

Total Versus Completion Thyroidectomy: A Multidimensional Evaluation of Long-Term Vocal Alterations

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Abstract

Background: Total thyroidectomy (TT) and completion thyroidectomy (CT) are two common surgical operations that are frequently followed by vocal symptoms despite preservation of the recurrent laryngeal nerve (RLN) and of the external branch of superior laryngeal nerve (EBSLN). The aim of this study was to analyze vocal alterations through endoscopic findings, video-laryngostroboscopy (VLS), acoustic vocal parameters and impact on patients' quality of life after surgery in the absence of laryngeal nerve injury. **Methods:** We enrolled 198 patients who underwent thyroidectomy by the same surgeon. One hundred twenty-six patients underwent TT (group TT) while 72 underwent CT (group CT). All patients underwent preoperative VLS and Voice Handicap Index (VHI) assessment and postoperative VHI, VLS and Acoustic Voice Analysis with Multidimensional Voice Program Analysis 12 to 18 months after surgery. **Results:** We observed a statistically significant higher rate of EBSLN injury in CT compared to TT. Even in the absence of RLN and EBSLN injury, patients who underwent TT and CT presented slightly worse acoustic vocal parameters and VHI scores compared to healthy controls. Interestingly, some acoustic vocal parameters and VHI scores were significantly worse in group CT compared to group TT. **Conclusions:** The higher rate of EBSLN injury in CT rather than in TT suggests a higher surgical risk in CT. The vocal parameters of loudness and self-perception of voice were significantly worse after CT, suggesting a larger trauma in patients' vocal outcome in CT if compared to TT, although these alterations were not reported as psychologically limiting daily life of patients. Nevertheless, the existence of multiple factors contributing to vocal alterations after thyroidectomy highlight the importance of a routine comprehensive functional voice analysis before and after surgery.

Keywords

total thyroidectomy, completion thyroidectomy, vocal alterations, laryngostroboscopy, VHI, acoustic voice analysis

Introduction

Dysphonia, one of the most relevant complications of thyroid surgery, may follow a damage of the recurrent laryngeal nerve (RLN) or of the external branch of the superior laryngeal nerve (EBSLN). In the latter case, the EBSLN may be injured when the superior thyroid artery is divided or after local cauterization, resulting in a reduced vocal tension generated by the decreased activity of the cricothyroid muscle. The cricothyroid muscle impairment can lead to reduced vocal ability, especially for high pitch sounds and pitch maintenance, resulting in a significant discomfort for patients, especially in professional voice users.¹⁻³

In the literature, the estimated rate of injury ranges from 0.3% to 13% for EBSLN and from 5% to 10% for RLN.⁴

Damages of the RLN can be easily diagnosed immediately after surgery through endoscopic visualization of altered vocal cord movement. Differently, injuries of the EBSLN are more challenging to recognize; posterior glottis rotation toward the affected side is frequently reported at endoscopy and patients

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complain of a weak, breathy, monotonous voice characterized by compression on the pitch range with inability to achieve high pitch tasks. However, some signs of EBSLN damage can be only highlighted with more specific exams such as electromyography, videolaryngostroboscopy (VLS), and Acoustic Voice Analysis (AVA).

Changes in vocal patterns after thyroidectomy may occur even in the absence of RLN or EBSLN damage.^{5,6} In most cases, such changes are transient and accompanied by subjective vocal and/or swallowing alterations in the days or weeks following surgery, although alterations up to 6 months have been reported in 11% to 15% of cases.⁷ In the recent years, an increasing number of studies focused on voice alterations after thyroid surgery in the absence of nerve injury; however, there is a lack of evidence and controversial data about long-term outcomes of these functional postthyroidectomy symptoms. Furthermore, only a few studies compared the incidence and characteristics of these symptoms in different types of thyroid surgery, such as total thyroidectomy (TT), the total bilateral extracapsular thyroidectomy, and completion thyroidectomy (CT), the surgical removal of the remnant thyroid tissue following procedures of less than total or near-TT.^{8,9}

The aim of this study was to analyze the long-term trend and evolution of vocal alterations in the absence of laryngeal nerve injury in patients undergoing TT and CT through endoscopic findings, VLS outcomes, acoustic vocal parameters, and subjective impact on quality of life.

Methods

One-hundred ninety-eight consecutive patients who underwent thyroidectomy between January 2016 and December 2017 in the Otolaryngology Department of our University hospital were enrolled in this study. Surgery was performed by the same team; 126 patients underwent TT and 72 underwent CT. In the latter case, a bilateral surgical revision without neck dissection was performed, independently from the previous surgery. All patients signed a written informed consent; the procedures performed were in accordance with the standards of the ethics committee on human experimentation of our University, that specifically approved this study, and with the Helsinki Declaration.

Inclusion criteria were adult age (≥ 18 years) and a histological result of multinodular goiters or low risk differentiated carcinoma (T1N0M0). Exclusion criteria were previous neck surgery except thyroidectomy, previous laryngeal lesions or vocal fold paralysis, neuromuscular disorders, lung diseases, history of prolonged intubation, cervico-mediastinal goiter, previous neck irradiation, preoperative functional voice disorders, and history of smoke/alcohol abuse.

All patients underwent preoperative VLS and Italian-validated Voice Handicap Index (VHI)¹⁰ assessment and postoperative VLS, VHI, and AVA with Multidimensional Voice Program Analysis (MDVP) 12 to 18 months after surgery. A postoperative laryngoscopy 1 month after surgery was also performed in all patients to highlight transient laryngeal palsy.

All examinations were executed in a single session by an expert otolaryngologist blinded on the type of thyroid surgery performed.

Videolaryngostroboscopy was executed using a 3.5 mm diameter fiberscope (Storz 11101RP) connected to a stroboscopic light source (ATMOS Endo-Stroboscope L) to evaluate the laryngeal morphology and motility and to study the vocal fold vibratory pattern (mucosal wave). The examination was video-recorded to appreciate symmetry and regularity of the vocal fold vibration and signs of functional dysphonia, so to identify possible preoperative alterations and postoperative injuries of the EBSLN. The criteria described by Arnold¹¹ in 1961 were used to diagnose the main laryngeal modifications visible in superior laryngeal nerve injury including absence of accentuated vocal fold opening and closure, shortening of the vocal fold ipsilateral to the damage, vertical asymmetry between vocal folds, mucosal wave asymmetry, and glottal torsion with deviation of the posterior commissure toward the affected side.

Acoustic Voice Analysis evaluated vocal quality through a software tool for quantitative acoustic assessment of voice quality (Kaypentax Mod. 5101).¹² The acoustic parameters measured in every patient were F0 (Fundamental frequency), Jitt (Jitter percent, the relative variability of the pitch in the short-term), sPPQ (smoothed Pitch Perturbation Quotient), Shim (Shimmer percent, the relative variability of the peak-to-peak amplitude in the short-term), and the sAPQ (smoothed Amplitude Perturbation Quotient). To improve the reliability of acoustic evaluation, we associated to the generally used relative variability of the pitch (Jitt) and of the peak-to-peak amplitude (Shim) in the short-term, the smoothed corresponding parameters (sPPQ and sAPQ).

Voice Handicap Index is a self-administered questionnaire containing questions on the discomfort related to the use of the patient's voice.¹⁰ The questionnaire, translated and validated in the Italian language by Schindler in 2010,¹⁰ includes 30 items, each scored on a 5-point scale ranging from "never" to "always," divided into 3 subscales including functional (*f*), emotional (*e*), and physical features (*p*) related to voice dysfunction. The range of normality and the threshold for significant changes were based on the values reported by Jacobson (a difference bigger than 8 points on the subscales and 18 points on the total score was considered to indicate significant vocal discomfort).

Statistical Analysis

Unless otherwise specified, all values are shown as means \pm SD. One-way analysis of variance was used to analyze differences in the selected MDVP variables in the following subgroups: men who had undergone TT versus healthy control; men and women who had undergone TT versus healthy control women; men who had undergone CT versus healthy control; men and women who had undergone CT versus healthy control women. *P* value was considered statistically significant if less than or equal to .05. Data were analyzed with a PC version of the Statistical Package for Social Sciences version 16.0 (SPSS, Chicago, Illinois).

Table 1. AVA in the male patient of the group TT with EBSLN injury.

| | Postoperative | Preoperative |
|-----------------|---------------|--------------|
| Shimmer percent | 7.75 | 2.523 |
| Sapq | 5.87 | 4.89 |
| Jitter percent | 2.74 | 0.5389 |
| sPPQ | 0.98 | 0.49 |
| F0 | 132 | 159 |

| AVA in the 4 female patients of the group CT with EBSLN injury (mean value) | | |
|---|---------------|--------------|
| | Postoperative | Preoperative |
| Shimmer percent | 5.37 | 1.981 |
| sAPQ | 5.91 | 2.71 |
| Jitter percent | 1.86 | 0.629 |
| sPPQ | 0.96 | 0.42 |
| F0 | 183 | 238 |

Abbreviations: AVA, Acoustic Voice Analysis; CT, completion thyroidectomy; EBSLN, external branch of superior laryngeal nerve; TT, total thyroidectomy.

Results

One-hundred sixty-four patients/198 (82.8%) matched the inclusion criteria and were included in the study. Patient age ranged between 23 and 75 years (median = 42 years); 33 (20.1%) were males and 131 (79.9%) were females.

Based on the type of surgery, patients were divided into 2 groups: the first included 111 patients that underwent TT (group TT), the second included 53 patients that underwent CT (group CT). Among the patients included in the study, 4 in group TT and 2 in group CT experienced a transient RLN palsy that recovered within one month after surgery and were subsequently excluded from further evaluation.

At postoperative evaluation, 1 (0.93%) patient (male) in group TT and 4 (7.84%) patients (all females) in group CT showed VLS findings and AVA modifications (reduction in F0; increasing of jitter percent and shimmer percent; Table 1) consistent with EBSLN damage.^{13,14} Alterations were significantly more common in group CT ($P < .05$), suggesting a higher incidence of EBSLN injury in CT compared to TT.

Patients without specific signs of EBSLN injury ($n = 153$; 106 in group TT and 47 in group CT) were further compared to healthy controls within same-sex subgroups. Patients that underwent thyroid surgery presented at AVA some acoustic parameters indicating altered amplitude micro-perturbations when compared with healthy controls; detailed results are shown in Table 2 and Table 3. Within female patients, the results of the Shim and sAPQ MDVP parameters were significantly higher in patients that underwent surgery, in both group CT and TT, compared to healthy controls ($P < .05$), while other parameters (F0, Jitt, sPPQ) were not significantly altered ($P > .05$). The same alterations were also found within male patients. Interestingly, the Shim and sAPQ MDVP values were also significantly higher in group CT when compared to group TT (Table 4), both in women and in men.

Table 2. AVA and VHI in female patients of the group TT vs healthy controls.

| | Patients | Controls | P Value |
|---|----------|----------|--------------------------------|
| Shim | 4.15 | 2.22 | $P < .05$ |
| sAPQ | 4.81 | 3.3 | $P < .05$ |
| Jitt | 0.93 | 0.64 | $P > .05$ |
| sPPQ | 0.76 | 0.44 | $P > .05$ |
| F0 | 218 | 239 | $P > .05$ |
| VHI p (perception of vocal Patients emission characteristics) | 4.33 | 0 | $P < .05$ |
| VHI f (daily activities impact) | 1.06 | 0 | $P > .05$ |
| VHI e (psychological impact) | 0.91 | 0 | $P > .05$ |

AVA and VHI between women of group CT vs healthy control women (One Way ANOVA)

| | Patients | Controls | P Value |
|---|----------|----------|--------------------------------|
| Shim | 5.27 | 2.22 | $P < .05$ |
| sAPQ | 5.99 | 3.3 | $P < .05$ |
| Jitt | 0.99 | 0.64 | $P > .05$ |
| sPPQ | 0.75 | 0.44 | $P > .05$ |
| F0 | 212 | 239 | $P > .05$ |
| VHI p (perception of vocal patients emission characteristics) | 4.49 | 0 | $P < .05$ |
| VHI f (daily activities impact) | 1.15 | 0 | $P > .05$ |
| VHI e (psychological impact) | 0.98 | 0 | $P > .05$ |

Abbreviations: ANOVA, analysis of variance; AVA, Acoustic Voice Analysis; CT, completion thyroidectomy; EBSLN, external branch of superior laryngeal nerve; TT, total thyroidectomy; VHI, Voice Handicap Index. Bold values are statistically significant values ($P < 0.05$).

At VHI, the VHI_p scale scores (measuring self-perception of voice emission characteristics) were significantly worse in patients undergoing surgery compared to healthy controls ($P < .05$), while no statistically significant differences were found between patients and controls for VHI_f (impact in the daily activities) and VHI_e (psychological impact), neither in women or men, in both groups. The comparison between VHI results between group TT and group CT showed statistically significant worse scores of the VHI_p scale in the group CT, compared to group TT, both in women and in men. No statistically significant differences were found for VHI_f and VHI_e between the 2 groups, independently from sex.

Discussion

In the recent years, new techniques and surgical instruments in thyroid surgery have improved the quality of the surgical procedures and reduced postoperative complications,¹⁵⁻¹⁸ including damage to the EBSLN, whose preservation is gaining increasing importance. Several approaches have been proposed to preserve the integrity of EBSLN, such as the isolation and individual ligation of superior pole vessels adjacent to the thyroid capsule, a careful identification of EBSLN prior lacing the vasculature and the neuro-monitoring of the EBSLN during surgery.¹⁸

Despite the increasing attention to preserve the EBSLN and RLN and avoidance of trauma to the cricothyroid muscle, vocal

Table 3. AVA and VHI in male patients of the group TT vs healthy controls.

| | Patients | Controls | P Value |
|---|----------|----------|-------------------|
| Shim | 5.06 | 2.89 | P < .05 |
| sAPQ | 6.01 | 3.43 | P < .05 |
| Jitt | 0.63 | 0.64 | P > .05 |
| sPPQ | 0.61 | 0.51 | P > .05 |
| F0 | 138 | 155 | P > .05 |
| VHI p (perception of vocal Patients emission characteristics) | 3.59 | 0 | P < .05 |
| VHI f (daily activities impact) | 0.48 | 0 | P > .05 |
| VHI e (psychological impact) | 0.16 | 0 | P > .05 |

AVA and VHI between men of group CT vs healthy control men (One Way ANOVA)

| | Patients | Controls | P value |
|---|----------|----------|--------------------|
| Shim | 6.18 | 2.89 | P < .05 |
| sAPQ | 7.36 | 3.43 | P < 0.05 |
| Jitt | 0.7 | 0.64 | P > .05 |
| sPPQ | 0.68 | 0.51 | P > .05 |
| F0 | 131 | 155 | P > .05 |
| VHI p (perception of vocal Patients emission characteristics) | 4.95 | 0 | P < .05 |
| VHI f (daily activities impact) | 0.53 | 0 | P > .05 |
| VHI e (psychological impact) | 0.12 | 0 | P > .05 |

Abbreviations: ANOVA, analysis of variance; AVA, Acoustic Voice Analysis; CT, completion thyroidectomy; EBSLN, external branch of superior laryngeal nerve; TT, total thyroidectomy; VHI, Voice Handicap Index. Bold values are statistically significant values ($P < 0.05$).

symptoms frequently occur after thyroidectomy.¹⁹ Such alterations may be due to other mechanisms that may also account for often reported postoperative voice changes. In fact, several studies have demonstrated that most of vocal alterations after thyroid surgery were not related to EBSLN or RLN damage, and described a highly variable spectrum of symptoms among patients, mainly during the early postoperative period.^{14,20,21} Among the causes, it has been hypothesized a transient neural conduction disorder involving the RLN or the EBSLN, an arytenoid trauma after endotracheal intubation, a cricothyroid muscle movement disorder, a mucosal damage, hematoma, inflammation or vocal fold edema induced by faulty venous or lymphatic drainage (or both), a surgical trauma with modification of vascular supply and venous drainage of the larynx, a surgical trauma with prelaryngeal muscle fixation to laryngo-tracheal axis with the consequent reduction in vertical movements, an intraoperative injury of fine anastomotic branches connecting RLN and EBSLN and/or one of their anastomosis with the sympathetic cervical chain, and an injury to perithyroid neural plexus.^{14,22-33}

Symptoms and endoscopic findings of EBSLN palsy are often underestimated both before and after surgery and can be better diagnosed with cricothyroid electromyography in addition to VLS. The most important sign of EBSLN injury is posterior glottal deviation toward the affected side at postoperative VLS,²³ as confirmed by electromyography,¹ even if

Table 4. AVA and VHI in female patients of the group CT vs female patients in the group TT.

| | Group CTT | Group TT | P Value |
|---|-----------|----------|-------------------|
| Shim | 5.27 | 4.15 | P < .05 |
| sAPQ | 5.99 | 4.81 | P < .05 |
| Jitt | 0.99 | 0.93 | P > .05 |
| sPPQ | 0.75 | 0.76 | P > .05 |
| F0 | 212 | 218 | P > .05 |
| VHI p (perception of vocal Patients emission characteristics) | 4.49 | 4.33 | P < .05 |
| VHI f (daily activities impact) | 1.15 | 1.06 | P > .05 |
| VHI e (psychological impact) | 0.98 | 0.91 | P > .05 |

AVA and VHI between men of group CT vs men of group TT (One Way ANOVA)

| | Group CT | Group TT | P Value |
|---|----------|----------|-------------------|
| Shim | 6.18 | 5.06 | P < .05 |
| sAPQ | 7.36 | 6.01 | P < .05 |
| Jitt | 0.7 | 0.63 | P > .05 |
| sPPQ | 0.68 | 0.61 | P > .05 |
| F0 | 131 | 138 | P > .05 |
| VHI p (perception of vocal Patients emission characteristics) | 4.95 | 3.59 | P < .05 |
| VHI f (daily activities impact) | 0.53 | 0 | P > .05 |
| VHI e (psychological impact) | 0.12 | 0 | P > .05 |

Abbreviations: ANOVA, analysis of variance; AVA, Acoustic Voice Analysis; CT, completion thyroidectomy; EBSLN, external branch of superior laryngeal nerve; TT, total thyroidectomy; VHI, Voice Handicap Index. Bold values are statistically significant values ($P < 0.05$).

some authors^{34,35} described posterior glottal deviation also in patients with uninjured EBSLN. This evidence, in addition to the invasiveness of cricothyroid electromyography, has led most clinicians to use VLS alone in the daily practice to evaluate signs of cricothyroid muscle dysfunction.^{33,36,37} However, a complete and accurate analysis of vocal quality through a software tool for quantitative acoustic assessment of voice quality could further help identify preoperative and postoperative EBSLN alterations and could safely replace the use of more invasive tests such as cricothyroid electromyography, especially in the preoperative phase. Previous reports have demonstrated that patients who undergo thyroidectomy may show at AVA significant alterations with decrease in Maximum Phonation Time (MPT),³³ F0^{7,20,38} and phonational frequency range.¹⁴ Nevertheless, in another study, Hong and Kim³³ did not find significant differences for MPT and other objective voice parameters in laryngeal nerves injury-free patients after thyroidectomy. In the present study, preoperative AVA in addition to VLS allowed an accurate exclusion of cricothyroid muscle damage,¹⁸ while postoperative evaluation allowed the identification of patients with EBSLN injury. Additionally, the absence of significant deterioration in mean vocal fundamental frequency (F0) among patients reporting voice changes without EBSLN injury confirmed indirect evidence of preserved superior laryngeal nerve integrity and function.³³

Many authors have studied the incidence of voice disorders in the postoperative period after thyroidectomy, but most of them analyzed the patients only in the early postoperative phase.^{5,20,27,39} On the contrary, only a few studies examined vocal alterations in the longer term (> one year).^{40,41} In a previous study,²³ we observed some vocal alterations in patients undergoing thyroidectomy despite intraoperative and postoperative integrity of both superior and inferior laryngeal nerves, hypothesizing that these alterations may follow an abnormal fibrosis of prelaryngeal muscles after dissection and suturing. In fact, after thyroid surgery, the laryngo-tracheal unit is supported only by the prelaryngeal muscles and an abnormal or excessive scarring after dissection and suturing or even simple manipulation during surgery may lead to a functional deficit.²⁴ Such vocal alterations, especially in the long term, may constitute a moderate to severe handicap in professional and social life of patients. Furthermore, the involvement of sternohyoid and sternothyroid muscular section in voice function is still unknown. Hong et al³³ used VLS and electromyography in patients whose strap musculature was not divided during thyroidectomy and who had normal laryngeal nerve function postoperatively, and hypothesized that laryngo-tracheal fixation of the extralaryngeal strap musculature could result in post-thyroidectomy voice alterations.

In our sample, we found a higher rate of EBSLN damage in patients undergoing CT compared to TT. Furthermore, 12 to 18 months after surgery, even in patients without RLN and EBSLN injury, we found some mild vocal alterations at AVA with MDVP in parameters concerning the variability of loudness (Shim and sAPQ) both in patients who underwent TT and CT when compared to the mean parameters of healthy controls. Another interesting finding was that these parameters (Shim and sAPQ) were significantly worse in patients undergoing CT than in those undergoing TT; these alterations were independent from sex.

At VHI, when comparing self-perception of voice (VHI_p) in patients who underwent total or CT and healthy control values, we found many patients that reported subjective changes in their voice perception, despite they did not report vocal problems in their daily activities (VHI_f) or psychological problems concerning their voice (VHI_e). Comparing the results of the VHI questionnaire between patients of group CT and group TT, we observed a significantly worse self-perception of voice in patients who underwent CT compared to TT, although these alterations were not referred as limiting for patients psychologically and in daily life. These alterations were independent from sex.

In the present study, thyroidectomy (both TT and CT) (1) was performed by the same surgeon with the same technique, thus eliminating a possible bias caused by different surgical techniques; (2) was done displacing the prelaryngeal muscles and never sectioning and resuturing them; and (3) in case of CT, consisted of surgical reintervention in patients treated in other hospitals at least 1 year before, thus a bilateral surgical revision of operating field without neck dissection was

performed. Our findings, particularly the alterations found in the acoustic parameters related to peak-to-peak amplitude variability, suggest that after thyroidectomy (and particularly in a completion surgery), in some patients the laryngeal muscles may be unable to maintain a stable voice emission. Although our data does not allow to establish a direct relationship, we can hypothesize that, despite the preservation of EBSLN, the routine surgical maneuvers performed on prelaryngeal muscles may induce fibrosis, altering the laryngeal muscle balance and consequently the voice emission of the patient. We can also suppose that surgery-related fibrosis might explain the persistence of vocal alterations in our sample for 1 year or more,⁵ especially when following a revision surgery that could further modify the laryngeal muscle balance and consequently slightly deteriorate the vocal emission. This is further supported by findings of previous studies that focused on timing of completion surgery and concluded that CT should be performed either within 10 days of the primary operation or beyond a minimum of 3 months in order to avoid strong postoperative adhesions.^{42,43}

The existence of other factors in addition to laryngeal nerve injury contributing to voice changes after thyroidectomy and the considerable variability of patient symptomatology highlight the importance of a routine comprehensive functional voice analysis before and after thyroid surgery to (1) detect unknown preoperative vocal modifications, (2) provide an early diagnosis of postoperative vocal disturbances, and (3) monitor the progress of laryngeal lesions. Moreover, a comprehensive functional voice assessment would allow early diagnosis of vocal disturbance and permit early voice rehabilitation. To further confirm our preliminary findings and eliminate potential sources of bias, future studies should compare pre and postoperative MDVP evaluation and cricothyroid electromyography to further define the physiology of post-thyroidectomy dysphonia.

Conclusions

To the best of the authors' knowledge, this is the first study that employs a complete functional voice assessment of postoperative vocal outcomes comparing TT and CT. In our study, we noticed a statistically significant higher rate of EBSLN injury in CT compared to TT, suggesting a higher surgical risk in CT. We also observed that, both in TT and in CT, even without RLN and EBSLN injury, there may be mild alterations in some parameters of AVA, such as the Shim and sAPQ, and in self-perception of voice (VHI_p) 12 to 18 months after surgery. An interesting finding was also that Shim and sAPQ and self-perception of voice (VHI_p) resulted significantly worse after CT rather than after TT, both in women and in men, suggesting a larger trauma in patients' vocal outcome in CT if compared to TT, although these alterations were not reported as psychologically limiting daily life of patients. The presence of multiple factors contributing to vocal alterations after thyroidectomy highlight the importance of a routine comprehensive functional voice analysis before and after surgery to (1) discover

unknown preoperative alterations, (2) permit an early diagnosis of postoperative vocal disturbances, (3) monitor the progress of laryngeal lesions, and (4) start an early voice rehabilitation.

Authors' Note

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request. All patients consented to publish their data. Our study received approval from the Ethics Committee Azienda Policlinico Umberto I, Rome. All patients declared their consent to participate to the study.


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