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Original Article

Optimal time to definitive enucleation of large cysts following marsupialization: A single center, retrospective study

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ABSTRACT

Objectives: Optimal time to enucleation following marsupialization of large odontogenic mandibular cysts is undefined. We aim to assess volume reduction throughout follow-up, to indicate optimal time to enucleation. Secondary objectives include the identification of factors influencing cyst reduction.
Study design: We retrospectively enrolled 15 patients with mandibular cysts of different histological types treated with marsupialization at our center between 2018 and 2022. Cyst volume was assessed with cone-beam computed tomography (CBCT) and a semi-automatic segmentation algorithm, at baseline and between 6 and 8 months post marsupialization.
Results: The overall mean cyst volume reduction percent (VR%) was 57.7 % or 0.2 % per day. VR% at 8 months was significantly higher than those assessed at 6 and 7 months (67.1% vs 47.1 %, $p = 0.003$). Time to CBCT was the only independent variable influencing cyst VR%.
Conclusion: Our study proves that the optimal time to enucleation for mandibular cyst is 8 months, independent of histological cyst type, patient age, baseline cyst volume and the number of pre-operative residual bone walls.

1. Introduction

Jaw cystic lesions are one of the most frequent diseases affecting oral and maxillofacial regions. The World Health Organization (WHO) Classification of Head and Neck Tumors updated diagnostic criteria for jaw cysts in March 2022 [1].

Surgery is the first choice to treat jaw cystic lesions. Several surgical methods have been described. The most invasive are enucleation and resection, which both remove cystic walls and contents entirely [2]. The alternative, more conservative surgical methods of marsupialization and decompression decrease intraluminal pressure allowing cystic volume reduction (VR) [3]. The marsupialization technique determines a communication between inner lumen of the lesion and the oral cavity through excision of part of the cystic wall suturing the remaining fibrous wall to the mucosal flap, followed by application of obturators or gauze [4]. Decompression is a technique that involves the use of a device, like a drainage tube, catheter or stent, sutured between lesion wall and oral mucosa and maintained *in situ* [5,6].

Both less invasive techniques improve bone healing and growth by intraluminal pressure decrease and minimize the risk of damage of surrounding structures [4,6]. If large cysts invade the adjacent structures, such as roots of teeth, nerves and blood vessels, maxillary sinus or nasal cavity, conservative surgical techniques are preferred [7]. A recent systematic review demonstrated that conservative therapies cannot be considered definitive. These techniques reduce cyst volume, which in turn reduces the invasiveness of secondary surgery of enucleation. This is especially important for large cysts [8]. Therefore conservative techniques reduce the aggressiveness of certain pathological types of keratocysts resulting in fewer recurrences following definitive enucleation [9].

The timing between a conservative intervention and definitive enucleation is at the discretion of the oral surgeon, as there are no current guidelines to support optimal timing. Currently, the oral surgeon bases this decision on cyst VR and distancing from major anatomical structures [10].

Despite a large heterogeneity of studies follow-up periods in literature [11,12], generally surgeons wait until the cysts are reduced by at least 50–60 % prior to enucleation. [13] The greatest VR seems to occur within the first 6–8 months after marsupialization [14,15],

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however, in order to gain the most momentum from the regenerative capacity of the bone, an earlier definitive surgical intervention would be preferred. [3] Therefore, evaluating the optimal time to VR of 50−60 % is essential to guide the creation of follow-up protocols.

Volumetric evaluation of cyst reduction with bidimensional panoramic radiography has been proven to be approximate at best [16]. To overcome bidimensional distortion of panoramic radiography, some more recent studies have investigated the adoption of three dimensional (3D) tools, such as cone beam computed tomography (CBCT) [17].

Further, factors influencing cyst reduction are controversial, as studies in literature report conflicting outcomes [18,19].

This retrospective study aims to investigate the cyst VR between 6 and 8 months following marsupialization of mandibular cysts with CBCT, prior to enucleation. Study secondary objectives include the comparison of cyst VR at 6−7 and 8 months and the identification of risk factors influencing cyst reduction.

2. Materials and methods

2.1. Study design and patient population

The study was performed retrospectively in a single center. All participants provided informed consent for the current study, which was carried out according to the Declaration of Helsinki. Ethical approval for this study was obtained from “Comitato Etico dell’Area Vasta Emilia Nord” AVEN (approval no. AOU 0005163/19).

Consecutive patients diagnosed with large mandibular cyst and treated with marsupialization at the Dentistry and Oral-Maxillofacial Surgery Unit, University of Modena and Reggio Emilia (Modena, Italy) between January 2018 and December 2022 were screened for the current study. Criteria for inclusion also specified the availability of preoperative cone-beam computed tomography (CBCT) assessment (Fig. 1) and post-surgical histological confirmation of odontogenic cyst. Criteria specified the exclusion of patients with (i) uncontrolled medical disorders, (ii) history of previous tumors or irradiation in the maxillofacial region, (iii) CBCT follow-up performed before or after 6−8 months post-operative period, and (iv) patients lost to follow-up.

Data obtained included patient demographic (age and sex), histological cyst type, number of residual bone walls, 3D cyst volume and time to CBCT. Two subgroups were created according to time to CBCT follow-up, of 6−7 months and 8 months, based on the distribution of follow-up.

2.2. Surgical procedures for marsupialization and 3-Dimensional (3D) volume measurements

Marsupialization is performed for all patients under local anesthesia (mepivacaine with 1:100,000 epinephrine injected around the lesion) followed by minimal incision and mobilization of full thickness vestibular flap. In the presence of tooth involvement, tooth extraction is performed. If the buccal cortical bone is intact, exposure of the cystic membrane tissue is performed using high-speed handpiece and round bur (Fig. 2A and B). A part of cystic wall is removed and fixed with formalin solution for histological analysis (Fig. 2C). The remaining lesion wall is sutured to flap borders or anchored to the vestibular bone to maintain communication of internal lumen of the cyst with the oral cavity (Fig. 2D and E).

Following suture, an iodoform gauze soaked with gentamicin is applied inside the new accessory pouch (Fig. 2F).

After surgery, all patients are prescribed oral amoxicillin associated with clavulanic acid (3 g/day for 7 days) and ibuprofen (600 mg/day), with 0.2 % chlorhexidine mouthwash (twice a day for 2 weeks).

Two weeks after surgery, gauze and sutures are removed. Irrigation of the surgical cavity with 0.2 % chlorhexidine is performed and new dressings (iodoform gauze soaked with gentamicin) is inserted. Irrigation and new dressings are programmed weekly for all patients. Weekly irrigation and new dressings are continued until the cyst size is clinically irrelevant or until CBCT follow-up confirms definitive surgery (enucleation).

3D CBCT data were obtained using a NewTom VGi scanner (Quantitative Radiology, Verona, Italy). The device is set for an exposure time of 15 s at 110 kV tube voltage and 8 mA tube current with a field

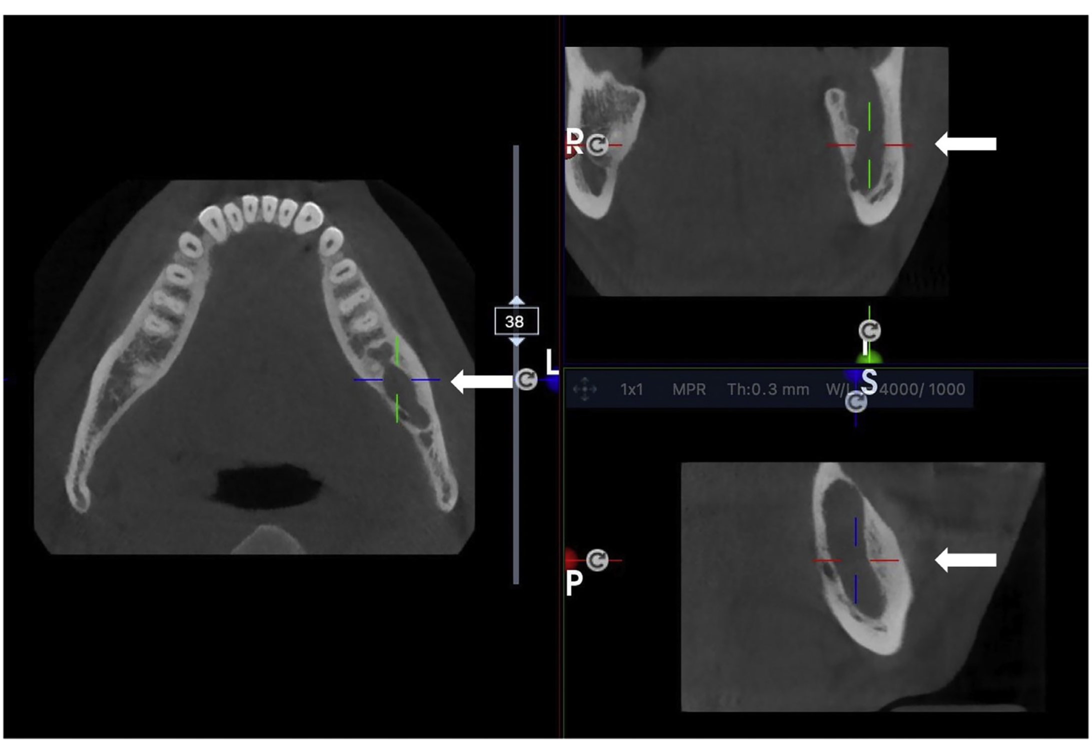

Fig. 1. Cone-beam computed tomography slices. White arrows indicate mandibular keratocyst before surgery.

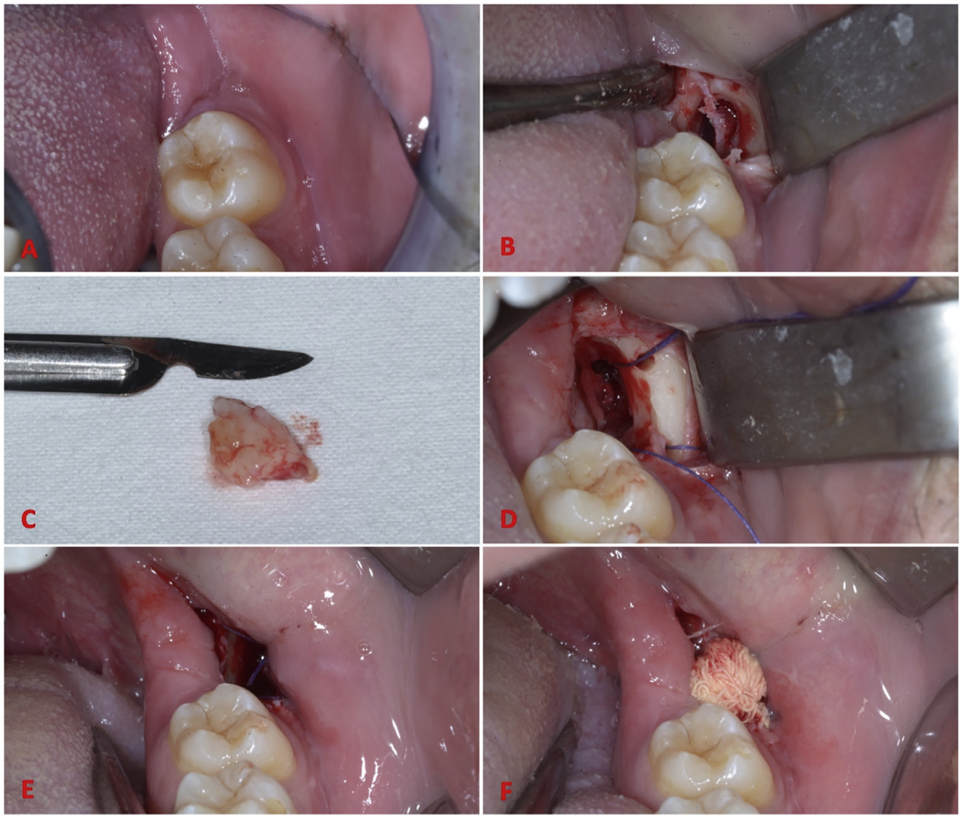

Fig. 2. Patient with marsupialized keratocyst included in the study. A: preoperative photograph; B: Intraoperative photograph showing removal of part of cystic wall for histological analysis; C: Intraoperative photograph showing osteotomy and opening of the cyst; D: Intraoperative photograph showing intrabone sutures; E: Intraoperative photograph showing opening of the marsupialized cyst; F: Intraoperative photograph showing gauze applied inside new accessory pouch.

of view of 120 × 80 mm and pixel size of 100 μm. A cut with a width of at least 0.5 mm was obtained from all contiguous cross-sectional images in 3 directions of space, *see* Fig. 1.

Our protocol prescribes pre- and post-operative (between 6 and 8 months) CBCT for all patients treated with marsupialization.

3D data from both pre- and post-operative CBCT of all patients were saved in DICOM (Digital Imaging and Communication in Medicine) file format and then stored in a workstation (MacBook Pro; 2,7 GHz; Apple Corp, Cupertino, CA).

The open-source image computing software 3D Slicer, version 5.2.2 (Brigham and Women's Hospital, Boston, USA) is used to visualize and analyze 3D data.

A semi-automatic segmentation is performed both manually, by drawing seed points or lines on the digital image to select cystic area in the 3 spatial projections (axial, coronal and sagittal), and automatically, by GrowCut segmentation algorithm with contemporary 3D visual examination. The tool "segment statistics - quantification" is used to obtain volume measurements in mm^3.

The segmentation procedure of a mandibular keratocyst with 3D Slicer software is shown in Fig. 3.

For each patient enrolled in the current study, preoperative or initial volume (V_0) and postoperative or follow-up volume (V_1) were calculated. Outcomes of marsupialization were calculated as follows:

- Percentage of VR (VR%) = $[(V_0-V_1)/V_1]$ x 100.
- Daily percentage of reduction (DR%) = $[1 - (V_0-V_1)/\text{time (in days)}]$ x 100.

Possible predictive variables influencing cyst VR included baseline pathologic volume, histological type, patient age, cyst shape (as number of preoperative residual bone walls) and time to follow-up.

2.3. Statistical analysis

Statistical analysis was performed using STATA® software version 17 (StataCorp. 2021. Stata Statistical Software, College Station, TX: StataCorp LLC.). Descriptive statistics were presented for baseline demographic clinical characteristics for the entire group and outcome. Continuous variables were presented as mean, standard deviation (SD), minimum (min), and maximum (max) and compared between subgroups using Unpaired Student's t-test. Analysis of variance (ANOVA) evaluated parameter differences for variables with ≥3 categories. Categorical variables are presented as frequency (N, percentage [%]) and compared using Pearson's chi−squared test. Pearson correlations coefficients were used to assess associations between outcome and preoperative volume, patient age and time.

Linear regression models evaluated associations between independent variables for outcome, with adjustment for relevant confounders. Multivariate regression used a stepwise selection method; first, the intercept−only model was fitted and individual score statistics for potential variables evaluated ($p < 0.05$ was considered significant). Margins statistics was used to obtain the predicted probability of previously fit model at fixed values of outcome and time of treatment for volume groups.

3. Results

A total of 15 mandibular odontogenic cysts in 15 patients (13 male, 2 female) met study inclusion. Median cohort age were 46.4 years (range: 16–85). Treated cyst types included keratocysts (n = 7), dentigerous cysts (n = 6) and radicular cysts (n = 2). Cyst shapes included 4 pre-operative residual bone walls (n = 3), 5 residual bone walls (n = 8), surrounded by bone without any cortical bone

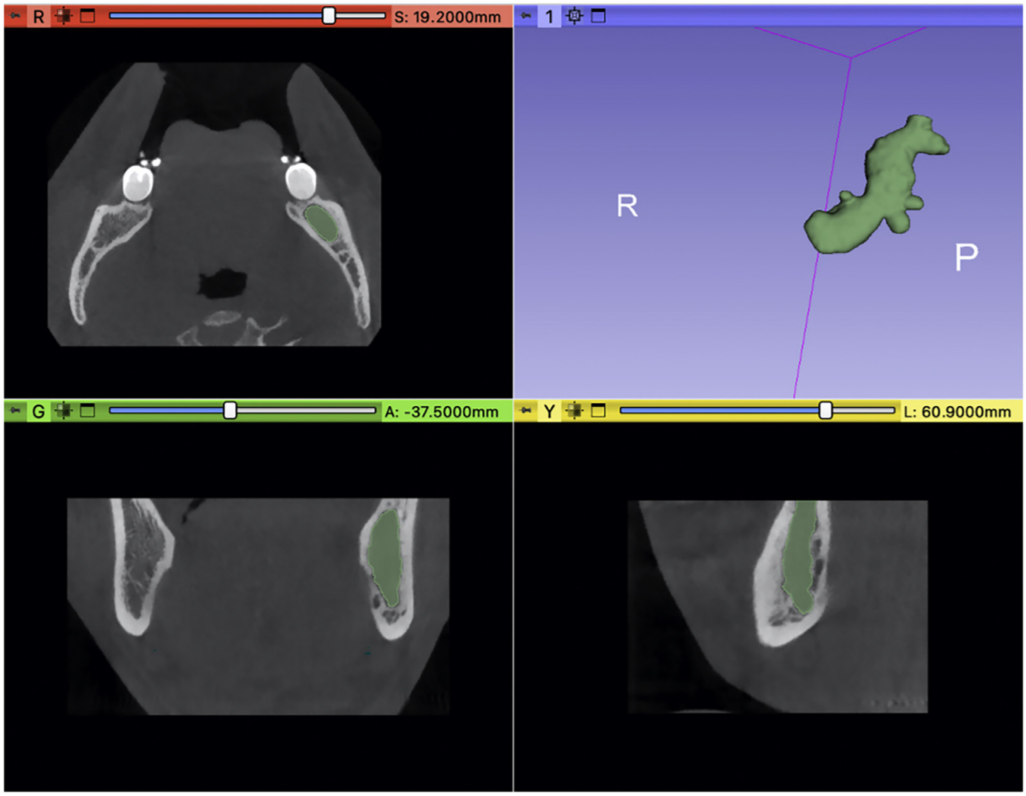

Fig. 3. Cone-beam computed tomography scan showing segmentation of the keratocyst before marsupialization with software 3D Slicer. The lesion is outlined on each slice in 3 spatial planes, by drawing seeds and lines. With GrowCut Segmentation algorithm the software automatically selects the cystic lesion providing a three-dimensional rendering of the cyst.

erosion (n = 4). Overall mean cyst volume at baseline was 6234.2 ± 3139.4 mm^3 (range: 3223.6–15312.7).

During the follow-up period, no post-operative complications were observed. Mean time to CBCT follow-up was 7.4 month (±0.8).

VR was observed in all cysts treated with marsupialization. Overall, median preoperative and postoperative cyst volume significantly reduced more than half (57.7 %), $p < 0.05$. DR% was 0.2 % ± 0.05 (0.16–0.33).

VR% was significantly different between volumes obtained at the 2 follow-up time points ($p = 0.003$). VR% at 8 months was 67.1 %, at 6 and 7 months was = 47.1 %. *[Table 1]*

Univariate analysis identified a correlation between patients' age and VR% (r = -0.604; p = 0.017) and DR% (r = -0.525; p = 0.044) was found. There was also a significant correlation between time to follow-up (in days) and VR% (r = 0.668, p = 0.006) but not with DR% (r = 0.295; p = 0.286). No correlations were found between VR% and DR%, and pathological type, number of residual bone walls and baseline cyst volume (data not shown). A scatterplot of the relationship between follow-up and VR% is shown in Fig. 4.

Table 1
Overall and daily volume reduction according to time to Cone Beam Computed Tomography (CBCT).

Volume reduction, months to CBCT	Mean percentage ±SD (range)	p-value
Overall		
6 and 7 (n = 7)	47.1 ± 10.4 (32.9–61.6)	0.003
8 (n = 8)	67.1 ± 10.6 (48.7–31.4)	
Daily		
6 and 7 (n = 7)	0.23 ± 0.05 (0.16–0.31)	0.127
8 (n = 8)	0.27 ± 0.04 (0.20–0.33)	

Multivariate regression analysis identified time to CBCT as the only predictive variable of cyst VR after marsupialization, see Table 2.

4. Discussion

This retrospective, observational study highlights that following marsupialization for mandibular cysts, a VR% of >50 % is observed at 8 months, but not at 6 and 7 months. Further, the only independent risk factor influencing cyst reduction identified was time to CBCT.

Although there is no consensus of the definition of a large cyst [20,21], the most appropriate treatment option is largely acknowledged to be two-stage surgery, consisting of initial marsupialization or decompression followed by a less invasive and definitive intervention, such as enucleation [22]. Cyst marsupialization enables cyst shrinkage, thereby reducing the risk of nearby anatomical structure involvement and the risk of cyst infection. Cyst infection is a frequent complication in large cysts direct enucleation [23].

Several studies have investigated cyst volume reduction over time following marsupialization and/or decompression, with heterogeneous results in heterogeneous patient populations reported in literature. VR% reductions have been reported in keratocysts following marsupialization. Zhao et al. [15] reported the relative VR% in 53 patients, grouped according to follow-up obtained before and after the 6 month post-operative period, revealing 65 % in patients with follow-up < 6 months and 80 % in patients with a longer follow-up (> 6 months). Shudou et al. [14] demonstrated a gradual VR% in 15 patients; increasing from 50 % at around 8 months, to 75 % at 16 months and 88 % at ∼2 years. Other types of cysts, treated with marsupialization, seem to report similar VR% reductions over time. Consolo et al. [3] analyzed 15 patients with mandibular cysts with a follow-up period ranging from 7 to 20 months post-operative. They reported a mean VR% of 64 % with a mean relative speed reduction of 0.17 %/day. Our results report a mean VR% of 57.7 % ± 14.5 %.

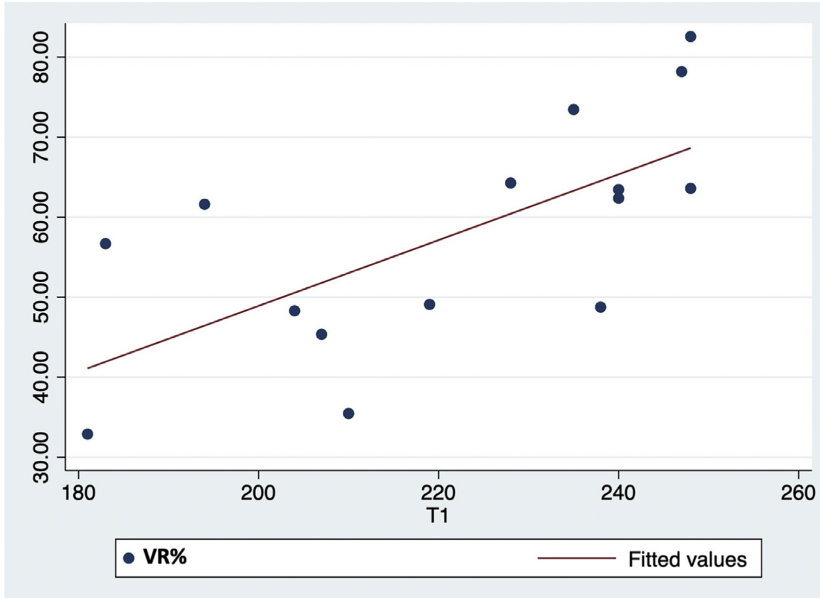

Fig. 4. Q-plot of the duration in days compared with the percentage of cystic volume reduction after marsupialization. The linear regression line is shown representing a linear correlation between duration of marsupialization and cyst volume reduction.

Several studies have reported similar VR% outcomes for decompression as the first stage surgical treatment for cysts. Xiong et al. [24], and Kivovics et al. [5] in a smaller study, both reported VR% at 6 months from decompression between 59 and 63 %. Kwon et al. [11] analyzed 50 decompressed mandibular cysts, revealing a similar VR% to that reported following marsupialization, of 55 % over a mean follow-up period of 9.4 months (3–27).

Few studies have investigated the optimal time of follow-up from marsupialization and/or decompression with different periods of follow-up [3,11,12]. Indications suggest that the ideal time to enucleation is when cyst VR% of at least 50–60 % is achieved [13]. However, definite surgical interventions are usually scheduled at the surgeons' discretion.

Studies have proven that VR is greater in the initial period following surgery. Over time, the speed of VR slows down, due to the reduced intracapsular pressure. Due to osteogenic differentiation, new bone apposition around marsupialized lesions is driven by negative pressure that decreases over time. [25] Xiong et al. [24] demonstrated that intracapsular pressure is highly influential in the initial phases of cyst VR following decompression.

We hypothesized that early surgical enucleation (within 3–6 months of marsupialization) should not be recommended because of low new bone density, poor distancing of the lesion from major anatomical structures and low VR. Therefore, based on previous studies, [14,15] we assumed that the greatest cyst reduction occurs in the first 6–8 months after marsupialization.

Regression analysis highlights that time is the only predictive variable for VR%. According to our analysis of the optimal time of follow-up time prior to enucleation, there is a significant difference between follow-up periods of 6 and 7 months and 8 months, demonstrating that the ideal period between marsupialization and enucleation is 8 months.

Many authors have analyzed correlations between VR and patient age, but the results are discordant. Most studies have reported that age and VR% are not correlate [26,27] However, Song et al. [19] reported a negative correlation between age and VR% for dentigerous cysts only. We report a negative correlation between age and both VR% and DR% ($p = 0.02$ and $p = 0.04$, respectively).

In our study, preoperative cystic volume was not correlated with outcomes. Jeong et al. [18] considered only the amount of VR and not its percentage and reported that the initial volume influenced the amount of cyst shrinkage, especially for large cysts with baseline volume > 10 mL. Kwon et al. [11] however, reported that baseline lesion volume was insignificant to volume reduction throughout the follow-up. Other variables analyzed in this study, including pathological type and number of preoperative bone walls, did not correlate with outcomes of marsupialization efficacy.

Volume variations of cysts are determined by different radiographical methods. Several studies based their volume valuations on two-dimensional radiographs, such as orthopanoramic radiography. This methodology has many limitations due to spatial distortion and the inability to perform three-dimensional reconstructions of the jaws.

Zhao et al. [15] assessed the cystic volume through the quantity of saline solution injected into the cavity. Recently, most studies have used 3D-CT images to allow more accurate volume assessments and exact association between cysts and adjacent anatomical structures [18,28]. As CBCT radiographs are less costly than regular CT scans, emit lower radiant doses and provide more accurate 3-D images [24,27], our study was performed exclusively with CBCT.

Cyst volume measurements by segmentation can be manual, automatic or semi-automatic, depending upon the CT-image software used. The automatic method, based on different HU (Hounsfield) values between cyst, bone and soft tissues, is the simplest

Table 2
Multivariate regression analysis. VR%, volume reduction percentage; DR%, daily percentage of volume reduction.

	Coefficient	*p*-value	[95 % CI]
VR%			
Baseline volume	0.001	0.124	(-0.003–0.0004)
Age	-0.28	0.082	(-0.61–0.04)
Histological cyst type	5.25	0.218	(-3.65–14.16)
Time to CBCT, days	0.32	0.034	(0.03–0.61)
DR%			
Baseline volume	-6.19e-06	0.136	(-0.0001–2.32e-06)
Age	-0.001	0.063	(-0.002–0.00009)
Histological cyst type	0.019	0.308	(-0.02–0.061)
Time to CBCT	0.0001	0.819	(-0.001–0.001)

methodology that does not require operator expertise. However, there is no uniformity in the calibration of the radiodensity in 3D radiographs [29]. Manual segmentation is commonly considered the most precise method, but it is highly operator dependent [30]. In this study, we analyzed images with the 3D Slicer software and GrowCut semi-automatic segmentation algorithm. A recent review demonstrated that GrowCut, a free-license software, is one of the two most accurate semiautomatic segmentation methods in cranio-maxillofacial complex, for surgical planning and outcome evaluation [17]. However, semi-automatic segmentation methods are limited when one or more of the bone walls are undefined (similar radiodensity of cyst cavity and surrounding soft tissue). In this case, manual corrections can be performed.

Our study is principally limited by the small sample size included in this retrospective study. Statistical comparisons may be underestimated, especially in the case of cyst shape or histological type, which may be proven by other larger, prospective studies. Further, our study includes a cohort with a large heterogeneity in cysts' preoperative volumes. However, a consensus definition of what is considered a "large" cyst is still missing in literature. This study only includes cysts located in the mandibular region and outcomes from this study may not be transferable to cysts located in other anatomical regions. As concomitant tooth extraction was not found significantly influence outcome in other studies, we did not evaluate this variable.

Our study proves that the optimal follow-up period between marsupialization and enucleation for mandibular cysts is 8 months. Our study implies that clinicians should anticipate this follow-up period, independent of histological cyst type, patient age, baseline cyst volume and the number of pre-operative residual bone walls. Further studies are required to investigate whether our results are transferable to patients treated with decompression and whether our data are confirmed in large, prospective studies.

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Statement of clinical relevance

The optimal time to definitive enucleation of large mandibular cysts following marsupialization is 8 months after first surgery independently of variables such as pathological cyst type, patient age, baseline cyst volume and cyst shape.

Declaration of competing interest

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 paper.

CRediT authorship contribution statement

Pierantonio Bellini: Methodology, Project administration. **Angelo Ricci:** Conceptualization, Data curation, Resources, Writing – original draft. **Giacomo Setti:** Investigation. **Federica Veneri:** Visualization. **Luca Losi:** Software. **Johanna Chester:** Writing – review & editing. **Ugo Consolo:** Supervision.

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