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RESEARCH ARTICLE

Influence of Methodological Variables on Fracture Strength Test Results of Intact Premolars: an ex-vivo study

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ABSTRACT

The present ex vivo study evaluated the influence of periodontal ligament simulation, load inclination, and tip morphology on fracture strength test results on intact premolars. Forty maxillary premolars were divided into four groups, Group 1, with a 90° load inclination, spherical tip with a diameter of 3mm and periodontal ligament simulation (PDL+); Group 2, with a 90° load inclination, flat tip with a diameter of 2mm, PDL+; Group 3, with a 45° load inclination, flat tip with 2mm of diameter, PDL+; Group 4, 90° load inclination, spherical tip with 3mm diameter, without periodontal ligament reproduction. Interactions among variables and intergroup significance were tested with Wilcoxon rank-sum and Kruskal Wallis's tests ($p \leq 0.05$). Statistically significant differences were found between groups B and C, but they were not found for the others. A 90° load inclination significantly increases fracture strength, while periodontal ligament simulation and tip morphology did not significantly influence the results.

Keywords: fracture strength, periodontal ligament, premolar, tooth fractures.

1. Introduction

Tooth fracture is a frequent dental problem influenced by several factors, such as tooth anatomy and cavity preparation procedures[1]. Fractures are more likely to occur in premolars due to their anatomical shape, crown volume, and unfavorable crown/root proportions[2,3]. From a functional point of view, premolars run a greater risk of damage because they are subjected to larger lateral forces during mastication and present a smaller amount of tooth structure compared to molars. It was demonstrated that the occlusal pressure raises progressively from the central incisors, reaches its peak at the premolars, and decreases steeply towards the second molars[4]. Moreover, caries-free intact premolars are the most easily available elements as their extraction is a widely accepted procedure to correct malocclusions[5].

Fracture strength is defined as the ability of a tooth to resist fracture under mechanical stress, and it is routinely assessed by simulated functional loading[6]. Teeth are loaded to fracture on a universal mechanical loading machine, and the maximum breaking load is recorded and expressed in Newton (kg x m/s^2). Despite this approach cannot faithfully reproduce an in vivo situation, several adjustments can be made to simulate physiological occlusal forces within the oral cavity, both during sample preparation and testing[7]. Test results can be influenced by variations in the loading pattern, such as the shape and diameter of the loading tip as well as its angulation with respect to the long axis of the tooth[8]. Moreover, it is possible to vary the site of application of the force on the occlusal surface of the tooth and simulate the periodontal ligament[9]. Periodontal ligament is a fundamental structure that plays a crucial role in supporting and cushioning teeth during chewing function, dissipating occlusal forces and protecting the teeth and the surrounding bone from damage[8]. A previous study emphasized the significance of including the simulation of the periodontal ligament in fracture strength tests using finite element analysis, to better understand the impact of occlusal forces on tooth strength and fractures resistance[9]. The influence that simulation of the periodontal ligament, load inclination, tip position, tip diameter, and thermocycling imparts on results of fracture strengths tests conducted on upper premolars was evaluated in a recent meta-analysis[8]. From these findings emerged that none of the above-mentioned methodological variables significantly influenced intact premolars.

To date, many studies follow different treatment protocols, highlighting a considerable lack of uniformity in the procedures and controversial results[8]. Moreover, data on periodontal ligament simulation, tip position, and diameter are limited. Precisely, no studies were conducted to test the influence of the aforementioned variables on the results of fracture strength tests conducted on non-treated extracted premolars (NTEPs). Standardization of these procedures is required to reduce the variability of fracture strength test results and to conduct more rigorous in vitro studies.

Therefore, the aim of the present study was to evaluate the influence of methodological variables, namely periodontal ligament reproduction, lead inclination (45°, 90°), and tip morphology (flat or spherical), on the fracture strength test of NTEPs. The null hypothesis was that periodontal ligament simulation, inclination, and morphology of the tip would not affect the fracture strength test in NTEPs.

2. MATERIALS AND METHODS

2.1 TIP PRODUCTION AND SELECTION

In order to select the tip diameter, spheres of various diameters were compared and tested on premolars with heterogeneous morphology and size (figure 1). The spheres with a diameter of 3mm and 3.5mm were found to make correct contact with almost all the tested elements. Following the selection of the samples, it was decided to use the 3mm diameter tip for the execution of the test, as it was able to exert the forces uniformly on both internal cusp sides of the selected samples. In addition to this innovative round-tipped tip, a classic 2mm-diameter flat-tipped was also used.

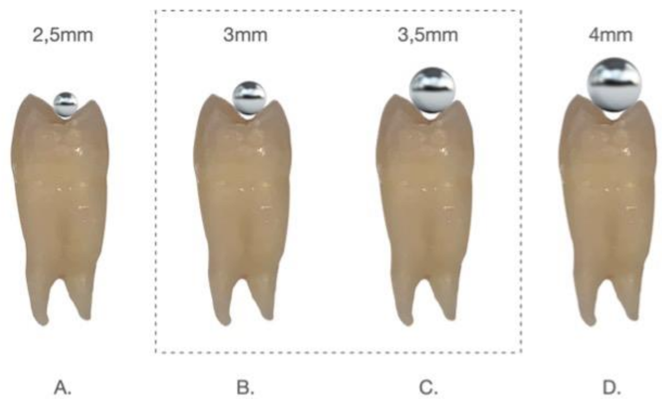

Figure 1. Tip selection procedure. Spheres of various diameters were compared and tested. B and C were found to make a correct contact with almost all the tested elements.

2.2 SAMPLE SELECTION

A total of forty teeth were collected from a group of freshly extracted, intact upper premolars extracted for either periodontal or orthodontic reasons after ethical approval (project no. 7/2021). All the teeth having signs of caries, cracks, fractures, and restorations were excluded from the study after being examined with a magnifier (10x). Moreover, all the teeth were extracted no more than two months prior to the study[10]. Before the test, all the residual periodontal tissue, plaque, and calculus were carefully removed from each tooth using both a manual scaler and periodontal curettes (Hu-Friedy, Chicago, IL). Afterward, the teeth were wiped using a rubber cup (Microdont, São Paulo, SP, Brazil) and fine pumice (Vigodent, RJ, Brazil) water slurry and placed in a numbered container containing saline solution (0.9%) and stored at a temperature of 37° C. The vestibulo-palatal (BL) and mesio-distal (MD) dimensions of each crown were recorded, as well as the distance between the CEJ and the incisal margin (CEJ) and the total length of the element (TL). These measurements were conducted using a digital caliper (Digimatic 500, Mitutoyo, Kanagawa, Japan). Furthermore, teeth were randomly divided into four distinct groups, as shown in figure 2 (n=10):

- **Group A (n=10):** load application at 90°, spherical tip diameter 3mm, reproduction of the periodontal ligament (figure 2A)
- **Group B (n=10):** load application at 90°, flat tip diameter 2mm, reproduction of the periodontal ligament (figure 2B)
- **Group C (n=10):** load application at 45°, flat tip diameter 2mm, reproduction of the periodontal ligament (figure 2C)

Group D (n=10): load application at 90°, spherical tip diameter 3mm, without reproduction of the periodontal ligament (figure 2D)

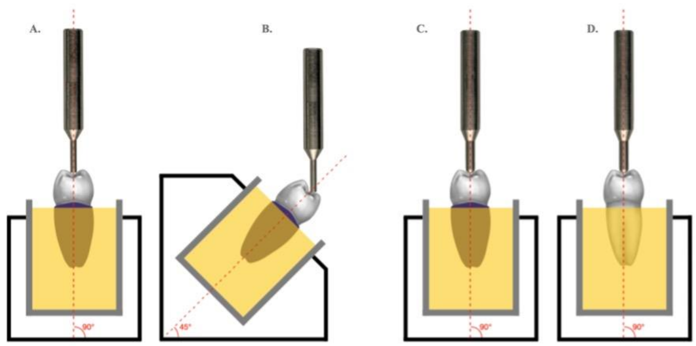

Figure 2. Schematic representation of the different methodological variables used to test fracture strength in the four different groups (A, B, C, D).

2.3 SAMPLE PREPARATION:

2.3.1 PERIODONTAL LIGAMENT SIMULATION

In Groups A, B, and C, the periodontal ligament was simulated following a previously validated procedure (figure 3) [11]. Each root was immersed in molten wax (TENATEX RED, kemdent) up to 2 mm below the CEJ to simulate the biological space and to create a 0.2-0.3mm thick wax layer (figure 3A and 3B). Each sample was then fixed with the crown pointing upwards by means of soft wax to the plexiglass plate of an embedding device (ED), making sure that it was positioned with the long axis perpendicular to the base of the support (figure 3C). The ED was composed of a metal base on which a threaded stainless-steel bar was inserted perpendicularly, anchored to the base through bolts. A perforated plexiglass plate inserted inside the threaded base is able to slide in order to position it at the desired height, depending on the tooth to examine. Its height can be adjusted by screwing or unscrewing a butterfly-shaped bolt placed at the top of the bar, whose position is maintained by a spring (figure 3D).

After fixing the tooth in the correct position, a metal cylinder (Tigre, Rio Claro, SP Brazil) was positioned over the ED, and self-curing acrylic resin was poured into it until it reached 1 mm from the CEJ to simulate the alveolar bone crest (figure 3E). Following resin polymerization, unscrewing the butterfly bolt, the wax-coated teeth incorporated into the resin were removed from the cylinders, and the wax was removed with hot water, leaving an impression in the acrylic resin (figure 3F). To simulate the periodontal ligament, the artificial alveolus created on the acrylic resin was filled with an elastomeric impression material (elite HD +, regular body, Zhermack) (figure 3G). The sample was then reinserted into the cavity, and excesses were removed with a scalpel blade (figure 3H). At this point, the samples were ready to be tested.

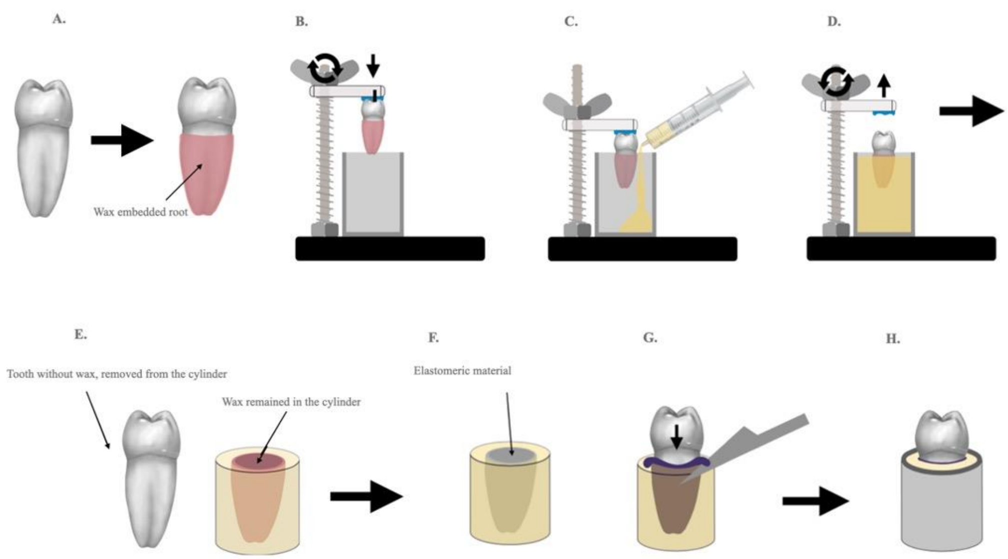

Figure 3. Schematic representation of root embedment and periodontal ligament simulation procedure.

2.3.2 PHASES OF "TRADITIONAL" EMBEDDING EXECUTION, WITHOUT SIMULATION OF THE PERIODONTAL LIGAMENT

Group D did not undergo periodontal ligament simulation. The samples were individually fixed, by means of soft wax, to the plexiglass plate of the ED, with the crown facing upwards, making sure that they were positioned with the long axis perpendicular to the supporting base. A metal cylinder was then placed underneath (figure 4A). By screwing the wing bolt of the ED, the element was immersed so that the resin reached 1mm from the enamel-cement junction (CEJ) in order to simulate the alveolar bone crest (figure 4B). The self-curing resin (ProBase Cold, Ivoclar Vivident AG) was then poured using a special syringe in order to control its flow (figure 4C). Once the resin was polymerized, the wing bolt was unscrewed in order to determine the detachment of the plexiglass plate from the tooth, which was instead incorporated into the resin (figure 4D).

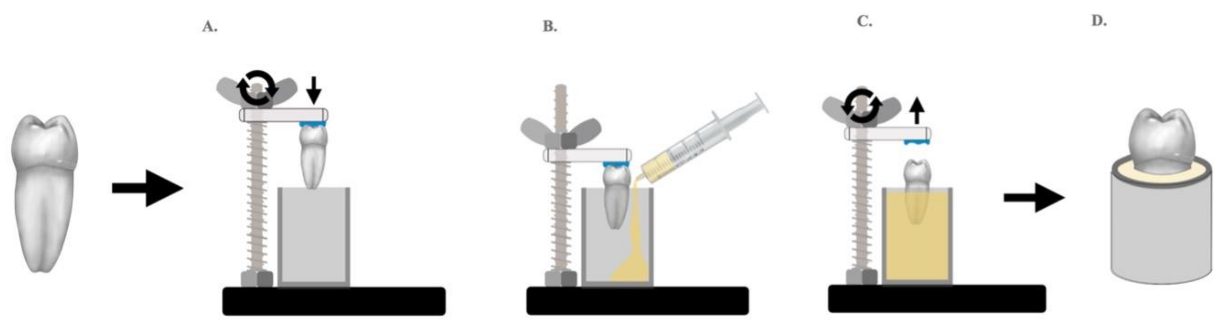

Figure 4. Schematic representation of root embedment without periodontal ligament simulation.

2.4 FRACTURE STRENGTH TEST

Each sample was fixed inside the universal loading machine (Triaxal Tester T400 Digital) by means of a goniometer with a clamp. The correct positioning of the machine was checked by means of a laser level. The load was applied at a speed of 1 mm/minute till fracture occurred and recorded in Newton (N) by a computer (Digimax Plus) connected to the machine. All tests were carried out on the same day.

2.5 STATISTIC ANALYSIS

Statistical analysis was performed using an ad hoc statistical software (STATA BE, version 17.1, StataCorp LP, TX, USA), setting the level of significance at $\alpha = 0.05$.

A total of samples were indicated as the ideal size required for noting significant differences using G∗Power v3.1 (Heinrich Heine, University of Düsseldorf, Düsseldorf, Germany)

Continuous variables were reported as Mean and standard deviations. After verification of data distribution, Kruskal–Wallis was applied to evaluate the difference in fracture strength (STRENGHT) between the four study groups. Wilcoxon rank sum test was performed to evaluate their inter-group differences: A *vs.* D (PDL), B *vs.* C (Load Inclination), and A *vs.* B (Tip Angulation).

3. RESULTS

Samples dimensions for each group are presented in Table 1. No statistically significant difference was observed in terms of tooth size between the four groups (Table 1). The four groups differ significantly in terms of fracture strength (P=0.0007). The mean load values are higher for group D and lower for group C (1354.92 ± 537.44 N and 477,47 ± 200.79 N, respectively). Additionally, the inter-group analysis disclosed statistically significant differences in fracture resistance between group couples (A *vs.* B; A *vs.* C; B *vs.* C; B *vs.* D; C *vs.* D). Comparisons among different couples of groups are shown in Table 2.

Table 1. Teeth dimensions (mesio-distal, bucco-lingual and root length) values for each subgroup

Groups	Teeth dimensions	Mean(SD) (mm)
A	MD[1]	6.91(0.33)
	BL[1]	9.51(0.25)
	TL[2]	21.82(2.09)
	CEJ[3]	8.08(0.68)
B	MD	6.64(0.31)
	BL	9.21(0.25)
	TL	21.09(2.04)
	CEJ	7.57 (0.45)
C	MD	6.37(0.27)
	BL	8.95(0.32)
	TL	20.88(0.977)
	CEJ	7.89(0.69)
D	MD	6.99(0.24)
	BL	9.6(0.32)
	TL	21.23(1.6)
	CEJ	7.95(0.68)

[1]Mesio-distal and Bucco-lingual dimensions were measured at the cement-enamel junction (CEJ).
[2]Tooth length was measured from the vestibular cusp to the root apex
[3]CEJ measured from the apex of the vestibular cusp to the CE

Table 2. Descriptive statistics per each group and differences between each group couple.

Group	N	PDL	Tip Design	Tip Diameter (mm)	Tip angulation	Mean(SD)[1] (N)	*P value**
A	10	+	Spherical,	3	90°	1298.59(583.20)[a]	a
B	10	+	Flat,	2	90°	818.05(398.22)[b]	b
C	10	+	Flat	2	45°	477.47(200.79)[b]	b
D	10	-	Spherical	3	90°	1354.92(537.44)[c]	c

*P-value<0.05
[1]SD, standard deviations.
Same letters indicate significance between the two groups (a,b,c).

The study showed that fracture load values from groups A, B, and C, in which the ligament was reproduced, did not differ significantly from those of group D, in which periodontal ligament simulation did not occur. Group A (PDL +) revealed lower strength values compared to group D (PDL-) (1298.59 ± 583,20 N and 1354.92 ± 537.44 N, respectively), however their difference in fracture strength was non-significant (P=0.9397) (figure 5). On the other hand, a statistically significant difference was detected comparing groups B and C, which differed for the load inclination (p = 0.0288). Group B (90 ° inclination of the tip) showed higher fracture load values compared to group C (45 ° inclination of the tip), as shown in Table 2. In group, C mean was 471.4 N, while in group B 725.3 N. Group B showed higher strength values compared to group C, as shown in Table 2 (477.47 N ± 200,79 and 725.3± 818,05 N).

Furthermore, fracture strength values between groups A and B characterized by different tip designs (3mm spherical tip and 2mm flat tip, respectively) are shown in Figure 2. Tip design is not significantly associated with changes in strength value. Indeed, no statistically significant difference (p = 0.0630) was disclosed between groups A and B.

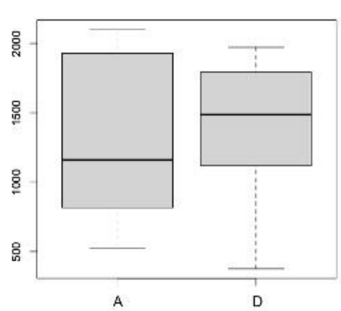

Figure 5. Box plot reporting fracture load values in group A and D differing in presence and absence of periodontal ligament simulation, respectively.

4. DISCUSSION

The aim of this ex-vivo study was to evaluate the impact of tip inclination (45° and 90°), tip design (spherical and flat), and periodontal ligament simulation on fracture strength test results conducted on uniformly selected NTEP.

The current study showed a statistically significant correlation between fracture resistance of NTEP and tip inclination; therefore, the null hypothesis was rejected for the tip inclination, while it had to be accepted both for periodontal ligament simulation and tip design. In fact, the simulation of the periodontal ligament and type of tip did not significantly affect the results of fracture strength test. On the contrary, fracture resistance significantly increased when the load was applied at 90° instead of 45°.

Teeth can fracture during normal function or due to traumatic occlusal contacts[12]. From a functional point of view, premolars run a greater risk of damage due to their anatomy and position in the oral cavity. Despite its limitations, fracture testing remains a common experimental method of evaluating fracture strengh[13]. Previous studies[8,14,15] revealed that intact premolars show higher fracture load values compared to teeth with fewer residual walls. Moreover, Gaeta *et al.* demonstrated that fracture strength is not affected by several methodological variables analyzed (*i.e.,* periodontal ligament simulation, tip diameter, position and inclination, and thermocycling in), partially confirming our results.

The periodontal ligament is an important structure that connects the tooth root and the alveolar bone, whose main function is the absorption of occlusal loads[11]. When a load is applied, tension is transmitted to the periodontal fibers, which compress and slightly displace the tooth, leading to alveolar bone deformation in the direction of the root movement. The resistance of the periodontal fibers to tooth displacement is initially low, but as soon as the root begins to compress the socket, their resistance progressively increases[16]. However, in the current study, periodontal ligament simulation did not significantly modify fracture load values. In accordance with our findings, a recent study by Marchionatti *et al.* showed that simulation of the periodontal ligament is unable to influence fracture strength, and therefore it may not be mandatory to reproduce this anatomical structure in fracture test studies. However, our results are in disagreement with previous studies, which demonstrated that fracture strength test requires periodontal ligament simulation to enable a more accurate in vitro reproduction of the oral environment[7,9,11,17]. Indeed, Soares *et al.*[11] proved through fracture mode analysis that periodontal ligament reproduction influenced the fracture pattern rather than the fracture load values by modifying stress distribution[11]. Precisely, the absence of periodontal ligament simulation led to a large number of fractures characterized by a failure at the interface between the resin cylinder and the coronal structure[11]. The divergent findings in previous research might be related to the inherent limitations of fracture strength testing, such as the use of extracted teeth, moreover, different teeth chosen for the test. In fact, this is the first study testing the influence of periodontal ligament of intact human premolars, while previous studies were conducted on bovine teeth[11,17].

The absence of a consensus among studies in the literature could be related to the intrinsic challenges and limitations of fracture tests, leading to a lack of consistency[7]. One of the hardest challenges in these tests is the use of extracted human teeth and precisely sample standardization. The main reason relies on their large anatomical variability, aging, and morphological alterations mainly caused by acid erosions, abrasions, and fatigue cracks. In the present study, to control for variables that could have affected the study outcomes, only maxillary premolars with similar dimensions (MD, BL, CEJ, TL) were selected, as shown in Table 1.

Although Group A reported higher fracture load values compared to Group B (figure 6), the difference is not statically significant. Therefore, according to our findings, the tip diameter and tip designs do not affect *ex vivo* fracture strength test results, as confirmed by a recent meta-analysis.

According to Yang *et al.*, load angulation influences stress distribution and accumulation both at the crown and root level confirming our results[18]. Indeed, in the present study, the tip inclination variable seemed to influence fracture resistance. Indeed, lower fracture load values were recorded when the force was applied at 45° instead of at a right angle (Figure 7). However, our findings are in disagreement with Gaeta *et al.*, who demonstrated that tip inclination does not affect fracture strength test in intact premolars; on the contrary, significant results emerged in premolars with a lower number of residual walls[8]. However, the authors reported that this lack of statistically significant difference in fracture strength could probably be due to the scarcity of data available regarding tip position and diameter. In contrast, our results are due to the different load application areas; it can be observed that in group B the tip, tilted with 90°angulation, contacted both sides of the cusp, distributing the forces more uniformly and resulting in higher fracture load values. On the contrary, in Group C (45 °), the tip was tilted by 45°, and it was able to contact only the palatine cusp, leading to fracture when lower loads were applied.

This is the first study investigating the influence of periodontal ligament simulation, load angulation, and tip designs on the results of fracture strength conducted on NTEP. Moreover, the literature supports a sharp decrease in microhardness of extracted teeth when stored for longer than two months[10]; for this reason, teeth only those teeth extracted more than two months prior to the study were excluded.

Nevertheless, this study has some limitations, such as the small sample size and the use of both second and first premolars. Indeed, despite the highly consistent fracture strength values obtained in this study, the inclusion of both first and second premolars could be a potential source of bias since differences in the shape of the cervical area between first and second premolars influence fracture susceptibility.

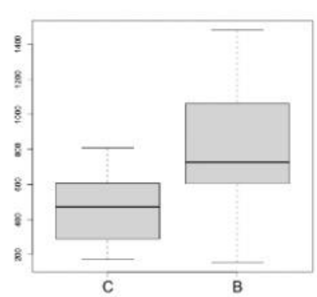

Figure 6. Box plot reporting fracture load values in group C and B differing for tip inclination.

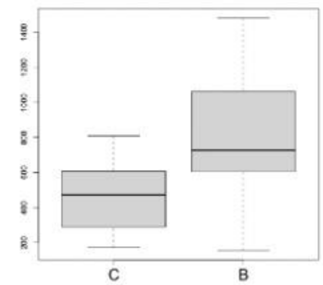

Figure 7. Box plot reporting fracture load values in group A and B differing for tip designs.

Conclusions

Considering the limitations of this in vitro study, it is possible to conclude that the tip inclination influences the resutls of fracture strenght test. On the contrary, fracture resistance is not modified by periodontal ligament simulation and tip design. Eventually, a rigorous standardization for *in vitro* studies would guarantee the quality and transparency of reported results. Further studies, including larger samples, should be conducted to examine the fracture type in addition to fracture strength when those methodological variables are applied to fracture strength test on NTEP.

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We declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

As Corresponding Author, I confirm that the manuscript has been read and approved for submission by all the named authors.

Data Availability Statement:

The data used in this research has been properly cited and reported in the main text. Further enquiries can be directed to the corresponding author.

Author Contributions:

Carlo Gaeta participated in the conceptual design of the work, data acquisition, and drafting of the manuscript. Giulia Malvicini participated in statistical analysis, data acquisition and revision of the manuscript. Emanuele Mignosa participated in data acquisition and conceptual design of the work. Gianmarco Cecot participated in data acquisition and drafting of the manuscript. Simone Grandini participated in conceptual design of the work and revision of the manuscript. Crystal Marruganti participated in the conceptual design and drafting of the manuscript.

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