A case of extreme skeletal class III Malocclusion beyond the envelope of discrepancy, managed effectively by a modified ortho-surgical protocol

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Abstract
Correction of severe anteroposterior skeletal discrepancy, as described in this case of Extreme Skeletal Class III Malocclusion, can be challenging and fraught with difficulties. Conventional, single stage Bi-jaw Orthognathic surgery, with pre-and post-surgical orthodontics is associated with drawbacks such as risk of relapse and an unsatisfactory outcome, with persisting occlusal discrepancies and skeletal abnormalities, especially when the magnitude of skeletal correction is large. Excessive mandibular setback restricts tongue space, narrows the posterior airway and pharyngeal spaces, and is prone to relapse from the forward pterygomaseteric pull; while large maxillary advancements are accompanied by wound dehiscence, bone exposure and delayed union at the site of pterygomaxillary disjunction, and risk of relapse due to backward palatopharyngeal pull. Bi-jaw surgeries invariably involve considerable blood loss and prolonged operating time with its attendant anaesthetic risks.

These drawbacks may be obviated by employing a two staged protocol of Bi-jaw surgeries allowing a minimum time period of 3 months to elapse between them, which allows the oral and maxillofacial musculature to adapt itself to the new jaw position following the first surgery, thus creating a better and more stable environment for the succeeding one. This reduces the chance

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of relapse thereafter, and produces more effective and stable long term results. The intervening time period also allows for observation of the repositioned jaw and arch relations achieved, and scrutiny for any positional changes in this post-surgical phase, which thereby allows modifications in the planned surgery of the next jaw, thereby achieving the most ideal final outcome.

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Introduction

In the present day scenario, severe maxillofacial deformity and asymmetry accompanied by compromised occlusion is one of the chief conditions necessitating correction by extensive orthodontic and maxillofacial surgical intervention. Although impaired esthetics is often the patient’s chief concern, it is almost invariably accompanied by functional debilitation such as difficulty in mastication, speech impairment, obstructive sleep apnoea; temporomandibular joint disorders, and psychosocial handicaps.1

Adult Skeletal Class III Malocclusion is one of the most severe and difficult to correct Maxillofacial deformities,2 involving multiple, complex, inter-related aspects such as cranial base abnormalities; maxillary and mandibular skeletal and dental components, which necessitate precise Orthognathic surgical repositioning of the jaws in conjunction with extensive pre- and post-surgical Orthodontic treatment, in order to achieve a functional occlusion and long-term stability in the maxillofacial skeleton.

Prevalence of Class III malocclusion in Caucasians ranges from 0.8 to 4% and up to 12% in Chinese and Japanese populations.3,4,5 More than 60% of them are due to skeletal discrepancies6,7 including mandibular prognathism, maxillary retrognathism and combinations of the two.8,9 These complex cases require careful and meticulous treatment planning, including predictive Cephalometric tracings, mock model surgeries, an integrated Orthodontic-Surgical approach and steady, uninterrupted patient compliance, motivation and cooperation.10

Depending on the extent of skeletal discrepancy, the management of skeletal ClIII malocclusion in adults,11 involves bi-jaw or single jaw surgery. The two most commonly employed surgical procedures to correct this craniomaxillofacial deformity are Bilateral Sagittal Split Ramus Osteotomy (BSSRO) with setback for the prognathic mandible, and Le Forte 1 Osteotomy with advancement for the retrognathic / retrusive Maxilla, which may or may not need to be accompanied by reduction Genioplasty for a prominent chin.

A major limitation of Orthognathic surgery for Class III malocclusions, is the possibility of postsurgical relapse.12 Common factors accounting for relapse are the amount of mandibular setback,13 reduced tongue space and stretching of the pterygomasseteric sling14,15 Excessive maxillary advancement may be accompanied by complications such as delayed union or non-union at the osteotomy sites, wound dehiscence at the pterygomaxillary disjunction sites and relapse due to posterior muscle pull.16,17

A case of a 19-year-old patient, with extreme Skeletal ClIII malocclusion beyond the envelope of orthodontic correction (Figure 1), with reverse overjet of 14 mm, ANB of −16 and Wits of 15 mm (BO ahead of AO) is described, which was effectively and successfully managed by a modified protocol of Two-Staged Orthognathic Bi-Jaw surgeries in conjunction with Pre- and Post-Surgical Orthodontic treatment.
Figure 1. Comparison of Pre-treatment, Post-Presurgical Orthodontics, Post-Maxillary advancement and Post- Mandibular setback phases of management. (A-D) Frontal face photographs upon presentation; following presurgical orthodontics, following the first surgical phase of maxillary advancement and following the second surgical phase of mandibular setback. Achievement of an excellent esthetic outcome, facial balance and symmetry. (E-H) Right Profile view prior to treatment, following presurgical orthodontics, following maxillary advancement and following mandibular setback, showing correction of the markedly concave profile to a normal appearing and aesthetically pleasing mildly convex one. (I-L) Left Profile view upon presentation, after presurgical orthodontics, after Maxillary advancement and after Mandibular setback phases, demonstrating the severe facial deformity owing to an excessively concave profile and increased lower anterior facial height, and its successful correction to an aesthetically appealing and balanced profile with ideal facial proportions. (M-P) Extreme reverse overjet of 16 mm, reduced after first phase of Maxillary advancement by 9 mm, corrected to a normal positive overjet of 2 mm following second surgical phase of Mandibular setback of 9 mm, and demonstrating stable results with nil relapse after two year’s follow up. (Q-T) Lateral Cephalograms prior to treatment, following the Maxillary advancement phase, Mandibular advancement phase, and two years following treatment demonstrating successful correction of the facial deformity and stability of results achieved with nil relapse after 2 years of follow up. (U-X) Orthopantomograms before and after each phase of treatment, demonstrating the fixation implants in situ.
The maximum quantum of Maxillary advancement possible and permissible, accounting for the expected relapse, is 7 mm and that for mandibular setback is 7 mm. The Case described, necessitated crossing these established limits of jaw movements (9 mm of Mandibular setback and 9 mm of Maxillary advancement), and yet provided stable and extremely effective results and a gratifying esthetic as well as functional outcome. Pushing the envelope of discrepancy was made possible by means of employing a protocol of staged Bi-jaw surgeries, with an intervening period of three months between the two surgeries. This enabled achieving jaw movements exceeding the hitherto advocated magnitude of maxillary advancement and mandibular setback, thus reaping rich dividends in this case of severe maxillofacial deformity.

Case report

Pre-treatment clinical assessment

A 19-year-old male patient reported with the chief complaint of an unsatisfactory facial appearance due to an excessively large size of the lower jaw. He also expressed a difficulty and lack of clarity in speech, with difficulty in articulation of words. Family history revealed that the patient’s paternal uncle as well as grandfather suffered from a similar maxillofacial deformity.

Upon extraoral examination, in the frontal view (Figure 1A), the patient exhibited a Leptoprosopic facial pattern, reduced malar prominence, paranasal, with potentially incompetent lips and a non-consonant smile arch. Vertical facial proportions revealed an increased lower anterior facial height. The patient presented with a severe Skeletal Cl III base, a grossly elongated mandible and an anteriorly divergent face. He exhibited a markedly concave facial profile (Figure 1E, I), obtuse mentolabial sulcus, an everted lower lip and an acute nasolabial sulcus.

Intraoral examination revealed an Angle’s Class III Malocclusion bilaterally (Figure 1M), a negative incisor overjet of 12 mm, with coinciding maxillary and mandibular dental midlines. Cl III molar and
canine relationships were observed bilaterally. The lower anteriors were retroclined, while the upper anteriors were proclined, demonstrating dental compensation for the severe Skeletal Class III base. The maxillary third molars were impacted.

**Pre-treatment skeletal assessment**

On lateral Cephalograms (Figure 1Q), the patient exhibited a severely proclined mandible, an excessively concave facial profile with extreme anterior divergence and Skeletal Cl III maxillofacial pattern with a prominent chin. Wits appraisal of −13 mm and ANB of −10.5 demonstrated a severe Skeletal Cl III jaw relationship due to combination of maxillary as well as mandibular skeletal malformation. As evidenced on the Orthopantomogram (OPG), the patient was in permanent dentition stage with no missing or supernumerary teeth (Figure 1U).

Lateral Cephalometric Analysis revealed a retrognathic maxilla and a grossly enlarged and prognathic mandible with ANB of −16 (SNA−79, SNB−95). The patient had a horizontal growth pattern with Frankfurt Mandibular Angle (FMA) of 36 (Table 1). The upper lip was retrusive in relation to Esthetic line by −15 mm and lower lip by −4 mm. Assessment of airway on lateral Cephalogram revealed no abnormality and no airway compromise and no predisposition to obstructive sleep apnoea. The Cephalometric findings included short anterior cranial base length, acute cranial base angle, a retrusive maxilla, proclined maxillary incisors, retroclined mandibular incisors, an excessive lower anterior face height and obtuse gonial angle.

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**Table 1**
Comparison of Pre-Treatment, Pre-Surgical (following Presurgical Orthodontics), Post Maxillary Advancement and Post Mandibular Setback Cephalometric Analyses.

| Parameters                        | Normal values | Pre-treatment values | Pre-surgical values | Post maxillary advancement values | Post mandibular setback values |
|-----------------------------------|---------------|----------------------|---------------------|-----------------------------------|-------------------------------|
| Steiner’s analysis                |               |                      |                     |                                   |                               |
| SNA                               | 82°           | 79°                  | 77°                 | 86°                              | 86°                           |
| SNB                               | 80°           | 95°                  | 95°                 | 95°                              | 85°                           |
| ANB                               | 2°            | −16°                 | −18°                | −9°                              | 1°                            |
| Occlusal plane angle              | 14°           | 17°                  | 16°                 | 15°                              | 14°                           |
| Mandibular plane angle            | 26° +/- 4°    | 36°                  | 36°                 | 35°                              | 31°                           |
| Upper incisors to NA (Degree)     | 22°           | 26°                  | 23°                 | 23°                              | 23°                           |
| Upper incisors to NA (Linear)     | 4mm           | 6mm                  | 5mm                 | 5mm                              | 5mm                           |
| Lower incisors to NB (Degree)     | 25°           | 17°                  | 22°                 | 22°                              | 22°                           |
| Lower incisors to NB (Linear)     | 4mm           | 2mm                  | 4mm                 | 4mm                              | 4mm                           |
| Inter-incisal angle               | 131°          | 152°                 | 138°                | 133°                             | 131°                          |
| Wits appraisal                    |               |                      |                     |                                   |                               |
| AO - BO                           | BO ahead of AO by 1mm | BO ahead of AO by 15mm | BO ahead of AO by 16mm | BO ahead of AO by 9mm | BO coinciding with AO |
| Tweed’s analysis                  |               |                      |                     |                                   |                               |
| FMA                               | 26° +/- 4°    | 36°                  | 36°                 | 35°                              | 31°                           |
| IMPA                              | 90°           | 79°                  | 87°                 | 87°                              | 87°                           |
| Maxillary & mandibular length analysis |       |                      |                     |                                   |                               |
| Mand Length (Co to Gn)            | 131+/- 4.6mm  | 140mm                | 140mm               | 140mm                            | 131mm                         |
| Max Length (Co to Point A)        | 100.9+/- 3.9mm| 86mm                 | 86mm                | 95mm                             | 95mm                          |
| Maxillo-Mandibular differential   | 30.1+/- 3.9mm | 54mm                 | 54mm                | 45mm                             | 36mm                          |
Diagnosis & treatment objectives

Problem list
1. Extreme Skeletal Class III Malocclusion.
2. Prognathic mandible.
3. Retrognathic maxilla.
4. Increased lower facial height.
5. Class III incisor and molar relationship.
6. Reverse Overjet by 14 mm.
7. Concave profile.
8. Compromised smile esthetics.
9. Compromised functional (masticatory) efficiency.
10. Compromised speech.

Treatment goals
1. To address the skeletal discrepancy, correct the severe facial deformity and improve facial esthetics by means of Ortho-surgical correction.
2. To correct the skeletal maxillo-mandibular relationship by means of staged Bi-jaw surgeries (Maxillary advancement by 9 mm and Mandibular setback by 9 mm).
3. To address increased lower facial height and achieve an ideal facial balance.
4. To achieve Class I molar and canine relation bilaterally and normal incisors axial inclination by means of dento-alveolar decompensation
5. To achieve an ideal functional occlusion by Pre-surgical decompensation and Post-surgical orthodontic settling.
6. To achieve normal overjet and overbite.
7. To improve his smile esthetics and achieve a pleasing esthetic profile.
8. To Improve functional efficiency in mastication and speech.

Treatment plan

Combined Orthodontic and Surgical line of treatment, involving four phases:

a) Presurgical Orthodontics phase: Pre-surgical orthodontic decompensation of the occlusal relationships, elimination of surgical occlusal interferences by intruding the over erupted teeth, attainment of an ideal dental arch alignment, and establishment of an ideal anteroposterior and vertical positions of the incisors. This procedure assists in producing a predictable and precise final outcome, so that patient’s function and facial harmony improve instantly after surgery.

b) Surgical Phase: Two separate surgical procedures, the first, surgery of the Maxilla, followed three months later of the Mandible.
   i) 1st Surgical phase: Le Fort I osteotomy for forward positioning of the maxilla by 9 mm.
   ii) 2nd Surgical Phase: Bilateral sagittal split osteotomy (BSSO) for mandibular setback by 9 mm.

c) Postsurgical Orthodontics phase: Settling and finishing of occlusal relationships and final retention plan, so as to achieve optimal functional efficiency, esthetic harmony and structural balance.

Treatment

Phase I: pre-surgical orthodontics

Presurgical orthodontics was begun with bonding of 0.022”×0.028” MBT pre adjusted edgewise prescription appliance. To achieve sufficient decompensation and ideal maxillary and mandibular incisor inclinations, existing spaces within the arches were utilized. Initial levelling and alignment was carried out using 0.016” NiTi, 0.016” ×0.022” NiTi and 0.019”×0.025” NiTi arch wire; followed by 0.019”×0.025” Stainless Steel for closure of residual spaces and correction of inclination of U/L incisors; and 0.021” × 0.025” Stainless Steel as final stabilizing wires.
At the end of the presurgical orthodontics phase, dental decompensations were eliminated and the patient developed a resultant reverse overjet of 14 mm (due to correction of axial inclination of U/L incisors) with super Class III molar and canine relationships. The upper third molars were extracted three months prior to the Surgical Phase I.

Presurgical phase records were repeated and compared (Figure 1B, F, J, N, R, V). Impressions were also taken and models were hand articulated for examining occlusal compatibility. Cephalometric prediction tracing was done.

Computed Tomographic scans were carried out together with 3-D reformattting of the images. After presurgical orthodontics, face bow transfer was done and maxillary relation to cranial base was recorded and transferred to a semi adjustable articulator. A recorded occlusal wax bite was utilized for the mandibular cast articulation. Mock surgery was performed on the articulated models and the individual dental casts were repositioned, simulating the movements of the jaws. An intermediate acrylic occlusal splint was fabricated after the maxillary cast was advanced by 9 mm on the articulator to oppose the mandibular cast, simulating the Maxillary advancement surgery.

Phase II: surgical phase
1st Surgical phase: maxillary advancement. Le- Forte I osteotomy was carried out, and the maxilla was repositioned 9 mm anteriorly (Figure 2A-D). The pre-fabricated intermediate Interocclusal wafer splint was placed which guided the positioning of the maxilla relative to the mandible. Semi-rigid internal fixation was carried out using 2 microplates at the bilateral pyriform apertures and 2 at the Zygomaticomaxillary crests, together with monocortical screws. Postoperative recovery was smooth and uneventful, and the patient was maintained on a soft diet for the first 2 weeks after surgery. No Maxillomandibular fixation was continued in the postoperative period, although the Interocclusal wafer splint was left secured to the maxillary arch for a month to provide stability during the healing and callus formation stage. CI III elastics were placed immediately post-operatively and maintained for the initial 4 weeks’ post-surgery, and thereafter discontinued when the Interocclusal splint was removed. Orthodontic dental levelling and alignment of the arches was continued after removal of the acrylic wafer splint. Radiographic and NCCT (Non-Contrast Computed Tomographic) records were taken.

At 3 months’ post maxillary advancement surgery, extra-oral records including photographs, Cephalogram and Radiographs were repeated and there was found an excellent stability of results with nil relapse (Figure 1C, G, K, O, S, W). Face bow transfer, articulation of models and mock surgery was carried out with fabrication of the final Interocclusal wafer splint, this time, for the Second surgical phase, namely the mandibular setback of 9 mm.

2nd Surgical phase: mandibular set back (BSSO). Bilateral sagittal split osteotomy was performed to set the mandible back by 9 mm (Figure 3A-D). The pre-fabricated final Interocclusal wafer splint was placed which guided the final positioning of the mobile distal segment in such a way that there was
a perfect fit of the splint with both the upper and lower dental arches occluding within it. Semi-rigid fixation was carried out bilaterally using a six-hole miniplate and monocortical screws at each of the mandibular osteotomy sites. The Interocclusal wafer splint was left secured to the maxillary arch for a month to provide stability during the healing and callus formation stage.

**Phase III: post-surgical orthodontics**

CI III elastics were placed immediately post-operatively and maintained for the initial 4 weeks' post-surgery, and thereafter discontinued when the Interocclusal splint was removed. Postsurgical intra- and extra-oral records were taken (Figure 1D, H, L, P). Following removal of the occlusal wafer splint, heavier 0.021" × 0.025" SS wires were replaced with smaller dimension 0.017" × 0.025" TMA in the upper arch and 0.016" SS round wire in the lower arch. Bilateral posterior box elastics with anterior vertical elastics were given. The patient was instructed to wear elastics full time for the next few weeks which were then weaned off, as further detailing of occlusion was taking place. After five months of active postsurgical orthodontic treatment, fixed appliances were debonded and post treatment retention phase was initiated with fixed lingual retainers in both U/L arches. Post treatment lateral Cephalogram and OPG were taken and evaluated for treatment changes by superimposition (Figure 1T, X).

**Results achieved**

Skeletal correction of the severe facial deformity was successfully accomplished, a straight facial profile, symmetry and balance was achieved (Figure 1D, H, L), and an ideal occlusion was established with proper overjet and overbite (Figure 1P). This produced an excellent and gratifying esthetics as well as functional outcome.

The cephalometric changes included an increased maxillary basal length, decreased mandibular body length, reduced Maxillomandibular differential resulting in an overall increase in the ANB angle, thereby achieving Skeletal Class I relationship (Figure 1T). There was good improvement in smile characteristics and achievement of positive overjet and overbite with CI I molar and canine relationship. Evaluation of the post treatment OPG showed the results achieved to be ideal, with adequate root parallelism and normal alveolar bone relationships (Figure 1X). NCCT of the Craniomaxillofacial complex post treatment demonstrated the extent of correction of the Maxillomandibular morphology that was achieved and the stable Skeletal Class I relation that could be attained using this management protocol of two-staged, surgical single-jaw procedures in conjunction with conventional pre- and post-surgical orthodontics.

At 1 year after debonding, the results were stable, and the patient was satisfied with his facial esthetics. His speech markedly improved in both clarity and enunciation, and masticatory efficiency increased significantly, thus achieving the goals of a successful esthetic as well as functional outcome.

**Figure 3.** Intraoperative photographs of Surgical Phase 2: Bilateral Sagittal Split Ramus Osteotomy and Mandibular Setback by 9 mm. (A) Retromolar area exposed using an extended Ward's incision. Osteotomy cuts marked and mandibular split completed on each side. (B) 9 mm wide cortico-cancellous segment of bone removed from the anterior end of the proximal mandibular segments bilaterally. (C) Mobile Distal segment of mandible set back by 9 mm, guided by the Interocclusal acrylic wafer splint which was secured to the upper arch. Semi-rigid fixation carried out bilaterally, using Titanium minibone plates and screws. (D) 9 mm wide cortico-cancellous bone segments which were removed bilaterally from the anterior ends of the proximal mandibular segment, to achieve mandibular setback of 9 mm.
Discussion

Cephalometric and geometric morphometric studies have shown that 63–73% of Skeletal Class III malocclusions are caused by developmental shortening and diminution of the palatomaxillary complex anteroposteriorly that allies with the vertical shortening of midfacial height anteriorly\textsuperscript{18,19,20} and elongation of the mandible anteroposteriorly, which leads to the typical appearance of a retrognathic midface and prognathic mandibular profile.\textsuperscript{21,22}

This case report describes the treatment of a adult male patient with extreme Skeletal and Dental Class III relationship. A modified Surgical-Orthodontic treatment was employed, pushing the envelope of discrepancy, by surgical advancement of the retrusive maxilla by 9 mm and setback of the mandible by 9 mm in two separate, single jaw surgical procedures with an intervening period of three months. This helped in achieving gratifying esthetic results, ideal skeletal and dental relations and a stable, functional Class 1 occlusion. Pre-surgical orthodontics helped eliminate all dental compensations and identified the location and extent of the skeletal discrepancies in both jaws. Normal skeletal base relationship was achieved by staged Bi-jaw procedure of first osteotomy and advancement of the maxilla, followed 3 months later, by setback of the prognathic mandible. Postsurgical orthodontics helped in the occlusal refining, by correcting emerging dental discrepancies and settling the occlusion to its final stable position.

It is proposed that when the skeletal discrepancy is excessive, as was in this case, a staged Bi-jaw procedure of Maxillary advancement followed 3 months later by the mandibular setback may be employed, which helps in achieving the desired magnitude of correction, with little or no relapse. Giving this period of time between the two surgeries helps the oral musculature adapt to the new position of each jaw better, with less likelihood of relapse and a stable long term overall result.\textsuperscript{23} By staging the two jaw surgeries, the surrounding mucosal and muscular tissues have a better chance to adapt to the skeletal changes brought about by each jaw repositioning, through progressive adaptation, rather than an immediate, transposition brought on by simultaneous repositioning of both jaws. This would particularly apply to the tongue musculature, which by the initial maxillary advancement, would be allowed time to re-adapt to a more superior position, so that when the patient is taken up for the second stage mandibular setback, it would not restrict the tongue space as severely, which could otherwise lead to pushing forward of the distal mandibular segment by the cramped tongue and varying amounts of relapse.

Furthermore, single stage Bi-jaw surgeries invariably entail a relatively greater blood loss, and a prolonged operating time with its attendant aesthetic risks including respiratory insufficiency which could result from atelectasis, pneumothorax, or pneumomediastinum. The risk of post-operative aspiration pneumonia, due to entry of food, saliva, or nasal secretions into the bronchial tree, has been shown to be higher following prolonged and extensive surgical procedures.\textsuperscript{24} Operator fatigue due to the prolonged surgery is another factor to be considered. Postoperative complications of nausea and vomiting have been related to prolonged orthognathic surgical procedures.\textsuperscript{25}

These disadvantages can be obviated by the proposed two-staged, shorter ‘Single-jaw at a time' operative procedures with an intervening period of three months between the two surgical phases.

Large mandibular setbacks often produce changes in the skeletal and soft tissues in the maxillofacial region, including the positions of the tongue and the hyoid bone, with consequent narrowing of the pharyngeal airway space, leading to Obstructive Sleep Apnoea.\textsuperscript{26} Our patient's final postoperative pharyngeal airway space (PAS) was normal, and he developed no obstructive sleep-related breathing disorders. This could be attributed to the positional conformation of the tongue, which did not encroach upon the Posterior Airway Space (as is otherwise usually the case with large mandibular setbacks), as it had already assumed a superior position following the first stage Maxillary advancement. Surgery. The post treatment oro-pharyngeal, intraoral and peri-oral musculature consequent to the modified, double staged surgical protocol, thus proved to be significantly superior to the conventional single staged Bi-jaw procedures.
Conclusion

There was achieved an effective and stable correction of the extreme Class III skeletal deformity & malocclusion, with a dramatic enhancement of facial balance, symmetry and proportion in this patient, following a modified Ortho-Surgical management protocol. The staged protocol of ‘Maxilla first and Mandible after’ Orthognathic surgery with conventional pre- and post-surgical orthodontics, helped in pushing the envelope of skeletal discrepancy correctable by orthognathic surgery, thereby achieving large quantum of jaw movements, with ideal and stable functional as well as esthetic results. This is suggestive of its efficacy and superiority over the hitherto employed single stage Bi-jaw procedures in the management of severe skeletal discrepancies.

Compliance with ethical standards

Research involving human participants and/or animals

All procedures performed on the patients (human participants) involved were in accordance with the ethical standards of the institution and/or national research committee, as well as with the 1964 Helsinki declaration and its later amendments and comparable ethical standards.

Declaration of Competing Interest

The author of this article has not received any research grant, remuneration, or speaker honorarium from any company or committee whatsoever, and neither owns any stock in any company. The author declares that she does not have any conflict of interest.

Ethical approval

This article does not contain any new studies with human participants or animals performed by the author.

Informed Consent

Informed consent was obtained from all the individual participants in this study.

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