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Laryngeal preneoplastic lesions and cancer: challenging diagnosis. Qualitative literature review and meta-analysis.

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ABSTRACT

Background: Multi-step cancerogenesis guides laryngeal cancer onset and it includes a wide variety of pre-cancerous lesions macroscopically challenging to identify and distinguish from initial cancerous foci.
Object: Different modalities of diagnostic techniques of laryngeal epithelial lesions exist and they do not offer a single system to make a differential diagnosis. Hence, this meta-analysis aimed to synthesize the validity of each single diagnostic tool to improve laryngeal patient management.
Methods: A systematic review of literature was led searching for articles mentioning the following terms: larynx, laryngeal precancerous lesions, laryngeal cancer, white light (WL) endoscopy, stroboscopy, contact endoscopy (CE), autofluorescence (AF), ultrasound (US), narrow band imaging (NBI), computed axial tomography (CAT), magnetic resonance imaging (MRI), positron emission tomography (PET), CAT/PET. Then, a quantitative analysis was carried on for paper published after 2005 onward.
Results: The search identified 7215 publications, of which 3616 published after 2005, with a final results of a total of 214 articles stratified and included by our selection criteria. 42 out of 214 articles were selected for quantitative synthesis. 25 out of 41 studies had a good quality score, 16 were fair.
Conclusions: A comprehensive overview of the most recent advances in laryngeal imaging technology combined with all of the information needed to interpret findings and manage patients with voice disorders can be found herein. Our flow-chart allows clinicians in risk-stratify patients and select proper examination modalities to provide appropriate care. Study limitations, together with possible clinical and research implications have been counted.

1. INTRODUCTION

In otolaryngology, hoarseness is the most common laryngeal symptom for which patients seek treatment and may be caused by a diversity of potential disorders. In this setting, visualization of the structure and function of the vocal folds has become an essential component of the clinical voice assessment protocol (Mehta and Hillman, 2008; Deliyski and Hillman, 2010), because the capacity of history and physical examination alone to yield adequate information to get the right diagnosis or even risk-stratify patients is only 5% compared with a 68.3% accuracy following an initial endoscopic laryngeal evaluation (Paul et al., 2013).

Because of the wide range of potential causes for the dysphonia, determining the precise etiology of the laryngeal/voice disorder is necessary to plan treatment. Since malignant transformation rate of premalignant lesions ranges from 6% to 22%, and it increases with the severity of the precancerous nature, their early detection is of paramount importance. In this setting, early detection and preoperative assessment are important to a curative and function-preserving therapy, because the treatment of laryngeal cancer and its precursor lesions has a great impact on important basic functions of daily life such as breathing, verbal communication and swallowing. Furthermore, delayed diagnosis, leading to loco-regional failure, and a high incidence of second primary are the two main reasons for poor outcome.

From these observations, it is obvious that obtaining images of high quality and resolution, revealing the detailed morphology of the glottal structures, is one of the main tasks in laryngeal imaging. Such images are essential in making correct diagnosis and choosing the treatment to gain the best result. Attempts to examine the human larynx date back more than 150 years, in fact, in 1854 Manuel Garcia, a vocal music teacher, examined his own larynx using a dental mirror and a hand mirror, and published his observations in 1855 (Alberti, 1996). Imaging technologies applicable in laryngology developed enormously since then. Nowadays, the diagnostic procedure of laryngeal diseases in clinical practice is rather complex and is based on the evaluation of patient's complaints, history, and data of instrumental as well as histological examinations. During the last two decades a variety of imaging techniques for the examination of the larynx and obtaining objective measurements of voice quality have been developed (Mafee et al., 2005; Uloza et al., 2005).

For instance, the term "laryngeal imaging" typically refers to the endoscopic imaging of vocal fold tissue clinical aspect and vibration via videoendoscopy and videostroboscopy; in addition, evaluation of larynx has improved significantly with the establishment of computed axial tomography (CAT) and magnetic resonance imaging (MRI), as these technologies provide insights into the endoscopically blind areas and reveal the depth of tumor infiltration (Gallivan and Gallivan, 2002). These technologies may be beneficial in staging laryngeal carcinoma and planning the most appropriate surgical procedure (Rumboldt et al., 2006; Hoorweg et al., 2006). Recently, ultrasonography has become useful as well in cases of larger laryngeal lesions and may have some role in screening for unilateral vocal fold pathologies. At the same time, further fine-tuning of the technique may be necessary (Rubin et al., 2004a; Schade et al., 2003).

Despite all of these efforts in achieving the perfect diagnostic tool, nowadays surgeons cannot trust one single imaging technique to plan laryngeal patients management and treatment; in fact, recent articles, published in literature, emphasized the need for further technological, methodological and clinical research on laryngeal imaging, including the development of clinical norms and objective image processing and measurement methods (Deliyski, 2007; Doellinger, 2009; Verikas et al., 2009).

This paper provides a review of the latest advances in laryngeal imaging over the last decade in order to give a comprehensive overview of the most recent progress in laryngeal imaging technology combined with all of the information needed to interpret findings and successfully manage patients with voice disorders.

2. LARYNGEAL DIAGNOSTIC SYSTEMS

In patients with dysphonia, the diagnostic accuracy from history and physical exam, excluding laryngoscopy, is only 5% compared with a 68.3% accuracy following an initial endoscopic laryngeal evaluation (Lichtenstein and Jaffee, 1943). Because of the wide range of potential causes for the dysphonia, determining the precise etiology of the laryngeal/voice disorder is necessary to plan treatment. Since malignant transformation rate of premalignant lesions ranges from 6% to 22%, and it increases with the severity of the precancerous nature, their early detection is of paramount importance. For instance, survival rates are significantly higher for early stage carcinomas, therefore, it is essential to concentrate on the

initial steps in tumor development in order to facilitate early detection and timely implementation of suitable therapy (Ferlito et al., 2000). Early detection and preoperative assessment are important to a curative and function-preserving therapy, because the treatment of laryngeal cancer and its precursor lesions has a great impact on important basic functions of daily life such as breathing, verbal communication and swallowing.

From these observations, it is obvious that obtaining images of high quality and resolution, revealing the detailed morphology of the glottal structures, is one of the main tasks in laryngeal imaging. Such images are essential in making correct diagnosis and choosing the treatment to gain the best result.

Nowadays, there is a wide choose of different diagnostic tools, but each of them presents specific properties and limits that influence their usage in common practice. Herein a summary of their characteristic.

2.1. Endoscopy

Over time, laryngeal photography was perfected, which enhanced diagnostic accuracy, documentation, and education. Shortly thereafter, in 1895, both stroboscopy and direct laryngoscopy were introduced, which dramatically advanced laryngoscopy and laryngology (Oertel, 1895). In the early 20th century, direct laryngoscopy and endolaryngeal surgery migrated to the operating room for the promise of improved surgical precision (Zeitels, 2004).

In laryngological and phoniatric diagnostics, white light laryngoscopy (WL) is the key procedure for functional investigation and early detection of neoplastic tissue (Krausert et al., 2011). In fact, at present, white light laryngoscopy combined with biopsy is the standard diagnostic procedure in the assessment of laryngeal cancer and precancerous lesions. However, white light laryngoscopy provides poor quality images and has difficulty identifying minute epithelial changes and directly differentiating benign from malignant tumors *in vivo*.

Furthermore, with imprecise laryngeal diagnostics and lack of clinical experience, very early stages of malignant lesions, such as dysplasia or carcinoma in situ (CIS), can be overlooked, because they often present themselves as low-contrast mucosal changes with superficial roughness or reddening and rarely have an obviously characteristic malignant aspect (Gugatschka et al., 2008).

2.2. Stroboscopy

Stroboscopy is considered to be an important part of diagnosing patients with laryngeal dysplasia. Nevertheless, we must note that a strict correlation between a vocal fold vibratory pattern and a certain type of lesion does not exist. Vocal fold pathology may produce changes in the appearance and vibratory patterns observed during stroboscopic examination. Interpreting the stroboscopic examination involves systematic judgment and describing the different vibratory pattern signs. These signs, which were first identified by Hirano and Bless (1993) included the fundamental frequency and periodicity, amplitude of horizontal excursion, glottal closure, symmetry of bilateral movement, mucosal wave, and non vibrating portions of the vocal fold.

Quantitative characterization of the vibratory behavior of the vocal folds is a pivot task to add to the diagnostic step. A well-known drawback of video-laryngo-stroboscopy (VLS) is the reliance on quasi-periodic voice signals (Kendall and Leonard, 2010) when producing a real-time slow-motion stroboscopic effect. Objective measurements of the vocal fold vibration pattern date back to the initial high-speed cinematography recordings of vocal fold vibration from the works of Moore and von Leden (1958), Timcke et al. (1958, 1959) and Timcke (1960).

Nowadays, because of the multiple factors related to the complex vocal fold vibratory system the actual clinical application of objective measurements has not yet been achieved.

Several indices, describing the glottal wave form, are usually used for the characterization. They provide mainly the "functional information" about the vibratory function of the vocal folds and the glottal closure, and when they are matched with direct endoscopic images, they provide together more detailed information about structural and morphological peculiarities which are both fundamental in making the correct diagnosis. In fact, this tool is able to reveal a number of abnormalities, including abnormalities of laryngeal structure, absence of vibration, and vibratory asymmetry. Flashing light is used to illuminate an object in stroboscopy. When the flashes are synchronized with the vocal fold vibrations, a stationary view of the vocal folds is obtained. However, the single-flash-timing laryngeal videostroboscopy has a limitation that it is effective only when vocal fold vibrations exhibit only one single fundamental frequency. Multiple tones (fundamental frequencies) may be recorded in the presence of some diseases, such as polyps, nodules, cysts or precancerous lesions (Deguchi et al., 2007).

Quantitative measures of motion and geometry of vocal folds can provide objective information and may be useful in planning medical treatment and tracing progress over time. Few studies have indicated which stroboscopic signs are more significant than others in evaluating the vibratory pattern of vocal folds with premalignant lesions. Despite there some known features that cannot be imaged with stroboscope, such as voice breaks, diplophonia, vocal function during voice onset and voice offset, vocal tremor and spasms, extremely rough voice quality, alternate laryngeal and pharyngeal sources of oscillation (Woo, 2014), the main limitation with the VLS remains the subjective nature of the interpretation of laryngeal phonatory function examination results, which significantly reduces the reproducibility and the use of VLS as a research tool or even as a quantitative instrument for comparing outcomes of treatment of voice and laryngeal disorders. In fact, there is a lack of data in the literature about the specificity and sensitivity of VLS parameters discriminating normal and pathological voices.

The discrepancy in diagnosis with VLS seems to be linked to certain key points: (1) during office endoscopy, tangential views of the medial surface of the glottis limit the diagnostic sensitivity; (2) sulci and mucosal bridges are most subject to this limitation; (3) informed consent should address the potential need for a change in intraoperative management. It is advisable to discuss the possibility for dissection in both vocal folds, even if a unilateral lesion is observed in the office; (4) suspension microlaryngoscopy (SML) is the final diagnostic step in the evaluation of glottic pathology (Dailey et al., 2007).

2.3. Contact endoscopy

Contact endoscopy (CE), first described in 1979 by Hamou, offers an additional *in vivo* diagnostic procedure based on the staining of the superficial mucosal layer and direct *in vivo* and *in situ* examination of the epithelial cells. The basic technique of CE involves staining of the superficial cells of the mucosa with 1% of non-toxic methylene blue before the magnification of the suspected areas through the direct contact of the tip of an endoscope to the mucosal surface to obtain cytological images (Puxeddu et al., 2015). CE enables visualization of the laryngeal mucosa pathology through high magnification and therefore detailed examination of cells and blood vessels without requiring tissue biopsy. This non invasive method allows *in vivo* visualization of vascular alterations of the mucosa and rates either as benign or malignant.

The use of contact endoscopy in otolaryngology was first described in 1995 by Andrea et al. however, up to now, only a few groups have evaluated this technique for *in vivo* histology

(Arens et al., 2003; Cikojevic et al., 2008; Andrea et al., 1995a,b). In fact, expansion of squamous epithelium from the vocal fold edges to the areas of columnar epithelium can be clearly visualized by contact endoscopy; hyperkeratosis (i.e. deposits of anuclear cells on the epithelial surface) or leukoplakia are clearly observed on contact endoscopy, and the grade of dysplasia that may occur in association with them is generally indicated by the impaired nucleus/cytoplasm ratio, nuclear hyperchromasia, and variation in the number and appearance of the nucleoli (Cikojevic et al., 2008).

False negative results may occur in case of incomplete penetration of the stain throughout the epithelial thickness thus hindering the identification of the grade of dysplasia; in this setting the presence of secretion decreases stain penetration, while the secretion itself precludes any direct contact between the endoscope and the mucosa epithelium. Another situation that may influence the incidence of false negative is the diagnosis of carcinoma *in situ*. Carcinoma *in situ* is characterized by heterogeneity of the cell population; however, angiogenesis is not present because the tumor process does not cross the basement membrane. It is therefore difficult to differentiate carcinoma *in situ* from invasive carcinoma. The finding of angioneogenesis definitely indicates carcinoma, whereas its absence does not exclude the possibility of invasive carcinoma (i.e.: the diagnosis of carcinoma *in situ* cannot be made with certainty). Finally, the presence of abundant necrosis in case of large tumor mass could cause bleed when touched by the endoscope thus impairing tissue staining and analysis of cell population.

For all these reasons, the reliability of contact endoscopy in literature is reported within 75% to 88% (Wardrop et al., 2000; Carriero et al., 2000).

In summary, CE can examine only limited cellular architecture of the epithelium. This is due to the poor penetration of methylene blue, which only dyes superficial layers and potential optical artifacts at high magnification due to glare from light reflected from cells that are not in focus (the focal distance of the endoscope is 80 mm at 603 magnification and 30 mm at 1503 magnification) (Ledda and Puxeddu, 2006).

In specific, difficulties of interpretation of the vascular patterns increase in the follow-up of the upper aero-digestive tract (UADT) cancers due to the changes of the surrounding tissues as a consequence of previous treatments such as radiotherapy. In order to improve these lacks, Storz Professional Image Enhancement System (SPIES) (KARL STORZ GmbH & Co., Tuttlingen, Germany) has been recently proposed as a novel digital technique providing specific color renderings that pronounce the spectral separation of the recorded broad visible spectrum within the high-definition camera system and it does not require a narrow band light source. SPIES enhances the appearance of the mucosal surface structures and subepithelial vasculature by selected wavelengths of light, providing in addition to the standard mode using white light (WL), five different defined spectral ranges (Clara, Clara1- Chroma, Chroma, Spectra A, Spectra B). The main target of analysis of these techniques is neoangiogenesis, a prerequisite for the progression of precancerous and cancerous lesions of the upper aero-digestive tract (UADT) (Puxeddu et al., 2015). SPIES, as well as narrow band imaging (NBI), allow for the recognition of the superficial changes of neoangiogenesis, and they require a certain degree of experience to avoid false positives, because the typical mucosal "spots" are not univocal and are challenging for clinical interpretation. It appears to have good sensibility (79.6%–94.7%), specificity (81%–95.5%), and accuracy (88%–94%) in the larynx (Arens et al., 2003) for distinguishing between benign and malignant mucosal lesions.

2.4. Autofluorescence

Autofluorescence (AF) is defined as natural fluorescence emission of tissue arising from endogenous fluorophores after exposure and activation by radiation of a suitable wavelength. In its resting state, a fluorophore is at a stable energy level at which it does not fluoresce. When a fluorophore is illuminated, its electrons are promoted to a higher energy level. In this excited state, the fluorophore is unstable and will quickly revert to a slightly more stable lower energy level by releasing heat. To return to its baseline the fluorophore emits light. Since some energy has already been released as heat, the emitted light is of lower energy and longer wavelength than that of the illuminating light (Hughes et al., 2010). Fluorophores are present at different concentrations in healthy and neoplastic laryngeal mucosa; for example, nicotinamide adenine dinucleotide (NADH) predominates within neoplastic cells in its dehydrogenated nonfluorescent form (Richards-Kortum and Sevick-Muraca, 1996; Schwartz et al., 1974). Several fluorophores are normally found within laryngeal mucosa including prophyrins, elastin, collagen, and NADH (Gillenwater et al., 1998; Malzahn et al., 2002).

Because each fluorophore has a specific wavelength at which its electrons are maximally excited, it is possible to target specific fluorophores, such as NADH, using an illuminating light of a single wavelength (monochromatic). The underlying fundamental principle of different fluorescence emission in AF is related to neoplasia-induced changes in terms of tissue morphology, optical properties, and concentration of endogenous fluorophores (Wagnieres et al., 1998). Autofluorescence diagnosis is based on the ability of oxidised flavin mononucleotide (FMN) in the normal cells to emit green fluorescence when exposed to blue light. Nicotinamide adenine dinucleotide plus hydrogen (NADH) and flavin adenine dinucleotide (FAD) are important intracellular fluorophores found in all tissue layers; their concentration is nearly 100 times lower in malignant tissue than in benign tissue (Uppal and Gupta, 2003), therefore, malignant cells do not have fluorescence to the same degree as benign cells (Baletic et al., 2004).

At present, autofluorescence endoscopy (AFE) is increasingly being used for early detection of malignant mucosal changes in diverse areas of localization and various medical specialties (Jacobson et al., 2012; Sieron-Stoltny et al., 2012). It is argued that the advantage of AFE compared to conventional white light endoscopy is based on the fact that premalignant and malignant lesions might be differentiated clearly from normal tissue because of decreased AF.

In 1924, Policard observed the ability of tissue to fluoresce under certain conditions. Alfano et al., in 1984, reported the possibility of differentiating between healthy and malignant tissue by means of their fluorescent characteristics. In 1933, Sutro and Burmann described the phenomenon of the different fluorescences of normal and tumor tissue.

Harries et al. (1995) first used autofluorescence to identify neoplastic cells within the laryngeal mucosa, he demonstrated in a pilot study of eight cases with carcinoma of the vocal folds concluded that the technique can increase the accuracy of staging of cancer of the larynx and allows earlier diagnosis of tumors and their recurrence. In the same year Chissov et al. (1995) reached the same conclusion. Malzahn et al. (2002) reported a sensitivity of 97.3% and specificity of 83.8% for this technique in detecting precancerous and cancerous lesions in 127 patients. Beneficial role for AF has been ask reported by other works (Zargi et al., 2000; Arens et al., 1999), even if it has been identified a high rate of false positive results, as well (Delank et al., 2000).

In summary, despite promising literature evidence for this technique, there are, however, several limitations to autofluorescence examination. First, the illuminating light does not penetrate through diseased epithelium (Baletic et al., 2004; Mostafa et al., 2007). As a result, epithelial hyperkeratosis may hide neoplastic changes within the basal mucosal layer (Arens et al., 2006a). Second, granulation tissue and teleangiectasia produce a sim-

ilar reduction in bright-green fluorescence, attributed to the absorbative properties of a heme molecule, which makes them indistinguishable from neoplasia by autofluorescence (Malzahn et al., 2002). Third, scar tissue, necrosis, and inflammation can unpredictably alter mucosal fluorescence (Malzahn et al., 2002; Zargi et al., 2000; Mostafa et al., 2007). Thus, several conditions in the larynx may produce false positive and false negative findings.

In conclusion, further studies to increase the sensitivity and specificity and investigation of technical solutions to reduce the number of false negative and false positive cases are required.

2.5. Narrow band imaging (NBI)

Narrow band imaging (NBI) is an optical image enhancement technology that enhances vessels in the surface of mucosa using the characteristics of the light spectrum (Sano et al., 2001). The NBI system consists of the same components as conventional videoendoscopic systems: a light source, a camera unit, and a camera head or chip-equipped videoendoscope. Additionally, the NBI system contains a special image processor and a lighting unit with special filters that narrow the frequency range of emitted light to 400–430 nm (centered at 415 nm, blue) and 525–555 nm (centered at 540 nm, green) bands. Since the blue light wavelength (415 nm) is absorbed by hemoglobin the capillary blood vessels are seen brown in the summary picture. Currently, available evidence indicates that NBI may be a promising approach in the diagnosis of laryngeal cancer. It allows to visualize the structure of the intraepithelial blood vasculature which cannot be seen with conventional white light (Irjala et al., 2011). In fact, the emitted light has less penetration and less scattering and is highly absorbed in hemoglobin, thus enhancing the image resolution. The reflection is captured by a charge-coupled device chip (CCD), and an image processor creates a composite pseudocolor image, which is displayed on a monitor, enabling NBI to enhance mucosal contrast without the use of dyes (Piazza et al., 2008). The detection of surface mucosal changes that are characteristic of neoplastic lesions (e.g., dysplasia, in situ carcinoma, and carcinoma), epithelial abnormalities (thickening and changes in the surface layer), and vascular changes can be best achieved with NBI.

In the NBI mode, normal laryngeal mucosa consists of submucosal vessels (appearing green) connecting with an arborescent vascular network (appearing dark brown). Abnormalities of these intraepithelial papillary capillary loop (IPCL), located beneath the basement membrane of epithelium, are usually classified in accordance with their shape changes; such changes have been found to predict the depth of superficial cancer invasion and are classified into five different categories (Kumagai et al., 2002; Ni et al., 2011). In type I, the intraepithelial papillary capillary loops are almost invisible, and oblique and arborescent vessels of small diameter can be clearly seen. In type II, the intraepithelial papillary capillary loops are also almost invisible, but the diameter of the clearly observed oblique and arborescent vessels is enlarged. In type III, the mucosa is white and the intraepithelial papillary capillary loops cannot be seen; if the white patch is thin, the oblique and arborescent vessels may be seen indistinctly, but if the white patch is thick the vessels will be obscured. In type IV, the mucosal intra- epithelial papillary capillary loops are visible with a relatively regular arrangement and low density, the capillary terminals are bifurcated or slightly dilated, and the intraepithelial papillary capillary loops appear as scattered, small, dark brown spots; the oblique and arborescent vessels are usually not visible. Type V changes are subdivided into types Va, Vb and Vc according to the shape, regularity and distribution of vessels. In type Va, intraepithelial papillary capillary loops are significantly dilated and of relatively high density, and appear to be solid or to have hollow, brownish, speckled features and various shapes. In type Vb, the intraepithelial papillary capillary loop itself is destroyed, with its remnants presenting in a snake-, earthworm-, tadpole- or branch-like shape, and the microvessels are dilated, elongated and 'woven' in appearance. In type Vc, the lesion surface is covered with necrotic tissue, and the intraepithelial papillary capillary loops present as brownish speckles or tortuous shapes of uneven density which are irregularly scattered on the tumor surface. Lesions viewed under NBI are usually recorded as: (1) malignant (i.e. type V); (2) suspected malignant (i.e. protuberant or ulcerative lesions covered with necrotic tissue, or leukoplakia of unknown type); or (3) benign (types I to IV) (Ni et al., 2011). In the literature, there exist only a few publications on the exclusive use of NBI in laryngology (Ni et al., 2011; Watanabe et al., 2009; Piazza et al., 2010a; Bertino et al., 2015a; Masaki et al., 2009; Piazza et al., 2012; Tjon Pian Gi et al., 2012; Imaizumi et al., 2012). However, several studies assessed the value of NBI in the head and neck also including the larynx (Irjala et al., 2011; Piazza et al., 2010a; Watanabe et al., 2008; Ugumori et al., 2009; Lin et al., 2010; Piazza et al., 2011; Shinozaki et al., 2012; Lin et al., 2012). NBI has shown in the larynx, over last years, improved sensitivity 61-91% and specificity 87-92% (Piazza et al., 2011; Yang et al., 2012) but false positives and false negatives have been reported, especially in the larynx, and increase with a short learning curve. This approach is promising for better discrimination of malignant and benign lesions as part of "prehistologic diagnosis" or "optical biopsy" (68). Nevertheless, there is no ultrathin, zooming, flexible videoendoscope available for ENT purposes; therefore, a combination of rigid telescopes and an HDTV camera head must be used to achieve sufficient resolution and ultrahigh magnification (Lukes et al., 2014). Although most authors concur that NBI with or without HDTV is a valid diagnostic tool, this method has some limitations. The most relevant is certainly the possibility of generating, at least in the early phase of the learning curve, an increased number of false positives with consequently unjustified biopsies. This is mostly related to acute inflammation and chronic post-RT changes. Therefore, we believe that the incidence of false positive findings was mainly related to the experience of the examiners more than to specific mucosal changes.

2.6. Ultrasound

Over the past 15 years, there has been an effort by head and neck surgeons to bring optical imaging technology from the research laboratory into the operating theater to improve their ability to identify the tumor margin in vivo to guide surgical excision (Hughes et al., 2010).

Sonography is regarded as the first imaging method routinely used for detecting cervical lymph node metastases from head and neck tumors (Ahuja et al., 2008; Chevallier et al., 2002) but it has rarely been used as an imaging technique for investigation of the larynx. Previous studies in the literature have yielded promising results for high-frequency sonography in diagnosis of laryngeal carcinoma (Kuribayashi et al., 2009; Desai et al., 2004; Gritzmann et al., 1989). However, the use of sonography in tumor staging is still limited.

Since the 1970s, B-mode US imaging has been used with some success in the laryngeal area to identify mass and cystic lesions at the vocal folds (Rubin et al., 2004b) and to detect vocal fold paralysis (Vats et al., 2004; Ooi et al., 1995); moreover, ultrasonography has been also used to evaluate laryngeal tumors in a few studies, and some of these have proved that ultrasonography could assist tumor staging in patients with advanced laryngeal cancer (Gritzmann et al., 1989; Loveday et al., 1994; Hu et al., 2012).

It has been demonstrated that ultrasound has a similar role in the visualization of hypopharyngeal tumor to CAT (Loveday, 2003), thus whether ultrasonography has similar ability in evaluating laryngeal cancer is worthy to be investigated. Thyroid cartilage, pre-

epiglottic space, paraglottic space, thyroid and cervical soft tissues, lying in the larynx anteriorly or superficially, are easy to be imaged with a high-frequency probe. Commonly, these structures are isoechoic or hyperechoic, which provide a contrast with a hypoechoic invading tumor (Loveday et al., 1994). Hence, a high sensitivity and specificity has been achieved by ultrasonography in the evaluation of involvement of these structures and reported in literature (Hu et al., 2012; Xia et al., 2013; Hu et al., 2011).

For instance, Kraft et al. (2010) established anatomical landmarks for laryngeal endosonography in order to allow a correct interpretation of sonographic images of its structures, and its known capacity to visualize critical regions such as the pre-epiglottic and paraglottic space endoscopically. Therefore, endolaryngeal ultrasonography seems to be able to predict the exact extension of a laryngeal tumor before surgery is performed, and to assist in finding the best therapeutic solution for the patient (Arens and Glanz, 1999). Furthermore, given its noninvasive nature and minimal disturbance to normal voice production, medical US imaging should be an ideal tool for studying vocal fold vibration. However, only a few studies have used dynamic US imaging to investigate the vibrating vocal folds (Hsiao et al., 2001; Hsiao et al., 2002). The body motion of the vocal fold, which is ruled by mechanical process described by Hirano (1974, 1975), presents a vibration frequency (f) >70 Hz which is higher than the frame rate (f_s) of dynamic B-mode US (<50 Hz). Under this condition, real-time dynamic motion pictures of vocal fold vibration cannot be obtained using B-mode imaging. If the phonation frequency is tuned to be close to an integer multiple of f_s, a slow motion montage of vocal fold vibration may be obtained with B-mode US imaging. Such motion pictures may provide valuable data for analyzing vocal fold vibration especially in the body. This unique method has been described for the first time by Tsai et al. (2009) who first attempted to represent a dynamic B-mode image of vocal fold vibration.

In conclusion, endosonography of the larynx produces horizontal slice images comparable with computed axial tomography (CAT) or magnetic resonance imaging (MRI) scans but with a higher resolution (Tsai et al., 2009). During the imaging process, it is essential to position the probe as centrally as possible, avoiding contact with the laryngeal wall, it allows to directly measure the antero-posterior and medio-lateral diameters of the laryngeal neoplasm. The orotracheal tube, together with laryngeal structures such as cricoid and thyroid cartilages, are useful anatomical landmarks. Hence, direct observation of sonographic images help in orientation, too.

2.7. Computed axial tomography (CAT) and Magnetic resonance imaging (MRI)

The radiologist makes a valuable contribution to the staging of laryngeal cancer and this has a direct influence on treatment planning. Since approaches with both computed axial tomography (CAT) and magnetic resonance imaging (MRI) are acceptable, the choice in practice depends on machine availability and local expertise as well as the ability of the patient to tolerate a prolonged MRI examination. Cross sectional imaging provides information on tumor volume, extension to and across the laryngeal ventricle, infiltration of pharynx, para-glottic, pre-epiglottic and extra-laryngeal spaces, all of which impact on the potential for voice conserving partial laryngectomies and response to radiotherapy. Together with an assessment of nodal and systemic metastases, this enables the radiologist to play an integral role in the multidisciplinary selection of treatment options.

In specific, radiologists have to describe if the clinical laryngeal lesions presents a deeper extension and if this extension affect main laryngeal structures. They do this by analyzing different parameters as following:

2.7.1. Relationship of the tumor to the ventricular complex

In order to define the tumor as supraglottic, glottic or subglottic the radiologist must determine the level of the ventricular complex (comprising the true cord, false cord and the intervening ventricle). These landmarks are essentially mucosal, and usually they are assessed endoscopically, however, sometimes a bulky tumor could hide its distal visualization. The superior aspect of the ventricular complex is defined by the superior margin of the arytenoids cartilages. The inferior aspect of the ventricular complex is defined by the true cords; radiologically identified by the transition of paraglottic fat to soft tissue in the wall of the larynx. This soft tissue represents the thyroarytenoid muscle. In addition, the true cord lies at the upper margin of the cricoid cartilage (at the level of the anteriorly pointing vocal processes of the arytenoid cartilages). The posterior commissure is seen between the arytenoids cartilages. The subglottic region has a characteristic appearance where the cricoid cartilage is seen as a complete ring providing the foundation of the laryngeal skeleton. Any soft tissue at the subglottic level is abnormal. The presence of an enlarged delphian node anterior to the trachea also indicates subglottic involvement.

2.7.2. Involvement of the submucosal spaces

An understanding of the pre-epiglottic and para-glottic spaces allows the radiologist to predict and identify patterns of tumor spread which have a critical impact on therapeutic decisions. Submucosal involvement of the pre-epiglottic and para-glottic spaces is difficult to evaluate clinically and endoscopically. The fatty C-shaped pre-epiglottic space is bounded anteriorly by the thyroid cartilage and thyrohyoid membrane and posteriorly by the epiglottis and quadrangular membrane. The pre-epiglottic space is continuous laterally with the paired para-glottic spaces.

CT and MRI are 88-93% accurate in the assessment of tumor extension within these spaces (Yeager et al., 1982).

2.7.3. Anterior and posterior extension

In addition to defining the craniocaudal extension within the submucosal spaces relative to the ventricular complex landmarks, it is important to evaluate the anterior and posterior extension of tumor. This is particularly important at the level of the glottis, where usually glottic carcinoma, grows towards the anterior commissure which is frequently associated with thyroid cartilage involvement of the base of epiglottis.

The cartilage is prone to invasion at this site because the internal perichondrium is deficient and the external perichondrium is thinner. The perichondrium covering the cartilage acts as a resistant barrier to invasion by tumor (Yeager et al., 1982). It has been shown that cartilage invasion mainly occurs where the attachment of the collagen bundles interrupts the perichondrial barrier. As the cancer cells multiply, they separate the collagen bundles, forming linear passageways through the perichondrium. Thus, the sites of attachment of the strongest membranes, such as the anterior commissure tendon, are also the most frequent sites of invasion. Invasion of the cartilage framework invariably takes place at the site of ossified or calcified cartilage (Gregor and Hammond, 1987). Non-ossified cartilage is resistant to tumor infiltration due to its capacity to release proteins that inhibit tumor angiogenetic factor and collagenases (Gallo et al., 1992). Recent published reports have shown that CT may yield acceptable sensitivity for detecting neoplastic invasion of laryngeal cartilage if the diagnostic criteria are selected and combined appropriately (Becker, 2000).

However, cartilage invasion is still sometimes overestimated, resulting in unnecessary total laryngectomies in some patients (Kuno et al., 2014).

Previous studies using single-slice spiral CT scanners have concluded that the CT criteria used for determining neoplastic invasion of the thyroid cartilage include erosion, lysis, and transmural extralaryngeal tumor spread (Sulfaro et al., 1989a; Zbaren et al., 1996; Becker et al., 1995; Becker et al., 1997; Beitler et al., 2010).

Tumor may also extend from the anterior commissure to an extra-laryngeal location caudal to the thyroid cartilage (via the cricothyroid membrane) and inferiorly to the subglottis. CAT is 75% accurate in predicting anterior commissure involvement (Barbosa et al., 2005). Predicting the presence of laryngeal cartilage invasion is a key aspect of the imaging assessment.

CAT and MRI are generally used to supplement microlaryngoscopy (MLS) in staging laryngeal cancer. Ideally, imaging should be able to predict the tumor size, the exact extension, a possible midline crossing, and cartilage infiltration in these lesions. Although CT proved to be helpful in the investigation of advanced malignancies (T2–T4), it often fails to show early cancer (Tis, T1 < 10 mm) due to the almost identical density of tumor tissue and the vocal muscle. With MRI, the same difficulties are encountered in demonstrating smaller tumors (Tis, T1 < 9 mm), but the latter can better differentiate cancer from muscle tissue and depict invasion of the laryngeal framework (Hermans, 2006). However, CT and MRI are expensive and cannot be performed during MLS. Further disadvantages are the radiation load of CT, the long acquisition times for MRI, and the need for contrast agents in both methods.

2.7.4. CAT

CAT is the preferred imaging method for staging of laryngeal and hypopharyngeal cancer. Multislice CAT allows the radiologist to evaluate almost all the relevant imaging issues. Voltage of 120-140 kVp, tube current of at least 180 mAs, display matrix of 512 × 512 pixels and a collimation of approximately 1 mm should be used. A single bolus of contrast medium is effective, however a biphasic contrast enhancement protocol may be employed. The images are obtained with the patient supine and during quiet respiration (not while holding the breath). The neck should be in slight extension, and the head is aligned along the cephalocaudal axis to allow comparison of symmetrical structures. A longitudinal field of view extends from the skull base to the sterno-clavicular joints with the patient breathing quietly and not swallowing. Malpositioning may create an appearance that simulates disease. Every effort should be made to make the patient feel comfortable. It may be helpful to perform an additional examination focused on the tumor with e-phonation (for better assessment of the laryngeal ventricle, anterior commissure and aryepiglottic folds) or modified Valsalva (for better assessment of the priform sinus and post cricoid regions) manoeuvres (Kuno et al., 2014). In addition to soft tissue windows, the bone windows (reconstructed with a bony algorithm) should be routinely evaluated if the tumor contacts ossified laryngeal cartilage. It should be ensured that axial images are reformatted in the plane of the larynx.

Spiral CAT is entirely dependent on the emergence of “slip ring” technology, which allows electrical energy to be transmitted to the gantry as it passes along the patient, allowing acquisition of CAT data in a helical fashion. It is also a faster process, with less discomfort to the patient and it is dynamic, with high resolution giving slices in continuity (Baum et al., 2000).

CAT provides a clearer illustration of bony structures and calcification than does MRI. Moreover, it is less expensive, faster and less susceptible to motion artifacts.Sclerosis is the most sensitive criterion but this often corresponds to reactive inflammation, particularly with respect to the thyroid cartilage. For instance, CAT signs of erosion and extra-laryngeal tumor of the thyroid, cricoid and arytenoid cartilages together with sclerosis of the cricoid and arytenoid cartilages result in a sensitivity of 64-72% and specificity of 86-94% for cartilage involvement, with an accuracy value of approximately 80% (Zbaren et al., 1996; Zbaren et al., 1997a; Zbaren et al., 1997b).

2.7.5. MRI

MRI is best performed with a high field MRI scanner. The MR examination requires a neck coil to obtain adequate resolution. Section thickness should be 4–5 mm for the neck coverage and 2–3 mm (0.1-0.2 mm interslice gap) for the focused study of the larynx. The display matrix size is 170 × 256 pixels minimum. A combination of T2-w sequences with fat saturation, T1-w sequences and T1-w fat saturated sequences with gadolinium, should be used in axial and coronal planes. The sagittal plane is also useful to assess potential tongue base involvement. Patients are asked to refrain from coughing and swallowing during the acquisition and MRI may produce poor results in the breathless or restless patient who is compromised by the tumor.

MR imaging is most frequently used if there is uncertainty in assessing cartilage involvement, when this is critical to therapeutic decisions. It may also better define the margin between the tumor and thyroarytenoid muscle and involvement of the tongue base.

MRI scanning has become increasingly involved as a sensitive imaging modality for detecting neoplastic involvement of the thyroid cartilage, primarily because of its high negative-predictive value. However, recently has been demonstrated MRI low positive-predictive value (68-71%), due to its failure in distinguish edema and inflammation surrounding the tumor from the true cartilage invasion (Becker, 2000; Zbaren et al., 1996; Becker et al., 1995). In addition, it could overestimate cartilage invasion (Declercq et al., 1998). MRI criteria for cartilage involvement by tumor are high signal on fat suppressed T2-w images and/or enhancement on post gadolinium fat suppressed T1-w images in the cartilage adjacent to the tumor, or the presence of extra-laryngeal tumor. The most recent studies describing radiological-pathological correlation demonstrated slightly improved sensitivity of 89-95% and reduced specificity of 74-84% relative to CAT; but accuracy still demonstrates value of about 80% as CAT (Zbaren et al., 1996; Becker et al., 1995; Zbaren et al., 1997a; Zbaren et al., 1997b). Moreover, MRI can be a lengthy and expensive procedure. Ana patient motion as simple as swallowing may generate significant artifacts.

Moreover, high-resolution MRI had been used to construct a three-dimensional anatomical model of the cartilages of the human larynx (Selbie et al., 2002), providing an anatomical framework for registering different larynges to the same coordinate space. Lately, a three-dimensional educational computer model of the larynx was developed from MRI scans (Hu et al., 2009). Two recent studies opened new directions for applying MRI for laryngeal imaging: MR microimaging of excised larynges confirmed valid high-resolution imaging of laryngeal tissue microstructure (Herrera et al., 2009), and dynamic MRI of laryngeal and vocal fold vibrations was realized for the first time for measuring of laryngeal structures and glottal parameters in dynamic function (Ahmad et al., 2009).

2.8. Positron emission tomography (PET, CT/PET)

Fluorine-18 fluorodeoxyglucose (FDG) positron emission tomography (PET) plays an increasing roll in the assessment of head and neck cancer, both for primary staging and for post-therapy management (Gordin et al., 2006a). PET is a functional imaging modality assessing the metabolic status of tumors and has proved superior to CT and MRI in differentiating recurrence from post-radiation effects or scar in patients with carcinoma of the larynx (Gordin et al., 2006a; Kresnik et al., 2001). A negative PET scan excludes recurrence with a high certainty. Positive PET scan findings should be assessed with biopsy and follow up PET imaging if this is negative. It should be noted that physiological uptake of [18F]fluorodeoxyglucose ([18F]FDG) is observed due to vocal

cord activity, so a "silent protocol" should be employed. Recent advances in technology make it possible to fuse anatomical images with functional images. The use of combined 18F-FDG PET/CT (PET/CT) fusion images has been shown to improve diagnostic accuracy. For head and neck cancers, the reported sensitivity of PET/CT is 98%, specificity of 92%, with an accuracy of 94% for the identification of a malignancy of the head and neck; this is a higher accuracy than with PET or CT alone (Jeong et al., 2008). Due to its high costs its use is advocated in case of uncertain results of CAT or MRI, and to complete pretreatment neck staging or in case of suspect for post-radiotherapy recurrence.

3. OBJECTIVE OF THE REVIEW

The clinical diagnosis of laryngeal pathology is important, as this primarily determines the next step towards the treatment. Unfortunately, it is not uncommon for different clinicians to use different nomenclature or to identify different stage for the same laryngeal lesion. This obviously makes evaluation and comparison among specialists indications difficult, and it does affect the treatment.

In order to make this inter-observer variability as much lower as possible, attempts should be made to improve diagnostic technology, stricter/more universally accepted definitions and supervised training of junior doctors in a voice clinic environment. This should in turn lead to useful outcome data and treatment recommendations.

The objectives of this systematic review and meta-analysis were to synthesize the key diagnostic management for laryngeal lesions through a review of the latest advances in laryngeal imaging modalities published in the past 10 years.

4. METHODS

The systematic review was written in accordance with PRISMA Statement (www.prisma-statement.org) (Moher et al., 2009; Shamseer et al., 2014), in order to guarantee a scientific strategy of research to limit bias by a systematic assembly, critical appraisal and synthesis of all the most relevant studies published on this topic (Sedgwick, 2015a; Goodman and Metabias, 2011).

The databases interrogated included PubMed Clinical Queries www.ncbi.nlm.nih.gov. Reference lists from identified articles were searched and cross-referenced to identify additional relevant articles, which we also include in this review, and relevant opinion leaders were contacted.

The search terms included the following various combinations to maximize the yield: larynx, laryngeal precancerous lesions, laryngeal cancer, white light endoscopy (WLE), stroboscopy, contact endoscopy (CE), autofluorescence endoscopy (AFE), ultrasound (US), narrow band imaging (NBI), computed axial tomography (CAT), magnetic resonance imaging (MRI), positron emission tomography (PET-CAT/PET).

The search was performed for the first time on January 2015 and was set to automatically update periodically until May 2015.

First, duplicates were removed electronically. Then, abstracts were reviewed to exclude obviously irrelevant articles. Experimental studies and papers dealing with pathologies other than precancerous and cancerous lesions of the larynx were excluded.

The inclusion criteria were deliberately kept wide to encompass as many articles as possible without compromising the validity of the results, and they are listed in Table 1.

We filtered the studies to ensure that only data from centers that had published on at least 10 patients were included in the review; this was done as a quality assurance measure as there are several case series in the literature, which have published the results of small numbers of cases spanning several years. Non-English language papers and duplicates were excluded.

Table 1
Inclusion criteria list.

	Inclusion criteria for paper selected for the review
1	Articles published from 2005 onward;
2	Published series of ≥10 patients;
3	Sensitivity and specifity clearly shown or detachable;
4	Clear description of selection criteria;
5	Diagnostic methods including: ultrasound analysis, autofluorescence and fluorescent analysis, contact endoscopy, endoscopy, stroboscopy, narrow band imagind (NBI), computed axial tomography (CAT), magnetic resonant imaging (MRI), positron emission tomography (PET);

The search excluded articles that were published before 2005 because we believe that it was pragmatic to include articles that were published on each diagnostic technique at least in the last decade, allowing time for the learning curve to be climbed for method such as narrow band imaging (NBI), came into clinical practice since 1997, and in order to try to compare data as much more recent as possible, due to the latest technology progression and the entrance in usage of more advanced instruments as magnetic resonance imaging (MRI) with high-resolution or diffusion-weighted imaging (DWI) MRI.

All data were independently extracted by two authors and quality assessed. Eligibility for inclusion was separately assessed and when in doubt discussed and decided by consensus.

A first qualitative and descriptive review-analysis of selected articles was carried on; whilst, exclusively, publications comparing diagnostic imaging findings with definitive histopathology results were included in our meta-analysis. For articles not reporting raw data, letters were sent to the corresponding authors requesting them, otherwise they were excluded from the quantitative analysis.

Raw data from the meta-analysis were entered into the appropriate contingency tables to allow calculation of sensitivity, specificity, accuracy, Jounden's index, positive and negative predictive values for each diagnostic imaging technique.

In accordance with the literature, malignant (i.e., carcinoma in situ, invasive cancer) were classified as positive whereas premalignant lesions (i.e., moderate and severe dysplasia) were classified as negative; benign lesions (inclusive of simple hyperplasia and/or mild dysplasia) were calculated as negative, as well.

Similar analyses were performed for the procedure type. Each diagnostic technique was evaluated for its capacity in distinguish premalignant from cancerous laryngeal lesions; when possible, every method was analyzed for its accuracy in identifying advanced stage cancers from early stages.

For this reason, in order to fulfill the need to identify the best diagnostic technique for every diagnostic step, we made two different meta-analysis:

1. On articles reporting diagnostic techniques able to make a differential diagnosis between preneoplastic and cancerous lesions: I group;
2. On those diagnostic methods that allow a more accurate analysis of tumor's volume and extension in order to refine the staging phase: II group.

4.1. Study characteristics and quality assessment

To our knowledge, no widely accepted measures of quality assessment of case series exist; here, all included papers were graded using the NICE scoring scale for retrospective case series (Available at:http://www.nice.org.uk/nicemedia/pdf/Appendix_04_qualityofcase_series_form_preop.pdf). This is a scor-

Table 2
Quality assessment scale.

	Quality assessment for case series
1	Case series collected in more than 1 center (i.e.: a multicentric study)
2	Is the hypothesis/aim/objective of the study clearly described?
3	Are the inclusion and exclusion criteria (case definition) celarly reported?
4	Is there a clear definition of the outcomes reported?
5	Were data collected prospectively?
6	Is there an explicit statement that patients were recruited consecutively?
7	Are the main findings of the study clearly described?
8	Are outcomes stratified (i.e.: by disease stage, abnormal test results, patient characteristics)?

ing scale with eight items, with each item scoring zero or one based on the study methods (Yes = 1; No = 0). Scores of ≥ 6 are considered to indicate a good quality study, scores between four and five as fair and those studies with a score of three are treated as poor quality (Table 2).

4.2. Statistics

Fisher's exact test was used for statistical analysis of categorical data for the descriptive review, and a value of $p < .05$ was considered significant.

Then, all the included studies for the meta-analysis, provided sufficient data (true-positive [TP], false-positive [FP], true-negative [TN], and false-negative [FN]) to permit calculation of sensitivity (Se) and specificity (Sp). Sensitivity and specificity at a 95% confidence interval (CI) were then pooled using a bivariate regression approach (Reitsma et al., 2005).

We constructed hierarchical summary receiver operating characteristic (SROC) curves to assess the interaction between sensitivity and specificity. The areas under the ROC curves (AUCs) were used to analyze the diagnostic precision of each diagnostic technique for differentiation of precancerous lesions from cancers and for distinguish advanced star tumors from early stage cases.

Heterogeneity (or absence of homogeneity) of the results between the studies is assessed graphically by forest plots and statistically using the quantity I^2 that describes the percentage of total variation across studies that is attributable to heterogeneity rather than chance (Higgins et al., 2003; Sedgwick, 2015b). This is a measure of heterogeneity between the studies and ranges from 0% to 100%; high figures indicate greater heterogeneity in the data.

When studies have low heterogeneity (pragmatically, $I^2 < 25\%$), the differences between reported outcomes can be explained simply by the observed natural differences between patients. In this case we can consider that all patients are part of the same larger pool. A fixed-effects meta-analysis is appropriate in which each *patient* is given approximately equal weight. However, with high heterogeneity, the studies differ by more than can be explained by intrapatients effects. This implies that there were differences in the patients studies, in the treatment interventions, or in the outcome measures. In this case, a random-effects meta-analysis is appropriate in which each *study* is given more equal weight.

All statistical analyses were performed using STATA software, version 12.1. In particular, "midas" routine (Dwamena, Ben A.(2007) midas: A program for Meta-analytical Integration of Diagnostic Accuracy Studies in Stata. Division of Nuclear Medicine, Department of Radiology, University of Michigan Medical School, Ann Arbor, Michigan.) was used to undertaking meta-analysis.

5. RESULTS

The search strategy identified 7215 articles in Medline from 1950s to the search date. 3616 articles published after the 2005 were selected, imported into Endnote, and the duplicates were removed. The removal of duplicates, non-English language works and of articles about other items rather than precancerous and cancerous laryngeal lesions yielded a total of 214 publications.

These 214 publications were considered for the qualitative systematic review of the literature, on the other hand, only 41 out of 241 were selected for the meta-analysis.

The various stages of systematically assessing the abstracts and reasons for exclusion from the review are described in Fig. 1.

23 articles (I group) out of 41 were addressed towards the differential diagnosis among precancerous and cancerous lesions, whilst the remaining 18 articles (II group) were analyzed for the assessment of the identification of advanced stage cancers reporting cartilage involvement against the diagnosis of early staged laryngeal tumors.

Quality score assessment and each article clinical characteristics selected for the meta-analysis are summarized in Tables 3 and 4.

5.1. group articles: cancerous vs. precancerous lesions assessment

The I group of articles reported an equal number of good (12) and fair (11) quality, in terms of determination of laryngeal cancerous lesions against precancerous lesions, a mean value for Se of 84.425 ± 19.098 SD (95% CI 78.640-90.210, median 90.5%) together with a similar mean value of 83.031 ± 17.559 SD for Sp (95% CI 77.089-88.972, median 88.5%). These results are referring to a good pool of laryngeal lesions analyzed (3301) with a total amount of 1386 cancerous lesions identified overall the studied populations. Any statistical significance was found among continuos data ($p = 0.7818$).

In specific, results about WLE precision on identify and distinguish superficial cancerous lesions from superficial precancerous lesions reported a very high I^2 value for both Se and Sp (81.38% and 70.82%, respectively), but with a AUC value of 91% (Fig. 2).

In regard to the diagnostic utility of WLE, Caffier et al. (2013) showed a higher accuracy for WL (97% vs. 81%) than AFE and AFE data analysis provided a reduction in specificity of 25% in comparison to WLS, together with a high false positive value (31%). These findings were in accordance with literature reports (Arens et al., 2007a; Kraft et al., 2011). On the other hand, even if Crosetti et al. (2012) confirmed that sensitivity and specificity of AFE in distinguish between benign and dysplastic/neoplastic lesions are negatively impacted by different features (i.e.: hypertkeratosis-leucoplakia, abnormal hyperplasia, hypervascularized lesions, chronic laryngitis and lesions with bacterial infections or previously treated by surgery or radiotherapy), their results were completely different from those reported by Caffier et al. (2013), indicating a very low accuracy in diagnostics for both these single used methods. They concluded supporting their hypothesis that a combination of both diagnostic tools allows a rise in sensitivity and specificity of about more than 35% and 25%, respectively. Thus, they proposed to combine endoscopic tools through a multistep system, in order to obtain a greater sensitivity and specificity, to improve definition in direct microlaryngoscopy and in follow-up searching for synchronous/metachronous tumors.

Only one full text article were selected for the qualitative and quantitative analysis of the stroboscope diagnostic methods, confirming the lack of a statistical and accurate analysis of this technique in literature. Uloza et al. (2013) found moderate-to-high rates of sensitivity (55.9–85.3%) and moderate rates of specificity (51.1–60.0%) of the basic VLS parameters discriminating laryngeal carcinoma and other mass lesions of vocal folds. However, opti-

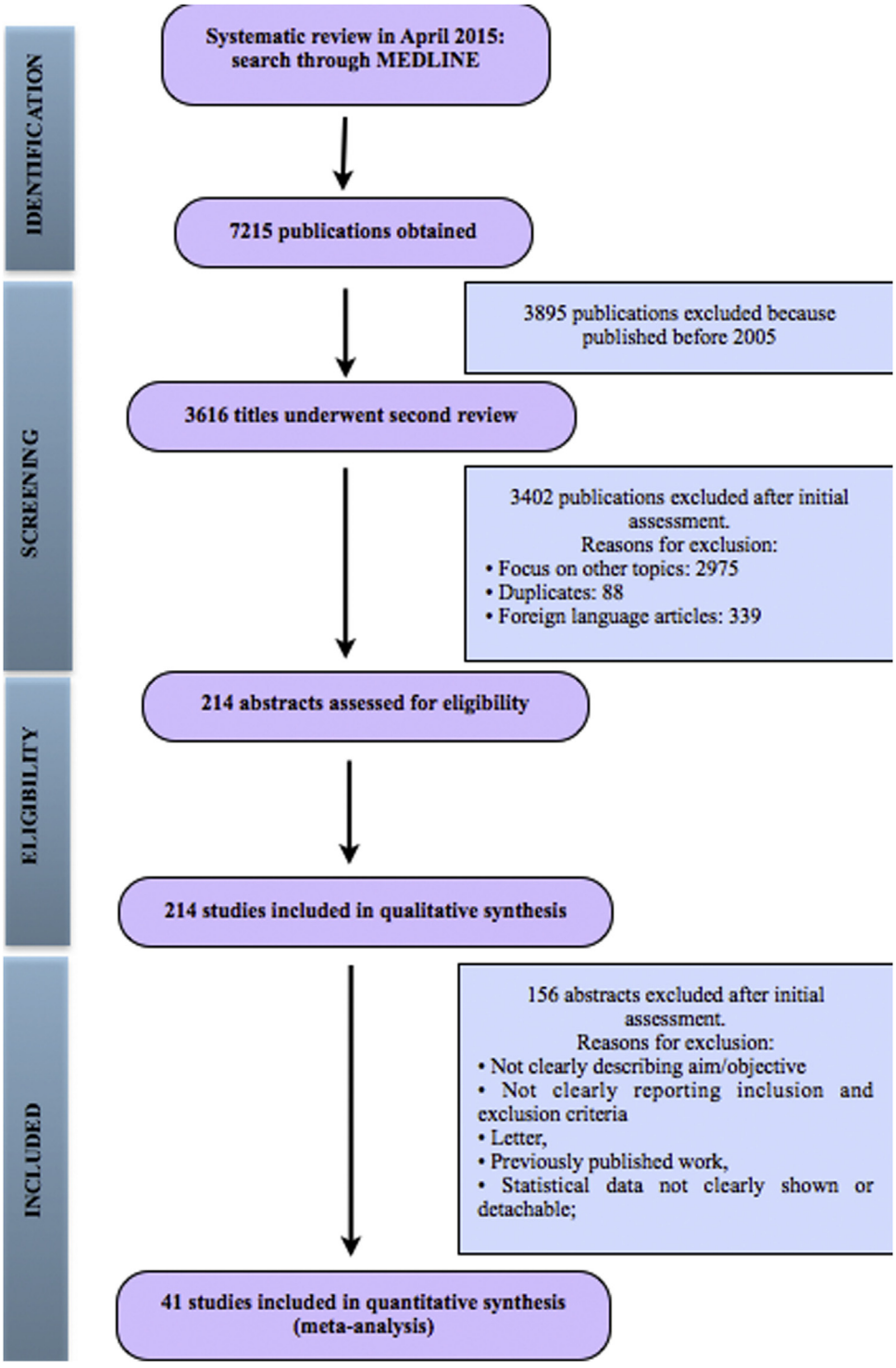

Fig. 1. This flow chart illustrates the process that was used to select articles for the review.

mum system of four following VLS variables—regularity, mucosal wave on the affected side, symmetry of vibration, and symmetry of glottal image — increased the sensitivity up to 85.3% and specificity up to 74.4%, and a final J index of 0.60.

Few studies on CE were selected, as well. A recent article published by Puxeddu et al. (2015) observed an accuracy of CE in the differential diagnosis between normal tissue and dysplasia versus carcinoma of 97.6%. Warnecke et al., 2010 declared a sensibility and specificity achieved by CE of 90% and 93.75%, respectively. But among these, only one study compared contact endoscopy with frozen section histopathology in 142 patients with lesions of the larynx, where CE yielded a sensitivity of 79.59% and a specificity of 100%, with a J index of 0.79 (Cikojevic et al., 2008). In summary we obtained a mean value of 89.0633 ± 9.0415 SD (95% CI 66.6031-111.5236, median 90%) for Se and of 97.200 ± 3.020 SD (95% CI 89.689-104.702, median 97.6%) for Sp, with a p value of 0.3992, showing anyway a higher NPV (97%) than PPV (82%) for CE.

About 10 years later the introduction of CE into the clinical practice, few publications about autofluorescence in laryngology came out; it was 1995 when Harries et al. demonstrated through their

pilot *in vivo* study the autofluorescence properties of the laryngeal tissue and the potential accuracy of this system of performing earlier diagnosis and staging of laryngeal tumors. Considering at least one decade to allow the flatting of the learning curve, our selected articles showed values of Se and Sp of 92.550 ± 3.611 SD (95% CI 89.531-95.569, median 93%) and of 78.850 ± 17.624 SD (95% CI 64.116-93.584, median 83%), respectively; with a not quite statically significant p value of 0.0583. We constructed hierarchical SROC curve to assess the interaction between Se and Sp and these were 93% and 80%, respectively with a AUC of 94%. (Fig. 3).

Our results confirmed data previously reported by Kraft et al. in their meta-analysis in 2011 (Kraft et al., 2011). We performed an up-date of Kraft's results and we obtained from their combination a final Se of 93% (95% CI 0.86-0.97) and a value of 80% (95% CI 0.68-0.89) for Sp, reporting a lower I^2 value for Se (67%) than for Sp (77%). (Fig. 4).

Table 3
Various contributions of the article selected for the meta-analysis addressed towards the definition of the diagnostic precision of white light endoscopy (WLE) (yellow), stroboscope (white), contact endoscopy (CE) (light blue), autofluorescence endoscopy (AFE) (grey), narrow band imaging (NBI) (red), computed axial tomography (CTA) (orange), magnetic resonance imaging (MRI) (green) for laryngeal cancer against precancerous lesions.
(Some articles analyzed more than one system and they are indicated by * or °;).

Author	Quality score	Number of study years	Number of patients	Number of diseased patients	Sensitivity (Se)	Specificity (Sp)	Younden's Index (J) (Se+Sp-1)
Bertino G. et al, 2015^^^^[129]	5	2	217	143	98.7%	33%	0.20
Kraft M et al., 2014*[130]	6	1	205	57	79%	95%	0.74
Dobre M et al., 2014**[131]	5	1	56	16	85%	75%	0.06
Caffier PP et al, 2013***[132]	7	1	32	17	100%	94%	0.94
Shang DS et al., 2013****[133]	6	2	50	33	81.8%	64.7%	0.46
Crosetti E et al., 2012°[134]	7	2	140	62	85.0%	40.0%	0.25
Piazza C et al., 2011°°[74]	5	2,5	444	279	41%	92%	0.33
Ni XG et al., 2011°°°[63]	7	0.5	85	45	68.9%	90%	0.59
Baletic N., 2010°°°°[135]	5	1	45	37	73%	98%	0.71
Piazza C et al., 2010^[65]	4	1	279	110	33.0%	95.0%	0.28
Watanabe A et al., 2008^^[71]	6	1	667	221	51.1%	99.7%	0.51
Saetti R et al., 2007^^^[136]	6	2	46	23	57.1%	84.8%	0.42
Uloza V et al., 2013[137]	6	2	159	34	85.3%	85.3%	0.60
Puxeddu R et al., 2015[31]	4	0,5	44	21	97.6%	97.6%	0.95
Warnecke A et al., 2010[35]	7	1	42	10	90.0%	94%	0.84
Cikojevic D et al., 2008[36]	5	1	142	49	79.59%	100%	0.79
Dobre M, 2014**[131]	5	1	56	16	92%	87%	0.08

Table 3 (*Continued*)

Author	Quality score	Number of study years	Number of patients	Number of diseased patients	Sensitivity (Se)	Specificity (Sp)	Younden's Index (J) (Se+Sp-1)
Kraft M et al., 2014*[130]	6	1	205	57	91%	84%	0.75
Succo G., 2014[138]	4	4	286	73	96.5%	98.5%	0.95
Caffier PP et al., 2013***[132]	7	1	32	17	94%	69%	0.63
Crosetti E et al., 2012°[134]	7	2	140	62	86.6%	41%	0.27
Baletic N., 2010°°°°[135]	5	1	45	37	89%	78%	0.67
Saetti R et al., 2007^^^[136]	6	2	46	23	94.3%	91.3%	0.85
Arens C et al., 2006[139]	6	1,5	42	30	97%	82%	0.79
Bertino G. et al., 2015^^^^[129]	5	2	217	143	97.4%%	85%	0.82
Lukes P et al., 2014[78]	5	3	109	43	100%	82%	0.82
Zobrodsky M et al., 2014[140]	4	4	66	14	92%	76%	0.68
Kraft M et al., 2014*[130]	6	1	205	57	97%	96%	0.93
Piazza C et al., 2011°°°[74]	5	2,5	444	279	97%	84%	0.81
Ni XG et al., 2011°°°[63]	7	0.5	85	45	89%	93.2%	0.82
Irjala H. et al., 2011[60]	4	0.12	73	35	55%	98%	0.53
Piazza C et al., 2010^[65]	4	1	279	110	98.0%	90.0%	0.88
Watanabe A et al., 2009[64]	6	1	34	23	91,30%	91,60%	0.83
Watanabe A et al., 2008^^ [71]	6	1	667	221	97.7%	98.9%	0.97
Beser M et al., 2009[141]	7	1	38	11	88%	50%	0.38
Shang DS et al., 2013****[133]	6	2	50	33	91%	76.5%	0.67

In specific, Succo et al. (2014) highlighted evidence of advantages in allowing precise calibration of the entity of superficial resection during trans-oral laser surgery (TLS) as already reported by Lucioni et al. (2012). Kraft et al. (2011) through their review of literature found a lower J value for AF (0.75) in comparison to the latest publications, but they concluded that FAE is highly effective in the early diagnosis of laryngeal cancer and its precursor lesions, and that can be applied in the primary detection of these lesions providing a better evaluation of their horizontal extension and delineation. However, it is equally recognized that this tool is not as yet qualified to substitute for histological work-up. Baletic et al. (2010) evidenced many conditions impacting AF features of laryngeal mucosa; while Rydell et al. (2008) confirmed a high value of J index for AF (0.89). Saetti et al. (2007) revealed a high sensitiv-

Table 4
Various contributions of the article selected for the meta-analysis addressed towards the definition of the diagnostic precision of ultrasound (US) (yellow), computed axial tomography (CTA) (orange), magnetic resonance imaging (MRI) (green) and position emission tomography (PET-CAT/PET) (purple) for advanced laryngeal tumors with cartilage involvement against early staged cancers. (Some articles analyzed more than one system and they are indicated by * or °;). (Amilibia et al., 2001; Celebi et al., 2012; de Souza et al., 2007; Fernandes et al., 2006; Wedman et al., 2009)

Author	Quality score	Number of study years	Number of patients	Number of diseased patients	Sensitivity (Se)	Specificity (Sp)	Younden's Index (J) (Se+Sp-1)
Xia CX et al., 2013*[90]	6	3	79	40	91.6%	95.6%	0.87
Hu Q et al., 2012**[88]	6	1	36	26	88.6%	85.5%	0.74
Kraft M et al., 2013***[142]	7	1	760	308	84%	93%	0.77
Allegra E et al. 2014****[143]	6	2	20	4	50%	100%	0.50
Han MW et al. 2013[144]	5	12	32	21	57%	94%	0.51
Hartl DM et al. 2013[145]	6	16	236	19	10.5%	94%	0.04
Kraft M et al. 2013***[142]	7	1	510	228	68%	84%	0.52
Xia CX et al. 2013*[90]	6	3	79	40	72.7%	66.7%	0.40
Hu Q et al. 2012**[88]	6	1	36	26	82.9%	91.6%	0.74
Celebi I et al. 2012[146]	6	1	27	18	88.9%	88.9%	0.77
Beitler JJ et al. 2010[108]	7	10	104	41	49%	92%	0.41
Just T et al 2010[147]	5	2	35	15	87%	95%	0.82
Beser et al. 2009[141]	6	1	38	26	88%	50%	0.38
Jeong HS et al. 2008[121]	7	2	114	55	82.5%	82.7%	0.65
de Souza RP et al. 2007[148]	7	5	60	14	100%	93.5%	0.93
Fernandes R et al. 2006[149]	5	1	27	15	80%	92%	0.72
Gordin A et al. 2006°[150]	6	2	51	25	88%	38%	0.04
Allegra E et al. 2014****[143]	6	2	20	4	100%	100%	1
Banko B et al. 2014[151]	6	2	40	19	79%	47%	0.26
Kraft M et al. 2013***[142]	7	1	150	70	63%	89%	0.52
Kinshuck AJ et al. 2012[152]	5	10	31	22	64%	67%	0.31
Banko B et al. 2011[153]	7	1	34	5	100%	93%	0.93
Becker M et al. 2008[154]	6	1	121	49	96%	75%	0.71
Wedman J et al. 2009[155]	4	1	10	7	90%	85%	0.75
Gordin A et al. 2006°[150]	6	2	112	66	96%	61%	0.57

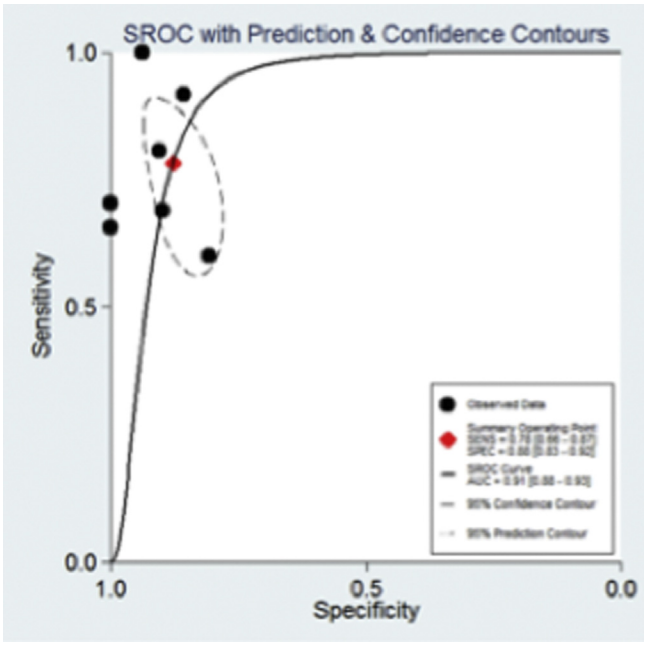

Fig. 2. SROC curve from bivariate model of WLE for cancerous lesions vs. precancerous lesions identification.

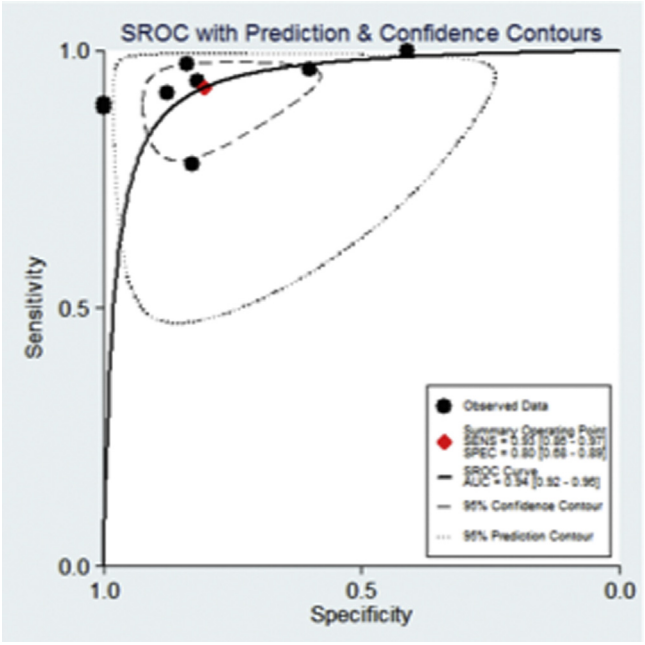

Fig. 3. SROC curve from bivariate model of AFE for cancerous lesions vs. precancerous lesions identification.

ity of the AF by the presence of demarcation of the cancer limits, with an increase in contrast between the normal and pathological mucosa. Here, the gap in sensitivity, between WL and AFE, resulted even more evident, about 37%, than as reported in literature; in fact, comparing confident intervals, AFE revealed a significantly higher sensitivity than standard WL ($p < 0.05$). Nevertheless, they insisted

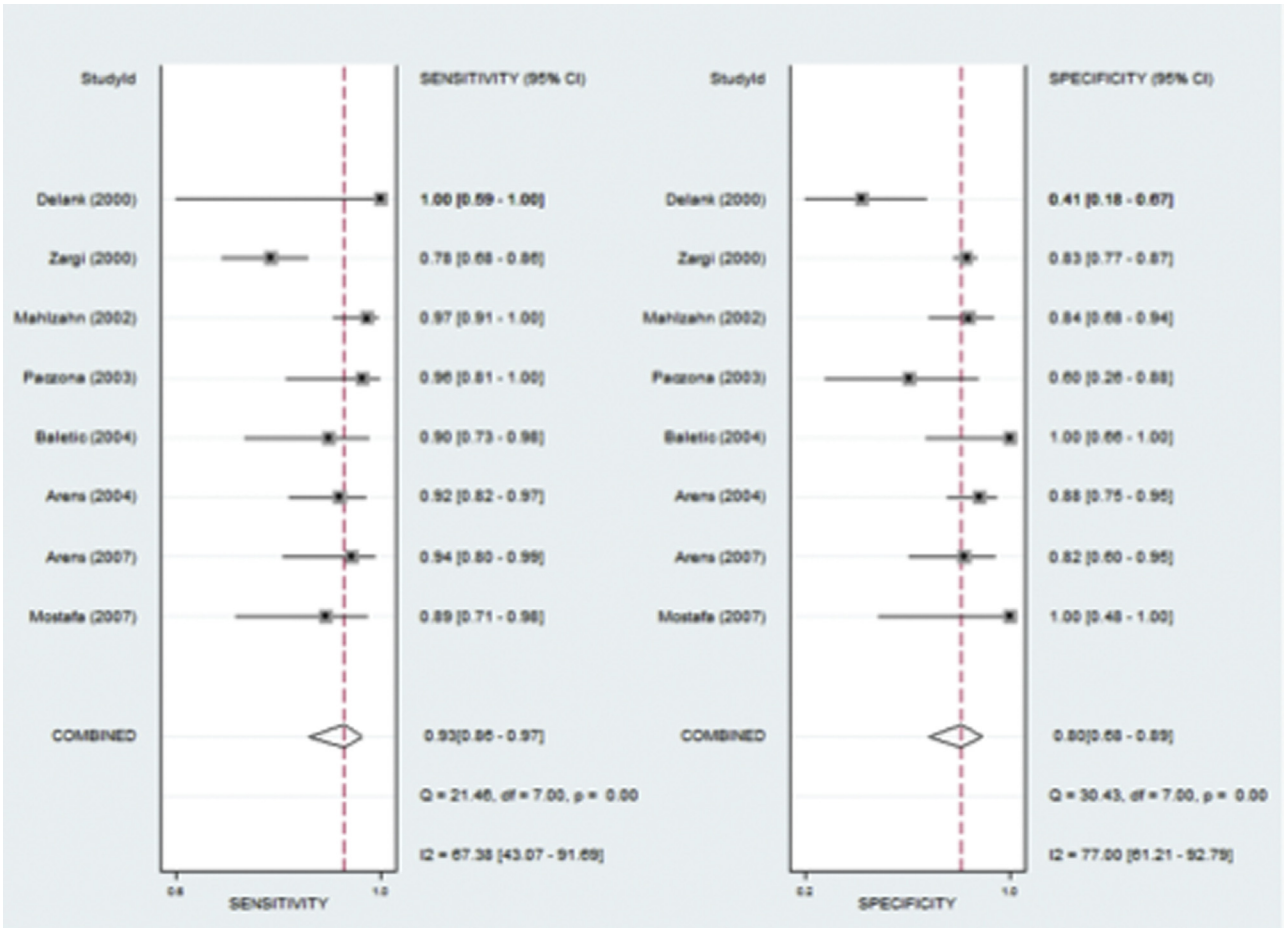

Fig. 4. Meta-analysis of sensitivity and specificity of AFE.

in specifying the need of a long learning curve and that risks are greatly operator-dependent.

The most recent diagnostic technique that has been introduced in the clinical practice is represented by the Narrow Band Imaging (NBI), in fact, first publications about this method are dated 2007. NBI has progressively showed very promising results of both Se and Sp for the differential diagnosis of laryngeal precancerous and cancerous lesions in the larynx thanks to its schematic classification of abnormalities of the intraepithelial papillary capillary loop (IPCL), that seems to have a direct matching with histological features, so far. In fact, Ni et al. (2011) through their proposal of classification of ICPL, obtained a J index for NBI much higher than WLE (0.82 vs. 0.59), reaching statistical significance in terms of accuracy ($p = 0.028$), sensitivity ($p = 0.020$), and negative predictive value ($p = 0.048$), in comparison to WL mode. Greater results, in terms of sensitivity, specificity and accuracy of NBI were obtained by Bertino et al. (2015b), who demonstrated the validity of this technique based on lesions' vascular pattern proposed by Ni and colleagues. Also Lukes and colleagues confirmed high values of sensitivity and specificity for squamous cell carcinoma (100% and 82%, respectively) when IPCL were visible (Lukes et al. 2014); and Kraft et al. (2014) confirmed the capacity of NBI to easily detect and distinguish malignant and premalignant tumors from benign lesions, with a final accuracy of 97%, with a J value of 0.93. Watanabe et al. (2009) achieved in their cohort of patients a high sensitivity for the NBI, almost equal to AFE (91.3%), but showed a higher specificity (91.6%) compared with AF method. In addition, they demonstrated that NBI system can evaluate recurrent laryngeal cancers as well as newly developed superficial cancers, which are one of the drawbacks to using autofluorescence endoscopy (Arens et al., 2007b). These results were confirmed by Zabrodsky et al. recently, in 2014 (Zobrodsky et al., 2014).

Irjala et al. (2011) supported same results about benefits of NBI accuracy by the addition of high-definition television (HDTV). In this respect, Piazza et al. (2010a) showed that the use of high definition television (HDTV) NBI system, helped in increasing the rate of diagnosis significantly, from 20.8% (without HDTV) to 42.7%, where the sensitivity rase up to 98% starting from 61%, and specificity increased by 3%, with a value of accuracy of 92% and a global J index of 0.88, almost doubled in comparison to NBI alone. Thus, it seems that HDTV-NBI proves a significant accuracy in recognizing true positives and in distinguishing, at the same time, true negatives.

From our study we can affirm that values of Se and Sp of 91.44 ± 13.279 SD (95% CI 81.941-100.939, median 97%) and of 89.470 ± 7.529 SD (95% CI 84.084-94.856, median 90.8%), respectively; with a difference between Se and Sp not statically significant ($p = 0.5527$). We constructed hierarchical SROC curve to assess the interaction between Se and Sp and these were 94% and 93%, respectively with a AUC of 97%. (Fig. 5).

Then, we obtained from their combination a final 95% CI 0.83-0.98 for Se and a value of 95% CI 0.88-0.96 for Sp, reporting a lower I^2 value for Sp (61.3%) than for Sp (87.48%). Here the hypothesis of hegemony among these different studies was about to be statistically significant ($p = 0.04$) (Fig. 6).

By comparing AFE and NBI values we can affirm that there is no statistical significancy in terms of both Se ($p = 0.7268$) and Sp ($p = 0.1543$), even if the AUC values revealed a slightly higher percentage for NBI (97%) than for AFE (94%).

If we make a comparison between AFE and WL, NBI and WL, we obtain interesting results. In fact, in terms of Se AFE showed a higher mean value of 91.914 ± 3.383 SD than WL (71.133 ± 21.579 SD) with a significant p value of 0.0229; while Sp did not show any statistical significant difference ($p = 0.8699$), with a mean value of 78.400 ± 18.987 SD for AFE and of 80.100 ± 22.750 SD for WL. If we make the same comparison between NBI and WL, there was a significant difference in terms of Se ($p = 0.0283$), referring to values of 88.422 ± 7.171 SD for NBI and of 71.133 ± 21.579 SD of WL; but, Sp did not show any statistical significance as well ($p = 0.3056$) where Sp of NBI (89.470 ± 7.529 SD) was higher than that one of WL (80.100 ± 22.750 SD).

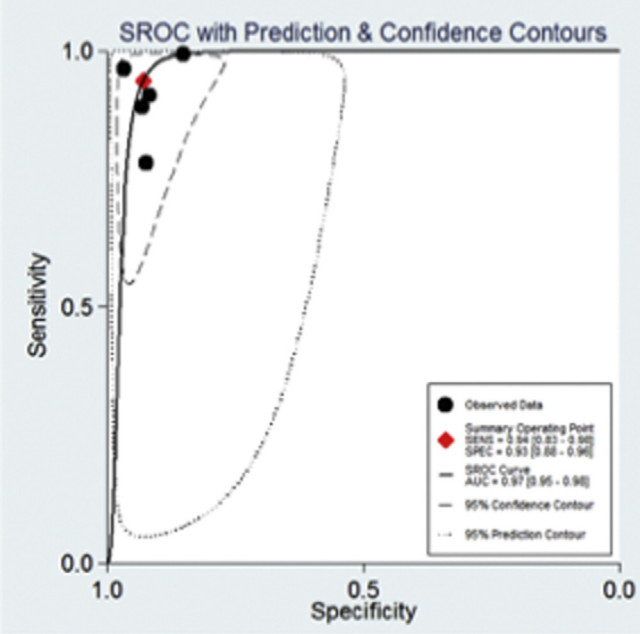

Fig. 5. SROC curve from bivariate model of NBI for cancerous lesions vs. precancerous lesions identification.

5.2. II group articles: advanced stage laryngeal tumor with cartilage involvement vs. early staged tumors assessment.

This group counted a higher number of good quality articles (13 vs. 5), where Se and Sp for determination of advanced laryngeal cancers with cartilage involvement presented a mean value of 78.268 ± 20.611 SD (95% CI 69.760-86.776, median 84%) and 82.140 ± 17.335 SD (95% CI 74.984-89.296, median 89%), respectively, calculated on a global number of patients of 1136 with an amount of 497 diseased populations (people with cartilage involvement). Any statistical significance was found among continuos data ($p = 0.3855$).

In the search for alternative imaging techniques, which could be performed by the treating physician, endosonography has become an important diagnostic tool from the 1960s'.

Preliminary results have reported a sensitivity of ultrasound in the assessment of laryngeal cancer of 84%, with a specificity of 93% and an accuracy of 89%; these values were statistical significant in comparison to CAT and MRI ($p < 0.0001$) in accordance with Kraft's results (Kraft et al. 2013).

Recent investigations on human laryngeal endosonography, have demonstrated a penetrating depth of 10 to 25 mm (depending on the applying frequency) and a high tissue resolution. In fact, in 1994, Zech et al. were first to report on this subject on cadavers; some years later, Arens et al. (1998, 1999) and Arens et al. (1998) published an experimental study on sonographic anatomy of the larynx and the first clinical experiences with this promising method in a variety of laryngeal lesions through the use of a flexible miniprobes, with a frequency of 10–30 MHz and a 360 field of view. Independently, Tamura et al. (2001, 2002) published similar clinical and experimental studies with the same probe. Tsui et al. in 2011 first characterized the lamina propria and the vocal muscle by ultrasound in order to describe biochemical properties of the vocal folds.

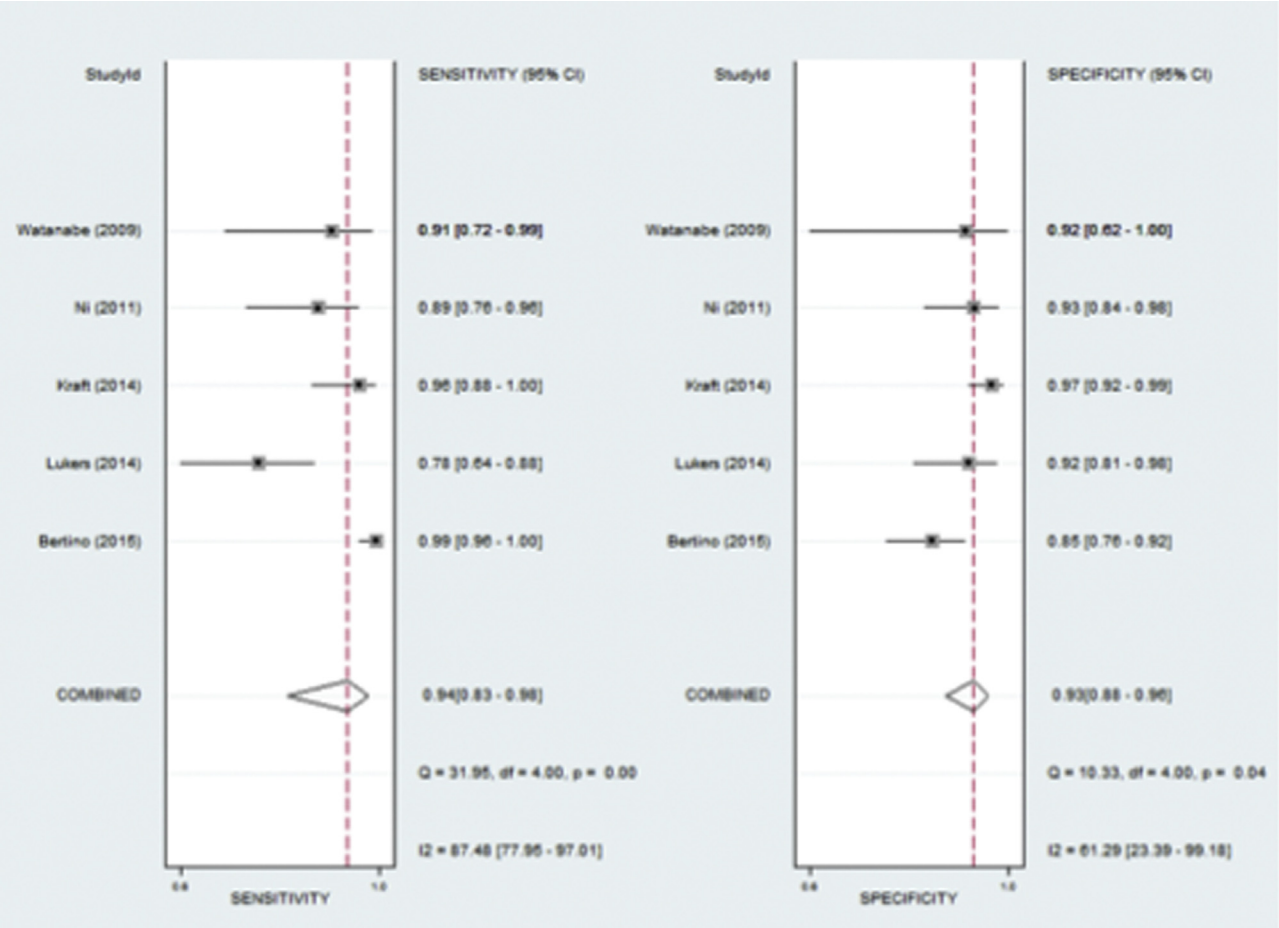

Fig. 6. Meta-analysis of sensitivity and specificity of NBI.

Thus the promising properties of ultrasonography of the larynx, some authors made comparison among this emergent tool and standard diagnostic images acquisition. In fact, Hu et al. compared sonography to MRI (91) and CAT (88) in the evaluation and staging of glottic carcinoma, and their results revealed that there was no statistical difference among these two technique in pre-therapeutic staging accuracy (80% vs. 76.7%; $p > 0.99$), and (83.3% vs. 88.8%; $p = 0.735$) respectively, suggesting ultrasonography as a noninvasive complementary modality for detection and initial staging of glottic carcinoma. In fact, when landmarks, i.e. ventricular bands and vocal cords, for localization are clearly identified by ultrasonography, laryngeal tumors could be correctly located ($p = 0.392$) (Xia et al., 2013). Moreover, they showed the utility of ultrasonography in diagnosing the neoplastic spread to the paraglottic space, evidencing its significative higher specificity in comparison to CAT (94.9% vs. 66.7%; $p = 0.001$). With a final J value for sonography of 0.907 against a value of 0.667 for CAT.

From our few selected articles we can affirm that we obtained a Se and Sp for US of 88.067 ± 3.828 SD and of 91.367 ± 5.244 SD, respectively, accordingly to recent reports.

About the predictability of CAT in the determination of laryngeal cartilage invasion it varies considerably among the most recent published reports (Table 5). With a low mean value for sensitivity of 64.194 ± 20.862 SD (95% CI 53.468-74-921, median 66%) in contrast to a higher mean value of 82.782 ± 22.289 SD for specificity (95% CI 72.322-95.242, median 91%). This comparison presents a significant p value of 0.0199.

We can see how different reports are in contradiction; for instance, Gordin et al. (2006b) showed a CAT sensitivity in identifying cartilage involvement of 88%, with a very low specificity (8%); results that have been disproved by Allegra et al. (2014) who found CAT values of 50% (sensitivity) and 100% (specificity), respectively for identification of cartilage invasion. Despite this conflicting final results, if we compare reports published before 2005 to the most recent works, we do not find any statistical significances among these two groups in terms of sensitivity ($p = 0.3367$) and specificity ($p = 0.2574$) for tumor cartilage involvement.

From our analyzed studies (see Table 4) we can affirm that values of Se and Sp of 71.750 ± 23.428 SD (95% CI58.223-85.277, median 81.25%) and of 83.029 ± 18.474 SD (95% CI 72.362-93.695, median 91.8%), respectively; with a difference between Se and Sp not statically significant ($p = 0.1691$).

The pooled sensitivity and specificity of CAT for advanced tumors with cartilage involvement rate were 74% and 84%, respectively, with a AUC of 85%. (Fig. 7).

The Fig. 8 provides the forest plot of studies that contributed to these results and the spread data with 95% CI for each study represented by horizontal lines. We obtained from their combination a final 95% CI 0.51-0.88 for Se and a value of 95% CI 0.55-0.96 for Sp, reporting a very high I^2 value for both Se (87.7%) and Sp (95.91%), where the lowest rates of Se were reported by Hartl et al. (2013) and Allegra et al. (2014), Beser et al. (2009), whilst Gordin et al. (2006b), showed the lowest rate for Sp.

Here the hypothesis of hegemony among these different studies was not statistically significant (Fig. 8).

It is obvious that over the last decade the sensitivity of CAT has improved considerably, rising its median value by about 10% and

Table 5
Sensitivity and specificity of CAT and MRI in identifying laryngeal cartilage invasion, various reports. (Han et al. 2013; Just et al. 2010; Sulfaro et al. 1989b)

Author	Year	Sensitivity (%)	Specificity (%)
Castelijns JA et al. [166]	1988	46	91
Sulfaro et al.[167]	1989	47	88
Becker et al.[106]	1995	66	94
Zbaren et al.[105]	1996	67	87
Becker et al.[107]	1997	61	92
Amilibia et al.[168]	2001	54	91
Gordin A et al.[150]	2006	88	8
Fernandes R et al.[149]	2006	80	92
Jeong HS et al.[121]	2008	83.3	83.3
Beser M et al.[141]	2009	88	50
Just T et al.[147]	2010	87	95
Beitler JJ et al.[108]	2010	49	92
Celebi I et al.[146]	2012	89	89
Kraft M et al.[142]	2013	68	84
Hartl DM et al.[145]	2013	11	94
Han MW et al.[144]	2013	57	94
Allegra E et al.[143]	2014	50	100

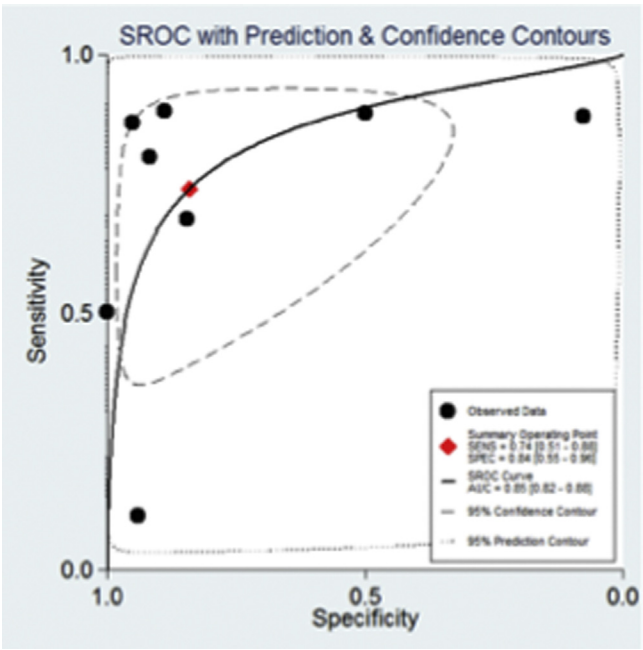

Fig. 7. SROC curve from bivariate model of CAT for advanced stage cancers with cartilage involvement vs. early staged cancers identification.

thus canceling the previous reported significancy between Se and Sp of CAT in identifying laryngeal cartilage involvement.

From the 1980s' the magnetic resonance imaging (MRI) saw it enter in clinical practice.

Table 6
Sensitivity and specificity of MRI in identifying thyroid cartilage invasions, various reports.

Author	Year	Sensitivity (%)	Specificity (%)
Becker et al. (2008)	2008	96	75
Lim et al. (2011)	2011	84.8	89
Banko et al. (2011)	2011	100	93
Kinshuck et al. (2012)	2012	64	67
Kraft et al. (2013)	2013	63	89
Shang et al. (2013)	2013	90.9	76.5
Banko et al. (2014)	2014	79	47
Allegra et al. (2014)	2014	100	100

Its predictability in the determination of laryngeal cartilage invasion varies considerably among published reports, as it has been shown of CAT as well (Table 6). With a mean value for sensitivity of 84.713 ± 14.958 SD (95% CI 72.207-97.218, median 87.85%) in contrast to a mean value of 79.563 ± 17.007 SD for specificity (95% CI 63.344-93.781, median 82.75%) This comparison did not present a significant p value (0.3321).

Those reports that were analyzed for the meta-analysis showed through the SROC curve an interaction between Se and Sp of 86% and 83%, respectively with a AUC of 91%. (Fig. 9).

The Fig. 10 provides the forest plot of studies that contributed to these results and the spread data with 95% CI for each study represented by horizontal lines. The pooled sensitivity and specificity of MRI for advanced tumors with cartilage involvement rate were 86% and 83%, respectively, with a final 95% CI 0.62-0.96 for Se and a value of 95% CI 0.64-0.93 for Sp. Moreover, heterogeneity of the studies included in this analysis was very high for both sensitivity (83.29%) and specificity (83.79%), where the lowest rates of Se were reported by Kraft et al. (2013), whilst Banko et al. (2014) showed

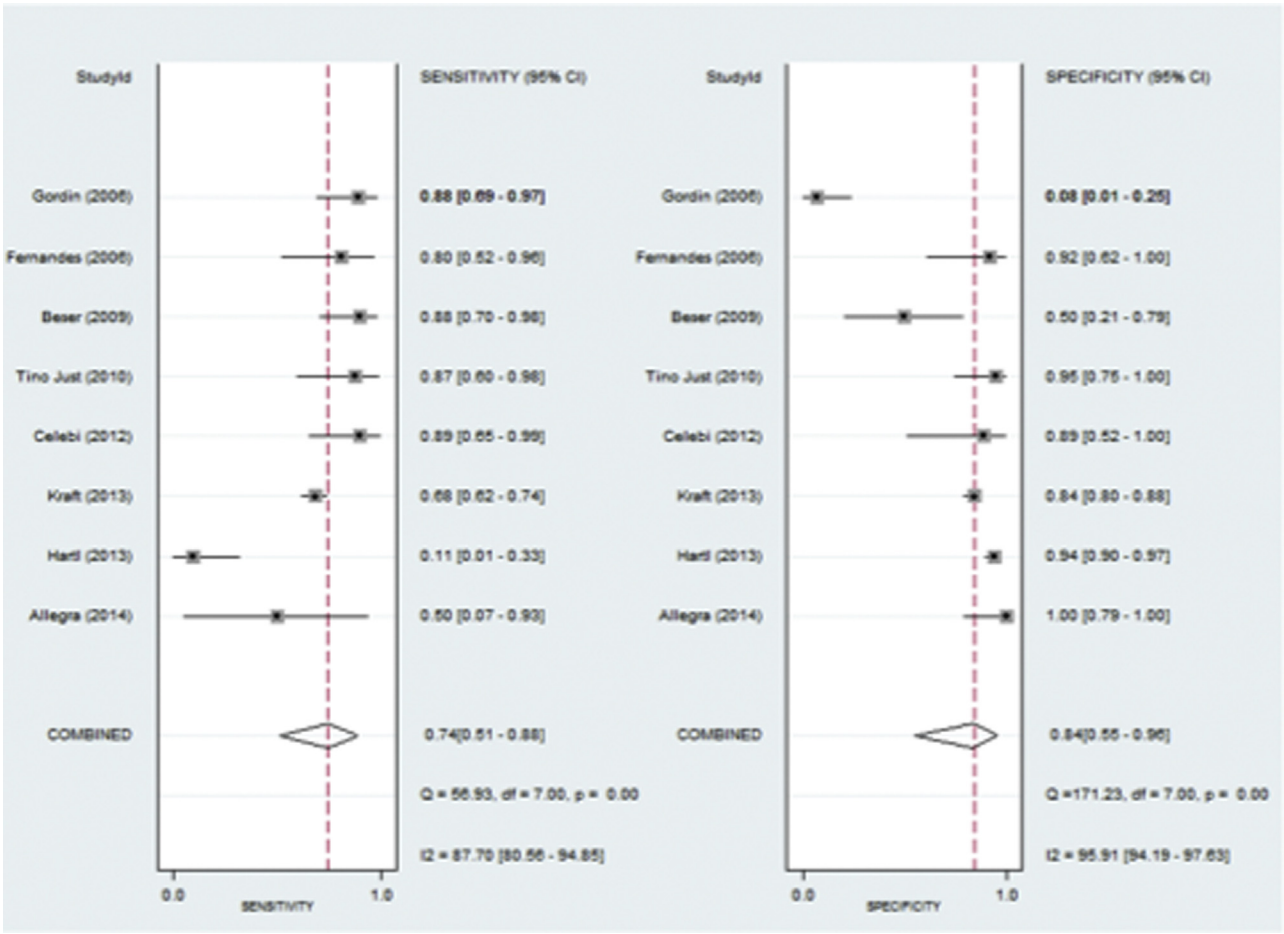

Fig. 8. Meta-analysis of sensitivity and specificity of CAT.

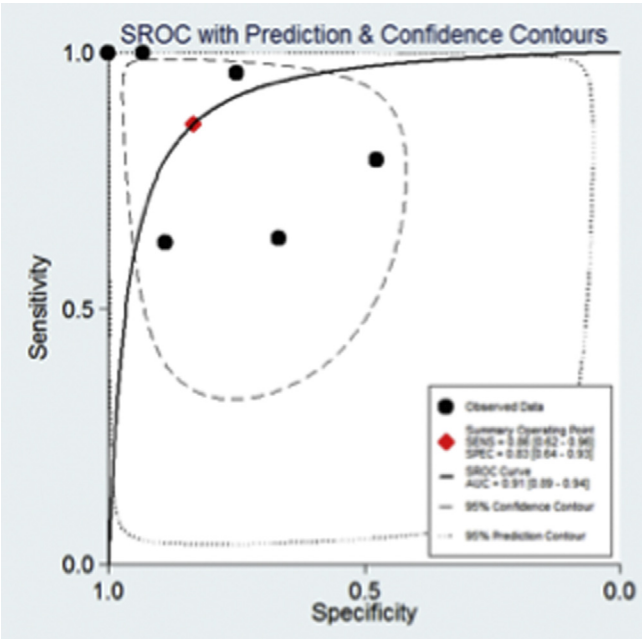

Fig. 9. SROC curve from bivariate model of MRI for advanced stage cancers with cartilage involvement vs. early staged cancers identification.

the lowest rate for Sp. Here the hypothesis of hegemony among these different studies was not statistically significant, too.

In the 1990s' PET and CAT/PET entered into the clinical practice and Gordin et al. (2006b) showed how the combination of CAT and PET can reach a better sensitivity and specificity in comparison to PET study by itself (92% and 96% vs. 92% and 73%, respectively).But, for the first time, Jeong et al. (2008) showed that PET/CT findings do not add to the conventional work-up for the initial evaluation of glottic cancer any further advantages that would support the additional cost of this exam.

By comparing CAT and MRI values we can affirm that there is no statistical significancy in terms of both Se ($p = 0.1708$) and Sp ($p = 0.6274$), even if the AUC values revealed a higher percentage for MRI (91%) than for CAT (85%).

6. DISCUSSION

Advanced image analysis procedures are used in laryngeal diagnostics with increasing frequency. Nonetheless, the image analysis procedures used are quite often limited to image visualization, for example, in indirect autofluorescence laryngoscopy. Quantification of color, texture, and shape of lesions and normal tissue could help in a more accurate categorization of lesions as well as in follow-up procedures.

6.1. Overview in relation to the literature

6.1.1. White light endoscopy and stroboscopy.

WLE together with stroboscopy remain the clinical key element for detecting and assessing vocal fold lesions, representing the essential diagnostic technique for evaluation of laryngeal mucosa

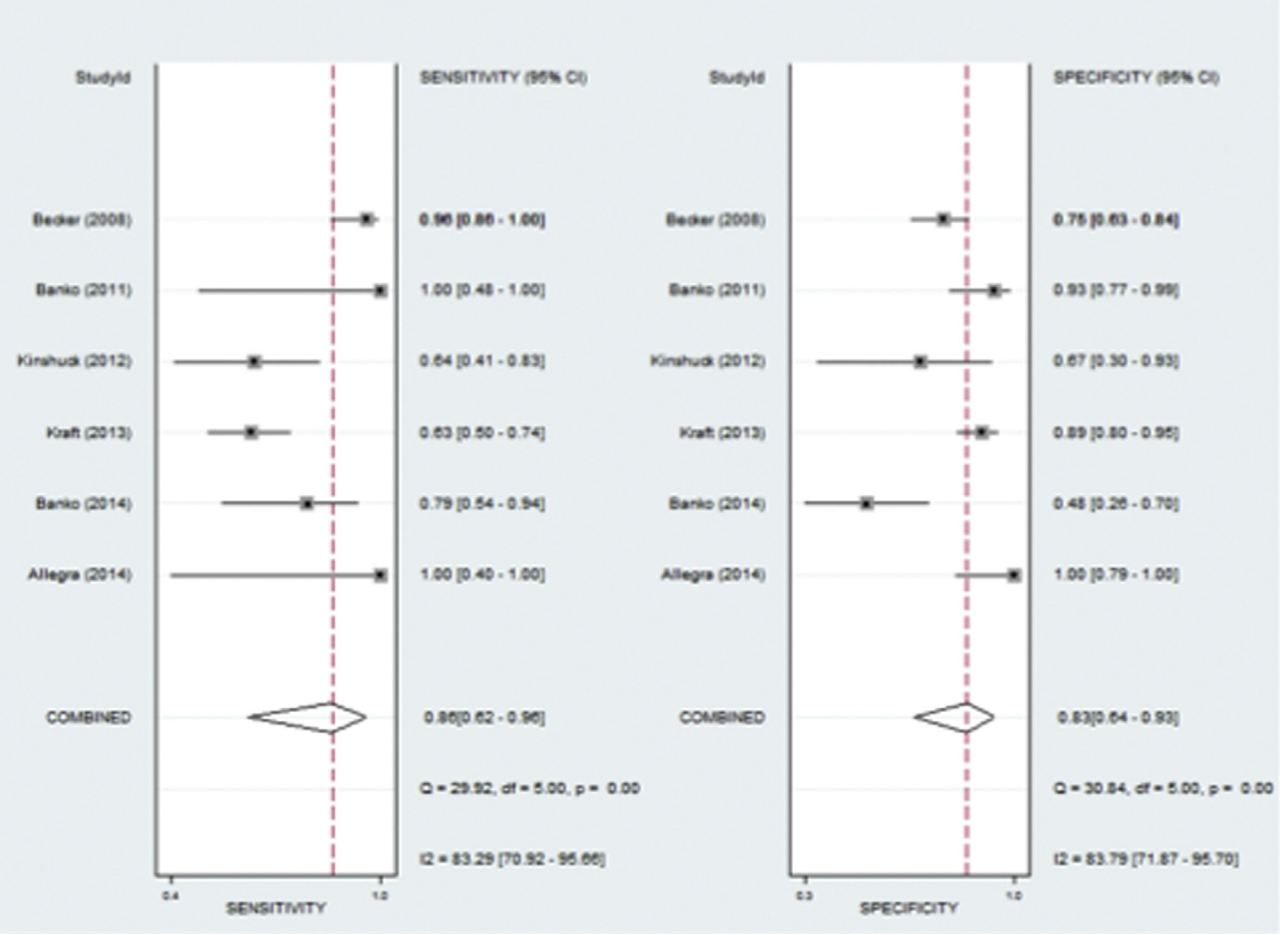

Fig. 10. Meta-analysis of sensitivity and specificity of MRI.

and vocal fold motion biomechanics. Videostroboscopy especially, is the most practical and useful procedure for clinical assessment of viscoelastic properties of the phonatory mucosa, seems to be the reason for the superior sensitivity and specificity of WLS in glottic lesions over other diagnostic methods with continuous non stroboscopic light sources such as AFE. It realizes beneficial visualization and quantification of mucosal wave properties and phonatory oscillations, allowing reliable conclusions in terms of clinical diagnosis.

In this setting the article written by Cohen et al. (2014) is worthy to be mentioned because, even if it did not meet all of the inclusion criteria to be enrolled for our meta-analysis, it showed a high quality score of 7 and gave the image of the actual skyline about general ear-nose-throat (ENT) practice in the U.S.A. analyzing factors influencing the common clinical practice and how this could engrave patients management.

It is well known that stroboscope is critical in the diagnosis of voice disorders, it can alter the treatment decision in 14% to 33% of cases in otolaryngology practice (Paul et al., 2013). Djukic et al. (2014) evidenced that the presence of some factors at the stroboscopic analysis placed patients at higher risk of recurrence and progression of the disease.

In clinical practice, decision-making is quite often based on subjective evaluation of video data, but stroboscopy will continue to be a useful tool for the clinical evaluation of the patient with dysphonia as long as the clinician is aware of the deficiencies of stroboscopy (Woo, 2014). In terms of quantitative evaluation of the VLS using basic parameters this tool seems to be reliable in clinical settings with high sensitivity and specificity distinguishing healthy and pathological voice patients groups, with a low interclass correlation (ICC) value, ranging from 0.2 to 0.9 (Rosen, 2005). Limitations of quantification of VLS variables originate from inherent restrictions of stroboscopic examination, which is basically an optical illusion made by the human eye, arising from the virtual reconstruction of adjacent phases of periodic oscillatory samples (Uloza et al., 2013).

Accordingly to these limits, a combination of VLS with other diagnostic procedures is advocated. Peretti et al. (2003) in their study showed 82% specificity and 100% sensitivity of VLS in diagnostic of glottal carcinoma; however, the combination of VLS with saline infusion into Reinke space raised the values of specificity up to 89% and sensitivity up to 100%. Gugatschka et al. (2008) demonstrated that the combination of cytology and pathological VLS allowed detection of glottic cancer with a sensitivity of 97%, in contrast to 74% as found by cytology alone.

6.1.2. Contact endoscopy.

CE allows for the detection of very early pathologic areas not visible with WL or not properly interpreted with image-enhanced endoscopy (IEE) alone by targeting biopsies, which reduces the chance of under treatment. Contact endoscopy has, in the hands of an experienced examiner, a high negative predictive value (97%); nevertheless, it must be measured against the current gold standard, the histopathological assessment of the biopsy samples.

This technique has shown a number of advantages: (1) ensures a non invasive and safe repeatable method; (2) when performed pre-operatively it provides an insight into the microscopic margins of the laryngeal mucosa lesion; (3) it can be used intra-operatively to ensure that the lesion has been completely removed; (4) it allows visualization of the microvascular net, which can assist the initial

diagnosis of early laryngeal lesions. As CE supplies intraoperative information on the type of laryngeal pathology, thus significantly influencing the subsequent operative course, the criteria for malignancy are set high in order to minimize the use of unnecessary operative procedures as already demonstrated by Puxeddu et al. (2015), and by Ledda and Puxeddu (2006), confirming the significant role of ECE for more comprehensive staging and management of early cancer of the UADT. In fact, false negative result will only delay optimal operative or other therapeutic intervention until histopathology results are obtained, whereas an operative procedure performed on the basis of a false positive result may imply major and irreversible error. In this view, CE can be very useful to the surgeon, and the operative procedure can be more safely continued on the basis of the findings obtained.

Warnecke et al. (2010), calculated the inter-rater reliability (k value) which was of 0.81 between the examiners rating contact endoscopic findings, thus showing an obvious correlation between the diagnosis of the experienced and inexperienced examiner. However, when compared to the current state-of-the-art diagnostic measure, histopathology, a stronger correlation with the otolaryngologist (kappa 0.81) than with the cytopathologist (kappa 0.61) was found. It is striking that the otorhinolaryngologist tended to overrate malignancies.

On the base of Cikojevic results (Cikojevic et al., 2008) obtained from contact endoscopy are therefore nearly as reliable as frozen section pathology. Intraoperative contact endoscopy shall be considered as an addition to histopathology rather than as a replacement. Accordingly, the advantages of contact endoscopy are the immediate results, its instant availability, and the possibility of examining many mucosal areas in a short time and directly during surgery.

6.1.3. Autofluorescence.

When imaged with an autofluorescence system, healthy laryngeal mucosa fluoresces bright green, whereas neoplastic mucosa appears red-violet (Arens et al., 2004), probably as a result of the thickened neoplastic epithelium preventing the illuminating light from reaching the basement membrane and collagen, which would normally provide fluorescence. In 2006, Arens and his colleagues (Arens et al., 2006b) affirmed that it is not possible to detect the responsible endogenous fluorophore from the remitted autofluorescence light because it is a summation spectrum, and in thickened precancerous and cancerous mucosa (>300 μm) the light beam is not able to penetrate. On the contrary, Wang et al. (1996) described a penetration depth of blue light (400 nm) in mucosal tissue of 500 μm; therefore, normal vocal fold mucosa with a thickness of 120 μm can easily be penetrated by the light beam.

Several studies have demonstrated that autofluorescence endoscopy and spectroscopy are better at identifying invasive carcinoma when compared with microlaryngoscopy alone (Malzahn et al., 2002; Baletic et al., 2004; Delank et al., 2000; Mostafa et al., 2007; Rydell et al., 2008); but Dobre et al. (2014), admitted to have problems in differentiating different grades of dysplasia, and to not be able to distinguish *in situ* carcinoma from invasive cancer.

In summary, despite the reported higher sensitivity value (93%) of AFE, which compared to the white light endoscopy Se has improved in percentage showing a statistical significancy of $p = 0.0229$, together with a better AUC value (94% for AFE vs. 91% for WLE), these mixed results of literature (Malzahn et al., 2002; Zargi et al., 2000; Delank et al., 2000; Arens et al., 2006b) image the high grade of heterogeneity found in our study, that could be linked the high incidence of false positive results with a still rather low Sp (80%).

In fact, due to the high absorption of excitation light by haemoglobin, false positives (decreased autofluorescence with a nonmalignant histopathology) occurred more frequently in the presence of highly vascularized lesions such as telangiectasic polyps, granulation tissue, papillomas; while they were relatively common in case of scar tissue or pronounced local inflammatory reactions of the mucosa. Bacterial plaques and necrotic tissue can lead to a defect of autofluorescence that leads to false positive findings.

On the other hand, false-negative findings [negative autofluorescence despite (pre)malignant histopathology] were generally rare and resulted mostly from pronounced hyperkeratosis of the examined areas that caused optical "masking" of clinically significant lesions. A dark field in an image may be the results of a shadow from an anatomic structure over this field. In attention to avoid false positive and false negative findings, we recommended simultaneous careful comparison of white light and autofluorescent images of same view.

Both effects: (1) epithelial thickening of (pre)malignant tissue with inhibited penetration of exciting light into submucous layers and (2) the altered metabolism of tumor cells with lower concentration of NADH and FAD, are claimed to be mainly responsible for the reduced total AF in neoplastic laryngeal tissue (Arens et al., 2007a).

These considerations are basic as far as concerns the application field of this method. In accordance with Delank et al. (2000), we consider that AFE is not useful in the evaluation of "frankly benign" laryngeal lesions.

6.1.4. Narrow Band Imaging.

The introduction of narrow band imaging (NBI, Olympus Medical System Corporation, Tokyo, Japan), already proven to be an useful screening method in other medical fields, has recently shown its potential in identifying carcinomas at an early stage in head and neck mucosal sites (Watanabe et al., 2009; Masaki et al., 2009; Muto et al., 2006; Kara et al., 2006). The NBI filter sets (415 nm, which is the hemoglobin absorption band, and 540 nm) are selected to obtain fine images of microvascular structures of different types tissue, which are necessary for diagnosing a tumor at early stage.

Ni et al. (2011) proposed a classification based on the superficial vascular patterns in laryngeal lesions enhanced with NBI, with a diagnostic accuracy of 90.4% (sensibility, specificity, and positive predictive value were respectively 88.9%, 93.2%, and 90.9%) in detecting malignant lesions from carcinoma in situ to invasive carcinoma; they correlated the superficial spots to vascular anomalies, but the technique with only NBI offers only a partial and sometimes subjective interpretation of the patterns, not allowing to recognize the deepest changes of the vasculature in the chorion. Previous studies have not described intraepithelial papillary capillary loop features in detail, and their data analysis indicated that intraepithelial papillary capillary loop features were closely related to histopathological findings with a statistical significance. Thus, the classification of intraepithelial papillary capillary loop features may facilitate the prediction of laryngeal cancer or precancerous lesions. However, the presence of necrotic tissue or a thick white patch on lesions may affect the evaluation of intraepithelial papillary capillary loop features, especially for invasive carcinoma, resulting in false negative findings.

From our results NBI showed a rise in Se in comparison to AFE (94% vs. 93%), together with an improvement of its Sp which increased of more than 10%, but without any statistical significancy ($p = 0.1543$). This could be explain by the high grade of I^2 for Sp (87.48% for NBI vs. 77% for AFE) which seems to be correlated to the unavoidable subjective interpretation of optical features requested by these two tools. If we compare Se of NBI and WL, a further significant result comes out ($p = 0.0283$), highlighting the evident and promising improvement in sensitivity and false-negatives incidence guaranteed by these two diagnostic techniques whose preliminary results have been first published in 1995

and 2007, respectively. Moreover, NBI showed a further increase in accuracy in comparison to AFE, reaching a value of AUC of 97%.

In specific, AFE in comparison with NBI, presents a lower specificity because some benign lesions also display a loss of green fluorescence; in addition, mucosal scars and inflammation often cause false positive results (Johnson, 2003) in contrast, Ni et al. (2011) indicated that these conditions, classified as type II or III under NBI, can be easily differentiated from type V lesions. Lukes et al. declared that their result may be influenced by very low numbers of benign lesions in their study group (Lukes et al., 2014), and that some of the associated endoscopic characteristics to each single laryngeal lesions, such as the surface, the presence of multiple lesions, and the spread to both vocal cords, may be taken as the criteria used for a more accurate diagnosis.

6.1.5. Ultrasound

In the literature, there exist only a few publications on endosonography of the larynx. Most of them were purely experimental studies on cadaver specimens (Arens et al., 1999; Arens and Glanz, 1999; Zech et al., 1994; Arens et al., 1998). In 1999, Arens et al. published the first clinical experiences in 38 patients presenting with 23 laryngeal cancers and 15 benign lesions. Two years later, Tamura et al. (Tamura et al., 2001) performed a retrospective study of 16 patients with 2 malignant and 14 benign vocal fold lesions. Both authors used 360° radial scanning flexible miniprobes with a frequency of 10 to 30 MHz. Because the interpretation of endolaryngeal sonograms can be influenced strongly when the definitive histopathology is already known, the predictive value of endosonography can be depicted only if an investigation is performed in a prospective and blinded manner. To our knowledge, the study of Kraft et al. (2013) is the only prospective clinical trial assessing the value of endosonography compared with CT and MRI in staging laryngeal cancer. For this reason, a real comparison of our results with the literature is not possible. Generally, there are no contraindications to perform endosonography of the larynx.

The two main limitations of laryngeal ultrasonography are the thyroid cartilage ossification and the air contained in the larynx (Hu et al., 2011). But modern real-time high-frequency sonography has remarkably improved the imaging resolution. Despite these limits, one study showed for sonography a sensitivity in detecting laryngeal cartilage invasion of 100% (Erkan et al., 1993), which is a well known crucial concern for patients outcome. Rothberg et al. (1986) stated that sonography was superior to unenhanced computed tomography for evaluation of cartilage invasion. Microscopic invasion of the cartilage may be missed at the ultrasonography. Several authors (Loveday et al., 1994; Hu et al., 2012; Hirano, 1974) reported an accuracy rate for detection of anterior commissure involvement of 86.7% for ultrasonography, 80% for MRI and 89% for CAT, without any statistical significance ($p = 0.688$); together with accuracy values on sonography of 83.3%, 80%, and 83.3% for supraglottic, subglottic and paraglottic areas respectively, on MRI of 90%, 83.3%, and 86.7%, and on CAT of 94.4%, 88.9%, 91.7% ($p > 0.99$).

Our results confirmed these promising prospectives showing a global Se value of 88% with a higher percentage of Sp (91%).

6.1.6. Computed Axial Tomography and Magnetic Resonance Imaging.

Both CAT and MR imaging are routinely used for detection of subtle cartilage invasion, but there is still controversy about which modality can most accurately detect cartilage invasion, and both modalities have shortcomings (Kuno et al., 2014).

CAT continues to be widely used for diagnosing cartilage invasion. In fact, although MRI is significantly more sensitive, it is less specific than CAT in detecting neoplasm cartilage invasion (Zbaren et al., 1996; Zbaren et al., 1997b). Advantages of MRI include superior soft tissue contrast and exact delineation of tumor margins (Hartl et al., 2013; Ljumanovic et al., 2008), where the cartilage invasions is usually overestimated for anterior commissure tumor.

Radiologic criteria for CAT diagnosis of cartilage invasion include sclerosis (increased density of the cartilage or high attenuation), lysis, erosion, and extra-laryngeal tumor spread (cartilage discontinuity with tumor extending through both the inner and outer cortices). When determining whether erosion or lysis is present or absent, differentiation from cartilage invasion may sometimes be difficult using conventional CT; some cases may be distinguished from erosion if two concurrent findings of negativity are identified: a perfect or almost continuously defined thin hypo-attenuating line between the tumor and the cartilage, and CT attenuation of non-ossifying cartilage that differs from that of the tumor. Demonstrating tumor invasion of non-ossified cartilage is problematic with CT due to similarity of the CT density (100 HU), making them almost indistinguishable, especially when the tumor is located adjacent to non-ossified cartilage. In addition, the appearance of laryngeal cartilage on CT varies widely according to differences in the proportions of hyaline cartilage (which ossifies with aging), cortical bone, and fatty marrow, which complicates interpretation.

On the other hand, MRI is more sensitive (sensitivity up to 96%) in this situation since tumor is of increased T2-w signal relative to non-ossified cartilage (Kuno et al., 2014). Unfortunately MRI has a tendency to overestimate cartilage involvement by tumor since it may be indistinguishable from that due to peri-tumoral inflammation. These changes are most commonly seen in the thyroid cartilage so specificity is lowest at this location. New criteria of moderately high T2-w signal and moderate enhancement as compared to the marked signal changes and gadolinium enhancement with inflammation, have been proposed to overcome this problem. Alternatively the lower specificity of MRI may be addressed by performing a corroborating CT scan in the presence of an MRI scan positive for cartilage invasion (Connor, 2007). The introduction of multi-slice CT has resulted in an increase of spatial and temporal resolution but has led to little progress in interpretation of cartilage invasion which is still sometimes overestimated. If the cartilage displays a signal intensity similar to that of the tumor, cartilage invasion should be suspected. Contrast-enhanced MR imaging is also useful when evaluations by CT alone are insufficient for excluding cartilage invasion, and have reached high sensitivity (96%) and high negative predictive values of up to 96%, but still maintaining a low specificity (Becker et al., 1995; Becker et al., 2008). However, the MR findings suggestive of cartilage invasion are not specific, and therefore may lead to a number of false positive signs. The reason is that reactive inflammation, edema and fibrosis in the vicinity of a tumor may demonstrate diagnostic features similar to those of cartilage invasion. Inflammatory changes are most common in the thyroid cartilage, and therefore the specificity of MR imaging for detecting invasion of the thyroid cartilage is only 56–65% (Becker et al., 1995). Furthermore, due to motion artifacts that degrade images resolution, together with lacks of thin sections and the easier incidence of overestimation of tumor extension, MRI is not a satisfactory first choice for imaging of laryngeal and hypopharyngeal cancer (Kuno et al., 2014). Despite these differences in Se and Sp, there is no statistical significance between CAT and MRI in cartilage invasion assessment (Kuno et al., 2014).

In literature, the pretherapeutic staging accuracy of laryngeal carcinoma has been reported to be 86 and 87.5% with CAT and MRI, respectively (Gilbert et al., 2010); moreover, MRI is more sensitive than CAT and presents h a high negative predictive value for detection of neoplastic cartilage invasion (Zbaren et al., 1996; Castelijns et al., 1988). On the other hand, CAT is more specific, but less sensitive method than MRI for the detection of neoplastic cartilage invasion (Becker et al., 2008). In this respect, cartilage invasion is critical not only for tumor staging, but also for outcomes (Becker et al., 2008).

Our results despite the higher accuracy value of MRI (91%) than of CAT (85%), no statistical significances were issued. This could be explain that further more accurate analysis on specific subgroup of patients with thyroid cartilage involvement should be carried on for both tools by using similar techniques and coils, especially for MRI.

7. LIMITS OF THE STUDY

All papers included in this study are retrospective works mainly from individual institutions and are prone to the bias involved with retrospective studies. While objections can be raised about the pooling of different diagnostic procedures under the same group and the high level of heterogeneity in the meta-analyses, the inclusion of over 4400 patients makes the results fairly robust. This study was unable to access non-English language papers owing to limited resources. The authors suspect that addition of this literature will not substantially alter the conclusions of this study, given the large numbers that have contributed to the results.

In addition a substantial cohort of patients in non-English language literature would have been duplicated in English language literature as these procedures are done by select centers who have published extensively in both English and non-English language.

8. CONCLUSIONS

Limitations of laryngoscopy and clinical examination include: submucosal intralaryngeal growth of tumors and invasion of the laryngeal cartilages and of the extralaryngeal soft tissues cannot be detected and large supraglottic and glottic tumors create difficulties in evaluating portions of the larynx more caudal in location.

The discrepancy in diagnosis between video-laryngostroboscopy (VLS) and suspension microlaryngoscopy (SML) highlights certain key points: (1) additional glottic lesions could be noted in less than 10% of cases; (2) SML does not alter diagnosis but might lead to undertake management changes; (3) accordingly, surgical consent must be exhaustive and specifically address the notion of "hidden" lesions, some of which may influence the extent of surgery performed. The patient age, treatment modality, and stroboscopic signs, such as abnormal amplitude of vocal fold vibration and the existence of non vibrating segment, can be considered as warning factors for recurrence and disease progression and discussion of possible contralateral vocal fold involvement is recommended.

In our opinion, the clinical experience of the examiner regarding precise application and evaluation of the available WLS appears to be more reliable in diagnosing vocal fold lesions than AFE, which is a relatively nonspecific method for diagnostics of mucosal tissue changes. AFE can be proposed as a promising noninvasive optical diagnostic procedure in more than 85% of cases, but the interpretation of its results requires knowledge of its basis principles and pitfalls of this technique.

Contact endoscopy offers a cost-saving, fast, repeatable, and risk-free examination that could considerably reduce the time to diagnosis or at least help to localize the region of interest to enable more specific biopsy sampling for histological evaluation. But, thus it gives information on only the three most superficial cell layers, due to the limitation in staining with methylene blue, further investigations are necessary to develop dyes that can also stain deeper cell layers.

The AFE procedure provides a better evaluation of the horizontal extension in cancerous lesions then WLE alone. It is short, easy to perform and without complication and biopsy is not required; thus, it could significantly improve the diagnostic efficacy of laryngeal pathology, due to a more precise assessment of boundaries of lesion with AFE, and the fact that AFE could serve as a guide for taking biopsy of lesion and endoscopic laser resection of laryngeal lesions. On the other hand, an intrinsic limit of the method is the impossibility to evaluate the extension of the carcinoma in the deeper tissues and, therefore, to differentiate between carcinoma *in situ* and invasive carcinoma.

NBI endoscopy can be performed during a normal daily work in outpatient clinic by pushing a fingertip control switch, without using special techniques, without drug application. The most prominent feature of this technology both in the pre- and intraoperative setting is a more accurate definition of the neoplastic superficial spreading with consequent improvement of the peripheral margins control and modulation of the custom tailored endoscopic treatment. It is, however, important to understand that it is not black and white reading and therefore one still needs an experienced clinician on the field and, even with an expert, a learning curve of the new technique has to be taken into account. During this period one has to be prepared for more time-consuming examination and very likely for some extra biopsies before the eye is trained for the NBI.

US present irrefutable advantages in comparison to CAT and MRI scans, which count: a lower benefit-costs ratio, absence of swallowing artifacts due to the general anesthesia, a real-time and repeatable mode, a closer relationship between diagnostics and therapy thus the treating surgeon performs the diagnostic technique at the same time. Then, it is suggested as a noninvasive complementary modality for the detection and initial staging of glottic carcinoma. On the other hand, disadvantages such as its limited availability, the need of performing this procedure under general anesthesia, lesions smaller than 3 mm are not detectable (anyway, still earlier than with CAT or MRI), together with its strong dependence on the examiner's experience, make the use of this tool optional and not straightforward.

Imaging, conventionally with computed axial tomography (CAT) and/or magnetic resonance imaging (MRI), is nearly always required as endoscopic staging alone may underestimate the extent of the tumor. Both can enable the accurate prediction of the site, size, and spread patterns of laryngeal tumors. MRI is more indicated for unilateral lesions due to its higher Se (96%) and NPV (96%) than CAT in identifying cartilage involvement, even if MRI presents a higher incidence of false-positives. In these cases a CAT scan could be performed in order to increase the specificity of the study. In case of bilateral vocal cords lesions, CAT seems to improve its Se and it could be proposed as first line diagnostic pre-treatment tool.

We could summarize this bullet list in the following flow-chart (Fig. 11).

9. CLINICAL IMPLICATIONS

- To avoid any condition that will obstruct visualization during AFE, SAFE-3000 system and high definition white light endoscopy camera (HDTV camera) are worthy to be used. The learning curve is rather rapid, but there is the need, especially in indirect laryngoscopy, to avoid any condition in fact, anatomical sites variation, such as an omega-shaped or retrograde-positioned epiglottis could imitate AF defects at the anterior commissure and anterior third of vocal folds due to shadows from these structures. Artificial defects of the AF signal also can result from hypertrophic ventricular folds, creating shadows over the lateral part of the vocal folds.
- *Compact endoscopy* is the latest modification of above techniques, which combines autofluorescence and contact endoscopy together.
- The future objective should be to introduce the NBI technique into routine laryngoscopy and to use an internationally accepted

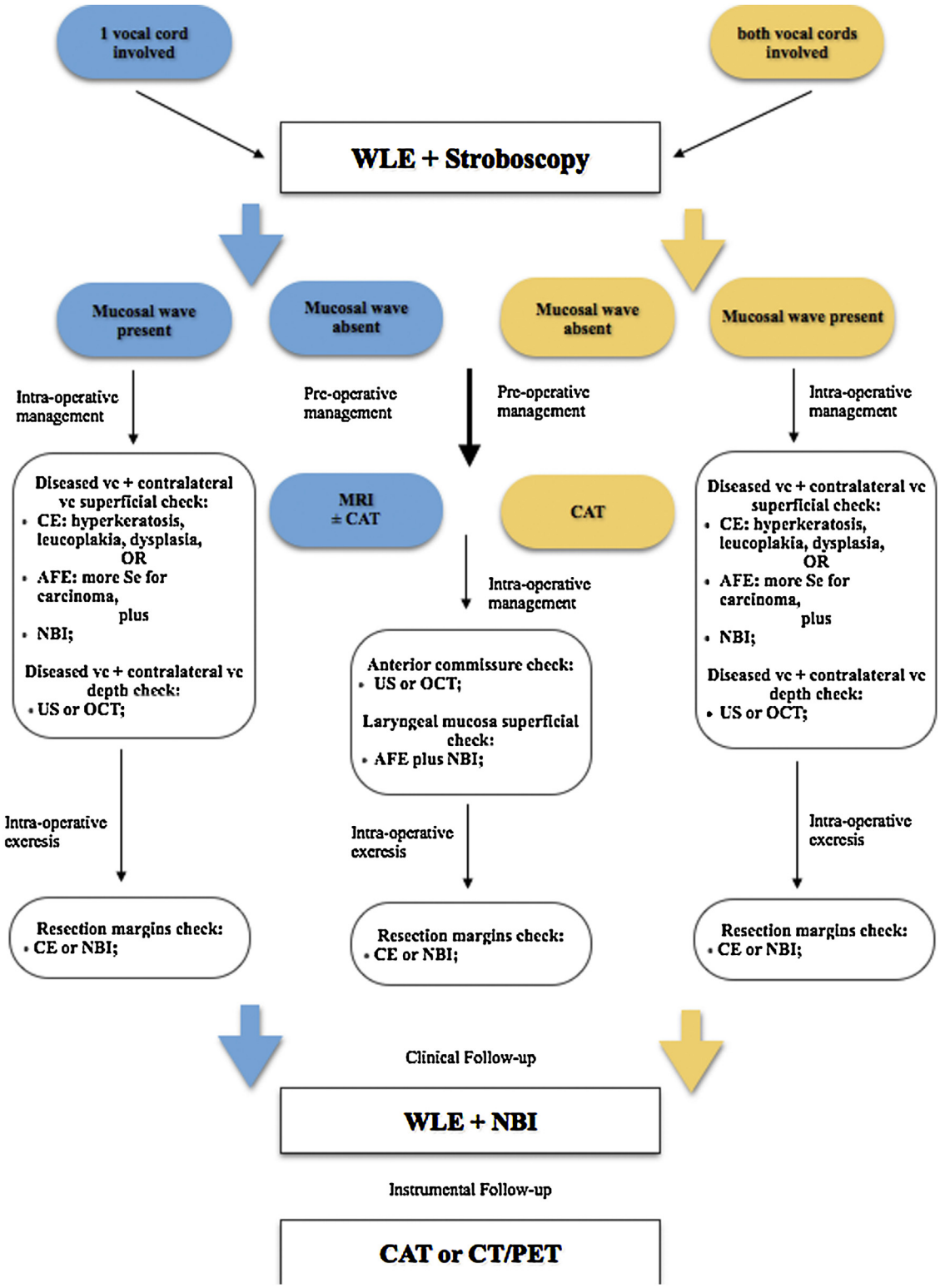

Fig. 11. This flow-chart images the diagnostic steps that would be suggested for the clinical assessment and management of laryngeal lesions suspicious for precancerosis or cancer. Two different types of scenario are reported: 1. Unilateral vocal cord lesion, and 1. Bilateral vocal cord lesion/s. Once white light endoscopy plus stroboscopic study represent standard office-based analysis, further following steps depends on the previous analysis result. The presence or absence of the mucosal wave at the stroboscopic test influences the management of the patient. In the first case an intraoperative phase is planned, on the other hand, the second scenario worths second level diagnostic tools, such as MRI and/or CAT. Then, an intraoperative superficial and/or three-dimensional visualization of the lesions is mandatory. One tool among CE, AFE and NBI is suggested, even if the most accurate result should come out from the combination of at least two out of them tests. If US or OCT is available its use could help in the lesions' volume identification, especially for bulky neoplasms that does not reach the inner cortex without any laryngeal movement impairment. The final resection margins check could be trust in CE or NBI help. For the clinical follow-up WLE and stroboscopic exams are always mandatory, together with a further second level instrumental diagnostic tool, which usually counts CAT and/or CT/PET, so far.

endoscopic evaluation of the vascular patterns of lesions of the head and neck area. In this way, case studies can be compared based on the same classification, which can contribute to the further definition of the diagnostic validity of the technique, and to the identification of any misleading case. Despite the high benefit of NBI in distinguishing precancerous lesions from cancerous lesions, there is little benefit of NBI in identifying advanced tumors, which are readily seen in conventional WLE. Nevertheless, NBI is able to better specify their exact extension and show infiltration of neighboring structures. Additionally, NBI might detect second primaries or field cancerization in these patients (Bertino et al., 2015b) and it presents a potential advantage in post-treatment surveillance (Muto et al., 2004; Lin and Wang, 2011; Piazza et al., 2010b). Compared to AFE, NBI endoscopy has the advantage of being able to obtain the same information using both a videoendoscope coupled with a HDTV camera and rigid telescopes (Piazza et al., 2010a).

- Compared with CAT and MRI, US has the advantage of earlier detection of smaller tumors (T1 >3 mm) as well as improvement in determining their exact size and extension at significantly lower costs. Smaller cancers (Tis, T1 <3 mm) and their precursor lesions can be better assessed through optical coherence tomography (OCT), whereas advanced tumors (T2–T4) still require additional imaging techniques such as CT or MRI (Kraft et al., 2008). Moreover, it permits observation of vocal cord mobility in real time (Desai et al., 2004; Hu et al., 2012; Xia et al., 2013). In this setting, it is a promising tool for studying the physiology of the vocal folds in phonation function. Further studies must be conducted to clarify the role of the vocal fold body in the vibration of vocal folds.

10. RESEARCH IMPLICATIONS

- Future prospective randomized clinical trials are necessary for a detailed assessment of the contribution from the most promising diagnostic tools, NBI and US, to the *in vivo* diagnosis of malignancies.
- In this setting, a noninvasive imaging technique that allows histologic diagnoses to be made *in vivo*, without taking a biopsy, will have potential significant benefits for the patient pathway. First, by focusing attention onto areas that have histologic features of malignancy this would reduce the risk of non diagnostic or unnecessary mucosal biopsies from healthy mucosa, which is particularly important for lesions on the vocal cords where preservation of the delicate superficial lamina propria is crucial for generating a normal voice. Second, it will potentially offer greater certainty about the position of tumor margins and facilitate decision-making during surgical tumor resection. Third, it may ultimately allow definitive histologic or molecular diagnoses to be made in the outpatient's clinic, removing the need for a general anesthesia and significantly shortening the patient's journey toward definitive treatment.
- Furthermore, primary research is required to validate other emerging techniques, such as Raman spectroscopy, and to develop their clinical applications in the larynx.
- The availability of powerful low-cost digital cameras and image-processing methods should help to standardize the analysis routines that may be used for clinical analysis. Collaboration between the clinician and the scientist in developing the software for imaging and analysis will go far in improving the knowledge base between clinical care and objective results.
- Finally, future automatic imaging analysis systems could perform the real quantification of images with diagnostic purpose. Essentially three-dimensional imaging of the superficial lamina propria is yet undeveloped. Toward that end, future evaluation about optical coherence tomography (OCT) as a new technique to evaluate the layered microstructure of the vocal fold could be implemented. OCT represents the optical analog of ultrasound and involves the detection of light backscattered off tissue boundaries (changes in refractive index). OCT has demonstrated to be potentially used intraoperatively for accurate staging of locally invasive laryngeal tumors, thanks to its spatial resolution of <10 μm (40). On the wake of promising OCT's results, the future deliver of an "optical biopsy" of the mucosa will improve the reach of a intraoperative diagnosis. Thus, OCT could be added to standard SML, allowing, in some instances, to remove the need for taking a tissue biopsy, maybe by replacing frozen section, and to guide surgeons towards more significant and representative areas of the suspect lesion. Moreover, OCT might become helpful during trans oral laser surgery since it provides a guide in tumor resection margins in three dimensions.
- Another important objective to be covered is the statistical analysis of the biomechanical parameters extracted from a large population database of voice samples, including different pathological cases as well as normal ones, taking into account side conditions such as sex, age, and habits.

DISCLOSURE

None to declare

CONFLICTING OF INTEREST STATEMENT

Author declares that there is no conflict of interest, financial of otherwise.

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