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ORIGINAL ARTICLE

Efficacy and safety of RD2 Ver.02, a whole blood clot therapy, coupled with a minimally invasive procedure in pilonidal sinus: a phase II study

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Abstract

Background PNS is caused by an infection in the sacrococcygeal area triggered by hair particle accumulation in skin tunnels, resulting in infection. Surgical options range from simple excision to complex flap constructions. Primary wound healing failure and recurrence rates contribute to the burden of PNS.

RD2 Ver.02, a novel autologous whole-blood clot product, demonstrated safety and efficacy in treating complex cutaneous wounds and was investigated for the management of PNS.

Methods A Phase II open-label, pilot, single-arm prospective study was conducted from May 2021 to May 2023 (Ethics Committee approval #7952-20). Patients with PNS underwent a minimally invasive trephine procedure under local anesthesia followed by RD2 Ver.02 instillation into the cavity. Primary healing was assessed at 3, 6, and 12 months. Secondary outcomes included the collection of adverse events.

Results Overall, 51 patients participated in the study. At 3 months, 42/51 healed (82.4%), 7/51 (13.7%) were granulating but not completely healed, and 2/51 (3.9%) failed to heal. At 6 and 12 months, 46/51 (90.2%) and 42/51 (82.4%) achieved complete healing, respectively. At 6 months, two PNSs recurred after initial healing and an additional four instances of PNS recurrence observed in 12 months, so a total of recurrence in six patients (11.8%). There were five adverse events (AEs) with no severe adverse events.

Conclusion RD2 Ver.02 is a safe and effective treatment of PNS when coupled with a minimally invasive trephine PNS procedure. Further comparative studies are needed to fully assess the role of this novel therapy for PNS.

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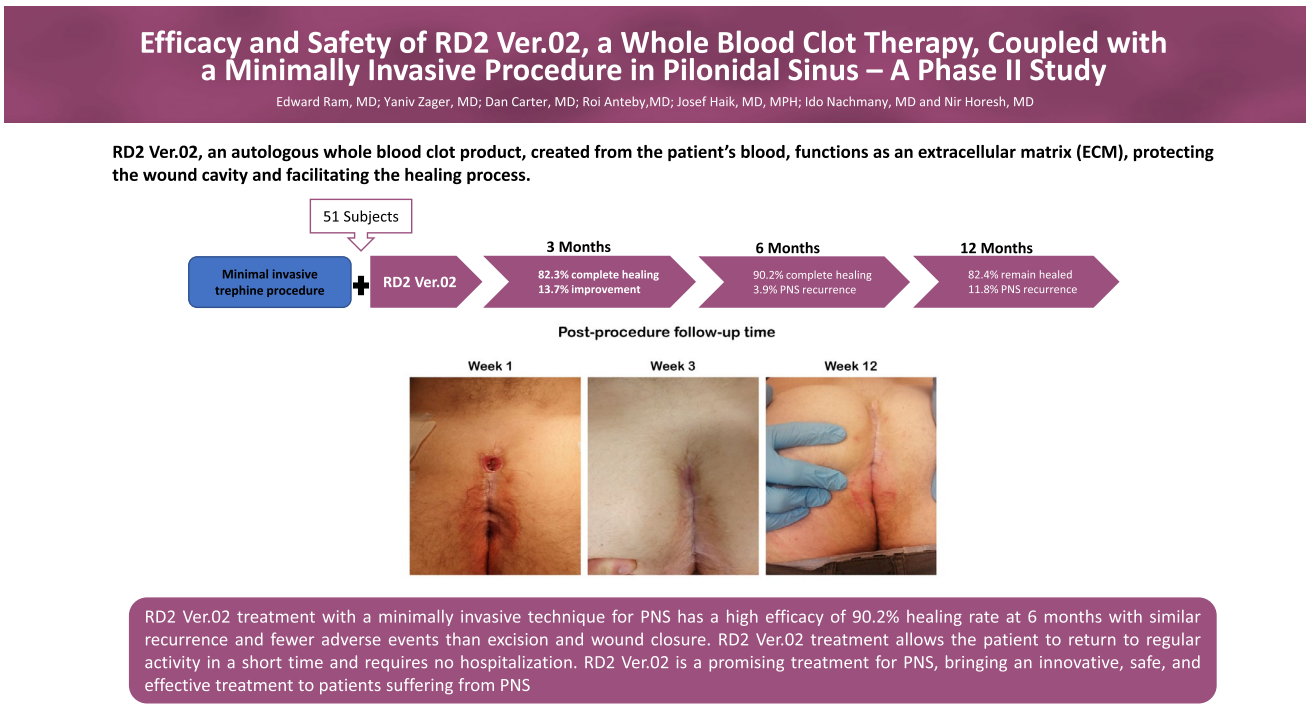
Graphical abstract



Keywords Pilonidal sinus disease · Blood clot · Procedure · Surgical outcomes

Introduction

Pilonidal sinus (PNS) disease is an infection in the sacrococcygeal region, thought to be caused by the buildup of hair particles within a small skin perforation, leading to discomfort and inflammation [1, 2]. PNS predominantly occurs in early adulthood exhibiting a higher prevalence in men compared to women, who account for 20% of the cases [3].

Traditionally, PNS is addressed surgically in a single stage either with primary surgical excision when inflamed and symptomatic or as a two-stage approach with initial incision and drainage followed by formal excision when the presentation is a pilonidal abscess. The specific options include excision with primary closure or planned healing via secondary intention, pit excision, Bascom procedure, or excision with one of several flap [4, 5]. Regardless of the surgical option selected, the patient faces significant morbidity due either to prolonged time for wound healing or from disease recurrence, which occurs in up to 40% of the cases [6, 7]. While wound closure techniques offer shorter healing times and lower indirect costs related to work absences, they are associated with a higher risk for recurrence and infection [8]. Several treatments have been reported, including phenol injection, fibrin glue, laser treatment, cryotherapy, Negative Pressure Wound Therapy (NPWT), and antibiotics, to augment surgical outcomes [4]. However, existing data fail to indicate substantial improvements in recurrence rates [9–11].

Minimally invasive procedures have gained traction in the medical community as a way to curtail costs and mitigate morbidity. For PNS, endoscopic techniques like endoscopic pilonidal sinus treatment (EPSiT) and video-asssisted ablation of pilonidal sinus (VAAPS) only require local anesthesia and simplified wound care, and they allow for a quicker resumption of daily activities compared to excision surgeries [12, 13]. While preliminary results in adult PNS patients are promising, their support is limited to a small number of clinical trials, and long-term outcomes remain unclear. In the pediatric and adolescent population, recurrence rates following EPSiT align with those from invasive procedures [14–16]. In addition, each of these techniques leaves a raw surface requiring primary healing of the wound cavity.

Wound healing post-surgical intervention via secondary intention can span from 2 to 6 months or even exceed 2 years [17], while incision closure significantly accelerates healing to 3–4 weeks [7]. The wound healing process is complex and involves stages of hemostasis, inflammation, proliferation, re-epithelialization, and remodeling [18]. Moreover, wound healing requires the regeneration of the damaged extracellular matrix (ECM), which offers not only structural support

for skin regeneration but also influences several cellular behaviors, including adhesion, proliferation, migration, and survival [19, 20].

RD2-Ver0.2 (RedDress Ltd., Pardes-Hanna, Israel) is an innovative whole-blood clot product that provides a functional ECM, protects the wound, and promotes the healing process. Patient-derived blood, combined with coagulation agents to quicken clot formation, is applied into wound cavities and completes coagulation ex vivo to offer a fibrin scaffold. This acts as a protective, provisional ECM promoting wound re-epithelialization [21]. This effect was observed in previous studies focusing on diabetic foot ulcers, pressure ulcers, complex and tunneling wounds, and surgical wounds. It has been proven to be safe and effective in managing wounds and achieving healing [22–27]. The formation of the clot establishes a biological system that facilitates the delivery of essential growth factors and triggers macrophages, cytokines, and mediators to enhance the healing process [20]. Utilizing the patient's own blood minimizes the risk of treatment rejection. Treatment with autologous blood clots, which serve as a temporary scaffold and stimulate surrounding cells to activate the wound healing process [20], presents a promising strategy in PNS management.

In this Phase II, prospective, open-label study, we aim to evaluate the safety and efficacy of RD2-Ver0.2 for achieving primary healing following a minimally invasive trephine approach to the PNS sinus tract.

Methods

A Phase II prospective single-arm feasibility study, to test the safety and efficacy of RD2 Ver.02 blood clot when coupled with minimally invasive technique, was conducted at the Sheba Medical Center (Ethics Committee approval # 7952-20) between May 2021 and April 2023. The primary endpoint of the study was achieving complete healing by 12 weeks in patients with pilonidal sinus who underwent a minimally invasive trephine pilonidal sinus procedure coupled with RD2 Ver.02.

Patients

Subjects were enrolled in the study after they were seen by an experienced senior colorectal surgeon in an outpatient clinic and were referred for an elective procedure to treat their PNS. The informed consent process included reviewing the informed consent with the patient and answering the patient's questions before signing the informed consent and scheduling the procedure. All the patients signed an informed consent agreeing to take part in the study. Of the patients who were enrolled, 69% (35 out of 51) had a history of PNS recurrence. The only inclusion criteria were patients aged > 18 years with PNS. Exclusion criteria were life expectancy < 24 months, cognitively impaired, cannot withdraw required amounts of blood, known coagulation problems (patients taking anti-coagulant agents were not excluded), pregnancy, breastfeeding, or receiving or scheduled to receive steroids (over 10 mg/day). No further exclusion criteria were part of the study.

Procedure

Following the screening, subjects underwent a minimally invasive trephine pilonidal sinus procedure using local anesthesia. The developed procedure is as follows (Fig. 1) [28, 29]:

1. Fistulous openings were examined for depth and direction of underlying tracts.
2. All visible median pits and lateral fistulous skin openings were excised using skin trephines of a suitable diameter.
3. In cases with fewer than two visible pits, a punch was utilized at the proximal part of the cyst to establish a tunnel. Additional punches were performed as necessary.
4. Upon reaching the pilonidal cavity, hemostat forceps and/or curette was deployed to eliminate all chronic inflammatory tissue within the cyst/sinus tracts including all hair and mucopurulent material.
5. The wound was then cleansed with hydrogen peroxide, followed by saline.
6. RD2 Ver.02 preparation. In brief, 18 ml of venous blood was collected into sterile acid citrate dextrose adenine (ACDA) vacuum tubes and activated ex vivo with calcium gluconate and kaolin powders (RedDress Ltd., Israel).
7. Following the activation of the blood, it was immediately applied into the sinus cavity using a 30-ml syringe, filling it completely and facilitating clot formation within the sinus (approximately 4.5 min). The surgical area was covered with a sterile non-adherent dressing and gauze (see technical description below) [21, 22].
8. Postoperative instructions encompassed prescription of antibiotics, maintaining the dressing dry for 24 h.

Healing assessment and follow-up

Patients were followed up 1, 3, 6, and 12 weeks post-procedure; however, healing assessment started on week 6. The data collected were sinus healing and adverse events. Long-term follow-ups were conducted remotely via telephone at 6 and 12 months post-procedure. Healing (defined as pit closure without pus or soiling on physical examination) was assessed clinically by the investigator during follow-up visits and by patient-reported symptoms via telephone

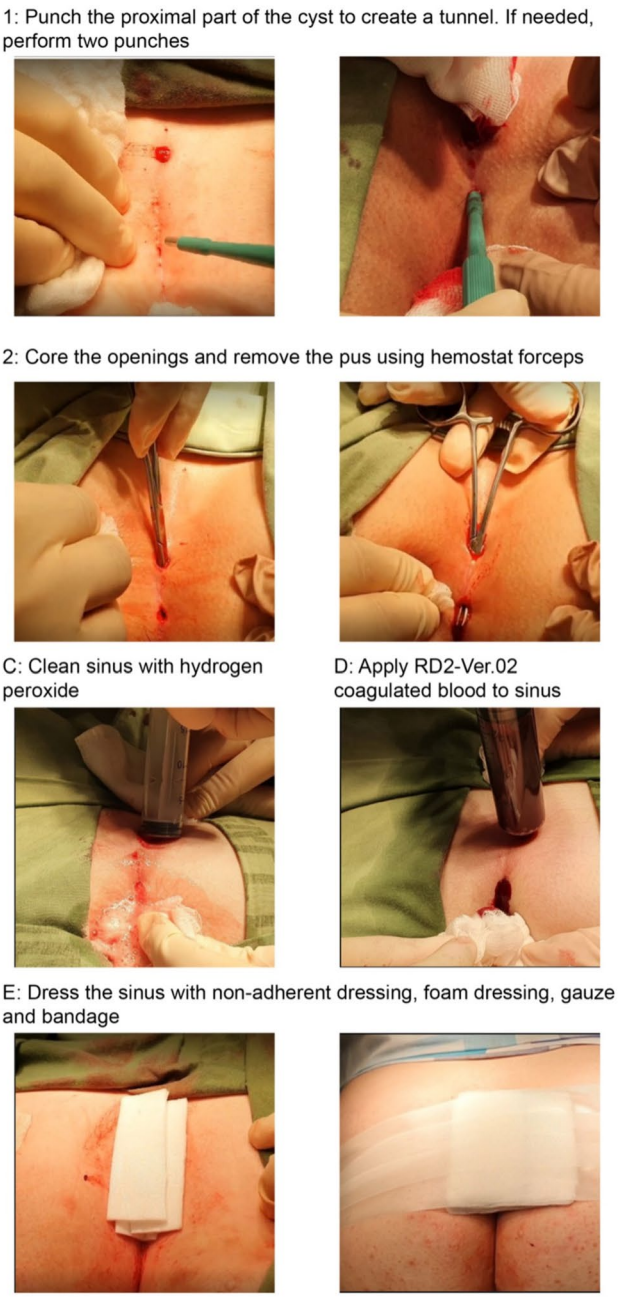


Fig. 1 Detailed step-by-step photos of the PNS procedure

during long-term follow-ups. Recurrence/non-healing was determined if abscess, drainage, pus, or soiling was observed from the PNS site in a clinical examination.

Adverse events

Adverse events were graded using the NCI CTCAEv5 scale [30]. Serious adverse events were described as any adverse change in the patient's health status that met any of the following criteria: results in death; is life-threatening; necessitates inpatient hospitalization or lengthens existing hospitalization; results in persistent or significant disability/incapacity (grades 3–5 using the NCI CTCAEv5.0 scale). Device-related adverse events (DRAEs) were defined as adverse events directly attributable to the use of RD2-Ver.02. DRAEs could include complications related to venipuncture (excluding lack of venous access), infections emerging within 2–4 days of device application, allergic reactions, and severe pain associated with product application.

Adverse events (AEs) encompass any adverse change in the patient's health condition, including any worsening or exacerbation of a pre-existing condition observed by the investigator or reported by the patient.

Table 1 Patients and sinus demographics, $N = 51$

Parameters	Value
Age (years)	25.4 (SD 7.07)
Gender	
Male	44 (86.2%)
Female	7 (13.8%)
Smoking history	23 (45%)
External pilonidal sinus openings per patient (median)	2 (range 1–4)
Sinus discharge	28 (55%)
Type of prior procedure (*some of the sinuses had undergone multiple various prior procedures)	
Pilonidal sinus excision	10 (19.6%)
Abscess drainage	13 (25.5%)
Pilonidal sinus laser surgery	7 (13.7%)
Number of prior procedures	
1 prior procedure at sinus	8 (15.7%)
2 prior procedures at sinus	10 (19.6%)
More than 2 prior procedures at sinus	6 (11.8%)
No prior treatment	27 (52.9%)

Statistical analysis

Statistical analysis was performed using SAS/STAT software version 9.4 (SAS Corp., Cary, NC, USA). Differences between qualitative variables were assessed using Fisher's exact test and χ^2 test, while quantitative variables were compared using the *t*-test. A p value < 0.05, based on a two-tailed analysis, was considered statistically significant.

Results

Fifty-one patients were included in the study. Patients' demographics and preoperative data can be seen in Table 1. The study population was predominantly male (86.2%) with a mean patient age of 25.4 ± 7.07 years; 47% (24 out of 51)

of patients had been previously treated surgically for PNS, with a number of patients having multiple prior attempts at PNS excision (Table 1). The median number of pilonidal sinus openings for each patient was 2 (range 1–4), with 16 patients exhibiting ≥ 3 openings; 28 (55%) patients exhibited sinus discharge, with the rest experiencing pain attributed to induration in their PNS.

At the 6-week follow-up, evaluation of healing took place. Unfortunately, 11 patients were absent, making it impossible to assess their progress at this specific time point. Of the remaining patients, 17 (42.5%) were confirmed to be healed, 21 (52.5%) exhibited ongoing signs of healing, and 2 failed to heal. At the 12-week follow-up, all 51 participants were assessed. By this point, 42 individuals (82.4%) were fully healed, while 7 (13.7%) were in the process of healing. Treatment was unsuccessful for two patients (3.9%), as determined by persistent non-healing PNS from early post-operative follow-ups (Fig. 2). At the 6-month mark, healing was noted in 46 of 51 (90.2%) participants, with 1 patient lost to follow-up (LTFU), and there were two instances of PNS recurrence. Durability for PNS healing at the 12-month follow-up was sustained in 42 of 51 participants (82.4%) and PNS recurrence in 6 patients (11.8%). A case study is presented in Fig. 3. A subgroup analysis of patients who had undergone prior attempts at PNS excision showed that 96% of them achieved complete healing within 3 months. Four patients who healed on the 6-month follow-up experienced recurrence at 12 months, bringing the total recurrence by 12 months to 11.6%. Among the patients who healed in the 6-month time point (46), 91% remained healed at 12 months.

Throughout the study, there were a total of five adverse events (AEs). This included two localized wound infections at the PNS site (one of which may have been device-related), one event of hydrocelectomy, one bleeding from the PNS site and one event of abscess. Of the five events, four were considered unrelated AEs. None of these were severe (see Table 2 for details). When AEs were classified based on severity, one event was deemed mild (2.0%), four were moderate (7.8%), and none were severe. None of the participants experienced AEs directly linked to the use of RD2-Ver.02. Among the AEs, one was an anticipated side effect of the minimally invasive trephine pilonidal sinus procedure of one case of sinus bleeding (2%) and one abscess at the pilonidal sinus site that required drainage (2%). There were no complications concerning venous access during the preparation of RD2-Ver.02.

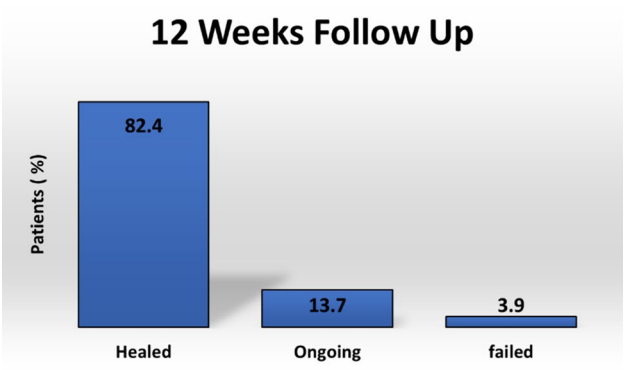


Fig. 2 Primary endpoint. Healing status at week 12

Table 2 Adverse events following pilonidal sinus procedure and RD2-Ver.02 application

AE category	*N* (% of total)
Abscess	1 (2%)
Bleeding at pilonidal sinus	1 (2%)
Infection at pilonidal sinus site	2 (3.9%)
Infection not at the pilonidal sinus site	1 (2%)
Total	5

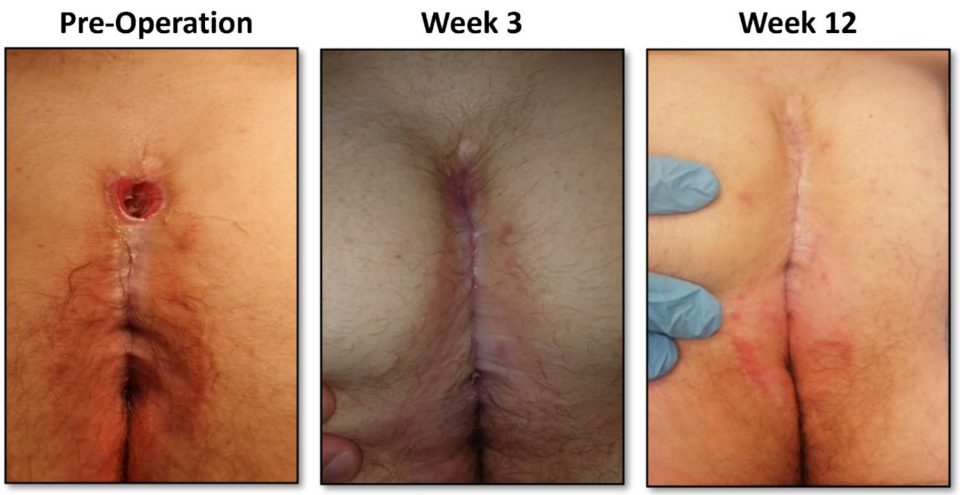


Fig. 3 Case study of a healthy 31-year-old male. Patient had a history of chronic PNS and had undergone three full excision surgeries. In two the sinus healed and reoccurred; one surgery failed. Patient presented with a complex infected cyst with three opening (left). Follow-up photos are presented 3 (middle) and 12 (right) weeks after procedure

Discussion

RD2-Ver.02 is an autologous blood clot that provides a fibrin scaffold acting as a protective, provisional extracellular matrix promoting wound healing [23, 24]. This clot adheres to the wound bed, molding itself onto the tissue, attracting necessary cytokines and chemokines, thereby inducing the healing cascade. Moreover, the clot serves as a physical structure supporting cell growth and suggests reducing bacterial bioburden, thus minimizing the risk of wound infection [31].

In this study, we demonstrate that the RD2-Ver.02 is a safe and effective treatment in PNS with a 82.4% healing rate at 12 months. Interestingly, in a prospective study that aimed to assess PNS recurrence rates post-procedure in a minimally invasive trephine technique, it was determined that, within 12 months after the surgery, 33% of the patients experienced PNS recurrence, highlighting the benefits of coupling the minimally invasive trephine technique with RD2 Ver.02 treatment [32].

The minimally invasive nature of this approach supplants excision of the PNS sinus/cyst with primary of secondary healing of the wound with closure of the debrided PNS sinus/cyst cavity with RD2 Ver.02. This approach can potentially reduce the complexity of the wound closure required and the time needed for wound management postoperatively. In addition, there is the option of repeating the treatment with RD2 Ver.02 without proceeding to a more complex excisional approach.

Currently, most treatments for PNS disease are surgical in nature and require general anesthesia and hospitalization. Following the procedure, patients often experience pain and discomfort, which can last anywhere from several days to weeks, meaning normal activity is only resumed after a period of 2–8 weeks [33]. Spinal anesthesia-related postoperative complications, which occur in approximately 12% of PNS cases, further contribute to extended hospital stays, increased expenses, and a longer interval before a return to routine activity [34]. A key advantage of the minimally invasive technique is its administration under local anesthesia, eliminating the necessity for post-procedure hospitalization. This also suggests that, due to the procedure's minimal invasiveness, the recovery time for patients and their return to normal activities may be shorter compared to more aggressive techniques.

The data demonstrated that trephine debridement of PNS followed by RD2 Ver.02 instillation provides a very safe and effective minimally invasive procedure for PNS. The reported 11.8% recurrence rate is acceptable compared to the rates associated with far more invasive and complex procedures. The possibility of repeat therapy might extend the benefits of this approach to PNS management without significant tissue trauma. The approach also reduces the morbidity and recovery phase compared to excisional approaches to PNS.

Therefore, RD2 Ver.02 emerges as a promising, innovative, safe, and effective solution, offering a new therapeutic option for patients suffering from PNS disease worthy of further investigation.

Study limitations

While our findings show promise, it is crucial to note this study's limitations. Our single-arm design lacks a control group, potentially limiting the definitive attribution of observed effects to RD2 Ver.02 treatment alone. Conducted in a single-center setting, our results may not fully represent diverse patient populations and varied clinical practices. Further multicenter trials and larger-scale randomized controlled trials would enhance the robustness and generalizability of our findings. Longer term follow-up is required to assure the absence of delayed recurrence events.

In this study, we have not assessed the cost-effectiveness of this procedure compared to other available treatments. The healing rate is among the variables crucial for defining the cost-effectiveness of the product and is still pending determination in future studies.

Conclusion

Our research indicates that the minimally invasive technique when coupled with RD2 Ver.02 shows promising outcomes for PNS treatment. Further comparative studies are needed to fully assess the role of this novel therapy for PNS.

Author contributions In this clinical study, all authors played pivotal roles in patient care and data collection. Furthermore, all authors played an integral role in the collection, analysis, and interpretation of clinical data. Professor Edward Ram was responsible for the performance of the minimally invasive procedure and RD2 Ver.02 application for all patients. All authors read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this paper.

Declarations

Conflict of interest Dr. Edward Ram is a consultant at RedDress Medical.

Ethical approval All procedures performed in the study involving human participants were in accordance with the ethical standards of the Sheba Medical Hospital Center and were in line with the principles

of the Declaration of Helsinki. The study was approved by the Sheba Medical Hospital Ethics Committee (#7952-20).

Informed consent Informed consent was obtained from all participants involved in the study.

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