

## ORIGINAL RESEARCH

# Use of combined laryngo-bronchoscopy intubation approach in a simulated difficult airway scenario with cervical stabilization

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**Abstract**

The occurrence of unexpected difficult airway management (DAM) during endotracheal intubation (ETI) attempts represents a life-threatening scenario. The management of such challenges may improve with training in simulated DAM scenarios. Moreover, simulation allows investigation at the potential value of new devices and techniques for DAM. The combined use of laryngoscopy with fiberoptic bronchoscope (CLBI) has been proposed in this regard, but its performance by novices facing DAM remain unexplored. We performed a randomized crossover simulation study evaluating the performance of ninety-six anesthesiology residents during ETI with four approaches: direct laryngoscopy (DL), Glidescope®, McGrath® and CLBI. Increased difficulty was produced by placement of a cervical collar. Residents had maximum of 3 attempts per device/technique (up to 60 seconds per attempt). The main outcomes were success rate (SR) and corrected time-to-intubation (cTTI, with 60 seconds added for each failed attempt). Subgroup analyses were performed separating residents according to their experience (junior, n = 60; senior, n = 36). The CLBI had significantly lower SR at both 1st and 3rd attempt (31% and 64%, respectively) as compared to DL (93% and 98%), Glidescope® (70% and 86%) and McGrath® (58% and 84%), with all  $p < 0.001$ . Moreover, CLBI had significantly longer cTTI (158.5 seconds; (54.3; 180)) than other devices: Glidescope® (37.6 seconds; (24.7; 88.2)), McGrath® (39.3 seconds; (20.6; 105.1)), and DL (19 seconds; (15.4; 27.2)), all  $p < 0.002$ . CLBI and McGrath® were the only approaches performing better in senior as compared to junior residents. In a DAM simulated setting, anesthesiology residents had lower SR and longer cTTI with the CLBI as compared to direct and video-laryngoscopy.

**Keywords**

Direct laryngoscopy; Endotracheal intubation; Fiberoptic bronchoscope; Manikin; Simulation; Video-laryngoscopy

## 1. Introduction

Endotracheal intubation (ETI) is a basic skill for anesthesiologists but it is performed also by other physicians, by paramedics and nurses [1, 2]. Indeed, ETI is used not only in the operating room but also under emergency conditions where it is needed to secure the airway and to provide effective ventilation [3, 4]. The occurrence of unexpected difficult airway management (DAM) during ETI attempts as part of the induction of general anesthesia represents one of the biggest life-threatening scenarios faced by anesthesiologists [5, 6]. Several conditions may complicate the airway management outside the operating room, as it happens in the case of patients with cervical spine immobilization [5, 7]. In such cases, the goal is to achieve sufficient laryngeal exposure during laryn-

goscopy, whilst avoiding cervical spine movements that may cause spinal cord injuries. The latter is mostly attained with the aid of a cervical collar. However, from practical perspectives the collar application may reduce the patient's mouth opening, rendering the introduction of the direct laryngoscope (DL) more challenging [8]. Moreover, the neck collar also produces an anterior displacement of the chin and larynx, with further difficulty added to the ETI procedure [8–10]. Therefore, the importance of preparedness and training in performing ETI under challenging situations cannot be overemphasized [11, 12].

Apart from the practical training obtained during residency, the use of simulation sessions has been gradually implemented to strengthen learning pathways and acquisition of skills in ETI [13]. The management of challenging scenarios with

simulated DAM may increase the confidence of the operators if and when a real DAM scenario occurs. Moreover, new devices (or techniques) have been developed to increase the chances of successful ETI in case of DAM; however, it is essential to preventively acquire confidence with such devices and approaches. In this regard, simulation has the advantage of allowing familiarization with devices and techniques.

The DL remains the most commonly used device, but video-laryngoscopes (VLS) have been introduced into clinical practice to ease ETI under challenging clinical conditions [9, 10, 14, 15]. In patients with limited cervical spine mobility the use of VLS provides better visualization of the airway, finally facilitating ETI [14, 16]; moreover, the learning curve with VLS seems faster than DL, especially in novices [17–19].

Fibreoptic-guided ETI is the elective approach for airway management under elective expected difficult conditions [20]. Conversely, the use of fibreoptic-guided ETI cannot be easily performed in unconscious patients due to loss of airway control and patency. For this reason, fibreoptic-guided ETI is not recommended in case of unexpected DAM. However, the simultaneous use of DL (or VLS) with the fiberoptic bronchoscope (FOB), also known as combined laryngo-bronchoscope intubation (CLBI), is a relatively newer approach that may overcome the challenges of introducing the FOB in unconscious patients [21–25]. Indeed, with such approach the laryngoscopy opens up the pharyngeal region for the introduction of the FOB, which in turn has the advantage of easier moves for directing the endotracheal tube (ETT) through the vocal cords.

Two simulation studies found promising results of the CLBI techniques in DAM in the hand of certified anesthesiologists [26, 27], but it is unknown if these results can be replicated in anesthesiology residents. Therefore, we conducted a study with simulated DAM (manikin with cervical collar) comparing the performances of different approaches (DL, VLSs and CLBI) for ETI in a population of anesthesiology residents.

## 2. Materials and methods

This study was designed as a randomized, crossover manikin trial, and conducted between January and February 2022 at the “Cristian Ilardi” Simulation Center of the School of Anaesthesia, Intensive Care and Pain Therapy of the University of Catania.

### 2.1 Study participants

Ninety-six anesthesia residents of the five years of residency in Anaesthesia, Intensive Care and Pain Therapy, and rotating at the University of Catania participated in this simulation study. All residents had variable clinical experience in adult ETI with the different airway devices, and they were asked to report the approximate number of procedures performed during their residency.

### 2.2 Study development

We used the same airway manikin (Larry Intubation Trainer, Armstrong Medical Inc., IL, US). The manikin was placed on a rigid table in a lighted room and positioned at the height of the xiphoid process of each participant. We artificially

increased the difficulty of the scenario by producing cervical immobilization applying a cervical collar to the manikin.

All the residents received a standardized 15-minute teaching on the manikin anatomy, on the study methods and on all the devices of the study (including video demonstrations on the use of these devices). After the teaching session, every participant had 15 minutes to practice ETI with the devices. Four devices were studied for ETI and the sequence followed a randomized order (sealed envelopes). Each participant was not allowed to watch each other in order to avoid any teaching bias. The following four techniques for airway management were tested:

A. DL using a Macintosh laryngoscope blade size 3 (Mercury Medical, Clearwater, FL, US);

B. VLS with distant monitor (VLS-DM) with Glidescope (Saturn Biomedical System Inc., Canada);

C. VLS with screen on device (VLS-SoD) with the McGrath MAC blade X3 (McGrath; Aircraft Medical Ltd, UK); and

D. CLBI approach with the same Macintosh DL and the use of a disposable bronchoscope (aScope™ 4 Broncho Regular endoscope, Ambu A/S Baltorpbakken 13 DK-2750 Ballerup, Denmark).

All ETI were performed using a lubricated ETT with a 7.5-mm internal diameter. The ETT was preemptively equipped with a hockey stick-shaped semi-rigid stylet for the intubations with VLS only. A stylet was available on request for the DL. The manikin and the ETT were periodically wetted with a lubricant. Six independent operators (LLV, SM, FM, FT, GS and FS) carried out the study. The same author (LLV) provided the standardized teaching sessions, while the others were in charge of organizing the flow of residents, performing the randomization and assessing performances in terms of success and time.

## 2.3 Outcomes

We tested two primary outcomes, (a) the success rate (SR) and (b) the corrected time to intubation (cTTI).

With regards to the SR, each participant had up to three attempts to perform ETI for each device/technique. Successful ETI was declared if confirmed by chest rise after bag insufflations by one of the research team. We registered ETI failure when the attempt lasted longer than 60 seconds or the ETT was placed in the esophagus. Regarding the cTTI, timing recording with a chronometer started when the operator grasped the device until the participant stated the ETT passed the vocal cords. The research team declared as successful the intubation attempt when bag inflation and manual ventilation produced effective chest rise. The absolute value of the time to intubation of the attempt was recorded and then corrected (cTTI) for the number of attempts by adding 60 seconds for each previously failed attempt. For example, an intubation occurring at the 21st second of the 2nd attempt had a cTTI of 81 seconds (21 + 60 seconds counted for the first failed attempt). In case of three failed attempts a count of 180 seconds was imputed.

As a secondary endpoint, we also analyzed the uncorrected TTI (uTTI) which is the absolute TTI taken by successful attempts only without correction for the SR. Our decision to use the cTTI instead of the uTTI is based on the importance of accounting for previously failed attempts. Indeed, each failure

exposes the patient to increased risks of desaturation, bleeding and secretions. For instance, using the uTTI a hypothetical device with low SR but short TTI would seem outperforming as compared to devices with longer TTI but greater SR.

Other variables recorded were: subjective estimate of the number of procedure performed with each device, glottic view in terms of Cormack-Lehane classification [28] and POGO (percentage of glottis opening, ranging from 0% to 100%) system [29]. Although not formally validated, we asked the Cormack-Lehane and POGO for the CLBI too. At the end of each airway scenario, each resident was asked to rate ease of ETI using all techniques on a 10-point Likert scale ranging from 1 (very easy) to 10 (very difficult).

## 2.4 Statistical analysis

Data distribution was investigated with Kolmogorov-Smirnoff test; data were reported as numbers (percentages) for the categorical variables, whilst the continuous variables were described in terms of mean and standard deviation or as median and interquartile range (IQR) according to their distribution. Differences for continuous variables (*i.e.*, cTTI) were investigated using the paired *t*-Student test or the Wilcoxon rank test for paired data according to data distribution, whilst analyses for categorical variables were conducted using the Fisher's exact test. Differences between groups were considered significant if the *p* value was below 0.05.

## 3. Results

The participants of the study had a mean age of  $29.7 \pm 3.1$  years and 39% were males ( $n = 37$ ). In particular, we enrolled 33, 27, 12, 11 and 13 residents for the first, second, third, fourth and fifth year of their training, respectively. The population characteristics and their experience with each device are reported in Table 1.

Of note, on average there was no experience with the CLBI approach in the overall population. The experience with VLSs was negligible in the junior resident population, whilst 5 to 10 VLS procedures were self-reported on average by the senior residents.

Table 2 describes the performances of each device in terms of SR, cTTI and uTTI. Results are shown for the overall participants. The highest SRs (at 1st, 2nd and 3rd attempt) were found for the DL, followed by VLSs (Glidescope® and McGrath®, respectively); the lowest SRs were recorded for the CLBI approach. Similar findings were observed for the cTTI.

Table 3 shows the analyses conducted comparing performances of devices (cTTI and SR both at 1st and 3rd attempt) in the overall population. All the analyses yielded significant results for the cTTI and the SR at 1st and 3rd attempt except for the comparison between McGrath® and Glidescope®, with the DL being the best performing device followed in order by Glidescope®, McGrath® and CLBI approach.

Table 4 shows the performances of each device in terms of SR, cTTI and uTTI for the two subgroups of junior and senior residents.

Table 5 reports the ease of use and the Cormack-Lehane and POGO scores for all the devices, both for the overall

participants and for the subgroups according to their training level.

The sensitivity analyses conducted on uTTI (time recorded for successful intubations not corrected for failures) confirmed the primary results obtained on the cTTI.

## 4. Discussion

We conducted a simulation study in an airway scenario at increased difficulty (cervical collar *in situ*) enrolling 96 anesthesiology residents at different stage of their training. We focused on the assessment of performances of the CLBI approach as compared to more commonly used devices, DL and VLSs. We secondarily evaluated performances dividing residents according to the stage of their training, arbitrarily separating them in groups (first two years of residency vs those from the third, fourth and fifth year). With such approach we aimed at gathering information on the best timing for the introduction of CLBI technique during the anesthesiology training.

We primarily found that CLBI had significantly lower SR and cTTI as compared to the other devices and the best performances were obtained by the DL which outperformed the VLSs. Although the SR of CLBI increased by the third attempt, this technique remained inferior to the others; indeed, roughly one in three participants failed to perform ETI with CLBI method by the third attempt.

We initially hypothesized that residents could have fast learning curves with the FOB, but our study failed to demonstrate advantages in the introduction of CLBI technique early during anesthesiology training. These negative findings are similar to the ones we obtained in a parallel study conducted in residents performing ETI in a normal airway scenario. Nonetheless, a previous simulation study performed in a difficult airway scenario, reported interesting results of the CLBI technique in the hands of experienced anesthesiologists [27]. Similarly, another study suggested that in a difficult airway scenario the TTI was shorter with the combined use of FOB and a VLS as compared to the VLS alone [26]. The CLBI technique has not been intensively studied yet and a part from the above mentioned studies, we found only one simulation study conducted in the setting of difficult pediatric airways. Eighteen anesthesiologists with at least 3 years of practice participated in this study, and the authors found that CLBI had the best performances as compared with FOB, Macintosh DL and Bullard laryngoscope [31]. Similar studies found promising results regarding the combined use of DL with a rigid bronchoscope [32] or a fiberoptic stylet [33].

Importantly, in our study the visualization of the vocal cords with the CLBI approach did not seem the limiting factor for the successful implementation of the technique; indeed, the median POGO score was 90% and lower only than McGrath median score, and higher than DL or Glidescope. Similarly, CLBI had the same median Cormack-Lehane scores than DL or Glidescope. Indirectly, the greater issue when attempting ETI with CLBI approach in the simulation setting of cervical immobility seems to properly direct the FOB tip towards the vocal cords. The CLBI approach reported the largest gaps in SR and cTTI between performances of junior and senior

**TABLE 1. Population characteristics and experience with each device.**

	Overall (n = 96)	Junior residents (n = 60)	Senior residents (n = 36)
Males	37 (39%)	26 (43%)	11 (31%)
Age	29.7 ± 3.1	28.8 ± 2.4	32.5 ± 3.3
Estimated number of procedures			
Direct Laryngoscope	43 (200)	15 (38)	325 (225)
Glidescope	0 (5)	0 (0)	5 (21)
McGrath	1 (8)	0 (1)	10 (16)
CLBI	0 (0)	0 (0)	0 (1)

CLBI: Combined laryngo-bronchoscopy approach.

**TABLE 2. Performances of the devices in the overall population of 96 participants.**

	Direct Laryngoscope	Glidescope	McGrath	CLBI
SR at 1st attempt	89/96 (93%)	67/96 (70%)	56/96 (58%)	30/96 (31%)
SR at 2nd attempt	92/96 (96%)	80/96 (83%)	73/96 (76%)	43/96 (45%)
SR at 3rd attempt	94/96 (98%)	83/96 (86%)	81/96 (84%)	61/96 (64%)
cTTI	19 (15.4; 27.2)	37.6 (24.7; 88.2)	39.3 (20.6; 105.1)	158.5 (54.3; 180.0)
uTTI	18.5 (15.2; 24.5)	30.4 (21.3; 41.0)	22.2 (16.5; 34.4)	43.6 (35.9; 53.8)

CLBI: Combined laryngo-bronchoscopy approach; SR: Success rate; cTTI: Corrected time to intubation; uTTI: Uncorrected time to intubation.

**TABLE 3. Statistical analyses of the performances of devices used.**

Comparison	cTTI	SR 1st attempt	SR 3rd attempt
Direct Laryngoscope vs. Glidescope	<0.001	<0.001	0.005
Direct Laryngoscope vs. McGrath	<0.001	<0.001	0.002
Direct Laryngoscope vs. CLBI	<0.001	<0.001	<0.001
Glidescope vs. McGrath	0.55	0.13	0.84
Glidescope vs. CLBI	<0.001	<0.001	<0.001
McGrath vs. CLBI	<0.001	<0.001	0.002

CLBI: Combined laryngo-bronchoscopy approach; SR: Success rate; cTTI: Corrected time to intubation.

**TABLE 4. Performances of the devices in subgroups of participants divided in junior (n = 60) and senior (n = 36) residents.**

		Direct Laryngoscope		Glidescope		McGrath		CLBI	
SR at 1st attempt	Junior	53/60 (88%)	0.040	40/60 (67%)	0.049	30/60 (50%)	0.040	12/60 (20%)	0.003
	Senior	36/36 (100%)		27/36 (75%)		26/36 (72%)		18/36 (50%)	
SR at 2nd attempt	Junior	56/60 (93%)	0.290	48/60 (80%)	0.040	41/60 (68%)	0.030	23/60 (38%)	0.140
	Senior	36/36 (100%)		32/36 (89%)		32/36 (89%)		20/36 (56%)	
SR at 3rd attempt	Junior	58/60 (97%)	0.530	49/60 (82%)	0.012	46/60 (77%)	0.008	32/60 (53%)	0.009
	Senior	36/36 (100%)		34/36 (94%)		35/36 (97%)		29/36 (81%)	
cTTI	Junior	20.3 (15.8; 33.7)	0.010	41.4 (26.7; 98.4)	0.006	66.3 (26.5; 174)	0.008	175.7 (93.4; 180)	0.008
	Senior	18.3 (14.7; 22.3)		32.6 (22.1; 64.5)		26.5 (17.2; 71.6)		45.2 (35.5; 52.1)	
uTTI	Junior	18.7 (15.5; 27.0)	0.038	31.2 (24.5; 43.1)	0.019	24.6 (17.5; 34.5)	0.330	73.4 (39.5; 180)	0.930
	Senior	18.3 (14.7; 22.3)		26.9 (19.7; 36.7)		21.5 (14.8; 32.4)		40.9 (36; 54.5)	

CLBI: Combined laryngo-bronchoscopy approach; SR: Success rate; cTTI: Corrected time to intubation; uTTI: Uncorrected time to intubation.



**TABLE 5. Ease of use, Cormack-Lehane score and POGO score.**

	Overall (n = 96)	Junior residents (n = 60)	Senior residents (n = 36)	p value
Difficulty (1 = very easy, to 10 = very difficult)				
Direct Laryngoscope	3 (3)	3 (4)	2 (2)	0.001
Glidescope	5 (4)	6 (3)	4 (4)	0.005
McGrath	4 (4)	5 (4)	2 (3)	<0.001
CLBI	7.5 (4)	8 (3)	5 (5)	<0.001
POGO (% of glottis exposure)				
Direct Laryngoscope	70 (50)	60 (42)	72.5 (60)	0.090
Glidescope	67.5 (40)	60 (40)	72.5 (40)	0.140
McGrath	100 (20)	95 (31)	100 (20)	0.440
CLBI	90 (45)	80 (50)	100 (20)	0.020
Cormack-Lehane (1, 2a, 2b, 3, 4)				
Direct Laryngoscope	2a (2)	2a (2)	2a (2)	0.060
Glidescope	2a (2)	2b (1)	2a (2)	0.190
McGrath	1 (1)	1 (1)	1 (1)	0.480
CLBI	2a (2)	2a (3)	1 (1)	0.006
Cormack-Lehane (1, 2a, 2b, 3, 4)				
Direct Laryngoscope	25–33–17–20–1	12–19–9–13–1	12–12–6–5–0	/
Glidescope	26–27–23–13–5	13–14–19–5–3	12–12–3–5–2	/
McGrath	46–35–3–1–0	29–22–2–1–0	23–10–1–0–0	/
CLBI	47–20–11–9–8	21–10–8–8–6	23–7–3–0–2	/

CLBI: Combined laryngo-bronchoscopy approach; POGO: percentage of glottis opening. Results are shown as median and interquartile range as appropriate. We report data for the overall population as well as for the subpopulation divided according to their level of training.

residents, supporting that greater experience is needed before implementing CLBI. Further, the CLBI was the technique with greatest subjective difficulty as stated by the participants (median score 7.5/10 as compared to median values of 3/10 to 5/10 for the others) and the only with significantly worse visualization of vocal cords (POGO and Cormack-Lehane scores) by junior resident as compared to the senior ones. All together these results suggest that the implementation of CLBI requires a structured training and possibly should be reserved for the last years of residency. Nonetheless, the CLBI approach seems conceptually promising and it has been implemented in several clinical circumstances of DAM [21–25], as it combines the advantages of DL/VLS for the glottis visualization with the chances to finely direct the ETT (flexibility and maneuverability of a FOB). Indeed, there has been a recent development on the market of devices combining these techniques (*i.e.*, Provu Video-stylet®).

Overall, junior residents had longer cTTI and lower SR as compared to senior ones; however, most of these comparisons failed to reach the statistical significance, albeit this result is likely due to a relatively low sample size. Similarly, they had worse POGO scores, but a statistically significant difference between junior and residents was found only for the CLBI approach. The comparison of the Cormack-Lehane scores produced similar results.

Interestingly, DL was the best performing device, both in term of SR and cTTI, despite the device did not achieve better results in terms of vocal cords visualization. The DL was still scored as the easier technique (median difficulty score 3/10), and probably this result is explained by the experience with the device by the participants and possibly by the ease in directing the ETT through the vocal cords. Even so, we did not expect that DL would have had extremely high SR in a scenario at increased difficulty with also shortest TTI, and in truth we hypothesized better performances of VLSs.

Considering the increased difficulty in airway management in a scenario with cervical immobilization it may seem surprising the 98% SR with DL by the third attempt. However, other groups have reported similar excellent performances by DL. Indeed, in three studies with similar setting the participants achieved a 100% SR [34–36] while other scientific groups reported slightly lower rates, but with the overall SR still over 90% [16, 37]. Moreover, in a simulated scenario of cervical immobilization with the added difficulty of ongoing cardiopulmonary resuscitation Bogdański *et al.* [7] showed a SR of 85% for DL and up to 94% for VLS.

As discussed, we reported better results for DL as compared with two types of VLSs. Although shorter TTI for DL as compared to VLS has been reported by Malik *et al.* [16] even under worse visualization conditions, several groups reported

that VLSs had better performances than DL (both MacIntosh or McCoy blade) [34] in case of cervical immobilization, both in the clinical setting or under simulated scenarios. It is likely that the better performances of DL in our study were driven by the greater experience with this device as compared to the VLSs as shown by the subjective estimation on the number of procedures performed during training. Considering that on average the visualization of the vocal cords with DL was similar to Glidescope and worse than McGrath, it is likely that VLSs techniques suffered from lower experience in directing the ETT after visualization of the glottis, even though a stylet was pre-inserted in the ETT. Indeed, the better performances of DL in novices as compared to VLS is not new [14, 17, 38], although it should be noted that the evidence is conflicting and several factors (type of VLS, skills of participants, airway difficulty, simulation or clinical setting, *etc.*) grossly influence the findings of each study [39–41].

Some strengths of our study deserve comments as well as the presence of several limitations.

The main strength was the relatively large size of participants, well above most of the studies conducted in the simulation setting, and investigating a relatively novel combined approach. Moreover, we think that the analysis conducted using the cTTI is another strength as this parameter accounts for failed attempts, thus avoiding a bias for devices with low SR but short TTI. Regarding this point, as shown by a recent meta-analysis most studies have been vague on the approach for handling the timing for failed attempts [42]. Finally, a methodological strength of our study was the randomization process for the order in the use of the devices.

Several limitations should be commented when interpreting our study. First of all, this is a single-center study requiring external validation. Second, despite our population seeming somewhat homogeneous (doctors in training), their experience in airway management was highly variable, as shown by the estimated procedures performed. Importantly, the experience with CLBI approach was almost negligible and this may explain the low SR achieved. We are planning a second session of retraining at 6 to 9 months to evaluate skill retention. It also must be noted that some residents were familiar with the simulator as they are faculty for advanced cardiac life support at our simulation center. Third, we did not explore the challenges in teaching the CLBI technique, but it should be noted that this technique theoretically allows a very good supervision during the ETI process as both mentor and resident shares the same view on the screen. Indeed, a previous study suggested that supervisors may teach and assess the success of the novices more easily when adopting the CLBI method [43]. On the other hand, DL does not allow simultaneous view of resident and teacher, and therefore its teaching could be more challenging. Fourth, our results have the intrinsic limitations related to the simulation environment. Despite the fact that the importance of simulation cannot be over-stated and a meta-analysis supports the role of simulation training for acquisition of skills in advanced airway management [39], simulation scenarios do not entirely reproduce some of the real-life challenges such as presence of secretions and bleeding (among others), as well as human factors like the emotional stress and/or anxiety due to progressive desaturation, brady-

cardia, *etc.* [44]. Furthermore, the similarity between the manikin's structure and the real upper airway anatomy has been consistently questioned both in adult and pediatric settings [45, 46].

## 5. Conclusions

In a simulation setting of increased difficulty in airway management due to presence of a cervical collar, anesthesiology residents had significantly lower success rate and longer time to intubation with the combined laryngo-bronchoscopy approach as compared to direct and video-laryngoscopy. The better performances achieved with direct laryngoscopy emphasize the importance of previous experience with the airway device.

## AVAILABILITY OF DATA AND MATERIALS

Data are available from corresponding author on reasonable request.

## AUTHOR CONTRIBUTIONS

LLV, SM and FM—designed the research study. FT, GS, FL, MS and ST—collected the data. LLV and FS—analyzed the data. SM, FM and FS—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final version of the manuscript.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study received a waiver from the local Ethical Committee (Comitato Etico Catania 1: waiver prot/01-18.07.2022). We obtained consent to participate from all subjects involved.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest. Filippo Sanfilippo is serving as one of the Editorial Board members of this journal. We declare that Filippo Sanfilippo had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to CSRG.

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