18 months after RP, clinical follow-up without prostate cancer relapse (PSA<0.01/ undetectable PSA).

Exclusion criteria were as follows: lack of sexual interest, use of anticoagulants and insufficient amount of subcutaneous fat (Figure 1).

Twenty-one patients with ED after RP were enrolled between May 2014 and September 2015 in this prospective open-label, single-arm and single-center study.

Our study was designed to include men early in the process in order to prevent corpus cavernosus fibrosis formation before the damage and ED is irreversible.

We included patients regardless of nerve-sparing surgery performed. Urinary continence was defined as pad-free status. All patients in the present study followed

a national sexual rehabilitation program, which included sexual counseling, pelvic floor exercises in combination with erectile treatment options marketed in Denmark e.g oral medication (phosphodiesterase type 5 inhibitor), intracorporeal injections (alprostadil or aviptadil combined with phentolamin) or intrauretral pellet (alprostadil) and lastly prosthesis implantation. The erectile aids (oral medication and injections therapy) were not effective prior to study enrollment in all men. We considered these inclusion criteria to indicate a very low likelihood of recovering sexual function. 1-3 weeks after baseline, patients returned to the clinic to receive the study treatment.

Approvals

The intracavernous injection of autologous non-cultured ADRC procedure is categorized as an advanced therapy medicinal product by the European Medicines Agency (European Union Regulation 1394/2007) since adipose-derived regenerative cells do not occur naturally in the penile corpora.

The study was approved by the Danish National Ethics Committee (no. 37054), The Danish Health and Medicines Authority (EUDRA-CT number 2013–004220-11) and the Danish Data Protection Agency (2008–58-0035). This study is registered at ClinicalTrials.gov (NCT02240823). The study was performed in accordance with the Declaration of Helsinki and the International Conference on Harmonization's Guideline for Good Clinical Practice (ICH-GCP) guidelines and monitored by the GCP Unit at Odense University Hospital. All patients provided written informed consent before participation. ADRC preparation was carried out in an authorized tissue establishment (Danish Health and Medicines Authority, Authorization no. 29035) for the handling of human tissues and cells at Odense University Hospital.

Tissue collection, ADRC preparation, and ADRC delivery

All procedures have been described in detail previously [13]. In brief, abdominal adipose tissue was collected under general anesthesia. Freshly isolated ADRC was obtained from the lipoaspirate and injected in the corpus cavernosum within 2 h after harvest.

Cell processing was performed using an automated processing Celution® 800/CRS system (Cytori Therapeutics, San Diego, California, USA). The cells were concentrated, washed, aseptically recovered and resuspended in 5 mL of lactated Ringer's solution. The final cell suspension was aspirated in a 5-ml syringe. Four milliliters were used for injection at 2 bilateral points in the distal and proximal parts of the corpus cavernosum in local anesthesia, after a tourniquet was applied at the base of the penis. The tourniquet was removed after 30 minutes.

One milliliter was used for cell characterization. Total viable nucleated cell recovery and viability percentage were determined using the Nucleocounter NC100 (ChemoMetec, Denmark). Within the crude ADRC, subpopulations were identified by flow cytometry analysis with a panel of cell surface makers (CD34+, CD90+, CD31+, CD73 and CD235a-CD45-CD31-CD34+ cells) in agreement with the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT) recommendations[15].

The ADRC dose varied as the quantity of subcutaneous fat available in men is individual, and the cell characterization was performed post-treatment.

Patients received one treatment with autologous ADRC by injection into the corpus cavernosum and were discharged on the same day.

Statistical Analysis

This was an exploratory pilot study and no sample size estimates were calculated. Continuous data are described either as mean, interquartile range (IQR), or standard deviation (SD). IIEF-5 outcomes were analyzed by repeated measurement (RM) two-way analysis of variance (ANOVA) with Sidak's post-hoc test for multiple comparisons, following log transformation of data. EHS outcomes were analyzed by Friedman's test for multiple non-parametric comparisons with Dunn's post-hoc test for multiple comparisons. International Consultation on Incontinence Questionnaire-Urinary Incontinence-Short Form (ICIQ-UI SF) outcomes were analyzed by Wilcoxon's matched pairs signed rank test. ADRC and volume of liposuction and donor age were analyzed by Pearson's product-moment correlation. ADRC phenotypes between groups were analyzed by the Mann-Whitney U test. All statistical analyses were performed using Prism 7 (GraphPad Software, La Jolla, CA, USA).

Primary and Secondary Outcomes

The primary endpoint was the occurrence of ADRC-therapy-related adverse events within 12 months after the injection. Adverse events were recorded at each visit by inspection of the injection site, followed by specific questions (Table 1).

Secondary endpoints (sexual function and urine continence) were evaluated using validated questionnaires; the IIEF-5 [16] and EHS [17]. Information on urinary incontinence was assessed by the ICIQ-UI SF [18]. Finally, the patients were asked whether they could achieve an erection sufficient for intercourse.

Patients were evaluated at inclusion, 1, 3, 6, and 12 months after the ADRC injection.

Results

A total of 21 men were enrolled in the study; 15 men were continent, while 6 were incontinent at inclusion; the mean age at baseline was 60.2 years (range 46-69). All patients reported an active sex life before RP, but 6 men stated usage of erectile aids prior to RP. Baseline characteristics including age, body mass index (BMI), smoking, alcohol intake, physical activity, degree of co-morbidities, pre-RP usage of erectile aids, nerve-sparing technique, and medications were similar for the 2 groups. The mean time between RP and ADRC treatment was 10.7 (range 6-15) months, although this differed significantly ($p=0.03$) (Mann-Whitney test) between continent and incontinent men (Table 2).

No serious adverse events were reported. Eight men experienced transient redness and swelling at the injection sites, 3 reported reaction in the penile area. Five men stated minor abdominal hematomas. One man had an abdominal hematoma which led to scrotal and penile hematomas. This patient had taken large doses of non-steroidal anti-inflammatory drugs (NSAIDs) for back pain in the days before the treatment. All reported hematomas resolved within 14 days without any sequela. Finally, 8 patients reported light abdominal discomfort after liposuction, and 4 reported sensitive abdominal skin, but only 1 patient needed analgesic drugs in the days following liposuction. No patient reported discomfort at the 1-month visit (Table 1). At the 3, 6, and 12-month evaluations, no patients reported any side or adverse events.

The characteristics of ADRC isolated by the automated Celution® system were comparable to those previously reported for other ADRC/stromal vascular fractions (SVFs) [19], including a mean yield of 1.5×10^5 ADRC/g fat tissue (SD 3.9×10^4), mean cell size of 10.6 (SD 0.2), mean viability of 84.8 (SD 2.7), and mean percentage of

fibroblastoid colony-forming units (%CFU-F) of 1.4 (SD 0.8) (Table 3). The mean liposuction volume was 207 ml (range 135-340 ml). The number of ADRCs obtained in each case was directly correlated to the amount of liposuction (Supplementary figure 2A), while age showed no relationship to ADRC amount or yield per gram fat tissue (Supplementary figure 2B). A large proportion of the freshly isolated ADRCs expressed the surface markers CD34 (stem/progenitor- and endothelial cells; 66.5%, 11.5; mean, SD) and CD90 (Thy-1, stromal-, stromal stem- and endothelial cells 69.8%, 8.2; mean, SD), whereas CD31 (endothelial (progenitor) cells) and CD73 (stromal stem cell subset) each defined smaller subpopulations (13.9%, 22.8% and 7.6, 13.5 respectively; mean, SD) (Table 3). The fraction of stromal stem cells as defined phenotypically by the markers CD235a-CD45-CD31-CD34+, encompassed approximately 26% of the parent ADRC, which is similar to results reported by others [19, 20].

Baseline median IIEF-5 scores (6.0; IQR 3) were unchanged 1 month after the treatment, but increased after 6 months to 7 (IQR 17, $p=0.002$). This improved erectile function was sustained at 12 months (median 8; IQR 14, $p=0.004$) (Supplementary figure 2C).

Eight out of 21 participants (38%) recovered erection sufficient for intercourse (ESI) in the 12 month observation time. All 8 men had insufficient effect of erectile aids at inclusion, and after treatment 3 men could complete intercourse without erectile aids, and the other 5 men using erectile aids 12 months after treatment. Two men used alprostadil injections, 2 men used PDE-5i, and 1 man used a penis ring. Six out of all the participants reported some degree of ED prior to RP (Table 2), and none recovered erectile function after stem cell treatment.

Post-hoc stratification according to urine continence at inclusion revealed an apparent association with refractory ED. Improvement in erectile function was solely demonstrated in the patients with normal preoperative erectile function that were continent at inclusion (Supplementary figure 2D). No significant relationship was seen between the nerve-sparing approach and erectile recovery. Eight out of 15 continent men (53.3%) recovered ESI. In the continent group, IIEF-5 scores were unchanged 1 month after the treatment (median 6; IQR 4), but significantly increased after 6 months to a median of 11 (IQR 17; $p=0.002$), and at 12 months to a median of 9 (IQR 13, $p=0.012$) (Supplementary figure 2E). The EHS data was also significantly increased at 6 months (median 3; IQR 2, $p=0.01$) and 12 months (median 2; IQR 2; $p=0.03$) as compared to inclusion (mean 1.2; IQR 1) (Supplementary figure 2F). In the group of incontinent men, the IIEF-5 scores were similar after 1, 3, 6, and 12 months and not different from the score at the time of inclusion (median 5; IQR 4; $p>0.99$) (Supplementary figure 2G). Likewise, EHS data for this group were unchanged throughout the study period (Supplementary figure 2H). There were no differences in ADRC characteristics between the groups of continent versus incontinent men (Table 3).

Additionally, urinary incontinence scores (ICIQ-UI SF) were not significantly lower 6 to 12 months after ADRC (Supplementary figure 2I).

Discussion

We previously reported 6 month outcome of the first 17 men included in the study and found that autologous, freshly isolated ADRC are safe and tolerable [13].

Here we report 12 month outcome of additional four men. Among the additional 4 men, two had some ED prior to RP and one was urine incontinent.

Improved erectile function was observed beginning at 6 months following treatment with 53% of the 15 patients that were continent (though incontinent patients had no improved erectile function) at 12 months.

Our results are in agreement with those reported by other researchers [10-12], who have also shown stem cell therapy to be safe and indicating improvement of erectile function. The use of fat as a source for autologous non-cultured adult stem cells for ED treatment is appealing as adipose tissue is obtainable in large quantities with minimal discomfort and allows for a safe, minimally invasive surgical procedure.

If speculating that ADRC therapy influenced erectile recovery, the differences in this trial are not likely to result from underlying differences in the amount or phenotype of the injected ADRC since ADRC yield, dosage, CFU-F, cell type composition (CD34, CD90, CD31, CD73), and viability showed no significant differences between continent and incontinent men (Table 3). Interestingly, stratification according to ESI also failed to unravel any underlying differences in the ADRC (Table 3). Rather than being the result of differences in the ADRC grafting material, the ability of some men to respond to treatment and others not, may rely on their intrinsic properties as a host instead.

We included patients regardless of nerve-sparing surgery performed. Nerve-sparing RP does not preclude irreversible ED, and even in studies with experienced surgeons, severe damage to cavernous nerves and arteries may occur unintentionally [21-23]. We found no difference in IIEF-5 score when comparing men having of nerve-sparing surgery performed to men with non-nerve-sparing surgery. Five out of six men with urine incontinence post-RP had severe reservoir voiding symptoms prior to RP (Table2). Urine incontinent men after RP are likely to represent individuals with more severe damage (e.g., advanced neurovascular degeneration) [24, 25]. Recent studies have shown erectile recovery beyond the first 12 months following RP, especially recovery in young healthy patients, but the probability of recovery is nevertheless associated with a high score at 12 months post-RP and thus early intervention is recommended in those with very low function [26, 27]. The fear of urine leaking, of smelling of urine and usage of diapers is humiliating to many men. Urinary incontinence has been rated a more bothersome outcome than ED [28]. The personal distress related to urine incontinence may lead to difficulties engaging in a sexual intimacy and not dispose toward sexual function recovery when the urine incontinence is prominent. Time between RP and cell therapy was significantly shorter among urine incontinent men (Table 2) thus the improvement seen in men who were continent only, could be due to spontaneous recovery and not due to therapy itself. None of the 21 men included, however showed progressive improvement prior to study enrollment and may represent a group with a poor prognosis of spontaneous return of erectile function.

Our study has several limitations. This pilot study was un-blinded and without a control group and only designed to demonstrate the safety of ADRC therapy. We cannot discern whether the positive effect is a result of the urologist's interviews or

the patients' own expectations of stem cell therapy, or whether the stem cells themselves have helped regenerate the erectile function [29, 30] or a paracrine mechanism is responsible [31] [32]. In general, reported degrees of ED after RP vary greatly (14–86%) depending on risk stratification and patient selection, the experience of the surgeon, type of operation, and the measure and definition of ED [33]. Results from our own department shows only 32% recover erectile function sufficient for intercourse 12 months after RP [34]. The result that 8/15 (53.3%) of the continent men with severe ED recovered erectile function after ADRC therapy is promising, although further placebo-controlled trials are needed to differentiate possible stem cell effects from spontaneous regeneration. We did not include objective measurement for the recovery of erectile function, such as measurements of penile hemodynamics or nerve impulse speed. In several cases, questionnaires have been found to correlate with objective measurements in optimal circumstances [35]. Both penile nocturnal tumescence measuring and color duplex Doppler ultrasound assessment on the response to erectogenic drugs have limitations, such as anatomic arterial variants, and differences in interpretation and execution of the scans, with both false negative and false positive results. An objective measure would have several advantages. Men suffering from ED are prone to low self-esteem, confidence, and sexual relationship satisfaction which is reflected in the IIEF-5 questionnaire confidence domain score.

Conclusion

In conclusion, our findings suggest that autologous, freshly isolated ADRCs are safe to use and the treatment tolerable in a 12 month follow up. The next step before stem

cell therapy can be made available to patients is to perform a randomized blinded and placebo-controlled trial among continent men.

Legends

Figures:

Figure.1. Flow-chart of inclusion process

Supplementary figure 2. Results

Table:

Table 1. Table 1 Advers events

Tabel 2 Demografic and Erctile Characteristica of the Patients

Table 3 ADRC Characteristics

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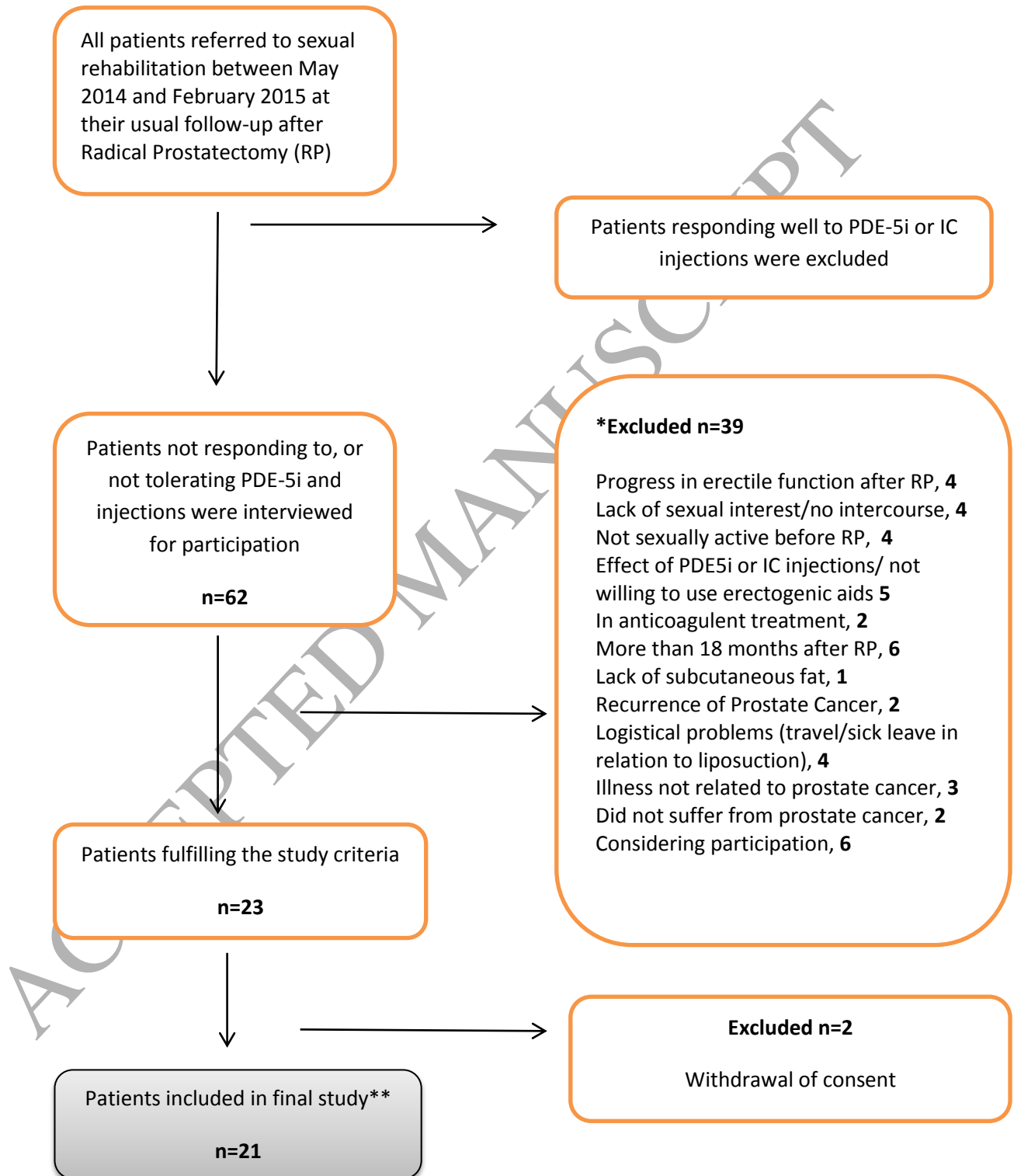
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Fig.1. Study overview.* Some patients were excluded based on several criteria.

(IC:Intracavernous; RP; Radical Prostatectomy).



Q no.	Adverse events related to liposuction and ADRC	No. events
1	Transient redness and swelling at the injection sites?	8
2	Local reaction in penile area?	3
3	Itching in penile area?	3
4	Infection?	0
5	Pain caused by liposuction demanding intake of painkillers?	1
6	Discomfort after liposuction?	8
7	Sensitive abdominal skin?	4
8	Hematoma?	5
9	Discomfort of different character?	0
10	Contact with the general physician?	0
11	Have you considered calling the hospital due to any symptoms?	0

Questions given to the patients.

Safety assessment of ADRC therapy. Minor events related to the liposuction and ADRC injection. Events were only reported within the first month following ADRC injections.

	All patients (n=21)	Continent (n=14)	Incontinent (n=7)	P-value ^b	ESI 6 and 12 months after ADR C's	No-ESI 6 and 12 months after ADR C's	P-value ^b
Age, years	61 (46-69)	60.8 (46-69)	60 (46-65)	n.s	59.5 (53-69)	61 (46-68)	n.s
Body mass index, kg/m ²	28.7 (23.3 - 34.9)	27.3 (24-31.5)	30.5 (23.2-34.9)	n.s	26.2 (24-31)	28 (23-35)	n.s
Preoperative PSA, ng/ml	8.2 (3.6-19.8)	8.2 (4-17)	8.3 (4.1-19.8)	n.s	6.1 (4.2-14)	8.2 (4.1-19.8)	n.s
Time between RP and ADRC inj. ^a	11 (6-15)	11.8 (6-17)	8.7 (6-11)	0.03	11.5 (8-15)	10 (6-14)	n.s
Erectile function							
Pre-RP							
EHS score	4 (3-4)	3.5 (3-4)	3.5 (3-4)		4 (3-4)	4 (3-4)	n.s
IIEF-5 score	24 (15-25)	24 (15-25)	24 (15-25)		24 (21-25)	24 (15-25)	n.s
Inclusion							
EHS score	1 (IQR 1)	2 (IQR 2)	1 (IQR 1.5)	n.s	3 (IQR 0.75)	0 (0-1)	n.s
IIEF-5 score	6 (IQR 2.5)	9 (IQR 14)	5 (IQR 3.75)	0,8073 n.s	16.3 (IQR 9.6)	5 (5-6)	n.s
Usage of erectile aids pre-	6	4	2		0	6	n.s

RP								
Hypertension	6	5	1	n.s		3	3	
Surgical approach								
Robotic prostatectomy	17	11	6	n.s		6	11	<0.05
Open retro prostatectomy	4	3	1			2	2	n.s
Bilateral NS RP	5	4	1	n.s		3	2	n.s
Unilateral NS RP	3	2	1	n.s		3	0	n.s
None NS RP	13	8	5	n.s		2	11	<0.05
Pathologic stage								
pT2c	18	14	4	n.s		7	11	n.s
pT3b	3	1	2	n.s		1	2	n.s
Severe LUTS ^c prior to diagnosis of prostate cancer.	5	0	5	<0.05		0	5	<0.05

Baseline characteristics of patients. Data represent as median and range. IQR, Interquartile range. ESI, Erection sufficient for intercourse. PSA, Prostate Specific Antigen. RP, Radical Prostatectomy. N.s, None significant^a Months, ^bMann Whitney test, ^c Severe LUTS defined as >15 DANPSS points. DANPSS, Danish Validated Questionnaire on LUTS and LUTS-bother

Cell parameter	All 21	Urinary continence			Sexual activity		
		Incontinent 6	Continent 15	<i>p</i> - value	- Coitus 13	+ Coitus 8	<i>p</i> - value
ADRC/g fat tissue	1.48x10 ⁵ (±3.9x10 ⁴)	1.36x10 ⁵ (±2.8x10 ⁴)	1.52x10 ⁵ (±4.3x10 ⁴)	n.s	1.40x10 ⁵ (±4.2x10 ⁴)	1.52x10 ⁵ (±3.8x10 ⁴)	n.s
Injected ADRCs	2.21x10 ⁷ (±8.7x10 ⁶)	2.60 x10 ⁷ (±9.8x10 ⁶)	2.06 x10 ⁷ (±8.0x10 ⁶)	n.s	2.09x10 ⁷ (±9.7x10 ⁶)	2.29x10 ⁷ (±8.3x10 ⁶)	n.s
Viability (%)	84.8 (±2.7)	84.0 (±3.7)	85.1 (±2.4)	n.s	85.18 (±2.5)	84.6 (±3.0)	n.s
% CFU-F	1.4 (±0.76)	1.7 (±0.7)	1.3 (±0.8)	n.s	1.4 (±0.9)	1.4 (±0.6)	n.s
Injected CFU-Fs	3.3 x10 ⁵ (±2.3x10 ⁵)	4.9 x10 ⁵ (±2.7x10 ⁵)	2.6x10 ⁵ (±1.9x10 ⁵)	n.s	3.0 x10 ⁵ (±2.2x10 ⁵)	3.5 x10 ⁵ (±2.6x10 ⁵)	n.s
% CD31	13.9 (±7.58)	13.9 (±5.8)	13.9 (±8.4)	n.s	17.0 (±6.6)	12.0 (±6.7.8)	n.s
% CD34	66.5 (±11.5)	68.8 (±5.6)	65.5 (±13.2)	n.s	66.9 (±13.8)	66.6 (±10.4)	n.s
% CD73	22.8 (±13.5)	26.0 (±10.9)	21.5 (±14.5)	n.s	22.0 (±14.1)	23.3 (±13.6)	n.s
% CD90	69.8 (±8.21)	70.6 (±5.2)	69.5 (±9.3)	n.s	74.1 (±6.3)	67.2 (±8.3)	n.s

Comparison of ADRC characteristics in patient groups following stratification according to urinary continence state or sexual activity. Significant differences compared to baseline are in bold type. The data are mean and standard deviation. N.s, non-significant (*p*-value >0.05).

ADRC: Adipose derived regenerative cell. CFU-F: fibroblastoid colony forming units . Cell surface marker: CD31, CD34, CD73, CD90.