# Polyphosphate (PolyP) for alveolar cleft repair, study protocol for a pilot randomized controlled trial.

**Salem Alkaabi** ( al_kaabi33@hotmail.com )
VUMC: Amsterdam UMC Locatie VUmc https://orcid.org/0000-0001-9450-6413

**Diandra S Natsir Kalla**
VUMC: Amsterdam UMC Locatie VUmc

**Abul Fauzi**
Hasanuddin University: Universitas Hasanuddin

**Andi Tajrin**
Hasanuddin University: Universitas Hasanuddin

**WEG Müller**
Johannes Gutenberg Universitat Institut fur Physiologie und Pathophysiologie

**HC Schröder**
Johannes Gutenberg University: Johannes Gutenberg Universitat Mainz

**XH Wang**
Johannes Gutenberg University: Johannes Gutenberg Universitat Mainz

**Tim Forouzanfar**
VUMC: Amsterdam UMC Locatie VUmc

**MN Helder**
VUMC: Amsterdam UMC Locatie VUmc

**M Ruslin**
Hasanuddin University: Universitas Hasanuddin

**Ghamdan Alsabri**
Amsterdam UMC - Locatie VUMC: Amsterdam UMC Locatie VUmc

**Study protocol**

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# Abstract

**Objective**: Bone grafting is an important surgical procedure to restore missing bone in patients with alveolar cleft lip/palate, aiming to stabilize either sides of maxillary segments by inducing new bone formation, and in bilateral cleft cases also to stabilize the pre- maxilla. Polyphosphate (PolyP), a physiological polymer composed of orthophosphate units linked together with high-energy phosphate bonds, is a naturally existing compound in platelets which, when complexed with calcium as Ca-polyP microparticles (Ca-polyP MPs), was proven to have osteoinductive properties in preclinical studies.

**Aim:** To evaluate the feasibility, safety and osteoinductivity of Ca-polyP MPs as a bone-inducing graft material in humans.

**Methods:** This prospective non-blinded first-in-man clinical pilot study shall consist of 8 alveolar cleft patients of 13 years or older to evaluate the feasibility and safety of Ca-PolyP MPs as a bone-inducing graft material. Patients will receive Ca-polyP graft material only, or Ca-polyP in combination with biphasic calcium phosphate (BCP) as a bone substitute carrier. During the trial, the participants will be investigated closely for safety parameters using radiographic imaging, regular blood tests, and physical examinations. After 6 months, a hollow drill will be used to prepare the implantation site to obtain a biopsy. The radiographic imaging will be used for clinical evaluation; the biopsy will be processed for histological/histomorphometric evaluation of bone formation.

**Discussion:** This is the first-in-man study evaluating the safety and feasibilty of the polyP as well as the potential regenerative capacity of polyP using an alveolar cleft model.

**Trial registration {31a}** The clinical trial protocol received a written approval by the ethical committee of Faculty of Medicine, Hasanuddin University, Makassar, Indonesia with code number 1063/UN4.6.4.5.31/PP36/2019. On completion of the trial, the results on safety, feasibility and bone formation with polyP as graft material will published.


# Administrative Information

**Trials guidance:**

| | |
|---|---|
| **Title {1}** | Polyphosphate (PolyP) for alveolar cleft repair, study protocol for a pilot randomized controlled trial. A total of eight patients, four patients (randomized) will receive Ca-PolyP MP as bone graft, and the other 4 patients will receive a combination of PolyP/BCP as graft material |
| **Trial registration {2a and 2b}.** | Trial registry: 1063/UN4.6.4.5.31/PP36/2019<br>Ethical committee of Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. |
| **Protocol version {3}** | Version 1.0, dated 28 May 2019 |
| **Funding {4}** | No funding was received |
| **Author details {5a}** | 1. Alkaabi SA & Natsir Kalla DS: Dept. of Oral and Maxillofacial Surgery/Oral Pathology, Amsterdam University Medical Centers and Academic Centre for Dentistry Amsterdam (ACTA), Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, Amsterdam, The Netherlands. Role: Main author and Conceptualization and Writing.<br>2. Alsabri GA: Dept. of Oral and Maxillofacial Surgery/Oral Pathology, Amsterdam University Medical Centers and Academic Centre for Dentistry Amsterdam (ACTA), Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, Amsterdam, The Netherlands. Role: Reviewer and editing.<br>3. Ruslin M , Fauzi A & Tajrin A: Dept. of Oral and Maxillofacial Surgery, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia. Role: Surgical procedures.<br>4. Ruslin M: Dept. of Oral and Maxillofacial Surgery, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia. Role: Correspondance.<br>5. Müller WEG, Schröder HC & Wang XG: Institut für Physiologische Chemie, Angewandte Molekularbiologie, Universitätsmedizin, Johannes Gutenberg-Universität Mainz, Mainz, Germany. Role: PolyP Inventor.<br>6. Forouzanfar T& Helder MN: Dept. of Oral and Maxillofacial Surgery/Oral Pathology, Amsterdam University Medical Centers and Academic Centre for Dentistry Amsterdam (ACTA), Vrije Universiteit Amsterdam, Amsterdam Movement Sciences, Amsterdam, The Netherlands. Role: Methodology and supervision. |
| **Name and contact information for the trial sponsor {5b}** | Muhammad Ruslin<br>Department of Oral and Maxillofacial Surgery<br>Faculty of Dentistry<br>Hasanuddin University<br>Kode Pos 90425<br>Makassar<br>Indonesia<br>Tel: +62-41-158-6012<br>Fax: +62-41-143-3015 |
| **Role of sponsor {5c}** | There was no sponsor. |

# Introduction

## Background {6a}

Alveolar cleft is a defect occurring as a result of failure of regular development during frontonasal prominence growth, which mostly affects the site between the lateral incisor and the canine (Von Eiselsberg F.,1901). In 1901, the alveolar bone cleft defect was first reconstructed by von Eiselsberg using an autogenous bone graft, while Lexer published in 1908 the first reconstruction with nonvascular graft material (Von Eiselsberg F., 1901; Lexer E., 1908). Autogenous bone most often derived from the cancellous iliac crest is still considered as a golden standard for the grafting procedure. Other sources such as the tibia, mandibular symphysis, rib, and the cranium are still being used by surgeon preference (Tomar K and Sahoo NK, 2018; Hughes CW and Revington PJ, 2002; Enemark H and Jensen, C, 2001; Witsenburg et al., 1990; Al-Sebaei MO et al., 2004). However, the drawback of autogenous graft is that it requires another surgical site, which may be associated with postoperative complications (Ilankoan V et al., 1998). Consequently, development of effective bone graft substitutes is currently being given high priority and attention (Lazarou SA et al., 2011; de Ruiter et al., 2014).

Müller and colleagues identified a new bone graft based on polyphosphate (polyp)(Müller et al., 2013; Müller et al., 2018). PolyP is a naturally existing compound in platelets (Ruiz FA et al., 2004); a physiological polymer composed of orthophosphate unites linked together with high-energy phosphate bonds similar to ATP (Wang XH et al., 2018). Complexed with calcium as Ca-polyP microparticles (Ca-polyP MPs), it was proven to have osteoinductive properties in preclinical studies (Müller WEG et al., 2011; Wang XH et al., 2012; Wang XH et al., 2018). PolyP is also used as a food additive (E 452) and in cosmetics (Smith J et al., 2003). As such, polyp is considered a safe material in current human applications (Tsutsumi K et al., 2014).

BCP is a mixture of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) with different ratios (Greenwald AS et al., 2001). BCP in some reports showed intrinsic osteoinductive properties causing ectopic bone formation (Yuan H et al., 2006; Habibovic P et al., 2004; Yuan H et al., 2001). While other reports such as de Lange et al showed that BCP has osteoconductive properties facilitating bone formation and remodeling in Maxillary sinus lift model (de Lange GL et al., 2014).

## Objective {7}

The protocol of this study as presented here is the first-in-human.

### Primary objective

To evaluate the safety trial using amorphous Ca-polyP MPs, employing the human alveolar cleft reconstruction model.

### Secondary objective:

To evaluate the feasibility and the potential regenerative capacity of polyP using an alveolar cleft model amorphous Ca-polyP MPs.

We hypothesize that the bony reconstruction with osteoinductive Ca-polyP MPs, either or not in combination with BCP granulate, will accelerate the quantity and quality of bone formation in a timely manner. Further, it will reduce the surgical time and morbidity by the absence of a donor site, thereby increasing the cost-effectiveness and quality of care.

## Methods And Design

### Ethics {9} {15}

The clinical trial was approved by the Ethics and Research Committee of Faculty of Medicine, Hasanuddin University, Makassar, Indonesia with code number 1063/UN4.6.4.5.31/PP36/2019. Participants will be recruited from general practices of Hasanuddin Dental Hospital and in the area around Makassar. The trial will be conducted in Hasanuddin Dental Hospital. All participants shall be asked to sign an informed consent. This study complies with the principles of the Declaration of Helsinki.

### Study design {8} {6b} {11c} {12}

This is a single-center prospective control clinical trial, assessing the safety and feasibility of Ca-polyP-MP (Calcium-polyphosphate microparticles, CAS No.: 13477-39-9, EC No.: 236-769-6) as bone graft material in an alveolar cleft model. The average MP particle size diameter is 280 ± 120 nm (Müller WEG et al., 2018). A total of 8 patients will be included in the trial. Four patients (randomized) will receive Ca-PolyP MP as bone graft, and the other 4 patients will receive a combination of PolyP/BCP as graft material. The primary endpoint will be set at 6 months. At each follow-up visit, AE and/or SAEs will be documented, and clinical assessments will be performed at time points specified in the Intervention section (below). All patients will be monitored closely using lab test (Complete blood count (https://doi.org/10.1053/jpan.2003.50013), others if needed), radiographs, and periodic physical examination (Table1). After these six months, a bone biopsy will be taken during dental implant preparation and processed for histological/histomorphometric analysis. Finally, a report on safety, feasibility, and potential efficacy with regard to bone formation will be made and published.

### Eligibility criteria {10}

### Inclusion and exclusion criteria {26a}

After written informed consent will be obtained by research team member, the participant will be screened further for eligibility. Patients should be ≥ 15 years healthy old male or female patients with an alveolar cleft bone defect, non-smoker, with no history of previous grafting procedure(s), a normal blood count, and with an ASA1 regarding anesthetic risks.

Patients will be excluded when they have poor oral hygiene with mouth plaque, systemic or local infection, have systematic disease, or received radiotherapy, chemotherapy, immunosuppressive or anticoagulant therapy recently. Other exclusion criteria comprise having received bone morphogenetic protein (BMP) growth factors or other bone growth promoting factor therapy, obvious malnutrition, and active influenza.

### Point {26b}:

N/A no additional consent is required at this level of the trial.

### Intervention {11a}

Under general anesthesia, and after local infiltration with adrenaline 1:100,000, an incision will be made at the cleft margin to create a pocket-like tissue towards the nose and the mouth in order to reconstruct the nasal floor as well as the palatal tissue. The goal of this approach is to get rid of the oro-nasal fistula and to expose the bony edges on both sides of the cleft. Under sterile conditions, either Ca-polyP MP alone (NanotecMARIN GmbH, Mainz, Germany) or a combination of BCP (Straumann Bone Ceramic, Villeret, Switzerland) and PolyP will be mixed with normal saline or blood in a ratio 1g:3-5 ml and 1g:1g:5-7 ml respectively. A homogenous mixture should be reached before placing the graft material into the cleft defect. A good adaptation of bone graft material should be considered while placing it in cleft defect. No membrane will be used unless absolutely necessary. A different graft quantity will be considered for larger defects, however, with the same mixing ratios. Absorbable sutures with 3/0 vicryl for mucosa, and 4/0 vicryl for nasal reconstruction will be used for closure.

Post-operative, suitable antibiotic and painkillers will be prescribed to all patients.

### Adverse Event (AE) and Serious Adverse Event (SAE) {11b} {11d} {30} {22}

Any adverse event will be graded with respect to intensity and classified as either serious or non-serious according to the World Health Organization Classification. Any change in health which occurs between screening examination and first administration of amorphous Ca-polyP microparticles or related procedures will be recorded as part of the subject’s medical history, and full medical care will be given to all participants. In the case of a SAE, the sponsor will be reported within 24 hours from the onset. If the SAE concerns severe toxicity or infection associated with the graft site, the trial will be terminated immediately.

### Sample size {14} {18b}

Since this is a first-in-man trial, the current trial sample size has been limited to only 2x4 patients, with the primary goal to gain a first insight on the feasibility and safety of the treatment with polyp. The number of patients has been kept low in order to minimize the risk of the graft exposure in case of any adverse effect.

### Randomization and treatment allocation {16a} {16b} {16c} {17b}

Because of this is a first in human study, it is not possible to keep all personnel and the participants to be blinded to assignment group. After written informed consent, randomization will be performed with regard to the treatment group, but the patients will be aware of the fact that they will be treated with polyp.

### Blinding {17a} {23}

The radiologist and the histopathologist will be kept blinded to the treatment when evaluating the data (Figure 1).

### Data collection {18a} {19} {27} {5d}

The rules and responsibilities will be provided to the research team. The doctors and nurses of the research team will collect the data according to the evaluation table 1. All research team members will receive training on how to collect data at all study visits. Each patient will be followed up for up to 6 months. The confidentiality of the participants will be well protected by the data manager.

### Outcomes

### Safety assessment based on physical examination and laboratory measurements

When a SAE occurs, it will be concluded that polyP is not (yet) safe in the current setting. For AEs, if they do not occur at a higher frequency than in patients treated with standard care (autologous bone) and/or can be resolved by non-invasive conventional methods (eg, analgesics, antibiotics), the polyP product will be considered safe. In all other cases, polyP will not be considered safe (yet).

### Radiographic evaluation

The Chelsea scale will be used to evaluate the bone graft and the level of the bone in comparison with the adjacent teeth. This scale starts with drawing an imaginary midline between the two teeth on either side of the cleft site. Each of those teeth (mesial and distal roots) will be divided starting from cemento-enamel junction to the root apex in four parts. A 0 score is given when no bone is present up till the midline, a 0.5 score is given when there is bone, but it fails to reach the midline, and a 1 score is given when the bone extends from the root surface to the midline (Witherow H et al., 2002).

### Histological and histomorphometric analysis {33}

The histological and histomorphometric analysis will be performed in at least 3 patients from each group. In those patients, the dental implant site will be prepared using a trephine burr (⌀ 2.0 mm × 10.0 mm in length) instead of a normal drill, thereby being able to collect a biopsy from the treated site without interfering with the normal procedure. The biopsies will be fixed in 10 % formalin and processed for embedding in methylmethacrylate for evaluation of hard tissue formation. After sectioning, different

stainings (Goldner's trichrome, Toluidin blue, Tartrate-resistand acid phosphatase (TRAP)) will be used, and histomorphometric parameters for bone formation will be analyzed. Two trained examiners, blinded for the treatment modality, will evaluate the images, and intra- and inter-observer reliabilities will be determined. In case of disagreement between observers, the specimen will be re-evaluated to reach consensus.

### Monitoring {21a} {21b}

Monitoring will be done constantly by internal monitors of the Ethics and Research Committee of Faculty of Medicine, Hasanuddin University, since there is a negligible risk a data safety monitoring board will not be formed. A safety report will be provided to the Medical Research Ethics Committee of the Ethics and Research Committee of Faculty of Medicine, Hasanuddin University every year. An interim analysis will not be conducted.

### Statistical analysis {20a}

A SPSS power analysis for parameter comparisons between the groups. A *p*-value less than 0.05 will considered statistically significant.

### {20b, 20c, 31c, 25}: N/D

Those points are not applicable in safety and feasibility in this small sample study.

## Discussion

This is the first-in-man study evaluating the potential regenerative capacity of polyP using an alveolar cleft model. PolyP represents a completely novel type of regenerative compound, since it can be considered as a rich energy source for tissue repair, which may be as pivotal for the bone regeneration process as the osteogenic factors, which are generally believed to be the primary active compounds (Wang XH et al., 2018). The high-energy phosphate bonds of polyP are identical to those present in the "common" cellular energy molecule ATP, and both serve as substrates for the enzyme alkaline phosphatase (ALP), a well-known marker for active bone formation (Müller W E G et al., 2018). PolyP has also been reported to promote mineralization (Lorenz B and Schröder HC, 2001) and to increase progenitor cell differentiation into osteoblasts ( Hacchou Y et al., 2007; Müller WEG et al., 2011). PolyP is present in platelets, which play an essential role in early wound repair. Interestingly, platelet rich plasma (PRP), a concentrate of platelet-rich plasma protein derived from whole blood and often used in bone repair strategies, therefore will also contain polyP. However, the efficacy of PRP to promote bone repair is nowadays questioned, since both positive and neutral/negative effects have been published recently (Kassolis JD et al., 2000; Roldán JC et al., 2004). We speculate that the much higher dose of polyP present in our preparations will be well above the bone regeneration threshold, and thus may have a positive effect on the bone repair process.

Calcium phosphate ceramics including biphasic calcium phosphates (BCPs) have been widely used as bone substitutes and tissue engineering scaffolds. Calcium phosphates are highly biocompatible, proven to be safe, and successfully used in many different clinical treatment modalities such as bone augmentation in spinal arthrodesis, maxillo- and craniofacial surgeries, orthopedics, periodontal treatment, and metallic implant coatings (Uzeda MJ et al., 2017; Oh JS et al,. 2019; Helder MN et al., 2018; Bouwman WF et al., 2017; Bouler JM et al., 2017; Kämmerer TA et al., 2017). Some reports describe that BCP may also have osteoinductive properties (Stähli C et al., 2010), which implies that BCP may add to the osteoinductivity as well. Moreover, a recent clinical study applying microstructured β-TCP for alveolar cleft repair demonstrated that calcium phosphate could be used safely and effectively for this purpose as well (Janssen NG. We are therefore convinced that the Straumann Bone Ceramic used in the current study will be a safe to use scaffold and may have a supportive or even synergistic effect on the bone formation when combined with the bioactive polyP.

For the clinical evaluation of bone formation, radiographic imaging will be applied. We are well aware that this will likely be relatively reliable in the case of the group that is treated with only with the (radiolucent) polyp microparticles but will not be easy with the BCP/polyP treatment group. The BCP scaffold will be radiopaque and cause signal scattering, which will preclude accurate visualization of new bone formation within the scaffold material. We will circumvent this limitation by our histological and histomorphometrical analysis of the biopsies taken at the 6 months follow-up time point, during dental implant placement. This will enable us to still evaluate the bone formation at the microscopic level, and to quantify multiple bone formation-related parameters and cellular activities as demonstrated before in other bone regeneration studies performed by our group (Helder MN et al., 2018; Farré-Guasch E et al., 2018; Bouwman WF et al., 2017; Prins HJ et al., 2016).

# Conclusion

With this protocol we summarized how we intend to evaluate the safety and feasibility of Ca polyP MP as a new grafting material in alveolar cleft model.

# Trial Status

Recruitment started in November 2019 and is planned to end in September 2020, with 8 patients randomized. The current protocol version is 1.0, dated 28 May 2019.

# Declarations

### Conflict of interest:

This article is free of conflict of interest and no funding was received.

# References

De Lange GL, Overman JR, Farré-Guasch E, Korstjens CM, Hartman B, et al. (2014) A histomorphometric and micro–computed tomography study of bone regeneration in the maxillary sinus comparing biphasic calcium phosphate and deproteinized cancellous bovine bone in a human split-mouth model. Oral Surg Oral Med Oral Pathol Oral Radiol 117: 8-22.

Enemark H, Jensen J, Bosch C (2001) Mandibular bone graft material for reconstruction of alveolar cleft defects: Long-term results. The Cleft Palate - Craniofac J 38:155-163.

Greenwald AS, Boden SD, Goldberg VM, Khan Y, Laurencin CT, et al. (2001) American Academy of Orthopaedic Surgeons; Bone graft substitutes: facts, fictions, and applications. J Bone Joint Surg Am 83:98-103.

Hacchou Y, Uematsu T, Ueda O, Usui Y, Uematsu S, et al. (2007) Inorganic polyphosphate: a possible stimulant of bone formation, J. Dent. Res 86; 893-897.

Hughes CW, Revington PJ (2002) The proximal tibia donor site in cleft alveolar bone grafting: Experience of 75 consecutive cases. J Craniomaxillofac Surg 30:12-17.

Ilankoan V, Stronczek M, Telfer M, Peterson LJ, Stassen LF, et al. (1998) A prospective study of trephined bone grafts of the tibial shaft and iliac crest. Br J Oral Maxillofac Surg 36: 434-9.

Kassolis JD, Rosen PS, Reynolds MA (2000) Alveolar ridge and sinus augmentation utilizing platelet-rich plasma in combination with freeze-dried bone allograft: case series. J Periodontol 71: 1654-1661.

Lexer E (1908) Die Verwendung der freien knochenplastik nebst versucher uber gelenkversteinfung und gelenktransplantation. Arch Klin Chir 86: 939-943.

Lorenz B, Schröder HC (2001) Mammalian intestinal alkaline phosphatase acts as highly active exopolyphosphatase. Biochim. Biophys. Acta 1547: 254-261.

Müller WEG, Achermann M, Wang S, Neufurth M, Muñoz-Espi R, et al. (20113) Inorganic polyphosphate induces accelerated tube formation of HUVEC endothelial cells. Cell. Mol. Life Sci 75: 21-32.

Müller WEG, Neufurth M, Wang S, Ackermann M, Muñoz-Espí R, et al. (2018) Amorphous, Smart, and Bioinspired Polyphosphate Nano/Microparticles: A Biomaterial for Regeneration and Repair of Osteo-Articular Impairments In-Situ. Int J Mol Sci 31;19(2). pii: E427.

Müller WEG, Wang XH, Diehl-Seifert B, Kropf K, Schloßmacher U, et al. (2011) Inorganic polymeric phosphate/polyphosphate as an inducer of alkaline phosphatase and a modulator of intracellular Ca2+ level in osteoblasts (SaOS- 2 cells) in vitro. Acta Biomater 7: 2661-2671.

Roldán JC, Jepsen S, Miller J, Freitag S, Rueger DC, et al. (2004) Bone formation in the presence of platelet-rich plasma vs. bone morphogenetic protein-7. Bone 34: 80-90.

Ruiz FA, Lea CR, Oldfield E, Docampo R (2004) Human platelet dense granules contain polyphosphate and are similar to acidocalcisomes of bacteria and unicellular eukaryotes. J Biol Chem 279: 44250-7.

Smith J, Hong-Shum L (2003) (Eds.), Sodium polyphosphate, in: Food Additives Data Book, Blackwell Science Ltd., Oxford, UK.

Stähli C, Bohner M, Bashoor-Zadeh M, Doebelin N, Baroud G (2010) Aqueous impregnation of porous beta-tricalcium phosphate scaffolds. Acta Biomater 6(7): 2760-72.

Tomar K, Sahoo NK (2018) Evaluation of graft uptake from the iliac crest in secondary alveolar bone grafting: Bergland's criteria revisited. J Oral Biol Craniofac Res 8(3): 171-176.

Von Eiselsberg F (1901) Zür technik der uranoplastik. Arch Klin Chir 64: 509-529.

Wang XH , Schröder HC , Müller WEG (2018) Amorphous polyphosphate, a smart bioinspired nano-/biomaterial for bone and cartilage regeneration: towards a new paradigm in tissue engineering. J. Mat Chem B 6: 2385-2412.

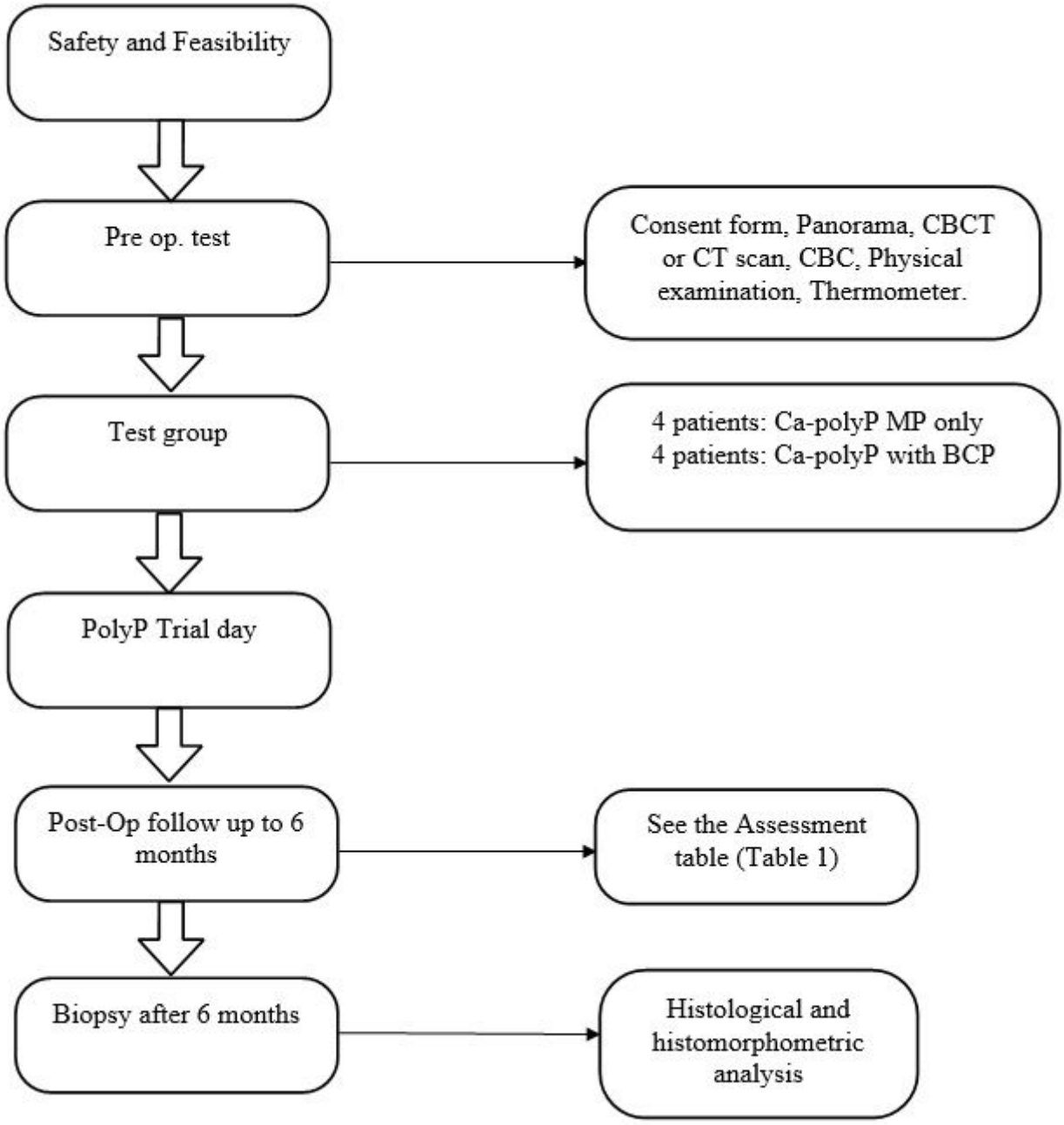
Witsenburg B, Peter H, Freihofer M (1990) Autogenous rib graft for reconstruction of alveolar bone defects in cleft patients: Long-term follow-up results. J Craniomaxillofac Surg 18: 55-62.

# Table

**Table 1:** Assessment table **{13}**

| | Consent form | Panorama | CBCT or CT | Physical examination | CBC | Thermometer | Biopsy |
|---|---|---|---|---|---|---|---|
| Pre operatively | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Operative day | | | | ✓ | | ✓ | |
| Post-op day1 | | ✓ | | ✓ | ✓ | ✓ | |
| Post-op day 8 | | ✓ | ✓ | ✓ | ✓ | ✓ | |
| Post-op day14 | | | | ✓ | | ✓ | |
| Post-op day 30 | | | | ✓ | ✓ | ✓ | |
| Post-op day 90 | | ✓ | | ✓ | | ✓ | |
| Post-op day 180 | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

## Figures




## Figure 1

Protocol flowchart