ORIGINAL ARTICLE

# Standardized intraoperative application of an absorbable polysaccharide hemostatic powder to reduce the incidence of lymphocele after kidney transplantation – a prospective trial

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The study was approved by the ethics committee of the Medical University of Vienna; EK 1125/2013).

## SUMMARY

We assessed whether standardized application of an absorbable polysaccharide hemostatic powder (HaemoCer™) has an effect on lymphocele rate after kidney transplantation. For this nonrandomized prospective trial, we first aimed to know our center-specific lymphocele rate diagnosed by ultrasound imaging. We retrospectively assessed all patient records of the elapsed year resulting in a center-specific rate of 20%, this was consistent with literature. The power analysis showed that 108 patients were required to detect a 50% reduction in lymphocele rate. During the prospective study period, 155 patients undergoing kidney transplantation were recruited to receive HaemoCer™ intraoperatively. In two patients, the product accidentally was not used. Six patients were excluded from analysis because of failure to complete follow-up (one early death and five early graft failures). Of the remaining 147 patients, 15 developed lymphoceles, which represents a rate of 10.2%; (95% CI: 6.3–16.2%). Compared to the expected occurrence, this was significantly lower ($P = 0.003$). Lymphoceles appeared to be associated with preoperative donor-specific antibody, retransplantation and immunoadsorption in HLA or ABO incompatible donors. At our institution, the frequency of lymphoceles after kidney transplantation appeared to be significantly reduced when HaemoCer™ was applied routinely. The magnitude of the effect warrants randomized evaluation.

## Introduction

Kidney transplantation has become the standard of care in most developed parts of the world and can be offered at a very high standard with excellent results. Patient and graft survivals are well over 70% at 5 years [1]. But several issues remain to be solved. Beside availability and quality of donor organs, premorbidity and presensitization of recipients as well as optimal immunosuppressive regimens, surgical complications interfere with

patient and graft outcome. Mainly, urinary and vascular problems, infectious complications and postoperative lymphoceles are relevant issues, the latter being often regarded as minor because of lack of symptoms and their often self-resolving nature. But as common risk factors are obesity and age of the recipient, nowadays we are looking onto a potential increase of the lymphocele problem.

In literature, varying incidences (0.6–38%) of lymphocele after kidney transplantation (KTx) were reported [2–7]. The variation may arise from the use of different definitions for lymphocele in the literature but also from dissimilar standards in routine follow up. Frequent use of ultrasound after KTx may increase the detection of asymptomatic lymphatic collections. Immunosuppressive therapies may also play a role [2,5,6,8]. However, the incidence of any lymphocele after kidney transplantation is consistently reported to be around 20% [6,9]. Numerous risk factors have been described [6,8,10,11]. Along with others, the most frequently discussed are age and elevated body mass index (BMI), repeated transplants, diabetes, polycystic kidney disease, rejection and certain immunosuppressive medication such as mTOR inhibitors. In our experience with highly immunized patients, fluid retention can increase during terms of immunoadsorption and could therefore contribute to lymphocele formation.

The main sources of relevant lymphorrhagia are the donor kidney lymphatics and lymphatics around the recipient's external iliac artery. Besides careful ligation of visible lymph vessels on dissection of the external iliac vessels and ligation of lymph vessels in the graft's hilum, there are no approved or standardized strategies to reduce lymphocele development.

We hypothesized that the standardized use of a spreadable hemostatic polysaccharide powder at the end of graft implantation may have a positive impact on lymph vessel sealing. The powder acts mostly by dehydration of blood and fluids in the wound and leads to a concentration of coagulation factors enhancing the natural clotting cascade. The result is an adherent gel matrix. We hypothesized that this could seal severed lymphatic outlets as an additive effect to hemostasis. We therefore initiated a prospective pilot study to get insight into the potential of HaemoCer™, available at our institution, to impact lymphocele formation after KTx. The results were to serve as a basis for a future randomized controlled trial. This is the first study to test HaemoCer™ for the prevention of lymphoceles after kidney transplantation.

## Patients, materials and methods

### Retrospective cohort and literature

The center-specific lymphocele rate after kidney transplantation was retrospectively assessed. Over a defined period (the year before starting the prospective study: April 2011–March 2012), the lymphocele occurrence was assessed from the database at the Division of Transplantation, Department of Surgery, Medical University of Vienna. Any lymphocele of a diameter larger than 2 cm was considered for analysis. Ultrasound examinations are performed routinely on postoperative day (POD) one and thereafter in a 2- to 3-day interval in case of delayed graft function (DGF) – or anytime when clinically relevant during the hospital stay at our center. After discharge patients are routinely checked at the nephrology outpatient clinic. On these occasions, graft ultrasound is performed and pathologies documented. For differential diagnosis computed tomography (CT) scan, puncture of the fluid collection and analysis of serum creatinine and urea were performed when necessary.

The center-specific lymphocele rate in the defined period was consistent with available literature. As a next step, a 50% reduction in lymphocele rate was defined as desirable difference and the number of patients to treat for detecting the proposed difference was calculated. Subsequently, a prospective study was conducted at the same center to evaluate the impact of the standardized use of the hemostatic powder HaemoCer™.

### Prospective cohort and study design

The study was approved by the ethics committee of the Medical University of Vienna; EK 1125/2013). Patients were included in the study after informed consent.

HaemoCer™ (BioCer Entwicklungs-GmbH, Bayreuth, Germany) is a plant-based, hemostatic polysaccharide powder. It creates hydrophilic biocompatible particles and dehydrates blood upon contact enhancing the natural clotting cascade. The concentration of coagulation proteins, platelets, and red blood cells is accelerated at the bleeding site. The result is a gel matrix that adheres to tissue. It contains no animal or human components, is degraded by histaminases and absorbed completely within 48 h. During this prospective single arm open single-center study, the product had to be applied as standard operating procedure to vessels and graft site at

the end of graft implantation in all included patients undergoing kidney transplantation (Fig. 1). The product was used according to the manual. The use of more than one pack or use of additional hemostatic substances was left to the discretion of the operating surgeon. We aimed at an inclusion period of 18 months.

### Immunosuppression and desensitization

Standard immunosuppression consisted of basiliximab induction with steroid taper, maintenance therapy included tacrolimus, mycophenolate, and steroids. A selected cohort of live donor recipients at low immunological risk received calcineurin-inhibitor-free immunosuppression based on belatacept. For ABO-incompatible (living) transplantation, we applied desensitization with ABO antigen-specific immunoadsorption (IA) [12]. Deceased donor recipients with preformed anti-HLA donor-specific antibodies (DSA) underwent desensitization with semiselective immunoadsorption and antithymocyte globulin induction as earlier described in detail [13].

### Postoperative course and monitoring

A suction drainage was routinely used in all patients and removed when drainage content was equal or below 100 ml/24 h, usually on POD two.

Postoperative routine assessment consisted of graft ultrasound on POD one and every other day thereafter



**Figure 1** Standard application of HaemoCer™ before closure of the retroperitoneum: powder was spread over vessel anastomoses and graft hilum as well as iliac vessels using applicator.

if the graft lacked function or whenever clinically indicated. After discharge patients were routinely checked at the nephrology outpatient clinic every month for the first 6 months. On these occasions, graft ultrasound was performed and pathologies documented. For differential diagnosis, a CT scan and/or puncture of the fluid collection were performed when necessary. For exclusion of urinoma, creatinine and urea were analyzed in the sample. We regarded patients who consented and received kidney transplantation as intention-to-treat group and patients who received HaemoCer™ intraoperative and did not experience graft loss or death within the first 90 days as per-protocol cohort.

### Power calculation and statistics

In the prestudy year (April 2011–March 2012), 183 patients were consecutively transplanted in our center. Thirty-seven lymphoceles (20.2%) had been documented in this period. This rate was also in concordance to literature. Thus, we assumed a 20% lymphocele rate for the prospective cohort at our center. A comparison with this reference value would need to include 108 patients to be able to detect a significant difference at a two-sided significance level of 0.05 and 80% power if the true lymphocele rate was 10% (representing a 50% reduction) when treated with HaemoCer™. It was assumed that at least 108 patients could be included with recruitment duration of 18 months.

Normally distributed continuous variables are presented described by mean and standard deviation or by median, 25% and 75% quartiles otherwise. Categorical variables are shown with absolute and relative frequencies. Group comparisons were performed by chi-square test or Fisher's Exact Test for categorical variables and group differences are summarized by odds-ratio (OR) and 95% confidence intervals. A $t$-test or Wilcoxon rank sum test is used to compare continuous variables between two groups. The occurrence of lymphoceles is tested to the reference value of 20% by a one-group chi-square test and described with absolute and relative frequencies and corresponding 95% confidence intervals according to Wilson. All tests base on a two-sided significance level of 5%.

### Results

During the prospective study period, 194 patients underwent kidney transplantation. All patients were approached for consent. One hundred and fifty-five patients signed the informed consent form and were

included in the study intention-to-treat analysis (Table 1). Of 155 patients, only two patients had a protocol violation for not receiving HaemoCer™ during surgery. In all other patients, the powder was used according to the study protocol. Median follow-up from transplantation was 21 months (18;25). We observed one death (0.6%) on day four following cardiac decompensation during dialysis. Five patients (3.2%) lost their transplants within 9 days because of vascular complications (no lymphocele detectable). This left 147 patients who were at risk to develop lymphoceles. No further grafts were lost until the end of study follow-up (Fig. 2). Demographics and outcome are summarized in Table 1. Immunosuppressive regimens did not contain mTOR inhibitors known to be an additional risk factor for the development of lymphoceles [5,8,14].

In the prospective period with standardized use of HaemoCer™, the lymphocele rate was found to be 10.2%. Of 147 transplanted patients in the prospective study period, 15 had a lymphocele. Of these, ten (7% of total group) were symptomatic and required therapy. All ten were drained, eight (5.4%) underwent laparoscopic or open reoperation). Patients with first transplant had 6.8% lymphoceles. Patients with preoperative DSA had lymphoceles in 20.6% of cases compared to 7.1% for patients without preoperative DSA. Two out of three patients with AB0-incompatible transplant experienced lymphocele formation (66.6%) compared to only 9.0% (8 out of 105 patients) with an AB0-compatible donor. Of patients, 27.6% (8 out of 29) who underwent Immunoadsorption (IA) suffered from lymphoceles compared to 5.9% (7 out of 111 patients) without this therapy. Other reported risk factors such as age, BMI, diabetes, and rejection episodes were not significantly associated with lymphocele occurrence in our cohort, although delayed graft function and living donation showed differences in occurrence. In comparison to the expected incidence of 20%, we found a significant reduction to 10.2% ($P = 0.003$, 95% CI 6.28–16.15%).

Of the two patients that had not received HaemoCer™ because of protocol violation, one had a lymphocele. To avoid selection bias, we also performed a sensitivity analysis including these two patients. Consequently, all lymphocele probabilities in untreated patients higher than 16.8% are significantly different from our study cohort with 10.2% lymphoceles ($P = 0.0047$, 95% CI 6.72–16.73%).

HaemoCer™ is primarily a hemostatic agent. Even though the focus of this study was lymphocele formation, it has to be noted that only 2% (3/155) patients had to be reoperated because of hemorrhage or hematoma in the prospective study cohort treated with HaemoCer™, whereas the rate was 7% (13/183) in the retrospective period without standardized HaemoCer™ treatment.

For the statistical setup of the prospective study, we required an estimation of the center-specific lymphocele rate. Therefore, as mentioned, a retrospective analysis had been performed including the elapsed year before starting the prospective study. The analysis revealed a 20.2% lymphocele rate comparable to literature. Thus, the prospective study was planned with an expected lymphocele rate of 20%.

The retrospective data set was not matched with our prospective study as data quality is different. However, when we reevaluated the retrospective cohort using the same stringent criteria for lymphocele as in the prospective cohort, we found four cases to be over-reported in the retrospective data set. Comparison of 15 occurrences in 147 treated patients with 33 occurrences in 183 "untreated" patients showed a significant reduction from 18.0% to 10.2% ($P = 0.0450$), Our data suggest that HaemoCer™ treatment is associated with a significant risk reduction for lymphocele formation (OR = 0.517; 95% CI 0.269–0.993). In the retrospective group, 26/33 lymphoceles were symptomatic and were drained (14.2% of total group), ten (5.5%) required operation.

## Discussion

Lymphoceles are a common finding after kidney transplantation, their importance for hospitalization and graft function should not be underestimated. Symptoms can range from none at all to suprapubic or lower abdominal swelling, ipsilateral leg edema and thrombophlebitis, perigraft fluid collection and pain, protracted output of clear liquid from wounds, fever and/or infection of the wound and graft site up to graft or bladder displacement, ureteral obstruction/compression, and deterioration of graft function [3,4,9,15,16].

To our knowledge, this is the first report on using a hemostatic powder to reduce lymphocele formation after kidney transplantation. The main finding is a significant reduction of the incidence of lymphatic collections when HaemoCer™ is routinely used before closure of the retroperitoneum. The application is very simple during kidney transplant. The powder can be applied via the applicator within several seconds. The substance is plant-based, not human and bears no risk of known or unknown infection. The dehydration of blood and therefore concentration of coagulation factors may occlude small blood vessels probably sealing severed

**Table 1.** Demographics and outcome data of study patients: basic data and outcome parameters of patients with and without lymphocele, significance of differences between groups and complete data of all recruited patients (intention-to-treat) including all excluded patients (statistically significant p-values in bold).

| | Patients with lymphocele [15] | Patients without lymphocele (132) | P | Recruited* (155) |
|---|---|---|---|---|
| Recipient age (mean ± SD) | 56.2 ± 13.7 | 53.1 ± 14.3 | 0.42 | 53.1 ± 14.3 |
| Gender | | | 0.39 | |
| Female | 8 (12.7%) | 55 (87.3%) | | 66 (42.6%) |
| Male | 7 (8.3%) | 77 (91.7%) | | 89 (57.4%) |
| Body mass index median, (q1;q3, – range) | 22.9 (21.6;25.6, range 19.3–37.6) | 25.5 (22.8;29.0, range 15.2–41.2) | 0.11 | 25.2 (22.5;28.7, range 15.2–41.2) |
| Diabetes | 4 (17.4%) | 19 (82.6%) | 0.26 | 24 (15.5%) |
| Number of transplants | | | **0.015** | |
| First | 8 (6.8%) | 110 (93.2%) | | 122 (78.1%) |
| Second | 6 (33.3%) | 12 (66.6%) | | 22 (14.2) |
| Third | – | 7 (100%) | | 8 (5.2%) |
| Fourth | – | 3 (100%) | | 3 (1.9%) |
| Fifth | 1 (100%) | – | | 1 (0.7%) |
| Preformed DSA | | | **0.046** | |
| Yes | 7 (20.6%) | 27 (79.4%) | | 36 (23.2%) |
| No | 8 (7.1%) | 105 (92.9%) | | 119 (76.8%) |
| ABO-incompatible transplant | | | **0.028** | |
| Yes | 2 (66.6%) | 1 (33.3%) | | 4 (2.6%) |
| No | 13 (9.0%) | 131 (91.0%) | | 151 (97.4%) |
| Recipient desensitization | | | **0.002** | |
| Yes | 8 (27.6%) | 21 (72.4%) | | 32 (20.6%) |
| No | 7 (5.9%) | 111 (94.1%) | | 123 (79.4%) |
| HLA mismatch (A, B, DR) | 3 (2,5) | 3 (2,4) | 0.31 | 3 (2,4) |
| Reoperated side | | | 1.00 | |
| Yes | 1 (9.1%) | 10 (90.9%) | | 12 (7.7%) |
| No | 14 (10.3%) | 122(89.7%) | | 149 (92.3%) |
| Living donation | | | 0.26 | |
| Yes | 4 (17.4%) | 19 (82.6%) | | 24 (15.5%) |
| No | 11 (8.9%) | 113 (91.1%) | | 131 (84.5%) |
| CIT, deceased donor organs (h) | 10.9 (3.2;18.6) | 14.1 (7.4;17.4) | 0.21 | 13.9 (7.2;17.3) |
| Delayed graft function | | | 0.11 | |
| Yes | 6 (17.6%) | 28 (82.4%) | | 34 (21.9%) |
| No | 9 (8.0%) | 104 (92.0%) | | 116 (74.8%) |
| Graft losses/PNF | – | – | | 5 (3.2%) |
| Rejection within 90 days | | | 0.41 | |
| Yes | 3 (15.8%) | 16 (84.2%) | | 19 (12.3%) |
| No | 12 (9.4%) | 116 (90.6%) | | 136 (87.7%) |

CIT, cold ischemia time; SD, standard deviation.

*Per-protocol analysis except (see Materials and methods).
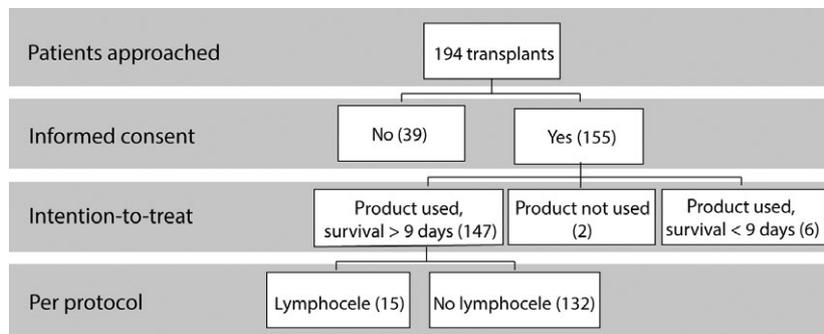
**Figure 2** Study cohort and participation: scheme of approach to include and analyze study population.

lymphatic outlets as an ancillary effect. There are no known side effects and the substance is quickly resorbed within 48 h. Meticulous surgical technique during organ recovery, preparation, and implantation is the golden standard and cannot be emphasized enough, but this protocol may contribute to reducing post-transplant lymphocele, as even clean surgery can miss small lymphatic vessels. These could be sufficiently sealed by the matrix established by this hemostatic substance. Literature is scarce on prevention strategies in the transplant setting. In a randomized trial, fibrin gluing did not turn out to be effective, a nonrandomized trial found class II compression stockings to be beneficial [17,18]. Other than that, only studies in nontransplant settings are available such as TachoSil® in groin lymphadenectomy [19], FloSeal in pelvic lymphadenectomy [20], or Arista™ AH in robotic prostate surgery [21], the latter being a cognate product to HaemoCer™. The results of these studies are variable. Also, the comparison of common surgical interventions with the transplant setting is limited for the unique features of rejection and immunosuppression in the latter. These factors could be influential as shown by the higher rate of lymphocele formation in repeated transplants and patients with DSA. We were able to show some potential risk factors for lymphocele in our prospective cohort being mainly associated with retransplantation and immunological high-risk constellations such as ABO- or DSA-incompatibility. We apply immunoadsorption on a regular basis in those recipients. Even though this therapy can lead to fluid stress on the patients' cardiovascular system, this alone is a minor problem nowadays. Reasons for an elevated lymphocele rate in IA patients can most likely be found in the accumulation of higher immunologic risk profiles, desensitization, more extended rates and longer duration of graft dysfunction and acute rejections as well as more need for dialysis in these recipients. Recipient age, body mass index, implantation on the side of previous kidney transplants, mismatch, delayed graft function or rejection did not influence the formation of lymphocele in this study. On the other hand, this trial was neither performed nor powered to clearly differentiate between risk factors.

The indwelling drainage has been shown to be an important tool for reduction of lymphoceles [22]. Derweesh *et al.* found a significant advantage from waiting for a discharge of less than 50 ml over two consecutive days. Others have also waited significantly longer [23]. We removed the drain after 2 days in all cases of less than 100 ml fluid discharge per 24 h as a center standard. This standard was identical for the historical and study groups. Nevertheless, early removal could have favored the generally rather frequent occurrence of lymphoceles at our center and made the problem more prevalent. A change in policy is currently under discussion.

We are aware of the limitations of the study being mainly the absence of randomization. However, the results of this pilot project allow an estimation of the magnitude of effect of HaemoCer™, which can serve for designing a prospective randomized trial.

Furthermore, the estimated lymphocele rate was based on a retrospective evaluation of a comparable, just elapsed time period of kidney transplantation at the same center but certainly the two cohorts were not matched.

Strengths are the prospective nature of the study, the large sample size within a short period and the small margin for error when using the study product because of its simple application.

In conclusion, we found a reduction of the occurrence of lymphoceles after kidney transplantation in association with the prospective use of HaemoCer™, a polysaccharide-based hemostatic powder. The results of our study may provide a valuable basis for a future randomized controlled trial to clarify the impact of HaemoCer™ on the incidence of lymphoceles.

## Authorship

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## Conflict of interest

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