Study Protocol

Efficacy of platelet- and extracellular vesicle-rich plasma in the treatment of chronic postoperative temporal bone cavity inflammations

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1 Introduction

Platelet-rich plasma is a blood product, prepared from peripheral venous blood, which possesses beneficial immune, hemostatic, and regenerative effects. Since it is rich in extracellular vesicles (EV), it can be called platelet- and extracellular vesicle-rich plasma (PVRP) (1). In addition to platelets, EVs are thought to be the main mediators of the regenerative effects of PVRP (2). EVs are a heterogeneous group of nanometrically-sized membrane structures that play an important role in intercellular communication and can, therefore, be used in diagnosis and treatment (3–5). Although PVRP has been used in medicine for tissue regeneration for decades, there is a lack of trials regarding EVs in association with PVRP so far (6).

Despite numerous clinical trials on the use of PVRP, research in otorhinolaryngology is currently scarce (7). A particular challenge is the treatment of chronic postoperative temporal bone cavity inflammation (CPTBCI). The postoperative temporal bone is most often the result of surgical treatment of cholesteatoma of the middle ear. Treatment of CPTBCI is often exhausted due to the failure of surgical procedures and standard conservative treatment methods. Up to 20 % of patients are expected to suffer from CPTBCI (8,9). Problems associated with CPTBCI not only impair quality of life, but also significantly impact the health care system (10–12).

We aimed to test the efficacy of PVRP in the treatment of CPTBCI after exhausted surgical and standard conservative treatment in a prospective randomized controlled clinical trial. The outcome of treatment was assessed by comparing the quality of life in patients treated with PVRP and patients treated with standard conservative methods throughout four otorhinolaryngological examinations (i.e., check-ups).

2 Methods

National Medical Ethics Committee, Republic of Slovenia approved the trial (No. 0120-146 / 2019/5).

The trial started by creating a list of patients or cases who met the inclusion criteria (i.e., recruitment) during visits at the Department of Otorhinolaryngology and Cervicofacial Surgery, University Medical Centre Ljubljana for six months (Table 1). CPTBCI on one ear (i.e., unilateral) was considered as one case of CPTBCI and CPTBCI on both ears (i.e., bilateral) as two cases of CPTBCI. Surgical treatment was considered as exhausted if an additional surgical treatment would not eliminate CPTBCI while preserving hearing. Standard conservative treatment was defined as exhausted if it was ineffective in treating CPTBCI for at least 8 weeks.

Table 1: Inclusion and exclusion criteria of the trial.

Inclusion criteria	**Exclusion criteria**
COM defined as a presence of ≥ 1 of:	venepuncture site inflammation
• *visible ear discharge*	pregnancy or breastfeeding
• *indirect ear discharge signs (e.g. on a pillow, clothes)*	chronic use of immunomodulatory and / or antimicrobial drugs
• *itching*	presence of systemic infectious disease
• *sensation of ear fullness*	presence of malignancy
• *clinical signs of inflammation exacerbation*	other experimental attempts to treat the consequences of radical COM surgery
exhausted surgical and standard conservative treatment	inability and / or refusal of the patient to participate in the trial
age > 18 years	presence of autoimmune disease

COM – chronic otitis media.

After recruitment, patients were randomly allocated one of two interventions: treatment with standard conservative methods (i.e., control group), and treatment with PVRP (i.e., PRVP group).

After recruitment and allocation patients were examined at the Department of Otorhinolaryngology and Cervicofacial Surgery, University Medical Centre Ljubljana when they attended their regularly scheduled check-up. We informed each patient about the allocation and re-checked the same inclusion criteria (Table 1). If the patient met these criteria and wanted to participate in the trial voluntarily, he/she signed an informed consent, which meant the enrollment in the trial. If the patient refused to participate, he/she did not enter the survey, but this did not affect the regularly scheduled treatment. The latter included the continuation of standard conservative treatment methods, described below. Patients enrolled in the trial started the treatment according to the treatment protocol, either with standard conservative methods (i.e., control group) or with PVRP (i.e., PVRP group). Only patients who completed treatment according to the protocol were included in the final analysis. The researchers who treated patients and patients were informed about the allocation, therefore the trial was not blind.

2.1 Treatment protocol

The treatment included four check-ups by the leading researchers at the Department of Otorhinolaryngology and Cervicofacial Surgery, University Medical Centre Ljubljana. Four-week intervals between individual check-ups were planned, so the treatment was expected to last 12 weeks. The treatment protocol for both groups is shown in Table 2.

Table 2: Chronic postoperative temporal bone cavity inflammation treatment protocol.

		Control group	PVRP group
1	A	review of inclusion and exclusion criteria	
	B	invitation to participate in the trial	
	C	enrollment in the trial	
	D	completion of the COMQ-12 questionnaire	

	E	toilet (i.e. cleaning) the middle ear		
	F_A	standard conservative methods	F	treatment with PVRP
2_A (~ 4th week)		1A, 1D, 1E, $1F_A$	2	1A, 1D, 1E, $1F_B$
3_A (~ 8th week)		1A, 1D, 1E, $1F_A$	3	1A, 1D, 1E
4_A (~ 12th week)		1A, 1D, 1E, $1F_A$	4	1A, 1D, 1E

Numbers (1-4) mark each check-up. Capital letters (A-F) mark interventions performed during the check-up, alphabetically. $_A$ refers to the control group check-up, $_B$ refers to the PVRP group check-up. Example: $1F_B$ stands for treating the patient in a PVRP group with a PVRP. 3_A contains measures 1A, 1D, 1E, $1F_A$. The second examination was scheduled for 4 weeks, the third 8 weeks and the fourth 12 weeks after the first examination. PVRP – Platelet- and extracellular vesicle-rich plasma; COMQ-12 – chronic otitis media questionnaire 12.

Patients in the PVRP group received PVRP at the first and second check-up (Table 2). We performed a cleaning (i.e., toilet) of the ear at each check-up. The other standard conservative methods, described below, were not used in PVRP group. At the third and fourth check-up, we performed ear toilet only. The discontinuation of other standard conservative methods at the third and fourth check-up in the PVRP group did not pose any risk, as the patients' problems were chronic.

Patients in the control group continued the treatment with standard conservative methods during each check-up. These methods included ear toilet, use of topical antimicrobial drugs, anti-inflammatory and antiseptic drugs (Table 2).

2.2 Outcome measures

The treatment outcome was measured with three outcome measures:

- Inflammation surface area in postoperative temporal bone cavity at each check-up. The surface area in milimetres was transformed to the ratio of surface area according to the first check-up (i.e., baseline evaluation). Therefore surfaces at the first check-up were represented as 100 % different than 100 % in subsequent check-ups.
- Slovenian version of COMQ-12 (Chronic Otitis Media Questionnaire-12) sum score (13) at each check-up. COMQ-12 is a chronic otitis media (COM)-related quality of life measure (12). It was completed by the patient himself. The questionnaire comprises 12 questions scored 0-5. Seven questions relate to the severity of COM symptoms, two questions to the impact of COM on lifestyle and work, two questions to the burden on the health system due to COM, and one question is general. The maximum possible sum score is 60. Since the CPTBCI is a type of COM, the higher COMQ-12 sum score signifies the worse quality of life associated with COM or CPTBCI.
- Microbiological analysis of temporal bone cavity ear swab performed at the first and third check-up. The cultivation of bacteria and fungi was performed.

2.3 Platelet- and extracellular vesicle-rich plasma preparation protocol

Blood was taken (i.e., venepuncture) at the Department of Otorhinolaryngology and Cervicofacial Surgery, University Medical Centre Ljubljana from the cubital vein by a vacuum method by a medical technician. 21 G wing needle (so-called butterfly) (Safety-Lok Blood Collection Set, BD Vacutainer, Becton Dickinson, USA) was used to draw blood into four 4.5 mL citrate tubes. (9 NC sodium citrate 0.105 M, BD Vacutainer, Becton Dickinson, USA). The tubes were stored at room temperature before and after venepuncture. PVRP was prepared according to the protocol described in Table 3 on the day of venepuncture in the Laboratory of Clinical Biophysics, Faculty of Health, University of Ljubljana, for each patient in PVRP group at the first and second check-up (Table 2).

Table 3: PVRP preparation protocol:

Step	Description
1	Venepuncture of cubital vein to draw blood into four 4.5 mL sodium citrate tubes
2	Weighing of four 4.5 mL citrate tubes. If the weights differed > 0.02 g (incomplete blood collection), they were balanced by the addition of counterweight tubes.
3	First centrifugation step: 300 g, 5 min and 18 °C.
4	Transfer of the supernatant (i.e., plasma or yellow top layer without buffy coat) with a sterile pipette from four centrifuged citrate tubes into two sterile polypropylene tubes.
5	Second centrifugation step: 700 g, 17 min and 18 °C.
6	Removal of approximately half of the supernatant (i.e., platelet-poor plasma) from two sterile polypropylene tubes with a sterile pipette.
7	Resuspension or homogenization of the remaining halves in sterile tubes with a sterile pipette to obtain half of PVRP.
8	Fusion of two halves of PVRP.
9	PVRP resuspension

Step 9 was followed by administration of PVRP in the ear via PVRP-soaked ear wick. Weighing was performed with Sartorius, T2145-OCE, Goettingen, Germany and centrifugation with Tehtnica, Centric 400R, Domel, d.o.o., Železniki, Slovenia. Termofisher scientific, USA (ref.: 225-1S, lot: 19100819) sterile pipettes and Simport scientific, Canada (ref.: T405-1A lot: 904175849) sterile tubes were used to prepare PVRP. PVRP – platelet- and extracellular vesicle-rich plasma.

PVRP was brought to a patient, waiting at the Department of Otorhinolaryngology and Cervicofacial Surgery, University Medical Centre Ljubljana. Immediately before administration, PVRP was resuspended, as it sedimented during the transport. Then the ear wick (i.e. gauze tamponade 1 cm x 10 m cut on a 7 cm long strip, Tosama d.o.o., Slovenia) was soaked with PVRP and inserted into the ear. The patient was instructed to pull the wick out of his ear after two days.

2.4 Statistical analysis plan

We used the computer program Microsoft Excel for Mac (versions 16.9.0-16.36) to record and edit the trial data, and the computer program SPSS (statistical package 23, IBM Corp.,

Armonk, New York, USA) for statistical analysis. The difference between the groups was defined as statistically significant if the probability of rejecting the null hypothesis was greater than 95 % ($p < 0.05$). Statistical analysis plan is in a separate document.

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