Efficacy of Platelet Enriched Plasma in preventing surgery for patients with chronic tympanic membrane perforation

Protocol Version 3; February 7th2; January 22, 2018

Background & Rationale

Tympanic membrane (TM) perforation is the leading cause of conductive hearing loss. TM perforations in adults typically arise from middle ear infections, trauma, or from iatrogenic cause. While approximately 80% TM perforations close with time and conservative managements, approximately 20% require surgical intervention[1-3]. Often before exploring surgical options some patients opt for trial for paper patch myringoplasty that can be performed in the clinic. Success rates with paper patch myringoplasty are variable and can range from 12 to 63% depending are whether the size of the perforation is large or small[4]. When patients fail conservative treatment with paper patch, patients will explore surgical options. Surgical success rates for myringoplasty vary and quoted ranging from 60 - 90% in adults to 34-85% in children, and often lower in anterior TM perforations secondary to more difficult exposure[1]. Of those patients that do require surgical intervention, costs of tympanoplasty can cost upwards of twenty-four thousand dollars[5].

Multiple different biologic materials have been heavily investigated in the literature including hyalornan, heparin, epidermal growth factor, fibroblast growth factor, platelet derived growth factor, and transforming growth factor with variable success[6]. Recently platelet enriched plasma (PRP) has been favored in multiple surgical procedures from orthopedics, ENT, to plastic surgery[7, 8]. The rationale behind its use are two fold; first platelets play a fundamental role in hemostasis and wound healing, and secondly the alpha granules contained within platelets contain various growth factors that play a fundamental role in wound healing[9]. Currently there are three different PRP that are FDA approved, SMARTPEP (SmartPEP, Harvest Technologies Corp, Norwell, MA) and Platelet Concentrating Collection Systems (3i/Implant innovations, Palm Beach Gardens, FL), and Arthrex (Arthrex Naples, FL) [2]. PRP is an autologous blood donation, where whole is blood is collected from the patient and subsequently isolated through differential gradient centrifugation in an anticoagulant citrate dextrose-A[2].

PRP is widely used throughout multiple surgical subspecialties daily including Otolaryngology, Orhtopedic surgery, Plastic surgery, Dermatology, OMFS, and Dentistry for over two decades. Despite its clinical advantages, to date there are no studies that have reliably investigated adverse events associated with PRP because of poor and inconsistent methodology[10]. However, there are intermittent case reports that report adverse events. A case report by Kaux *et al* (2014) found one adverse event out of 1000 injections performed. They reported one PRP injection into a 35-year-old male with diabetes caused an exuberant inflammatory reaction, characterized by swelling, pain, and erythema[11]. However, a large Cochrane data base review looking at PRP in musculoskeletal injuries examined over 19 trials covering over 1000 patients and found no difference in adverse events (including pain, infection, tendon rupture, and anaphylactic reaction) [12]. A separate systematic review examining six studies found no difference in complication rates between PRP and controls for acute muscle injuries (including hematoma, discomfort, hyperesthesia at injection site)[13]. Another Cochrane database review looking at PRP effectiveness for sinus lift procedures for dental implant rehabilitation examined over 29 trials and found no difference in complication rates or adverse events with PRP compared to controls (including sinusitis, infection, nerve injury, hematoma, perforation into sinus) [14].

Recently a study by Erkilet (2008) investigated the use of platelet enriched plasma (PRP) on 88 TM on 44 rats, treating one ear with PRP while the contralateral ear was left to heal spontaneously[9]. They

demonstrated that those tympanic membranes treated with PEP had faster healing times both clinically and on histopathology. In a separate study, acute TM perforations were created in rabbits and PRP was shown to significantly quicken time to heal without any difference on a microscopic level[15]. While PRP has been shown to be effective as sole treatment for animal models, it has yet to be investigated independently humans. A study by El-Anwar (2015) looked at concurrent use of PRP with myringoplasty, which involves a post-auricular incision to inlay a medial TM graft, and found statistically improved graft take and healing[16]. However, the use in this case is in an intraoperative setting with an already established surgical procedure.

We wish to pursue and investigate the use of PRP in a clinical setting as the sole treatment for small tympanic membrane perforations. While no studies exist on the specific use of PRP as sole treatment modality, multiple studies have shown success with various growth factors such as PDGF and ECF[17]. PRP is promising because its ability to use a variety autologous growth factors stored within their alpha granules, negating the need for exogenous growth factors. Ultimately if PRP can increase the rate of tympanic membrane perforation healing thus negating the need for surgical intervention, PRP can potentially save the health care industry millions of dollars every year.

Specific Aim

The goal of our proposed prospective project is to investigate the efficacy of platelet enriched plasma (PRP) as a treatment for patients with chronic tympanic membrane perforation. Our primary outcome includes the prevention of patients requiring surgery for their perforation.

Inclusion Criteria

- Patients aged 18 or older
- Patients who have a chronic anterior tympanic membrane perforation that is <50% of the eardrum who meet criteria for tympanoplasty procedure
- Patients willing to comply with the protocol and attend all study visits.
- Patients is able to provide written informed consent

Exclusion Criteria

- Patients who are aged 17 or younger
- Patients who have previously undergone middle ear or lateral skull base surgery
- Patients who have a chronically draining ear who would not qualify for a tympanoplasty
- Any type of platelet disorder, cancer, or ongoing systemic infection.
- Any type of hemodynamic instability, septicemia, infection, tobacco use, any use of steroids to the ear drums, and patient unwilling to accept the risks of the procedure.
- Type I diabetes or other autoimmune pathology (based on case report by Kaux et al (2014) [11])

Benefits and Risks

There are some risks associated with a tympanoplasty, however, these risks are not associated with this study and will be covered in a separate consent for surgery.

There is a minimal risk for patients associated with the possibility for an unintentional breach of confidentiality. All records will be adequately protected to assure that this risk is minimized to the best of our ability.

Potential benefits of this study include the possibility of avoiding surgery for this subset of patients. This will decrease overall cost for the patient and avoid the inherent complications and pain associated with tympanoplasty surgery.

Study Procedures

During routine clinical care visits, the patients will be screened for selection according to the inclusion and exclusion criteria. Each patient matching these criteria and deemed eligible for the study by the study personnel will be approached for participation in the study. Study personnel will consent the patients in the clinic space behind closed doors. All patients will be given the opportunity to determine their interest in participation and ask any questions necessary to the study personnel. We will stress to the patient's that involvement in this study will not delay standard of care and will not delay or negatively impact eligibility for any type of surgical intervention. Dr. Staecker is currently scheduling cases out by about 3 months, which is beyond the 6 week window we expect to complete this study. The only way involvement in this study will change any surgical plans is if PRP is able to successfully heal the tympanic membrane perforation.

This study consists of an initial visit, followed by three additional follow up visits. The initial visit may last up to one hour and will include consenting the patient for the procedure, a blood draw for PRP extraction, completing a short questionnaire (attached), history and physical, taking pictures of the perforation and application of the PRP. Patients will be scheduled for surgery approximately 5-8 weeks after their initial appointment. Prior to surgical intervention, they will be seen in clinic for follow up and given the same questionnaire (attached). The three additional visits will consist of shorter visits to evaluate the perforation size and need for surgery. Patients will be given compensation of $10 for each visit they participate in in the study.

Patients will only be treated once with the PRP isolate. During the initial visit, the patient's blood will be drawn by study personnel. While the PI is completing other parts of the initial visit, the blood will be drawn in the clinic and centrifuged down into PRP in the clinic. This will take approximately 15 minutes. Please see below for step by step. We have also provided the Arthrex handout information sheet for PRP. The PRP has a gel-consistency that will be applied to the patient's tympanic membrane using a cotton tip swab.

After the five of the initial patients are treated we will review the treatment outcomes. Should all five fail to receive any benefit from the PRP treatment or if some other unanticipated serious adverse events emerges during the study the study team will consider the utility of moving forward. Given the history of the routine use of PRP in multiple other subspecialties including otolaryngology facial plastics surgery we do not anticipate any systemic or local reactions or problems. While this study is not without risks, patients are already scheduled for surgery at the time of enrollment. Participation in the study does not delay routine care in this patient population as surgery is universally scheduled 5-8 weeks after initial consultation. The likelihood of failure and/or success is not known and therefore stopping the study after 5 patients may prevent us from seeing the positive impact of this treatment. Prevention of even one out of 10 patients proceeding to surgery would be financially justifiable given the expense of surgery and the relative low cost of PRP treatment. After 10 patients are enrolled and treated the study team will again revisit the utility of proceeding with the study. At that point if no patients have any measurable benefit from the treatment the study will be stopped and carefully reviewed for futility.

Adverse events will all be documented using the CTCAE 4.0 Protocol and the PI will assess the association with the study drug. Any adverse events will be reviewed with the independent safety monitor who will have the authority to stop the study if the adverse events are associated with the investigational treatment and deemed a significant risk likely to occur in future participants. The standard serious adverse events of death, life-threatening complication, inpatient hospitalization or prolongation, or persistent or significant disability will all be considered events worthy of stopping the study. Again, this is an autologous therapeutic and there is no evidence of serious adverse events or adverse events beyond placebos or alternative therapies reported in the PRP literature for any of the approved uses.

Protocol for Preparation:
Each kit will contain the following: Double syringe, double syringe luer cap, tourniquet (disposable and latex free), alcohol pad, angel wing infusion set 19G, Gauze sponge (2x2in), Band-Aid (latex free), and patient label. All samples will be processed at KUMC in the ENT clinic.

Step 1: Prior to withdrawing, prime the outer and inner syringes by pulling each plunger completely back and forward before starting the process. Withdraw approximately 1.5ml ACD-A into the syringe. However, if the ACP is going to be used within 30 minutes of blood withdrawal, the use of ACD-A is not required.
Step 2: Blood withdraw. Place the tourniquet along the proximal arm and prep the skin where the desired vein to withdraw blood is located. Slowly withdraw by pulling back on the wings that are colored red. Fill the syringe with a maximum of 16cc of venous blood at a rate of 1cc every 2 seconds and seal the syringe with the red cap. Of note, blood will be collected with a single uninterrupted venipuncture with minimal damage to and manipulation of the donor's tissue. The 19 gauge butterfly needle will be used to withdraw blood, which is located in the kit.

Step 3: The centrifuge will be located in the clinic. Gently rotate the syringe in order to mix the blood and the ACD-A. Place the syringe into a bucket and an appropriate size counterbalance in the opposite bucket of the centrifuge. For the sake of this study, we will centrifuge the blood immediately upon collection. Recommendations are that it is done within 4 hours and stored at a temperature of 20 to 24 degrees Celsius.

Step 4: Run the centrifuge at 1500 rpm for 5 minutes. Remove the syringe, taking care to keep it in an upright position to avoid mixing the plasma and red blood cells.

Step 5: Transfer 4-7ml of the ACP from the larger outer syringe into the small inner syringe that comes with the kit. Slowly push down the outer syringe's red wings, while slowly pulling up the plunger of the small inner syringe. Final concentration of platelets is estimated around 600 x 10^3 / ul.

Step 6: Unscrew the small inner syringe. The ACP is ready for use at the point of care. The ACP can also be transferred into a sterile cup on the sterile field and transferred into a 10ml syringe for use. The ACP should be used within 4 hours after blood draw. For this procedure, we will use immediately after isolating.

Step 7: one the ACP is isolated and ready to use it will be applied directly to the small tympanic membrane perforation with the use of the otomicroscope.

Patient follow up. We will ask patients to remain in the clinic for 30 minutes after the injection to assure the safety in the acute post-treatment period including bleeding, vertigo, ear fullness, pain, erythema, swelling, or other signs of inflammation or infection. We will conduct a phone interview between 3-5 days after application of PRP. During this phone interview conducted by study team members, we will again evaluate any concerning side effects with questions about the same issues mentioned above and any worsening of hearing loss or discomfort. Each subsequent follow-up visit will evaluate the safety of the treatment with the same endpoints and additionally include an assessment of efficacy.

The objective study outcomes will be in terms of success of healing of tympanic membrane, and pictures taken in clinic will be the data collected. The pictures taken of the perforation will be standardized to the size of the malleus in order to compare images between different visits.

Patients who do not have complete closure of the tympanic membrane will proceed to surgery as originally planned and resume routine follow-up. Should any patients achieve full closure and healing they will have their surgery cancelled and resume routine follow-up.

A full schedule of events for each study visit is included below.

Schedule of Events

	Treatment Visit	Post injection Follow up phone Call	Follow up Visit 1	Follow up Visit 2	Final Follow
	Day 0	Day 3-5	Day 14	Day 28	Day 42
Visits	1	N/A	2	3	4
Medical History and Current Medications	X				
Ringing in the Ear Evaluation	X	X	X	X	x
Overall Exam	X		X	X	x
Blood Samples	X				
Questionnaires	X	X	x	X	X
Hearing Test	X				X
Ear Exam	X		X	X	X
Eardrum Mobility Test	X				X
Balance Testing	X		X	X	X
Study Treatment Application	X				
Review Current Medications and Side Effects	X		X	X	X
Signs of Infection, edema, or Inflammation	X	X	X	X	X
Photographs of Eardrum	X		X	X	X
Approximate Study Visit Length (in hours)	2 hours	5-10 minutes	0.25 hour	0.25 hour	0.25 hour

While the principal investigator, Dr. Staecker, has had limited exposure to the use of PRP we as ENT residents have use it on a consistent basis during our facial plastics procedures. More specifically we apply it after rhytidectomy (face lift) to help with healing and decrease post-operative hematomas. We perform about 15-20 rhytidectomies a year. Furthermore, we will not have a control arm because those subsets of patients already exists in a day to day basis in the Neurotology clinic. The control arm would be standard patients that do not wish to participate in the study and simply with to schedule surgery for tympanoplasty. Our aim with this initial study is to understand and evaluate the potential efficacy of platelet enriched plasma (PRP) as a treatment for patients with chronic tympanic membrane perforation. If preliminary data does suggest PRP may be beneficial, our next step is to perform a larger randomized prospective study.

Data Management and Security

To ensure the privacy and confidentiality of data collected from patients, data will be stored in REDCap, a password protected database accessible only by the relevant study personnel. No identifying information such as medical record number, date of birth or social security number will be collected.

Monitoring and Reporting Unanticipated Problems

The attached questionnaire will be utilized to identify any problems with the study that may be causing patient discomfort of problems. The results of these questionnaires will be discussed at regular meetings with the study personnel to determine to continue or discontinue the study. Any adverse events noted during the follow-up will also be reviewed. Any problems identified will be promptly reported according to the requirements of the KU Human Subjects Committee standard operating procedures.

This study will only be stopped prior to consenting all patients if there is a serious adverse event deemed to be connected with the application of the PRP. Patient's will be followed closely, including a follow up phone call 3-5 days post application, at the pre-arranged clinic visits, or at any point if a patient has significant concerns or symptoms. Multiple large systematic reviews and Cochrane data base reviews have found no associated complications with PRP compared to controls. However there are case reports with possible adverse events, including inflammatory reaction, in patients with autoimmune issues such as diabetes type I. In both the phone interview and clinic follow up we will ask and examine for any signs of excessive inflammation or reaction; specifically looking for erythema, drainage, swelling, edema, pruritus, or other inflammatory symptoms. After five subjects have been enrolled, we will look at cumulative adverse events and the number of subjects needed to proceed. We have appointed Dr. James Lin, a Neurotologist, who will serve as an independent safety monitor to review any adverse events. If after five subjects or any other point the study is deemed unsafe or delaying standard of care, we will terminate the study.

Statistical Analysis Plan and Sample Size

Chi-square and Fischer exact tests will be used to test for whether or not patients were able to avoid surgery. Time to healing analysis will also be completed. Statistical significance will be based on an *a priori* designation of $p <= 0.05$. Demographic variables and clinical variables will be summarized using descriptive statistics.

A minimum of 10 patients will be enrolled in this pilot study. Patients will not be randomized as a part of this study. Due to the pilot nature of this study and the absence of any prior data to build sample size calculations, this exploratory group should provide data for future studies and justify future sample size calculations.

Bibliography

1. Aggarwal, R., S.R. Saeed, and K.J. Green, *Myringoplasty.* J Laryngol Otol, 2006. **120**(6): p. 429-32.
2. Arora, N.S., et al., *Platelet-rich plasma: a literature review.* Implant Dent, 2009. **18**(4): p. 303-10.
3. Kristensen, S., *Spontaneous healing of traumatic tympanic membrane perforations in man: a century of experience.* J Laryngol Otol, 1992. **106**(12): p. 1037-50.
4. Golz, A., et al., *Paper patching for chronic tympanic membrane perforations.* Otolaryngol Head Neck Surg, 2003. **128**(4): p. 565-70.
5. Crowson, M.G., et al., *Cost analysis and outcomes of a second-look tympanoplasty-mastoidectomy strategy for cholesteatoma.* Laryngoscope, 2016. **126**(11): p. 2574-2579.
6. Ma, Y., H. Zhao, and X. Zhou, *Topical treatment with growth factors for tympanic membrane perforations: progress towards clinical application.* Acta Otolaryngol, 2002. **122**(6): p. 586-99.
7. Eppley, B.L., W.S. Pietrzak, and M. Blanton, *Platelet-rich plasma: a review of biology and applications in plastic surgery.* Plast Reconstr Surg, 2006. **118**(6): p. 147e-159e.
8. Pietrzak, W.S. and B.L. Eppley, *Platelet rich plasma: biology and new technology.* J Craniofac Surg, 2005. **16**(6): p. 1043-54.
9. Erkilet, E., et al., *Platelet-rich plasma improves healing of tympanic membrane perforations: experimental study.* J Laryngol Otol, 2009. **123**(5): p. 482-7.
10. Foster, T.E., et al., *Platelet-rich plasma: from basic science to clinical applications.* Am J Sports Med, 2009. **37**(11): p. 2259-72.
11. Kaux, J.F., et al., *Exuberant inflammatory reaction as a side effect of platelet-rich plasma injection in treating one case of tendinopathy.* Clin J Sport Med, 2014. **24**(2): p. 150-2.
12. Moraes, V.Y., et al., *Platelet-rich therapies for musculoskeletal soft tissue injuries.* Cochrane Database Syst Rev, 2014(4): p. Cd010071.
13. Grassi, A., et al., *Is Platelet-Rich Plasma (PRP) Effective in the Treatment of Acute Muscle Injuries? A Systematic Review and Meta-Analysis.* Sports Med, 2018.
14. Esposito, M., et al., *Effectiveness of sinus lift procedures for dental implant rehabilitation: a Cochrane systematic review.* Eur J Oral Implantol, 2010. **3**(1): p. 7-26.
15. Karatayli Ozgursoy, S., et al., *Platelet-Rich Plasma Application for Acute Tympanic Membrane Perforations.* J Int Adv Otol, 2017.
16. El-Anwar, M.W., et al., *Topical use of autologous platelet rich plasma in myringoplasty.* Auris Nasus Larynx, 2015. **42**(5): p. 365-8.
17. Hong, P., M. Bance, and P.F. Gratzer, *Repair of tympanic membrane perforation using novel adjuvant therapies: a contemporary review of experimental and tissue engineering studies.* Int J Pediatr Otorhinolaryngol, 2013. **77**(1): p. 3-12.