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Staphylectomy with Onemytis Airplasma versus carbon dioxide laser to correct elongated soft palate: A prospective, randomized, clinical, histological and immunohistochemical trial in dogs

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ABSTRACT

Elongated soft palate is a pathological condition that frequently affects dogs and is often treated surgically by staphylectomy. The aim of the study was to compare clinical efficacy and tissue invasiveness of two staphylectomy techniques: Onemytis® with Airplasma® technology versus CO_2 laser.

Thirty-six dogs with elongated soft palate were included in a prospective, randomized clinical trial and allocated into two groups: 18 in Group OM (Onemytis®) and 18 in Group CO2 (CO_2 laser). Outcomes assessed included clinical parameters (clinical score, surgical duration, owner satisfaction), histological features (edema, thermal damage, hyperemia, margin regularity), and immunohistochemical markers (Caspase-3, Heat Shock Proteins, T.U.N.E.L expression) to evaluate surgical efficacy and iatrogenic injury.

The clinical outcome showed satisfactory and similar clinical efficacy for both surgical techniques ($P > 0.05$). The duration of surgery with Onemytis® was significantly shorter ($P = 0.0004$). Histological ($P < 0.0001$) and immunohistochemical analyses (HSP and T.U.N.E.L: $P < 0.0001$; Caspase-3: $P > 0.05$) consistently indicated that Onemytis® was less invasive than CO_2 laser, causing reduced collateral tissue damage.

Both instruments are suitable in performing staphylectomy in dogs with elongated soft palate, however the results of the present study suggest that Onemytis® is faster and induces less trauma on pharyngeal tissue compared to CO_2 laser.

1. Introduction

Elongated soft palate (ESP) is a congenital airway abnormality frequently found in brachycephalic dogs, but it can occasionally occur in mesocephalic and dolichocephalic breeds (Bright, 1998). It often results in airways obstruction, inflammation and edema. ESP may be accompanied by other primary anatomical abnormalities such as stenotic nares and nasal cavities, rostral or caudal aberrant turbinates, nasopharyngeal mucosal hyperplasia, macroglossia, and tracheal hypoplasia (Coyne and Fingland, 1992; Jones et al., 2020; Oechtering et al., 2016; Poncet et al., 2006; Ševčíková et al., 2022). The resulting excessive negative inspiratory pressure can lead to secondary alterations, including eversion of the laryngeal saccules, tonsillar hypertrophy and eversion, laryngeal, tracheal and bronchial collapse, but also upper gastrointestinal tract pathologies, such as gastroesophageal reflux, regurgitation, vomiting, ptyalism and hiatal hernia (Cook et al., 2015; Dupré and Heidenreich, 2016; Krainer and Dupré, 2022; Poncet et al., 2006; Poncet et al., 2005). The combination of these abnormalities is known as brachycephalic airway obstructive syndrome (BAOS). Clinical presentation may include snoring, coughing, respiratory wheezing/stridor, inspiratory dyspnea, respiratory distress, reduced exercise and heat tolerance, and, in more severe cases, cyanosis, collapse, syncope. The clinical condition is usually progressively worsening until it becomes life-threatening (Hedlund, 2008; Krainer and Dupré, 2022; Riecks et al., 2007; Torrez and Hunt, 2006). The symptoms are often exasperated during exercise or excitement by the resulting edema and inflammation (Torrez and Hunt, 2006).

A recent report showed that ESP and concurrent airway abnormalities were also common among non-brachycephalic dogs, similarly to

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brachycephalic dogs (Himel et al., 2023). A previous study reported that the prevalence of ESP in non-brachycephalic dogs may currently be underestimated (Tambella et al., 2013). Although less prevalent and typically presenting with milder clinical condition, the ESP can be found even in non-brachycephalics, more often as a single alteration. The clinical condition is usually represented by respiratory stridor associated to a reduction of sports or working performances. Non-brachycephalic dogs usually benefit from surgical treatment with a low rate of complications (Himel et al., 2023; Tambella et al., 2013). Furthermore, in non-brachycephalic dogs only an elongation of the soft palate is usually observed, not accompanied by an evident increase in its thickness, as occurs instead in brachycephalic dogs, where it could be explained by a combination of muscular hypertrophy of the palatal muscle and mucosa edema, induced by the increased resistance to air flow in the airways (Grand and Bureau, 2011; Kim et al., 2019).

The most commonly used surgical technique for ESP correction is the staphylectomy: it is aimed at shortening the soft palate by resecting its caudal portion to prevent the obstruction of the glottis rim during inspiration (Krainer and Dupré, 2022). Other techniques are also described to correct excessive thickness and length of the soft palate, such as the folded flap palatoplasty (Findji and Dupré, 2008).

The classic staphylectomy technique imply a staged resection of the soft palate with curved Metzenbaun scissors and immediate resynthesis of the oropharyngeal and nasopharyngeal mucosa flaps by a continuous suture with absorbable monofilament material. The procedure described above is repeated until the complete removal of the surplus portion of the palate (Bright, 1998; Davidson et al., 2001).

Several biomedical devices have been used to perform staphylectomy, including monopolar electrocautery (Dunié-Mérigot et al., 2010), bipolar sealing device (BSD) (Brdecka et al., 2007, 2008; Kirsch et al., 2019), harmonic scalpel (Conte et al., 2022; Michelsen, 2011), CO_2 laser (Clark and Sinibaldi, 1994; Davidson et al., 2001; Kirsch et al., 2019; Tambella et al., 2013), diode laser (Conte et al., 2022; Tamburro et al., 2019), airplasma device (Tamburro et al., 2019; Vezzoni et al., 2016). The literature on the use of Onemytis® with Airplasma® technology in dogs is quite recent and limited in number (Collivignarelli et al., 2022; Lacitignola et al., 2020; Tamburro et al., 2019; Vezzoni et al., 2016). In particular, a general surgery case series reported two staphylectomies with a favorable outcome (Vezzoni et al., 2016). A pilot study compared the clinical complications and histological thermal injuries resulting from Onemytis® and diode laser in dogs that underwent staphylectomy (Tamburro et al., 2019). However, none of the previous studies on the treatment of ESP were designed as a comparative, prospective, randomized study, and none jointly considered clinical, histological and immunohistochemical evaluations in a canine population spontaneously affected by ESP and whose sample size was calculated a priori, as was done in the present study.

The aim of the study was to compare the effect of two surgical instruments, the CO_2 laser and the Onemytis® with Airplasma® technology in performing staphylectomy in dogs with elongated soft palate. The clinical effect, potential complications, and the severity of the iatrogenic lesion resulting from the surgery were compared between the two procedures by performing clinical, histological and immunohistochemical evaluations.

2. Materials and methods

The Animal Welfare Body of the University of Camerino approved the study (protocol n. 5/2022, 1st June 2022) after assessing its scientific merit and legal compliance with the National Legislative Decree 4 March 2014 n. 26, implementation of EU Directive 2010/63/EU.

2.1. Inclusion criteria and allocation in groups

Client-owned dogs of any age, breed, or sex presenting with clinical signs consistent with elongated soft palate, and for which the owners expressed specific informed consent, were included in the study.

All dogs showing other anomalies of the upper airways (including laryngeal collapse, tracheal hypoplasia, eversion of the laryngeal saccules) were excluded from the study to eliminate potential sources of bias that could alter the outcome of the study.

The population was randomly divided into 2 groups with attribution of the surgical technique: Group CO2 (staphylectomy performed with CO_2 laser) and Group OM (staphylectomy performed with Onemytis®). A simple randomization with an allocation ratio 1:1 was performed using the random number generator GraphPad QuickCalcs Software (GraphPad Software Inc., San Diego, CA, USA). Each group was stratified by skull type into brachycephalic (Subgroup B) and non-brachycephalic (Subgroup NB) categories.

2.2. Preoperative clinical evaluation

As a result of clinical history, all dogs potentially eligible for the study underwent physical examination, complete blood count, serum biochemistry, and electrocardiography. If indicated based on initial clinical assessment, general anesthesia was administered to perform laryngoscopy and direct radiographic study of the cervical and thoracic region.

The association of clinical symptoms (including snoring, stertorous gurgling breath sounds, inspiratory difficulty breathing, reduced exercise and heat tolerances) with an extension of the caudal edge of the soft palate more than 5 mm beyond its joint with the epiglottis allowed the diagnosis of elongated soft palate and gave the indication for corrective surgery.

2.3. Surgery procedures

All dogs included in the study underwent the same anesthetic protocol. The premedication was carried out with a sedative-analgesic mixture of dexmedetomidine (1 μg/kg) (3 μg/kg) and methadone (0,3 mg/kg) intramuscularly. Pre-oxygenation was carried out for 5 min using a face-mask and each patient was induced with intravenous propofol (2–4 mg/kg). Once a good muscle relaxation was achieved, endotracheal intubation was performed. General anesthesia was maintained with isoflurane in pure oxygen.

All surgeries were performed with the dog in dorsal recumbency, mouth open, tongue everted and moved using a tongue depressor or a traditional laryngoscope to ensure visualization of the oropharynx.

The caudal edge of the soft palate was grasped with DeBakey anatomical forceps and flexed cranially. The lateral edges of the excess palate were bilaterally anchored with monofilament suture thread (USP 3/0). During the operation, an assistant moderately pulled rostrally the ends of these anchoring sutures to facilitate the exposure of the palate.

With the tongue in a relaxed position, the surgeon estimated the amount of tissue to remove identifying the exact point where the incision should be made: the central portion of the arc-shaped incision was at the point where the tip of the epiglottis touches the soft palate, i.e. the area coinciding with an imaginary line that joins the middle points of each tonsil, as described by (Berger and Eeg, 2006). The goal of the surgery was to adapt the soft palate to the shape of the epiglottis and barely make contact with it.

The palatal resection was performed using the Onemytis® with Airplasma® technique (Group OM) or the CO_2 Laser technique (Group CO2), based on the allocation group.

The duration of surgery and any clinical manifestations of intraoperative complications were recorded. For the purposes of clinical evaluations, the duration of surgery was considered as the time elapsed between the start of the palatal exeresis and the end of the hemostasis and visual control phase of the operative site.

To carry out histological and immunohistochemical evaluations, the excised palatal portion was immersed in 10 % buffered formalin.

During the operation, to avoid the outflow of blood or biological fluids into the respiratory tract and possible tissue damage and combustion, moistened gauze was placed around the endotracheal tube, as described by Davidson and collaborators (Davidson et al., 2001).

Two curved hemostatic forceps were applied to the soft palate at the level of the caudal edge of the tonsillar crypt to allow delimitation of the amputation line bilaterally. These forceps were kept in place throughout the resection phase (Bjorling et al., 2000). The use of hemostatic forceps allows to keep the oropharyngeal and nasopharyngeal mucosa aligned during the cutting phase; this avoids the sliding of the two mucous membranes on each other and allows to obtain an action of the instrument perpendicular to the target surface.

2.4. CO_2 laser technique

In Group CO2, a modified surgical technique described for the first time by Clark and Sinibaldi was performed (Clark and Sinibaldi, 1994). In this study, a SWOT (Shanghai Wonderful Opto-ElecricsTech.Co., Ltd) carbon dioxide laser surgical system, model ML015-CA, was used.

During the palatal exeresis, the laser system was used at a power of 9 ± 2 W, with a focused beam and in superpulse mode.

An arch-shaped incision starting from the lateral edge of the soft palate until reaching the contralateral portion was performed. The laser beam progressively deepened through the nasopharyngeal, muscular and oropharyngeal layers of the soft palate until complete resection was achieved.

Following the resection, keeping the hemostatic forceps in place and setting the laser beam at a lower power (3–4 W), with defocused spot and in continuous mode, a coagulation of the incisional margin was performed, obtaining the introflexion of the ventral and dorsal mucosa; this helped avoid placing sutures on the resection margins. To achieve defocusing, the distance between the end of the handpiece and the target tissue was increased, thereby reducing the power density and increasing the diameter of the spot.

A careful examination of the resection margins followed the removal of the delimiting hemostatic forceps, evaluating the adequacy of the palatal length obtained, the degree of carbonization, adhesion and possible bleeding. An additional defocusing step was performed when necessary.

2.5. Onemytis technique

In Group OM, the staphylectomies were performed using a plasma electrosurgery instrument, the Onemytis® 2 Airplasma® (Otech Industry s.r.l., Alessandria, Italy).

The anesthesia protocol, the positioning of the animal, the methods of visualization of the oropharynx, the identification of the resection limits, the positioning of the limiting forceps and the moistened gauze around the tracheotube were the same as in the Group CO2.

To obtain the soft palate resection, the device was used in continuous mode at a power of 70–80 %, progressively acting on the same layers encountered with the CO_2 laser, i.e.: nasopharyngeal mucosa, muscular layer and oropharyngeal mucosa.

Once the exuberant portion of the palate was removed, keeping the hemostatic forceps in place, Onemytis® was used to coagulate the incision margin with introflection of the ventral and dorsal mucosa. The power was set at a lower level and the handpiece was directed orthogonally to the cutting plane and longitudinally to the axis of the palate.

The limiting forceps were removed and the resection margins were subjected to careful examination to evaluate the presence of charring, hemorrhage and adhesion, as well as the adequacy of the length obtained. No sutures were placed on the resection margins. In most cases the surgery was bloodless; however, when a slight hemorrhage occurred, hemostasis was performed using Onemytis® with soft localized touches.

2.6. Postoperative care and clinical follow-up

After surgery, food intake was prohibited for 12 h. For the first 3 days, only soft canned commercial food was allowed. The dogs were hospitalized for at least 24 h.

Each dog underwent clinical evaluation at the beginning of the study (T0) and during the postoperative *follow-up* period, 24 h (T1), 30 days (T2) and 6 months (T3) after surgery.

2.7. Clinical evaluation

To assess the severity of the condition, a six-levels clinical scoring system was used (Tambella et al., 2013): 0 = health, full recovery; 1 = snoring breathing (frequent or manifest only after intense physical exercise), reduction of exercise tolerance and physical performance; 2 = recurrent snoring with mild dyspnea upon exercise or following particular environmental conditions, stress, or excitement; 3 = snoring breathing, episodes of mild dyspnea at rest; 4 = snoring breathing, average permanent dyspnea at rest; 5 = snoring breathing, severe dyspnea, hypoxia, cyanosis and syncope.

2.8. Degree of owner satisfaction

Six months after surgery (T3), each owner's perception of the overall results of surgery was collected according to the criteria used by Dunié-Mérigot and collaborators (Dunié-Mérigot et al., 2010): *excellent*: marked clinical improvement with limited or no residual clinical signs and no activity restriction; *good*: clinical improvement with residual signs and restriction of activity; *fair*: unchanged clinical condition; *poor*: worsening of the clinical condition.

2.9. Histological evaluation

The excised portion of the soft palate was kept in a 10 % buffered formalin solution for 24 h. Then the samples were dehydrated with alcoholic solutions of increasing concentration, and cleared in with an ethanol/xylene solution before embedding in paraffin wax. Three-μm thick histological sections were stained with Hematoxylin-Eosin and mounted for the observation under the optical microscope (Leica DM2500) using different magnifications.

To histologically quantify the severity of the iatrogenic lesion induced by surgery, a score from 0 to 3 points was assigned for the following four parameters:

Edema (0 = no edema; 1 = mild edema, localized in the perivascular area, in the absence of lymphangiectasia; 2 = moderate superficial edema with diffuse sub-epithelial localization and with mild lymphangiectasia; 3 = severe, diffuse, superficial and deep edema, generalized to the whole sample and with moderate-severe lymphangectasia);

Burning (0 = no burning; 1 = slight presence of some areas of tissue carbonization on the excision line; 2 = presence of a moderate condition of carbonization in some points of the excision edge; 3 = severe charring along the entire line of excision, with the characteristic two areas of the wall);

Hyperemia (0 = absence of hyperemia; 1 = slight hyperemia with capillaries in some points without red blood cells and with a tense wall; 2 = presence of a moderate condition of hyperemia, with all the capillaries filled with red blood cells and with a dilated wall; 3 = severe condition of hyperemia with all highly dilated capillaries, filled with red blood cells and with high diapedesis of red blood cells through the vessel wall, infiltrating the perivascular connective tissue; frankly hemorrhagic tissue);

Margin (0 = regular margin; 1 = margin slightly irregular, with areas of coarctation of the tissue; 2 = moderately irregular margin with areas of tissue distress and tissue coarctation; 3 = strongly irregular margin, sometimes not evaluable and with frayed edge, with high coarctation of the tissue).

2.10. Immunohistochemical and T.U.N.E.L evaluation

The same paraffin embedded soft tissue samples used for histological evaluation were sectioned to obtained three-μm thick histological section and placed on polarized slides. For the evaluation of total amount of apoptotic cells, the sections were stained with DeadEnd™ Colorimetric *Terminal deoxynucleotidyl transferase dUTP nick end labeling* (T.U.N.E.L) System (*Promega* Italia Srl, Italy) according to the manufacturer instructions, and with a Rabbit Polyclonal anti-Caspase-3 antibody, (diluted 1:100, ab2302, Abcam, Cambridge, UK).

Thermal stress was evaluated via Hsp70 expression using a monoclonal antibody (diluted 1:300, ab 5439, Abcam, Cambridge, UK). For each sample, close to the cut line, three microscopic fields were evaluated using a light microscope (Carl Zeiss, Jena, Germany), a × 40 objective, a × 10 eyepiece, and a square eyepiece graticule (10 × 10 squares, having a total area of 62,500 μm^2). Ten appropriate fields were chosen for each compartment of the excised soft palate portion, and arithmetic means were calculated for each tissue region. Results were expressed as IHC positive cells per 62,500 μm^2.

2.11. Statistical analysis

To calculate the sample size a priori, the Wilcoxon-Mann-Whitney test was used, setting an alpha error of 0.05 and a power of 80 %, obtaining Cohen's d from the results of the study by Brdecka and collaborators (Brdecka et al., 2007). A post-hoc power analysis was performed with the data (effect size and sample size) achieved at the end of the study. The power and sample size analyses were performed with the G*Power software (version 3.1.9.6).

The following comparative analyses were performed on the data:

- Comparison of the clinical score between groups and between subgroups at each observation time;
- Clinical trend over time in groups and subgroups;
- Comparison between groups and between subgroups in relation to reaching the "0" score during follow-up.
- Comparison between groups and subgroups in relation to the time to complete the intervention.
- Comparison of the total histological score between groups and between subgroups;
- Comparison of the number of cells positive cells for immunohistochemical markers between groups and between subgroups.

Data deriving from categorical variables were evaluated with Fisher's exact test.

The ordinal variables were summarized using median and range and analyzed with the Mann-Whitney rank sum test (inter-group/−subgroup comparisons at each observation time) and with the Friedman test and Dunn's post-hoc test for multiple comparisons (evaluation of the trend over time).

The cardinal variables were analyzed for the assumption of normality of the data distribution and homoscedasticity using the Shapiro-Wilk test and the F-test respectively; they were summarized using the arithmetic mean and the standard error of mean (sem) and analyzed using the Student's *t*-test (comparison between groups/subgroups).

P values <0.05 were considered statistically significant. All data were analyzed using GraphPad Prism 10 statistical software for MacOS, version 10.1.1–270 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

A total of 36 dogs were included in the study and underwent staphylectomy to correct the elongation of the soft palate; 18 dogs were allocated in Group CO2 and 18 dogs in Group OM.

The final sample size (n = 36) exceeded the minimum sample size (n = 34) estimated a priori for adequate statistical power of a reliable randomized controlled study (effect size d = 1.017827; alpha error = 0.05; power = 80 %). At *post-hoc* analysis, the power of the study reached the level of 99.84 % with a total sample size of 36 dogs, effect size d = 3.499937, alpha error = 0.05.

Baseline demographic data did not show statistical differences between groups, nor between subgroups (Table 1).

The baseline clinical findings were normal and the results of the hematological analyses performed before the anesthesia were within the normal reference ranges. No tracheostomy was required in any dog, and all maintained orotracheal intubation during surgery. No anesthetic complications occurred. Four dogs per group showed mild intraoperative bleeding, which was quickly resolved with temporary pressure with a gauze pad. In all other dogs, the operating field was completely bloodless during the entire procedure. No dog required suturing of the edges of the palatal incision. Four dogs in the Group CO2 and two dogs in the Group OM showed mild signs of post-operative edema, which was resolved by prolonging treatment with dexamethasone sodium phosphate (1 mg/kg) for 3 days.

Table 1
Demographic data of the population under study.

A. Age

Group/Subgroup	Both subgroups	B	NB	Statistics between subgroups
CO2	33.44 ± 5.21	24.88 ± 6.06	40.30 ± 7.59	t = 1.528, P = 0.1462
OM	34.0 ± 4.47	38.40 ± 6.88	28.50 ± 5.04	t = 1.107, P = 0.2845
Statistics	t = 0.0809, P = 0.9360	t = 1.435, P = 0.1707	t = 1.224, P = 0.2388	

B. Body Weight

Group/Subgroup	Both subgroups	B	NB	Statistics between subgroups
CO2	18.78 ± 2.52	20.25 ± 5.09	17.60 ± 2.25	t = 0.5113, P = 0.6161
OM	16.06 ± 2.74	12.80 ± 2.67	20.13 ± 5.03	t = 1.363, P = 0.1918
Statistics	t = 0.7318, P = 0.4693	t = 1.373, P = 0.1886	t = 0.4922, P = 0.6292	

C. Sex

Group/Subgroup	Both subgroups	B	NB	Statistics between subgroups
CO2	10 M, 8F	4M, 4F	6M, 4F	P > 0.99
OM	9 M, 9F	4M, 6F	5M, 3F	P = 0.6372
Statistics	P > 0.99	P > 0.99	P > 0.99	

D. Breed

Group/Subgroup	B	NB
CO2	8	10
OM	10	8
Statistics	P = 0.7395	

Mean values ± sem of age (months) [Table 1A], body weight (kg) [Table 1B]. Frequencies of sex (number of dogs) [Table 1C] and breed [Table 1D] in Subgroups within each Group. B: Subgroup B (brachycephalic subgroup); NB: Subgroup NB (non-brachycephalic subgroup); CO2: Group CO2 (CO_2 laser); OM: Group OM (Onemytis); M: male; F: female. P: P-value; t: result of the t-test.

3.1. Comparison of the clinical score between groups and within each group

The clinical score reported before surgery (T0) in the Group CO2 (median: 2, range: 2–4) and in the Group OM (median: 2, range: 2–4) showed no statistically significant differences (U = 149; $P = 0.7097$).

Even during *follow-up*, all other comparisons did not show a statistically significant difference between groups (Fig. 1A):

- At T1: CO2 (median 1.5, range 0–4) vs OM (median 2, range 0–3); U = 159; $P = 0.9151$;
- At T2: CO2 (median 0, range 0–3) vs OM (median 0.5, range 0–2); U = 160.5; $P = 0.9476$;
- At T3: CO2 (median 0, range 0–2) vs OM (median 0, range 0–1); U = 159; $P > 0.99$.

Within the Group CO2, a statistically significant improvement in the clinical trend was observed over time ($\chi^2_r = 45.95$; $P < 10^{-4}$) with significant difference showed in the following comparisons (Fig. 1A): T0 vs T2: $P < 10^{-4}$; T0 vs T3: $P < 10^{-4}$; T1 vs T2: $P < 0.048$; T1 vs T3: $P = 0.003$.

Within the Group OM, a statistically significant improvement in the clinical trend was observed over time ($\chi^2_r = 45.52$; $P < 10^{-4}$) with significant difference showed in the following comparisons (Fig. 1A): T0 vs T2: $P < 10^{-4}$; T0 vs T3: $P < 10^{-4}$; T1 vs T3: $P < 0.0179$.

3.2. Comparison of the clinical score between brachycephalic (B) and non-brachycephalic (NB) dogs in each Group at each observation time

3.2.1. Group CO2

In the Group CO2 the clinical score reported at T0 in the subgroup B (median 2.5, range 2–4) was not significantly different (U = 26; $P = 0.18$) from that reported in the subgroup NB (median 2, range 2–3). At T1, the comparison between subgroups B (median 2, range 1–4) and NB (median 0.5, range 0–2) highlighted a statistically significant difference (U = 12.50; $P = 0.0127$), as well as at T2 (B: 1, 0–3; NB: 0, 0–1; U = 7.5; $P = 0.0012$) and at T3 (B: 1, 0–2; NB: 0, 0–0; U = 10; $P = 0.0015$) (Fig. 1B).

3.2.2. Group OM

In the Group OM the clinical score reported at T0 in the B subgroup B (median 3, range 2–4) was not significantly different (U = 25; $P = 0.15$) from that reported in the subgroup NB (median 2, range 2–3), as well as at T1 (B: 2, 0–3; NB: 2, 0–2; U = 33.5; $P = 0.58$), at T2 (B: 1, 0–1; NB: 0, 0–2; U = 34; $P = 0.637$) and at T3 (B: 0.5, 0–1; NB: 0, 0–1; U = 25; P = 0.15) (Fig. 1B).

3.3. Evaluation of the clinical trend over time in the two subgroups (brachycephalic and non-brachycephalic dogs)

The 8 B-dogs in Group CO2 showed a statistically significant improvement in the clinical trend over time ($\chi^2_r = 20.28$; $P = 0.0001$) with significant differences from T0 (2.5, 2–4) to T2 (1, 0–3; $P = 0.0299$) and from T0 to T3 (1, 0–2; $P = 0.0010$) (Fig. 1B).

The 10 B-dogs in Group OM showed a statistically significant improvement in the clinical trend over time ($\chi^2_r = 26.29$; $P < 10^{-4}$) with significant differences from T0 (3, 2–4) to T2 (1, 0–1; $P = 0.0004$) and from T0 to T3 (0.5, 0–1; $P = 0.0003$) (Fig. 1B).

The 10 NB-dogs in Group CO2 showed a statistically significant improvement in the clinical tend over time ($\chi^2_r = 24.13$; P $< 10^{-4}$) with significant differences from T0 (2,2–3) to T2 (0, 0–1; $P = 0.0032$) and from T0 to T3 (0, 0–0; P = 0,0012) (Fig. 1B).

The 8 NB-dogs in Group OM showed a statistically significant improvement in the clinical trend over time ($\chi^2_r = 19.59$; $P = 0.0002$) with significant differences from T0 (2, 2–3) to T2 (0, 0–2; $P = 0.0221$) and from T0 to T3 (0, 0–1; $P = 0.0014$) (Fig. 1B).

3.4. Evaluation of the zeroing of the clinical score during the follow-up

During follow-up there was a progressive increase in the frequencies of clinical score zeroing with no significant differences between the two groups at each of the postoperative times ($P > 0.99$) (Table 2).

Comparing the subgroups within each group, the frequency of resetting was always higher in NB dogs, with a non-significant difference in the Group OM, while it was significant within the Group CO2 ($P = 0.0359$ in T1; $P = 0.0029$ in T2; $P = 0.0015$ in T3).

Table 2
Distribution of the population (absolute frequencies, number of dogs) after attribution of the clinical score at each time point summarized by group.

Group	*Clinical score*	*0*	*1*	*2*	*3*	*4*	*5*
CO2	*T0*	0	0	12	4	2	0
	T1	2	7	6	2	1	0
	T2	10	5	1	2	0	0
	T3	12	5	1	0	0	0
OM	T0	0	0	10	7	1	0
	T1	3	4	9	2	0	0
	T2	9	8	1	0	0	0
	T3	12	6	0	0	0	0

CO2: Group CO2 laser; OM: Group Onemytis; T0: baseline, before surgery; T1: 24 h after surgery; T2: 1 month after surgery; T3: 6 months after surgery. The legend of the scoring scale is shown in the text.

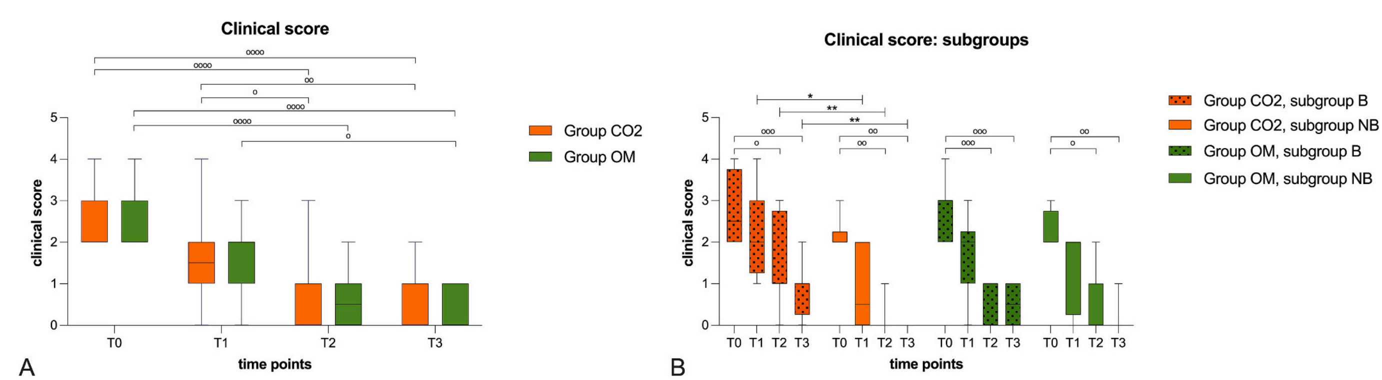

Fig. 1. Boxplot showing the clinical scores in the two groups [Fig. 1-A] and in subgroups [Fig. 1-B] at baseline (T0) and during follow up: 24 h (T1), 30 days (T2) and 6 months (T3) after surgery. Group CO2: CO_2 laser group; Group OM: Onemytis group. B: Subgroup B (brachycephalic subgroup); NB: Subgroup NB (non-brachycephalic subgroup). The ends of the whiskers show minimum and maximum score values; boxes show the median, the first and the third quartile. Asterisks indicate significant differences between groups. Circles indicate significant differences within each group. *P*-values, */°: $P < 0.05$; **/°°: $P < 0.01$; °°°: $P < 0.001$; °°°°: $P < 0.0001$.

3.5. Time required to complete the surgery

Staphylectomy in the Group OM (mean time in seconds ± sem: 254.1 ± 7.6) was significantly faster than in the Group CO2 (313.5 ± 12.9) ($t = 3.965$; P = 0.0004) (Fig. 2A).

Similar results were obtained considering the subgroups separately. In B dogs, the duration of surgery was significantly shorter when the Onemytis® technique was used (mean time in seconds: 263.0 ± 9.6) compared to the laser technique (mean time in seconds: 353.8 ± 17.8) ($t = 4.731$; P = 0.0002). Also in NB dogs, the duration of surgery with Onemytis® (mean time in seconds: 242.9 ± 11.5) was significantly shorter than with the laser technique (mean time in seconds: 291.3 ± 13.7) ($t = 2.623$; $P = 0.0185$) (Fig. 2B).

Within Group CO2, subgroup B (mean time in seconds: 353.8 ± 17.8) required a longer duration of surgery compared to subgroup NB (mean time in seconds: 291.3 ± 13.7); the difference between subgroups was statistically significant ($t = 2.830$; $P = 0.0121$). Within Group OM, no statistically significant difference was observed ($t = 1.352$; $P = 0.1953$) comparing subgroups B (mean time in seconds: 263.0 ± 9.6) and NB (mean time in seconds: 242.9 ± 11.5) (Fig. 2B).

3.6. Degree of owner satisfaction

The Group CO2 was rated *good* in 7 cases and *excellent* in 11 cases. The Group OM reported *good* in 6 cases and *excellent* in 12 cases. The owners' perception of the overall effectiveness of the procedures was therefore very favorable and with no significant differences between the two groups ($P > 0.99$).

3.7. Histological comparison between groups and between subgroups

The values of the total histological score obtained in the Group OM (median 6, range 3–10) are significantly lower (U = 38.50; $P < 10^{-4}$) than the Group CO2 (median 9, range 7–11) (Fig. 3A).

Comparing the two groups for individual parameters (edema, burning, hyperemia and margin) better results were always reported by Group OM. The difference between groups was significant for Burning (Group CO2: 2, 1–3; Group OM: 1, 0–3; U = 49; $P = 0.0001$) and Margin (Group CO2: 3, 0–3; Group OM: 2, 0–3; U = 64; $P = 0.0009$) (Fig. 3B).

Considering just the subgroup B, the degree of histological lesion was significantly lower when the OM technique was used (median 6.5, range 3–10) compared to the CO2 technique (median 9, range 7–10) (U = 17; $P = 0.0392$) (Fig. 3C). A similar result (U = 0.0; $P < 10^{-4}$) emerged considering only subgroup NB (OM-NB: 5, 3–6; CO2-NB: 9, 7–11) (Fig. 3C).

Within Group OM, subgroup B (median 6.5, range 3–10) showed a higher degree of histological injury than subgroup NB (median 5, range 3–6) (U = 17; $P = 0.0347$). Within Group CO2, no significant difference emerged between subgroups (B: 9, 7–10; NB: 9, 7–11; U = 40; P > 0.99) (Fig. 3C).

3.8. Immunohistochemical comparison between groups and between subgroups

The Group OM showed a clear reduction in the expression of all stress and thermal damage markers, with a notable reduction in the fraction of apoptotic cells compared to the palate samples of the CO2 Group (Fig. 4).

3.8.1. Caspase-3

The number of positive cells for the anti-Caspase 3 antibody in the Group CO2 (mean ± sem: 17.44 ± 3.61) and in the Group OM (11.40 ± 1.15) did not show statistically significant differences ($t = 1.596$; $P = 0.1198$) (Fig. 5A).

There were no significant differences between the surgical techniques even when considering separately only B-dogs (CO2-B: 22.0 ± 6.97; OM-B: 11.90 ± 1.66; $t = 1.563$; $P = 0.1377$), or only NB-dogs (CO2-NB: 13.80 ± 3.28; OM-NB: 10.78 ± 1.62; $t = 0.7639$; $P = 0.456$) (Fig. 5B).

Subgroup comparisons did not show significant differences for Caspasi 3, neither within the Group CO2 ($t = 1.138$; $P = 0.2717$) nor within the Group OM ($t = 0.4760$; $P = 0.6405$) (Fig. 5B).

3.8.2. HSP

A higher number of positive cells for HSP were counted in Group CO2 (mean ± sem: 55.50 ± 4.44) than in Group OM (21.27 ± 1.40), with a significant difference between groups ($t = 7.353$; P < 10^{-4}) (Fig. 5C).

Similar result emerged considering subgroup B (CO2-B: 54.90 ± 7.20; OM-B: 19.28 ± 1.97; $t = 5.267$; P < 10^{-4}) or subgroup NB (CO2-NB: 55.98 ± 5.89; OM-NB: 23.75 ± 1.69; $t = 4.742$; $P = 0.0002$)

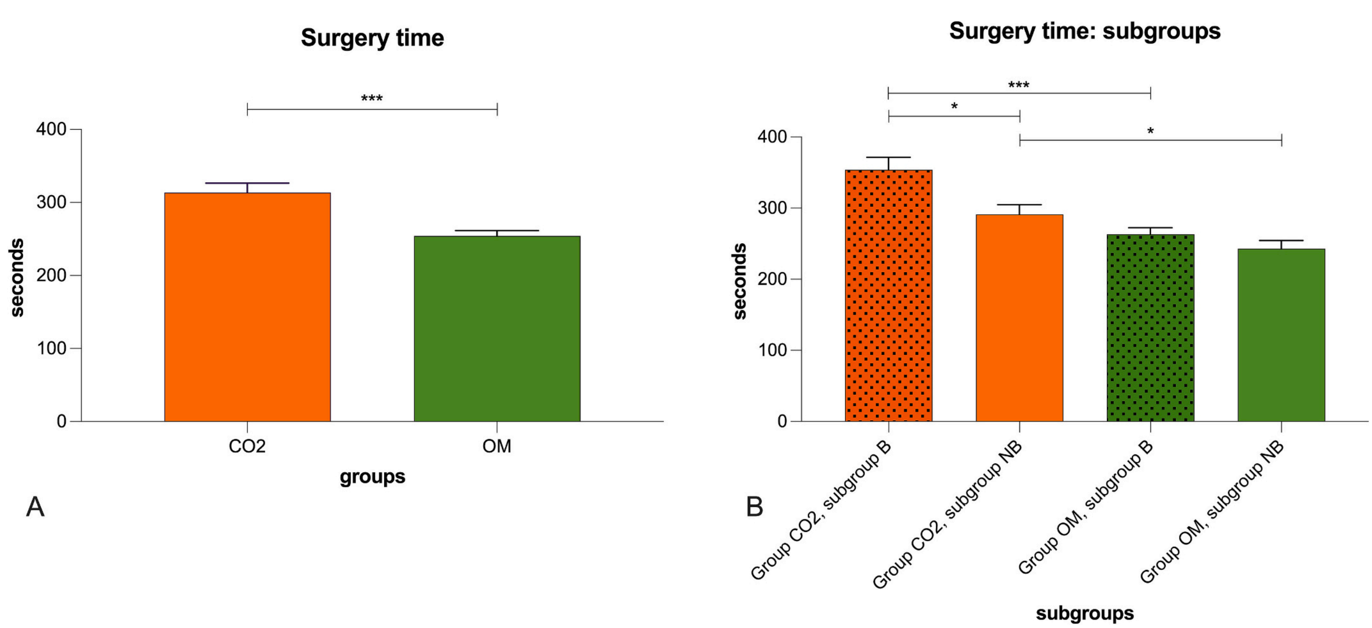

Fig. 2. Mean time ± standard error (in seconds) required to complete surgery in the Group CO2 (CO_2 laser group) and in the Group OM (Onemytis group) [Fig. 2-A] and in subgroups [Fig. 2-B]. Asterisks indicate significant differences. *P*-values, *: $P < 0.05$; ***: $P < 0.001$.

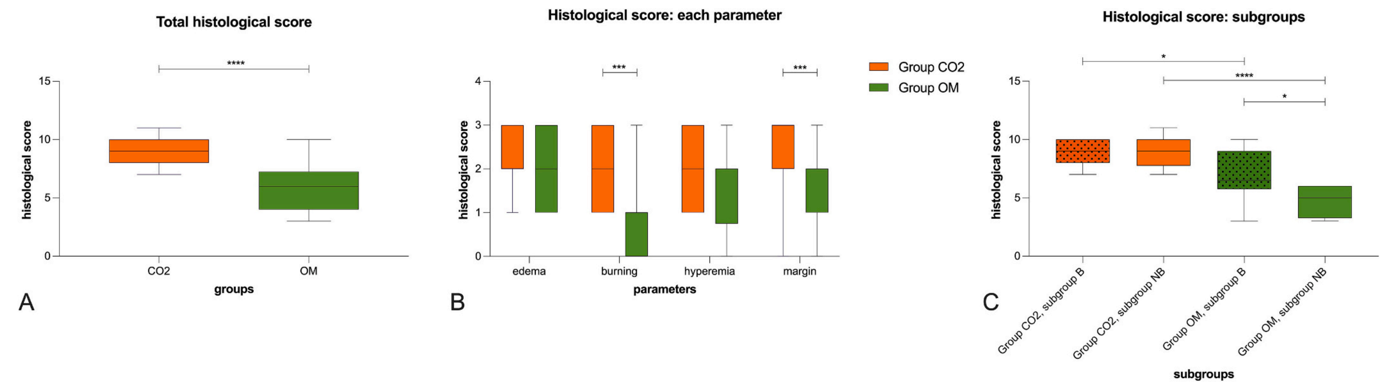

Fig. 3. Boxplot showing the total histological score detected in the Group CO2 (CO_2 laser group) and in the Group OM (Onemytis group) [Fig. 3-A], the histological scores in Group CO2 and in the Group BSD for each parameter (edema, burning, hyperemia, margin) [Fig. 3-B], and the total histological score in subgroups, within each group [Fig. 3-C]. B: Subgroup B (brachycephalic subgroup); NB: Subgroup NB (non-brachycephalic subgroup). The ends of the whiskers show minimum and maximum score values; boxes show the median, the first and the third quartile. Asterisks indicate significant differences. P-values, *: $P < 0.05$; ***: $P < 0.001$; ****: $P < 0.0001$.

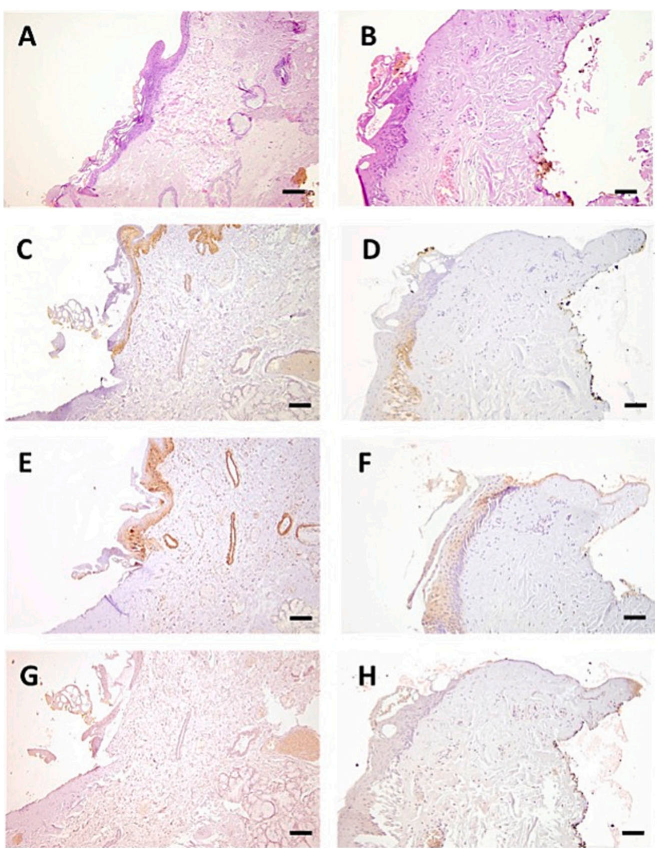

Fig. 4. Portion of the palate excised with CO2 Laser (A) and Onemytis® (B), morphological appearance. (C—H), TUNEL and IHC results of different antibodies applied to histological sections. TUNEL expression in epithelial and mesenchymal cells of soft palate removed with CO_2 laser (C) and Onemytis® (D), with evident differences in apoptosis rate. Similar differences in terms of HSP70 positive cells are evident in tissue removed with CO_2 laser (E) and Onemytis® (F). No differences are present in terms of Caspase-3 expression in mesenchymal or epithelial cells between CO_2 laser (G) and Onemytis® (H). (A, B), H&E, 10×; (C,D) *Terminal deoxynucleotidyl transferase dUTP nick end labeling* (TUNEL); Mayer's nuclear counterstain. (E,F,G,H) IHC stain; Mayer's nuclear counterstain, 10×; (Scale bars = 300 μm).

(Fig. 5D).

No significant differences were shown between subgroups B and NB, neither within the Group CO2 ($t = 0.1173$; $P = 0.9081$) nor within the Group OM ($t = 1.672$; $P = 0.1139$) (Fig. 5D).

3.8.3. Tunel

Considering Tunel, a higher number of positive cells were counted in Group CO2 (mean ± sem: 69.23 ± 6.11) than in Group OM (23.84 ± 1.52), with a significant difference between groups ($t = 7.207$; $P < 10^{-4}$) (Fig. 5E).

A significant difference between the two groups were shown both in B dogs (CO2-B: 70.85 ± 10.99; OM-B: 20.22 ± 1.66; $t = 5.10$; $P = 0.0001$); and in NB dogs (CO2-NB: 67.94 ± 7.17; OM-NB: 28.38 ± 1.72; $t = 4.818$; $P = 0.0002$) (Fig. 5F).

Within the Group OM, a significant difference between B- and NB-dogs was shown ($t = 3.380$; $P = 0.0038$), while no significant difference was shown within the Group CO2 ($t = 0.2299$; $P = 0.8211$) (Fig. 5F).

4. Discussion

In this randomized study, the plasma surgery qualities of the Onemytis® in comparison with the CO_2 laser instrument were investigated in dogs.

Onemytis® has already been evaluated in performing staphylectomy (Tamburro et al., 2019) but with a smaller series, comparing it to the diode laser, and with a main histomorphological outcome. In the present study, clinical, histomorphological and immunohistochemical outcomes were considered to compare OM and CO_2 laser. Immunohistochemistry is considered a very accurate technique in assessing thermal damage induced in tissues by electrosurgery instruments. Nonetheless, immunohistochemical analyses are rarely encountered in these types of studies; this aspect further characterizes the present study.

Non-brachycephalic dogs with elongated soft palate, unlike B dogs, are only rarely suffering from other primary upper respiratory conditions (Tambella et al., 2013), although they may have secondary obstructive conditions such as everted laryngeal saccules, laryngeal or tracheal collapse (Himel et al., 2023). To reduce confounding factors, dogs with concurrent upper airway anomalies were excluded from the study. While the exclusion of dogs with concurrent airway anomalies improves internal validity, it also limits the generalizability to the broader brachycephalic population, where comorbidities are common. The narrowness of the inclusion criteria influenced the overall level of severity of clinical conditions by excluding complex and severe conditions. In fact, at T0 only 3 dogs (two in the Group CO2 and one in the Group OM) recorded a 4-points and no dogs recorded 5-points clinical score. Among B dogs, this aspect limited the number of includable dogs, as the elongated soft palate falls within the brachycephalic airway

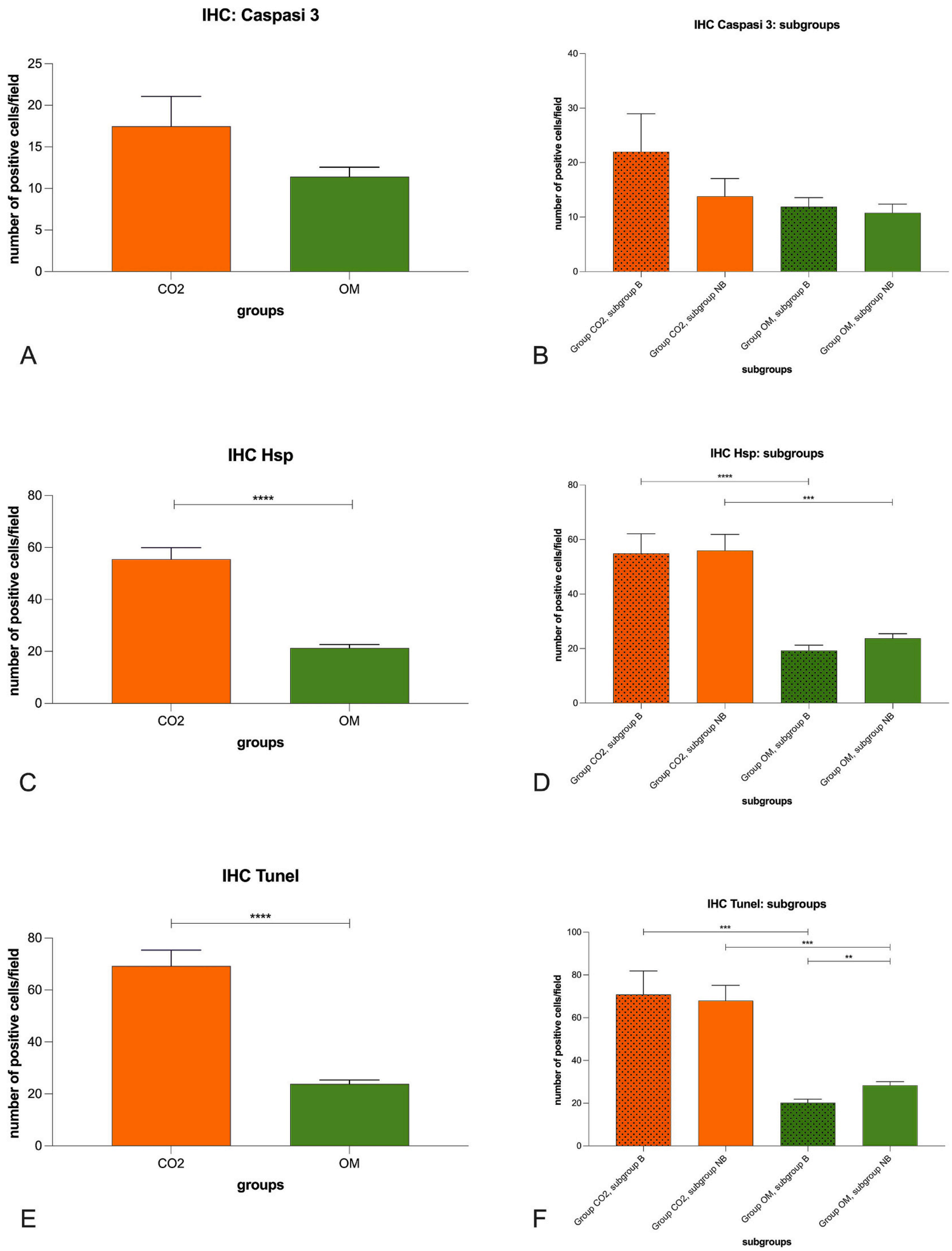

Fig. 5. Mean values ± standard error of the number of positive cells to Caspasi 3 antibody [Fig. 5, A and B], HSP antibody [Fig. 5, C and D], and TUNEL antibody [Fig. 5, E and F] in groups [Fig. 5, A,C,E] and subgroups [Fig. 5, B,D,F]. Group CO2: CO_2 laser group; Group OM: Onemytis group. B: Subgroup B (brachycephalic subgroup); NB: Subgroup NB (non-brachycephalic subgroup). Asterisks indicate significant differences. P-values, **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$.

syndrome (BAOS) where various primary and secondary alterations usually coexist.

The minimum sample size of the population was calculated a priori in order to determine the number of dogs required to increase the strength of the study and obtain statistically detectable differences between the groups.

The comparison between the B and NB subgroups is a further strength of this study, since the scientific literature considering the elongated soft palate in NB dogs is rather limited (Arai et al., 2016; Himel et al., 2023; Koch et al., 2014; Tambella et al., 2013).

Baseline demographic and clinical data were sufficiently homogeneous between groups to design a quality comparative study.

Most authors, when describing the staphylectomy technique, place the patient in ventral decubitus. In our case, on the contrary, dorsal recumbency was preferred. This recumbency helped to move the endotracheal tube away from the area of action of the two instruments and therefore limit the risk of its perforation, an event that could create serious accidents.

Regarding the feasibility of use, the articulated arm reduces the handling of the CO_2 laser. Onemytis® is very easy to handle: the small size of the handpiece (with electrodes of various shapes and lengths) allows the surgeon to easily operate in the oral cavity, and it is likely that this advantage could arise in other types of surgery, even in other districts.

When using the laser, all safety precautions must be taken, including the use of specific protective glasses (Brdecka et al., 2007). In contrast, there are no such prescriptions when using Onemytis®.

From the results of the study, it is possible to state that both surgical techniques are excellent therapeutic solutions for the elongated soft palate. The evaluation of the clinical score showed no significant differences between the two techniques. This confirms the already demonstrated validity of electrosurgery devices in staphylectomy in reducing bleeding, the duration of surgery and intra- and post-operative complications. While in the traditional "cut and over-sew" technique intraoperative bleeding is frequently encountered (Conte et al., 2022), in our study it occurred mildly in 4 dogs in the Group CO2 and 4 dogs in the Group OM.

The use of an electric scalpel could therefore also be proposed, allowing a quick technique and reducing intraoperative bleeding. However, it is often associated with the development of post-operative swelling (MacPhail, 2012), while with the devices evaluated the post-operative edema was occasional and mild in 4 dogs in the Group CO2 and in 2 dogs in the Group OM.

It has already been proven that using electrosurgery techniques, palatal excision occurs more quickly than the traditional technique (Conte et al., 2022); the suturing phase is in fact completely avoided with the electrosurgical techniques used. The duration of surgery was significantly shorter for the Group OM compared to the Group CO2. To justify the longer surgical times observed in B-dogs of Group CO2, we must consider that the lengthening of the soft palate in this subgroup is often concomitant with its thickening (Krainer and Dupré, 2022) therefore multiple passages of the laser beam are necessary to complete the resection. Furthermore, these outcomes can be partly explained by the greater manageability of the Onemytis® handpiece in the narrow oral cavity of brachycephalic dogs.

Considering the clinical follow up in subgroups, statistically significant differences were found between B-dogs and NB-dogs, within the Group CO2 (at T1, T2 and T3), showing a higher score in brachycephalic dogs, due to their well-known and typical tendency to respiratory stress. However, the clinical trend of each subgroup during follow up was still oriented towards significant improvement and often towards resolution of the clinical condition for both devices used.

The owner's perception of the overall effectiveness of the surgery was very positive and comparable between the two techniques.

No significant difference between the Groups CO2 and BSD appeared in the frequency of zeroing of the clinical score, demonstrating a similar therapeutic effectiveness. Considering the analysis between subgroups, the outcome of zeroing was always higher in non-brachycephalic dogs with significant difference within the Group CO2.

The results of histological evaluations reveal the superiority of Onemytis®, in terms of surgery-induced lesions. The total histological score was significantly lower in the OM group, and also in all histological parameters (edema, burning, hyperemia and margin) the results were better for Onemytis®, with statistically significant differences between groups for burning and margin. Another study, however, showed a similar palatal histolesivity of Onemytis® compared to the diode laser (Tamburro et al., 2019).

The results of the immunohistochemistry agreed with those of the histological score, clearly showing a lower iatrogenic lesion in the Group OM compared to the Group CO2. This difference can be explained by the different type of interaction of the two devices with biological tissues.

The CO_2 laser produces a strong photothermal action at the tissue level, with induction of effects linked to the temperature reached in the tissues, such as coagulation, vaporization, carbonization and fusion (Berger and Eeg, 2006). When tissue ablation is achieved with the laser, the tissue damage can be more or less evident also based on the thermal relaxation time (TRT). The TRT is the time necessary for target tissue to cool down by 50 % through transfer of heat to surrounding tissues (Berger and Eeg, 2006). In this study, to obtain an efficient photothermal ablation, minimizing collateral damage to the surrounding tissue as much as possible, a superpulse mode was used during palatal exeresis. The superpulse mode is bursts of short laser pulses with very high peak power that are spaced far enough apart, much more than TRT, for efficient tissue cooling between pulses (Berger and Eeg, 2006).

The Onemytis® device produce the effects on the tissues using the Airplasma® technology. The construction components, performance and functioning of the Onemytis® are identical to the corresponding device for human surgery (Oneyonis®). The Airplasma® technology is based on the principle of transforming air into an adequate conductor for energy (plasma). Plasma is defined as an ionized gas in which a significant percentage of electrons have been removed from the respective atoms thanks to the application of a high-voltage electromagnetic pulses using high- frequency sinusoidal oscillator. The energy transferred to the tissues in the form of plasma is visible in the form of a glow; it is associated with a high control power without reaching dangerous temperature peaks. This process allows operating at a temperature < 50 °C, limiting tissue damage in the surrounding tissue (Lacitignola et al., 2020; Tamburro et al., 2019). The deeply different interaction of the two devices with the tissues produced the histomorphological differences observed in the study, clearly in favor of Onemytis®, not only in terms of structural tissue modifications but also in terms of tissue damage markers expressed. Indeed, avoiding photothermal, photochemical and photomechanical phenomena, which are induced with CO_2 laser treatment, Onemytis® results in a significant reduction in evident in cellular mortality in the tissue undergoing surgery. TUNEL together with Casp-3 will account for the fraction of total cell death related to ablative damage. There is evidence of decreased pro-apoptotic cell activity both at the cut line and in the immediate surrounding tissue area, demonstrated by the reduced count of TUNEL-positive cells in palates undergoing surgery with Onemytis®. The absence of significance of the Cas-3 marker is indicative of the fact that the cells that were not TUNEL positive at the time of tissue fixation, were not so abundant and heavily damaged that they had already started their death process. The correlation between the pro-apoptotic effect of the two ablative modalities and the extrinsic damage is evidenced by the expression in tissue of the marker *Heat Shock Prote*ins' (HSPs). These proteins, normally housed within the mitochondria, are expressed in the event of major mitochondrial alteration when they de-localise to extra-mitochondrial, cytoplasmic locations, and indicate a response mechanism to cellular stress that protects intracellular proteins from harmful events. The ablation modality of Onemytis® allows for a low-temperature palatal excision with a significant reduction in HSP expression in the tissue, whereas significant upregulation was evident after CO_2 laser treatment. The highest expression of HSP70 was observed in the epidermal compartment, with no substantial differences between the squamous cell and basal cell layers. Expression was lower in the dermal papillary layer, around salivary glands and blood vessels, and very weak or absent in the squamous layer, fibroblasts and adipose tissue. The upregulation of HSP70 is often considered preventive for apoptosis. However, it has been observed that in many cases, heat-induced damage causes increased expression of Hsp70, upregulation of p53 and immunoprecipitation of chromatin combined with increased binding of p53 to the consensus sequence of the TRAIL-R2 gene, all of which induce apoptosis (with increased T.U.N.E.L.), but not caspase-3 expression, as observed in this study. From the point of view of the immunohistochemical evaluation of the samples, therefore, the fact that the number of TUNEL positive cells was significantly lower in the group of dogs treated with OM, in which a significant reduction in HSP70 expression was also observed, indicates that this treatment, operating at lower temperatures than CO_2 laser treatment, manages to preserve a greater number of live and above all vital cells (absence of Cas-3 expression). These cells, after treatment, are therefore able to survive, resulting in a strong reduction in the post-operative inflammatory response, and above all, accelerating the healing times of the treated area.

Although it was not the specific focus of this study, the reduced invasiveness of Onemytis® might influence long-term outcomes, in terms of healing times, quality of repaired tissue, complication rates, and could increase its potential for broader surgical applications.

5. Conclusion

The present study suggests that staphylectomy, performed with Onemytis® or with CO_2 laser, may result in a favorable prognosis in dogs with elongated soft palate. Clinical findings of the two surgical techniques were statistically comparable. Considering the duration of the surgery, the safety for the operator, the manageability and versatility of the handpiece, Onemytis® was better than CO_2 laser. The histological and immunohistochemical evaluations agreed in defining Onemytis® as less invasive than CO_2 laser during ablation, considering the lower iatrogenic damage caused to the surrounding tissues, the lower rate of induction of cellular apoptosis and heat shock proteins in the treated tissues, factors which likely contribute to reduced postoperative inflammation and enhanced tissue healing. Interesting foundations arose from this study, allowing to address the daily clinical use and the development of further clinical studies using Onemytis®, a helpful surgical device recently introduced in veterinary surgery; exactly the same technology is applied in human surgery with a specific medical device (Oneyonis®).

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Ethics and animal welfare statement

The project underwent ethical review by the Animal Welfare Body of the University of Camerino and was given approval (protocol n. 5/2022, 1st June 2022), as it was compliant with the National Legislative Decree 4 March 2014 n. 26, implementation of EU Directive 2010/63/EU. All institutional and national laws, guidelines and policies for the care and use of animals were followed. The research in this manuscript used a high standard of veterinary care and involves informed client consent. Written informed consent was obtained from the owners for the participation of their animals in this study.

CRediT authorship contribution statement

Adolfo Maria Tambella: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Writing – original draft. **Mario Procaccini:** Data curation, Investigation, Visualization, Writing – original draft. **Lucia Biagini:** Data curation, Investigation, Methodology, Writing – review & editing. **Livio Galosi:** Investigation, Methodology, Validation, Writing – review & editing. **Stefano Martin:** Formal analysis, Visualization, Writing – review & editing. **Cecilia Vullo:** Methodology, Supervision, Writing – review & editing. **Fabrizio Dini:** Methodology, Resources, Writing – review & editing. **Giacomo Rossi:** Data curation, Funding acquisition, Methodology, Resources, Supervision, Writing – original draft.

Declaration of competing interest

The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest or bias to the work.

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