ACTA Otorhinolaryngologica Italica

Official Journal of the Italian Society of Otorhinolaryngology Head and Neck Surgery

Organo Ufficiale della Società Italiana di Otorinolaringoiatria e Chirurgia Cervico-Facciale

Volume 40
June 2020
3

PACINI EDITORE MEDICINA

www.actaitalica.it

POSTE ITALIANE SPA - Spedizione in Abbonamento Postale - D.L. 353/2003 conv. in L. 27/02/2004 n° 46 art. 1, comma 1, DCB PISA - Iscrizione al tribunale di Pisa al n. 10 del 30-07-93 - Finito di stampare presso IGP Pisa - Giugno 2020 - ISSN: 0392-100X (Print) - ISSN: 1827-675X (Online)

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Publisher
Pacini Editore Srl
Via Gherardesca, 1 - 56121 Pisa, Italy
Tel. +39 050 313011 -Fax +39 050 3130300
info@pacinieditore.it - www.pacinimedicina.it

Acta Otorhinolaryngologica Italica is cited in Index Medicus, MEDLINE, PubMed Central, Science Citation Index Expanded, Scopus, Open-J Gate, Free Medical Journals, Index Copernicus, Socolar

2019 Journal Impact Factor, Journal Citation Reports (Source Clarivate, 2020): 1.510
Acta Otorhinolaryngologica Italica is available on Google Scholar

Volume 40
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3

www.actaitalica.it

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Articles in open access, information for authors including editorial standards for the preparation of manuscripts and submission and Publisher's note available on-line: www.actaitalica.it

ACTA OTORHINOLARYNGOLOGICA ITALICA 2020;40:157-163; doi: 10.14639/0392-100X-N0674

Review

Are errors in otorhinolaryngology always a sign of medical malpractice? Review of the literature and new perspectives in the SARS-CoV-2 (COVID-19) era

Gli errori medici in otorinolaringoiatria sono sempre indici di colpa medica? Revisione della letteratura e nuove prospettive nell'era SARS-CoV-2 (COVID-19)

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SUMMARY

In medical practice, during certain procedures that usually are not regarded highly demanding, some skill-based errors, that might not be considered as medical malpractice, may occur. In fact, such errors can be caused by factors beyond the physician's control.A review of Greek case law regarding medical malpractice in otorhinolaryngology was performed to identify cases of lawsuits that concerned medical errors during routine procedures. The analysis of the cases showed that some medical errors may cause serious complications, even if deviation from the standard of medical care is minimal. Thus, in some cases it may be difficult to make a distinction between preventable and unpreventable complications. Certain medical errors from routine medical procedures might be considered unpreventable and, therefore, classified as almost no-fault errors. A brief commentary regarding opportunities to further improve the medical liability system after the SARS-CoV-2 emergency is also given.

KEY WORDS: medical error, medical malpractice, negligence, otorhinolaryngology, COVID-19

RIASSUNTO

In ambito medico legale, durante alcune procedure non particolarmente complesse, possono verificarsi errori che potrebbero non essere necessariamente ricondotti a colpa medica. La ragione è che tali errori possono essere causati da fattori che vanno oltre il controllo del medico. È stata fatta una revisione della legislazione greca, riguardante casi di colpa medica in otorinolaringoiatria, allo scopo di identificare i casi giuridici riguardanti errori medici durante tali procedure. L'analisi dei casi ha evidenziato che alcuni errori medici possono causare complicanze serie, anche in caso di errori minimi che deviano di poco dallo standard di trattamento. Per questo motivo, in alcuni casi può essere difficile distinguere tra complicanze prevedibili e non prevedibili. Alcuni errori, che si verificano durante procedure mediche di routine, potrebbero essere considerati non prevedibili e per questo classificati come errori "quasi" senza colpa. Viene infine fornito un breve commento sulle opportunità di migliorare ulteriormente il sistema di responsabilità medica dopo l'emergenza SARS-CoV-2.

PAROLE CHIAVE: *errore medico, colpa medica, negligenza, otorinolaringoiatria, COVID-19*

Introduction

In Ear-Nose-Throat (ENT) surgery, errors can be committed even by a skillful surgeon. However, otorhinolaryngology is a medical specialty with a low rate of malpractice. In a recent study of the American College of Surgeons, it

Received: February 7, 2020
Accepted: May 5, 2020
Published online: June 10, 2020

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Funding
This work has been supported by Linea D1 Fondi di Ateneo Università Cattolica del Sacro Cuore to Antonio Oliva.

Conflict of interest
The Authors declare no conflict of interest.

How to cite this article: Voultsos P, Oliva A, Grassi S, et al. Are errors in otorhinolaryngology always a sign of medical malpractice? Review of the literature and new perspectives in the SARS-CoV-2 (COVID-19) era. Acta Otorhinolaryngol Ital 2020;40:157-163. https://doi.org/10.14639/0392-100X-N0674

was found that only 12% of otolaryngologists had received claims against them in the past two years [1], while a Danish study found an increasing trend in the number of otorhinolaryngology malpractice claims.

In this specialty, complications are seldom severe, but are strongly surgery-related [2], and, therefore, proper technical and non-technical skills and full compliance with guidelines and international standards are pivotal to avoid malpractice litigation [3]. "ENT Today" reported in October 2013 that, during the period 2007-2011, 53% of allegations against otolaryngologists were associated with "improper performance of surgery". Among 40 claims lodged in the UK for malpractice related to tonsillectomy (TE) during the period 1995-2010, the most common injury was postoperative bleeding, followed by nasopharyngeal regurgitation (a potential injury of the glossopharyngeal nerve that may occur during ENT surgery) [4]. A German study, which included the 50 most common inpatient ENT surgical procedures (septoplasty, TE with or without adenoidectomy (AE), etc.), detected "surgical malpractice" in 6.1% of all cases [5]. The complexity of ENT surgery, along with individual anatomical variations and the close proximity to critical anatomical structures, may explain why some severe complications should be considered inevitable, even for the most skilled – and experienced surgeon, especially in the case of transnasal surgery and functional endoscopic sinus surgery (FESS) [6]. In these cases, such errors may be classified as "almost system errors" and considered as unpreventable adverse events. In this paper, we discuss three Greek cases of errors in Otorhinolaryngology, showing the main technical and medico-legal issues. Our aim is to underline that not all technical errors should be always considered as medical malpractice.

Case descriptions

A review of the Greek legal database was performed over the period of the past 15 years. Three cases of medical malpractice, due to erroneous maneuvers of ENT surgeon, were identified (summarised in Tab. I). More specifically, two concerned erroneous surgical maneuvers resulting in injury to anatomical structures. One case concerned medical maneuvers (which caused laryngeal injury and, hence, a fatal reflex reaction) performed in the post-operative phase and under emergency conditions.

Case 1

Judgment No. 1135/1993 of the Greek Supreme Court ("Areios Pagos") regards the case of an otolaryngologist who was trying to stop a heavy nasal bleeding that occurred during a septoplasty. Therefore, he was putting pressure using forceps directly on a piece of gauze, which he had inserted into the nasal cavity, against the bleeding area. He applied too much pressure on the forceps, causing its shift towards the upper part of the nasal cavity, breaking the cribriform plate of the ethmoid bone. This resulted in a hole through which the endocranial and nasal cavities communicated. Thus, a large volume of air entered the endocranial cavity, causing swelling of nasal mucosa and the right sinus, resulting in radiological finding of an hyperdense mass at the right caudate nucleus of the endocranial cavity, communicating hydrocephalus of the lobe, meningoencephalitis and inflammation of the brain ventricles due to antibiotic-resistant staphylococcus and Candida fungus. The otolaryngologist who performed the operation abandoned the patient without informing the director. Diagnosis of the complication was done by CT performed six days after surgery. The surgeon was aware of what had happened, and hence, he should have informed the other physicians treating the patient. The Court sentenced him to a term of 18-months detention.

Case 2

Judgment No. 3127/2009 of the Three-Member Court of Appeal of Thessaloniki concerned an excessive tissue resection during a TE under local anesthesia that resulted in impaired function of the glossopharyngeal nerve (with subsequent rhinolalia aperta, reduced mobility and sensory disturbances to the soft palate and facial arches). The injury of the glossopharyngeal nerve was established through an expert report two years after the surgery. The surgeon was found guilty.

Case 3

In Judgment No. 4639/2002 of the Three-Member Court of

Table I. Case summaries.

Cases	Procedure	Claimed error	Conviction
1	Septoplasty	Rupture of the cribriform plate of the ethmoid bone	18-months detention
2	Tonsillectomy	Excessive tissue resection resulting in impairment of the glossopharyngeal nerve function	Guilty (unknown)
3	Abrupt insertion of the suction device	Fatal injury of the laryngeal aperture (stimulation of the vagus nerve and cardiac arrest)	1-year detention

Appeal of Athens, a physician on duty was sentenced to a term of 1-year detention for having inserted, in an "abrupt and unskillful manner", the metal nozzle of the tube of a suction device used to remove excretions that had accumulated postoperatively in the airways of a 16-year-old female patient who underwent surgery for turbinate hypertrophy and removal of a small nasal spine. Insertion of the nozzle may cause complications such as injuries and uncontrolled stimulation of the vagus nerve that may lead to cardiac arrest and death, which is the case in question. Post-mortem examination revealed injuries to the laryngeal aperture, confirming their iatrogenic nature.

Discussion

Regarding case 1, septoplasty is one of the most common operations in ENT surgery [7] and cerebrospinal fluid (CSF) leak is one of its possible complications [7]. When it occurs after septoplasty, it is mainly attributed to a cribriform plate defect inadvertently caused by a physician during the surgical procedure (iatrogenic complication) [7,8]. Fractures of the cribriform plate are related to poor technique or inadvertence, such as in the following cases: poor angling of dissection forceps, elevation of forceps beyond the ethmoid roof [9] and forceful removal of the perpendicular plate [10] of the ethmoid (by applying a multidirectional force). "Slit-shaped dehiscence at the horizontal lamella of the cribriform plate" may also be observed [10]. Surgeons should consider the occurrence of an undiagnosed encephalocele [11] or meningoencephalocele formation after septoplasty [12].

In Judgment No. 1135/1993 of the Supreme Court, the surgeon's inadvertence resulted in perforation of the cribriform plate in attempting to control bleeding that occurred during septoplasty. In our opinion, this case is exemplary because the serious complication might be viewed as being "in all likelihood unavoidable", provided that the subjective perception of the surgeon (who was under stress) played an essential role. To avoid the occurrence of a CSF fistula, multidirectional forces should not be applied and accurate preoperational knowledge of the possible anatomical variations would be essential [13]. Importantly, the ethmoid roof level may be different on each side (right and left) [14]. Bony structures in the anterior cranial fossa are very thin and dura mater is tightly attached to them [7]. In the case of a CSF fistula, the symptoms appear immediately after septoplasty. CSF leakage typically occurs after 12-22 weeks [10]. Notwithstanding, Soni et al. reported a case of CSF leakage that occurred 2 weeks after septoplasty [11]. During septoplasty (especially endoscopic septoplasty), a surgeon may use some anatomical landmarks in order to reduce the probability of complications. Interestingly, Seth et al. stated that "the inferior turbinate and vertical middle turbinate attachment may be used to guide the extent of cartilage resection" [15]. Some of these landmarks may not be totally reliable. Schultz-Coulon recommend the use of a microscope to obtain optimal visualisation and sparing of the junction area between the lamina quadrangularis and perpendicularis. It is not clear whether under those particular circumstances it would be possible even for a very skillful, experienced and diligent surgeon to be aware of the borderline between due and excessive pressure exerted on the cribriform plate of the ethmoid bone. In addition, it is worth mentioning that the cribriform plate is so thin that it can be broken during the intra-operative phase without the surgeon noticing it.

Regarding case 2, nerve lesions may occur during ENT surgery due to errors in surgical procedures, resulting in deterioration of the patient's quality of life. In TE with or without adenoidectomy (AE), injuries of the shaft or tonsillar or lingual branches of the ninth cranial nerve may develop, resulting in dysgeusia and ageusia as well as motor disorders of the soft palate, resulting in rhinolalia aperta, regurgitation, or a combination of both [16]. Velopharyngeal insufficiency following TE is reported in the literature, and, hence, preoperative evaluation of the anatomical variations in the velopharynx is recommended [16]. A very rare but distressing type of lesion to the ninth cranial nerve due to TE is the underdiagnosed secondary glossopharyngeal neuralgia. When the dissection starts in the incorrect surgical plane during TE, injury of the ninth nerve may possibly occur because of the proximity of the nerve's course to the tonsillar fossa [16]. Not only there is a close proximity between the cranial nerves and the area where TE is performed, but there are also different motor and sensory pathways in the same nerves [16]. Therefore, the same medical error may result in complications that could be classified into different severity. Lesions of the superficial petrol nerve endings may occur during TE. Lesion to the hypoglossal nerve occurs less frequently during TE in comparison with lesion of the glossopharyngeal nerve because of the deep anatomical position of the nerve. A lesion to the hypoglossal nerve can hardly ever occur without serious concurrent bleeding, given the proximity of the nerve to carotid artery branches. During TE, an injury of the aberrant courses of the internal carotid artery may occur.

As in this specific case, "excessive tissue resection" may occur in TE, resulting in nerve injuries. However, there may be cases where it may be (almost) impossible for a very skillful, experienced and diligent surgeon to distinguish the due tissue resection from the excessive one. It is known that in the area of the pharynx, inside the same nerves, there are different sensory and motor pathways and it is difficult

to make accurate detection of the anatomical course of a nerve, especially of its branches and endings. Therefore, it is necessary having a great awareness of which nerves are at risk. It is also to be noted that aberrant vessels may run close to the oropharynx, rhinopharynx and the tonsil fossa. Thus, some nerve injuries in ENT surgery might be classified as 'in all likelihood unpreventable'.

In case 3, the Court of Appeal of Athens concluded that the defendant conduct was neither as diligent nor as accurate as it should have been. In fact, the physician inserted abruptly the nozzle of the suction device tube. The negligence was considered gross since the breach of duty consisted in a clear and significant deviation from the standard of care and occurred in a phase of the procedure in which no particular technical or non-technical skills were required to achieve a good outcome (the consequences of error would have been likely avoidable if the required attention had been paid).

General medico-legal concerns

Negligence usually includes doing something that an ordinary, reasonable and prudent practitioner would not do, or not doing something that a person like that would do considering circumstances and knowledge. In case that the "objective bystander" reconstruct (ex post) the micro-movements of a particular surgical procedure, some of them might be found to be erroneous, while they are not, in all likelihood, foreseeable from the perspective (ex ante) of an ordinary physician of the relative specialty, because of their high complexity. A physician who committed an erroneous maneuver should be regarded as he made a "mistake of fact", by reducing or eliminating the physician's civil liability or criminal culpability, only in the case in which an ordinary, reasonable, and prudent physician, working under similar circumstances, could not adapt micro-maneuvers to the conditions of the particular patient. There are cases of erroneous medical maneuvers, occurring during routine procedures, in which a sharp line of distinction between medical negligence and no-fault error may be extremely difficult to be drawn. It is difficult to rule out with certainty that a given erroneous medical maneuver was practically unavoidable and hence constitutes a "no-fault error". The preventability of an erroneous maneuver may be established with the probability, according to which a maneuver might be classified as (almost) negligence (in case of high degree of preventability) or (almost) no-fault error (in case of low degree of preventability).

In cases 1 and 3, the adverse event may be viewed as "in all likelihood unpreventable", whereas in case 2 it may be considered "in average likelihood" unpreventable. The line drawn between unwitting no-fault error and inadvertent negligence-based error may be blurry. This may be due to a variety of factors such as: fallibilities and risks inherent in excellence of a medical specialty, fallibilities inherent in the physician's mind and environmental factors that may influence the physician, as well as the interaction among these factors. Subjective perception plays a leading role, for example, when the physician should have performed a "careful penetration of an instrument" or should have exerted a "mild" pressure on a delicate and brittle anatomic structure to stop the bleeding. A surgeon may be involved in medical litigation for an unavoidable complication due to "unpredictable" situations (e.g. those due to the "idiosyncrasy", the particularities of the patient, or spontaneous movement of the patient's body during a surgical procedure). Additionally, a physician may find himself acting under the influence of situational factors (e.g. conditions of extreme stress). Regarding the case of a physician who exerts a certain pressure on an anatomical structure, the focus should be put on the physician's awareness of that pressure. We may assume that, when pressure has been exerted on a thin bone surface, the degree of awareness may be determined by analogous situations experienced before. Moreover, such influences may result from dynamic and complex interactions between factors such as the physician's biorhythm, stress, distress and other (mostly environmental) factors. These factors may deprive a physician of abilities not only to perfectly reflect and ponder, but also to be fully aware of what he/she is doing.

Certain routine medical error cases should not be considered medical malpractice because of their complexity and difficulty. In order to classify them as "too much complex and difficult", every single event of the medical procedure should be strictly analysed, as well as the circumstances under which the procedure was carried out. The adverse events that result from erroneous medical maneuvers considered "in all likelihood unpreventable" should be classified as almost no-fault errors. Sohn remarks that negligence is not at the centre of most medical errors [17], thus implying that most of them are, in reality, system errors.

The no-fault compensation system

The no-fault compensation system seems to better serve the purposes of civil medical liability, which is focused on the patient (namely, on restoration of damage) rather than the physician (namely, on indictment and sentence or payment of compensation). Sohn stated that probably "a more rational system would focus more on the goals of compensation and improvement, rather than on punishment for those who err" [17]. Notwithstanding, medical negligence is considered a failure to meet a requisite standard of care [17]. This

is probably the main reason why French Jurisprudence of the courts oscillated between two positions: the obligation of the physician to guarantee a safe result by correctly carrying out a surgical procedure, and the obligation to satisfy the standard of care, which correspond to the rules of "good medical practice" and prudence [18]. In Italy, medical tort and criminal law were radically reformed by Law N. 24/2017. This law can be considered a "safe harbour law": when a physician commits an error and the error causes injury and avoidable harm to the patient, he cannot be considered criminally liable if full compliance with national guidelines or international/national best practices (e.g. international guidelines) is proven. Obviously, this "safe harbour" cannot be granted in cases of gross negligence (when the conduct significantly deviated from the standard of care). Regarding tort law, when the defendant committed the error in a public or private hospital, the plaintiff (the patient) must prove breach of duty (while, before the Law N. 24/2017, in many cases the defendant had to prove his innocence). This rule is not valid in cases of lawsuits directly against hospitals: when this occurs, the hospital has to prove that the claim is unfounded. This shift of the burden of proof is substantial, because it aims to deflate the lawsuits against physicians but, at the same time, allows patients to obtain compensation directly from hospitals [19].

It is important to mention a current and critical problem worldwide, namely severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2). This outbreak began with a cluster of cases of pneumonia in Wuhan (December 31, 2019) and increasingly spread globally, with the World Health Organization declaring a pandemic (March 11, 2020). During this critical period, the Italian Government introduced Decree-Law N. 18 (March 17, 2020) in order to reduce the impact of Covid-19 and strengthen Public Health through its reorganisation. In fact, in the most highly affected regions of the country, many hospitals (both public and private) have been turned into Covid-centers [20]. However, Decree-Law N. 18 provides nothing about medical liability, and therefore an amendment has been recently proposed. This concerns the abolition of both civil and criminal liability for medical errors, occurring in this critical time, except in cases of serious professional misconduct and willful misconduct. Moreover, according to this proposal, cases of serious professional misconduct would be assessed by taking into account the number of patients in need of care and availability of medical resources (health professionals, medical devices) considering the emergency situation in which the medical staff is working. However, this amendment is currently under consideration, and has not been approved.

In general terms, since unpreventable (or in all likelihood unpreventable) technical medical errors resulting from routine medical procedures should not be regarded as medical negligence, the implementation of the "no-fault" system would be considered as a necessary reform of the medical malpractice system. Importantly, the particularities of each single case should be examined carefully and precisely in order to establish if the given case of medical error would be eligible for compensation through the so-called "no-fault compensation system" or not. The so-called "system errors" would be more fairly addressed through the "no-fault" compensation system. Generally speaking, system errors are errors for which the responsibility is institutional rather than individual. Sohn states that system error is an "occasional", "simple", "unwitting", "unavoidable" human error [17]. A system error is attributable to the healthcare system or bureaucracy, such as organisational error (staffing, failure to have expert mentorship, etc.) or improper processes (drug carts set out improperly, etc.). A kind of system error might be physicians having to be on call for significant periods of time without enough rest. Importantly, the line of distinction between system error and individual error may be blurry when it comes to technical human errors resulting from difficult, complicated and complex surgical procedures such as those examined before. It is argued in the literature that the "no-fault" system has more benefits compared to the negligence-based model, by reducing the costs of litigation and improving patient care [(21)]. Moreover, the "no-fault" system serves the interests of all the stakeholders involved in medical malpractice: patient, physician, healthcare system and the whole community. It is further argued that there is a strong public interest in the implementation of the "no-fault system" [17]. Not surprisingly, the "no-fault" system seems to be better applied when it comes to injuries caused during ultra high-risk surgical procedures, in which surgeon negligence is difficult to ascertain [22]. This may happen even if the surgeon is experienced, skillful and prudent [23].

As emerged from the documents retrieved from the proceedings of the trials, the overriding and ultimate goal of all the claimants was not economic, namely, they wanted to achieve the punishment of physicians who erred. Some claimants wanted to find out what really happened. Thus, in case of a medical error that might be viewed as almost "no-fault" error, the claimants' goal may not be, most likely, the punishment of the physician. As a consequence, many claimants might seek noneconomic types of redress. In addition, the amount of the compensation sought might be lower and there might not be criminal cases against physicians. Interestingly, according to the "no-fault system" the extent of compensation is generally lower than that concerning the tort system, and therefore with budgets similar

to the costs of the tort system more patients would be compensated. Finally, it is crucial to bear in mind that, contrary to the negligence-based system that "objectifies" medical liability and according to the "no-fault system", physicians might be unpunished, and hence would be strongly discouraged from practicing "defensive medicine" [17], which represents a huge cost for Public Health [24]. The "no-fault system" benefits both physicians and patients, and fosters a good relationship between them [21]. In this perspective, the patient's trust in the doctor would be strengthened, leading to an improvement in the quality of healthcare. Moreover, it also promotes the public interest by reducing the huge costs of litigation and those of "defensive medicine" [21,25,26]. These two aspects especially concern surgeons compared to clinicians, since they have a higher risk of medico-legal events [27] since they are involved in surgical procedures which, in most cases, are at high risk of errors due to their complexity and difficulty. Moreover, in support of the no-fault compensation system, the 1982 President's Commission for the Study of Ethical Problems in Medicine and Biomedical Behavioral Research stated that "a successful compensation system would treat like cases alike, make fair payment for the harm sought to be remedied, and disburse funds with maximum efficiency and minimum administrative cost" [28].

Conclusions

There are routine medical procedures in which erroneous medical maneuvers may cause serious complications even though the deviation from the standard of medical duty of care (or diligence/prudence) was only slight. Under certain circumstances, it may be extremely difficult to draw a sharp line of distinction between avoidable and unavoidable complications caused by such maneuvers. Additionally, under particular circumstances, it may be very difficult or impossible to make "ex post" effective and reliable judgment about a physician's negligence. Skill-based medical failures may be caused by situational factors that can strongly influence the physician's control over his/her abilities. In conclusion, there are technical medical errors resulting from routine medical procedures that are unavoidable or in all likelihood unavoidable. These errors might be classified as almost no-fault errors. The adoption of the "no-fault compensation system" by the medical liability system seems to address the aforementioned errors in a fair manner. In Greece, the implementation of the no-fault system should be supported within a narrow range inclusive of "in all likelihood unpreventable" human errors resulting from routine medical procedures. Compensation of such errors through the no-fault system would offer significant advantages (e.g. compensation in a timely manner, disclosure of the errors). Of note, however, the "no-fault" system's alleged disadvantages would in all likelihood remain unobserved, provided that the tort system will keep compensating the majority of medical errors. Our ambition is to offer an instrument to make better judgments about medical liability. However, more work is needed to increase awareness of this topic, especially after the SARS-CoV-2 emergency. During this critical period, the Italian Government introduced Decree-Law N. 18 (March 17, 2020) in order to reduce the impact of Covid-19 and strengthen Public Health through its reorganisation. This document also concerns the proposal regarding the abolition of both civil and criminal liability for medical errors, occurring in this critical time, except those cases of serious professional misconduct and willful misconduct. Although this amendment is currently under consideration and is not still approved, it represents a good opportunity to further improve the medical liability system in Italy as well.

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ACTA OTORHINOLARYNGOLOGICA ITALICA 2020;40:164-172; doi: 10.14639/0392-100X-N0409

Review

A review of the "OMICS" for management of patients with obstructive sleep apnoea

Una review sulle scienze OMICHE nella gestione del paziente con sindrome dell'apnea ostruttiva del sonno

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SUMMARY

Obstructive sleep apnaea (OSA) syndrome is a condition characterised by the presence of complete or partial collapse of the upper airways during sleep, resulting in fragmentation of sleep associated with rapid episodes of intermittent hypoxia (IH), activation of the sympathetic nervous system and oxidative stress. OSA is associated with a broad spectrum of cardiovascular, metabolic and neurocognitive comorbidities that appear to be particularly evident in obese patients, while affecting both sexes in a different manner and varying in severity according to gender and age. In recent years, studies on OSA have increased considerably, but in clinical practice, it is still a highly underdiagnosed disease. To date, the gold standard for the diagnosis of OSA is nocturnal polysomnography (PSG). However, since it is not well suited for a large number of patients, the Home Sleep Test (HST) is also an accepted diagnostic method. Currently, the major aim of research is to identify non-invasive methods to achieve a highly predictive, non-invasive screening system for these subjects. The most recent reports indicate that research in this field has made significant progress in identifying possible biomarkers in OSA, using -OMIC approaches, particularly in the fields of proteomics and metabolomics. In this review, we analyse these OMIC biomarkers found in the literature.

KEY WORDS: OMICS, proteomics, metabolomics, OSA

RIASSUNTO

La sindrome da apnea ostruttiva nel sonno (OSA) è una condizione caratterizzata dalla presenza di completo o parziale collasso delle vie aeree superiori durante il sonno, con conseguente frammentazione del sonno associata a rapidi episodi di ipossia intermittente (IH) e attivazione del sistema nervoso simpatico e dello stress ossidativo. L'OSA è associata ad un ampio spettro di patologie cardiovascolari, metaboliche, neurocognitive e comorbidità che appaiono particolarmente evidenti nei pazienti obesi, interessando entrambi i sessi in modo diverso e variando la gravità a seconda del sesso e dell'età. Negli ultimi anni, gli studi sull'OSA sono aumentati considerevolmente, ma nella pratica clinica, si tratta ancora di una malattia altamente sottodiagnosticata. Ad oggi, il gold standard per la diagnosi di OSA è la polisonnografia notturna (PSG). Tuttavia, poiché non è adatto ad un gran numero di pazienti, anche l'Home Sleep Test (HST) è un metodo diagnostico accettato. Attualmente, l'obiettivo principale della ricerca è quello di identificare metodi non invasivi per ottenere un sistema di screening altamente predittivo e non invasivo per questa categoria di soggetti. I lavori più recenti indicano che la ricerca in questo campo ha compiuto progressi significativi nell'identificazione di possibili biomarcatori in OSA, utilizzando approcci OMICI, in particolare nel campo della proteomica e della metabolomica. In questa review, analizziamo una lista di questi biomarcatori presenti in letteratura.

PAROLE CHIAVE: *OSA, scienze omiche, proteomica, metabolomica*

Received: July 30, 2019
Accepted: September 21, 2019

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Funding
None.

Conflict of interest
The Authors declare no conflict of interest.

How to cite this article: Conte L, Greco M, Toraldo DM, et al. A review of the "OMICS" for management of patients with obstructive sleep apnoea. Acta Otorhinolaryngol Ital 2020;40:164-172. https://doi.org/10.14639/0392-100X-N0409

Introduction

Obstructive sleep apnoea (OSA) is considered by far the most important form of sleep disturbance in breathing. It is caused by increased collapsibility or insufficiency/loss of muscular dilation capacity of the upper airways, leading to repeated pharyngeal constriction (hypopnoea) or closure (apnoea), therefore resulting in decreasing oxyhaemoglobin saturation and with increasing partial pressure of carbon dioxide in arterial blood [1]. To restore pharyngeal patency, patients experience recurrent awakenings, resulting in fragmented sleep, followed by reduced cognitive performance and, in some cases, diurnal sleepiness episodes.

Despite its high prevalence and the high burden of morbidity, OSA remains a significantly underdiagnosed disease worldwide. The Hypnolaus study estimated that the prevalence of moderate-to-severe sleep-disordered breathing (≥ 15 events per h) was 23.4% (95% confidence interval (CI), with a range of 20.9-26.0) in women and 49.7% (with a range of 46.6-52.8) in men [2], whereas according to the American Academy of Sleep Medicine [3], only 20% of patients are diagnosed (about 6 million of a total of 24 million) in the US. The annual cost for an undiagnosed patient is estimated at around $ 5,500 (considering direct and indirect health costs), while it decreases to $ 2,100 per year for diagnosed patients [4]. On this basis, it is evident that OSA is not only a serious health problem, but also a socio-economic issue.

OSA is also becoming dangerously frequent in children, associated with adenotonsillar hypertrophy [5] as well as high rates of overweight and obesity in children in Western countries. These trends will have disastrous long-term consequences for global health and life expectancy if solutions are not taken to correct erroneous lifestyles from the earliest age [6]. These data also suggest that the only way to make the costs of OSA sustainable is through prevention.

To date, the gold standard for diagnosis of OSA is nocturnal polysomnography (PSG). This sleep examination utilises electroencephalography, electrooculography in both eyes, sub-mental electromyography, nasal airflow, snoring sounds, electrocardiography, thoracic/abdominal movements, pulse oxygen saturation and body position to measure various parameters. The PSG indices included are apnoea-hypopnoea index (AHI) and oxygen desaturation index. However, since it is not well suited for a large number of patients, the Home Sleep Test (HST) is also an accepted diagnostic method [7,8]. Given the difficulty of applying the HST to the population as a screening system due to high costs and examination timing, researchers are currently focusing on identifying new biomarkers for early diagnosis of OSA [9]. In the case of sleep disorders and lung diseases, traditional biomarker research techniques have proved to be not particularly well performing. Studies based on proteomics and metabolomics, however, are more sensitive, although, to date, the number of molecules potentially available for clinical application in the context of OSA is still limited. The development of new technologies is therefore necessary, also to provide a greater understanding of the biochemical mechanisms involved in OSA.

In Table I, the list of proteins and metabolites differently expressed in OSA subjects identified in the literature is reported.

Proteomics approaches

The study of the proteome in OSA patients has been broadly assessed. Many studies have reported that OSA patients express increased levels of mediators of systemic inflammatory response. Zhang et al. [10] used, for the first time, a proteomic approach to detect protein profiles of serum extracellular microvescicle proteins in an intermittent hypoxia (IH) rodent model [11]. Extracellular microvescicles are vesicles released from cells into the extracellular fluid environment, including serum. Their potential utility in clinical diagnosis is well documented, since vesicles are reported to reflect the physiological or pathological status of the tissue from which they arise. They found 4 differentially expressed proteins in serum extracellular microvesicles compared to control: C-reactive protein (CRP), haptoglobin (HP), fibronectin (FN1) and platelet factor 4 (PF4). In addition, Nadeem et al., through meta-analysis of the literature [12], confirmed altered levels of CRP and other systemic inflammatory mediators, including intercellular adhesion molecules (ICAM), coagulation factors (factor VIII, tissue factor) and a significant increase in serum levels of tumour necrosis factor alpha (TNF-α), interleukin 1β (IL-1β) and interleukin 6 (IL-6) in patients with OSA. The excessive infiltration of inflammatory cells is also highlighted by the formation of subepithelial oedema in OSA patients as documented by histology. Among these proteins, circulating CRP is an important predictive factor of cardiovascular risk involved in the onset and progression of atherosclerosis [13,14]. Its pro-inflammatory and atherogenic properties have been found in endothelial cells, both smooth and striated muscle cells and macrophages. Its levels, as well as those of IL-6, are strongly associated with oxidative stress or anoxia [10,15]

A similarly important role in the clinical picture of the OSA patient is the high level of TNF-α observed; it is, in fact, a pro-inflammatory cytokine with an important role in the host defence, which at the same time mediates the onset of a series of pathological processes including atherosclerosis, septic shock and autoimmune diseases. The release of TNF-α is mediated by IL-6, as well as by other pro-inflammatory cytokines such as IL-2, IFN-γ and by TNF-α itself through a positive feedback process [16].

Table I. Metabolites and proteins found in OSA patients through OMICS approaches.

Reference	Sample	Number of participants	Proteins/ metabolites	Differently expressed biomarkers
Chen et al. 2017 [69]	Peripheral blood mononuclear cells	48 patients with sleep-disordered breathing	Proteins	Angiomotin (AMOT), pleckstrin homology, MyTH4 and FERM domain containing H3 (PLEKHH3), adenosine deaminase RNA specific (ADAR), baculoviral IAP repeat containing 3 (BIRC3), and galectin 3 (LGALS3) proteins
Krishna et al. 2006 [64]	Urine	11 paediatrics OSA and 11 controls	Proteins	Gelsolin, Perlecan (a heparan sulfate proteoglycan), Albumin, Immunoglobulin
Shah et al. 2006 [65]	Serum	20 paediatrics OSA and 20 controls	Proteins	3 proteins with molecular masses of 5896, 3306 and 6068 Da
Gozal et al. 2009 [66]	Urine	30 paediatrics OSA and 30 controls	Proteins	Uromodulin, Urocortin-3, Kallikrein, Bikunin, Tenascin, Human Tribbles homolog-2, Zinc finger protein-81, 36/1, Orosomucoid-2, a1-Microglobulin, PCAF histone acetylase, Prolyl hydroxylase domain
Becker et al. 2014 [70]	Urine	14 paediatrics OSA and 13 controls	Proteins	30-fold more candidate biomarkers
Jurado-Gamez et al. 2012 [33]	Serum	30 OSA and 10 controls	Proteins	30 proteins
Seetho et al. 2014 [67]	Urine	27 OSA and 25 controls	Proteins	15 peptides
Zheng et al. 2014 [30]	Saliva	20 Non-CVD OSA and 18 CVD OSA	Proteins	Fibrinogen alpha chain (FGA), Alpha-2-HS-glycoprotein (AHSG), Tubulin alpha-4A chain (TUBA4A) and other 7 differentially expressed peptides still to be identified
Ferrarini et al. 2013 [34]	Plasma	18 OSA severe and 15 OSA non severe	Metabolites	Phosphatidylcholine (PC), Phosphoserine (PS), Lysophosphatilysophosphatidylcholines (LPC), Lysophosphatidylethanolamine (LPE), LPA, PE methyl-hydroperoxy-octadecatrienoate, PGF2-alpha diethyl amide Pipecolic acid, Arg, Phe, His
Kawai et al. 2013 [3]	Saliva	20 male OSA	Metabolites	Phosphatidylcholine (PC)
Engeli et al. 2012 [37]	Plasma	29 OSA, 26 OSA type II diabetes, 21 controls	Metabolites	Anandamide; (AEA), Arachidonoylglycerols; (AG), Oleoyl ethanolamide; (OEA), Arachidonic acid (AA), increase in the total monounsaturated fatty acids (MUFA)
Ezzedini et al. 2013 [39]	Tonsillar tissue	114 pediatrics OSA and 92 recurrent tonsillitis	Metabolites	Palmitoleic acid, Oleic acid, Stearic acid
Papandreu et al. 2013 [38]	Adipose tissue	63 OSA	Metabolites	Myristic, Palmitic, Stearic, Oleic acid, n-6 fatty acids n-3 (precursors of prostaglandins and serotonin) and n-6 fatty acids
Fletcher et al. 1987 [60]	Urine	8 severe OSA and 5 HTN and obese non OSA patients	Metabolites	Epinephrine (E), Norepinephrine (NE), Metanephrine (MN), Normetanephrine (NMN)
Paci et al. 2000 [45]	Plasma	10 male OSA (8 normotensive and 2 untreated HTN) and 11 controls	Metabolites	Norepinephrine (NE), Epinephrine (E), Dopamine (DA), Endogenous digitalis-like factor (EDLF)
O'Driscoll et al. 2011 [62]	Urine	70 snorers and 26 controls	Metabolites	Epinephrine (E), Norepinephrine (NE), Dopamine (DA), Noradrenaline, Adrenaline
Paik et al. 2014 [61]	Urine	49 OSA (of which 23 with insomnia)	Metabolites	Homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC)
Gislason et al. 1992 [63]	Cerebrospinal fluid	15 OSA and 18 healthy controls, 12 patients with suspected neurological disease	Metabolites	5-hydroxyindoleacetic acid (5-HIAA, serotonin metabolite), Homovanillic acid (HVA, DA metabolite), 3-methoxy-4-hydroxyphenyl glycol (MHPG)
Dikmenoglu et al. 2006 [47]	Plasma	11 OSA and 11 controls	Metabolites/ proteins	Malondialdehyde (MDA), fibrinogen
Stanke-Labesque et al. 2009 [51]	Urine	40 non obese OSA and 20 controls	Metabolites	Leukotriene E(4) (U-LTE (4)), 11-dehydroTXB2

Continues

Table I. *Follows.*

Barcelò et al. 2012 [31]	Saliva	119 OSAS and 35 controls	Metabolites	Gamma glutamyltransferase (GGT), Fetuin-A,
Zhang et al. 2018 [10]	Serum extracellular microvescicles	20 OSA and 20 controls	Proteins	Haptoglobin, C-reactive protein (CRP), Platelet factor 4 (PF4), Coagulation factor XIII (F13a1), Fibronectin (FN1)
Lebkuchen et al. 2018 [35]	Plasma	37 OSA and 16 controls	Metabolites	Deoxy sugar; 2,6-diphenyl-1,7-dihydrodipyrrolo[2,3-b:3′,2′-e] pyridine; 9-hexadecenoic acid (Z), Arachidonic acid (AA), 5,5′-biphthalide, L-glutamine, Glycerophosphoethanolamines (PE), Monoacylglycerophosphocholines (lyso-phosphocholines) (LPC), sphingomyelin (SM), diacylglycerols (DAG), glycerophosphocholines (PC), glycerophosphates (PA), Glutamic acid, Methyl cysteine, Serine
Xu et al. 2016 [50]	Urine and plasma	60 OSA, 30 simple snorers and 30 controls	Metabolites	2-hydroxy-3-methylbutyric acid, 3,4-dihydrxoybutyric acid, 3-hydroxybutyric acid, 4-hydroxypentenoic acid, cytidine 5'-diphosphocholine, ethanolamine, myo-inositol, 2,3-dihydrxoypropanoic acid, arabinose, arabitol, cellobiose, maltose, threitol, alanine, isoleucine, serine, threoninyl-methionine, trimethylamine N-oxide, valine, 5-hydroxyindoleacetic acid, lactic acid, glycochenodeoxycholate-3-sulfate, putrescine, 4-hydroxybutyric acid, vanillic acid, hypoxanthine, inosine, xanthine

An analysis of whole-genomic microarrays recently carried out by Yung-Che et al. found overexpression of angiomotin (AMOT), pleckstrin homology, MyTH4 and FERM domain containing H3 (PLEKHH3), adenosine deaminase RNA specific (ADAR), baculoviral IAP repeat containing 3 (BIRC3) and galectin 3 (LGALS3) proteins in treatment-naïve OSA patients [17]. LGALS3 has shown to be involved in cancer, inflammation and fibrosis, heart disease and stroke. Studies have also suggested that expression of galectin-3 is implicated in a variety of processes associated with heart failure, including myofibroblast proliferation, fibrogenesis, tissue repair, inflammation and ventricular remodelling [18].

Expression of AMOT in endothelial cells and its level is associated with proliferation and invasion of breast tumours [19].

ADAR are double chain RNA editing enzymes responsible for post-transcriptional modification of mRNA transcripts by changing the nucleotide content of the RNA. The conversion from A to I in the RNA disrupts the normal A:U pairing which makes the RNA unstable [20]. ADAR is considered to be involved in the insurgence of cancer. Studies in the sleep field also revealed that the ADA G22A polymorphism (c.22G > A, rs73598374) is associated with fewer awakenings throughout the night, and a higher duration of slow wave sleep (SWS), as compared to the normal ADA G22G genotype [21].

BIRC3 is a downstream effector of the ubiquitous hypoxia-inducible factor (HIF-1α) that is involved in pro-survival and inflammatory responses induced by the docosahexaenoic acid/neuroprotectin D1 pathway under oxidative stress in an ischaemia-reperfusion stroke model. HIF-1α functions as a principle regulator activity of cellular and systemic homeostatic response to hypoxia. This heterodimer is composed of an alpha and a beta subunit that can activate the transcription of many genes, including those involved in energy metabolism, apoptosis and angiogenesis, as well as other genes whose protein products increase oxygen delivery and facilitate metabolic adaptation to hypoxia. Since many studies have shown that OSA is associated with an imbalance between oxidant production and antioxidant activity, this fact, combined with an overabundance of oxidants, can be linked to the multifactorial aetiology of metabolic disorders, including insulin resistance [22].

Almendros et al. [23] examined the correlation between HIF-1α factor and vascular endothelial growth factor (VEGF) expression in patients with cutaneous melanoma. Interestingly, they found in a large prospective study that the expression of HIF-1α was an independent factor associated with nocturnal IH measures of respiratory disturbance during sleep in patients affected by cutaneous melanoma [23], meaning that it has a significant contribution to the disease. Notably, the risk of melanoma was significantly higher in patients with OSA (HR = 1.14, 95% CI 1.10-1.18), along with pancreatic and kidney cancer [24]. In recent years, other potential associations between OSA and cancer have been reported, principally ascribed to an effect of IH on tumour biology [25-27].

A significant correlation between OSA and increased cardiovascular risk and hypertension (HTN) is strongly reported in the literature [28,29]. Mass spectrometry was performed on salivary samples of OSA patients with cardiovascular diseases (CVD) compared to non-CVD OSA patients [30]. A panel of 11 biomarkers were identified as differentially expressed between the two groups. It was found that the level of alpha-2-HS-glycoprotein (AHSG) peptide was significantly lower in the OSA-CVD group compared to the non-CVD group. A reduced level of AHSG had already been reported in severe OSA patients [31] at metabolic level [32]. AHSG protein is synthesised by hepatocytes and is involved in different process such as formation of brain and bone and endocytosis. Interestingly, lack of this protein is involved in leanness.

Metabolomics approach

The field of metabolomics, and the consequent search for potential biomarkers in OSA patients, is beginning to be explored only in recent years. The lipidomic profile in OSA patients reported in the literature mainly reveals alterations in phospholipid biosynthesis and fatty acids. One of the major studies using mass spectrometry has allowed to identify, both at a serum and urinary level, as many as 103 proteins that are differently expressed in adult OSA patients compared to controls, all potentially associated with imbalances in lipid metabolism and alterations in the vascular system [33]. Among phospholipids, glycerophosphocholines (PC), lysophosphatidylcholines (LPE), glycerophosphoethanolamines (PE), lysophosphatidylethanolamine (LPA), phosphoserine (PS), and lysophosphatidic acids, along with glycerophosphates (PA), monoacylglycerophosphocholines, lyso-phosphocolyne (LPC) and sphingomyelin (SM) classes were found to be up-regulated in patients with OSA compared to controls [34,35]. Increased PC expression at the salivary level was also reported using LC-MS/MS methods [36,37].

Alterations in fatty acids have also been detected. Among those that are significantly increased in OSA compared to normal subjects, circulating anandamide (AEA), 2,4-dihydroxybutyric acid, 2-hydroxy-3-methylbutyric acid, 3,4-dihydrxoybutyric acid, 6-aminocaproic acid, pentanoic acid, and glyceraldehyde, 3-methyl-3-hydroxybutyric acid, and 4-hydroxypentenoic acid were up-regulated, whereas bile acid and glycochenodeoxycholate-3-sulphate (GCDCA-3-sulphate) were decreased [36-38]. Other groups, using GC-LC techniques, found that palmitoleic and oleic acid levels were lower, while stearic acid levels were higher in the tonsillitis tissue of infant control subjects compared to the hyperplastic tissue typical of the diseased counterpart [39].

Other research groups observed that in OSA patients levels of 1/2-arachidonoylglycerols (AG), and oleoyl ethanolamide (OEA) in plasma are higher compared to controls. It is interesting to note that arachidonic acid (AA) concentrations and eicosanoids[34,35] were also up-regulated in OSA patients, suggesting a role for the endocannabinoid system in regulating blood pressure in patients with high risk OSA for HTN and CVD [36,37,40].

The endocannabinoid system is, in fact, based on lipid molecules produced by the body in response to various stimuli that bind specific membrane receptors associated with the protein G, called cannabinoid receptors type 1 and 2 (CB1 and CB2) [41]. The endocannabinoid system represents a neuromodulation system, playing a role in the control of pain at the level of the central nervous system, in regulation of cell proliferation and in modulation of the immune response. Interestingly, it also seems to play a role in mechanisms that modulate appetite and therefore obesity [37]. The endocannabinoid system also plays an important role in the release of adipokines. Recent research has shown that the pharmacological blockade of CB1 by an antagonist, named *Rimonabant,* stimulates the release of adiponectin, which is normally inhibited. Adiponectin is a circulating hormone secreted by adipose tissue, with anti-atherogenic and antidiabetic properties that can reduce liver glucose production, as well as suppress lipogenesis and activate oxidation of fatty acids [42]. How endocannabinoids regulate metabolism are still only partially understood, despite the fact that their role in controlling hunger and satiety acts mainly in hypothalamic structures through activation of neurons capable of stimulating the action of neuropeptides [43]. Alterations in the endocannabinoid system therefore affect and alter energy metabolism of the body and homeostasis of lipids, as suggested by Di Marzo and Matias, who were the first to formulate the increasingly valid hypothesis that obesity can be associated with pathological hyperactivation of the endocannabinoid system [44]. All these conditions can be associated with an increased risk of cardiometabolic diseases such as type 2 diabetes, dyslipidaemia, arterial hypertension, myocardial infarction and stroke, conditions normally found in OSA patients.

Mediators involved in the systemic inflammatory response and oxidative stress have also been reported in OSA. Among the metabolites associated with oxidative stress, urinary 15-F2t- isoprostane, one of the most sensitive metabolites correlated with lipid peroxidation, is positively linked to thickness of the intima-media carotid tunic [45]. These molecules were shown to be a specific, chemically stable, quantitative marker of oxidative stress *in vivo*. In particular, F2t-isoprostanes are prostaglandin isomers synthesised *in vivo* through free radical catalysed peroxidation of AA in biological membranes, independently of the activity of cyclo-oxygenase. Increased urinary excretion or plasma concentrations of 15-F2t-isoprostane has been observed in many conditions including smoking, diabetes and cardiovascular diseases [46].

Another important biomarker of oxidative stress, malondialdehyde (MDA), is present at significantly higher concentrations in patients with OSA vs. control [47]. MDA is the result of lipid peroxidation of polyunsaturated fatty acids. It is an important product in the synthesis of thromboxane A2 in which cyclooxygenase 1 or cyclooxygenase 2 metabolises AA into prostaglandin H2 and ROS degrade polyunsaturated lipids to form MDA [48]. This compound is a reactive aldehyde and is one of many reactive electrophilic species that causes toxic stress in cells and reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts; it can thus be used as a biomarker to measure the level of oxidative stress in an organism [49].

Arguably, the tricarboxylic acid cycle (TCA) and its mediators tend to increase in OSA [50], suggesting augmentation of oxidative stress.

Among metabolites that are potential pro-inflammatory markers, Stanke-Labesque et al. [51] found leucotriene E4 (U-LTE4), an inflammatory molecule associated with cysteinyl leukotriene production, whose elevation in urinary concentration has been demonstrated in patients with OSA. Recently, Gautier-Veyret and his group have shown that activation of this pathway contributes to OSA-induced atherogenesis, and its blockade could therefore represent a new therapeutic target for reducing CVD [52]. It is also interesting to note that Continuous Positive Airway Pressure (CPAP), a respiratory ventilation method mainly used in the treatment of sleep apnoea, reduces the urinary concentration of U-LTE4 by up to 22%, but only if the treatment is carried out in patients with a normal body mass index (BMI) [51].

Arguably, CPAP treatment reduces also serum levels of homocysteine (Hcy) by almost 30%, which, along with plasma levels, were found to be significantly higher in patients with OSA compared to controls [38,39,53]. In addition, neural-like cell exposure to Hcy for a period of 5 days resulted in a 4.4-fold increase in production of reactive oxidative species (ROS) [54]. Hcy is known to mediate adverse effects on the cardiovascular endothelium and smooth muscle cells with resultant alterations in subclinical arterial structure and function [55], leading to CVD and its complications, such as heart attack and stroke [56]. Moreover, hyperhomocysteinaemia leads to enhancement of the adverse effects of risk factors like HTN, smoking, and lipid and lipoprotein metabolism, as well as promotion of inflammation [57]. Another study demonstrated that Hcy is capable of initiating an inflammatory response in vascular smooth muscle cells by stimulating CRP production, which is mediated through the NMDAr-ROS-ERK1/2/p38-NF-κB signal pathway [58]. CRP expression was also found to be altered in the proteome of OSA patients (see previous section).

Some studies also suggest that elevated Hcy levels may be associated with alterations in mental health such as cognitive impairment, dementia, depression, Alzheimer's and Parkinson's disease [59] through its capacity to act as a neurotransmitter. In particular, Hcy may act either as a partial agonist at glutamate receptors or as a partial antagonist of the glycine co-agonist site of the NMDA receptor. As such, in the presence of normal glycine levels and normal physiological conditions, Hcy does not cause toxicity but in case of head trauma or stroke, there is an elevation in glycine levels in which instance the neurotoxic effect of Hcy as an agonist outweighs its neuroprotective antagonist effect. This neuronal damage following a stroke has been attributed to the over stimulation of excitatory amino acids such as glutamate and aspartate through activation of NMDA receptors [55]. Ganguly et al. [55] have investigated how Hcy is able to selectively stimulate the release of these excitatory amino acids in stroke and concluded that they may trigger the release of catecholamine, resulting into detrimental effects in the brain and cardiovascular system. Interestingly, in OSA patients, glutamate metabolites were also found to be significantly altered [50].

The study of catecholamine metabolites and derivatives as potential predictors of the onset of the pathological process seems particularly promising. Fletcher et al. [60], for example, observed that norepinephrine (NE) and normetanephrine levels were significantly higher in the urine of patients with OSA than those in obese HTN controls, as well as epinephrine (E) levels, at the plasma level [45], who also found higher levels of dopamine (DA) in the comparison of 10 male patients with OSA and 11 controls. HPLC observations revealed a significant increase in all urinary catecholamines in OSA children, and the levels of NE and E during the night were strongly related to the severity with which patients manifest the altered phenotype. Paik et al. [61], after studies carried out using GC-MS to detect metabolites of urinary neurotransmitters, demonstrated that homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC), both dopamine metabolites, were increased in sleepy patients with OSA, suggesting that excessive daytime sleepiness in these subjects is probably caused by an increase in night-time activity of the dopaminergic and sympathetic systems [62]. Although this theory seems intriguing, the results of several other studies question it. Paci et al. have reported that E and DA levels did not vary significantly between OSA patients and controls. In addition, the results of the studies of Gislason et al, found 5-hydroxyindo-lacetic acid (5-HIAA), HVA and 3-methoxy-4-hydroxyphe-glicolenyl glycol (MHPG) in the cerebrospinal fluid of 15 patients with OSA and 18 controls; however, even in this case, the levels of all these biomarkers were similar in patients with OSA and control subjects [45,63].

The inconsistency of the results obtained from the studies on catecholamine metabolites in patients with OSA may be due to various factors such as the heterogeneity of the analytical platforms used by the various research groups, the different biological matrices taken into account, small size of the cohorts and the different protocols used for sample collection. All these elements may also affect the reproducibility of studies.

The first studies aimed at finding differentially expressed metabolites at the urinary level in children with OSA was carried out by Krishna et al. [64]. They adopted a mass spectrometry technique on a cohort of 22 subjects, who demonstrated an alteration in glomerular and tubular filtration of the kidneys compared to healthy counterparts. High levels

of proteins such as jasmine, perlecan (a heparan sulphate proteoglycan), albumin, and immunoglobulin were detected in urine. These results suggested increased catabolic activity of some proteins in OSA patients [64]. In the same period, Shah et al. also identified three proteins of 5,896, 3,306 and 6,068 kDa that were differently expressed in pathological children, which were capable of discriminating the latter from healthy patients with 90% specificity and 93% sensitivity [65]. Three years later, Gozal et al., using a method based on the use of 2-Dimensional DIfference Gel Electrophoresis and Mass Spectrometry (2D-DIGE-MS), were able to identify 16 metabolites differently expressed in the urine of OSA patients compared to controls. In particular, the analysis of concentrations of some of these, including uromodulin, urocortin-3, orosomucoid-1, and kallikrein, were able to identify the pathogenic phenotype with a sensitivity of 95% and a specificity of 100% [66].

The contribution of Seetho et al. and Zeng et al. in the field of research into potential OSA biomarkers is extremely interesting, with the former, focusing on polypeptides using urine of obese OSA patients as a biological matrix, and the second, looking for proteins differently expressed between OSA patients suffering from CVD in saliva. The work of the two groups allowed identification of 27 potential biomarkers, fibrinogen alpha chain (FGA), tubulin alpha-4A chain (TUBA4A) and AHSG. More specifically, AHSG has been shown to be expressed at lower levels in OSA frameworks associated with changes in cardiovascular function [30,67].

Alterations in amino acid biosynthesis were also reported in OSA using a metabolomics approach. Xu et al. identified 21 differentially expressed urinary metabolites among a simple snoring group and controls, including aspartyl-serine, isoleucine-threonine (Ile-Thr), and methionine, whereas levels of 3-hydroxyanthranilic acid and 5-hydroxytryptophan decreased. Hydroxyprolyl-methionine, hypoxanthine, Ile-Thr, indole-3-acetamide, isoleucine, lactic acid, myo-inositol, pentanoic acid, threitol, threoninyl-methionine, trimethylamine N-oxide (TMAO), uridine, and valine were consistently higher or lower [50]. Other groups have also reported that methylcysteine and serine decreased in OSA [36,37].

The metabolomics profiling of spermine biosynthesis, indoles and tryptophan metabolism, tyrosine metabolism as well as porphyrin metabolism were also altered significantly [38,50].

Conclusions

OSA is characterised by recurrent episodes of collapse of the upper airways during sleep, which are reflected in a desaturation of haemoglobin that leads to the awakening of affected subjects. The chronic IH registered in this condition leads the body to enact molecular adaptations to the low-oxygen conditions to which it is subjected [68]. Despite this, sleep fragmentation results in a dangerous condition of excessive sleepiness during the rest of the day. In addition to the long-term problems mentioned, sleep fragmentation is a daily danger for the individual linked to the increased risk of road or work accidents. The body responds to chronic fatigue through compensatory mechanisms that evoke inflammatory responses, hyperactivation of the sympathetic system and alteration of endothelial function, such as regulation of tight junctions; these events have an important role in promoting the onset of atherosclerosis and, in the long term, cardiovascular and cerebrovascular diseases [12]. Recent studies also show a significant correlation between OSA and metabolic and neurocognitive risk as well as an association with cancer mortality.

In the literature, proteomics and metabolomics approaches were used to detect change in physiological or pathological status of OSA patients compared to controls, in order to discover new mediators that can be used as biomarkers of the disease. Notwithstanding, OSA and therapies related to this disease [71-73], are a somewhat ‘new’, and there are many proteins and metabolites that are associated with the disease, in particular those involved in inflammation and oxidative stress, in line with the clinical IH that patients undergo in OSA.

Lipid dysmetabolism in OSA reflects alterations in phospholipids biosynthesis, steroidogenesis and fatty acids. This may influence cell membrane formation, augmenting lipid uptake, atherogenesis and inflammation. In addition, alterations in amino acids, nucleic acids and some mediators that act as neurotransmitters, such as Hcy and the endocannabinoid system, have been seen in OSA patients, suggesting an increased risk of cardiometabolic diseases such as type 2 diabetes, dyslipidaemia, arterial HTN, myocardial infarction and stroke, conditions normally found in OSA patients.

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ACTA OTORHINOLARYNGOLOGICA ITALICA 2020;40:173-180; doi: 10.14639/0392-100X-N0538

HEAD AND NECK

Platysma myocutaneous flap revised in the free flaps era: clinical experience in 61 patients

Tecnica alternativa per il prelievo del lembo di platisma miocutaneo nell'era dei lembi liberi: esperienza clinica in 61 pazienti

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SUMMARY

Reconstruction of oral cavity and oropharyngeal defects following radical surgery for squamous cell carcinoma (SCC) can be achieved by a variety of options. In selected cases myocutaneous platysma flap (MPF) may be a valid choice. However, several anatomical and oncological controversies on the use of this flap are debated. A retrospective study on 61 patients treated between January 2005 and December 2017 in two referral centres in which MPF was used for the reconstruction of defects following surgical resection of SCC of the oral cavity and oropharynx was conducted. The technique of flap harvesting with anatomic details is described. In all cases the submental artery was sacrificed preserving the facial artery. All clinical data were collected. Tumours involved the oral cavity in 95.1% of cases, and the oropharynx in 4.9%. Pathological staging (TNM 7th edition) of tumours was: pT1 (42.6%), pT2 (39.3%), pT3 (4.9%) and pT4a (13.1%). Success rate of the flap was 93.4%. Four (6.5%) patients developed a partial necrosis of the skin paddle without platysma muscle involvement; none required surgical revision. The mean follow-up was 69 months (5-153 months). Thirteen patients (21.3%) developed a local recurrence, and in 1 patient was associated with contralateral neck metastasis. The MPF can be a suitable option in head and neck reconstruction of small or medium-sized defects in selected cases. The vascular pedicle can be provided by branches of the facial artery achieving both oncological radicality and optimal flap vascular supply.

KEY WORDS: platysma flap, myocutaneous flap, reconstructive surgery, oral cavity reconstruction

RIASSUNTO

La ricostruzione della cavità orale e dei difetti orofaringei a seguito di un intervento chirurgico radicale per carcinoma a cellule squamose (SCC) può essere ottenuta con varie opzioni. In alcuni casi selezionati il lembo miocutaneo di platisma (MPF) potrebbe essere una valida alternativa ai lembi liberi. Tuttavia, sono sorte negli anni numerose controversie anatomiche e oncologiche sull'uso di questo lembo. È stato condotto uno studio retrospettivo su 61 pazienti trattati tra gennaio 2005 e dicembre 2017 in due centri di riferimento, in cui è stata utilizzato il MPF per la ricostruzione dei difetti risultanti dalla resezione chirurgica di carcinomi squamosi della cavità orale e dell'orofaringe. Viene descritta la tecnica chirurgica di prelievo del lembo con particolare attenzione ai dettagli anatomici: in tutti i casi trattati l'arteria submentale veniva sacrificata preservando l'arteria facciale ed il peduncolo vascolare è stato basato su rami collaterali dell'arteria stessa. Le neoplasie coinvolgevano la cavità orale nel 95,1% dei casi e l'orofaringe nel 4,9%. La stadiazione patologica (TNM 7a edizione) dei tumori era: pT1 (42,6%), pT2 (39,3%), pT3 (4,9%) e pT4a (13,1%). Il tasso di successo del lembo è stato del 93,4%. Quattro (6,5%) pazienti hanno sviluppato una necrosi parziale della padella cutanea senza coinvolgimento del pia-

Received: November 8, 2019
Accepted: November 22, 2019

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Funding
None.

Conflict of interest
The Authors declare no conflict of interest.

How to cite this article: Calabrese L, Accorona R, Gazzini L, et al. Platysma myocutaneous flap revised in the free flaps era: clinical experience in 61 patients. Acta Otorhinolaryngol Ital 2020;40:173-180. https://doi.org/10.14639/0392-100X-N0538

no muscolare del platisma; in nessun caso si è resa necessaria una revisione chirurgica del lembo. Il follow-up medio è stato di 69 mesi (5-153 mesi). Tredici pazienti (21,3%) hanno sviluppato una recidiva locale, in 1 paziente questa era associata alla presenza di una metastasi del collo controlaterale. L'MPF può essere un'alternativa nella ricostruzione di difetti di piccole o medie dimensioni della testa e del collo in casi selezionati. Il peduncolo vascolare può essere fornito da rami dell'arteria facciale, raggiungendo così sia una corretta radicalità oncologica, sia un'ottimale vascolarizzazione del lembo.

PAROLE CHIAVE: *lembo di platisma, lembo miocutaneo, chirurgia ricostruttiva, ricostruzione del cavo orale*

Introduction

Reconstruction of oral cavity defects following radical surgery for SCC can be achieved by a variety of techniques [1-6]. The choice is influenced by several factors, including size and location of the defect, patient characteristics, donor site morbidity, and functional and aesthetic outcomes [1-13]. Nowadays microvascular free flaps, for instance anterolateral tight (ALTFF) and radial forearm (RFFF), are considered the gold standard among head and neck surgeons for restoration of oral defects. However, in some particular situations, as in compromised patient in which a time-consuming procedure is at high risk, a pedicled flap could be a better choice [9-13]. Moreover, in small defects the donor site morbidity and aesthetic outcomes have to be taken into account [2,4]. Many loco-regional pedicle flaps are available for head and neck reconstruction [9-15]; among these, the MPF presents some advantages, for instance appropriate thickness, ideal pliability, wide arch of rotation (almost 180°), and good colour match with facial skin in case of external reconstruction. Furthermore, donor site morbidity is low, and it is easy to access in the same operative field, reducing the operation time [1-5]. Consequently, MPF can be an excellent choice in reconstruction of oral cavity, oropharynx, and low face defects of small to medium size (15-75 cm^2) [1-8]; however, some anatomical and oncological controversies on the use of this flap are still debated. In particular, several authors have stated that it is difficult to perform a safe oncological neck dissection and at the same time preserve effective vascularisation of the flap [7-12].

The aim of this paper is to analyse the use of MPF for reconstruction of post-surgical defects in 61 cases of oral cavity and oropharyngeal tumours. A detailed technique of flap harvesting is described step-by-step. Additionally, the main indication and controversies reported in the literature, are critically analysed.

Materials and methods

A retrospective review on all patients in which a MPF was used for reconstruction of defects following surgical resection in oral cavity and oropharynx, between January 2005 and December 2017, was conducted at the Head and Neck Surgery Department of the European Institute of Oncology (Milano) and at the Division of Otorhinolaryngology of "San Maurizio" Hospital (Bolzano).

Inclusion criteria was:

- affected by SCC of the oral cavity or oropharynx.

Exclusion criteria were:

- submitted to prior surgery with neck dissection;
- submitted to prior radiation or chemoradiation therapy.

The surgical technique was standardised for the entire series of patients. Demographic data, risk factors and tumour characteristics were collected.

Intraoperative data were type of surgical approach (pull through, mandibulotomy, mandibulectomy), need for monolateral or bilateral neck dissection and intraoperative complications.

During follow-up, patients were evaluated daily until hospital discharge and with regular clinical controls during long-term follow-up. Data on total hospital stay, local postoperative complications (diastasis, fistula, and partial or total necrosis), adjuvant therapies and disease progression were reported.

The clinical pathological staging of the disease was performed according to the 7th edition of the AJCC/UICC TNM and clinical staging system.

Surgical indications

This flap was chosen in patients with the subsequent characteristics:

- defect in oral cavity and oropharynx (without indication for compartmental surgery) that could not be closed by a primary suture, up to 75 cm^2;
- need for neck dissection;
- compromised patients that could not bear the longer length of general anaesthesia required for a free flap (i.e. American Society of Anaesthesiologists [ASA] physical status classification class 3 or 4, poorly controlled diabetes mellitus or hypertension, chronic obstructive pulmonary disease [COPD], morbid obesity body mass index > 40, moderate or severe reduction of cardiac ejection fraction, implanted pacemaker, end stage renal disease [ESRD] undergoing dialysis, recent history of myocardial infarction, coronary stents, transient ischemic attach [TIA] or stroke).

Surgical technique

The surgical technique of MPF harvesting is well described

in the literature. In the present study, we describe a modified technique to harvest the superiorly based MPF, developed by the senior authors. The vascular support of the flap, in this variant, is not provided by the submental artery as usually described in the literature. Conversely, it is ensured by constant branches of the facial artery anastomosed in the paramandibular area with the orbicular artery system. We point out several technical details in flap elevation and its insetting.

Surgical steps

Step #1

A U-shaped flap is drawn including a skin island centred on the lower portion of the platysma muscle just above the clavicular insertion. An adequate length of the horizontal axis of the skin paddle is required to close the donor site with minimal tension (Fig. 1A).

Step #2

Sharp dissection of the flap is conducted in a subcutaneous plane until the level of the hyoid bone. The clavicular insertions of the platysma muscle are divided and the muscle with the overlying skin island is elevated. The external jugular vein is ligated and harvested with the flap, while the portion of the external cervical fascia overlying the sternocleidomastoid muscle is not included (Fig. 1B, 1C).

Step #3

The elevation of the flap is then conducted toward the mandible dividing the segmental vessels of the middle part of the muscle. A key point of this surgical step is to identify and preserve the marginal branch of the facial nerve. Any perforator skin vessel arising from the cranial portion has to be preserved to ensure adequate vascularisation of the distal part of the flap and minimise the risk of skin necrosis. The perforators are located in the adipofascial tissue; therefore, the dissection should be conducted in a deeper plane to preserve these vessels. After this step, platysma muscle is dissected anteriorly and posteriorly to achieve full mobilisation of the flap (Fig. 1D).

Step #4

Submandibular gland dissection allows identification and preservation of the common trunk of the facial artery while all the cervical branches, including the submental artery, are ligated during level IA dissection ensuring oncological radicality. This is the key point of the whole surgical procedure, because in the present technique the vascular arterial supply of the flap is provided by small calibre constant branches of the facial artery that are routinely identified in the submandibular area. These branches are direct to platysma and anastomosed in the paramandibular area with the orbicular artery system and not from the submental artery as widely described in literature (Fig. 2). This step is contraindicated if massive metastases at level IB are present. Neck dissection, either radical or selective, is then completed (Fig. 1E). If possible, the facial vein is preserved.

Step #5

After tumour resection in pull-through or mandibulotomy, the defect is reconstructed rotating the MPF into the oral cavity. Any twisting, excessive traction, or stretching against the inferior edge of the mandible should be avoided. Moreover, the tunnel should be of adequate width in order to avoid vascular supply impairment. During the flap insetting, the sutures at the proximal aspect of the flap have to be cutaneous only, without involving the subcutaneous and muscular layers, in order to maximise blood flow through the muscle pedicle to the skin paddle.

Step #6

The neck donor site is than closed primarily, usually without tension (Fig. 1F).

Results

A total of 61 patients, 37 men (60.6%) and 24 women (39.4%), matched the inclusion criteria and were analysed in the present study. Median age was 68 years (25-87). Table I summarises the demographic data and patient characteristics. In the large majority of cases the disease involved the oral cavity with 58 (95.1%) cases, while only 3 (4.9%) patients presented oropharyngeal cancer fit for MPF reconstruction. Regarding subsites, in the oral cavity 29 (47.95%) patients presented with oral floor SCC, 15 (24.6%) with cheek mucosa SCC, 10 (16.14%) with gum SCC, 2 (3.3%) with lateral border of the tongue SCC, and 2 (3.3%) with retromolar trigone SCC: concerning oropharyngeal SCC, all the 3 (4.9%) patients presented with lateral wall SCC. Pathological staging of the tumour was: pT1 in 26

Table I. Preoperative characteristics of patients treated with radical surgery followed by reconstruction with platysma (n = 61).

Characteristics	N (%)
Age (years)	
Median (range)	68 (25-87)
Gender	
Male	37 (60.6)
Female	24 (39.4)
Alcohol consumption	
No	20 (32.8)
Yes	40 (65.6)
Former	1 (1.6)
Smoking	
No	16 (26.2)
Yes	29 (47.5)
Former	16 (26.3)

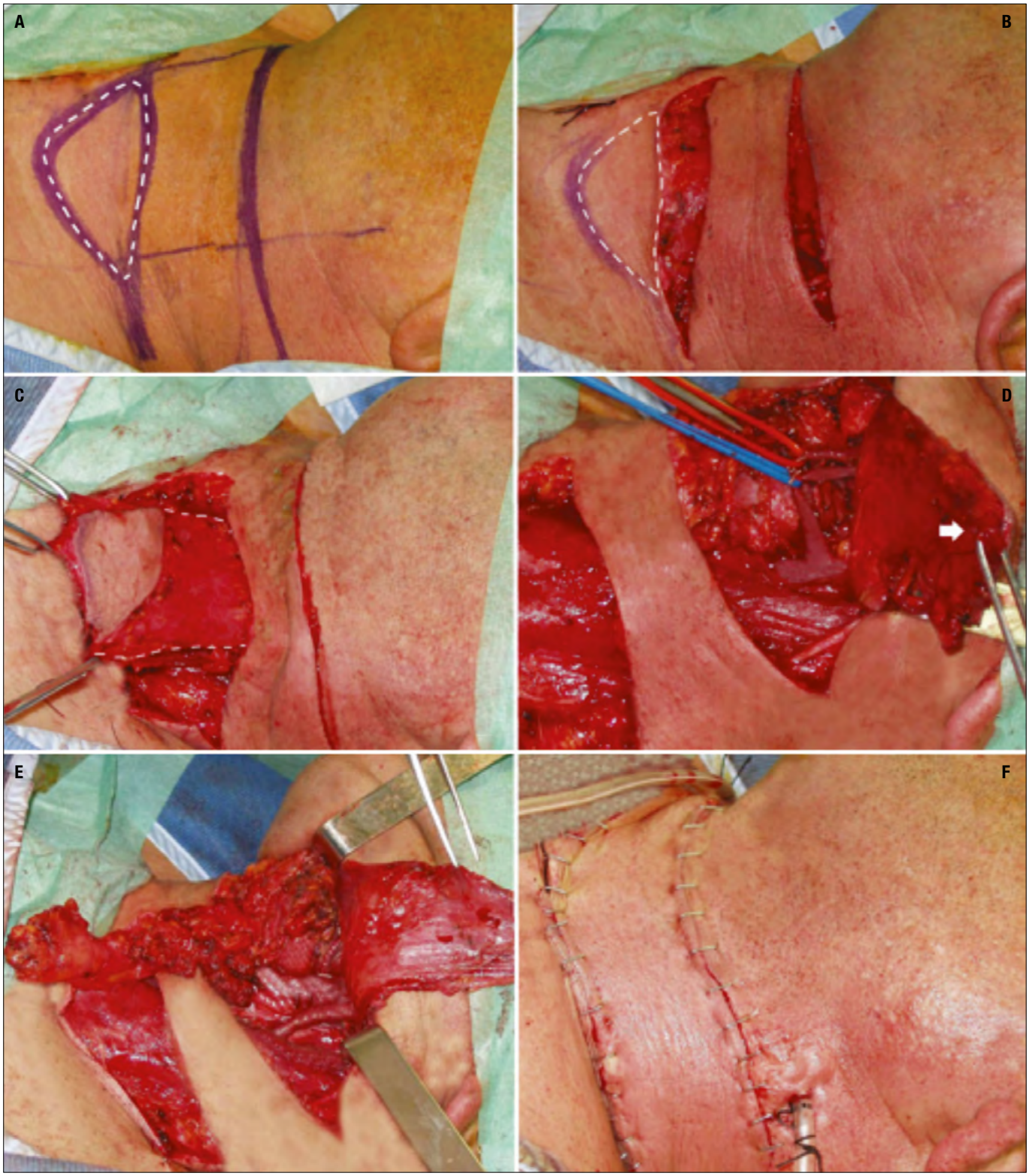
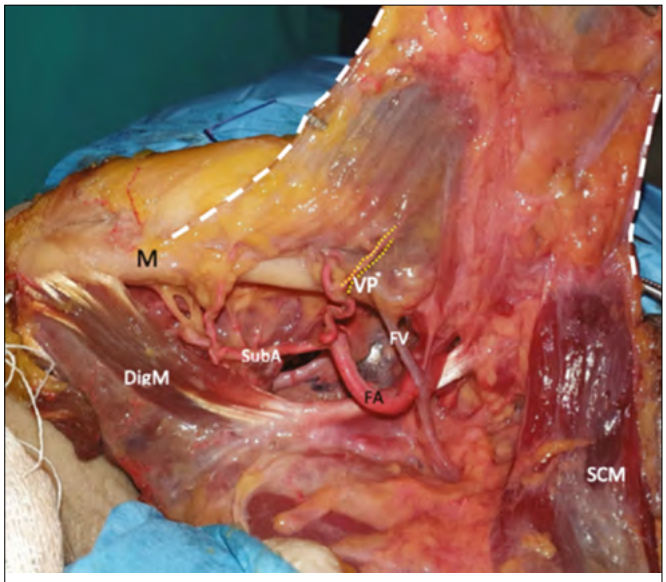


Figure 1. Surgical steps. **Panel a:** Drawing of the flap. Dotted line: Skin paddle of the platysma myocutaneous flap. **Panel b:** Cervical incision and identification of the platysma muscle. Dotted line: skin paddle of the platysma myocutaneous flap. **Panel c:** Harvesting of the platysma myocutaneous flap detaching it from the deep dissection plane. Dotted line: anterior and posterior limit of the incision of the platysma muscle. **Panel d:** Identification of the facial artery (red vessel loop) and vein (blue vessel loop). White arrow: platysma myocutaneous flap rotated in order to expose the submandibular region. **Panel e:** Neck dissection of level I is completed by preserving the facial vessels and sacrificing the submental pedicle. **Panel f:** Closure of the neck incision and the skin donor site.



DigM: anterior belly of the digastric muscle; M: mandible; FV: facial vein; FA: facial artery; SubA: submental artery; VP: vascular pedicle; SCM: sternocleidomastoid muscle.

Figure 2. Anatomy of the submandibular region and vascularisation of the platysma myocutaneous flap. Vascular pedicle of the flap arising from the facial artery in submandibular area and direct to the platysma muscle. (yellow dotted line). White dotted line: anterior limit of the platysma muscle.

cases (42.6%), pT2 in 24 (39.3%), pT3 in 3 (4.9%), and pT4a in 8 cases (13.1%). Moreover, the pathological staging of the neck was: pN0 in 38 cases (62.2%), pN1 in 13 cases (21.3%), pN2 in 10 cases (16.4%), and pN3 in none. Level IB was involved by lymph node metastasis in 5 (8.2%) patients at pathological examination. In 59 of 61 patients, tumour-free margins (R0) were achieved, while in the remaining 2 patients, microscopic positive margins (R1) were found.

The surgical approach consisted in pull through procedure in 43 (70.5%) cases, mandibulotomy in 15 (24.6%) cases, and mandibulectomy in 3 (4.9%) cases (Tab. II).

The mean hospitalisation time was 14 days (2-28 days) with a mean discharge time of 12 days after surgery. All patients were able to start oral feeding before they were discharged.

Regarding local early post-operative complications, flap failure or other major complications did not occur in any case. Nevertheless, 4 patients developed a necrosis of the skin paddle without impairment of the flap because the underlying platysma muscle remained vital; the skin necrosis was managed with daily bedside debridement achieving healing by second intention. Among these, 1 patient developed diastasis of the wound and 1 a salivary fistula, both treated successfully conservatively.

Adjuvant treatment was required in 17 (27.8%) patients: 4 (6.5%) patients received concomitant chemoradiation therapy, while 13 (21.3%) radiation therapy alone. In none of these patient faliure or necrosis of the flap was observed. During long-term follow-up 12 (19.7%) patients required local anaesthesia for a tongue synechia with ankyloglossia. Regarding the neck scar, none of the patients complained of impairment of neck movements or aesthetic dissatisfaction. The mean follow-up was 69 months (5-153 months). Twelve (19.7%) patients developed a local recurrence, and in 1 patient the recurrence was associated with neck metastasis. All these patients were consequently surgically re-treated. Moreover, in 2 (3.3%) cases the disease spread with distant metastasis. Finally, 8 (13.1%) patients presented a second metachronous tumour at follow-up (Tab. III).

Table II. Patient and tumour characteristics after surgery (n = 61).

Characteristics	N (%)
Type of surgery on tumour	
Mandibulectomy	3 (4.9)
Mandibulotomy	15 (24.6)
Pull through	43 (70.5)
Type of neck dissection	
Monolateral	41 (67.2)
Bilateral	20 (3)
Site of tumour	
Gum	2 (3.3)
Tongue	22 (36.1)
Cheek mucosa	10 (16.3)
Retromolar trigone	24 (39.3)
AGP	3 (4.9)
Histology	
Squamous cell carcinoma	61 (100)
Pathological status of tumour	
T1	26 (42.6)
T2	24 (39.3)
T3	3 (4.9)
T4a	8 (13.1)
Pathological status of neck	
N0	38 (62.2)
N1	13 (21.3)
N2 (a,b,c)	10 (16.4)
N3	0
Grading	
G1	12 (19.7)
G2	32 (52.5)
G3	17 (60.7)
Not evaluable	1 (1.6)
Defect dimension	
< 25 cm^2	56 (91.8)
26-50 cm^2	4 (6.6)
> 50cm^2	1 (1.6)

Discussion

The platysma is a thin quadrangular-shaped muscle that lies in the superficial fascia of the neck. The muscle fibres arise from the superficial fascia of the pectoralis

Table III. Outcomes, adjuvant treatments, follow-up and failures (N = 61).

Characteristics	N (%)
Total hospital stay (days)	
Mean (range)	14 (2-28)
Post-surgery stay (days)	
Mean (range)	12 (2-27)
Local postoperative complications	
Yes	6 (9,8)
Partial skin necrosis (no reintervention)	4
Diastasis	1
Fistola	1
Debridement	
Yes	12 (19.7)
Adjuvant treatments	
None	44 (72.1)
Chemoradiotherapy	4 (6.5)
Radiotherapy	13 (21.3)
Follow-up	
Median (months)	69
Range (months)	(5-153)
No evidence of disease	44 (72.1)
Alive with disease	1 (1.6)
Died of disease	8 (13.1)
Died of other tumour	5 (8.2)
Died for other causes	3 (4.9)
Failures	
Local	13 (21.3)
Locoregional	1 (1.6)
Distant metastasis	2 (3.3)
Second tumour	8 (13.1)

major and deltoid muscles, cross the clavicle and run obliquely inserting at the angle of the mandible, inferior part of the cheek, and depressor muscles of the lip [1-8,16-21]. The main artery supplying the platysma is the submental branch of the facial artery [3,4]. Furthermore, other vessels provide blood supply to the muscle: branches of transverse cervical artery inferiorly, branches from occipital and posterior auricular arteries posteriorly and branches from superior thyroid artery anteriorly [3,4]. Venous drainage is ensured primarily by the external jugular vein, but it is also provided by the medial jugular veins, submental vein, facial vein, and anterior communicating veins [3,4].

Futrell was the first to describe a true muscular-cutaneous cervical flap composed of a lower cervical skin paddle and platysma muscle with the mandibular insertions preserved [16], while other authors in the past described only random cervical platysma flaps [22,23].

Considering the main vascular pedicle, two different variants of MPF can be harvested for head and neck reconstruction: the posterior flap based on branches of the occipital artery and the superior or vertical flap based on the submental branch of facial artery; the latter has the widest diffusion in surgical practice [2-4]. MPF has applications in reconstructing a large variety of head and neck defects and, in particular, has shown to be a good surgical option for the reconstruction of small to medium, and, rarely, large sized (15-75 cm^2) mucosal defects of the oral cavity [1-8,16-21]. The thinness and pliability of the muscle-skin paddle unit make the platysma flap particularly suitable for tailoring defects of floor of the mouth, cheek mucosa, and gum to prevent post-operative functional impairment, due to primary closure with excessive tension [1,2]. According to the literature, other sites suitable for reconstruction with MPF are the lip, lateral wall of the oro-hypopharynx, and low facial skin defects [1-8,16-21]. When compared to other flaps such as the ALTFF, RFFF, and pectoralis major myocutaneous pedicle flap, the MPF is less bulky, with better skin colour match; the time for harvesting is shorter, and donor site morbidity is lower [2,3]. However, several anatomical and oncological controversies have limited its use [1-8,16-21].

The first matter of debate regards blood supply of the flap. Indeed, the submental artery is usually ligated during neck dissection of submandibular area to achieve oncological radicality [3-5]. Several authors studied the anatomical vascular supply of the platysma muscle in relation to harvesting technique of MPF [3]. Some concluded that preserving the submental artery is not crucial to ensure survival of MPF [3,17,22,23]. This conclusion was supported by McGuirt et al. and Ruark et al. who first reported their experiences on 19 and 41 cases respectively, in which the anterior portion of facial artery was sacrificed without flap failure [22,23]. More recently, Huang et al. stated that submental artery preservation is not a critical step of flap harvesting, reporting a good surgical result in a study on 68 patients [2]. The hypothesis is that the vascularisation is converted to a random pattern after ligation of submental artery [2,22,23]. Indeed, the large number of anastomoses with the homolateral and contralateral lingual artery, inferior labial artery and superior thyroid artery, replace the submental artery, ensuring sufficient blood supply to the platysma [1-3,22,23].

In the present study, resection of oral cavity tumours has been associated with an oncologically-safe neck dissection including levels IA and IB, sacrificing the submental artery. The main trunk of the facial artery was, conversely, preserved; in this way, the vascular supply of the flap is provided by branches of the facial artery anastomosed in the paramandibular area with the orbicular artery system, which is usually spared in a routine procedure of neck dissection. This is also supported by Cormak et al., who demonstrated the presence of small calibre vessels arising from the facial artery in the submandibular area, direct to platysma [24-26]. These findings were also observed in the present study: the branch of the facial artery providing the vascular supply to platysma was identified in all the flaps in this series.

In addition, several authors reported that the mainstay of MPF success is preservation of an adequate venous drainage. Tension or kinking, especially when the flap is rotated under the mandible, in association with surgical closure of the main venous pedicle of the flap, can result in venous impairment with subsequent flap suffering and, in the worst hypothesis, failure [4,5]. For that reason, authors recommended to design the skin paddle at an adequate distance from the mandible to allow safe transfer of the flap into the oral cavity, with adequate rotation arch. Particular attention should be paid to include the external jugular vein in the deep surface of the flap [1,2,4,24-26]. Moreover, in N0 neck cases, the authors preserved the facial venous system in order to reduce the risk of venous complications.

Post-operative complication reported in the literature are highly variable, ranging from 0 to 45% [1-8]. Huang et al., in a study on 68 patients, described 10 (14.7%) events of flap venous congestion, among which 7 (10.3%) suffered from partial necrosis of the flap; moreover, the authors reported 4 (6%) cases of donor site dehiscence [2]. In a study by Koch et al. on 70 patients, 27% of cases developed a local complication, from partial to total necrosis of the flap, with 12% salivary fistula [8]. These extremely variable data are probably related to different harvesting techniques. In our experience, a MPF harvested with the technique described is highly reliable with a total flap survival rate of 93.4%. In only 4 patients partial necrosis of the skin paddle was observed and the underlying platysma muscle remained viable in all cases. No major post-operative complication such as total necrosis of the flap were reported, and no patient required revision surgery.

Another very relevant issue is the oncological safety of MPF [5]. Some skills must be obtained during harvesting, since it has to be performed simultaneously with neck dissection. Indeed, it is oncologically unsafe to manipulate the lymphatic vessels during isolation of the vascular pedicle without performing lymph node dissection. Furthermore, the cervical fascia overlying the sternocleidomastoid muscle should be not included in the flap: in this way, flap elevation does not interfere with normal neck dissection.

In the present study, a median follow-up of 69 months (range 5-153 months) suggests that this reconstruction does not worsen long-term oncological results, which is consistent with the literature [11]. In fact, 13 patients reported local recurrence at the level of the primary tumour subsite (tongue, retromolar trigone, cheek mucosa), 1 patient developed a locoregional recurrence (contralateral lymph-nodes relapse) and 2 patients had a distant metastasis relapse. Moreover, 8 patients developed a second tumour in a different subsite during follow-up. These latter cases are not related to treatment of the primary tumour, but conversely, can be related to prolonged exposure of these patients to risk factors such as smoking and alcohol abuse, with consequent field cancerisation of the oral cavity and upper respiratory-digestive tracts.

In our opinion and according to the literature, the presence of positive lymph nodes is not a contraindication for the use of this flap [4,5]. The facial artery is normally preserved during routine neck dissection, while preservation of the submental artery is technically possible, but oncologically unsafe, especially in the case of tumours involving the floor of mouth or tongue. In our study, we found positive neck lymph nodes in 23 patients (pN1 in 13 cases, pN2 in 10 cases), and level IB was involved by lymph node metastasis in 5 (8.2%) cases at pathological examination. Nevertheless, none of the patients in the present study developed a homolateral neck recurrence, and only one patient had a contralateral lymph node relapse at follow-up, demonstrating the oncological safety of this procedure in our series. Nonetheless, it is fundamental to highlight that this technique is contraindicated if massive infiltrative metastasis at level IB is present.

Regarding the neck scar, it does not usually affect neck movements; moreover, since it is very low in the neck, it is uncommon for patients to complain of aesthetic dissatisfication [1-5,17].

Small skin paddle and thinness limit the use of MPF, especially when large resection or tongue compartmental surgery are required. Moreover, previous treatments such as surgery, radiotherapy and chemoradiotherapy are main contraindications for this flap [3-8,17].

Conclusions

MPF is simple, versatile, and could be valued as a reconstructive alternative, with a low rate of complications. It is a good therapeutic option for reconstruction of the oral cavity and oropharynx with small- and medium-sized defects. The neck scar is not compromising from functional or aesthetic points of view.

The vascular supply of the MPF can be provided by specific branches of facial artery achieving both oncological radicality and a high flap success rate.

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ACTA OTORHINOLARYNGOLOGICA ITALICA 2020;40:181-189; doi: 10.14639/0392-100X-N0411

Head and neck

Prevention of fistulas after salvage laryngectomy using temporoparietal fascia free flap

L'utilizzo del lembo libero di fascia temporoparietale per la prevenzione delle fistole dopo laringectomia di salvataggio

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SUMMARY

We conducted a retrospective review to assess the role of the temporoparietalis fascia flap (TPFF), comparing rates of postoperative pharyngocutaneous fistula (PCF) and functional outcomes with those of pectoralis major myocutaneous flap (PMMF) and primary closure of the pharynx, in a population of patients treated with salvage total laryngectomy (STL). Patients were divided in three groups depending on the pharynx reconstruction technique after primary closure: no vascularised tissue augmentation (group 1), PMMF patch (group 2), or TPFF patch (group 3). The main outcomes analysed were overall fistula rate, fistula requiring reoperation and speech and swallowing function at 6 months. Factors influencing the incidence of fistulas were also evaluated. 39 patients respected inclusion criteria: 14, 11 and 14 patients in the three groups, respectively. Nine patients of 39 (23.1%) experienced a PCF. No statistically significant differences were noted between the three groups, except for a longer surgical operation time and a trend for better functional results in group 3. None of the factors analysed significantly influenced the overall rate of fistula. TPFF patch thus represents a reliable alternative to PMMF in preventing PCF in the setting of STL, with minor donor-site morbidity and good functional outcomes.

KEY WORDS: laryngectomy, flaps, surgery, fistulas

RIASSUNTO

È stata condotta una revisione retrospettiva per valutare il ruolo del lembo di fascia temporoparietale (TPFF), confrontando i tassi di fistola faringocutanea postoperatoria (PCF) e gli esiti funzionali con quelli del lembo miofasciale di gran pettorale (PMMF) e la chiusura primaria del faringe, in una popolazione di pazienti trattati con laringectomia totale di salvataggio (STL). I pazienti sono stati divisi in tre gruppi a seconda della tecnica di chiusura del faringe dopo sutura diretta dello stesso: nessun rinforzo con tessuto vascolarizzato (gruppo 1), patch con PMMF (gruppo 2) o patch con TPFF (gruppo 3). I principali risultati analizzati sono stati la frequenza complessiva della fistola, la necessità di re-intervento e gli esiti funzionali inerenti linguaggio e deglutizione a 6 mesi. Sono stati anche valutati i possibili fattori predisponenti l'incidenza delle fistole. 39 pazienti hanno rispettato i criteri di inclusione: rispettivamente 14, 11 e 14 pazienti nei tre gruppi. In nove pazienti su 39 (23,1%) il decorso è stato complicato dallo sviluppo di PCF. Non sono stati osservati risultati statisticamente diversi tra i tre gruppi, fatta eccezione per un tempo di intervento chirurgico più lungo e una tendenza a risultati funzionali migliori nel gruppo 3. Nessuno dei fattori analizzati ha influenzato in modo significativo la frequenza complessiva della fistola. L'utilizzo del TPFF rappresenta un'alternativa affidabile al PMMF nella prevenzione della fistola faringocutanea nel contesto delle laringectomie di salvataggio, con morbilità minore nel sito donatore e buoni risultati funzionali.

PAROLE CHIAVE: *laringectomia, lembi, chirurgia, fistole*

Received: July 31, 2019
Accepted: December 8, 2019

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Funding
None.

Conflict of interest
The Authors declare no conflict of interest.

How to cite this article: Pellini R, Zocchi J, Pichi B, et al. Prevention of fistulas after salvage laryngectomy using temporoparietal fascia free flap. Acta Otorhinolaryngol Ital 2020;40:181-189. https://doi.org/10.14639/0392-100X-N0411

Introduction

The advent of chemoradiotherapy (CRT) protocols has shifted the treatment paradigm of advanced laryngeal cancer from primary surgery to organ preservation options, based on the combination of CRT and salvage surgery [1,2]. The literature evidence, starting from the pioneering works by Veteran's Affairs Cooperative Laryngeal Cancer Study Group and Radiation Therapy Oncology Group (RTOG) 291-11 trials [3,4] has shown that radiotherapy (RT) and CRT are associated with comparable control rates as primary surgery with the additional value of a functional organ preservation rate ranging from 60 to 100% [1,5,6].

Although partial laryngectomy can be used in selected laryngeal relapses [7], most patients with persistent/recurrent disease or dysfunctional larynx undergo salvage total laryngectomy (STL) [8], which is performed in approximately 31-36% of cases following RT and in 16-28% of patients treated with CRT [3,9]. Despite being commonly used, the procedure is characterised by a high risk of post-operative complications, including the common and fearsome pharyngocutaneous fistula (PCF), reported in approximately 30% of cases [7]. Interestingly, several factors have been described to be independently associated with the occurrence of PCF, including chronic obstructive pulmonary disease, low hemoglobin level (< 12.5 g/dL) prior to surgery, need for blood transfusion, advanced primary tumour, supraglottic subsite, hypopharyngeal tumour site, positive surgical margins and the addition of neck dissection [10,11]. Previous RT or CRT are also associated with healing complications and fistula formation: hypoxia resulting from microvascular damage induced by radiation impairs wound healing [12] and chemotherapy exacerbates this effect, producing endarteritis and fibrosis [13]. Notably, the occurrence of complications seems to be higher if STL is performed within the first year after CRT [14].

Some studies have suggested that the use of well-vascularised, non-irradiated tissue may reverse the negative effects of CRT and prevent the occurrence of PCF. In fact, the flap reconstruction technique has been associated with reduced risk of fistula formation and better tendency to spontaneous healing compared with primary closure following STL [7,14,15]. Moreover, a recent systematic review by Paleri et al. concluded that better functional outcomes are achieved with the introduction of vascularised tissue from outside the irradiated field [6]. This is particularly important considering that the main goals of reconstruction should not be limited to reduce wound complications, but also to maximise post-operative function, mainly swallowing and phonation [16].

Today, no clear indication exists regarding the type of pharynx reconstruction (primary closure, pedicled flap, or free flap) should be adopted in various patients [17], and the different techniques available are chosen on a case-by-case basis, depending on patient's comorbidities, donor site morbidity, availability of technical experts for microvascular anastomosis [14] and the preferences of the surgeon and institution. Pectoralis major myofascial flap (PMMF), first described in 1979 [18], has been the most widely used flap reconstruction technique for several years, but nowadays is being replaced by more popular options, such as the on-lay or patch flap [16]. Tissue transfer, with a variety of donor sites available, is also commonly used in pharyngeal reconstruction after STL [19,20].

Temporoparietal fascial flap (TPFF) has been suggested as a valuable alternative to the one more commonly used (PMMF) in STL [21]. However, this evidence is reported only by a single study on a limited number of patients and the advantages, limits and functional outcomes of this technique compared with other flaps are still poorly characterised in the current literature. As we recently adopted this alternative technique in our surgical strategy for STL, we aimed to assess the feasibility of TPFF, comparing rates of postoperative PCF and functional outcomes with those of PMMF and primary closure of the pharynx after STL.

Patients and methods

Patients and study characteristics

In this retrospective study, we collected data on a consecutive series of patients who underwent total laryngectomy for persistent or recurrent laryngeal squamous cell carcinoma (SCC) after RT/CRT with curative intent at IRCCS "Regina Elena" National Cancer Institute, between July 2010 and January 2018. The study was approved by the Institutional Ethic Committee (RS1167/18). No other inclusion criteria were applied, while exclusion criteria were primary laryngectomy (no previous RT/CRT), any kind of partial or circumferential resection of the hypopharynx and use of any other flap than PMFF or TPFF.

Patients were divided into three groups according to pharynx closure; namely primary closure without vascularised tissue augmentation (group 1), primary closure and PMMF patch (group 2) and primary closure and TPFF patch (group 3).

After the surgical reconstruction, a nasogastric tube (NGT) was used in all patients until oral diet intake was restored. Broad spectrum antibiotic therapy was also administered to all patients postoperatively. We did not proceed directly with tracheoesophageal puncture (TEP) in any case, but all patients were followed by a professional teacher of oesophageal voice technique.

Surgical technique of TPFF flap harvesting

TPFF is based on the superficial temporal artery (STA) and vein. The vascular pedicle is usually checked and marked preoperatively using a portable colour flow Doppler. Digital palpation to feel superficial vascular pulsation can help for a continuous check during incision and dissection. The STA usually runs through the retromandibular parotid gland, crosses the posterior root of the zygomatic bone, taking a more superficial course into the fascia at the level of the zygomatic arch [22], with a mean distance from the tragus of 16 mm [23]. One or two veins usually accompany the STA [22]. A variety of surgical incisions have been described [24]; we prefer the Y-shaped incision, which allows better exposition of the surgical field when harvesting the flap. The incision begins at a pretragal level and ends at the level of the temporal line. It is possible to find the vascular pedicle anteriorly to the surgical skin incision [25]. The dissection proceeds in the subdermal subfollicular plane, superficially to the musculoaponeurotic system. Both incision and dissection are performed rigorously with a cold scalpel technique in order to avoid damage to the vascular pedicle and hair follicles through the electric scalpel. The frontal branch of facial nerve, coursing just under the temporoparietal fascia, is recognised after it crosses the superficial surface of the zygomatic arch [23,24] and spared. Consequently, once the frontal branch of the facial nerve is identified, the anterior incision of the fascia can be made immediately posteriorly to its course. Posterior incision is usually performed posteriorly to the vein, thus avoiding damage to the vascular network, while the superior one is conducted at the level of the temporal line. The deep landmark plane for the surgeon is the temporalis fascia. The detachment of the flap from the deep temporal fascia is started from superior to inferior and conducted through the avascular areolar tissue that separates the two fascial layers. The definitive dimension of the flap is approximately 12 × 10 cm, with a thickness of 2-4 mm (Fig. 1).

Once tracheostomy is harvested and primary closure of the pharynx is completed via a continuous Connel suture, similar to the PMMF technique, the free flap is applied directly over the pharyngeal closure with an on-lay technique, wrapping the pharyngeal mucosa and fixing it to the base of the tongue superiorly, to the prevertebral fascia laterally and to the tracheo-oesophageal septum inferiorly. Anastomosis is usually performed with superior thyroid artery and internal jugular vein with a Prolene 9/0 suture (Ethicon Sàrl, Neuchâtel, Switzerland).

Parameters evaluated

For each patient, sex, age, smoking habit, medical history, postoperative outcomes and postoperative complications were recorded. Patients were followed for at least 12 months after surgery. Clinical evolution and postoperative complications included total hospital stay; starting soft food oral intake without complications; need for surgical revision; and incidence of PCF and incidence of minor and major complications, such as wound dehiscence, minor and major haemorrhage, haematoma, flap necrosis and donor site morbidity. Patients developing PCF were not considered for time to oral intake recovery analysis.

Presence of comorbidities (vascular and heart diseases, pulmonary diseases or diabetes), pre-treatment clinical staging, dose of radiation received (Gy), the interval from RT/CRT, technique of reconstruction, surgical procedure time, concurrent mono- or bilateral neck dissection, vascular anastomosis time (when free flap was adopted) and pathology report (cancer site, TNM stage group) were registered and analysed as possible risk factors for PCF formation.

Postoperative functional results were also assessed by evaluating swallowing and voice outcomes. Swallowing outcomes at 6 months were routinely evaluated in all patients. The analysis was then elaborated using a 3-point scale in which patients were categorised as: 1) taking nothing by mouth; 2) oral intake with a liquid/soft diet; and 3) oral intake without limitation. Voice outcomes were evaluated through understandability of speech analysis at 6 months using a 5-point scale adopted by the Microvascular Committee of the American Academy of Otolaryngology-Head and Neck Surgery [15], in which patients were categorised as: 1) never understandable; 2) difficult to understand; 3) usually understandable but may need face-to-face contact; 4) understandable most of the time but may need repetition; and 5) always understandable.

Statistical analysis

Descriptive statistics were used to describe patient characteristics. The association between variables was tested by Pearson Chi-Square test or Fisher's Exact test. The comparison between groups was performed by Mann-Whitney U test or Kruskal-Wallis nonparametric test, when appropriate. A p-value < 0.05 was considered as statistically significant. The SPSS (21.0) statistical programs was used for all analyses.

Results

Relapse characteristics

A total of 39 patients were included in the study, 32 males and seven females with a median age of 67 years (range: 49-86). In total, 28 of 39 patients had at least one co-pathology in past medical history: seven had chronic obstructive

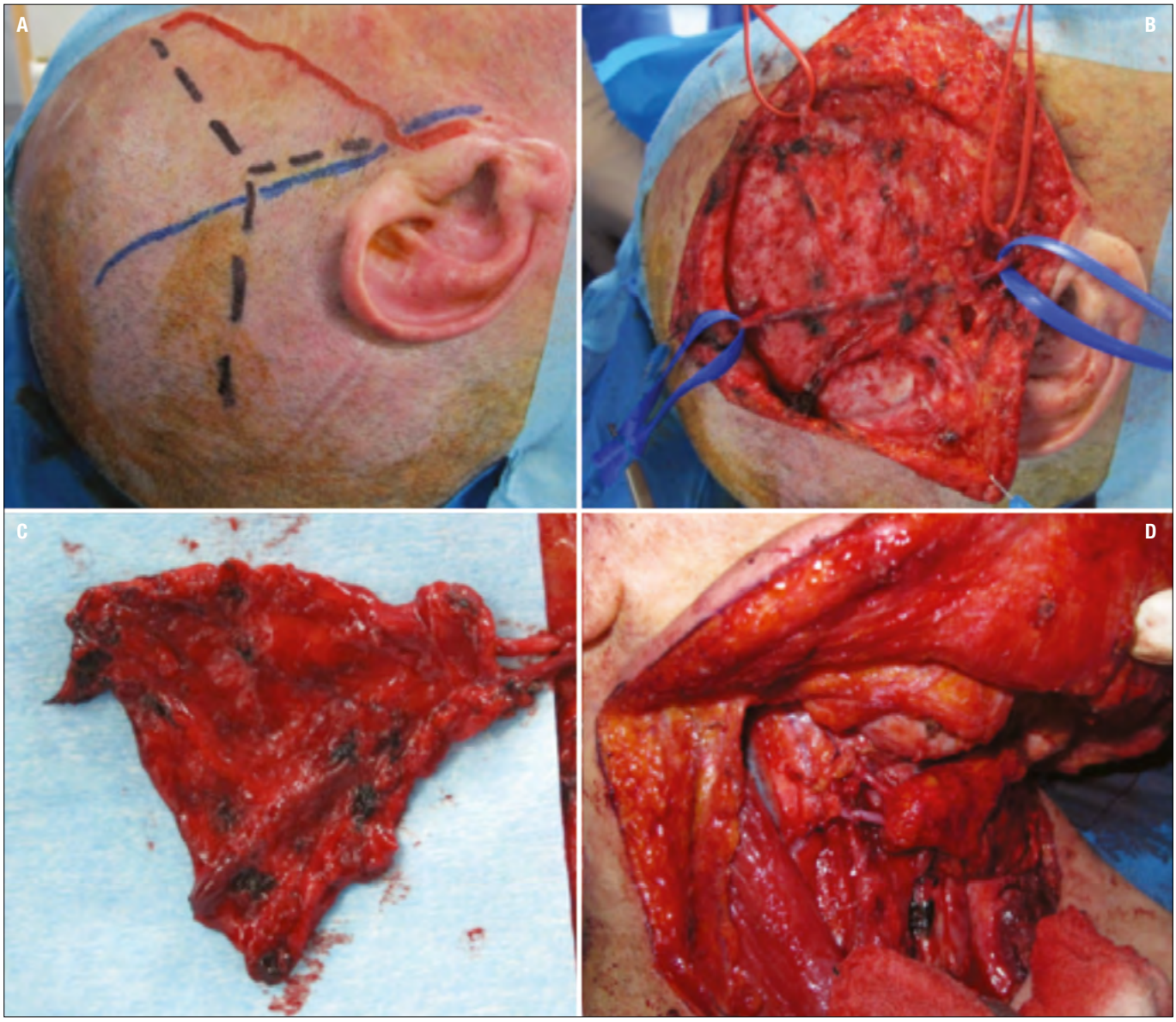

Figure 1. TPFF harvesting. **A:** Drawing of superficial course of the STA, collateral vein and Y-shaped cutaneous incision; **B:** identification and isolation of pedicles through subfollicular, supra-superficial musculoaponeurotic system plane dissection; **C:** definitive harvesting of the flap with a usual dimension of 12x10 cm; **D:** microanastomosis with superior thyroid artery and internal giugular vein and on-lay application of the flap over the pharyngeal closure.

pulmonary disease, 20 had vascular or heart disease, and seven had diabetes. A total of 12 patients had more than one disease. Pre-radiotherapeutic treatment clinical T staging was: 6 T1 (3 T1a, 3 T1b), 17 T2, 14 T3 and 2 T4a. Median radiation dose received was 70 Gy (66-70) on T. Pathological staging showed eight cases with an early-stage relapse (rpT1–T2) and 30 patients had an advanced rpT3–T4 lesion, while one patient necessitated total laryngectomy for dysfunctional larynx following chondroradionecrosis.

In total, 14 patients received pharyngeal reconstruction through a primary closure (group 1), 11 were treated with PMMF (group 2) and 14 with TPFF (group 3). The three surgical groups were homogeneous from epidemiological and clinical points of view (age, sex, time from primary treatment to salvage surgery). Overall, 14 patients had previous CRT, while 25 had only RT. Median interval from previous RT/CRT to salvage surgery was 10 months (range: 3-276), with no significant differences among groups. A total of 16 and 22 patients had monolateral and bilateral neck dissection, respectively; only one patient was not subjected to this procedure.

Major features of the three groups are summarised in Table I.

Post-surgical complications and PCF

The main clinical outcomes in the three surgical groups are summarised in Table I.

Six patients (15.3%) necessitated surgical revision: three patients in group 1 underwent either PMMF (n = 2) or direct suture (n = 1); two patients in group 2 were treated with a sternocleidomastoid muscle flap (n = 1) or direct suture (n = 1), and one patient in group 3 was revised through a PMMF. Time to wound closure was 45, 28 and 14 days, respectively, in the 3 groups. Only three patients (one for each group) obtained closure through a medical treatment only; the remaining patients underwent surgical revision.

Overall, three patients experienced minor complications (immediate postoperative bleeding) and three patients (two in group 1 and one in group 2) were discharged with a NGT. No major or minor intra- or post-operative complications were noted in the TPFF group; in particular, none of the patients in this group experienced alopecia, facial nerve deficit (frontalis branch), or other donor site complications (dehiscence, keloid), nor necessitated flap revision.

Nine of 39 patients (23.1%) experienced a PCF in the postoperative period, 4 (28.6%), 3 (27.3%) and 2 (14.3%) patients, respectively, in the three groups (p = 0.62). None of the potential risk factors considered (age, sex, comorbidity, CRT, radiation dose; mono- or bi-lateral neck dissection) influenced the incidence of PCF. The time interval between

Table I. Comparison of baseline characteristics and clinical outcomes between the three surgical groups (primary closure, PMMF, TPFF).

	Total	PC; group 1	PMMF; group 2	TPFF; group 3	P-value
Patients (n)	39	14	11	14	-
Age, median (range); years	67 (49-86)	68 (51-86)	61 (49-72)	69.5 (61-80)	**0.03**
Smoking status, n (%)					
Yes	27 (69.2)	11 (78.6)	6 (54.5)	10 (71.4)	0.42
No	12 (30.8)	3 (21.4)	5 (45.5)	4 (28.6)	
Comorbidity, n (%):					
Yes	26 (66.7)	10 (71.4)	6 (54.5)	10 (71.4)	0.60
No	13 (33.3)	4 (28.6)	5 (45.5)	4 (28.6)	
Primary cancer site, n (%)					
Glottic	16 (41.0)	8 (57.1)	1 (9.1)	7 (50.0)	
Glottic-hypoglottic	10 (25.6)	3 (21.4)	3 (27.3)	4 (28.6)	0.15
Glottic-supraglottic	1 (2.6)	0	1 (9.1)	0	
Supraglottic	12 (30.8)	3 (21.4)	6 (54.5)	3 (21.4)	
TNM stage*, n (%)					
T1	2 (5.1)	2 (14.3)	0	0	
T2	6 (15.4)	1 (7.1)	0	5 (35.7)	**0.004**
T3	12 (30.8)	6 (42.9)	1 (9.1)	5 (35.7)	
T4	18 (46.2)	5 (35.7)	10 (90.9)	3 (21.4)	
Primary to salvage treatment (months), median (range)	10 (3-276)	12.5 (4-276)	11 (3-108)	14 (85-108)	0.95
Neck dissection, n (%)					
None	2 (5.1)	1 (7.1)	0	1 (7.1)	0.16
Monolateral	21 (53.8)	11 (78.6)	6 (54.5)	5 (35.7)	
Bilateral	16 (41.0)	2 (14.3)	5 (45.5)	8 (57.1)	
Surgical time (minutes)	240 (100-440)	187.5 (100-270)	240 (210-300)	309 (200-440)	**< 0.0001**
Time to oral feeding (days)	20.5 (13-42)	20.5 (16-24)	20 (13-42)	20.5 (13-23)	0.98
Hospitalisation (days)	24 (15-129)	24.5 (18-90)	23 (15-129)	24 (17-39)	0.85
PCF	8 (22.8)	4 (28.6)	3 (27.3)	2 (14.3)	0.62
Nutritional score					
1	2 (5.1)	1 (7.1)	1 (9.1)	0	0.66
2	9 (23.1)	3 (21.4)	3 (27.3)	3 (21.4)	
3	28 (71.8)	10 (71.4)	7 (63.6)	11 (78.6)	
Speech score					
1	3 (7.7)	3 (21.4)	0	0	
2	7 (17.9)	4 (28.6)	3 (27.3)	0	**0.03**
3	15 (38.5)	4 (28.6)	4 (36.4)	7 (50.0)	
4	14 (35.9)	3 (21.4)	2 (18.2)	6 (42.9)	
5	3 (7.7)	0	2 (18.2)	1 (7.1)	

*Values are median (range) or numbers (%). * One patient in TPFF group underwent total laryngectomy for dysfunctional larynx. Bold values represent p-values that are statistically significant.*

primary treatment (RT/CRT) and salvage surgery was the only element that was close to being an independent factor for PCF in the entire population (p = 0.13), although it did not reach statistical significance (Tab. II).

No statistically significant differences were noted between groups in terms of hospitalisation time (p = 0.85) and time to oral feeding (p = 0.98), while surgical time varied significantly between the three techniques (p < 0.0001) (Tab. II)

Postoperative outcomes: swallowing and phonation.

At 6 months from surgery, swallowing outcomes were similar in the three groups (p = 0.66) with all patients but two having re-established "per os" feeding and reported "nutritional mode" scores of 2 or 3. Two patients (one in group 1 and one in group 2) remained dependent on a NGT (score 1); both patients were considered frail subjects who did not begin rehabilitation therapy and precociously died due to disease relapse. Two patients in the PMMF group required an esophageal dilatation procedure to resolve solid consistence dysphagia.

Considering speech intelligibility, 35.7% of patients in group 1, 18.1% in group 2 and 42.8% in group 3 reached a score of 4 (understandable most of the time but may need repetition) or 5 (always understandable). Statistical analysis showed a significant difference between the three groups, favoring TPFF (p = 0.03). The majority of patients (43.5%), however, were classified as score 3 (usually understandable but may need face-to-face contact).

Table II. Main clinical factors associated with the incidence of PCF.

Patient characteristics	Pharyngocutaneous fistula		P-value
	Yes	No	
Comorbidity			
Yes	5 (17.9)	23 (82.1)	0.24
No	4 (36.4)	7 (63.6)	
Sex			
Male	8 (25)	24 (75)	0.99
Female	1 (14.3)	6 (85.7)	
Neck dissection			
Monolateral	3 (18.8)	13 (81.3)	0.99
Bilateral	5 (22.7)	17 (77.3)	
Prev. treatment			
RT	7 (28)	18 (72)	0.44
RCT	2 (14.3)	12 (85.7)	
RT dose (Gy)	70 (66-70)	70 (60-70)	0,81
Time from RT (months)	6 (3-37)	12 (3-276)	0.13
Age (years)	67 (51-86)	65 (49–86)	0.44

Values are median (range) or numbers (%).

Discussion

Our study focused on the use of TPFF as a possible alternative to PMMF or other free flaps in reconstructive surgery after STL in patients with recurrent laryngeal cancer.

PMMF is historically the most widely used flap for head and neck reconstruction, with a reported flap necrosis rate of only 2.3%, which makes it possibly the most reliable reconstructive method, even in the setting of STL. Notably, a systematic review by Guimarães reported a decreased incidence of PCF of approximately 22% in 742 patients treated with PMMF [17] compared with primary closure alone. This technique presents several advantages, including easy harvesting and constant and predictable pedicle, robust vascularisation, reduced operative time, abundance of tissue, allows a unique surgical field and does not require a separate reconstructive team or microvascular experience. The richer and more robust vascularisation compared with the peripheral edges of a free flap may also increase the ability to seal off the pharyngotomy [17]. Furthermore, the deep fascia surrounding the pectoralis muscle is rich in hyaluronan, which may have an important role in the earliest stages of wound healing [26]. On the other hand, harvesting a PMMF exposes the patients to some implicit and constant consequences, partly due to its bulkiness, and partly to donor site morbidity [27].

Considering the disadvantages associated with PMMF, in the last years we decided to shift to a free flap procedure, consisting in the use of the temporoparietal fascia (TPF). This fascial layer represents a continuation of the superficial musculoaponeurotic system, is 2-3 mm in thickness and can comprise an area of 17 × 14 cm. This flap is characterised by predictable vascularisation, furnished by the STA and collateral veins; it also receives branches from the deep temporal artery, branch of internal maxillary artery [28], thus offering rich vascularity, which makes it ideal when a highly vascularised tissue is required in the surgical bed. Moreover, the TPF is characterised by good pliability, and reduced volume and encumbrance, making it a good candidate for reconstruction after STL. The use of TPF as a flap has been reported in a variety of reconstructive settings [29]; however, only a very limited number of case reports have described the use of TPFF after STL [29,30]. The largest experience is that reported by Higgins et al., who registered only one failure and two minor complications in 12 patients treated with TPFF [21].

Surgical outcomes and PCF occurrence

In our retrospective study, we reported no major differences in terms of intra- or postoperative surgical complications in patients treated with TPFF compared with primary closure or PMMF. Regarding PCF formation, which is the most fearsome complication after STL, we did not find any significant difference between the three groups, even if a

somewhat minor incidence was noted in the TPFF group, where only two patients (14%) developed a PCF (Tab. II). Vascularisation provided by the TPFF can explain the low rate of PCF observed in our series; although, contrary to PMMF, the presence of experts in microsurgical procedures is needed for microvascular anastomosis. Of note, the relative lack of useable vessels for microvascular anastomosis reported in patients previously treated with CRT [31] did not constitute a real contraindication to free flap use in our experience, nor did it lead to a higher incidence of flap failures compared with patients who are not irradiated.

Moreover, none of the patients in the TPFF group experienced donor site morbidity, such as alopecia or frontal branch weakness. Donor site morbidity can be strongly reduced by respecting some critical surgical steps such as the use of cold surgical instruments and bipolar electrocautery, which must be limited to control haemostasis in case of minor bleeding. The damage to frontal branch of facialis nerve can also be avoided by limiting the flap dissection posteriorly to the Pitanguy line which connects a point 0.5 cm below the tragus and 1.5 cm lateral to the superior brow. In order to obtain a much longer length of the vascular pedicle, it is possible to continue the dissection of the temporal vessels downwards along the tragal region. Even with this expedient, the length of TPFF pedicle is quite short, but in the setting of STL this does not represent a real limit.

Our analysis showed a significant difference in operating time between the three groups, with a mean surgical time of 309 minutes for TPFF, compared with 187.5 minutes for primary closure and 240 minutes for PMMF. This difference is consequent to the obvious extension of surgery when a microvascular flap is harvested, but can also be explained by the different incidence of lateral neck dissection in the three groups. In our experience, TPFF harvesting does not allow two surgical teams to work together, contrary to what described by Higgins et al. [21]. In spite of this, median extra time compared with PMMF harvesting was only 69 minutes, including a median of 30 minutes spent for vascular anastomoses. This is an acceptable and justified extension of surgical time if accompanied by beneficial effects such as reduction in hospital stay, need for second surgical procedure, or postoperative complications. It could thus be speculated that, even from an economical point of view, the use of the TPFF does not add any expenses compared to the major pectoralis myofascial flap.

In our opinion, TPFF may be associated with minor morbidity even in a hypothetical comparison with other free flaps. Radial forearm free flap (RFFF) has similar tissue qualities with a longer pedicle, although if a skin paddle is harvested the forearm donor site generally requires skin grafting, which can be complicated by tendon exposure or forearm stiffness [24]. This aspect can be overcome if the RFFF is harvested as a fascial-only flap as described by Fung et al. [19]. Anterolateral tight flap (ALT) has meaningless donor site morbidity, with the only lasting sequelae represented by a vertically oriented scar along the thigh and thigh numbness [24]. However, we believe that the characteristic thickness of adipose tissue limits its use in STL, as already shown by a randomised study comparing RFFF with ALT [31]. Notably, the results of RFFF and ALT strongly depend on body habitus of the patient, while TPFF has the advantage of being independent of the patient's anatomy.

Finally, it should be noted that no flap has shown a significant advantage about PCF formation in the literature. This is consistent with the rationale that any non-irradiated vascularised tissue may be beneficial in aiding wound healing. Therefore, the choice of flap should consider other factors such as performance status, donor site morbidity, functional outcomes and the availability of technical expertise for microvascular anastomosis.

Functional and aesthetic outcomes

No studies exist on functional responses after TPFF. The recent multicentre study by the Microvascular Committee of the American Academy of Otolaryngology-Head & Neck Surgery [15] clearly showed that vascularised tissue augmentation with muscle leads to worse speech and swallowing function compared to primary closure or vascularised tissue augmentation without muscle. Another study on alternative free flaps concluded that fasciocutaneous free flap guarantees better swallowing functional outcomes and similar rates of postoperative complications compared with PMMF [27].

Our study showed similar swallowing outcomes between the three groups. Except for two frail patients who never re-established oral intake alimentation, all patients ate by mouth at the 6-month follow-up and only two patients in the PMMF group underwent a procedure of oesophageal dilatation for dysphagia with food with a solid consistency, while none of the patients in the TPFF group required this operation. Empirically, muscle fibrosis and atrophy consequent to denervation and scarring process, can lead to pharyngeal constriction and consequent dysphagia when PMMF is used, while this effect can be insignificant for a thin fascial layer such as that provided by the TPF.

We report less remarkable outcomes regarding speech intelligibility at 6 months, with few patients reaching a satisfying "speech score" (score 4 or 5) and mot patients were classified as group 1 or 3. This can be in part explained by the less reproducible results achieved through oesophageal voice training even with long logopedic rehabilitation. Fung et al. found no difference in voice-related quality of

life between patients treated with primary total laryngectomy and STL with a fascial free flap; this suggests that an additional fascial layer over the pharyngeal closure does not impede the vibratory (pharyngoesophageal) segment [20]. These results are attributed to the pliability and thinness of the flaps applied. Considering that TPFF is the thinnest flap described in the human body [29], characterised by a tissue composition that is associated with good pliability and easy draping without architectural distortion, we could assume that comparable functional results would be achieved.
Finally, concerning motility and aesthetical outcomes, TPFF can be considered as a less invasive procedure compared with PMMF as long as the correct technique is applied. In fact, PMMF pedicle encumbrance determines unaesthetic bulging in supraclavicular region and in the neck, with possible distortion and stenosis of tracheostomy, making skin closure demanding when the muscle is excessively bulky. Moukarbel et al. also clearly showed a detectable limitation in shoulder and neck function, unavoidably associated with chest deformity, subsequent to PMMF harvesting [32].

Limitations

We acknowledge some limitations of our study, including the retrospective nature of the analysis, the limited number of patients and lack of more thorough functional analysis. Despite the modest size of the population, it is important to underline that this is the largest experience reported to date on patients with advanced laryngeal cancer treated with TPPF, since this aspect has been only marginally described in literature. Moreover, the retrospective approach is intrinsically linked to the type of study and surgical procedure performed.

Conclusions

The reinforcement of pharyngeal suture with non-irradiated well vascularised tissue seems to slightly reduce the rate of PCF after STL. The use of TPFF as an overlay technique, although increasing the surgical time compared with PMMF, seems to be at least equally efficacious in reducing PCF compared with other techniques. We conclude that TPFF is a reliable alternative flap in case of STL, which should also be considered for its minor donor site morbidity and better functional outcomes in selected patients.

Acknowledgements

Editorial assistance was provided by Ambra Corti, Luca Giacomelli, PhD, and Aashni Shah; this assistance was supported by internal funds.

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ACTA OTORHINOLARYNGOLOGICA ITALICA 2020;40:190-197; doi: 10.14639/0392-100X-N0311

Thyroid

Ki-67 and CK-19 are predictors of locoregional recurrence in papillary thyroid carcinoma

Ki-67 e CK-19 sono fattori predittivi di ricaduta locoregionale nel carcinoma papillare della tiroide

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SUMMARY

Most patients with papillary thyroid carcinoma have good prognosis; however, recurrence rates and the need of salvage treatment remain a significant problem for 5-40% of patients. Although several risk classifications based on clinicopathological prognostic factors are used, it is not possible to predict which patients will have a higher risk of recurrence. The objective of the study is to analyse the impact of cytokeratin-19 and Ki-67 immunoexpression as predictive markers of the risk of recurrence in papillary thyroid carcinoma. This is a retrospective case-control study, including 42 patients with papillary thyroid carcinoma and 42 controls. The groups were matched by gender, age and pathological staging T and N. Slides were made by the microarray tissue system. Multivariate logistic regression was applied to identify an independent risk factor for recurrence. Of the 42 selected cases, 30 patients (71.4%) were female and 12 (28.6%) were male, ranging in age from 10 to 80 years (median of 39 years). Most patients (64.3%) had tumors at initial T staging (T1-T2). Half of the sample was classified as low risk according to the American Thyroid Association (ATA) risk stratification. Follow-up time ranged from 46 to 196 months, with time to recurrence from 2 to 106 months (median, 30 months). CK-19 and Ki-67 immunoexpression had a statistically significant association with the risk of recurrence (p = 0.029 and p = 0.007, respectively). In multivariate logistic regression analysis, immunoexpression for these markers was an independent risk factor for locoregional recurrence (OR-9,64; CI-1.14-81.01 and OR-3,21; CI-1.32-7.94, respectively). The immunohistochemical analysis of the Ki-67 and CK-19 markers is useful to predict tumour recurrence in patients with papillary thyroid carcinoma.

KEY WORDS: thyroid neoplasms, immunohistochemistry, recurrence, biomarkers, carcinoma papillary

RIASSUNTO

La maggior parte dei pazienti affetti da carcinoma papillare della tiroide godono di una prognosi favorevole tuttavia il 5-40% di essi possono essere colpiti da ricaduta di malattia e dover affrontare una chirurgia di salvataggio. Nonostante la presenza di diverse classificazioni di rischio e fattori prognostici clinicopatologici, non è possibile identificare con certezza i pazienti con più alto rischio di ricaduta. Lo scopo di questo studio è analizzare Ki-67 e CK-19 come fattori predittivi di ricaduta nel carcinoma papillare della tiroide. Abbiamo effettuato uno studio retrospettivo caso controllo che ha incluso 42 pazienti affetti da carcinoma papillare della tiroide e 42 controlli. I gruppi sono stati stratificati per genere, età, staging del T e N. Dei 42 pazienti, 30 erano di sesso femminile e 12 di sesso maschile, con un'età compresa fra i 10 e 80 anni (media 39 anni). Il 64,3% dei pazienti erano affetti da tumori T1-2. Metà del campione è stato classificato come a basso rischio secondo la classificazione della American Thyroid Association (ATA). Il tempo di follow-up è variato dai 46 a 196 mesi, con un periodo libero da malattia compreso fra i 2 e 106 mesi (media 30 mesi). L'immunoe-

Received: May 15, 2019
Accepted: January 9, 2020

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Funding
The authors thank to the AC Camargo Cancer Center Pathology Department for bearing the costs of immunohistochemical slides.

Conflict of interest
The Authors declare no conflict of interest.

How to cite this article: Viana AOR, Gonçalves Filho J, Francisco ALN, et al. Ki-67 and CK-19 are predictors of locoregional recurrence in papillary thyroid carcinoma. Acta Otorhinolaryngol Ital 2020;40:190-197. https://doi.org/10.14639/0392-100X-N0311

spressione di CK-19 e Ki-67 è associata in maniera statisticamente significativa con il rischio di ricaduta (p = 0,029 and p = 0,007, rispettivamente). Abbiamo effettuato un'analisi di regressione multivariata in cui si è evidenziato che l'immunoespressione di questi due marcatori è risultata un fattore di rischio indipendente per le recidive locoregionali (OR-9,64; CI-1,14-81,01 e OR-3,21; CI-1,32-7,94, rispettivamente). L'analisi immunoistochimica di Ki-67 e CK-19 è utile allo scopo di predire il rischio di ricaduta nei pazienti affetti da Carcinoma papillare della tiroide.

PAROLE CHIAVE: *neoplasie tiroidee, immunoistochimica, recidiva, biomarkers, carcinoma papillare*

Introduction

Papillary carcinoma is the most common well-differentiated thyroid carcinoma, corresponding to more than 80% of cases. It usually has a slow growth rate and can metastasise to cervical lymph nodes without affecting, however, overall survival rates [1-3]. In most series of patients with papillary thyroid carcinomas, the reported specific disease survival rate is up to 98% and 93% at 5 and 10 years, respectively. However, in long-term follow-up, the recurrence rate is about 28% [4-6].

Several clinical and pathological features have been shown to predict the more aggressive behaviour of thyroid carcinoma, but the most useful prognostic factors in well-differentiated thyroid carcinoma are patient age, tumour size, tumour invasion, presence of distant metastasis and tumour dedifferentiation [7]. However, a significant number of cases without these characteristics can present locoregional recurrences. Thus, predicting outcomes in thyroid neoplasms is not reliable using clinicopathological information alone.

In order to search for new tools to improve the ability to predict which patients will have a better or worse outcomes, immunohistochemical (IHC) has been used to evaluate different markers in papillary thyroid carcinoma, both as a diagnostic tool and as a prognostic factor [8]. Cytokeratin-19 (CK-19) and Ki-67 are some of the markers used in prognostic evaluation in papillary thyroid carcinoma [7,9-12]. Currently, Ki-67 is regarded as one of the most promising markers for assessing cell proliferation activity. In clinical practice, it is used to estimate the prognostic factor in several different malignant tumours, such as mammary, thyroid and neurological tumors [11]. In thyroid neoplasms, there are studies that analyse both the diagnostic and prognostic value of this marker. Dwivedi et al. [13] analysed the expression of Ki-67 and observed greater expression of this marker in papillary thyroid carcinomas in relation to non-neoplastic lesions. Miyauchi et al. [14] found that Ki-67 was an independent prognostic factor for disease-free survival.

Ck-19 is present in the simple or glandular epithelium; however, enhanced immune expression is seen in some pathological conditions, as in tumours of epithelial origin. It is a sensitive marker for papillary carcinomas, usually with a strong diffuse plasma reactivity, while in benign thyroid lesions and normal thyroid tissue, a focal and light reactivity is observed [15,16]. Cheung et al. [15] demonstrated diffuse immune reactivity in 66% of papillary thyroid carcinomas. While the significance of CK-19 immunoexpression for differential diagnosis of thyroid lesions has been widely used and debated, its value as a prognostic factor in papillary carcinoma is still uncertain. In hepatocellular carcinoma and intrahepatic cholangiocarcinoma, it is well recognised that patients with immunoexpression for CK-19 have worse prognosis, with the level of immunoexpression significantly related to tumour aggressiveness and postoperative recurrence [17,18].

Most studies that investigate immunohistochemical markers for papillary thyroid carcinoma do so in order to differentiate between benign and malignant neoplasms, and few studies look for prognostic markers [7,9,11,12]. The aim of this study was to analyse the impact of the immunoexpression of CK-19 and Ki-67 on the risk of patients with papillary thyroid carcinoma.

Materials and methods

This is a retrospective case-control study including patients with thyroid papillary carcinoma who were treated surgically for curative purposes from January 1, 2000 to July 31, 2010. The case group was formed of all patients who presented locoregional tumour recurrence and underwent surgical salvage treatment. The control group was formed by patients who did not present locoregional recurrence. Data from the control group were matched with those from the case group according to age, gender and T and N staging. Exclusion criteria were: 1) patients submitted to initial treatment or salvage treatment at another institution; 2) unresectable locoregional relapse; 3) only distant recurrence; 4) biochemical relapse, with no identifiable site of recurrence; 5) records with incomplete information about the disease; 6) less than two years post-recurrence follow-up; 7) loss of follow-up was considered when the patient did not return on a scheduled appointment twice in the stipulated period.

All tumours were staged according to the American Joint Committee on Cancer tumor-node-metastasis (TNM) staging system (7th edition). The case group had analysed

samples of the initial tumour and the specimen from the salvage surgery. The control group had only the specimen of the primary surgery analysed. The study was evaluated and approved by the Research Ethics Committee of the A.C. Camargo Cancer Center. All demographic, clinical, pathological and therapeutic information were collected from the electronic medical records from the A.C. Camargo Cancer Center by a single investigator (AORV), using a standardised form.

A TMA Block was constructed using 1.0 mm diameter representative samples of each tumour tissue taken from the recipient paraffin block, with papillary thyroid carcinoma samples in the case group at initial (n = 42) and relapse (n = 42) and in the control group (n = 42). All specimens were collected in duplicate. The slides were stained by IHC CK-19 (Clone RCK107, Dako), Ki-67 (Clone MIB-1, Dako). The reaction for labeling the samples was performed by dewaxing and hydrating the histological sections, with subsequent antigenic recovery in the pressure chamber in citrate solution (pH 6.0). Exposure to hydrogen peroxide 3% for 5 min; incubation of the slides with primary antibodies diluted in previously established titers in PBS buffer; incubation of the slides with Post Primary Block (Novolink Max Polymer-Leica Biosystems) for 30 minutes; Incubation of slides in substrate solution with chromogen diaminobenzidine (DAB, Sigma) 5 minutes; Fractionation with Harris haematoxylin for 30 seconds, followed by washing with distilled tap water; Assembly of the slides in Entellan neu (Merck, 1.07961.0100, Darmstadt, Germany). The negative control was obtained through the same tissue analysed, included in each series of immunohistochemical staining, with the omission of the primary antibody. The standardisation of pathological interpretation took into account the expected positivity for each marker: Cytokeratin 19 shows cytoplasmic positivity and Ki-67, nuclear positivity. The absence of staining was considered nonspecific and negative. Cytokeratin-19 was analysed by a score that classified the labeling in three categories: (0) no cell marking, (1) < 25% positive, (2) > 25% positive. Ki-67 was also classified into three categories: (0) no cell marking, (1) < 10% positive, (2) > 10% positive [19,20].

The information collected was stored in a computerised database made specifically for the study and statistical analysis was performed using SPSS (Statistical Package for Social Science, SPSS version 18.0 for IOS) statistical software. The categorical qualitative variables were presented through descriptive statistics (numbers and percentages), while continuous quantitative variables were presented by means of the median, mean and standard deviation. For analysis of the association of immunoexpression of biological markers (CK-19 and Ki-67) with the clinicopathological aspects of the patients, as well as the comparison between cases and controls, the χ^2 Test or the Fisher's exact test were used as appropriate. The McNemar test was used to ascertain the differences in the comparison between two paired qualitative samples (before and after). The Student's T test was used to compare the means between the continuous variables, whose normality distributions were confirmed through the Shapiro-Wilk Test.

Overall and disease-free survival were analysed using the Kaplan-Meier method. The follow-up time was considered from the date of surgery until the date of the last information and, in the cases of death, until the date of death. The period between the date of surgery and the occurrence of relapse was considered to calculate the probability of disease-free (or recurrence-free) survival.

Multivariate logistic regression was performed aiming to identify possible independent associations. All the significant associations observed in the univariate analyses were included in the regression model. Odds ratio (OR) values were determined to investigate the intensity of associations. In all tests statistical significance was considered for a value of $p \leq 0.05$.

Results

During the 10 year period, 2140 thyroidectomies were performed for thyroid carcinoma in the institution, in which, 1743 were histologically diagnosed as papillary thyroid carcinoma. Cases with incomplete clinical or pathological information (n = 178) or lost to follow-up (n = 305) were excluded. Of the remaining cases, 82 presented recurrence, but 40 cases had to be excluded because they did not meet the inclusion criteria. At the end of this process, 42 cases were obtained with all clinical information on treatment and follow-up present, as well as paraffin blocks suitable for the available immunohistochemical analysis.

The clinical characteristics of cases and control groups are described in Table I. The follow-up time of the 42 patients in the study group ranged from 46 to 194 months (median, 117 months). The time to recurrence ranged from 2 to 106 months (median, 30 months). The site of locoregional recurrence was the lateral chains of cervical lymph nodes [25 patients (59.5%)], followed by cervical lymph nodes of the central compartment [10 patients (23.8%)]. Three patients (7.1%) presented recurrence in the thyroid bed and three patients also presented local and lateral recurrences simultaneously. One patient presented recurrence in the thyroid bed and bilateral lateral lymph nodes.

All patients with recurrence were submitted to salvage surgical treatment according to the site of recurrence.

Table I - Clinicopathological characteristics of selected cases and controls

Variable	Categories	Cases N (%)	Control N (%)	p
Age	Variation Median	10-80 39	14-77 39	0.659
Gender	Female Male	30 (71.4) 12 (28.6)	30 (71.4) 12 (28.6)	1.000
Size of node	Variation Median	2-50 mm 12 mm	2-80 mm 10 mm	0.669
Multifocality	Yes No	18 (42.9) 24 (57.1)	13 (31) 29 (69)	0.366
Extrathyroid extension	Yes No	16 (38.1) 26 (61.9)	11 (26.2) 31 (73.8)	0.350
Blood Vascular Invasion (BVI)	Yes No	2 (4.8) 40 (95.2)	0 (0) 42 (100)	0.494
Lymphatic Vascular Invasion (LVI)	Yes No	4 (9.5) 38 (90.5)	2 (7.1) 39 (92.9)	1.000
Perineural Invasion (PNI)	Yes No	3 (7.1) 39 (92.9)	0 (0) 42 (100)	0.241
Pathological T staging	pT1-T2 pT3-T4	27 (64.3) 15 (35.7)	27(64.3) 15 (35.7)	1.000
Pathological N staging	pN0 pN1a pN1b	21 (50) 18 (42.9) 3 (7.1)	25 (59.5) 15 (35.7) 2 (4.8)	0.476
ATA risk classification	Low risk Intermediary risk High risk	21 (50) 17 (40.5) 4 (9.5)	30 (71.4) 9 (21.4) 3 (7.1)	0.129
Type of surgery	Total thyroidectomy Partial thyroidectomy	42 (100) 0 (0)	40 (95.2) 2 (4.8)	0.494
Iodine (I131)	Yes No	35 (83.3) 7 (16.7)	33 (78.6) 9 (21.4)	0.782
Recurrence	Local Level VI Levels II-V Local + level VI + levels II-V Level VI +levels II-V Local + distant Without recurrence	3 (7.1) 11 (26.2) 24 (57.1) 1 (2.4) 2 (4.8) 1 (2.4) 0 (0)	(0) (0) (0) (0) (0) (0) 42 (100)	0.000

P-value obtained by the Chi-Square Frequency Test or Fisher's Exact.

Of the three patients with recurrence in the thyroid bed, two required the use preoperative radioactive technetium injection and a probe for identification of local recurrence, which was confirmed in pathological examination, with no evidence of lymph node involvement. One patient had local recurrence with invasion of the cricoid cartilage and underwent right vertical hemilaryngectomy. There were also no lymph nodes involved in the specimen. There was no association of locoregional recurrence with distant metastasis.

As for the pathological analysis of the recurrences, all confirmed the diagnosis of papillary carcinoma. The number of lymph nodes dissected in lateral neck dissections (levels II to V) ranged from 17-97 (mean of 49 lymph nodes). Positive lymph nodes found in the specimen ranged from 1-10 (mean of 3.3 lymph nodes). The number of lymph nodes dissected in the central compartment dissections (level VI) ranged from 3-10 (mean of 5.3 lymph nodes), with positive lymph nodes found in the specimen ranging from 1-3 (mean of 1.8 lymph nodes).

Twenty-eight patients (66.7%) underwent adjuvant radioiodine therapy with doses varying from 108 to 389 mCi (mean of 239.6 mCi). Only the patient that underwent partial laryngectomy was submitted to adjuvant teleradiotherapy. This patient had no previous iodine uptake prior to salvage surgery (diagnosed through increased thyroglobulin and local uptake seen in PET-CT). No patient in the study group underwent chemotherapy.

Regarding the status at the end of the follow-up, 39 patients (92.9%) were alive and without evidence of disease, 2 patients (4.8%) died due to other causes (both with second primary tumors – lung and breast – and died from complications of these neoplasms). One female patient had recurrent disease in the lateral cervical levels (new relapse), but she was pregnant and decided to undergo salvage surgery after puerperium. The patient continues regular prenatal and follow-up examinations and no lymph node uptake has been observed so far.

Overall survival at 10 years for the case group was 95.2% (Fig. 1). In the control group, follow-up ranged from 51 to 205 months, with a median time of 112 months. No patient presented locoregional recurrence. Two patients (4.8%) died due to causes not related to thyroid cancer (1 breast cancer and 1 CNS cancer). Overall survival for the control group at 10 years was also 95.2%. Disease-free survival in the group that presented recurrent disease was 21.4% at 5 years.

There was a significant association between the presence of higher Ki-67 expression in the group that presented relapse, compared to the control group, where the majority did not present expression for this marker or, when present, was classified as weak ($p = 0.007$). Similarly, CK-19 was immunoexpressed in both, but in the case group, a greater number of patients with higher grades of immunoexpression were shown ($p = 0.029$) (Tab. II).

There was no significant association among clinical and histopathological variables (gender, age, staging, multifocality, extrathyroidal extension, blood vascular invasion, perineural invasion and ATA risk classification). There was a significant association between CK-19 immunoexpression and lower lymphatic vascular invasion (LVI) rate ($p = 0.046$).

Multivariate analysis by logistic regression of

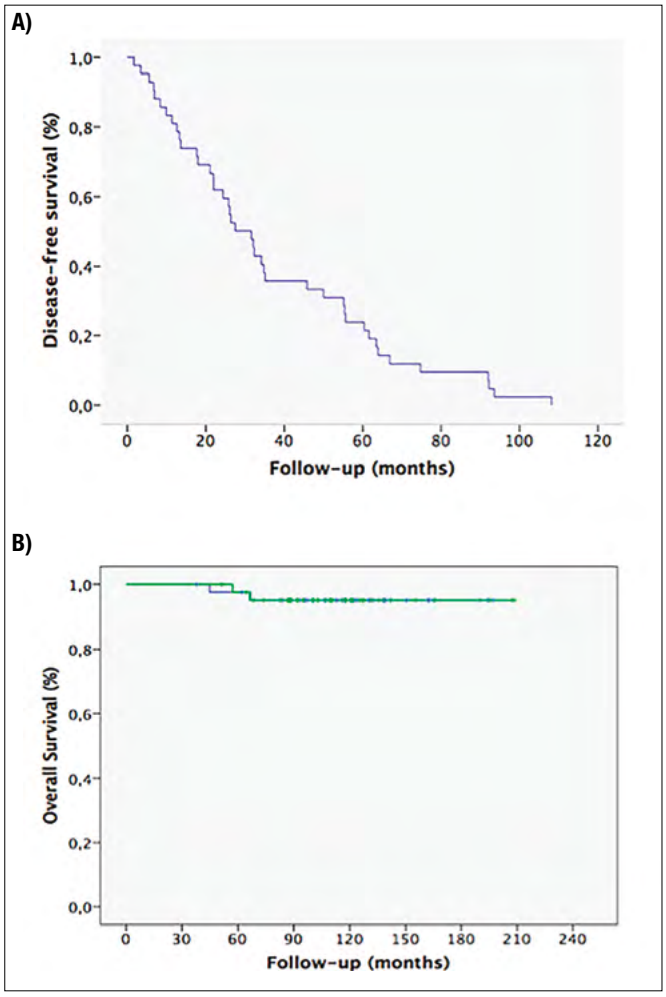

Figure 1. A) Recurrence-free survival of 42 patients with carcinoma papillary thyroid cells included in the group of cases; **B)** Global survival between the studied case and control groups. (Method of Kaplan-Meier).

Table II. Immunoexpression of CK-19 and Ki-67 in the case and control groups.

Markers IHQ	Cases (%)	Control (%)	p
CK-19:			
negative	1 (2.4)	8 (19.0)	0.029
< 25%	5 (11.9)	3 (7.1)	
> 25%	36 (85.7)	31 (73.8)	
Ki-67:			
negative	14 (33.3)	26 (61.9)	0.007
< 10%	25 (59.5)	16 (38.1)	
> 10%	3 (7.1)	0 (0)	

P-value obtained by Fisher's Exact Test.

immunoexpression of the cytokeratin-19 and Ki-67 markers showed that the presence of immunoexpression of CK-19 and Ki-67 were significant independent predictors of the risk of recurrence, with a confidence interval of 1.14-81.01 for CK-19 and 1.32-7.94 for Ki-67 (Tab. III). There was no significant association between ATA classification and CK-19 or Ki-67 immunoexpression (Tab. IV).

We also performed an analysis combining risk stratification according to ATA and Ki-67 immunoexpression. The cases were reassembled as follows: a) low risk: patients classified as low risk ATA/Ki-67-; b) intermediate risk: patients with low risk ATA/Ki-67+, intermediate risk ATA/Ki-67- or + and high risk ATA/Ki-67-; c) high risk: patients high risk ATA/Ki-67+. We observed that in the group of cases, 76% of the patients classified as low ATA risk were reclassified as intermediate after the new stratification with the inclusion of Ki-67. In the control group, 3 patients classified as high risk were reclassified as intermediate risk. When we analysed the locoregional recurrence rate after the new stratification, we observed that 100% of patients reclassified as high risk presented tumour recurrence (Tab. V).

Discussion

Papillary thyroid carcinoma is the most common histological subtype among malignant thyroid neoplasms, and usually has an indolent clinical course in most cases. In our study, we had a 6.5% rate of tumour recurrence, consistent with the literature reporting rates of 5-30% [21,22]. The follow-up period in this series was almost 10 years (118 months), which is necessary for proper evaluation because of the indolent tumour behaviour [23]. Possibly our low rate of relapse, close to the lower margin described in the literature, can be due to the fact that the majority of operated cases were pT1 (61.9%), also consistent with the significant change in the profile of cases of papillary carcinoma in the last decades, with a significant decrease in the number of advanced tumours treated [24,25]. Despite this, we had a significant number of early recurrences (median of 30 months), although all patients were staged during initial evaluation with preoperative cervical ultrasonography to identify probable lymph node metastases, and most were classified as low risk (50%) or intermediate risk (40.5%) by ATA risk stratification.

Although uncommon, relapses and deaths have been reported with some cases of papillary thyroid carcinoma [24,25]. Several clinical or histopathological prognostic factors are predictors of survival (TNM, ATA, MACIS, AGES, GAMES, EORTC, among others) and are not predictors of recurrence in patients with papillary carcinoma [26]. With this in mind, several authors have sought to identify molecular markers that could, in addition to the classic clinical and histopathological parameters, distinguish low- and high-risk patients for recurrences aiming to minimise the risk of overtreatment for low-risk patients [10-12,27-29].

Table III. Logistic regression analysis of CK-19 and Ki-67 immunoexpression between the case and control groups and the risk of recurrence.

Markers IHQ	Cases (%)	Control (%)	Odds Ratio	95% IC	p
CK-19:					
negative	1(2.4)	8 (19.0)	1	1.14-81.01	0.037
imunoexpress	41(97.6)	34 (81)	9.64		
Ki-67:					
negative	14 (33.3)	26 (61.9)	1	1.32-7.94	0.010
imunoexpress	28 (59.5)	16 (38.1)	3.25		

P-value obtained by Fisher's Exact Test.

Table IV. Relationship of expression of markers with ATA risk classification.

Markers IHQ	ATA risk classification (%)			p
	Low	Intermediary	High	
CK-19:				
negative	1 (2.4)	0 (0)	0 (0)	0.655
< 25%	3 (7.1)	1 (2.4)	1 (2.4)	
> 25%	17 (40.5)	16 (38.1)	3 (7.1)	
Ki-67:				
negative	5 (11.9)	0 (0)	0 (0)	0.380
< 10%	15 (35.7)	7 (16.7)	3 (7.1)	
> 10%	1 (2.4)	2(4.8)	0 (0)	

Table V. Locoregional recurrence rate among all patients studied (cases and controls) after a new risk stratification (ATA + Ki-67).

ATA + Ki-67 risk classification		Locoregional recurrence (%)
Low risk	Low ATA/Ki-67-	5/23 (21.7)
Intermediary risk	Low ATA/Ki-67+ Intermediary ATA/Ki-67+ or - High ATA/Ki-67-	34/58 (58.6)
High risk	High ATA/Ki-67+	3/3 (100.0)

In 2009, ATA published a risk stratification for patients with well differentiated thyroid carcinoma undergoing thyroidectomy, in order to select those patients with a higher risk of recurrence and/or disease persistence, with update of this guideline in 2015 [30]. There were three categories: low risk, intermediate risk and high risk of recurrence. Low-risk patients were defined as having intrathyroidal DTC with no evidence of extrathyroidal extension, vascular invasion, or metastases. Intermediate-risk patients demonstrated either microscopic extrathyroidal extension, cervical lymph node metastases, RAI-avid disease in the neck outside the thyroid bed, vascular invasion, or aggressive tumour histology. High-risk patients had gross extrathyroidal extension, incomplete tumour resection, distant metastases, or inappropriate postoperative serum Tg values. In our series, we were unable to identify patients at greater risk for tumour recurrence, since 90.5% of the cases belonged to the low an /or intermediate risk groups.

We evaluated the immunoexpression of two biological markers (Ki-67 and CK-19) in 42 patients with papillary thyroid carcinoma who presented with locoregional recurrences during the follow-up period. Cytokeratin-19 is one of the most widely used immunohistochemical markers for thyroid neoplasms, and is not usually produced by healthy thyroid cells. Its presence is therefore related to neoplastic transformation. There is strong evidence in the literature reporting weak positivity for CK-19 in benign lesions, but levels of variable expression in malignant thyroid lesions [7]. Although CK-19 immunoexpression has been used in the differential diagnosis of neoplastic thyroid lesions, its use as a prognostic factor is not well known. However, CK-19 has a well-defined prognostic value in liver tumours, where patients with strong immunoexpression for CK-19 have a significantly worse prognosis, characterising greater tumour aggressiveness in those with higher expression of this marker [17,18]. In the case of thyroid neoplasms, there is little information about the carcinogenic role of CK-19 or its value as a prognostic factor [7]. In our study, the immunoexpression of CK-19 was strongly expressed and significantly more evident in patients who presented recurrence of the disease compared to the control group. Only one case in the sample did not have immunoexpression for CK-19. Paradoxically, most patients in the case group with strong immunoexpression for CK-19 did not present lymphatic vascular invasion ($p < 0.05$). We did not find in the literature any correlation between lymphatic vascular invasion and CK-19 immunoexpression in papillary thyroid carcinoma.

Some studies have reported a significant correlation of the level of CK-19 immunoexpression in thyroid papillary carcinoma with extrathyroidal extension and pTNM staging [7]. These studies demonstrate that the high expression of CK-19 in tumour thyroid cells is associated with worse prognosis, which was also seen in our study; however, we did not find correlation of these levels with staging and extrathyroidal extension.

Ki-67 is a DNA-binding protein that is found primarily in the nucleus and is related to cell proliferation, being an important tumour marker. Its high immunoexpression is associated with worse prognosis in breast cancer and prostate cancer [31,32]. In thyroid, few studies have analysed the use of this marker as a prognostic factor. Ito et al. [10] demonstrated that Ki-67 was an independent prognostic factor for disease-free survival in patients with papillary thyroid carcinoma. In the present study, there was a significant association between the presence of Ki-67 expression in the group that presented recurrence,

compared to the control group, where the majority did not present expression for this marker or, when present, presented weak immunoexpression. No association was found for this marker with gender, age, T and N staging, multifocality, extrathyroidal extension, lymphatic vascular invasion, or ATA risk classification.

Tang et al. [12] showed higher immunoexpression of Ki-67 in papillary thyroid carcinomas > 1.0 cm and in the presence of thyroiditis, but without relation with multifocality, extrathyroidal extension and lymph node metastasis. Zhou et al. [11], on the other hand, observed a significant correlation between increased Ki-67 immunoexpression and extrathyroidal extension and lymph node metastasis. Pan et al. [20], in a meta-analysis on 51 studies in thyroid cancer, showed that patients with higher Ki-67 immunoexpression had worse survival. It was also associated with tumour size, lymph node metastasis and extrathyroidal extension.

In multivariate analysis, we observed that Cytokeratin-19 and Ki-67 were independent risk factors for locoregional recurrence in papillary thyroid carcinoma. It is, therefore, suggested that cell proliferative activity in the primary lesion is an important factor that reflects the probability of carcinoma recurrence. Therefore, these markers may be useful in clinical practice in order to predict tumour recurrence in patients with papillary thyroid carcinoma.

The proposed risk classification combining the expression of Ki-67 and ATA classification seems to have the potential to increase the ability to predict recurrences. In the group of cases, only 5 patients remained at low risk and 37 patients (88.1%) were classified as intermediate or high risk for recurrence. All the patients reclassified as high risk presented locoregional recurrences. Therefore, the use of this marker associated with the ATA classification must be tested in a larger independent sample to confirm its predictive value on the risk of recurrence.

Conclusions

Ki-67 and cytokeratin 19 immunoexpression is related to locoregional recurrence in papillary thyroid carcinoma, and these two markers are independent risk factors for tumour recurrence. The combination of Ki-67 immunoexpression may contribute to improve the predictive value of ATA recurrence risk classification, although these findings need to be confirmed in a larger independent patient sample.

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ACTA OTORHINOLARYNGOLOGICA ITALICA 2020;40:198-203; doi: 10.14639/0392-100X-N0284

LARYNGOLOGY

Demographics and coexisting tremor, cervical dystonia and vocal fold disorders in a group of patients with spasmodic dysphonia

Dati demografici e presenza di tremore, distonia cervicale e patologie delle corde vocali in un gruppo di pazienti con disfonia spasmodica

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SUMMARY

The primary aim of this study is to describe the demographic and clinical characteristics of a group of patients with spasmodic dysphonia (SD). As a secondary aim, we examined associations of age at SD diagnosis and sex with co-existing cervical dystonia and nonvocal tremor; as well as association of vocal tremor with sex and nonvocal tremor. Seventy-four consecutive patients who were treated for SD at the Mayo Clinic in Jacksonville, Florida between October 1, 2015 and March 31, 2018 were included in this retrospective study. Information was collected regarding sex, age at SD diagnosis, BMI, SD diagnosis type, recent history of major stress/depression, recent history of upper respiratory tract infection (URTI), co-existing neurological diseases, and co-existing vocal disorders. The majority of patients were female (75.7%) and median age at SD diagnosis was 61 years (range: 17 – 80 years). The median BMI was 25.7 (range: 16.9 – 63.7). The most common diagnostic combinations were adductor dysphonia only (52.7%), adductor dysphonia and MTD (18.9%), and adductor dysphonia and tremor (17.6%). Co-existing tremor was present in 36.6% of patients and cervical dystonia was present in 15.5%. Co-existing vocal disorders were observed as follows: paresis/paralysis (3.1%), cyst (3.1%), mass (4.7%), polyp (1.6%), and anterior glottis web (1.6%). Sex was not notably associated with either cervical dystonia or nonvocal tremor (all $P \geq 0.30$). Older age at SD diagnosis was significantly associated with cervical dystonia ($P = 0.049$), but not nonvocal tremor ($P = .22$). Other than co-existing tremor, most patients had no co-existing neurological diseases or vocal disorders. Additionally, patients who were older at SD diagnosis were significantly more likely to have co-existing cervical dystonia.

KEY WORDS: spasmodic dysphonia, demographics, risk factors, neurologic disorders, vocal fold pathologies

RIASSUNTO

L'obiettivo principale di questo studio è quello di descrivere le caratteristiche demografiche e cliniche di un gruppo di pazienti affetti da disfonia spasmodica (SD). Come obiettivo secondario, abbiamo valutato l'età dei pazienti al momento della diagnosi associandola al sesso e tremore non cordale. Sono stati valutati retrospettivamente settantaquattro pazienti consecutivi, trattati per SD alla Mayo Clinic di Jacksonville dall'1 ottobre 2015 al 31 marzo 2018. Sono stati raccolti dati riguardanti sesso, età alla diagnosi, BMI, tipo di SD, storia personale di stress maggiore/depressione, recenti infezioni delle vie aeree superiori (URTI), presenza di patologie neurologiche o delle corde vocali coesistenti. La maggioranza dei pazienti è risultata di sesso femminile con un'età media alla diagnosi di 61 anni (17-80). Il BMI medio è 25,7 (16,9-63,7). le associazioni diagnostiche più comuni sono state disfonia da iperadduzione e MTD (18,9%); disfonia da iperadduzione e tremore (17,6%). La disfonia da iperadduzione isolata è stata trovata nel 52,7% dei casi. Il 36,6% dei pazienti era affetto da tremore mentre il 15,5% da distonia cervicale. Sono state identificate patologie delle corde vocali come paresi/paralisi (3,1%), cisti (3,1%), neoformazioni (4,7%), polipi (1,6%), sinechie glottiche anteriori (1,6%). Non è stata riscontrata alcuna

Received: May 6, 2019
Accepted: October 18, 2019

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Funding
None.

Conflict of interest
The Authors declare no conflict of interest.

How to cite this article: Karatayli Ozgursoy S, Vargas ER, Heckman MG, et al. Demographics and coexisting tremor, cervical dystonia and vocal fold disorders in a group of patients with spasmodic dysphonia. Acta Otorhinolaryngol Ital 2020;40:198-203. https://doi.org/10.14639/0392-100X-N0284

associazione fra sesso e distonia o tremore non cordale. L'età avanzata alla diagnosi ha mostrato un'associazione statisticamente significativa con la distonia cervicale (P = 0,049) ma non con il tremore non vocale (P = 0,22). In questo gruppo di pazienti affetti da SD la maggior parte dei casi non mostra contestuali patologie neurologiche o patologie delle corde vocali. I pazienti più anziani alla diagnosi di SD hanno più probabilità di essere affetti da distonia cervicale.

PAROLE CHIAVE: *distonia spasmodica, caratteristiche demografiche, fattori di rischio, malattie neurologiche, patologie delle corde vocali*

Introduction

Spasmodic dysphonia (SD) is a voice disorder with an unknown pathogenesis. SD is considered to be an adult-onset focal dystonia and mainly diagnosed in two forms. Adductor SD is the most common, diagnosed in 90% of patients [1]. Epidemiologic, genetic and neurologic risk factors for SD have been addressed in several studies [2-5]. Most recent evidence suggests that SD is a type of focal dystonia [6,7]. Although the neuropathophysiologic mechanism still remains unclear, three involved neurologic mechanisms are proposed: reduced cortical inhibition, sensory-processing disturbances, and functional neuroanatomic changes in SD [8].

In a three-center study, 74 of 4,447 (1.7%) patients with dysphonia had SD [9]. In another large study group who sought voice therapy, 21 of 821 (2,6%) patients had SD [10]. Risk factors for SD such as stress, upper respiratory tract infection (URTI) and coexistent neurologic diseases have been investigated in several studies [2,11-15]. Up to date, the relationship between age at SD diagnosis and sex with co-existing cervical dystonia (CD), nonvocal and vocal tremor has not well been studied. SD was reported to be significantly more common in females than in males in a study population who sought voice therapy [10]. In our study, we describe the demographics of SD, examine the associations of age at SD diagnosis and sex with coexisting CD, and evaluate associations of sex and nonvocal tremor with vocal tremor in a small group of SD patients.

We hypothesize that SD is more common in females and is commonly seen in patients with vocal tremor and/or other dystonias.

Materials and methods

Study patients

A retrospective chart review of 74 consecutive patients who were treated for SD at Mayo Clinic in Jacksonville, Florida, between October 1, 2015 and March 31, 2018 was performed. Diagnosis of SD was made based on patient history, speech examination findings and laryngoscopic examination. This study was approved by the Mayo Clinic Institutional Review Board.

All adult patients who had adductor or abductor SD with or without any accompanying muscle tension dysphonia (MTD), vocal or non-vocal tremor, and SD were included in this retrospective study. It is not always easy to distinguish SD and MTD since the features are not always black and white and might be overlapping. Diagnosis was made by history, comprehensive speech evaluation and endoscopic visualisation of the larynx during a dynamic voice assessment using a flexible laryngoscope. Information was collected from the medical records regarding patient sex, age at SD diagnosis, body mass index, SD diagnosis type, recent history of major stress/depression, recent history of URTI, coexisting neurologic diseases and coexisting vocal disorders. A relatively large amount of data was unavailable for recent history of major stress/depression (n = 38 missing) and for recent history of URTI (n = 41 missing). Data was missing for fewer than 15 patients for all other variables.

Statistical analysis

Continuous variables were summarised with sample median and range. Categorical variables were summarised with number and percentage of patients. Associations of sex and age at SD diagnosis (dichotomised at the median) with coexisting CD and nonvocal tremor were examined using Fisher's exact test. Associations of sex and nonvocal tremor with vocal tremor were also evaluated using Fisher's exact test. P values of 0.05 or lower were considered statistically significant and all tests were 2-sided. All statistical analyses were performed using R Statistical Software (version 3.2.3; R Foundation for Statistical Computing, Vienna, Austria).

Results

A summary of demographic and clinical features is provided in Table I. The majority of patients were women (n = 56; 76%), and the median age at SD diagnosis was 61 years (range, 17-80 years). The median body mass index was 25.7 (range, 16.9-63.7). The most common diagnostic combinations were adductor SD only (n = 39; 52.7%), adductor SD and MTD (n = 14; 18.9%) and adductor SD and tremor (n = 13; 17.6%). Recent history of major stress/depression and URTI were observed in 58% (21/36) and 21% (7/33) of patients, respectively. Coexisting tremor (as a neurologic disease) was present in 36.6% of patients (n = 26), and cervical dystonia was present in 15.5%

Table I. Demographic and clinical features of the overall cohort.

Variable	Number of records found	Summary[a,b]
Sex (male)	74	18 (24.3)
Age at diagnosis, y	72	61 (17-80)
BMI, kg/m^2	63	25.7 (16.9-63.7)
Diagnosis:	74	
adductor SD only		39 (52.7)
adductor SD and MTD		14 (18.9)
adductor SD and tremor		13 (17.6)
abductor SD only		3 (4.1)
adductor SD, MTD, and tremor		3 (4.1)
adductor SD and abductor SD		1 (1.4)
adductor SD, abductor SD and tremor		1 (1.4)
Recent history of major stress/ depression	36	21 (58.3)
Recent URTI	33	7 (21.2)
Coexisting neurologic disease (can have more than one):	71	
Parkinson disease		0 (0.0)
cervical dystonia		11 (15.5)
nonvocal tremor		26 (36.6)
other		0 (0.0)
none		37 (47.9)
Coexisting vocal disorders (can have more than one):	64	
paresis/paralysis		2 (3.1)
polyp		1 (1.6)
cyst		2 (3.1)
anterior glottic web		1 (1.6)
mass		3 (4.7)
none		56 (87.5)

BMI: body mass index; MTD: muscle tension dysphonia; SD: spasmodic dysphonia; URTI: upper respiratory tract infection; [a]: median and range are presented for continuous variables; [b]: number and percentage are presented for categorical variables.

(n = 11). Coexisting vocal disorders were observed during laryngoscopy as follows: vocal mass (n = 3; 4.7%); vocal fold paresis/paralysis (n=2; 3.1%); cyst (n = 2; 3.1%); anterior glottis web (n = 1;1.6%); and polyp (n = 1; 1.6%). Only 1 patient had more than 1 coexisting vocal disorder and had anterior glottis web, right vocal fold paresis and granuloma (mass). Other patients had only 1 coexisting vocal disorder, if any.

Table II displays associations of sex and age at SD diagnosis with coexisting CD and tremor (as a neurologic disease). Diagnosis of 'CD' and 'tremor' were driven from the clinical notes of neurologists in patient charts. Sex was not notably associated with either CD or tremor; CD was present in 3 (16.7%) of men and 8 (14.3%) of women (P = 1.00) and tremor was present in 4 (22.2%) men and 22 (39.3%) women (P = 0.30). CD was significantly associated with age at SD diagnosis, being present in 2 (5.6%) patients with an age at diagnosis 60 years or younger (n = 36) compared to 9 (25.0%) patients diagnosed after age 60 (n = 36) (P = 0.049). Nonvocal tremor was not strongly associated with age at diagnosis, occurring in 10 (27.8%) patients with an age at diagnosis of 60 years or younger (n = 36) and 16 (44.4%) patients diagnosed after age 60 (n = 36) (P = 0.22).

Associations of sex and nonvocal tremor with vocal tremor are shown in Table III. There was no notable association between sex and vocal tremor (P = 1.00), although there was a significant association between nonvocal tremor and vocal tremor (P = 0.001). Specifically, of 26 patients with nonvocal tremor, 12 (46.2%) had vocal tremor compared to only 5 (11.1%) patients in the subgroup without nonvocal tremor (n = 45).

Table II. Associations of patient sex and age with coexisting neurologic disease.

	Association with CD		Association with nonvocal tremor	
Variable	**No. (%) with CD (n = 74)**	**P value[a]**	**No. (%) with nonvocal tremor (n = 74)**	**P value[a]**
Sex:		1.00		0.30
male (n = 18)	3 (16.7)		4 (22.2)	
female (n = 56)	8 (14.3)		22 (39.3)	
Age at diagnosis:		0.049		0.22
≤ 60 (n = 36)	2 (5.6%)		10 (27.8%)	
> 60 (n = 36)	9 (25.0%)		16 (44.4%)	

CD: cervical dystonia; [a]: P values result from Fisher exact test.

Table III. Associations of sex and nonvocal tremor with vocal tremor.

Variable	No. (%) with vocal tremor (n = 74)	P value[a]
Sex:		1.00
Male (n = 18)	4 (22.2)	
Female (n = 56)	13 (23.2)	
Nonvocal tremor:		0.001
Yes (n = 26)	12 (46.2)	
No (n = 45)	5 (11.1)	

[a]: P values result from Fisher exact test.

Discussion

SD, also known lately as laryngeal dystonia, has no known aetiology and cure to date. Risk factors for SD and associations between other neurologic diseases and SD have attracted the interest of laryngologists and neurologists. In our group of SD patients, as well as demographics and risk factors, we examined the relationship between age at diagnosis and sex with CD, nonvocal and vocal tremor.

The majority of our patients were women (76%), in concurrence with the literature. In a review of epidemiology in SD patients by Tanner, the female:male ratio was 3:1 [1]. Patel et al reported 77.6% of patients were women in an SD group of 718 patients [15], and Schweinfurth et al reported 79% female dominancy in their study of 168 patients with SD [3]. Female predominance in SD might be attributed to genetic, hormonal, or autoimmune factors, but this remains unknown.

Median age at diagnosis was 61 years in our cohort (range, 17-80 years). In other studies, median age was from 43 to 59 years [2,3,13,15-18]. The older age of onset in our patient cohort might be attributed to delay in diagnosis as well as delay in consulting to a tertiary care clinic. Creighton et. al. reported in their study on 107 patients using a questionnaire that it took 4.43 years to be diagnosed with SD after first going to a physician with vocal symptoms [19]. They concluded that 'objective criteria for the diagnosis of SD and increased clinician education are warranted to address this diagnostic delay.'

The most common diagnostic combinations in our study group were adductor SD alone (53%), adductor SD and MTD (19%) and adductor SD and tremor (18%). Adductor SD incidence among all SD patients is reported to be between 82% and 91.8% in the literature [1,13,15,16,20]. In our study cohort, 95.9% of the patients had adductor SD (alone or combined with abductor SD, MTD and/or tremor). Abductor SD (alone or combined with adductor SD and/or vocal tremor) constituted 6.8% of all patients.

Vocal tremor is not a rare finding in SD patients. Patients with SD were reported to be 12.8 times more likely to have vocal tremor than the control group in a study by White et al. [17] In another study, vocal tremor was confirmed with electromyogram in 29% of SD patients [21]. Vocal tremor prevalence was between 26% and 32% in other studies [13,16,17]. In our study, vocal tremor prevalence was 23.0%; 23.2 % of female patients and 22.2% of male patients had vocal tremor. This is in agreement with, but slightly lower than other studies. The highest vocal tremor incidence among SD patients was reported by Patel et al., being 54.5% in their study group; 60.0% among women, and 32.8% among men [15]. Vocal tremor is a clinical diagnosis. The variety of incidences in different studies might be attributed to the lack of objective diagnostic measures for vocal tremor.

Recent history of major stress/depression and URTI prior to onset of symptoms was observed in 58% and 21% of patients, respectively, in our SD patients. In 1983, Schaefer documented that SD symptom onset followed URTI in a small group of patients [11]. In 1984, Izdebski et al, comparing 200 SD patients with 200 case-controls, found no statistically significant precipitating factors for SD including URTI, stress, occupation, and voice use patterns [2]. In a chart review of 350 SD patients, Childs et al. identified the most common risk factors for SD as stress (42%), URTI (33%) and pregnancy/parturition (10%) [12]. In 2012, Tanner compared 150 SD patients with 136 patients having other voice disorders using a questionnaire, and reported that SD is uniquely associated with a personal history of sinus and throat illnesses, mumps and rubella [1]. Schweinfurth et al, in their study on 168 SD patients who completed a questionnaire, reported that 30% of patients related the onset of symptoms to URTI and 21% to stress [3]. White and colleagues, in 2012 investigated the prevalence of anxiety and depression in SD, comparing 128 SD patients with 146 case-controls with other voice disorders. The results showed that individuals with SD are no more likely to have anxiety or depression than those with other voice disorders. However, there was association between anxiety and depression and patients with voice disorders [14].

Coexisting nonvocal tremor in our SD group was present in 37% of patients, and coexisting CD was present in 16%. Schweinfurth et al. reported that essential tremor was found in 26% of their 168 SD patients [3]. In 2011, Tanner et al. compared 150 SD patients with 150 case controls with normal voices, and SD was reported to be related to personal history of tremor, as well as family history of tremor [13]. In contrast, patients with SD were no more likely to have nonvocal tremor than the control group in a study by White et al. [17]. SD patients were more likely to have CD than the patients with other vocal disorders in another study by Tanner et al. [4]. Increased incidence of other dystonias, especially blepharospasm, and writer's cramp was also seen in SD patients [13]. Patel et al. reported that other movement disorders (CD, blepharospasm, limb dystonia, oromandibular dystonia) were found in 5.2 % of their group of 718 SD patients [15].

Coexisting vocal disorders were observed as follows: vocal mass (5%), vocal fold paresis/paralysis (3%), cyst (3%), anterior glottis web (2%) and polyp (2%). Tanner et al. reported that risk factors for SD included occupational and avocational voice use as well as family history of voice disorders [13]. In another study, a history of frequent,

occupationally intense voice use was prevalent in both SD and voice disorders case-control group. However, those in the SD group had been employed in this type of job for more years [4]. We did not encounter any SD studies in the English literature reporting simultaneous vocal disorders with SD.

Sex was not notably associated with either CD or nonvocal tremor in our SD patient group. In the literature, predictive factors associated with increased nonvocal tremor severity include older age, longer disease duration, presence of vocal tremor and a longer follow-up duration [22]. CD was significantly associated with age at SD diagnosis, with a higher incidence in patients diagnosed after age 60; however, nonvocal tremor did not reveal an association with age at diagnosis.

SD patients with vocal tremor have shown higher associated nonvocal tremor [15]. In our group, there was a significant association between nonvocal tremor and vocal tremor ($P = 0.001$). Specifically, of the patients with nonvocal tremor, 46% had vocal tremor compared to only 11% of patients in the subgroup without nonvocal tremor. White et al. stated that 'the presence of comorbid nonvocal tremor in patients with vocal tremor is > 50% in both controls and patients with SD'; therefore the authors recommended referral of all patients with SD and/or vocal tremor to a neurologist for a thorough evaluation [17].

Our study did not fully examine family histories of neurologic disorders and voice disorders, or personal history of infectious disease as identified by Tanner and Schweinfurth, or the gradual or sudden onset as identified by Childs et al. [1,3,12]. Several limitations of this study are important to bear in mind. First, the retrospective design introduces biases into data collection and yielded a large amount of missing data for some variables. Additionally, we did not include a control group of non-SD patients and are therefore unable to properly evaluate risk factors for SD. Finally, the sample size of the study was relatively small, resulting in a lack of precision in the descriptive summaries presented.

In the literature, there are not many studies regarding the risk factors for and co-existence with SD. More prospective studies are needed in order to better understand SD and thus to improve the clinicians' approach to SD patients. We believe this study will raise more questions and interest on the subject.

Conclusions

In this group of SD patients, a majority of patients were women and presented with adductor SD. Other than coexisting tremor, most patients had no coexisting neurologic diseases or vocal disorders. Additionally, patients who were older at SD diagnosis were significantly more likely to have coexisting CD. However, this finding may simply reflect the tendency to experience more coexisting health conditions as patients age. SD patients should be evaluated for coexisting tremor or dystonias, as treating them can improve vocal outcomes.

Acknowledgements

This retrospective study was approved by the Mayo Clinic Institutional Review Board.

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ACTA OTORHINOLARYNGOLOGICA ITALICA 2020;40:204-210; doi: 10.14639/0392-100X-N0477

Laryngology

Upper dysphagia in patients affected by systemic sclerosis: prevalence and features

La disfagia orale e faringea in pazienti affetti da sclerosi sistemica: prevalenza e caratteristiche

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SUMMARY

Herein, we describe the prevalence and features of dysphagia in patients affected by systemic sclerosis (SS). We analysed the data of 19 patients obtained by administering the M.D. Anderson Dysphagia Inventory (MDADI) scale that measures dysphagia symptoms and by physical assessment consisting of judging specific lip, mandible and tongue performances (scale 0-3) and diadochokinesis, respiratory and phonatory functions (scale "poor", "fair", "good", "normal") according to Robertson's method. Subjects also underwent flexible endoscopic examination of swallowing. MDADI showed that 74% of answers were included in "*mild*" class of disability, 21% as "*moderate*" and 5% as "*severe*". The performance of lips, mandible and tongue that most frequently scored 1 were the opening (52.6% for the lips and 47.4% for the mandible) and the pop of the tongue (52.7%). The percentage of compromised respiratory, phonatory and diadochokinesis tests ("poor" or "fair") was 81%, 70.1% and 74%, respectively. Flexible endoscopic examination of swallowing revealed pharyngolaryngeal sensory deficit and signs of oropharyngeal dysphagia in more than half of cases (58% and 53%, respectively). This study highlights the presence of dysphagia in SS patients and demonstrates the importance of a multidimensional approach that includes subjective and objective evaluation to characterise specific features of swallowing alterations that have a high-impact on upper dysphagia.

KEY WORDS: dysphagia, systemic sclerosis, upper dysphagia, flexible endoscopic examination of swallowing

RIASSUNTO

Scopo dello studio è stato quello di valutare, per mezzo di un campionamento trasversale, la prevalenza e le caratteristiche dei disturbi di deglutizione in pazienti affetti da sclerosi sistemica (SS). Abbiamo analizzato i dati ottenuti da 19 pazienti sottoposti a tests soggettivi, clinici e strumentali. Per la valutazione soggettiva è stato utilizzato il questionario di autovalutazione "M.D. Anderson Dysphagia Inventory". Per l'esame clinico ai pazienti veniva chiesto di eseguire specifici movimenti e prassie delle labbra, della lingua e della mandibola (score da 0 a 3), performances vocali, respiratorie e di diadococinesi in accordo con il sistema Robertson's ("insufficiente", "quasi sufficiente", "sufficiente" e "normale"). Infine ciascun paziente veniva sottoposto ad esame fibroendosopico della deglutizione con test della sensibilità. Risultati dell'MDADI: il 74% dei pazienti mostrava un'alterazione "lieve" della deglutizione, il 21% ed il 5% rispettivamente un grado "moderato" e "severo" di disfagia. Le performances più compromesse erano l'apertura della mandibola e delle labbra (52,6% and 47,4%) e lo schiocco della lingua (52.7%). La voce, la respirazione e la diadococinesi erano alterate in più del 70% dei casi. La FEES ha dimostrato un'alterazione della fase faringea e la presenza di deficit della sensibilità faringolaringea in più della metà dei pazienti (58% e 53%). Lo studio mette in evidenza l'elevata prevalenza della disfagia alta nei pazienti affetti da sclerosi sistemica e dimostra l'importanza di una valutazione multidimensionale che coinvolga entrambi il logopedista ed il foniatra in grado di eseguire un esame clinico specifico e strumentale mirato, indispensabili per contribuire a riconoscere la sede della disfagia e con essa caratteristiche non altrimenti rilevabili.

PAROLE CHIAVE: *disfagia, sclerosi sistemica, disfagia orale e faringea, valutazione fibroendoscopica della deglutizione*

Received: September 18, 2019
Accepted: March 20, 2020

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Funding
None.

Conflict of interest
The Authors declare no conflict of interest.

How to cite this article: Galli J, Marchese MR, De Canio C, et al. Upper dysphagia in patients affected by systemic sclerosis: prevalence and features. Acta Otorhinolaryngol Ital 2020;40:204-210. https://doi.org/10.14639/0392-100X-N0477

Introduction

Systemic sclerosis (SS) is a rare autoimmune disorder characterised by alterations in humoral and cellular immunity, leading to fibroproliferative alterations in microvasculature which in turn causes abnormal collagen deposition in the skin and internal organs [1,2]. The gastrointestinal tract is one of the most commonly affected organ systems, involved in approximately 90% of SS patients. The specific changes contribute to autonomic dysfunctions and dysmotility [4] that cause a variety of morbid symptoms including dysphagia [2-4]. The pathogenesis of dysmotility is related to progression of myopathy, neuropathy and fibrosis leading to abnormalities in compliance and contractility of the GI tract wall [4]. In the literature, dysphagia is reported as a rare presenting complaint of scleroderma in which symptoms occur as the oesophagus becomes more severely affected. When the oesophagus is compromised, the disease process is usually diffuse with involvement of multiple levels of the gastrointestinal tract [5]. Nevertheless, as demonstrated in a previous study, dysphagia (oropharyngeal) is actually not so rare in immunomediated diseases, particularly in cases affected by SS [6]. The recent literature offers no specific studies that are capable of ruling out the presence of primary alterations of swallowing in SS. In addition, almost all published studies describe dysphagia in SS as lower or non-specific dysphagia. Up to now, except for a case reported in 1981 [7], only Rajapakse et al. [8] presented a case series affected by dysfunction of the pharyngoesophageal region. Therefore, based on the available literature, oropharyngeal dysphagia (OD) is infrequent, poorly recognised and poorly documented.

It is known that aspiration, pneumonia, malnutrition, increased mortality, prolonged hospitalisation, advanced disability and declining quality of life may be the consequences of OD. Since early dysfunction is still very responsive to appropriate management, it is clear that early diagnosis and treatment are fundamental issues in preventing such life-threatening complications.

In the light of all the aforementioned considerations, the primary objective of this study was to investigate the prevalence of swallowing dysfunction in SS patients using self-assessment questionnaires in addition to physical evaluation that included clinical and instrumental approaches. The secondary aims were to describe the features of dysphagia focusing on the site and characteristics of symptoms and to provide a detailed description of the structural and functional abnormalities.

Materials and methods

From January to June 2018, at the clinics and rheumatology department of St. Carlo's Hospital at Potenza, we recruited patients affected by SS disease. The inclusion criterion was clinically and laboratory-defined SS disease. The exclusion criteria were thyroid, laryngeal, oesophageal (all except GERD), gastric, respiratory diseases or previous surgery, inability to cooperate, and past or present swallowing rehabilitation therapy. All patients routinely underwent ENT evaluation including flexible fiberoptic rhinolaryngoscopy to evaluate the anatomical integrity of pharynx and larynx. Comorbidities were recorded and patients were asked to answer specific dysphagia symptoms listed in the dysphagic adults assessment questionnaire developed by Travalca Cupillo-Castellini [12] (Tab. I). All persons gave their informed consent prior to inclusion in the study.

In order to assess the impact of dysphagia on the quality of life, we selected the "MDADI" M. D. Anderson Dysphagia Inventory translated into Italian [9,10]. It is a long-standing validated screening self-reported measure of a patient's perceived handicap or impairment from their swallowing. We opted for MDADI because of its simplicity, limited number of questions and direct scoring to assess the handicapping effects of OPD. In order to calculate the prevalence of symptoms (items), we divided the answers into two classes: "No *symptomatic"* that included the "never" and "almost never" answers and "*Symptomatic*" that included the "almost always" and "always". Moreover, we calculated the total score between 0 and 60 and, to obtain a grading scale of dysphagia, we divided the distribution of the scores into four classes of disability: 0-2 (*absent*), 3-14 (*mild*), 15-29 (*moderate*) and 30-60 (*severe*). One additional item is present, whose score is not computed in the total of the MDADI score but which accounts for the general (G) distress reaction to symptoms ("*Does your swallowing problem interfere with your quality of life?").* The interviews were carried out by two trained speech pathologists (DC, MM). With respect to bedside swallowing evaluation (BSE) [11], clinical signs closely related to dysphagia and aspiration were considered: presence of "wet voice", post-swallow residue in the mouth and post-swallow cough. Moreover, using a scale from 0 (not able) to 3 (good) the performance of the lips, mandible and tongue was tested according to the protocol of Travalca Cupillo-Castellini [12], and, diadochokinesis, respiratory and phonatory functions according to Robertson's method [13] (scoring "poor", "fair", "good" and "normal"). Finally, we performed flexible endoscopic examination of swallowing with a sensory test according to Rees [14] and Langmore [15]. The sensory test was performed by lightly touching the aryepiglottic fold or the tip of the epiglottis with the tip of endoscope and ask the patient if he/she feels it. We considered normal subjects who answered affirmatively or who coughed.

Table I. Prevalence of swallowing symptoms and comorbidities in decreasing order.

Symptoms	No. cases	%	Comorbidities	No. cases	%
Food or liquid come back up into the throat	13/19	68	Sicca syndrome	9/19	47
Frequent throat clearing	13/19	68	Osteoarthrosis	8/19	42
The amount of saliva is decreased	12/19	63	Arterial hypertension	8/19	42
Food or liquid come back up into the mouth	12/19	63	Gastroesophageal reflux disease	7/19	37
Globus pharyngeal	11/19	58	Sjogren syndrome	4/19	21
Feeling of food remaining in the upper throat	11/19	58	Hiatal hernia	4/19	21
You clear your throat when you swallow food	10/19	53	Hypovitaminosis D	3/19	16
Difficulty to start swallowing	9/19	47	Thyroid nodules	3/19	16
Cough when you swallow food	8/19	42	Hashimoto's thyroiditis	3/19	16
Feeling of food remaining in the mouth	8/19	42	Hypothyroidism	3/19	16
Loss of saliva during the night	6/19	32	Pulmonary arterial hypertension	3/19	16
Decrease in body weight	6/19	32	Osteoporosis	3/19	16
History of pneumonia by bacterial infection	6/19	32	Dyslipidaemia	2/19	11
Feeling of food in the lower throat	5/19	26	Venous chronic insufficiency	2/19	11
The amount of saliva is increased	4/19	21	Esophagitis	1/19	5
Increase in body weight	4/19	21	Hypercholesterolaemia	1/19	5
Leakage of food or fluid from the mouth	3/19	16	Diabetes type 2	1/19	5
Food or liquid come back up into the nose	3/19	16			

Written informed consent was obtained from all participants included in the study. Statistical analysis was performed using commercially available software (Excel – Microsoft Corporation, Redmond, Washington, USA). Continuously distributed outcomes were summarised as means and categorical outcomes with frequencies and percentages. The numerical data and categoric variables were compared by applying a Student's t test and chi-square test, respectively. The level of significance was set at $p < 0.05$.

Results

From a series of 28 patients, 9/28 met exclusion criteria and 19/28 were considered. Seventeen cases were female and two were males with a mean age of 58.9 years (min. 30 max 78; SD = 13.5). Three of 19 (16%) casess were affected by diffuse cutaneous SS and 16/19 (84%) by limited cutaneous SS.

Comorbidities and dysphagia-specific symptoms

The principal comorbidities and respective prevalence are listed in Table I. Sicca syndrome was the most prevalent occurring in 9/19 (47%) of cases, followed by osteoarthritis 8/19 (42%), arterial hypertension (8/19; 42%), gastro-oesophageal reflux (7/19; 37%) and fibromyalgia (5/19; 26%). The prevalence of specific dysphagia symptoms is shown in Table I. The symptoms referred by more than half of cases were "*Frequent throat clearing*" (13/19; 68%), "*Food or liquid come back up into the throat*" (13/19; 68%), "*Food or liquid come back up into the mouth*" (12/19; 63%), "*The amount of saliva is decreased*" (12/19; 63%), "*Feeling of food remaining in the upper throat*" (11/19; 58%), "*You clear your throat when you swallow food*" (10/19; 53%).

M.D. Anderson Dysphagia Inventory (MDADI)

The total mean score was 11.42 ("mild" dysphagia). In particular, 74% of answers were included in "*mild*" class of disability, 21% as "*moderate*" and 5% as "*severe*". The partial scores for each group of questions were 7.68, 2.42 and 1.31 for the Physical, Emotional and Functional sections, respectively. The score of the Physical (P) section was the highest and significantly greater compared with the other sections ($p < 0.05$). Finally, the mean score of Emotional (E) sub-items was significantly higher than the Functional (F) one ($p < 0.05$). The mean percentage of "Symptomatic" answers was 17.58%, 10.53% and 6.32% for P, E and F group of sub-items, respectively. Nevertheless, these frequencies were significantly less ($p < 0.05$) compared with those of answers with a score between 0-1 (82.42%, 89.47%, 93.68% and for the physical, emotional and functional group of sub-items respectively). Table II showed the frequency of all items in decreasing order. Regarding the item for general (G) distress reaction to dysphagia, 26.3% of answers were included as "Symptomatic".

Bedside swallowing evaluation

The tongue appeared atrophic in 17/19 (89%) of patients, while they were normal in the remaining two cases. On-

ly 2/19 (10%) patients had normal teeth, while the other 17 patients (89%), partially or totally edentulous, used dental prostheses. Three of 19 (16%) patients showed a "wet voice", while post-swallow residue in the mouth was observed in 12/19 (63%) of cases and in only 1/19 (5.2%) case was post-swallow cough present. As shown in Table III the performance of the lips most frequently scoring as 1 was the opening (52.6%). The remaining dynamic tests of the lips were performed almost normally (score 2) in the more than half of cases (54.7%). The opening of mandible and the pop of the tongue were most frequently compromised since a score of 1 was present in 47.4% of patients and in 52.7%, respectively. The percentage of compromised respiratory tests ("poor" or "fair") was 81% and significantly higher compared with "good" or "normal" performances (18.9%) ($p < 0.05$). The test most frequently judged "poor" was "*take a deep breath, then make the /s/ sound again, but start at a whisper then get louder*". The frequencies of the remaining tests are showed in Table IV; 70.1% of phonatory performances were abnormal ("poor" and "fair") and 29.8% were "good" or "normal". The difference was statistically significant ($p < 0.05$). The first two recurrent tests with "poor" score were, respectively, "*Take a deep breath and during expiration say /a/ how long is possible*" and "*Take a deep breath and say a sustained /a/ as soft and as loud possible*". Finally, 76.3% of diadochokinesis tests were impaired ("poor" and "fair"). In particular, 74% of performances were "fair" (Tab. IV).

Flexible endoscopic examination of swallowing

The findings of flexible endoscopic examination are shown in Table V. The oral phase lasted a mean of 21.8 seconds (SD 5.5).

Discussion

Among musculoskeletal diseases, dysphagia is best known as a complication of scleroderma. Nevertheless, the literature refers almost exclusively to the dysfunction caused by the oesophageal abnormalities [16]. In reality, the SS has numerous deleterious effects that compromise more than one stage of the swallowing process. Salivary dysfunction can be seen in up to half of patients with SS as demonstrated by Baron et al. [17]. Consistent with the literature, we observed the co-occurrence of Sicca syndrome in 47% of cases and 63% complained of a sensation of "*dry mouth*". Microstomia (decrease of the mouth opening) and microcheilia (decrease of the lip opening) are common manifes-

Table II. Mean prevalence of MDADI items with score > 1 in decreasing order.

		Prevalence (%)
Physical		
P7	It takes me longer to eat because of my swallowing problem	42
P4	I feel that I am swallowing a huge amount of food	26.3
P5	I limit my food intake because of my swallowing difficulty	26.3
P6	Swallowing takes great effort	21
P8	I cough when I try to drink liquids	21
P2	Swallowing is more difficult at the end of the day	15.8
P3	People ask me, "Why can't you eat that?"	15.8
P1	I cannot maintain my weight because of my swallowing problems	10.5
Functional		
F5	My swallowing difficulty has caused me to lose income	15.8
F2	I feel free to go out to eat with my friends, neighbors, and relatives	10.5
F3	My swallowing problems limit my social and personal life	5.3
F1	People have difficulty cooking for me	0
F4	I feel excluded because of my eating habits	0
Emotional		
E4	I am upset by my swallowing problem	26.3
E7	I do not feel self-conscious when I eat	15.8
E6	I have low self-esteem because of my swallowing problems	10.5
E2	I am embarrassed by my eating habits	5.3
E3	Other people are irritated by my eating problem	5.3
E5	I do not go out because of my swallowing problem	0

tations of SS that are reported to be present in 50-80% of cases [18-20]. Specifically, we observed microstomia in 47% of subjects. Erosions and resorption of mandible and temporomandibular joint involvement are common findings among SS patients and may explain the previously mentioned changes [21]. Overall, reduced oral opening and xerostomia interfere with speech, mastication and oral hygiene predisposing to oral and dental disease. In this regard, it is

Table III. Prevalence of score 0, 1, 2 and 3 for performance of the lip, mandible and tongue.

	0	1	2	3
Lips				
Opening	-	52.7%	36.8%	10.5
Extension	5.3%	26.3%	57.9%	10.5
Protrusion	-	31.6%	57.9%	10.5
Ability to hold a depressor between the lips	-	10.5%	68.5%	21
Exert force against resistance	5.3%	26.3%	52.7%	15.7
Mandible				
Opening	-	47.4%	42.1%	10.5%
Lateralisation	5.3%	36.8%	36.8%	21.1%
Protrusion	5.3%	36.8%	47.4%	10.5%
Tongue				
Protrusion	-	15.8%	57.9%	26.3%
Lateralisation	-	15.8%	57.9%	26.3%
Tongue tip elevation out of the mouth	31.5%	26.3%	36.9%	5.3%
Tongue tip elevation into the mouth	10.5%	26.3%	52.7%	10.5%
Circular movements around the lips	-	15.8%	63.2%	21%
Pop of the tongue	21%	52.7%	10.5%	15.8%
Vertical resistance	36.8%	36.8%	26.3%	-
Lateral resistance	5.2%	26.3%	47.4%	21.1%
Central resistance	-	15.8%	42.1%	42.1%

Table IV. Distribution of respiratory, phonatory and diadochokinesis performance.

	Poor	Fair	Good	Normal
Respiratory				
Take a deep breath, then make the /s/ sound for as long as you can	42.1%	37%	0	21.1%
Take a deep breath, then make the /s/ sound again, but start at a whisper then get louder	73.7%	21.1%	0	5.2%
Take a deep breath, then make the /s/ sound again, but start at a whisper then get softer	52.7%	42.1%	0	5.2%
After a deep breath say repeatedly /s/	47.3%	37%	10.5%	5.2%
Phonatory				
Take a deep breath, then make the /a/	26.3%	21.1%	36.8%	15.8%
Take a deep breath, then make the /a/ sound for as long as you can.	42.1%	36.8%	15.8%	5.3%
Take a deep breath, then make the /a/ sound for as aloud as you can.	26.3%	36.8%	26.3%	10.5%
Begin at your conversational level of speech, say /a/ and sing up a scale	36.8%	47.4%	10.5%	5.3%
Begin at your conversational level of speech, say /a/ and sing down a scale	42.1%	36.8%	10.5%	10.5%
Take a deep breath, then make the /a/ repeating the sound (a-a-a)	10.5%	57.9%	15.8%	15.8%
Diadochokinesis				
Open and close the mouth as many times as you can in 5 seconds	10.5%	79%	10.5%	0
Protrude and retract lips as many times as you can in 5 seconds	31.6%	63.1%	5.3%	0
Protrude and retract tongue as many times as you can in 5 seconds	82.4%	10.5%	5.3%	0
Raise and lower the tongue as many times as you can in 5 seconds	94.7%	5.3%	0	0
Move tongue from side to side as many times as you can in 5 seconds	89.4%	5.3%	0	5.3%

Table V. Percentage endoscopic of fiberoptic evaluation of swallowing findings.

Findings	N. cases	%
1. Laryngopharyngeal sensory deficit	11	58
2. Repeated dry swallows	10	53
3. Residue post-swallow	10	53
4. Mucous secretions in the pharynx and larynx	5	26
5. Facilitating manoeuvres	5	26
6. Dryness appearance of oropharyngeal mucosa	5	26
7. Deficit of oral bolus propulsion	4	21
8. Glottic incompetence	3	16
9. Delayed swallow initiation	2	11
10. Abnormal pharyngeal squeeze	2	11
11. Aspiration or penetration pre-, intra-, post-swallow	0	0

interesting to note that almost all our cases were edentulous and showed atrophic tongues.

Our results were not differentiated based on the stage of the disease. Nevertheless, the percentage of subjective swallow abnormalities resulted from the anamnestic list of symptoms was higher: dysphagia was present in 40-50% of cases and was mostly related to oral and oropharyngeal dysphagia. Among all symptoms, globus pharyngeal was reported in 58% of the sample versus 5-45% estimated in the general population, respectively, for persistent and intermittent globus pharyngeal [22,23]. We hypothesise that the prevalence increases in SS patients because of disease-related xerostomia, pharmacotherapy and other less well-understood processes involving immune-mediated mucosal changes and altered sensory perception. In our sample, the symptoms (i.e. "*feeling of food remaining in the mouth*" and "*feeling of food remaining in the lower throat*", "*difficulty to start swallowing*") are consistent with objective findings. First, the BSE showed post-swallowing oral residue, and, moreover, flexible endoscopic examination revealed dry swallows and post-swallow pharyngo-laryngeal residue in 53% of cases and deficit of oral bolus propulsion in 21%. Similarly, Montesi et al. [7] found abnormalities in the oral and pharyngeal phases during videofluoroscopy (VFS) in SS patients. Using VFS, Russo et al. [24] demonstrated the presence of intraswallowing laryngeal penetration caused by altered epiglottal motility in 57.8%, and pooling of contrast agent in the valleculae and/or pyriform sinuses in 51.1%. Nevertheless, it cannot be excluded that upper esophageal sphincter dysfunction is possible in SS or secondary to GERD which may cause this finding.

Gastro-oesophageal reflux (GERD) occurs in over 50% of SS patients, which causes symptoms mimicking swallowing disorder. In our sample, 37% of patients had a diagnosis of GERD and almost 70% complained of symptoms suggestive of GERD (raclage, sensation of food backing up into the throat or mouth). Thonhofer et al. [25] found a high prevalence of oesophageal disease in asymptomatic patients. However, further research specifically oriented to clarify the role of GERD on dysphagia in SS is necessary. Forty-two percent of cases reported onset of cough during swallowing and one-third of patients had had at least one episode of pneumonia by bacterial infection. This may be correlated to the multifactorial increased risk of aspiration in SS. First, we found "poor" or "fair" performances of respiratory, phonatory and diadochokinesis in about 80% of patients. Normally, eating, swallowing and breathing are tightly coordinated; the coordination of breathing and swallowing reveals a well-timed pattern between physiological respiratory events and related swallowing events, and vocal fold closure might be part of a protective mechanism that involves swallowing apnoea [26]. Secondly, it is known that the physiologic breathing cycle is not simply repressed during swallowing; it is substituted by a different and well-controlled behaviour pattern [27] that is sensitive to variations in bolus volume [28-30] and viscosity [31,32]. Moreover, direct stimulation of the laryngeal vestibule produces a reflex apnoea with abrupt vocal fold closure. In about 60% of SS patients, we demonstrated a decrease of laryngeal mechanosensitivity, probably resulting from GERD [33] that impairs the perception of bolus characteristics, which may consequently alter these mechanisms of control and increase the risk of aspiration. Thirdly, decreased pharyngeal muscular performance as demonstrated by the high percentage (53%) of dry swallows and pharyngo-laryngeal residue contribute to a further increase of the risk of post-swallow aspiration. Nevertheless, the absence of signs of aspiration or penetration was probably because the residual strength was sufficient to ensure good control of the bolus.

Although swallowing alterations are common in the immunomediated population [6], as seen herein, they are often overlooked by patients as well (general distress was clinically significant in 26.3% of cases with a mean "mild" impact) probably because of the predominance of other discomforts. Nevertheless, both early diagnosis and treatment of swallowing alterations is an issue that must be considered carefully. Towards this objective, it is important to evaluate the scores from questionnaires not in absolute terms, but in relation to the clinical background. Moreover, the risk in underestimating dysphagia may be reduced by associating physical and instrumental assessment to characterise the abnormalities in the oral and pharyngeal stages of swallowing [34].

In conclusion, this study is the first to highlight the importance of a multidimensional approach in swallowing evaluation in SS patients, which should include subjective and objective evaluation (the latter by a speech pathologist and phoniatric consultant). It also demonstrates specific features

of swallowing alterations to consider when addressing the high impact of the upper dysphagia in SS.

Several limitations should be considered when interpreting this investigation. In addition to the cross-sectional nature of the study, a control group is also lacking. In this regard, this work should be seen as preliminary, but can increase awareness in taking an otorhinolaryngologic approach to dysphagia within the complex framework in patients with SS. It can also encourage ENT specialists and rheumatologists to consider oropharyngeal dysphagia in the evaluation of patients suffering from SS.

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ACTA OTORHINOLARYNGOLOGICA ITALICA 2020;40:211-216; doi: 10.14639/0392-100X-N0382

Rhinology

Correction of alar rim retraction by lateral crural extension graft

Correzione della retrazione alare mediante il lateral crural extension graft

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SUMMARY

Alar rim retraction is a deformity of the alar conformation that can primarily occur in patients who have not undergone surgery or it can represent the outcome of a previous rhinoplasty surgery. Several surgical techniques for the treatment of alar retraction have been described. This study describes the lateral crural extension graft, a versatile and simple graft to correct alar retraction. Between 2015 and 2017, 47 patients who presented alar rim retraction underwent open septorhinoplasty surgery using the lateral crural extension graft. The retraction was assessed by using the classification systems by Kim for frontal view and Gunter for profile view. Postoperative photos with a minimum follow-up of 12 months were compared with preoperative photos by measuring in millimeters the improvement of alar rim retraction. The mean distance between the alar rim and the long axis of the nostril was reduced by 2.7 mm on average (range, 2.1 to 3.8 mm), showing an objective effectiveness of the procedure. In 7 cases, the correction was incomplete due to excessive cutaneous scarring retraction which caused partial recurrence of alar rim retraction. On the basis of a VAS rating scale, 32 (68%) of 47 patients said they were very satisfied with the outcome, 9 (19%) were satisfied and 6 (12%) were not very satisfied. The lateral crural extension graft is a simple and reliable method for correcting alar rim retraction. In cases of severe skin deficiency, it is not sufficient and a composite graft reconstruction must be used.

KEY WORDS: alar retraction, lateral crus retracted, extension graft, alar rim retraction

RIASSUNTO

La retrazione dell'ala nasale è un'alterazione della conformazione alare che può ritrovarsi sia in rinoplastiche primarie che in rinoplastiche di revisione. Sono state descritte diverse tecniche chirurgiche per la soluzione del problema. In questo studio descriviamo il "lateral crural extension graft", un innesto versatile e semplice da utilizzare. Tra il 2015 e il 2017, 47 pazienti presentanti retrazione dell'ala nasale sono stati sottoposti ad intervento chirurgico di settorinoplastica open con utilizzo del "lateral crural extension graft". La retrazione è stata determinata in base alle classificazioni di Kim per la visione frontale e di Gunter per la visione di profilo. Le foto post-operatorie con un follow-up minimo di 12 mesi sono state paragonate con quelle preoperatorie misurando in millimetri il miglioramento della retrazione alare. La distanza media tra l'alar rim e l'asse maggiore della narice è stata in media ridotta di 2,7 mm (range, 2,1 to 3,8 mm), indicando un'oggettiva efficacia della procedura. In 7 casi la correzione è stata incompleta per presenza di retrazione cicatriziale cutanea che ha causato una parziale recidiva della retrazione alare. Sulla base di una scala di valutazione VAS, 32 dei 47 pazienti (68%) erano molto soddisfatti del loro risultato, 9 (19%) erano soddisfatti e 6 (12%) poco soddisfatti. Il "lateral crural extension graft" è un metodo semplice ed affidabile per la correzione della retrazione dell'ala nasale. Nei casi in cui sia presente un deficit cutaneo importante, esso non è sufficiente e bisogna far ricorso al "composite graft".

PAROLE CHIAVE: *retrazione alare, retrazione crus laterale, retrazione rima alare*

Received: July 5, 2019
Accepted: October 27, 2019

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Funding
None.

Conflict of interest
The Authors declare no conflict of interest.

How to cite this article: Marianetti TM, Moretti A. Correction of alar rim retraction by lateral crural extension graft. Acta Otorhinolaryngol Ital 2020;40:211-216. https://doi.org/10.14639/0392-100X-N0382

Introduction

Alar retraction (AR) is a common clinical-surgical problem that is very difficult to resolve. It can be either primary or resulting from aggressive cephalic trim of the lower lateral cartilage during a previous rhinoplasty surgery.

In its primary forms, the alar rim is related to congenital cartilaginous and/or cutaneous deficiency or to the particular conformation and positioning of the lateral crura. More often, it is the outcome of previous rhinoplasty surgery with excessive removal of the alar cartilage and/or vestibular skin. The outcome of an aggressive surgery on the alar cartilage and adjoining skin, together with excessive resection, usually brings about weakening of the cartilage that ends up retracting upwards, causing exposure and alteration of the alar-columellar unit [1].

In addition to provoking severe aesthetic problems, alar retraction associated with overresection or malpositioning of the lateral crura may also be responsible for functional problems related to the collapse of the external nasal valve. Several classifications of alar rim retraction have been proposed, hence the altered relationship between the alar rim and the long axis of the nostril. The most well-known and universally accepted classifications are Gunter's for the lateral view [2], which defines discrepancies in the alar-columellar relationship, and Kim's for the frontal view [3]. In these classifications, the authors distinguish alar retraction in medial, central and lateral, depending on whether the retraction affects the most medial portion of the alar rim (alar notching), the midpoint or the alar base. The techniques which involve correction of alar retraction are even more numerous.

Alar contour graft or alar rim graft is a commonly used graft which allows correction of mild cases of alar retraction. When this deformity is caused by the weakness of the soft triangle, due to malpositioning of the lateral crura, it is appropriate to correct this conformation of the alar rim through caudal repositioning of the lateral crura associated or not to the lateral crural strut graft which depends on the integrity of the cartilage. The alar spreader graft and the upper lateral-alar interposition graft may be useful, but can often have the secondary effect of creating a bulk on the tip of the nose in the central or lateral portion respectively. In the most serious cases of alar retraction, the most appropriate solution is represented by a composite graft harvested from the cymba concha [4].

In this study, we describe, propose and analyze the function, method and aesthetic impact of the lateral crural extention graft (LCEG), which enables the caudal extension of the lateral crura of the alar cartilage to improve the morphology and stability of the alar rim.

Materials and methods

We have analysed pre-, post- and intraoperative photos of all patients who underwent a primary or revision septorhinoplasty surgery in the three-year period from 2015-2017. A total of 310 patients were treated. The mean follow-up period was 17 months (range, 12-23 months). Forty-seven patients whose preoperative photos presented an alar retraction greater than 2 mm in frontal or lateral projection were selected and the retraction was determined based on the classifications of Kim [3] for frontal view and Gunter [2] for profile view.

All patients underwent septorhinoplasty with the open technique and in all cases an alar extention graft was bilaterally grafted (Fig. 1).

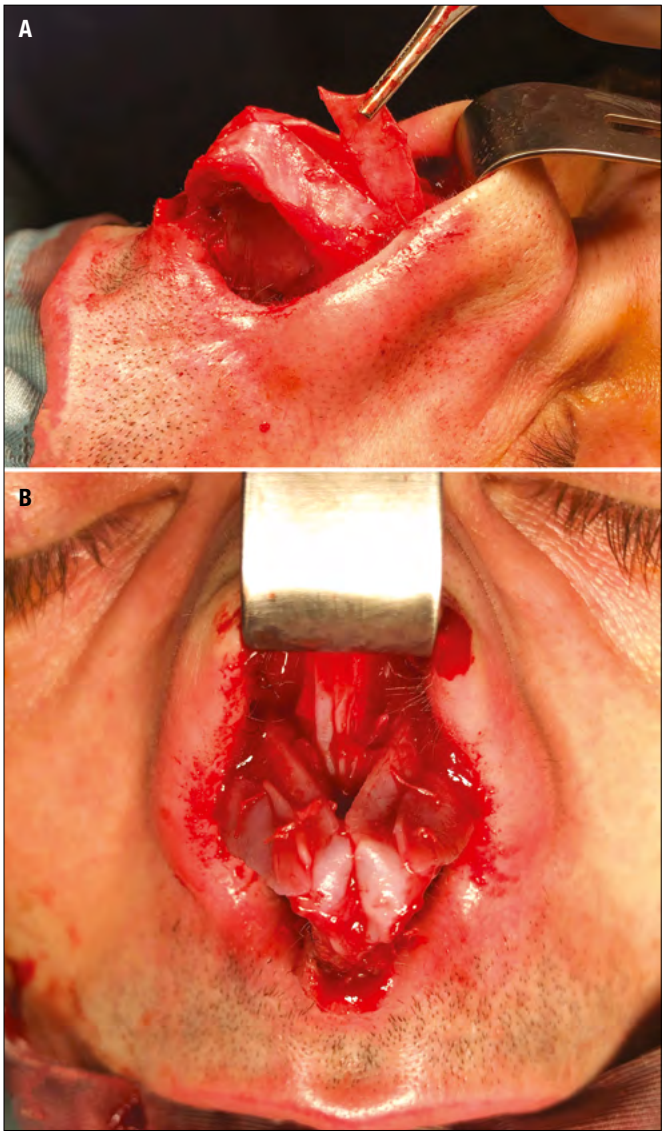

Figure 1. A) Intraoperative image showing the harvesting of the cephalic portion of the lateral crus used as grafting material for lateral crus extension graft. **B)** Intraoperative view of the bilateral lateral crural extension graft.

Photographs were taken in classic poses with a Canon EOS 60D digital single-lens reflex camera with a 105-mm macro lens. Postoperative photos with a minimum follow-up period of 12 months were compared with preoperative photos by measuring improvement of alar retraction in millimeters. Pre- and postoperative photographs were compared digitally by overlapping tragus and the external cantus in order to have a reliable comparison in measurements. Data on surgical technique, which were applied to individual cases and adapted with respect to the entity of the deformity to be corrected, were extracted from the worksheet on the surgical maneuvers performed and attached to medical records and intraoperative photos. Through a patient survey, based on a VAS scale (with scores from 0 to 100), patient satisfaction was evaluated and was categorised into three groups: very satisfied (VAS score range 90-100), satisfied (VAS score 50-89) and unsatisfied (VAS score less than 50).

Surgical technique

By using an open approach, an incision closer to the alar rim is made rather than making it along the caudal border of the alar cartilage. After exposing the cartilages of the tip, a sufficient amount of vestibular skin is dissected in the caudal portion, creating a large pocket inside the alar cutaneous margin. During this phase, particular care must be taken in the dissection of the area with maximal retraction since it must be well detached to allow subsequent extension by the graft.

In fact, before dissection the skin in this region, especially in revision rhinoplasty, is firm and tightly adherent and does not enable positioning of the extension graft. Next, depending on the lateral crura, the graft is made and can be harvested from the cephalic border of the alar cartilage, especially in primary cases or from the cymba concha in revision rhinoplasty. In particular, the cavum conchae, having a slight natural concavity, lends itself as an ideal donor site for reconstructing the ala.

The graft, which should be at least 6 mm wide, is then placed at the level of the caudal margin of the lateral crus with a 2 mm overlap to allow stabilisation of the same through mattress sutures in PDS 6.0. The overlapping and mattress sutures ensure greater graft stability together with a greater ability to stretch the retracted ala compared to edge-to-edge sutures. Finally, the graft is inserted into the mucocutaneous pocket created in the alar margin and the vestibular skin is sutured with rapid 5-0 Vicryl, which should not be too tight.

Results

Thirty-three patients were women and 14 men with a mean age of 32 years (range, 21-49 years), all Caucasians, were assessed. In 13 cases, there was congenital alar retraction, while the remaining 34 patients had undergone previous rhinoplasty surgery. According to Kim's frontal classification system, 28 of 47 patients affected by alar retraction fell into Type 2 (central retraction), 13 into Type 3 (lateral retraction) and 6 into Type 1 (medial retraction). Taking into account Gunter's classification system for profile view, the distance between the alar rim and the long axis of the nostril, which was measured by the lateral projection photos, ranged from 2.8 to 5.9 mm, with an average value of 3.4 mm.

All patients underwent septorhinoplasty with the open technique and in all cases an alar extention graft was bilaterally grafted. Thanks to the use of the LCEG the distance between the alar rim and the long axis of the nostril was reduced on average by 2.7 mm (range, 2.1 to 3.8 mm), indicating an objective efficacy of the procedure (Figs. 2-5). No complications were observed except for one complaint of a small palpable cartilaginous step-off on the alar margin. In 7 cases,

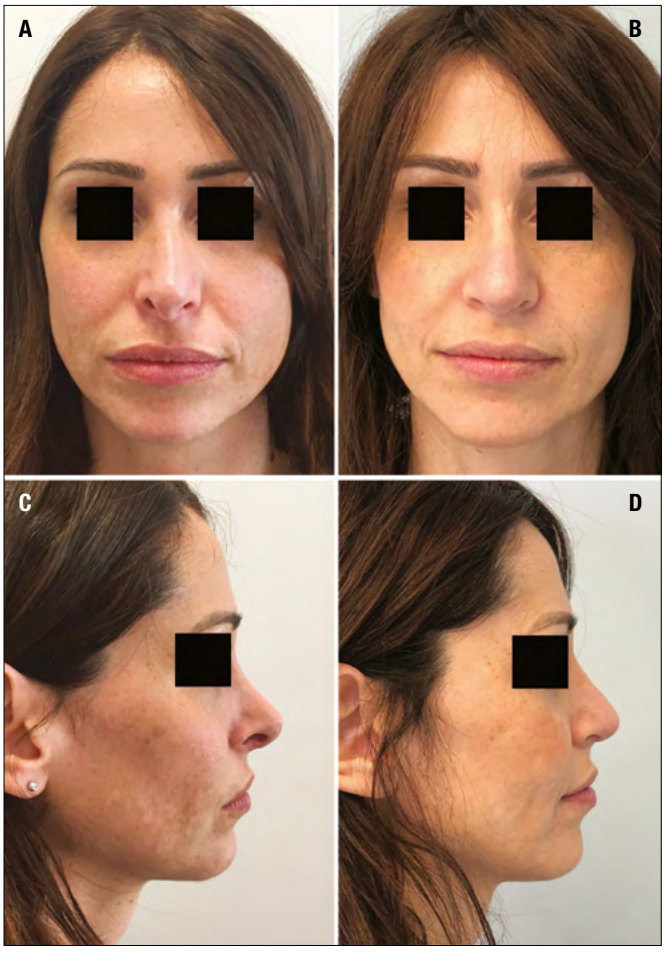

Figure 2. CASE 1:Patient affected by secondary alar retraction corrected by bilateral lateral crura extension graft. **A)** Preoperative frontal view. **B)** Post-operative frontal view. **C)** Preoperative right profile. **D)** Post-operative right profile.

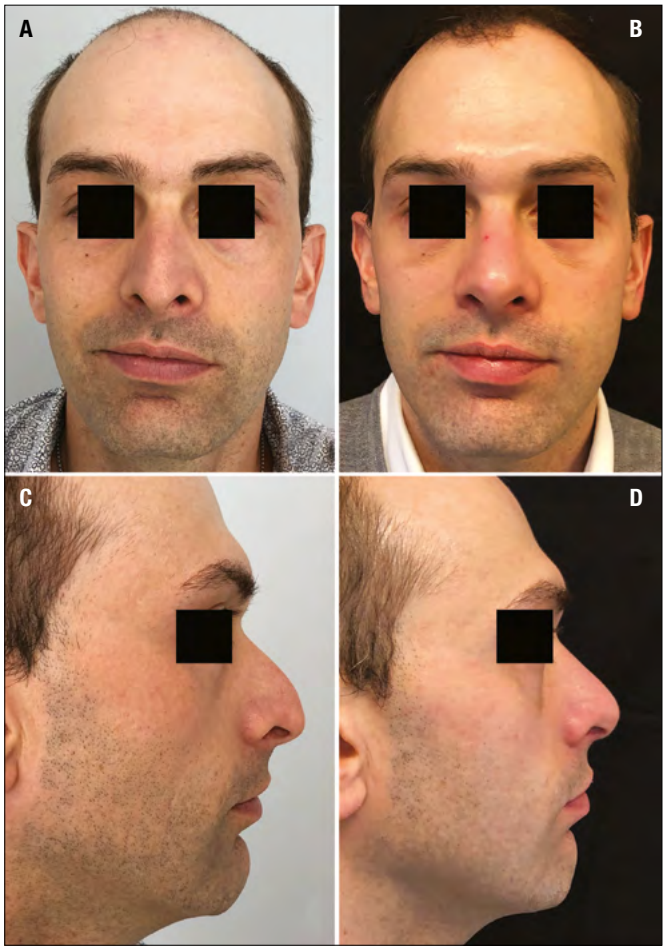

Figure 3. CASE 2: Patient affected by primary alar retraction corrected by bilateral lateral crural extension graft. **A)** Preoperative frontal view. **B)** Post-operative frontal view. **C)** Preoperative right profile. **D)** Post-operative right profile.

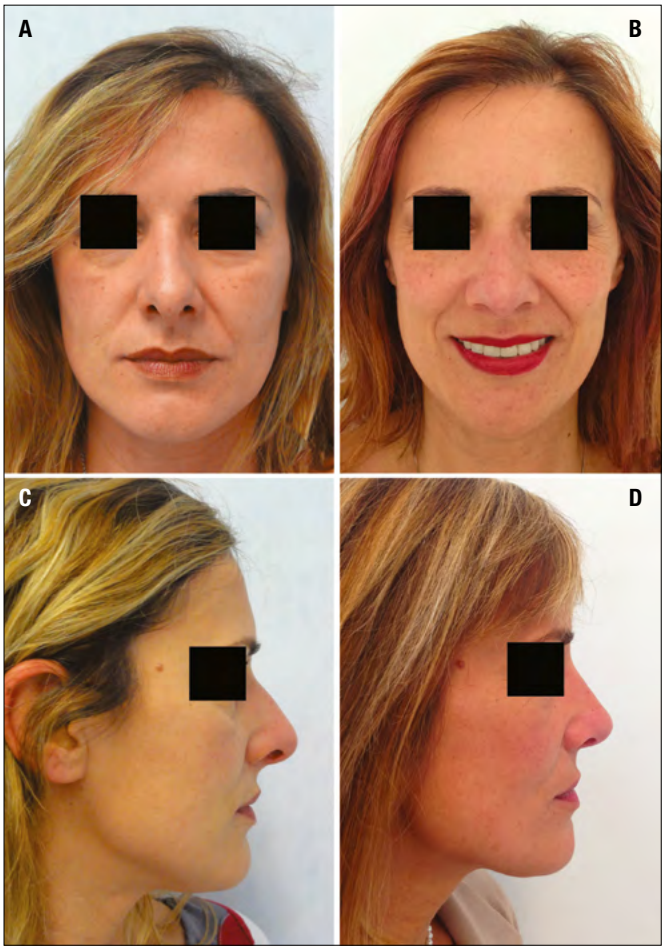

Figure 4. CASE 3: Patient affected by primary alar retraction corrected by bilateral lateral crural extension graft. **A)** Preoperative frontal view. **B)** Post-operative frontal view. **C)** Preoperative right profile. **D)** Post-operative right profile.

the correction was incomplete due to the presence of cutaneous scars which caused a partial recurrence of the alar retraction. On the basis of a VAS rating scale, 32 (68%) of 47 patients said they were very satisfied with the outcome, 9 (19%) were satisfied and 6 (12%) were not very satisfied.

Discussion

For the rhinoplasty surgeon, the alar retraction, both unilateral and bilateral, represents a challenge that is difficult to manage, especially when it is the outcome of excessive and aggressive surgical surgery at the level of the lower lateral cartilage structures and adjacent soft tissues. Treatment is simpler in primary cases or when there is no evidence of a very strong deformity. In the diagnostic phase, it is appropriate to have an evaluation in the lateral view following Gunter [2], but this information should also be coupled with evaluation in the frontal view [3].

In our experience, we observed that in iatrogenic cases the deformity is basically correlated to a pre-existing alar retraction at the first surgery that remained unnoticed because it was minor. This is especially true if associated with malpositioning of the lateral crura that wasn't corrected by surgery, and which is usually the stigma of overly aggressive treatments with abundant resection and interruption of the alar cartilage. Other elements that can contribute to alar retraction are the integrity of cartilage and the manipulation of alar soft tissues, especially when the skin is thin.

As part of possible correction treatments for alar retraction, it is possible to state that the surgical strategy to be adopted is closely linked to the entity of the deficiency to be corrected. For example, mild and medium-sized alterations can be resolved with an alar rim graft. This is a graft that can be easily inserted at the level of the alar contour, with both closed and open approaches. The technique includes positioning of a strip of cartilage, which is usually a septal one,

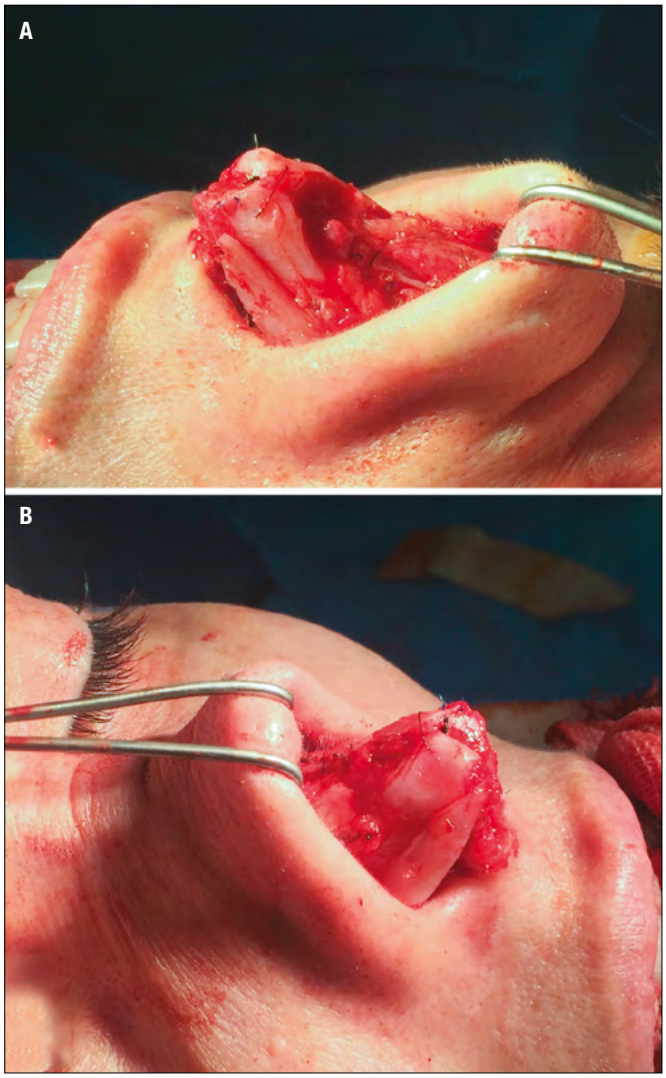

Figure 5. Intraoperative view of the patient showed in Fig. 2. **A)** Left lateral crus extension graft. **B)** Right lateral crus extension graft.

into a pocket harvested from the alar rim where there is no cartilage tissue. The success of this procedure depends on soft tissues and degree of extension of the alar rim [5].

In cases where the defect appears to be more complex and considerably evident, retraction and pinching are related to excessive rotation of the tip, which results from inaccurate resection and excessive medialisation of the alar cartilage.

This condition can be effectively solved using an alar spreader graft, but the graft must include complete detachment with release of tissues at the level of the scroll area in order to be able to slide down the positioning of the lateral crura. This graft, which is placed and fixed between the septum-upper lateral cartilage junction in the most caudal portion and the cephalic border of the alar rim, must always provide for wide detachment of the tip's support structures, especially between the lateral cartilages, so as to obtain a more caudal positioning of the alar rim.

Sutures must be well positioned to prevent extension of the nose and a hanging columella due to excessive sliding downwards resulting from detachment and tissue release. This is useful when correcting moderate and severe alar retraction with a rotated and pinched tip, but a visible bulbous evidence on the supratip area may remain.

The lateral crural strut graft proposed by Gunter in 1997 [6] represents a possible resolution to alar retraction associated with malpositioning because it allows to place the entire base of the nostril and the alar rim in a more caudal position. The lateral crural strut graft is inserted below the alar margin and the lateral crus, which is totally detached up to the level of the piriform aperture. In case of isolated and localised alar retraction, this procedure is not recommended due to prolonged oedema and possible and unpredictable reconstructive outcomes on the alar rim.

As an alternative to the lateral crural strut graft, it is possible to use an intercartilaginous graft between the lower margin of the upper lateral cartilage and the upper margin of the lateral crura of the alar cartilage [7]. This procedure involves repositioning of the lateral crus downwards by inserting a spacer coupling after extensive detachment. The intercartilaginous graft can be more easily inserted into iatrogenic ARs since the upper and lower lateral cartilages usually show separation as a result of the modeling procedures that allows placement and stabilisation of the graft.

In primary alar retractions, on the contrary, the accommodation for the graft must be created by separating the two cartilages. The intercartilaginous graft enables the correction of significant alar retraction of medium-severe degree, but it is not possible to place it in cases of absence or excessive resection of the alar cartilage or when there is a simultaneous retraction of soft tissues with insufficient skin.

In these cases, it is essential to use a composite chondrocutaneous graft [8]. This is usually harvested from the auricular basin and allows correction of complex alar retraction in which loss of substance due to excessive scar retraction has occurred. The grafted cartilaginous component, which is usually smaller than the cutaneous one, must provide complete coverage even by the recipient tissues to avoid excessive phenomena of reabsorption. Another critical point in the use of this graft involves the symmetry of the nasal orifices, especially when there is bilateral reconstruction. This drawback is closely linked to the unpredictability of post-surgical healing and tissue repair processes.

According to our experience, the LCEG offers a valid alternative for cases in which it is necessary to provide the cartilage support necessary for the retracted ala. It is a simple and enduring technique, and can often replace the caudal

repositioning procedure of the lateral crura in case of their malpositioning [9]. In fact, in these cases the same cartilage which has been removed from the cephalic portion of the crura can be placed caudally to obtain a different orientation of the lateral crura at the end of the procedure. However, the LCEG offers greater guarantees of symmetry and is easier to achieve at a technical level compared to repositioning of the crura. Moreover, it is a graft that allows obtaining a more solid and predictable cartilage support than the alar rim graft.

The cartilage necessary for the graft to stretch the lateral crura can also be obtained from the auricular concha [4,10], especially in secondary cases where it is not possible to harvest it from the nose due to the small amount of cartilage available or in order not to weaken the pyramid structure. However, in cases where alar retraction is provoked by a cutaneous deficiency as well as a cartilaginous one, a composite graft is the only treatment option.

Conclusions

Alar retraction can be corrected with different surgical methods. The more caudal alar portion has thicker skin and is not supported by cartilage. To obtain caudal extension of the vestibular skin of the margin and the alar skin, it is also necessary to take into account the relationship between the tip and the width of the alar base; frontal classification of alar retraction is very useful when choosing the surgical method.

The outcomes of this study objectively confirm that excessive surgical manipulation of the lateral crura can lead to alar retraction and that the LCEG has a measurable improvement effect on this retraction. This graft can also be used in primary cases of alar retraction or for prophylactic purposes to prevent it by using the cephalic trim of the lateral crura.

In most cases, correction of the retraction with an alar graft or an LCEG is very effective, although when the retraction is more lateral it should be coupled with reduction of the alar base. On the other hand, when the retraction is also related to a deficiency of the vestibular and alar soft tissues, it is appropriate to use a composite graft.

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ACTA OTORHINOLARYNGOLOGICA ITALICA 2020;40:217-223; doi: 10.14639/0392-100X-N0357

Rhinology

Effects of liposomal nasal spray with vitamins A and E on allergic rhinitis

Effetti dell'applicazione di spray liposomiale con vitamine A ed E nelle riniti allergiche

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SUMMARY

The aim of this study was to investigate the relationship between nasal obstruction and nasal cytology in patients with allergic rhinitis (AR) treated with a liposomal based nasal spray containing vitamins A and E. This is a prospective double-blind, controlled study. A total of 106 patients with AR, who rejected anti-allergic therapy, were randomly divided into two groups: G (study group, n = 53) received liposomal nasal spray and C (control group, n = 53) received 0.9% sodium chloride solution nasal spray. Both nasal sprays were applied two times a day, in the morning and at night, in both nasal cavities. The study lasted for 30 days. The first ENT evaluation was performed the first day (T0) and the second evaluation was performed at the end of the study (T1). Symptoms (SNOT-22 test with VAS) and signs (nasal cytology) of both groups were recorded at T0 and T1. Liposomal nasal spray was effective in improving both nasal symptoms and cytology in patients suffering from perennial AR. Treatment with liposomal nasal spray with vitamins A and E was followed by a significant improvement of VAS scale ($p < 0.0001$), a significant decrease in SNOT-22 ($p < 0.0001$) and a significant decrease in inflammatory cell count ($p < 0.0001$). In conclusion, our study provides evidence that liposomal nasal spray improves the nasal symptoms of AR. The patients were compliant to this therapy because of limited side effects. The reduction in inflammatory cells count was remarkable and confirmed the close association between eosinophil infiltration and nasal airflow impairment. These results may have implications for clinical practice.

KEY WORDS: allergic rhinitis, liposomal nasal spray, vitamins A and E, nasal cytology, VAS, SNOT-22

RIASSUNTO

Scopo del nostro studio è stato indagare la relazione tra ostruzione nasale e citologia nasale in pazienti con rinite allergica trattati con spray nasale liposomiale con vitamine A ed E. Lo studio è prospettico, controllato in doppio cieco. I 106 pazienti affetti da rinite allergica, che hanno rifiutato la terapia anti-allergica, ammessi nello studio sono stati assegnati a uno dei due gruppi: G (gruppo di studio, n = 53) che ha assunto uno spray nasale liposomiale con vitamine A ed E e C (gruppo controllo, n = 53) che ha assunto uno spray di soluzione salina (NaCl) 0,9%. Entrambi gli spray nasali sono stati assunti mattina e sera per 30 giorni. La prima visita è stata effettuata al tempo zero (T0) e la seconda visita al termine dello studio (T1). In entrambe le visite sono stati valutati i segni (citologia nasale) e i sintomi (SNOT-22 e VAS) della rinite allergica. Lo spray nasale liposomiale con vitamine A ed E è risultato efficace nel migliorare la sintomatologia nasale e nel ridurre la conta cellulare in pazienti affetti da rinite allergica. Nel nostro studio abbiamo evidenziato un miglioramento significativo della scala VAS ($p < 0,0001$), un'importante riduzione del punteggio del test SNOT-22 ($p < 0,0001$) e una rilevante riduzione della conta delle cellule infiammatorie della mucosa nasale, soprattutto neutrofili ed eosinofili ($p < 0,0001$). Alla luce dei risultati rilevati è possibile affermare che lo spray nasale liposomiale si è dimostrato in grado di migliorare i sintomi della rinite allergica. I pazienti hanno manifestato un'ottima compliance al trattamento proposto per la percezione di un favorevole rapporto tra efficacia e scarsi effetti collaterali. La riduzione della conta cellulare infiammatoria è risultata rilevante, confermando la stretta associazione tra infiltrazione eosinofila e ostruzione nasale. Questi risultati potrebbero avere implicazioni per la pratica clinica.

PAROLE CHIAVE: *rinite allergica, spray nasale liposomiale, vitamina A ed E, citologia nasale, VAS, SNOT-22*

Received: June 9, 2019
Accepted: October 27, 2019

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Funding
None.

Conflict of interest
The Authors declare no conflict of interest.

How to cite this article: Lauriello M, di Marco GP, Necozione S, et al. Effects of liposomal nasal spray with vitamins A and E on allergic rhinitis. Acta Otorhinolaryngol Ital 2020;40:217-223. https://doi.org/10.14639/0392-100X-N0357

Introduction

Allergic rhinitis (AR) is a serious public health problem worldwide and one of the most common chronic diseases that affects the daily life of patients with severe symptoms and major disabilities. It is a common disorder that affects up to 40% of the population of all ages [1].

Its high and still increasing prevalence, socio-economic burden, frequent association with asthma and significant impairment of quality of life (QoL) make it a disease of substantial importance [2]. Treatment options for allergic rhinitis include allergen avoidance, pharmacological therapy and immunotherapy. Pharmacological therapies include intranasal corticosteroids, anti-leukotrienes and antihistamines: the latter is the most common medication, but is burdened by side effects which often lead to a rejection of the therapy [3].

The aim of this study was to investigate the relationship between nasal symptoms, mostly nasal obstruction, and nasal cytology in patients with AR treated with a liposomal based nasal spray containing vitamins A and E. These patients had rejected any other anti-allergic therapy.

The rationale for the use of a liposomal nasal spray is that liposomes support the cleansing, lubrification and hydration of nasal mucosa. Moreover, the rationale for the use of vitamins A and E is based on the role of vitamin A in the immune response and the role of vitamin E as an antioxidant.

Materials and methods

Patients

106 patients (50 males and 56 females, 18 years old or older) with symptoms of allergic rhinitis and positive RAST (radioallergosorbent test) and skin prick test to common perennial allergens: *Felis domesticus*, *Canis familiarus*, *Dermatophagoides spp.*, *Alternarian alternate*, *Aspergillus fumigatus* were studied. These patients had rejected anti-allergic therapy for several reasons, mainly side effects such as drowsiness, dizziness and dry mouth.

Exclusion criteria were the presence of airway infection, sinusitis, tumour of sinuses, adenoidal hypertrophy, previous nasal surgery, nasal fracture in the previous three months, untreated asthma, sarcoidosis, Wegener's granulomatosis, previous head and neck irradiation, smokers and subjects younger than 18 years of age. The ethics committee of San Salvatore Hospital approved the study and all patients enrolled gave their written informed consent (ID of the protocol: 29/2017.18).

Study design

This was a prospective, double-blind, randomised, controlled study carried out at the Department of Otorhinolaryngology of San Salvatore Hospital in L'Aquila, Italy, between February 2017 and August 2018. The study lasted for 30 days.

Patients were randomly divided into two groups: G (study group, n = 53) and C (control group, n = 53). Group G received a commercially available liposomal [1.000% p/p (100 g)] vitamin A [0,058% p/p (100 g)] and vitamin E [0.018 % p/p (100 g)] nasal spray. Other components of the nasal spray are hydrogenated phospholipids, monobasic and bibasic phosphate sodium, N-hydroxymethylglycinate, EDTA (ethylenediaminetetraacetic acid), sodium chloride and purified water. The control group received 0.9% sodium chloride nasal spray. Both nasal sprays were applied two times a day, in the morning and at night, in both nasal cavities, for 30 days.

A detailed clinical history and a complete ENT physical examination, including nasal endoscopy, was obtained for each patient at first evaluation (T0), before using the nasal spray, and at the second visit (T1) performed at the end of the study, after 30 days of nasal spray usage. Symptoms in both groups were evaluated at T0 and T1 by Sinonasal Outcome Test-22 (SNOT-22) and by a visual analogue scale (VAS); nasal inflammation was evaluated by nasal cytology.

All subjects had to answer an examiner-guided questionnaire, the Italian-SNOT-22 [4], about their clinical history and nasal symptoms which have a profound influence on the rhino-QoL in people suffering from persistent allergic rhinitis that is closely related to chronic rhinosinusitis [5]. It is a simple and fast questionnaire structurally composed of 22 rhinitis-related items that evaluate the severity of complaints at first visit. All items are scored from 0 (= no problem) to 5 (= problem as bad as it can be). The sum of each item results in a maximum score of 110. High score indicates a poor outcome. The items composing the SNOT-22 can be divided into two categories: 12 questions about physical symptoms which cover rhinologic symptoms as well as ear and facial symptoms, and 10 questions about health and QoL which cover sleep function and psychological issues [6].

The VAS scale is an instrument widely used in the rhinology: it is a simple and quantitative method that can be used for quantitative evaluation of severity of allergic rhinitis, according to ARIA [7]. It represents a 10 cm horizontal scale from 0 to 10: patients are instructed to indicate the point on the line that best corresponds to their status about nasal obstruction. (0 = no nasal obstruction, 10 = complete nasal obstruction) [8].

Cytology was performed by analysing nasal scrapings obtained from the mucosal surface of the inferior turbinate and samples were stained using haematoxylin and eosin. By using a microscope, inflammatory cell count was done,

including eosinophils, neutrophils and mast cells [9]. The cytology score was obtained using the high-power field method.

Statistical analysis

The statistical software package used to analyse data was *SAS (*Statistical Analysis System). The discrete variables (sex, recent or chronic obstruction, smoking, previous surgery, snoring, sinusitis, polyps, injuries, allergy, nasal septum deviation, turbinate hypertrophy) were analysed with chi-Square or Fisher's exact tests as appropriate.

The normality of continuous variables (age, cytology, VAS, SNOT-22 test) were analysed with the Shapiro Wilk-Test. In consideration of non-normality of distributions, Wilcoxon rank-sum test was used or the variables were transformed logarithmically as appropriate.

Analysis of variance for repeated measures was used to test differences in the variations in the time of the variables between the two groups. The presence of a significant interaction time*groups demonstrates a difference between groups. Spearman nonparametric correlation was used to evaluate correlations between differences in the variations in the time of the variables. Finally, the relation between two variables (cytology and VAS, SNOT-22 test and VAS) was presented in a scatter diagram with a regression line.

Results

Regarding demographic data, no significant differences between groups in the baseline data of age or gender distribution were noted (Tab. I).

The continuous variables (age, cytology, VAS, SNOT-22 test) were identified by mean, standard deviation and p-value for each group at T0 and T1 (Tab. II).

VAS

At T0, the VAS score was almost the same in the two groups (6.57 ± 1.68 for G and 6.47 ± 1.88 for C; p = 0.785). At T1, subjects in group G had less severe nasal obstruction, while the comparison between T0 and T1 was not significant in group C (3.75 ± 1.41 for G and 6.51 ± 2.00 for C; p < 0.0001) (Fig. 1A). Allergic rhinitis can be divided into three groups according to the VAS score: mild (0-3), moderate (3.1-7) and severe (7.1-10). Before treatment, both groups had a moderate VAS score. After treatment, G became a class among mild and moderate instead C remained into the same class (moderate). The Group x Factor interaction detected a significant difference of the variations in the VAS score at T0 and T1 between groups (p < 0.001).

Cytology

At T0, significant differences in cytology values were seen between groups, e.g. G had a higher cell count, including eosinophils, mast cells and, above all, neutrophils, than C (10.21 ± 7.02 for G and 8.11 ± 1.69 for C; p < 0.001) (Fig. 2). At T1, subjects in G had a significant decrease in cell count until values lower than C; otherwise, no significant differences between the two measurements could be found in C (5.57 ± 2.22 for G and 8.70 ± 3.45 for C; p < 0.0001) (Fig. 1B).

The Group x Factor interaction detected a significant difference of the variations at T0 and T1 between groups (p < 0.001). A significant difference (p < 0.0001) in cytology values at T0 and T1 was found in G: only 4 subjects had a higher cytology score after treatment, while 47 patients decreased their cytology score and 2 had the same score; the large sample test statistic Z was 5.6147. No significant differences (p = 0.1894) in cytology at T0 and T1 were

Table I. Demographic data.

Demographic data	Study group (G) n = 53	Control group (C) n = 53	P-value
Male/female, n (%)	24/29 (22.64/27.36)	26/27 (24.53/25.47)	0.697 [a]
Age, mean (SD)	39.66 ± 15.20	42.21 ± 12.35	0.238 [b]

[a] *Chi-Square test;* [b] *Wilcoxon rank sum test.*

Table II. Continuous variables.

Variable		N	Study group (G) mean (± SD)	Control group (C) mean (± SD)	P*
Cyto	T0	53	8.11 ± 1.69	10.21 ± 7.02	< 0.001
	T1	53	8.70 ± 3.45	5.57 ± 2.22	
VAS	T0	53	6.47 ± 1.88	6.57 ± 1.68	< 0.001
	T1	53	6.51 ± 2.00	3.75 ± 1.41	
SNOT	T0	53	44.08 ± 17.49	49.70 ± 12.88	< 0.001
	T1	53	43.81 ± 17.16	34.32 ± 12.79	

**: ANOVA for repeated measures time groups interactions.*

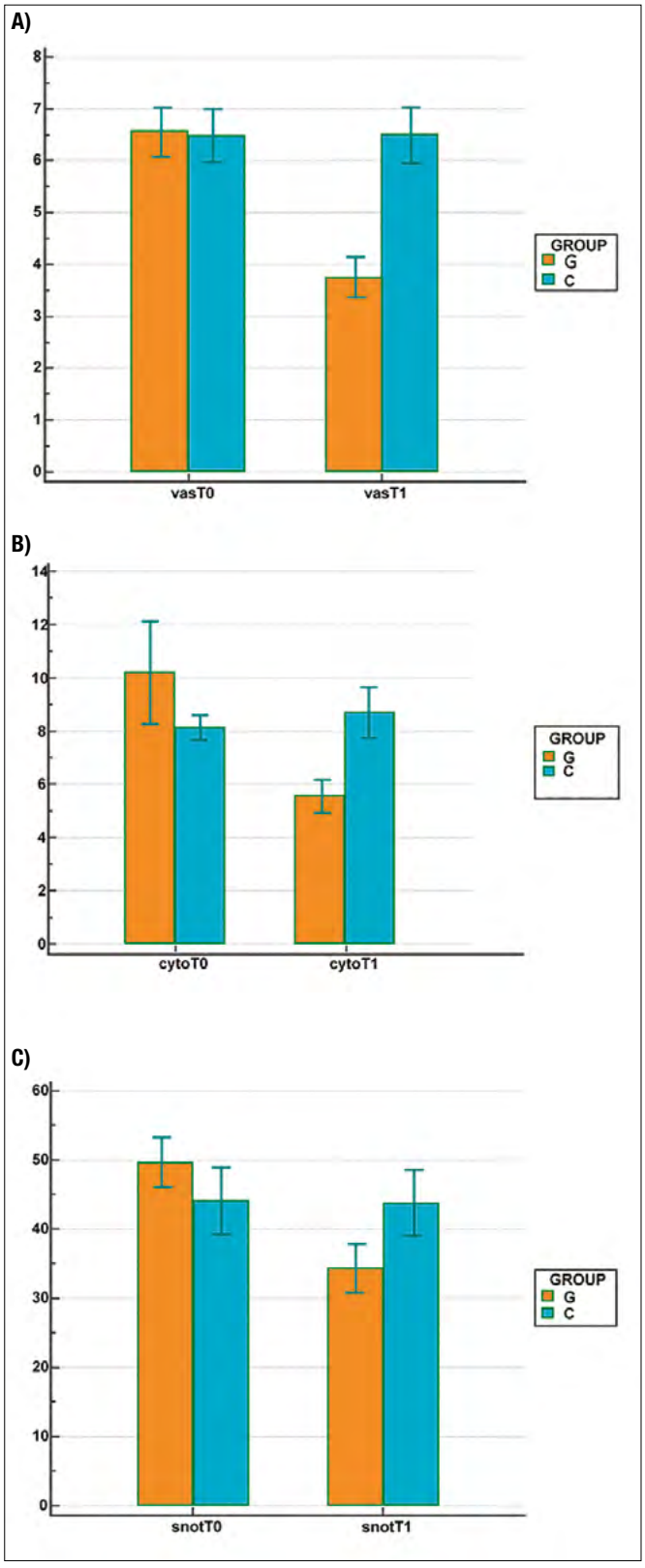

Figure 1. (**A**) VAS T0/T1 in the treated group (G) vs no treatment (C); (**B**) Cyto T0/T1 in treated group (G) vs no treatment (C); (**C**) SNOT T0/T1 in the treated group (G) vs no treatment (C).

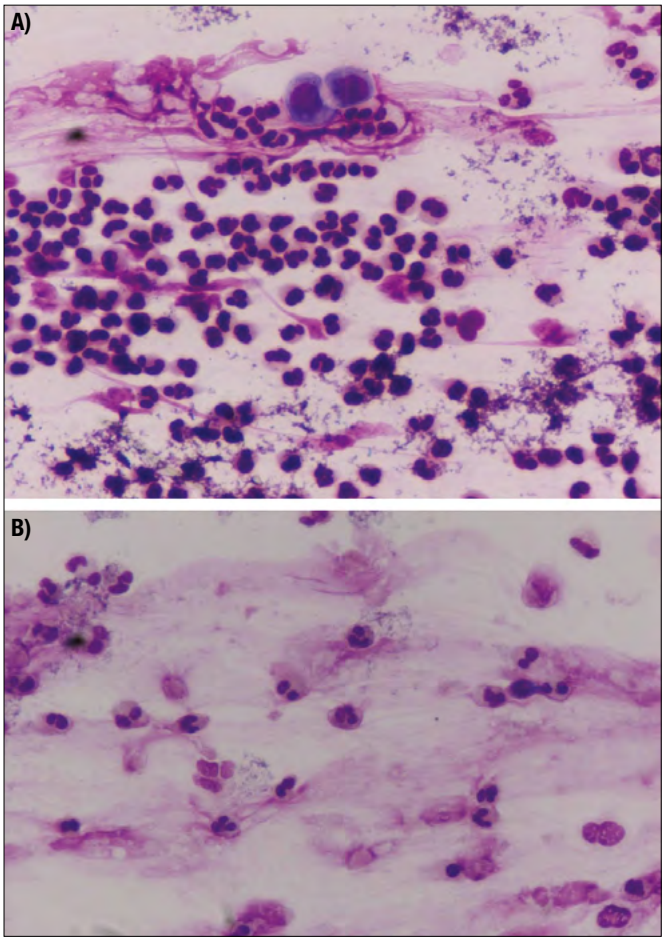

Figure 2. Neutrophils in the nasal scraping in the treated group before (**A**); and after (**B**) treatment.

found in C: 22 patients had a higher cytology score, 16 lower and 15 had the same score.

SNOT-22 test

At T0, SNOT scores were similar in the two groups, but a trend towards a higher score was seen in G (49.70 ± 12.88 for G and 44.08 ± 17.49 for C; p = 0.06). At T1 a significant difference between the two groups was detected (34.31 ± 12.79 for G and 43.81 ± 17.16 for C; p = 0.0017) (Fig. 1C).

The Group x Factor interaction detected a significant difference of the variations at T0 and T1 between groups ($p < 0.001$). A significant decrease ($p < 0.0001$) in SNOT score at T0 and T1 was found in G. Only 1 subject had a higher SNOT score after treatment compared to 52 patients with a lower score; the large sample test statistic Z was 6.3164. No significant differences (p = 0.9881) in SNOT score at T0 and T1 were detected in C: in that group, 24 patients had a higher SNOT score, 25 patients lower and 4 had the same score.

In our study, we obtained three new results from values T0 - values T1 for each variable: Δvas = 1.38679 ± 2.11377, Δcyto = 2.02830 ± 5.92727, Δsnot = 7.82075 ± 9.42115. Spearman's index improves the correlation model: the results were the same as Pearson's coefficient, and in fact there was a moderate correlation between Δcytology and Δvas ($r = 0.488$; $p < 0.0001$), between Δcytology and Δsnot ($r = 0.570$; $p < 0.0001$) and, finally, between Δvas and Δsnot ($r = 0.656$; $p < 0.0001$).

The relations between the variables (cytology, VAS and SNOT) were presented in scatter plots with a regression line. (Figs. 3-5).

Discussion

Our study was designed to evaluate a liposomal based nasal spray containing vitamins A and E, at the doses with the greatest potential for clinical use, in a large group of adult patients with allergic rhinitis who had rejected anti-allergic therapy for several reasons, mostly side effects such as drowsiness, dizziness and dry mouth.

Usually, pharmacological therapy of allergic rhinitis must take into account the severity and duration of symptoms, efficacy, availability, cost of the drugs and patient choices. The World Health Organization's guidelines on allergic rhinitis and its impact on asthma (ARIA) provide a progressive algorithm for treatment, recommending systemic or topical antihistamines or glucocorticoids with chromones as an alternative and then, if necessary, leukotriene antagonists and decongestants [3]. Unfortunately, both classic and new-generation antihistamines significantly increase daytime sleepiness and seem to have a negative influence on mood states [10].

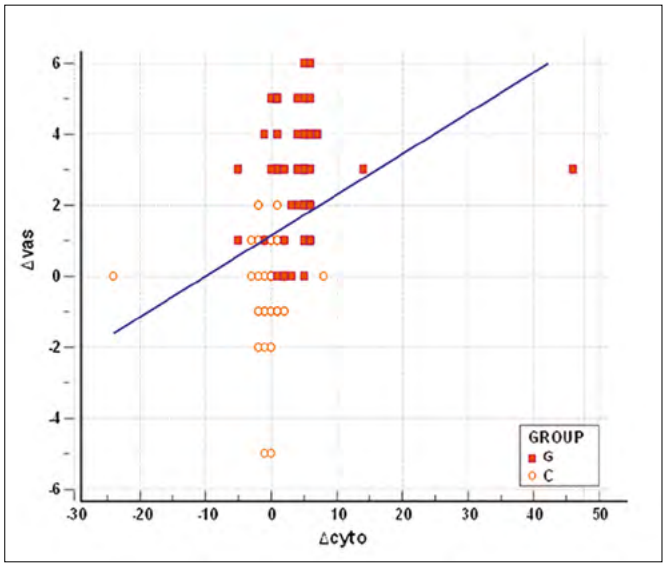

Figure 3. Regression between variations in cytology and variations in VAS.

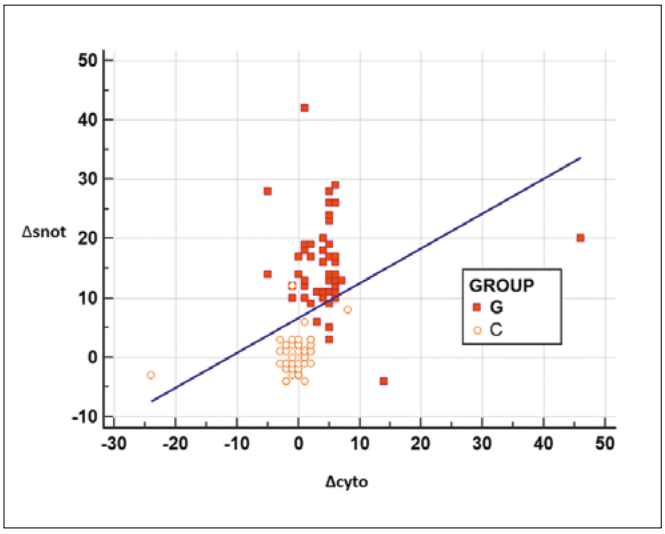

Figure 4. Regression between variations in cytology and SNOT.

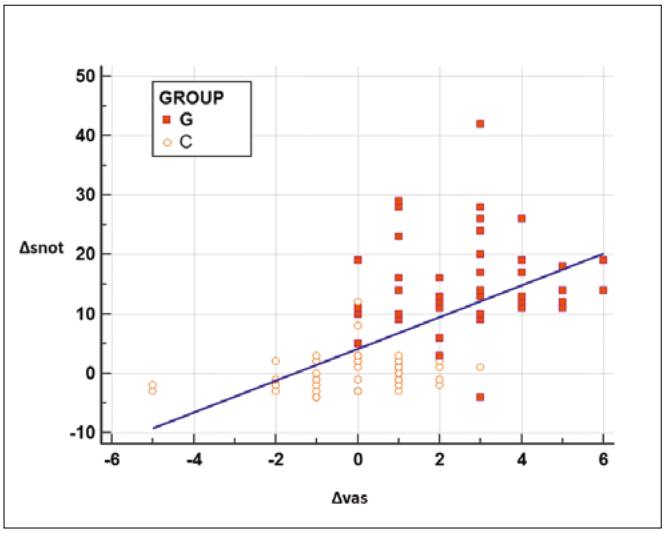

Figure 5. Regression between variations in VAS and SNOT.

In the last few years, topical nasal corticosteroids have been established as first-line treatment for allergic rhinitis. However, nasal corticosteroids can produce hypothalamic-pituitary-adrenal axis suppression and other adverse effects. Fluticasone causes a reduction in endogenous cortisol secretion and there is little evidence that skeletal growth is restricted by the administration of topical nasal steroid sprays [11]. A high-dose and long-term nasal steroid administration may cause iatrogenic Cushing's syndrome that is characterised by complications of glucocorticoid excess, as well as serious and even life-threatening complications of adrenal insufficiency [12]. Gross et al. demonstrated the efficacy and safety of a nasal spray

composed by the association antihistamine-corticosteroid, which minimises the undesirable effects leaving the effectiveness of the active ingredients unaltered [13].

A recent study analysed the montelukast effectiveness in improving oculonasal symptoms, patient-reported outcomes and eosinophilic biomarkers in responder patients: a significant reduction of eosinophils in nasal mucosa were observed after treatment [14]. Another study revealed significant vasodilation of human nasal mucosa after use of montelukast nasal spray in patients with allergic rhinitis, probably because of its α-adrenoceptor antagonism [15].

Chronic use of nasal decongestants is not recommended because their overuse can lead to rhinitis medicamentosa [16].

In our trial, we used a nasal spray composed of a solution of vitamin A, vitamin E and liposomes.

We used a liposomal nasal spray because liposomes, when applied in rhinitis, support the cleansing, lubrification and hydration of nasal mucosa. Liposomes consist of phospholipids that make up 75% of the protective nasal surfactant layer, so they are useful in treating mucosal barrier disorders that play an important role in the pathogenesis of allergic disease [17]. Liposomes are small sphere-shaped vesicles that can be created from cholesterol and natural phospholipids. An explanation for the mechanism of action of liposomes assumes that they stabilise the nasal mucosal barrier and treat its dysfunction by integrating in the damaged cell membrane protecting upper airways against pathogens [18] thus stabilising respiratory barriers and strengthening their anti-inflammatory and wound-healing capacities [19]. Andersson et al. [20] assumed that liposomes absorb and thus inactivate allergens. Liposomes are able to entrap proteins in the aqueous interior and have been widely used as drug delivery carriers due to their high biocompatibility [21]. Aliu et al. investigated the possibility of developing liposomes as a new allergen delivery system [22] encapsulating allergens: it offers the ability to protect the allergen from degradation, potentially aids transport within tissues, and in turn targets APCs [23]. Several studies [24-26] demonstrated that the symptoms of allergic rhinitis are effectively reduced by nasal application of liposomes, which have been available in the German pharmaceutical market since 2007, on inflamed nasal mucosa [25]. In the open, monocentric, prospective study by Weston et al. [26], a liposomal nasal spray led to a significant reduction of AR symptoms of allergic rhinitis and improvement of QoL, comparable to the effect of a standard treatment with an antihistamine/glucocorticosteroid spray. The prescription-free liposomal based spray, as shown by Bohm et al. [27] has an appreciable potential to reduce allergic rhinitis symptoms comparable to the established cromoglycate combination therapy, which is known to be evidence-based, albeit weakly effective allergy medication.

The nasal spray used in our study is also composed of vitamins A and E because of their important role in nasal mucosa: they are widely used in clinical practice for prevention and treatment of several medical conditions, especially in allergic symptoms.

Vitamin A has important effects on the immune response: low vitamin A (retinoic acid) levels are associated with less IFN-γ and tendency for more viral detection, which may explain the association with vitamin A deficiency and rhinitis exacerbations. Indeed, IFN-γ is a critical molecule in immune system with multiple functions, mostly related to Th1 response against bacterial, viral and fungal infections. Elenius et al. [28] provided new evidence suggesting that vitamin A may have antiviral effects.

Vitamin E acts as an antioxidant in cellular membranes and scavenges free radicals by blocking the peroxidation of poly-unsaturated fatty acids. It also modifies prostaglandin formation and thereby enhances the production of prostaglandin I2, which inhibits the effects of histamine. In addition, the antioxidant role played by vitamin E reduces the level of immune inflammation. Zheng et al. [29], using a model of induced nasal allergy in mice, demonstrated that a diet supplemented with high-dose vitamin E led to significant reduction in all the outwardly observable markers of nasal allergy compared with a diet supplemented with low-dose vitamin E. They demonstrated the improvement of symptom scores in patients with seasonal allergic rhinitis.

At the end of the present study, we observed, in group G, a significant improvement of nasal obstruction, evaluated by VAS, and a significant decrease in cell count analysed by nasal cytology, mostly neutrophils and eosinophils (Pearson coefficient $r = 0.323$, $p = 0.0007$; Spearman index $s = 0.488$, $p < 0.0001$). The relationship between nasal obstruction and cytology has not been proposed in other studies. Gelardi et al. [30] pointed out that allergic patients to pollens displayed higher levels of cellular infiltration (eosinophils, neutrophils and mast cells) and a greater increase in nasal resistance in comparison with perennial allergic patients. In our study, the scores recorded in the control group did not differ significantly comparing T0 and T1 data ($p = 0.845$ for VAS and $p = 0.189$ for cytology score in *Wilcoxon rank sum test*).

A significant improvement of QoL, evaluated by SNOT-22 test ($p < 0.0001$), was detected after treatment in the study group. No significant differences in SNOT score were detected in the control group ($p = 0.9881$).

These significant and innovative results are a valid answer to the question about the efficacy of the proposed nasal spray as a complementary rather than alternative therapy in patients suffering from persistent allergic rhinitis.

Conclusions

In conclusion, our study provides evidence that this liposomal nasal spray is able to improve nasal symptoms of AR, and thus significant improvement of QoL. Patients were compliant with this therapy because of minor side effects compared with anti-allergic therapy. The effectiveness of this liposomal nasal spray in terms of reduction of inflammatory cell counts is remarkable. It confirms the close association between inflammatory cell count and nasal obstruction. These results may have implications for clinical practice, and in fact the use of liposomal nasal spray with vitamins A and E can be considered a complementary therapy in patients suffering from persistent allergic rhinitis. Our findings highlight the importance of this liposomal nasal spray in improving nasal symptoms and local inflammation, but further experimental studies are needed to better define the mechanisms.

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ACTA OTORHINOLARYNGOLOGICA ITALICA 2020;40:224-229; doi: 10.14639/0392-100X-N0139

Rhinology

Clinical comparison of the efficacy of spirulina platensis and cetirizine for treatment of allergic rhinitis

Studio comparativo di efficacia sulla spirulina platensis e cetirizina nel trattamento della rinite allergica

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SUMMARY

The aim of this study is to compare the efficacy provided by spirulina platensis and cetirizine for treatment of allergic rhinitis (AR). A randomised controlled clinical trial was performed on 53 patients with AR divided into experimental and control groups in an accredited tertiary academic centre. Subjects in the experimental group were treated with spirulina (2 g per day) and the control group was treated with cetirizine (10 mg per day) for 2 months. Symptoms were assessed based on a standard questionnaire and inflammatory mediators, and the results were compared before and after treatment. There were 23 men and 30 women with a mean age of 26.75 years (SD 9.26; range 8-58). The differences between the two groups in terms of age and gender were not significant. There was no significant difference between the two groups in clinical presentations before the intervention ($P > 0.05$). The prevalence of rhinorrhoea ($P = 0.021$), nasal obstruction ($P = 0.039$) and smell reduction ($P = 0.030$) in the experimental group improved significantly compared to those seen in the control group after intervention. Sleep condition, daily working and social activity improved significantly in the experimental group ($P < 0.05$). Furthermore, there was no significant difference between groups regarding inflammatory mediators before treatment; however, after 1 month of treatment, the levels of interleukin (IL)-1α ($P < 0.001$), IL-1β ($P < 0.001$) and IL-4 ($P = 0.008$) were all significantly lower, and IL-10 levels were significantly higher in the experimental group, compared to those in the control group. In conclusion, spirulina is more effective than cetirizine in improving cardinal symptoms of AR patients. Furthermore, spirulina can be considered as an alternative treatment in patients with AR.

KEY WORDS: allergic rhinitis, therapeutics, medical therapy of chronic rhinosinusitis, spirulina

RIASSUNTO

Lo scopo di questo studio è quello di paragonare l'efficacia della spirulina platensis e della cetirizina nel trattamento della rinite allergica (AR). Questo trial clinico randomizzato controllato riguarda 53 pazienti affetti da AR, suddivisi in due gruppi, sperimentale e di controllo afferenti ad un centro terziario accademico accreditato. I pazienti del gruppo sperimentale sono stati trattati con Spirulina (2 g/die) mentre il gruppo controllo con cetirizina (10 mg/die) per due mesi. Sono stati valutati i pazienti attraverso un questionario standard sui sintomi e sui mediatori dell'infiammazione, prima e dopo il trattamento. Sono stati valutati 23 uomini e 30 donne con età media di 26,75 anni (SD 9,26; range 8-58). La differenza nei due gruppi in termini di età e genere non sono significative. Non è emersa differenza significativa fra i due gruppi in termini di presentazione clinica prima della terapia ($P > 0,05$). La prevalenza della rinorrea ($P = 0,021$), ostruzione nasale ($P = 0,039$) e iposmia ($P = 0,030$) nel gruppo sperimentale è migliorata significativamente se paragonata al gruppo controllo, grazie alla terapia. La condizione di sonno, attività lavorativa giornaliera e attività sociale è migliorata nel gruppo sperimentale ($P < 0,05$). In più, non vi è alcuna differenza dopo 1 mese nei livelli di IL-1 fra i due gruppi per quanto riguarda i mediatori dell'infiammazione prima del trattamento; i livelli di interleuchina (IL)-1α ($P < 0,001$), IL-1β ($P < 0,001$), e IL-4 ($P = 0,008$) sono significativamente più bassi nel gruppo sperimentale e quelli di IL-10 più alti. In conclusione, la Spirulina è più efficace della cetirizina nel trattamento dei sintomi della rinite allergica e può essere considerata una valida alternativa terapeutica.

PAROLE CHIVAE: *rinite allergica, terapie, terapia medica della rinite allergica, spirulina*

Received: March 2, 2019
Accepted: October 27, 2019

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Funding
None.

Conflict of interest
The Authors declare no conflict of interest.

How to cite this article: Nourollahian M, Rasoulian B, Gafari A, et al. Clinical comparison of the efficacy of spirulina platensis and cetirizine for treatment of allergic rhinitis. Acta Otorhinolaryngol Ital 2020;40:224-229. https://doi.org/10.14639/0392-100X-N0139

Introduction

The prevalence of allergic rhinitis (AR) is on the rise on a global scale, and has major impact affecting the quality of life in large populations. Based on various reports its prevalence in Asia ranges from 27% in South Korea to 32% in the United Arab Emirates [1]. According to the recent study in Iran, the prevalence of allergic rhinitis in Tehran (capital of Iran) and Mashhad (Northeast Iran) was reported to be 28.3% and 22.4%, respectively [2,3]. The main medical procedure for treatment of AR involves the administration of antihistamines and corticosteroids, which mainly reduce the signs and symptoms of the disease [4]. However, the long-term intake of these medications can cause serious side effects for patients. Current therapeutic methods have failed to secure a definite treatment for AR, and the need for continuous medical treatment has raised concerns about side effects in patients. Therefore, alternative therapeutic options are required in this regard. The use of complementary and alternative medicine (CAM) has been a rising trend over the past decades. Given the increasing prevalence of AR, it is necessary for ear, nose and throat (ENT) specialists, as well as other physicians involved in treating this condition, to manage CAM therapies used by patients [5]. Spirulina as one of the drugs used in CAM treatment of AR that is a type of blue-green algae that belongs to cyanobacteria and grown on a large scale. Few studies have investigated the effect of spirulina on control and management of AR. The use of spirulina has been widely commercialised as a nutritional supplement to balance the function of the immune system and as treatment for a range of diseases [6]. Today, spirulina is predominantly utilised as a nutritional supplement in the pharmaceutical industry in the form of pills or powder. The effect of spirulina on inflammatory mediators in human samples has been limited to the results of one study up to now [7]. To the best of our knowledge, no study has simultaneously examined the clinical and laboratory effects of spirulina. The aim of the present study was to determine the therapeutic effect of spirulina platensis on AR.

Materials and methods

This single-blind randomised clinical trial was carried out on 65 patients with persistent AR from October 2015 to March 2016. The allergic patients were those with clinical symptoms and signs of AR that showed a positive reaction to at least one allergen during a skin prick test. All of the participants had persistent allergic rhinitis. The usual pollen calendar in Iran is from May to July. However, by selecting an appropriate timeframe, we attempted to reduce the impact of seasonal factors on severity of allergic symptoms.

The effect of spirulina was investigated on the signs and symptoms of AR according to the visual analog scale (VAS). In addition, daily working, sleep condition and social activity of patients in both groups were assessed before and after the intervention based on VAS. Subjects were divided into two groups, namely the experimental and control groups with daily doses of 2 g spirulina and 10 mg cetirizine both for 2 months, respectively.

Patients were randomly assigned to each group based on a simple randomisation method using a random number table. The symptoms were compared between the two groups before and after treatment. Informed consent was obtained from each participant prior to participating in the study. Moreover, the study protocol was approved by the Local Institutional Board with the ethics code of 940150.

Patients with signs and symptoms of AR visiting either allergy or ENT clinics were enrolled in this study. A full medical history was taken, and thorough ENT examination was performed to confirm the clinical diagnosis of persistent AR and rule out other possible causes of rhinitis. Next, subjects underwent a skin prick test for the identification of common aeroallergens; accordingly, the diagnosis could be further confirmed by the presence of related antibodies.

All patients with systemic diseases, using anticoagulants or any drugs with anti-inflammatory effects, with an intolerance to the side effects of spirulina (e.g., nausea, constipation, and mild fever), using immunosuppressive drugs, with prior immunotherapy, using blood glucose or blood pressure controlling drugs, as well as pregnant or lactating women, and asthmatic patients were excluded.

Sample size calculation

In this study, the objective is to show that spirulina is at least as good as cetirizine as standard therapy in reducing the cardinal symptoms of AR. After assessing similar studies [5] with a clinically significant effect of 0.4 or more over the 2 weeks, a two-sided significance of 0.05 and a power of 0.9, a total of 26 participants would be required in each arm assuming a standard deviation of 0.51 and 0.55. We anticipated a 15% percent withdrawal rate. Thus, the total primary sample size was considered to be 65.

Clinical assessment

The overall discomfort experienced by the patients related to cardinal symptoms of rhinitis and factors related to the subjects' quality of life were recorded and compared after the intervention within and between groups. Clinical symptoms were recorded using a VAS for five cardinal rhinitis symptoms, including sneezing, nasal congestion, rhinorrhoea, smell disturbance and nasal itching. Furthermore, several factors related to patients' quality of life, including sleep condition, working life and social activity, were evaluated based on VAS. The symptoms were recorded according to patients' perception and scored from 0 to 5 (i.e., 0 and 5 indicative of no symptoms and the most severe symptoms, respectively).

Laboratory assessment

Before and after intervention, all patients were referred to the immunology laboratory, and 5 ml of blood was taken from each participant. Next, blood samples were centrifuged, and the serum was separated. The levels of interleukin (IL)-10, IL-4, IL-1 and interferon-gamma (IFN-γ) were measured using a microarray technique before and after treatment in both groups, and the results were compared.

Statistical analysis

The collected data were analysed using SPSS software (version 16). Due to the fact that the study was a randomised controlled clinical trial, the normality test was first checked and then an independent t-test or paired t-test was used. The paired-sample t-test was utilised for intergroup comparisons. For subgroup analysis in each group, the t-test was used for assessing quantitative variables. In addition, a P-value less than 0.05 was considered statistically significant for all the above-mentioned tests.

Results

In this randomised controlled clinical trial, 65 patients were enrolled among whom 11 were excluded due to a negative prick test or withdrawal from intervention. The remaining 53 participants were divided into the experimental (n = 26) and control (n = 27) groups. One patient in the experimental group was excluded due to the refusal to continue the treatment course. The mean age of participants was 26.75 ± 9.26 (mean ± SD) years (age range: 8-58).

Based on statistical findings, no significant difference was observed between the two groups regarding age or gender (Fig. 1).

Results of VAS test showed that Salsala was identified as

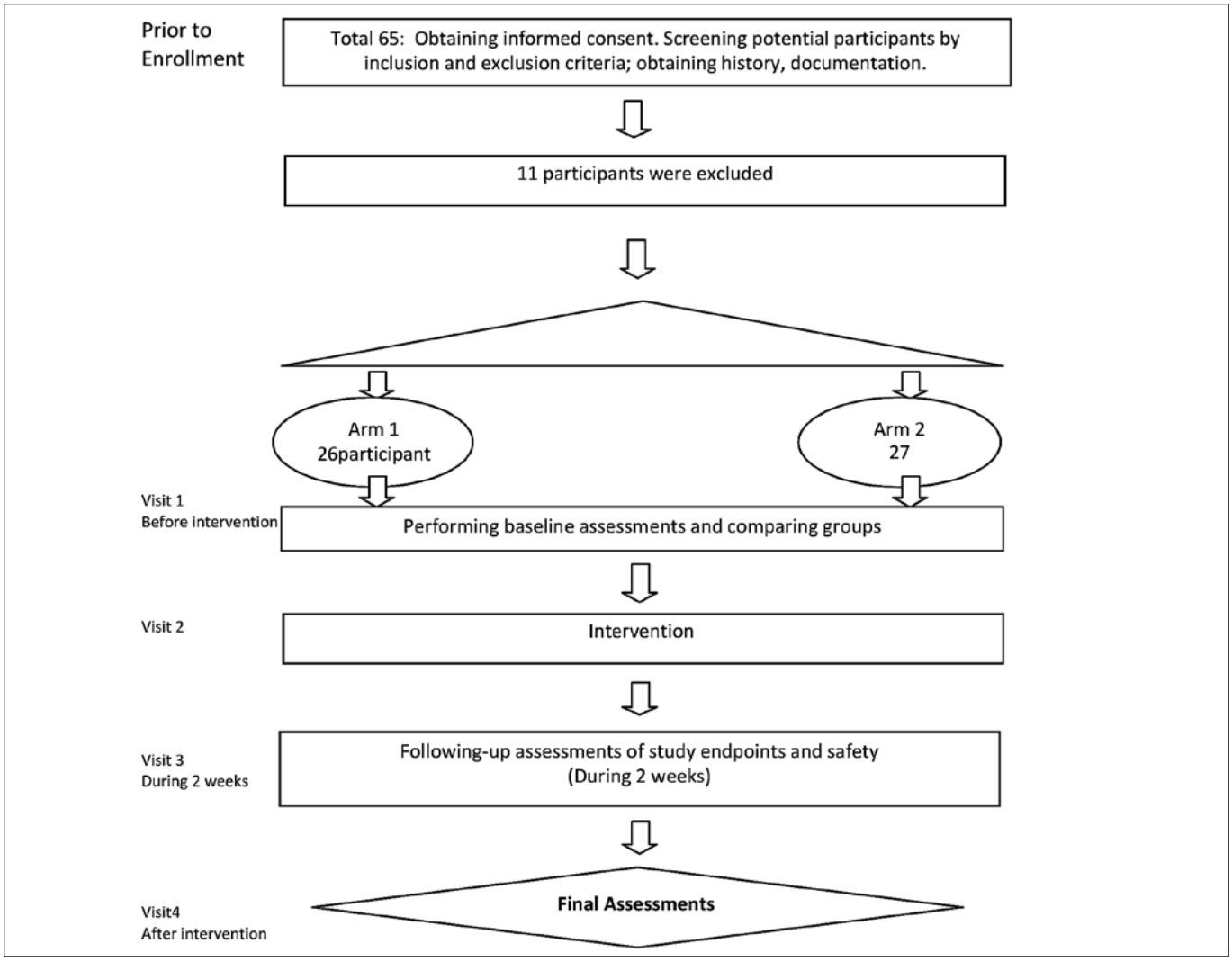

Figure 1. Enrolment of patients.

the most common allergen among participants and the least prevalence was related to feather. Before treatment initiation, there was no difference in symptoms between the two groups (Tab. I). After the therapeutic course, improvement of symptoms was significantly higher in the experimental group than in the control group for all symptoms except nasal itching, sneezing, as well as and INF-γ.

The prevalence of rhinorrhoea (P = 0.021), nasal congestion or obstruction (P = 0.039) and loss of smell (P = 0.030) were significantly lower in the experimental group than in the control group; however, no significant changes were observed regarding the prevalence of sneezing (P = 0.096) and nasal itching (P = 0.099) between the two groups. Sleep condition, working life and social activity improved in both groups, and the improvement was significantly higher in the experimental group. In addition, the levels of IL-1α (P < 0.001), IL-1β (P < 0.001) and IL-4 (P = 0.008) were significantly lower in the spirulina group in comparison to those in the control group. (Tab. II).

Investigations showed that a reduction was observed in the severity of all symptoms in both groups after intervention (Figs. 2, 3) and these differences were significant for all factors in the spirulina group and for all factors in the cetirizine group except IL-1β (P = 0.109), IL-4 (P = 0.078) and IL-10 (P = 0.68) (Tab. III).

Discussion

In the present study, based on intragroup evaluation, treatment resulted in a significant decrease in all rhinitis symptoms and improved the patients' quality of life. The use of spirulina was associated with significant improvement in the severity of most rhinitis symptoms, including rhinorrhoea, nasal congestion and smell disturbance. In addition, the quality of life in the experimental group enhanced significantly in comparison to that in the control group.

In the comparison between the two types of treatment, despite the fact that the levels of mediators were not different at the beginning of the study, the use of spirulina led to a significant decrease in the levels of IL-α, IL-β and IL-4, as well as a significant increase in the level of IL-10. According to the decreasing effect of spirulina on pro-inflammatory cytokines (e.g., IL-α, IL-β, and IL-4) and the increasing effect on the immunosuppressive cytokine (i.e., IL-10), it seems that relief of symptoms may be due to the anti-inflammatory effects of this agent.

Mao et al. assessed the mechanism of spirulina on AR pa-

Table I. Comparison of cardinal allergic rhinitis symptoms in two groups before intervention.

Type of symptoms	Allergic rhinitis symptoms	Treatment methods		P-value
		Spirulina (n = 26)	Cetirizine (n = 27)	
Clinical presentation	Nasal itching	2.08 ± 1.60	2.37 ± 1.25	0.438
	Rhinorrhoea	2.42 ± 1.47	2.48 ± 1.22	0.876
	Nasal obstruction	3.08 ± 1.30	3.59 ± 1.57	0.191
	Sneezing	2.31 ± 1.12	2.44 ± 1.31	0.686
	Smell reduction	2.96 ± 1.89	2.52 ± 1.83	0.389
Quality of life factors	Sleep condition	1.73 ± 1.28	1.48 ± 1.40	0.502
	Daily working	2.38 ± 1.10	2.85 ± 1.56	0.215
	Social activity	1.69 ± 1.32	1.96 ± 1.43	0.477
Inflammatory mediators	INF ϒ	1.56 ± 2.38	0.75 ± 1.46	0.07
	IL-1α	0.55 ± 1.19	0.18 ± 0.28	0.583
	IL-1β	0.84 ± 1.15	0.86 ± 1.38	0.795
	IL-4	1.55 ± 1.45	1.24 ± 0.57	0.587
	IL-10	1.3 ± 1.05	1.83 ± 1.43	0.135

Data are reported as "mean ± standard deviation"; significance level is 0.05.

Table II. Comparison of cardinal symptoms of allergic rhinitis in the two groups after intervention.

Type of symptoms	Allergic rhinitis symptoms	Group		P-value
		Spirulina	Cetirizine	
Clinical presentation	Nasal itching	1.08 ± 0.89	1.52 ± 1.01	0.099
	Rhinorrhoea	0.96 ± 1.00	1.63 ± 1.04	0.021*
	Nasal obstruction	1.46 ± 0.95	2.15 ± 1.26	0.039*
	Sneezing	0.96 ± 0.72	1.37 ± 1.01	0.096
	Smell reduction	0.81 ± 0.9	1.44 ± 1.30	0.030*
Quality of life factors	Sleep condition	0.77 ± 0.59	1.48 ± 1.37	0.018*
	Daily working	0.88 ± 0.65	1.56 ± 1.31	0.023*
	Social activity	0.50 ± 0.58	1.11 ± 1.31	0.034*
Inflammatory mediators	INF ϒ	0.47 ± 0.75	1.22 ± 1.63	0.583
	IL-1α	0.08 ± 0.16	0.56 ± 0.49	< 0.001*
	IL-1β	0.08 ± 0.27	1.28 ± 1.34	< 0.001*
	IL-4	1.05 ± 1.06	1.64 ± 0.79	0.008*
	IL-10	2.44 ± 1.49	1.7 ± 1.17	0.049*

Data are reported as "mean ± standard deviation"; significance level is 0.05.

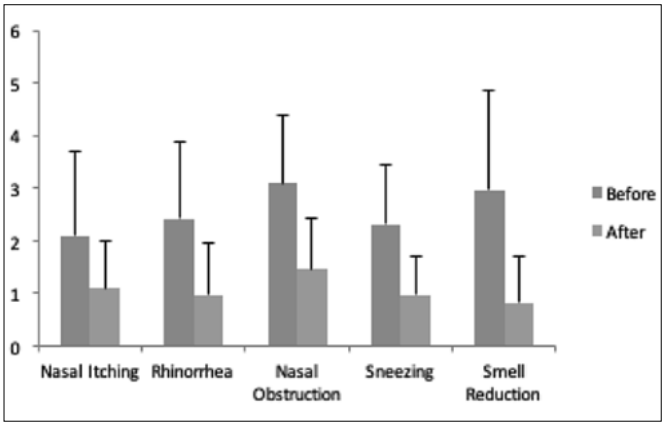

Figure 2. The effect of intervention on cardinal symptoms of allergic rhinitis in *spirulina group.*

Table III. Comparison of cardinal allergic rhinitis symptoms before and after intervention in separate groups.

Type of symptoms	Allergic rhinitis symptoms	P-value	
		Spirulina	Cetirizine
Clinical presentation	Nasal itching	< 0.000*	< 0.000*
	Rhinorrhoea	0.003*	< 0.000*
	Nasal obstruction	< 0.000*	< 0.000*
	Sneezing	0.037*	< 0.000*
	Smell reduction	< 0.000*	0.001*
Quality of life factors	Sleep condition	0.023*	0.012*
	Daily working	0.003*	< 0.000*
	Social activity	0.016*	0.043*
Inflammatory mediators	INF ϒ	0.003*	0.053*
	IL-1α	0.01*	0.005*
	IL-1β	0.005*	0.109
	IL-4	< 0.001*	0.078
	IL-10	< 0.001*	0.68

Data are reported as "mean ± standard deviation"; significance level is 0.05.

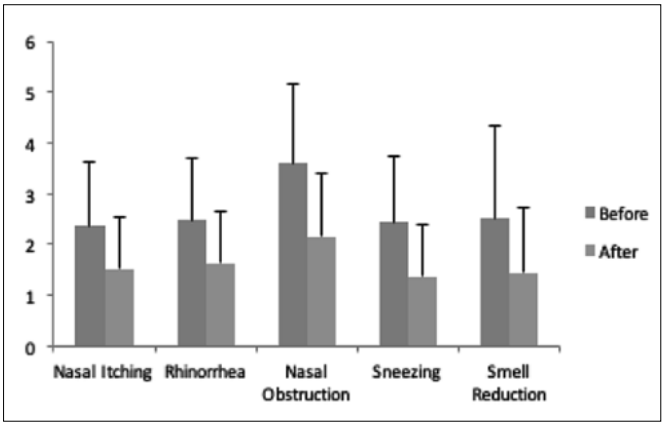

Figure 3. The effect of intervention on cardinal symptoms in the *cetirizine group.*

tients [7]. In this regard, they reported that daily consumption of 2,000 mg spirulina decreases IL-4 levels by 32% in phytohemagglutinin-induced cells. These findings show that Spirulina can improve the T helper (Th) profile by suppressing Th2 cell differentiation (which is mediated to some extent by suppressing the production of IL-4) in AR patients. The aforementioned study was the first human nutritional study to demonstrate the protective effects of spirulina on AR. The present study confirmed these positive findings from both clinical and laboratory aspects. The difference between the two studies is probably due to differences in the duration of treatment and follow-up periods. In the present study, patients were followed up for a shorter time; furthermore, the use of spirulina was compared with another standard treatment.

In a study by Cingi et al. (2008), the effects of spirulina were also studied on AR [8]. The results of a double-blind placebo-controlled study investigating the efficacy and tolerability of spirulina treatment on AR indicated a significant improvement in clinical symptoms, such as rhinorrhoea, sneezing, nasal itching and congestion in the spirulina group in comparison to the control group ($P < 0.001$). Spirulina was clinically more efficient in treating AR compared with placebo. In the above-mentioned study, it was proposed that further studies are required to better clarify the mechanism of the mentioned effect. The findings of a study by Cingi et al. in terms of the efficiency of spirulina in controlling the symptoms of AR are consistent with the results of the present study. The mechanism of this effect was also surveyed by investigating the related cytokines; nevertheless, Cingi et al. only focused on the clinical effects of spirulina.

In a systematic review (2006), Karkos et al. examined the supplements commonly used in complementary medicine in the field of otolaryngology [9]. In this study, the evaluations were only conducted on clinical trials, and animal studies were not included. Three studies were observed regarding the effect of spirulina on allergy, rhinitis and immune response modulation.

One of the above-mentioned trials was a randomised double-blind placebo-controlled study on AR patients, which showed the positive effects of spirulina dietary supplementation for 12 weeks. Although the other two were non-randomised studies, they reported the positive effects of spirulina on mucosal immunity. Karkos et al. concluded that there is acceptable evidence regarding the positive effects of spirulina on AR, although further trials are required in this regard.

In addition to human studies, investigation in animal models have also examined the effect of spirulina platensis on IL-4 and IFN-γ expression in the serum of a laboratory rat affected with AR [10,11]. They concluded that spirulina platensis is very effective in the treatment of AR through the regulation of IL-4 and IFN-γ expression, as well as the adjustment of the imbalance in the Th1/Th2 cytokine network. These findings were also confirmed by a recent study that showed immulina exhibits anti-inflammatory properties and inhibits the release of histamine from mast cells [12]. The present study on the effect of spirulina on AR patients confirmed this effect on human beings.

There are a few major aspects of using spirulina, which further highlight the effectiveness of this intervention. Given the results of multiple studies performed on rodents, as well as the long and historical use of spirulina algae by humans, the Food and Drug Administration has approved the safety of spirulina [13]. Therefore, there are no concerns regarding the administration of spirulina.

On the other hand, in addition to the therapeutic applica-

tion, spirulina can have invaluable effects as a nutritional and pharmaceutical supplement. In previous studies, it was shown that spirulina contains high levels of proteins, essential fatty acids, essential amino acids, micro- and macrominerals, vitamins and polysaccharides; in other words, it offers a favourable combination of all components required by the human body [14-16].

It should be noted that considering the nature of AR and effect of exposure to allergens on the severity of clinical symptoms, the different environmental conditions of the two groups could have affected the results of the present study. However, by conducting a randomised trial and selecting an appropriate timeframe, we tried to reduce the impact of seasonal factors on the severity of allergic symptoms. One of the advantages of the present study was the laboratory assessment of patients by measuring inflammatory mediators; consequently, the mechanism of its effect could be judged more precisely.

Spirulina treatment has a principal downside in terms of the high dose required; a patient needs to take 2 g of spirulina equivalent in four daily capsules. In practice, many patients will resist taking such a high volume of medicine. Therefore, it is recommended to perform further studies with a longer follow-up period to investigate the effect of the drug on the relapse of clinical symptoms and ensure patients that there are no side effects of spirulina. Also, patients were regularly monitored for side effects during the study. However, more thorough studies are recommended to more accurately identify any potential side effects.

Conclusions

The use of spirulina seems to be more effective than the administration of cetirizine in improvement of both clinical presentations and inflammatory mediators of AR patients. Spirulina should be considered as an alternative treatment in patients with AR.

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ACTA OTORHINOLARYNGOLOGICA ITALICA 2020;40:230-237; doi: 10.14639/0392-100X-2432

AUDIOLOGY

Psychometric properties of the Italian Tinnitus Functional Index (TFI)

Proprietà psicometriche della versione italiana del Tinnitus Functional Index (TFI)

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SUMMARY

Various questionnaires are used to assess the impact of tinnitus on the quality of life. The Tinnitus Functional Index (TFI) has excellent properties for scaling the severity of tinnitus and treatment-related changes in both clinical and research settings. The aim of this study was to evaluate the psychometric properties of the Italian version of the TFI with particular emphasis on factor analysis, internal consistency, reliability and validity. The original English version of the TFI was translated into Italian using the translation/back - translation process; 137 participants who were recruited at the Tinnitus Clinic in Milan and had suffered from tinnitus for at least three months (39.4% females, age: 18-80 years, mean age: 48.26, SD: 14.08) completed the Italian version of the TFI, the Tinnitus Handicap Inventory (THI), the Beck Depression Inventory - Primary Care Version (BDI-PC) and the Numeric Rating Scale of annoyance (NRS-A). Of these patients, 57 completed the TFI again at a second visit 7-14 days later, before undergoing any intervention, in order to provide data for reproducibility assessment. The psychometric properties were investigated using exploratory factor analysis and internal consistency and test-retest reliability instruments. The convergent validity of the TFI was evaluated using correlation coefficients obtained from the remaining measurements. The Italian TFI has a four-factor structure that was somewhat different from the original. The internal consistency proved to be good ($0.92 \leq \alpha \leq 0.96$) as did the test-retest reliability ($0.79 \leq \alpha \leq 0.85$). In terms of convergent validity, the TFI showed high correlations with the THI ($r = 0.77$) and the NRS-A ($r = 0.70$) scores, and moderate correlations with the BDI-PC scores ($r = 0.46$). The difficulties encountered when attempting to reproduce the original eight-factor structure were consistent with other studies in which the TFI was translated into European languages. In spite of this, the factorial structure of the Italian version of the TFI was characterised by high levels of reliability and validity. Overall, the Italian adaptation of the TFI was shown to be suitable to measure the impact of tinnitus on the daily lives of individuals.

KEY WORDS: tinnitus, exploratory factor analysis, convergent validity, reliability, outcome instruments

RIASSUNTO

Diversi questionari sono utilizzati per valutare l'impatto dell'acufene sulla qualità della vita. Il Tinnitus Functional Index (TFI) proposto da Meikle et al, nel 2012, ha dimostrato proprietà eccellenti per misurare la gravità e le modificazioni indotte dal trattamento degli acufeni, sia in ambito clinico che di ricerca. Lo scopo di questo studio è stato valutare le proprietà psicometriche della versione italiana del TFI, in particolare, l'analisi fattoriale, la consistenza interna, l'affidabilità e la validità. La versione originale inglese del TFI è stata tradotta in italiano secondo la procedura translation - back translation; 137 partecipanti con acufeni da almeno 3 mesi (39,4% femmine, età: 18-80 anni, età media: 48,26, SD: 14,08), reclutati presso la Tinnitus Clinic di Milano, hanno completato la versione italiana del TFI, il Tinnitus Handicap Inventory, la Beck Depression Inventory - Versione Primary Care e la scala di valutazione numerica per il fastidio. Una parte del campione, 57 pazienti, ha completato la versione italiana del TFI in una seconda visita, dopo 7-14 giorni, prima di ricevere qualsiasi tipo di trattamento, per ricavare i dati per la valutazione della

Received: November 17, 2018
Accepted: April 13, 2019

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Funding
None.

Conflict of interest
The Authors declare no conflict of interest.

How to cite this article: Barozzi S, Del Bo L, Passoni S, et al. Psychometric properties of the Italian Tinnitus Functional Index (TFI). Acta Otorhinolaryngol Ital 2020;40:230-237. https://doi.org/10.14639/0392-100X-2432

riproducibilità. Le proprietà psicometriche sono state studiate attraverso un'analisi fattoriale esplorativa ed il calcolo di misure di consistenza interna e affidabilità test-retest. La validità convergente è stata valutata mediante i coefficienti di correlazione con le restanti misure. La versione italiana del TFI ha mostrato una struttura a quattro fattori, parzialmente diversa dalla struttura originale a otto fattori. L'adattamento italiano del TFI ha rivelato buoni livelli di consistenza interna (0,92 ≤ α ≤ 0,96) e affidabilità test-retest (0,79 ≤ α ≤ 0,85). In termini di validità convergente, ha mostrato buone correlazioni con i punteggi del THI (r = 0,77) e della scala del fastidio (r = 0,70) e correlazioni medie con i punteggi del BDI (r = 0,46). Le difficoltà nel riprodurre la struttura originale a otto fattori sono coerenti con altri studi di validazione del TFI nelle lingue europee. Nonostante tali discrepanze, la versione italiana del TFI ha mostrato una struttura fattoriale caratterizzata da alti livelli di affidabilità e validità. Nel complesso, l'adattamento italiano di TFI si è rivelato idoneo a misurare l'impatto degli acufeni sulla vita quotidiana degli individui.

PAROLE CHIAVE: *acufene, analisi fattoriale esplorativa, validità convergente, affidabilità*

Introduction

Tinnitus is generally defined as the perception of sound in the absence of a corresponding external stimulus. It is a common auditory complaint with prevalence rates ranging from 2.4% to 20.1% [1]. Some people can ignore this phantom sound, while others suffer considerably and consequently, with negative impact on the quality of life. Tinnitus is a purely subjective phenomenon and lacks any objectively identifiable markers; moreover, tinnitus may affect different domains of well-being (i.e. sleep, concentration, hearing, emotions). For these reasons, quantifying the severity of this symptom and how it changes over time and with treatment is challenging.

Several questionnaires have now been developed and validated for assessment of tinnitus complaints: Tinnitus Handicap Questionnaire [2], Tinnitus Reaction Questionnaire [3], Tinnitus Handicap Inventory (THI) [4], Tinnitus Questionnaire (TQ) [5] and its short form, Mini-TQ [6] and the Tinnitus Primary Function Questionnaire [7]. The THI and Mini-TQ have also been validated in their Italian language versions [8,9]. More recently Moschen et al. validated the Italian version of the Chronic Tinnitus Acceptance Questionnaire (CTAQ-I) [10].

In 2012, Meickle et al. proposed the Tinnitus Functional Index (TFI) [11]. The TFI has been shown to be a sensitive tool to rate the severity and negative impact of tinnitus and is especially effective in evaluating the effects of treatment. Since its publication, the TFI has received considerable international attention in both clinical and research settings and has become the new "gold standard". It has been translated into and validated in a number of languages including Dutch [12], Polish [13], Swedish [14], German [15,16], Hebrew [17] and Chinese [18,19]. To our knowledge, there has been no validated Italian version to date.

The TFI consists of 25 items, each rated on a 11-point Likert scale. Patients rate each item according to how they have felt over the past week. The total score varies from 0 to 100 and is classified according to five levels of clinical severity [20]: not a problem (0-17); small problem (18-31); moderate problem (32-53); big problem (54-72); very big problem (73-100). The TFI incorporates eight subscales named: Intrusive (items 1-3), Sense of Control (items 4-6), Cognitive (items 7-9), Sleep (items 10-12), Auditory (items 13-15), Relaxation (items 16-18), Quality of Life (items 19-22) and Emotional (items 23-25). In addition to the total score, the subscale scores can be calculated. A reduction of 13 in the TFI score is considered a significant reduction in discomfort [20].

The aim of this study was to evaluate the psychometric properties of the Italian TFI (I-TFI). More specifically, we investigated the factor structure of the instrument together with its internal consistency, test-retest reliability, convergent and discriminant validity.

Materials and methods

Procedures

The study was carried out in accordance with the Declaration of Helsinki. For this trial, participants were recruited at the Tinnitus Clinic in Milan where they had referred a complaint of tinnitus. Patients were included in this study if they had had tinnitus for a minimum of three months, were fluent in the Italian language, were at least 18 years old and had normally cognitive function and preserved reading skills. All of the subjects enrolled in the study gave written informed consent. None of the patients was undergoing any type of treatment for tinnitus at the time of evaluation.

During their first examination at the Tinnitus Clinic (T0), all participants underwent complete audiological evaluation including full medical anamnesis, otomicroscopy and pure-tone audiometry in a sound attenuated room. Hearing levels were measured in each ear separately at 0.25-8 kHz at half octave steps for air conduction and at 0.5-4 kHz for bone conduction. The Pure Tone Average (PTA) was measured at 0.5, 1 and 2 kHz. After audiological assessment, the participants completed the I-TFI, THI, Beck Depression Inventory - Primary Care Version (BDI-PC) [21] and a Numeric Rating Scale of annoyance (NRS-A). Of the patients who took part in the first survey, a group was selected for test-

retest reliability analysis. For this purpose, these patients were asked to complete the I-TFI a second time within a 1-2-week interval (T1) and before undergoing any intervention, in order to provide data for reproducibility assessment. A variation of two days before or after the requested one/two-week long interval between trials was considered acceptable in compliance with the patients' needs. No access to the responses given in the first questionnaire was allowed to patients when filling out the second I-TFI. All of the participants included in the study completed the I-TFI without assistance. The time required to fill out the questionnaire was approximately 10 minutes.

The study consisted of two different phases: a translation and cross-cultural adaptation process (*phase 1*) and analysis of psychometric properties (*phase 2*).

Participants

One hundred and fifty subjects (40% females) attending the Tinnitus Clinic of Milan were asked to participate in this study; 13 patients refused to participate and therefore 137 adults aged 18-80 years (39.4% females M_{age} = 48.26, SD_{age} = 14.08) suffering from tinnitus were included. All were examined by an ENT physician, underwent the audiometric tests described above and completed the I-TFI, I-THI, BDI-PC and NRS-A. Of the 137 patients who took part in the first survey, 70 were selected for the second survey based on the I-TFI questionnaire alone. 57 participants completed the second questionnaire within the accepted period and their scores were therefore included in the test-retest reliability analysis (10 patients did not complete the I-TFI within the accepted period of time and three did not complete the second questionnaire).

Measures

1. The TFI [11] consists of 25 items: the patient answers each question by giving a score ranging from 0 to 10 (except for items 1 and 3, which are expressed as a percentage ranging from 0% to 100%). The overall TFI score is calculated by multiplying the mean of all questions by 10 (questions 1 and 3 must be converted into a 0-10 scale) or dividing the sum of all of the scores by 2.5, to give a global score out of 100. Subscales are calculated as the mean of the answered questions multiplied by 10.
2. The THI [4] is one of the most commonly used questionnaires for assessing tinnitus distress and reporting treatment outcomes. The THI consists of 25 items that can be divided into three subscales: Functional, Emotional and Catastrophic. Patients score their symptoms on a three-point scale. The total score ranges from 0 to 100; a higher score is indicative of greater tinnitus distress. Good reliability (0.94) and validity were reported for the total score of the Italian THI [8,22].
3. The BDI-PC [21] is a screening instrument for depression that minimises the possibility of yielding spuriously high estimates of depression for patients with medical problems by focusing on symptoms of sadness, pessimism, past failure, loss of pleasure, self-dislike, self-criticalness and suicidal thoughts or wishes. Its seven items are taken from the Beck Depression Inventory-II [23]. Each item is rated on a 4-point scale ranging from 0 to 3, and the BDI-PC score is calculated by summing up all of the highest ratings for each of its seven items. The respondents are asked to describe their feelings "over the past 2 weeks, including today."
4. *NRS-A*: the patient is asked to rate tinnitus-related annoyance from 0 (minimal annoyance) to 10 (maximum annoyance). Of the 137 participants, only 125 answered this scale at T0.

Translation and cultural adaptation process (phase 1)

A cross-cultural adaptation process of translation and back-translation was followed in accordance with the "Principles of Good Practice for the Translation and Cultural Adaptation Process for Patient-Reported Outcome Measures" [24]. Two professional translators carried out independent forward translations of the original TFI into Italian (forward translation). Discussion of the translated manuscripts with an otorhinolaryngologist and an audiologist with extensive experience in tinnitus resolved discrepancies between the original independent translations and ensured a single forward translation (reconciliation). This new and final version of the I-TFI was then translated back into English by a qualified professional translator (backward translation). The same professional translator compared this back-translation to the original manuscript; no signs of incongruent translation were noted as every item was semantically identical to the original English manuscript (back translation review). The final I-TFI was tested for cognitive equivalence by a group of tinnitus patients who discussed the wording and the meaning of each item of the I-TFI with the clinicians. Subsequently, the wording of the questionnaire was modified on the basis of the suggestions given by patients (cognitive debriefing and finalisation).

Analysis of psychometric properties (phase 2)

Construct validity

An exploratory factor analysis was carried out to investigate the I-TFI structure with the aim of replicating the questionnaire's original eight-factor configuration.

The final number of factors to be retained was identified by two selection methods: Kaiser-Guttman criterion [25] and

Cattell's Scree test; as a consequence, only factors with eigenvalues greater than one or positioned to the left of the inflexion point on the eigenvalue plot were inspected further. Parallel analysis was also carried out in order to corroborate the identified factor solution.

Since Mardia's multivariate omnibus test showed violation of the assumption of multivariate normality, a Principal Axis Factoring (PAF) analysis was carried out [26]. The direct oblimin rotation method made it possible to correlate the factors.

Internal consistency and test-retest reliability

Internal consistency and test-retest reliability were investigated using inspection of Cronbach's alphas and test-retest α coefficients respectively. In line with literature, values between 0.70 and 0.79 were considered acceptable, scores between 0.80 and 0.89 good, and values higher than 0.90 were deemed excellent.

Convergent and discriminant validity

As a final step, the instrument's convergent and discriminant validity were investigated using Pearson bivariate correlation coefficients. In order to evaluate convergent validity, the I-TFI total and subscale scores were correlated with the global scores from the THI and the NRS-A scale. Similarly, in order to evaluate discriminant validity, the total and subscale scores of the I-TFI were correlated with the global BDI-PC score. In line with literature, values below |0.30| were interpreted as indices of absence of meaningful association between the involved constructs; values between |0.30| and |0.49| were deemed as indices of low correlation, values between |0.50| and |0.69| as indices of moderate correlation and values above |0.70| as indices of high correlation between constructs [27].

Results

A total of 54 females and 83 males completed the I-TFI questionnaire at T0. The mean PTA for the right ear was 16.35 dB HL (SD: 12.45) and for the left ear 18.23 dB HL (SD: 13.34). Descriptive statistics for all of the I-TFI items at T0 were subsequently calculated (Tab. I). No univariate outlier emerged in item distribution, although the results of Mardia's multivariate omnibus test indicated a violation of the assumption of multivariate normality. For this reason, exploratory factor analysis (EFA) with Principal Axis Factoring rather than Principal Component Analysis was preferred.

Factor analysis

All of the I-TFI items were initially checked to make sure that the EFA assumptions were met. Sampling adequacy was verified using the Kaiser-Meyer-Olkin measure (KMO). The total KMO value was 0.92 and all of the KMO values for the individual items were higher than 0.77, and therefore above the threshold value of 0.60 [28]. Bartlett's test of sphericity ($c^2(300 = 4060.38\ p < 0.001)$ indicated that between-item correlations were sufficiently large to perform EFA.

Table I. Descriptive statistics of the I-TFI.

Item	Min-Max	M (SD)	Item	Min-Max	M (SD)
1.	1-10*	6.42 (2.82)	14.	1-10	2.51 (2.95)
2.	1-10	5.88 (2.20)	15.	1-10	2.69 (3.13)
3.	1-10*	5.56 (3.06)	16.	1-10	3.79 (3.43)
4.	1-10	6.68 (3.18)	17.	1-10	4.62 (3.15)
5.	1-10	6.38 (2.93)	18.	1-10	5.45 (3.38)
6.	1-10	6.34 (2.83)	19.	1-10	3.30 (3.24)
7.	1-10	4.15 (3.06)	20.	1-10	3.64 (3.58)
8.	1-10	3.66 (3.21)	21.	1-10	3.28 (3.25)
9.	1-10	3.72 (2.94)	22.	1-10	3.35 (3.18)
10.	1-10	4.23 (3.52)	23.	1-10	4.69 (3.39)
11.	1-10	3.72 (3.45)	24.	1-10	5.69 (3.12)
12.	1-10	3.73 (3.57)	25.	1-10	3.93 (3.44)
13.	1-10	2.80 (3.02)			

*N: number of participants; M: mean; SD: standard deviation; *1-100% scores converted to 1-10.*

Both Kaiser-Guttman's criterion and Scree plot inspection suggested a four-factor solution, together explaining 75.8% of the variance. The four-factor solution also showed eigenvalues higher than the cut-off value suggested by Parallel Analysis [29]. These results show that the I-TFI factor structure differs from the original eight-factor solution observed by Meikle et al. [11].

Table II shows the I-TFI factor loading matrix.

Overall, all items showed primary loadings higher than 0.60 on one of the four factors, with the exception of items 18 (0.43), 21 (0.57), and 24 (0.42); at the same time, items 18 and 24 were the only items, (together with item 17) that had cross-loading values higher than 0.30 with a second factor.

As Table II shows, the first extracted factor of the I-TFI (Factor 1) combined items from the Intrusive (I) and Sense of Control (S) subscales of the original instrument's structure, together with item 24 whose primary loading in the eight-factor structure was on the Emotional subscale (E). With the exclusion of item 24, our results showed that items belonging to the Cognitive (C), Quality of life (Q) and Emotional (E) factors of the original structure collapsed into the second I-TFI factor (Factor 2). Auditory (A) was the only factor that completely mirrored the solution proposed by Meikle et al. [20]. Finally, the fourth I-TFI fac-

Table II. Exploratory factor analysis (principal axis factoring): oblimin-rotated factor loadings.

Item	Factor 1 (I)ntrusive/ (S)ense of Control/ (item 24)	Factor 2 (C)ognitive/ (Q)uality of life/ (E)motional	Factor 3 (A)uditory	Factor 4 (SL)eep/ (R)elaxation
1 (I)	**0.71**			
2 (I)	**0.78**			
3 (I)	**0.77**			
4 (S)	**0.78**			
5 (S)	**0.75**			
6 (S)	**0.78**			
7 (C)		**0.90**		
8 (C)		**0.96**		
9 (C)		**0.83**		
10 (SL)				**0.87**
11 (SL)				**0.96**
12 (SL)				**0.92**
13 (A)			**0.84**	
14 (A)			**0.98**	
15 (A)			**0.91**	
16 (R)				**0.93**
17 (R)		0.32		**0.51**
18 (R)	0.35			**0.43**
19 (Q)		**0.64**		
20 (Q)		**0.66**		
21 (Q)		**0.57**		
22 (Q)		**0.68**		
23 (E)		**0.64**		
24 (E)	**0.42**	0.37		
25 (E)		**0.79**		

*N: number of participants; **Bold**: item highest factor loading; loadings below 30 are not reported; (I): Intrusive; (S): Sense of control; (C): Cognitive; (SL): Sleep; (A): Auditory; (R): Relaxation; (Q): Quality of Life; (E): Emotional.*

tor (Factor 4) combined the Sleep (SL) and Relaxation (R) items from the original factor structure.

As for the level of association among the four I-TFI factors, Table III shows the correlation values together with descriptive statistics.

In general, negligible or low correlations were found among factors, with the exception of factor 2 which showed moderate levels of association with both factor 1 and factor 4.

As a last step, an additional EFA with a fixed eight-factor structure was performed in order to test the original structure suggested by Meickle and colleagues. All of the items showed primary loadings on the expected items with the exception of item 16 that loaded on the Sleep factor (*data are available from the corresponding author upon request)*.

Table III. I-TFI factor correlation and descriptive statistics.

N = 137	1.	2.	3.	4.
1. (I)ntrusive/(S)ense of Control/(item 24)	-			
2. (C)ognitive/(Q)uality of Life/(E)motional	0.54	-		
3. (A)uditory	0.35	0.39	-	
4. (SL)eep/(R)elaxation	0.49	0.61	0.14	-
Min-Max	6-70	0-88	0-30	0-60
M	42.95	33.71	8	25.52
SD	16.47	25.21	8.75	18.58

M: Mean; SD: Standard Deviation; (I): Intrusive; (S): Sense of control; (C): Cognition; (SL): Sleep; (A): Auditory; (R): Relaxation; (Q): Quality of Life; (E): Emotional.

Table IV. Internal consistency and test-retest reliability.

	αT0 (N = 137)	αT1 (N = 57)	α(T0-T1) (N = 57)
1. (I)ntrusive/(S)ense of Control/(item 24)	0.91	0.91	0.79
2. (C)ognitive/(Q)uality of Life/(E)motional	0.96	0.95	0.81
3. (A)uditory	0.96	0.97	0.85
4. (SL)eep/(R)elaxation	0.96	0.94	0.84
I-TFI total score	0.96	0.95	0.83

αT0: Cronbach's alpha at T0; αT1: Cronbach's alpha at T1; α(T0-T1): Test-retest alpha; (I): Intrusive; (S): Sense of control; (C): Cognition; (SL): Sleep; (A): Auditory; (R): Relaxation; (Q): =Quality of Life; (E): Emotional.

Internal consistency and test-retest reliability

Internal consistency was evaluated using Cronbach's alpha indices calculated for each of the four factors and for the total I-TFI score at T0 (N = 137) and T1 (N = 57); the test-retest alpha coefficient [30] was employed to evaluate test-retest reliability (T0-T1, N = 57).

The results are reported in Table IV. All of the Cronbach α indices showed very high levels of internal consistency (> 0.90). As for test-retest reliability, it ranged from 0.79 (Factor 1), to 0.85 (Factor 3) indicating acceptable to good levels of reliability.

Convergent and discriminant validity of the I-TFI

Both the convergent and discriminant validity of the instrument were assessed at T0 using Pearson's correlation coefficients calculated between the total I-TFI score and: a) the THI; b) the BDI-PC, c) the NRS-A. The descriptive statistics and correlation matrix of all of the instruments employed are reported in Table V.

The total I-TFI score showed a high positive correlation with both the THI and the NRS-A, demonstrating acceptable levels of convergent validity with measures related to

Table V. I-TFI convergent and discriminant validity: Pearson's correlation indices and descriptive statistics.

	1.	2.	3.	4.
TFI (total score)	-			
THI	**0.77****	-		
NRS-A	**0.70****	0.64**	-	
BDI-PC	<u>0.46**</u>	0.63**	0.43**[a]	-
Min-Max	3-95	0-94	0-10a	0-17
M	44.08	37.64	5.92a	2.31
SD	23	21.75	2.09a	2.98

*M: Mean; SD: Standard deviation; **Bold**: Convergent validity; <u>Underlined</u>: Discriminant validity; ** $p < 0.01$; [a]N: 125.*

tinnitus construct. Moreover, acceptable levels of discriminant validity are suggested by the low association between the total I-TFI score and that of the BDI-PC. Even though both depression and tinnitus can be considered weakly related, as both are an expression of the health-illness continua, each dimension is characterised by distinctive features.

Discussion

This is the first study aimed at examining the psychometric properties (i.e. the factor structure, internal consistency and test-retest reliability, and validity) of the Italian TFI in a clinical sample of Italian adults experiencing tinnitus. It is important to carry out thorough evaluation of the psychometric properties of a questionnaire in order to assess whether this instrument is a reliable and valid form of measurement.

Unlike the original structure, analysis of the I-TFI demonstrates that the best factorial structure is four factors. Factor 1 includes items that originally loaded on Intrusiveness (1-3) and Sense of Control (4-6), and item 24 ("*how bothered or upset have you been…*") which originally loaded on Emotional distress. Factor 2 comprises the original Cognitive (7-9), Quality of Life (19-22) and Emotional (23, 25) items. Factor 3 includes the three Auditory items (13-15). Factor 4 contains the Sleep (10-12) and Relaxation items (16-18).

Similarly, the evaluations of TFI versions translated into other languages show some uncertainty regarding its factor structure: using the eigenvalues > 1 criterion, the Dutch version found 7 factors, the Swedish 6 factors and the Polish version identified 5 factors; the German TFI extracted 5 factors adopting the less restrictive Jolliffe's criterion (eigenvalues > 0.7).

Similar to the German validation, our study groups the original Intrusive and Sense of Control items in Factor 1, but with the addition of item 24 (Intrusive/Sense of Control/item 24). A possible explanation of the attribution of item 24 to the same factor loading Intrusiveness and Sense of Control items could be the Italian translation: in fact, the Italian translation for "bothered" (item 24) is similar (but not the same) as "annoyed" (item 3). Furthermore, in our study item 24 showed comparable loadings with factor 2, which included Emotional items.

Factor 2 of our extracted factors included three subscales of the original TFI: Cognitive, Quality of Life and Emotional. This result is supported by a similar finding during the validation of the German version for Switzerland; the Dutch version also combined the two subscales, Cognitive and Quality of Life, in one factor. This result can be justified by examining the content of the items involved in the Emotional and Cognitive scales. The former presents the topics of activation (two items) and depressed mood (one item), while the latter includes items concerning attention capacity. A link could be hypothesised if we were to consider the influence of hyperactivation on attention skills. The item concerning mood in the Emotional scale, together with items about activation, could have some concept points in common with the scale of Quality of Life, where items are about the pleasure of being involved in social activities or other commitments, linking anxiety and depressive moods with a decreased hedonia.

Factor 3 is the only factor to be defined individually and corresponds to the Auditory subscale of the questionnaire; moreover, it has high factor loadings (0.84, 0.98, 0.91). Validations in other languages, even those that did not confirm the eight-factor structure, also found the Auditory factor to be separate. Recently, Fackrell et al. suggested that the Auditory subscale does not contribute to the level of global functional impact of tinnitus and proposed a modified seven-factor model, without the Auditory items, which fits the variance in the patients' scores better [31].

Factor 4 incorporates the Sleep and Relaxation items. Herein, features of tinnitus often disturb the falling asleep phase, during which the transition from the vigil state of consciousness to the state of sleep requires relaxing parasympathetic processes.

An EFA with the superimposed eight-factor structure was also performed to investigate whether the original structure suggested by Meickle et al. could be confirmed. In this case, primary loadings of the items loaded on the original factors with the exception of item 16 that loaded on Sleep. Furthermore, the definition "*quiet resting activities*" (item 16) in the Relaxation subscale, is translated as "*sleep*" in the Italian version. This result suggests that further data must be collected to combine the results of a confirmatory analysis with those of the exploratory analysis.

The Italian version of the TFI shows very high levels of

internal consistency with Cronbach's alpha: above 0.90 for the overall TFI and for each of the four factors.
The test-retest reliability analysis was carried out on a subgroup of patients and the results showed acceptable to good levels of test-retest reliability ($0.79 \leq \alpha \leq 0.85$).
In order to assess convergent and discriminant validity, the I-TFI was compared with the original THI, the most widely used tinnitus questionnaire in Italy, which has already been validated in the Italian language and used in other validation studies. The results show a strong positive correlation with the THI, thus confirming that the I-TFI and the THI measure a similar construct.
The I-TFI also shows a high convergent validity with the scores for tinnitus annoyance; this was predictable as the TFI includes aspects related to the annoyance experienced by patients with tinnitus. Instead, the global I-THI score showed marginal correlations compared with the BDI-PC scores, thus indicating acceptable levels of discriminant validity.
There are some limitations in this study. First of all, the number of patients recruited was limited and insufficient to enable us to perform a Confirmatory factor analysis; further studies on a larger number of subjects suffering from tinnitus are needed. Secondly, we did not evaluate the I-TFI responsiveness to treatment-related changes and therefore further research is needed to address this point.

Conclusions

The difficulties in reproducing the original eight-factor structure are consistent with other TFI validation studies on European language versions of the questionnaire, which also failed to reproduce the original eight-factor structure. In spite of this, the factorial structure of the Italian version of the TFI is characterised by high levels of reliability and validity. Overall, the Italian adaptation of the TFI is suitable for measuring the impact of tinnitus on the daily lives of individuals.

Acknowledgements

We would like to thank Michele Gunness from Oregon Health & Science University for providing us the licence of this version.

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ACTA OTORHINOLARYNGOLOGICA ITALICA 2020;40:238-239; doi: 10.14639/0392-100X-2150

Letter to the Editor

Proposal for a new nomenclature of tracheo-oesophageal puncture: a different perspective

Proposta di una nuova classificazione delle fistole tracheo-esofagee per il posizionamento della protesi fonatoria: una nuova prospettiva

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KEY WORDS: tracheo-oesophageal puncture, total laryngectomy, voice and pulmonary rehabilitation, new classification

PAROLE CHIAVE: *protesi tracheo-esofagea, laringectomia totale, riabilitazione fonatoria e polmonare*

Dear Editor,

The management of laryngeal cancer is focused on improving survival while preserving function; nevertheless, total laryngectomy is often required for primary and recurrent disease [1].

While total laryngectomy is undoubtedly an effective oncological surgery, it profoundly alters speech, respiration and sense of smell and taste. Specifically, the loss of voice has the impact on the psychosocial and economic consequences following laryngectomy [2 3].

There are three methods of voice rehabilitation after total laryngectomy: electrolarynx, oesophageal speech and tracheo-oesophageal (TE) speech. Historically, oesophageal speech was the method of choice by which all others were compared, and patients who could not master oesophageal speech used the electrolarynx. In 1969, Staffieri [4] introduced a surgical voice restoration technique called "phonatory neoglottis surgery": this was a personal technique that allowed one-way air transit from the lungs to the hypopharynx or oesophagus through a fistula between the trachea and the hypopharynx or oesophagus. In 1977, Amatsu [5] with a different surgical technique that included a posterior tracheal wall flap, namely the "Amatsu tracheo-oesophageal shunt", achieved similar results. Both authors addressed the issue of frequently occurring aspiration and the use of a trachea-oesophageal prosthesis placement to manage failures. In 1972, Mozolewski first described a TE shunt prosthesis with a valve function. In 1980, Singer and Blom [6] proposed a simplified endoscopic method for voice restoration. They addressed the problems of aspiration and stenosis (of the fistula) by means of a valved prostheses placed inside the TE fistula. This procedure was initially proposed as a secondary salvage technique for patients who failed oesophageal speech or those who were displeased with the electrolarynx voice. Maves and Lingeman [7] and Hamaker et al. [8] were the first to introduce TE puncture with voice prosthesis as a primary technique, performed at the time of laryngectomy.

Nowadays, TEP with voice prosthesis is the gold standard for voice rehabilitation after total laryngectomy [9].

Received: March 14, 2018
Accepted: June 15, 2018
Published online: August 8, 2019

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Funding
None.

Conflict of interest
The Authors declare no conflict of interest.

How to cite this article: Leone CA, Capasso P, Russo G. Proposal for a new nomenclature of tracheo-oesophageal puncture: a different perspective Acta Otorhinolaryngol Ital 2020;40:238-239. https://doi.org/10.14639/0392-100X-2150

In the literature, there is little agreement on the nomenclature of tracheo-oesophageal puncture (TEP): the terms currently used mostly include *primary* or *secondary* TEP. In the first case, the puncture is performed at the time of the laryngectomy, while in the latter the puncture is performed at a later time. Regarding the voice prosthesis, it can be placed at the same time of the puncture (primary voice prosthesis placement) or after a period of temporary stenting of the fistula using a catheter or feeding tube before initial voice prosthesis insertion.

Unfortunately, *secondary TEP* is a heterogeneous group in which at least two subgroups of patients are present: the first (A) concerns patients with planned secondary TEP after pharyngo-laryngectomy, flap reconstruction, or adjuvant radiotherapy in whom TEP is postponed because of the high risk of complications or dissection of the TE space (e.g. gastric pull-up), and therefore the TEP is performed after healing of the surgical wound. In these cases, the surgeon has planned voice rehabilitation with the voice prosthesis prior to surgery and has discussed this with the patient. The second subgroup (B) includes patients who underwent total laryngectomy in the past and are dissatisfied with (oesophageal) speech. In most of these cases, at the time of total laryngectomy the surgeon did not anticipate the use of heat and moisture exchangers (HMEs) and voice prosthesis, and the stoma might be more difficult for TEP voicing. In some of these cases, a stomaplasty might help to create a better stoma for HME use and occlusion.

We believe that these two subgroups of patients among the so-called "secondary TEP" are different from each other in terms of surgical planning of the TEP (refinements or not) and timing of TEP after total laryngectomy (months or years). In order to distinguish these two subgroups of patients, to better understand their follow-up and to highlight the criterion of time in the planning of the TEP, we propose a different nomenclature for TEPs:

- simultaneous TEP (former primary TEP);
- sequential TEP (former secondary TEP, subgroup A);
- delayed TEP (former secondary TEP, subgroup B).

We also believe that the surgeon should try to create a flat regular stoma and prevent hypertonicity (cricopharyngeal muscle myotomy, circular stoma, sternal head of sternocleoidmastoid muscle section) regardless of the voice rehabilitation chosen after total laryngectomy. A regular flat stoma is required for pulmonary rehabilitation with HME filters for all patients.

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In Memoriam of

Roberto Molinari (1933-2020)

"I am immensely saddened to hear the terrible news of the passing of our dear friend Roberto. He was a giant in our field. I will pray to the almighty God, to rest his soul in peace, and give his family and our fraternity the courage to bear this loss."

This is the memory of Roberto Molinari by Jatin Shah.

We who have had the privilege of working with him for many years want to express our gratitude for all that we have learned by sharing daily clinical and research activities with him. Dr. Molinari worked with great passion, patience, and determination to realize his dream: a head and neck department of excellence. When he started working at the National Cancer Institute in Milan, the surgery department consisted of a single room with dozens of beds in which patients were hospitalized without distinction. The opening of the new monobloc gave space to his project that has become an international reference centre over time. He motivated a group of enthusiastic young people, with whom he developed demolitive and reconstructive surgical techniques.

He supported and disseminated the concept of multidisciplinarity, collaborating with radiotherapists and oncologists. He devised a polychemotherapy schedule (VBM) and tested the potential of preoperative endoarterial chemotherapy, thanks to a consolidated collaboration with Dr. Bonadonna, encouraged by the personal friendship that saw them visiting many European countries together with their families during the summer holidays. He took care to train head and neck oncologists thanks to several initiatives of which he was a tireless protagonist: the monthly meetings on Monday evening in Varese to define head and neck guidelines with surgeons, radiation therapists and members of the GLOCC (Gruppo Lombardo di Oncologia Cervicocefalica); the live surgery courses; chapters in the handbooks of medical and surgical oncology; meetings of the national task force of the head and neck; coordination of the cooperative group head and neck of the EORTC. His department had really become a "school" and many of today's Italian and European head and neck surgeons and oncologists owe him a lot.

He deeply loved his profession; apparently reserved, he was instead open and available to any request and occasion for meeting and sharing. In the department there was a climate of friendship and collaboration that was achieved not only in clinical activity and research, but also in opportunities for aggregation outside the work environment: it was a tireless median, pillar of the football team of the institute; he played tennis with his collaborators early in the morning before starting daily practice.

He loved to sing and had a wonderful voice that all the participants at conferences of the institute had learned to know. In fact, he presented and commented on the live surgery courses (not only those head and neck) with his unmistakable voice that will always remain in our memory.

Fausto Chiesa