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RESEARCH ARTICLE

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Efficacy and Toxicity of Calcitonin Treatment in Children with Cherubism: A Single-Center Cohort Study

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ABSTRACT

Cherubism is a rare autosomal dominant disease characterized by expansile osteolytic jawbone lesions. The effect and safety of off-label calcitonin treatment during the progressive phase of the disease are not well described. In this retrospective study, we present data on the radiological response and adverse effects of subcutaneously administered calcitonin in a cohort of nine cherubism children (three female, six male). Two of the nine patients underwent two separate treatment courses with a significant off-treatment interval in between; therefore, a total of 11 treatment courses with a mean duration of 17.9 months (range <1 to 35, SD 10.8) were studied. To measure the response, the cumulative volume of cherubism lesions was calculated from available three-dimensional imaging. The primary outcome was the change in the volume of lesions during calcitonin treatment and only assessed for the eight treatment courses with a minimal duration of 6 months. A statistically significant reduction in the mean cumulative volume of lesions was seen regardless of treatment duration. Average volume reduction was highest in the first half year of treatment, with a gradual, ongoing reduction thereafter. For the secondary outcome, the change in the cumulative volume of lesions after treatment cessation was assessed for the seven treatment courses with follow-up imaging available. After six of these seven treatment courses, the cumulative volume increased again but remained undoubtedly smaller than the initial volume at the start of therapy. Adverse effects were assessed for all 11 treatment courses and occurred in 73% of them. Most adverse effects were mild and low grade, with the most severe being one grade 3 symptomatic hypocalcemia requiring hospitalization and early treatment termination. Calcitonin treatment seems effective and tolerable in treating actively progressing cherubism in children. However, further research is required to better understand the pharmacological treatment of cherubism, including also other drugs, dosing, and protocols. © 2023 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: Therapeutics—calcitonin; Diseases and disorders of/related to bone—cherubism; Molecular pathways—development; Dental biology; Cells of bone—osteoclasts

Introduction

Cherubism (OMIM 118400) is a rare autosomal-dominant disease affecting the jawbones.[1-3] It is caused by a germline pathogenic variant in the *SH3BP2* gene[4-6] and is classified as one of the giant cell lesions of the jaw (GCLJ).[7] Clinically, bilateral expanding inflammatory/fibrous lesions may develop during early childhood, which are expected to regress spontaneously after adolescence.[2,8] It is hypothesized that the inflammatory/fibrous osteolytic lesions are initiated and amplified by

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molecular changes following dental and maxillofacial osseous development, tooth eruption, and in response to early changes in the oral microbiome.[1,9]

During the progressive phase of the disease, the osteolytic jawbone lesions may experience aggressive growth. Left untreated, this can lead to severe damage, including permanent functional impairment, visual loss, respiratory distress, disrupted dental development, and severe aesthetic and psychological concerns.[2,3] As surgery is preferably delayed until the growth of lesions stagnates after adolescence,[2,3] systemic treatment options targeting the lesional microenvironment seem a more viable option in this phase.

Among various drugs, calcitonin has incidentally been applied off-label for this purpose.[3] Calcitonin inhibits bone resorption through direct inhibition of the osteoclast-like multinucleated giant cells[10-12] and has been successfully used in treating solitary GCLJ since 1993.[13] However, its effectiveness in treating other GCLJ, such as cherubism, has been scarcely documented.[8,14-19] Only five case reports described its use in children with cherubism,[8,14,15,17,19] and treatment duration varied greatly from 6 to 30 months.[8,14-19] Postsurgical follow-up has been reported,[8,18] but only one case report covers follow-up after calcitonin treatment is stopped and surgery withheld.[14]

Therefore, this study aims to objectivate, in a standardized manner, the response to calcitonin treatment in a single-center cohort of cherubism patients. We aimed to quantify the treatment response through three dimensional radiological volume measurements of cherubism lesions during and after treatment and report on the occurrence and severity of treatment-related adverse effects.

Material and Methods

Study design and population

A retrospective cohort study was designed. Patients diagnosed with cherubism were selected from the local GCLJ registry at the Department of Oral and Maxillofacial Surgery of the Amsterdam University Medical Centers (UMC). In all cases, the clinical diagnosis was genetically confirmed by DNA analysis, demonstrating a heterozygous germline pathogenic variant for the *SH3BP2* gene known to be associated with cherubism. Only those treated with calcitonin according to the local treatment protocol described below, between January 2012 and January 2022, were included in this study.

For data collection, the medical files and radiological imaging were reviewed. Data were collected on patient and treatment characteristics, including age at disease onset, age at diagnosis, pathogenic genetic variant, age at the start of treatment, indication and duration of treatment, and reported adverse effects as well as imaging response during treatment and follow-up until January 2023. Disease severity was classified according to Motamedi-Raposo.[20,21]

Treatment protocol

All included patients were treated according to the local treatment protocol of the Amsterdam University Medical Centers. This protocol is based on the hypothesis that the growth of cherubism lesions results from molecular changes following dental and maxillofacial osseous development and tooth eruption[1,9] and that treatment initiation should, if possible, be guided by the dental transitional phases.

Pharmacological intervention is considered, preferably during dental transitional phases, when lesions are actively progressing and one of the following criteria is fulfilled: (1) severe malformation with functional impairment or endangered vital structures (e.g., intra-orbital expansion or airway compromise), (2) severe disruption of dental development expected to lead to long-term oral disability, or (3) impaired psychosocial development related to disease-associated facial deformities. The pharmacological intervention studied involves daily subcutaneous administration of 100 IU calcitonin (Calcitonin EssPharma, Essential Pharma Ltd, Birkirkara, Malta). For patients under the age of 4 years, the daily dose is lowered to 50 IU. The intended duration of is 2 years for every patient. This is based on the mean treatment duration found to be effective in treating solitary GCLJ.[12,22] Pharmacological intervention for this specific indication, including the use of calcitonin, is considered off-label. Therefore, the prescription and monitoring of calcitonin for cherubism patients in our center is restricted to a pediatric oncologist (EE, HB) with experience in administering this medication for solitary GCLJ.

Treatment monitoring consists of frequent clinical evaluations, laboratory investigations, and a radiological response evaluation by (cone-beam or multislice) CT at the start of calcitonin administration ($T = 0$), 6 months into treatment ($T = 0.5$), after 1 year of treatment ($T = 1$), and every full year ($T = 2$, $T = 3$) thereafter until calcitonin administration is stopped. After completion of calcitonin treatment, all patients are followed in a multidisciplinary surveillance program until young adulthood. This program consists of clinical examinations by a team of dental and medical professionals and two-dimensional radiographic imaging to assess disease progression and dental development. Three-dimensional imaging is only requested when strictly indicated to minimize radiation exposure.

Outcome measurement

The primary outcome measure of this study was the change in the cumulative volume of lesions in response to calcitonin during its administration. Only patients with a minimal treatment duration of half a year and radiological response evaluation while on treatment at the aforementioned moments of radiological assessment were included for the primary outcome (“on-treatment”) assessment.

Secondary outcome measures were the change in the cumulative volume of lesions during follow-up and the occurrence and severity of treatment-related adverse effects. Secondary outcome volume measurement (“off treatment”) was only executed if follow-up imaging was available at least 6 months after cessation of calcitonin administration. The duration of follow-up at measurement was determined as the interval between the last dose of calcitonin and the date imaging was performed, rounded to (a multiple of) half a year. The occurrence and severity of adverse effects were only collected for the on-treatment periods.

For volume measurement, Brainlab iPlan Cranial was used (version 3.0, Brainlab AG, Munich, Germany) (Fig. 1). Lesion borders were manually traced in every axial slice of every CT or CBCT scan, enabling the creation of a 3D reconstruction of the lesions. To enhance accuracy, the maxillofacial bones were reconstructed at >400 Hounsfield units (HU) and subtracted from the manually annotated lesions. The resulting volume was deemed the true lesion volume and was used for comparison and statistical analysis. To reduce the risk of bias, all lesions were traced manually in random order, three-dimensionally reconstructed, and measured by two researchers independently (WHS, EBM).

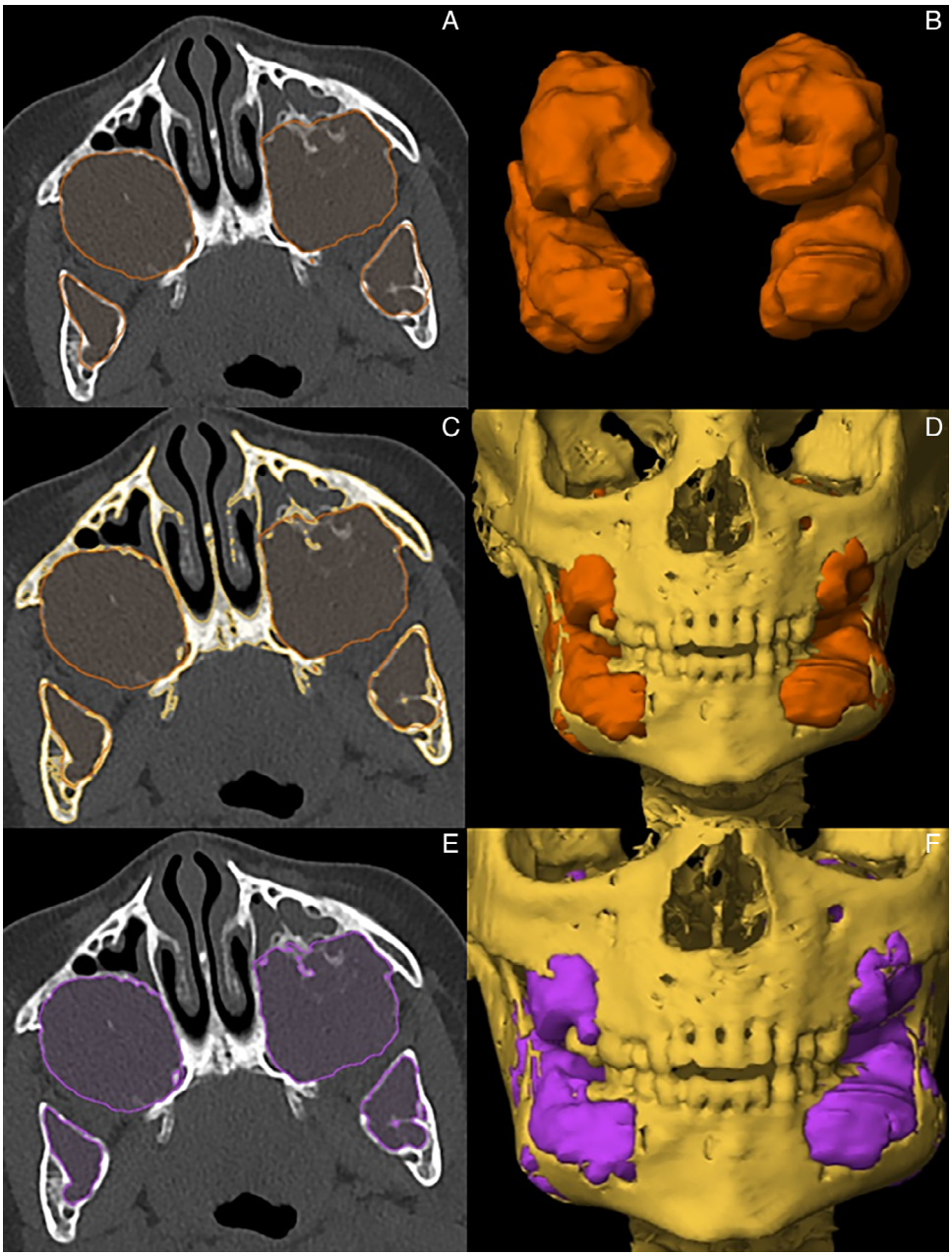

Fig. 1. Volume measurements. For volume measurements, first the lesions were manually traced (1A, orange) on axial slices, making it possible to render a 3D reconstruction of lesions (1B). Thereafter, maxillofacial bones as well as teeth were segmented automatically at >400 HU in every axial slice (1C, yellow), creating a 3D reconstruction of all maxillofacial hard tissues (1D). To enhance accuracy, the area of bone and teeth overlapping the manually annotated lesions was subtracted from the lesions in every axial slice, creating the true lesional volume (1E, purple), which was also 3D reconstructed (1F). The volume of this reconstruction was used for comparison and statistical analysis.

Before annotations were performed, three random scans were discussed among accessors in an online calibration session to agree on definitions.

The reported adverse effects were recorded and classified according to the National Cancer Institute Common Terminology Criteria for Adverse Effects (CTCAE) version 5.0 as grade 1 (mild), grade 2 (moderate), grade 3 (severe), or grade 4 (life-threatening). If available, required adjustments for pediatric patients were applied.

Statistical analysis and study execution

Statistical analysis was executed using SPSS 26 (IBM, Armonk, NY, USA). Descriptive analyses were used for characteristics of the study population as well as the adverse effects and follow-up measurements. The intraclass correlation coefficient (ICC) was calculated for volume measurements to determine the agreement among the two observers. If ICC was >0.70, the average of the two observers' measurements was used for further

statistical analysis. The normality of data was tested according to Shapiro–Wilk. For volume measurement during treatment ("on treatment"), quantitative analysis was executed using repeated measures ANOVA and pairwise comparison if data were normally distributed. Separate paired sample *t* tests were used to compare the difference between $T = 0$, $T = 0.5$, and $T = 1$. The significance alpha was adjusted based on Bonferroni correction. That is, the significance alpha (0.05) was divided by the number of pairwise comparisons ($N = 3$). Therefore, the adjusted significance alpha was 0.017.

This study was executed and written in accordance with the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement for cohort studies. As this was a retrospective study, exemption from the need for official approval was confirmed by the Medical Ethics Commission of the Amsterdam UMC, University of Amsterdam, as confirmed in their letter (IRB No. W21_105#21.121). The study was compliant with the Declaration of Helsinki.

Results

In total, 19 children were selected from the GCLJ database with a DNA-confirmed diagnosis of cherubism. Between January 2012 and January 2022 nine patients, eligible for pharmacological treatment according to the criteria mentioned earlier, started with calcitonin treatment and were included in this study. Six patients completed a minimal course of 6 months with response imaging at $T = 0.5$ and could be included for primary outcome volume measurement ("on-treatment"). Two of these six individual patients underwent two distinct rounds of calcitonin treatment during respectively the first and second dental transitional phases with a significant off-treatment interval in between. As a result, a total of eight distinct treatment courses were included for primary outcome volume measurement ("on treatment"). After seven of these eight distinct treatment courses follow-up imaging was available at least 6 months after the last calcitonin administration. The follow-up imaging made after these seven treatment courses was used for secondary outcome volume measurement ("off treatment"). The occurrence and severity of adverse effects were reported for all nine patients. Again, since two patients underwent two separate rounds of calcitonin treatment at different time points, this led to a total of 11 distinct treatment courses to be analyzed for toxicity (Fig. 2).

Patient and treatment background

The majority of patients were male (Table 1). In one patient, only the mandible was affected, classified as a Motamedi-Raposo[20,21] grade III-5 (1/9, 11%); all others had disease manifestations in both maxilla and mandible, classified as either grade IV-1 (4/9, 44%) or grade IV-3 (4/9, 44%). The mean age of disease onset when the first enlargement had been observed was reported to be 4.0 years (range 1 to 8, SD 2.9), while the diagnosis was confirmed by DNA analysis at the mean age of 5.3 years (range 1 to 9, SD 2.8). Three different *SH3BP2* mutations located on exon 9 of chromosome 4p16.3 were found, either c.1244G > A (6/9 pts, 67%), c.1253C > A (1/9 pt, 11%), or c.1258G > A (2/9 pts, 22%), the latter two patients being brother and sister. In all children, the disease resulted from a heterozygous mutation inherited from their father. The mean age at the start of calcitonin treatment was 8.2 years (range 2.1 to 14.3, SD 3.6), while the mean duration of therapy was 17.9 months (range <1 to 35, SD 10.8). Two treatment courses were initiated with patients still having their deciduous teeth before dental transition, five treatment courses during the first transitional period, and four treatment courses during the second transitional period. In all patients, the decision to start treatment was a result of shared decision-making with parents based on multiple individual disease characteristics. The main decisive factors for therapy initiation were the severely progressive growth of lesions in 73% of the treatment courses, the severe disruption of dental development and displacement/damage of tooth buds by the expansile lesions in 55%, and/or the severity of disease advancement at a young age in 36%. One patient had developed psychosocial and developmental problems as a result of bullying due to his appearance.

Primary outcome: Volume measurement during treatment

Interobserver conformity was moderate to excellent (Table 2), so the average of measurements of the two independent observers was used for further statistical analysis. Measurement data were normally distributed at $T = 0$, $T = 0.5$, $T = 1$, and $T = 2$ based on the Shapiro–Wilk test ($p < 0.05$). All patients showed a reduction in the cumulative volume of their lesions (Fig. 3). The mean cumulative lesion volume at baseline was 80.85 cm^3 ($N = 8$, SD 21.85), $T = 0.5$ 42.53 cm^3 ($N = 8$, SD 12.56), $T = 1$ 31.03 cm^3 ($N = 7$, SD 9.62), and at $T = 2$ 24.53 cm^3 ($N = 6$, SD 8.77). For the six treatment courses lasting 2 years, there was a significant effect of the treatment time on the mean volumes based on repeated measures ANOVA ($F_{(3,15)} = 30.689$, $p < 0.01$), with mean volume reduction over 70% after 2 years of treatment. Paired analysis (Table 3) with Bonferroni correction showed a significant reduction ($p < 0.05$) in mean volumes between the start of treatment and all other moments of measurement. Reduction in mean volume between 0.5 years of treatment and 1 year was also significant; however, no significant difference ($p > 0.05$) could be observed between lesion volumes at 0.5 years and 2 years of treatment as well as 1 and 2 years of treatment. Paired sampled *t* test, evaluating the paired mean volume differences of all treatment courses, including those with a duration of 1 year or less, also showed a significant reduction after 1 year of treatment (mean difference 50.76, $p = 0.002$), as well as in the first half year (mean difference 38.32, $p < 0.001$) and the second half year of treatment (mean difference 11.87, $p = 0.004$) (Table 4).

Secondary outcome: Volume measurement during follow-up

Patient age at treatment termination ranged from 3 to 14 years, with five patients being in the mixed dentition phase and one patient almost having completed dental eruption. Based on the availability of follow-up imaging, cumulative volume assessment after treatment termination ("off treatment") could be performed after seven distinct treatment courses (Fig. 4). One treatment course had a follow-up CT available 6 months after treatment cessation, four treatment courses 1 year after treatment cessation, and two treatment courses 2 years after treatment cessation. After six out of these seven treatment courses, the cumulative volume increased during follow-up. The increase ranged from 28% in 6 months to 41%, 88%, and 121% in 1 year,

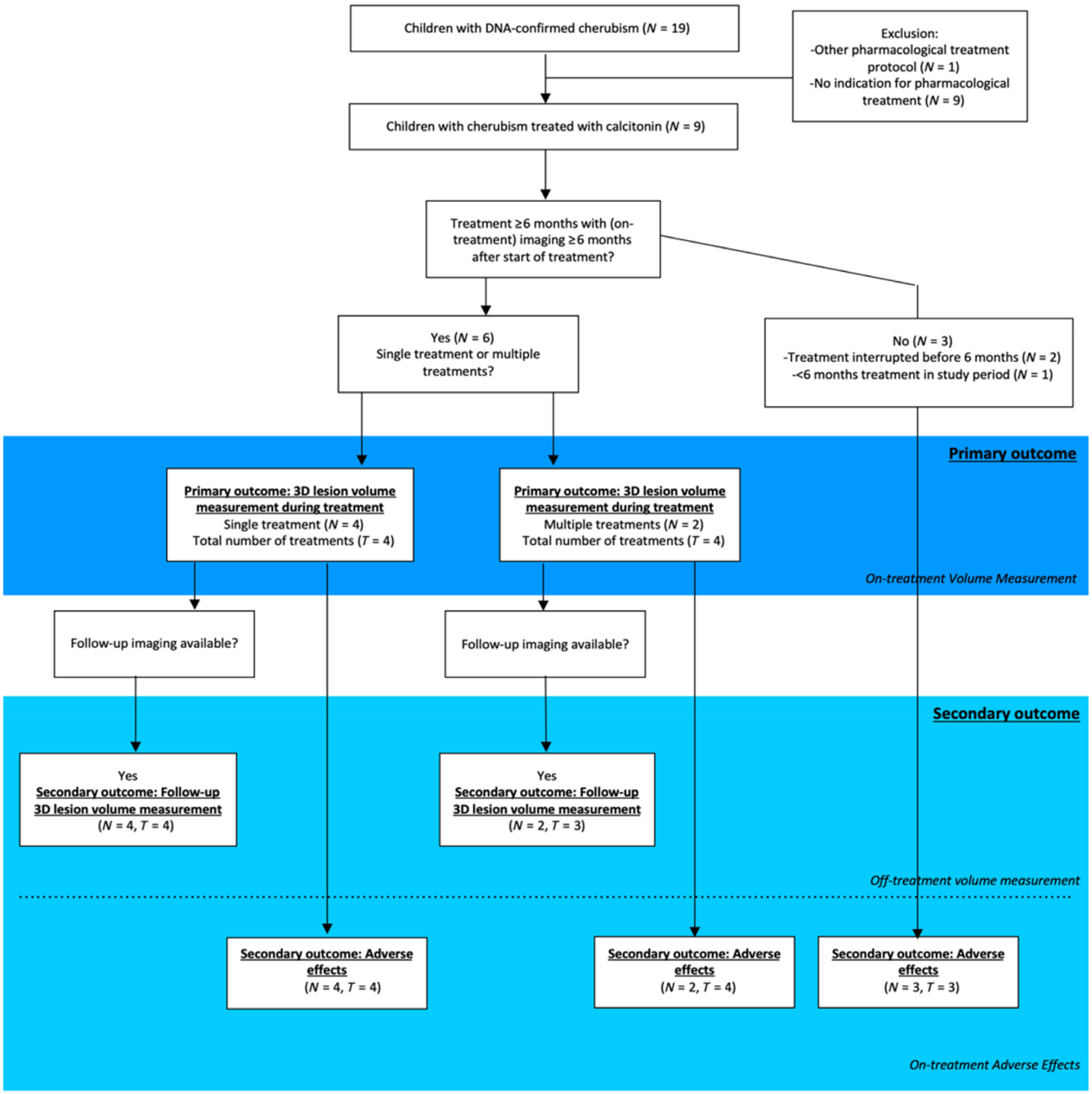

Fig. 2. Consort flow diagram. This diagram illustrates the eligibility of nine included patients and the 11 distinct courses of calcitonin treatment they underwent. In total, eight distinct treatment courses of calcitonin treatment were analyzed for changes in the cumulative volume of lesions while "on-treatment" (primary outcome). In total, seven distinct courses of calcitonin treatment were analyzed for changes in the cumulative volume of lesions during follow-up (secondary outcome). A total of 11 distinct courses of calcitonin treatment were analyzed for the occurrence and severity of adverse effects (secondary outcome). N = number of patients; T = number of distinct treatment courses.

to 151% and 172% in 2 years. One patient showed a volume decrease of 46.9% 1 year after treatment was stopped.

Adverse effects and toxicity

Adverse effects occurred in 73% of treatment courses. The majority of adverse effects were mild (Table 5). The most frequently occurring grade 1 side effects were nausea (45%), vomiting (27%), and injection site reactions consisting of painful, erythematous irritation (27%). In 18% of the treatment courses, flushing occurred after injections (either grade 1 or 2), while in another 18% of treatment courses, complaints of headache or fatigue arose (either grade 1 or 2). Only one other grade 2 adverse effect was

Table 1. Patient and disease background

Patient	M/F	Motamedi/ Rapo-so-Amaral grade	Age at first observed enlargement	Age at diagnosis (through DNA analysis)	Genetic mutation	Origin of mutation	Age at start of therapy	Transitional phase at initiation	Indication for therapy	Duration of therapy
1	F	IV-3	2 years	3 years	c.1244G > A (p.Arg415Gln)	Father	6 years and 3 months	First	Rapid size progression during active surveillance, severely disrupted development of permanent tooth buds	22 months
2	M	IV-1	8 years	8 years	c.1244G > A (p.Arg415Gln)	Father	8 years and 9 months	First	Disrupted development of permanent tooth buds, disrupted tooth eruption	5 months
3	M	IV-1	7 years	9 years	c.1244G > A (p.Arg415Gln)	Father	8 years and 11 months	First	Rapid size progression	<1 month
4	M	III-5	6 years	7 years	c.1253C > A (p.Pro418His)	Father	11 years and 8 months	Second	Rapid size progression during active surveillance, psychosocial problems due to bullying	35 months
5	F	IV-1	2 years	2 years	c.1258G > A (p.Gly420Arg)	Father	4 years and 6 months	Pre	Size progression during active surveillance, disrupted development of permanent tooth buds	24 months
6	M	IV-3	1 year	1 year	c.1258G > A (p.Gly420Arg)	Father	2 years and 1 month	Pre	Severity at young age	18 months
7	M	IV-1	7 years	7 years	c.1244G > A (p.Arg415Gln)	Father	10 years and 6 months	Second	Severity at young age, disrupted development of permanent tooth buds, disrupted tooth eruption	ongoing
8 (first treatment course)	F	IV-3	1 year	6 years	c.1244G > A (p.Arg415Gln)	Father	6 years and 10 months	First	Severity/progression of disease, severely disrupted development of permanent tooth buds, disrupted tooth eruption	22 months
8 (second treatment course)	-	IV-3	-	-	-	-	14 years and 3 months	Second	Rapid size progression 5 years after first treatment termination	6 months
	M	IV-3	2 years	5 years	c.1244G > A (p.Arg415Gln)	Father	5 years and 3 months	First	Severity/progression of disease, disrupted	23 months

(Continues)

Table 1. Continued

Patient	M/F	Motamedi/ Rapo-so-Amaral grade	Age at first observed enlargement	Age at diagnosis (through DNA analysis)	Genetic mutation	Origin of mutation	Age at start of therapy	Transitional phase at initiation	Indication for therapy	Duration of therapy
9 (first treatment course)									development of permanent tooth buds	
9 (second treatment course)	-	IV-3	-	-	-	-	11 years and 3 months	Second	Size progression 4 years after first treatment termination; prophylactic to prevent further progression during second transitional period	24 months

Note: This table illustrates the patient and disease characteristics of all included patients.
Abbreviation: F = female; M = male.

Table 2. Intraclass correlation coefficient (ICC)

ICC calculated at	ICC of single measures
start of treatment	0.820 (95% CI: 0.168 to 0.964)
T0.5	0.725 (95% CI: −0.065 to 0.951)
T1	0.838 (95% CI: 0.070 to 0.973)
T2	0.751 (95% CI: −0.087 to 0.964)
T3	NA
Follow-up T0.5	NA
Follow-up T1	0.940 (95% CI: 0.207 to 0.996)
Follow-up T2	0.903 (95% CI: 0.12 to 1.000)

Note: Intraclass correlation coefficient was used to determine interobserver conformity.
Abbreviation: CI = confidence interval.

Fig. 3. Lesion volume during calcitonin treatment. This graph illustrates the cumulative volume of lesions for all patients eligible for the primary outcome ("on-treatment").

recorded, consisting of an erythro-papillomatous rash most likely to be eczema resolved with local treatment.

One patient developed an oral abscess unrelated to systemic treatment, but due to an underlying dental problem, requiring surgical incision and drainage.

One patient developed a grade 3 symptomatic hypocalcemia with paresthesia and vomiting after the first injection. This led to brief hospitalization for treatment and subsequent lowering of calcitonin doses and vitamin D supplements. Due to the induced anxiety in the child and parents, treatment was terminated within a month. Apart from an increased serum PTH (grade 1), mild hypokalemia (grade 1), and mild vitamin D deficiency (grade I), no other abnormal laboratory results were observed.

Lastly, injections were considered painful by most patients. Reduced compliance became an issue in two treatment courses after patients had been treated for a longer time. One treatment course was terminated early after 6 months as wished by the patient and her parents. In another patient, adverse effects were too burdensome, leading to early treatment cessation after 5 months.

Table 3. Pairwise analysis of repeated measures ANOVA

Volume at time	Volume at time	Mean difference	SE	Significance[a]	95% confidence interval for difference	
					Lower bound	Upper bound
Vol T0	Vol T0.5	42.603[b]	8.022	0.019	8.757	76.449
	Vol T1	56.134[b]	9.130	0.010	17.613	94.655
	Vol T2	62.409[b]	10.430	0.011	18.403	106.415
Vol T0.5	Vol T0	−42.603[b]	8.022	0.019	−76.449	−8.757
	Vol T1	13.531[b]	2.411	0.015	3.358	23.703
	Vol T2	19.806	5.440	0.089	−3.147	42.759
Vol T1	Vol T0	−56.134[b]	9.130	0.010	−94.655	−17.613
	Vol T0.5	−13.530[b]	2.411	0.015	−23.703	−3.358
	Vol T2	6.276	4.109	1.000	−11.062	23.613
Vol T2	Vol T0	−62.409[b]	10.430	0.011	−106.415	−18.403
	Vol T0.5	−19.806	5.440	0.089	−42.759	3.147
	Vol T1	−6.276	4.109	1.000	−23.613	11.062

Note: Including all six treatment courses with measurements at $T = 0$, $T = 0.5$, $T = 1$, and $T = 2$.
[a]Adjustment for multiple comparisons: Bonferroni.
[b]The mean difference is significant at the 0.05 level.

Table 4. Paired sample *t* tests

Pair	Mean	SD	SE mean	95% confidence interval of difference		*t*	*Df*	Significance (two-tailed)
				Lower	Upper			
Pair 1 ($N = 8$) Vol T0–Vol T0.5	38.323	18.958	6.703	22.474	54.172	5.718	7	<0.001
Pair 2 ($N = 7$) Vol T0–Vol T1	50.755	24.884	9.405	27.741	73.770	5.396	6	0.002
Pair 3 ($N = 7$) Vol T0.5–Vol T1	11.817	7.044	2.662	5.302	18.332	4.439	6	0.004

Note: Including all eight treatment courses of patients eligible for primary outcome, also those excluded for repeated measures ANOVA. Based on the Bonferroni correction, the adjusted significance alpha is 0.017.

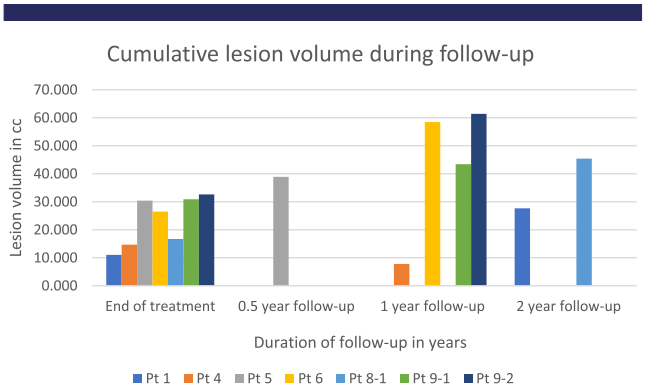

Fig. 4. Lesion volume during follow-up. This graph illustrates the cumulative volume of lesions after treatment cessation for patients eligible for the secondary outcome ("off-treatment").

Discussion

The evolution of cherubism lesions after natural regression or surgery has been reported based on 3D imaging.[2,21,23,24] However, there is very little information on radiological changes that occur after pharmacological therapy.[3,18] Therefore, this study objectively assessed radiological changes to measure the response to calcitonin therapy and demonstrated that calcitonin can be effective in treating actively progressing cherubism in children. It prevented not only further growth in all patients treated for at least 6 months but also significantly reduced the cumulative volume of lesions regardless of treatment duration. Average volume reduction was highest in the first half year of treatment, with a gradual ongoing reduction thereafter. Compared to the volumes at the start of treatment, there was a statistically significant mean volume reduction at all durations of treatment. However, the reduction was not statistically significant between 0.5 and 2 years of treatment, as well as between 1 and 2 years of treatment.

The study has limitations due to its retrospective design and small sample size, given the rarity of the condition and restricted indication for intervention. Also, the lack of biopsy samples precluded the exploration of changes on tissue level. Nonetheless, the observed trends are valuable in understanding the biology of the disease and its response to therapy. Most patients showed a slight, though nonsignificant, reduction in volume after 1 year of treatment, while one patient had an increase in volume at the end of his second treatment likely due to poor compliance. This noncompliant patient could be a strong confounder in the results on average volume reduction after 1 year of treatment, but this observation also suggests that poor compliance can influence the therapeutic effect and lead to tolerance of calcitonin. Also worth noting is that both patients who received a second treatment course after an off-treatment interval of 48 and 66 months experienced a more profound response with a greater volume reduction than at the end of their first treatment course. This observation might suggest that multiple, shorter (1-year) treatment cycles are more effective than one treatment cycle with a long duration.

The main strong point of this study is the standardized volumetric assessment of lesions to objectively measure response. Apart from volumetric changes, we also observed other clinically relevant and positive effects, such as reparation and thickening of the cortices, delineation of the parts of lesions expanding into surrounding soft tissues, and emergence of intralesional ossifications (Fig. 5). These changes are similar to effects seen in other GCLJ following systemic therapy and can aid in the safe curettage of remaining lesions.[22] While not a study endpoint, these features are clinically significant for treatment planning and play a role when choosing the optimal imaging modality for response assessments. As such, we recommend the use of conventional (low-dose) CT, which provides the benefit of both multiplanar reformatted images and good resolution of bone and soft tissues outweighing the disadvantage of additional radiation exposure compared to other imaging modalities.[23-29]

Following treatment cessation, the volume of the lesions increased in most patients but remained undoubtedly smaller than the initial volume at the start of treatment. In one patient, a further decrease was observed. This could be explained by his stage of development at the time of treatment cessation, considering that dental eruption was nearly completed and the natural course of cherubism starts to show stabilization and spontaneous regression at the age of 14.[2] The 86% of patients with an increase were younger and still in the mixed dentition phase at treatment termination. These clinical observations

Table 5. Adverse effects and toxicity

Reported Adverse Effect	Grade 1	Grade 2	Grade 3	Grade 4
Noncardiac chest pain	1 (9%)			
Eczema		1 (9%)		
Fatigue	1 (9%)	1 (9%)		
Flushing	1 (9%)	1 (9%)		
Gait disturbance	1 (9%)			
Headache	2 (18%)			
Injection site reaction	3 (27%)			
Muscle cramps	1 (9%)			
Nausea	5 (45%)			
Oral fistula			1 (9%)	
Paresthesia	1 (9%)			
Tremor	1 (9%)			
Vomiting	3 (27%)			
Hypocalcemia			1 (9%)	
Hyperparathyroidism	1 (9%)			
Hypokalemia	1 (9%)			
Vitamin D deficiency	1 (9%)			

Note: This table illustrates the reported and graded side effects of systemic treatment according to the National Cancer Institute Common Terminology Criteria for Adverse Effects version 5.0. Side effects occurred in 73% of treatment courses. Grade 1 (mild) effects occurred in 55% of treatment courses, while grade 2 (moderate) side effects occurred in 27%. One patient had a grade 3 (severe) hypocalcemia requiring hospitalization. No grade 4 (life-threatening) adverse effects were recorded.

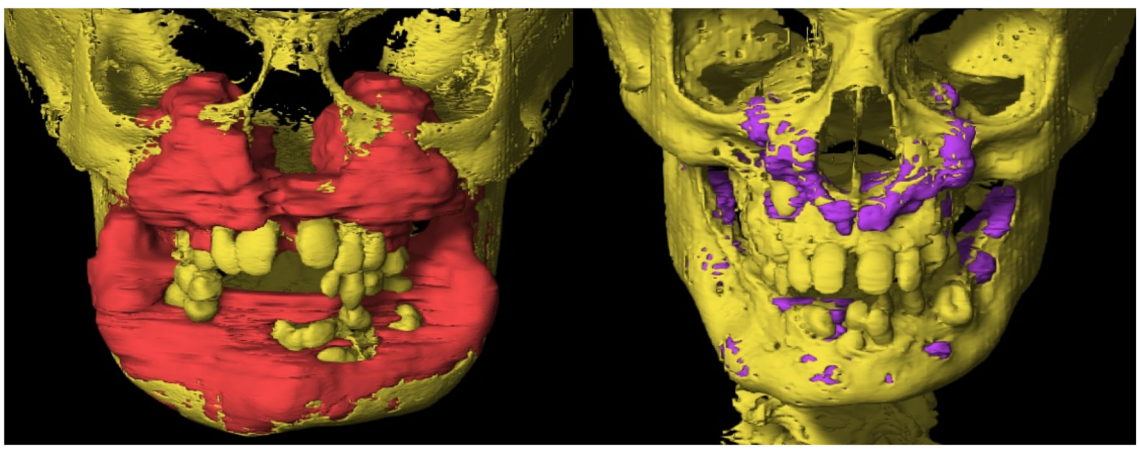

Fig. 5. Treatment response. This figure illustrates the treatment response of patient 8. Reconstruction of lesions at the start of treatment and after 22 months demonstrates the largest observable reduction. Next to a vast reduction of volume of lesions, repair and thickening of cortices and intralesional ossification eventually resulted in more normalized contours of the maxilla and mandible.

support the hypotheses that growth in cherubism may result from molecular and pathophysiological processes related to dental transition and development.[1,9]

The adverse effects occurring in 73% of treatments were predominantly mild and low grade, with the most severe being hypocalcemia requiring hospitalization and ultimately leading to early treatment termination in a single patient. The observed hypocalcemia was probably related to an intramuscular administration by an inexperienced nurse. This divergent route of calcitonin administration, which is known to be associated with a higher incidence of adverse effects,[30,31] immediately highlights the importance of a dedicated, well-trained team supervising the treatment and actively monitoring the introduction phase of the intervention.

Most patients experienced the treatment as burdensome due to painful injections. This could explain the association between a long duration of treatment and reduced compliance or early treatment termination. Therefore, the earlier suggested multiple shorter treatments would be an alternative to diminish noncompliance. Another reason to limit the treatment duration is the reported small increased cancer risk in adults associated with extended calcitonin use. However, malignancies related to the use of calcitonin were specific for adults above 50 years of age, and the European Medical Agency (EMA) concluded that this association was most likely the result of increased tumor growth acceleration rather than de novo oncogenesis.[32] From these data, we consider that calcitonin use in our pediatric population is safe regarding the occurrence of malignancies. Still, we recommend carefully assessing the risk–benefit ratio of treatment in every patient and only proceeding with treatment when the growth of cherubism lesions is expected to cause irreversible long-term sequelae.

Although we chose calcitonin due to our positive experience with this drug in other GCLJ, off-label use of other pharmacologic agents has been reported for cherubism based on the molecular and cellular characteristics of its pathophysiology.[9,33-40] Inhibitors of tumor necrosis factor α, produced by hyperactive macrophages,[37,38] were proposed but failed to improve cherubism in three pediatric cases.[41,42] Clinical outcomes after targeting the hyperactive osteoclasts in eight pediatric and two young adult cherubism cases by either calcineurin inhibitors (tacrolimus)[43] or receptor activator of NF-κB ligand inhibitors (denosumab)[44-47] were positive, while for bisphosphonates[16,42,44,48] results varied. An attempt to mitigate the protein product of the mutated *SH3BP2* gene by a c-abl inhibitor (imatinib) demonstrated a partial regression of lesions in four pediatric cherubism cases.[49,50] Most treatments were found to be well tolerated, and many of the known adverse effects[49,51-56] 56] were not observed, apart from a serious hypocalcemia and rebound hypercalcemia that occurred in three denosumab cases.[46,47,57] Although the drugs with positive outcomes mentioned in these incidental reports are promising, it is difficult to make a meaningful comparison with our results due to the lack of a comparative and systematic analysis. To select the ideal drug, dosing, and treatment protocol that considers both clinical outcomes and long-term safety data in this vulnerable pediatric population, larger studies need to be designed. To achieve this, future collaboration within rare disease networks is an absolute necessity.

In conclusion and based on the significant reduction of lesion volumes independent of treatment duration seen in this study, calcitonin treatment seems effective in treating actively progressing cherubism in children. Although adverse effects occur in most patients, they are generally mild if appropriately treated. Still, further research is required for better insight into the systemic treatment of cherubism. Based on the greatest volume reduction in the first half year of treatment and increased response after an off-treatment interval observed in this study, a new treatment protocol consisting of shorter but more frequent treatment courses might seem an interesting approach for future studies, possibly improving tolerance and compliance. The current hypothesis that the growth of cherubism lesions results from molecular processes accompanying dental transition and jaw development could further guide treatment planning.

Author Contributions

Willem H. Schreuder: Conceptualization; data curation; formal analysis; writing – original draft; methodology; investigation; supervision; project administration; writing – review and editing; visualization. **Ethan B. Meijer:** Data curation; formal analysis; methodology; investigation; project administration; writing – original draft; visualization. **Arjen H.G. Cleven:** Conceptualization; formal analysis; methodology; writing – review and editing. **Esther Edelenbos:** Writing – review and editing; investigation. **Cornelis Klop:** Visualization; writing – review and editing; methodology; software. **Ruud Schreurs:** Methodology; visualization; writing – review and editing; software. **Renate T. de Jong:** Writing – review and editing. **Merel C. van Maarle:** Writing – review and editing. **Roy B.G. Horsthuis:** Writing – review and editing. **Jan de Lange:** Writing – review and editing; conceptualization; supervision. **Henk van den Berg:** Supervision; conceptualization; data curation; writing – review and editing.

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Data Availability Statement

The data supporting this study's findings are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Disclosure

None. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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