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Objective quantification of the vocal fold vascular pattern: comparison of narrow band imaging and white light endoscopy

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Abstract No clinical standard procedure has yet been defined to quantify the vascular pattern of vocal folds. Subjective classification trials have shown a lot of promise. Narrow band imaging (NBI) as an endoscopic imaging tool is useful, because it shows the vascular structure clearer than white light endoscopy (WL) alone. Endoscopic images of 74 human vocal folds (NBI and WL) were semi-automatically evaluated after image processing with respect to pixels of vessels and mucosa by the software MeVisLab. The ratios of vessel/mucosa pixels were compared. Using NBI, more vocal fold vessels are visible compared with WL alone ($p = 0.000$). There may be a difference between the right and left vocal folds due to the handedness of the examiner ($p = 0.033$) without any interaction between the method (NBI/WL) and the side (right/left) ($p = 0.467$). MeVisLab is a suitable tool for the objective quantification of the vessel/mucosa ratio for NBI and WL endoscopic images. NBI is an appropriate endoscopic tool for examination of diseases of vocal folds with changes in the vascular pattern. There is evidence that the handedness of the examiner may have an influence on the quality of the examination between the right and left vocal folds.

Keywords Vocal fold · Blood vessels · Narrow band imaging · MeVisLab · Quantification

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Introduction

Laryngoscopy is a standard tool for the routine examination and screening of pathological lesions of the larynx. It is also used in follow-up examinations after the treatment of malignant diseases. In the case of tumor growth or an inflammatory lesion, an increase in vessel density and vascular changes can be observed [1–3]. Premalignant or malignant lesions can be detected by endoscopic visualization of vascular changes in tumors [4, 5].

To get the full benefit of the laryngoscopy for diagnostics and treatment, it is necessary to work with special endoscopic imaging tools, such as narrow band imaging (NBI) [6].

The NBI light source only emits wavelengths of blue (390–445 nm, peak 415 nm) and green (530–550 nm, peak 540 nm) light. It is a useful imaging tool for an improved visualization of the subepithelial vascular pattern. Blue light only penetrates the epithelium and represents the capillary vessels in a specific brown color. Green light is able to penetrate deeper to the subepithelial vascular plexus and represents the vessels in a blue color [7–9].

NBI is added to an HDTV or 4K endoscopy system and can be switched on just by pressing a button. This method exploits the fact that tumors are highly vascularized and, therefore, are well suited for a hemoglobin-specific imaging system.

The endoscopic examination of the vascular pattern of the vocal folds for premalignant, malignant, and inflammatory lesions can be simplified and made more effective using NBI.

The benefit of NBI in comparison with white light (WL) endoscopy and other endoscopic tools, such as autofluorescence for the detection of premalignant and malignant lesions, is well reported [10–14]. It can be demonstrated

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that the use of NBI increases the sensitivity from 80–90 to 97–100 % and specificity from 75 to 99 % [15, 16] for the detection of recurrent respiratory papillomatosis and nasopharyngeal carcinoma. A similar result was achieved by Kraft et al., which compared NBI and WL for the diagnosis of tumor-specific neoangiogenesis [17].

In addition, NBI has shown its value as a screening tool for follow-ups for carcinomas of the esophagus. It has been demonstrated that the use of NBI significantly increases the sensitivity and specificity as well as the positive and negative predictive values, in comparison with white light endoscopy alone [18].

Most of these studies required the examiner to evaluate the tissue alternatingly with NBI and WL endoscopy. The examiners choose tissue, which was suspicious for a malignant lesion, and took a sample that was tested for suspicious cells by the institute of pathology. Initial screenings by examiners and their outcome are dependent on his/her subjective opinion and his/her experience.

Subjective classification of the beginning of (longitudinal) vascular changes of vocal folds is possible [19, 20]. There has never been a study to objectively prove that NBI represents more vessels in comparison with WL.

In addition, there is no method described, which can measure blood vessels or the ratio between vessels and tissue automatically, as seen during endoscopy. An automated tool is needed that has the ability to detect, measure, and analyze the number of vessels seen in an endoscopic image to fill this gap. After a one-time calibration, the program must be able to search and select the vessels without human intervention.

A tool that fulfills these expectations was used by Turkmen et al. [21] for videolaryngoscopy. This method works by utilizing RGB-signals that represent the three basic colors red, blue, and green. For the segmentation of glottis, the RGB-signal was split, and only the red channel was used, since it showed the highest contrast between glottis and vocal fold.

Turkmen et al. used software that detected the edges and set the reference point on its own, just using the green channel.

Mizuno et al. [22] used a CD34-marker for vessel detection in colorectal lesions. For this procedure, a biopsy had to be taken. The use of a biological marker and the need for a biopsy did not seem to be practical for everyday examinations or screenings.

The vessel detection via CT and Xenon inhalation to measure the blood flow in pancreatic tumors was reported by Kubota et al. [23]. The results from the CT scans were compared with the CD34 marked histological samples. A correlation of $r = 0.885$ suggested that the blood flow could be measured non-invasively using a CT scan. Further studies using CT scans were done by Bai et al. [24] and Jiang et al. [25] to measure the microvessel density (MVD) in lung and liver lesions. Due to the radiation exposure and the smaller size of the tissue of the vocal fold, this method does not seem to be a useful tool for the screening of the vocal folds.

A different approach was used to calculate the hemoglobin indices of subepithelial lung carcinoma [4] with and without NBI. Irregular vessel patterns and sizes were detected. The hemoglobin indices of the subepithelial carcinoma were significantly higher compared with the regular tissue.

All of the methods described above are either not practical or involved radiation exposure. Only the method used by Turkmen et al. [21] worked with endoscopic images alone. No biopsy had to be taken, and no radiation exposure was required.

The aim of this study, therefore, was to find and evaluate a novel and non-invasive detection tool that can be used in everyday medical analysis. This could be achieved using a method that can automatically detect the visible vessels of the vocal folds using NBI and WL.

Materials and methods

The study was conducted in accordance with the guidelines proposed in the Declaration of Helsinki and was approved by the Local University and Hospital Ethics Committee (Project Number: 107/14). All patients gave their informed consent.

74 human laryngoscopic images of patients from the Departement of ENT of the Otto-von-Guericke University Magdeburg, Germany, were retrospectively selected. Vocal folds with normal tissue and vascular structure were included in this study. NBI and WL images from the vocal folds were created using EVIS EXERA III, CV-190, CLL-S1, and ENF-VH (Olympus, Nikkei, Japan). The footage was recorded in HD. Comparable photographs of NBI and WL images were stored.

The right and left vocal folds of 47 subjects could be included. From 14 subjects, only the left, and from 13 subjects, only the right could be used due to bad light conditions, lesions, or artifacts.

After the selection of endoscopic pictures, image processing had to be performed. The freeware GIMP 2.6 (https://www.gimp.org/, GNU Image Manipulation Program) was used to cut out the membranous parts of the vocal fold and adjust the NBI and WL image in size, length, and angle (Figs. 1, 2).

Using the program ImageJ (http://imagej.nih.gov/ij/index.html, a freely available software tool) the artifacts, e.g., reflections of the light or saliva, could be erased in both images (Fig. 4). To remove the artifacts, a white plane was placed over the images of NBI and WL, and only the relevant structures of the vocal cord were visualized. Following that, the regions without artifacts became visible.

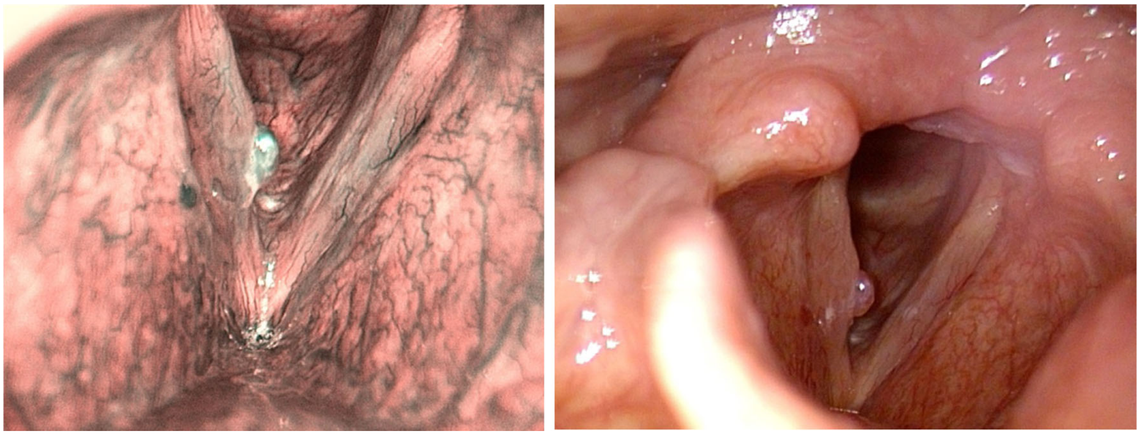

Fig. 1 Original images from the videolaryngoscopy of a patient with recurrent respiratory papillomatosis (*left* NBI, *right* WL)

Fig. 2 Split vocal fold after removal of artifacts through use of a *white plane*

After processing the images, a few test runs were made by creating histograms and inverted pictures. The use of histograms, inverted images, and optical evaluation with GIMP or ImageJ did not lead to satisfying results. An automatic determination of vessel and epithelium was not possible.

The requirements for the software were to detect the epithelium and the vessels as well as to distinguish between epithelium and vessel. The software had to be able to count the pixels of the tissue as well as the pixels of the vessel and to calculate a ratio between them. The preferences for NBI and WL and for all pictures of all subjects needed to remain stable and unaltered after initial adjustment.

Region of interest

The footage of the videolaryngoscopy showed that the vocal folds exhibit a convexity in the coronary cut when they are relaxed, but even under tension, while phonation the vocal folds still showed a rest of convexity. This partial convexity led to an uneven illumination of the vocal fold. The lateral and medial edges of the vocal folds appeared darker than the center of the tissue. Using an optical software for vessel detection, a reference point within the vessel was utilized and compared with the pixels surrounding it; the darker areas of the epithelium often showed up as dark as a vessel. When performing an automatic segmentation, the dark areas of the epithelium were mixed up with the vessels. To avoid this problem, a region of interest (ROI) for every vocal fold was created (Fig. 3). The ROI is defined as a homogeneous, illuminated area without any artifacts. The only dark spots in the ROI are now created by the vessels.

The visualization program was already successfully used for the automatic segmentation of liver veins [26] and the volumetry of vertebral bodies of the human spine [27]. Therefore, the method appears suitable for the segmentation of the vocal fold.

Fig. 3 Region of interest (ROI) without any artifacts and with homogeneous illumination

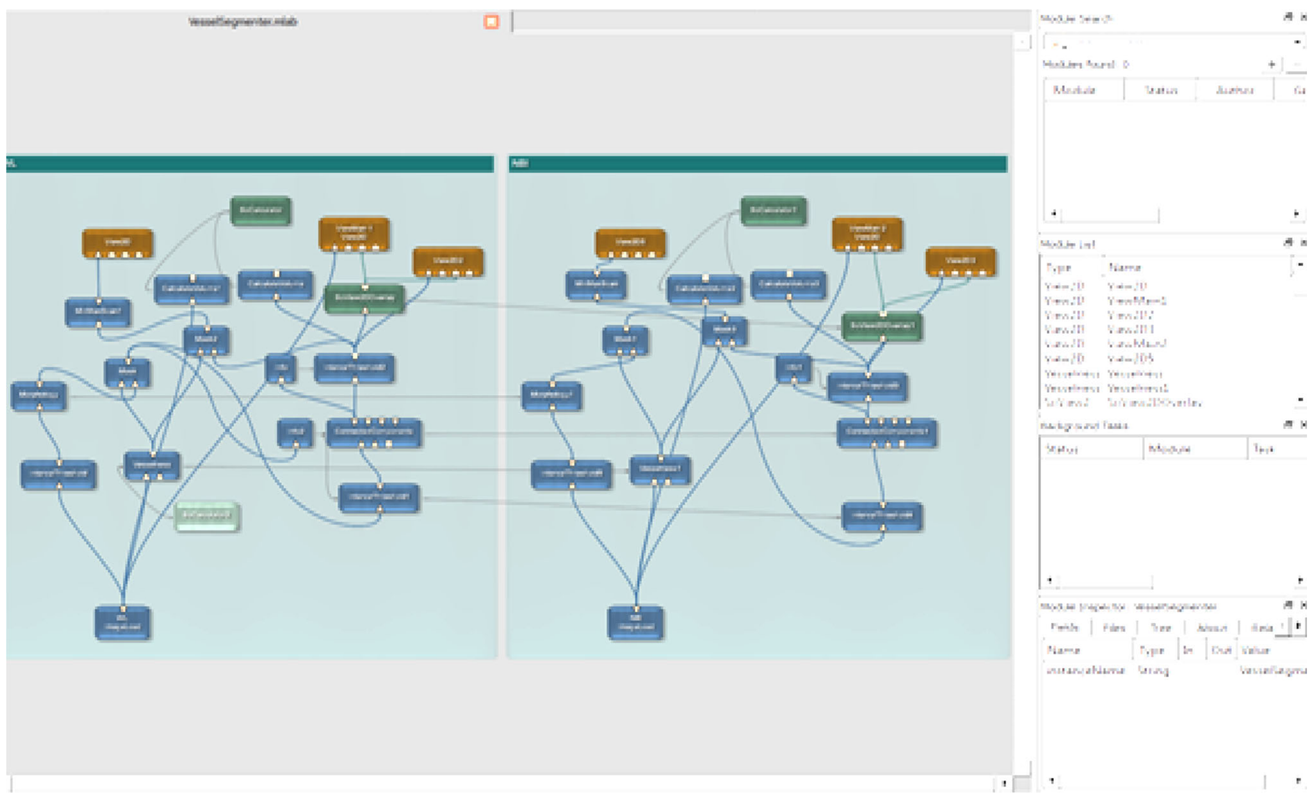

Fig. 4 Screenshot of the algorithm embedded in MeVisLab

MeVisLab is a freely available platform for medical image processing and visualization developed by MeVis Medical Solutions AG and the Fraunhofer MEVIS, Bremen, Germany [28].

MeVisLab enables the development of new algorithms for image processing and includes modules for segmentation, quantitative, and functional analysis. Visual programming is supported by an integrated development environment (IDE) with databases and toolkits. MeVisLab allows for the creation of individual algorithms and software demonstrators, as was performed for this study (Fig. 4).

Standard image format for MeVisLab is Digital Imaging and Communication in Medicine (DICOM). Other formats, such as TIFF, JPG, or BMP, are also accepted.

MeVisLab is written in C++, which enables it to load and read images independently from other platforms.

Integrated in MeVisLab is the open-source software "Insight Toolkit" (ITK), which is used for registration and segmentation. Segmentation can be based on pixel, edges, surfaces, or models [29, 30]. The purpose of all segmentation tools is to summarize contiguous pixels to a connected image area.

The segmentation tool used for this study utilizes a filter that separates objects in the image and defines each pixel either as part of a structure (e.g., a vessel) or not. The result of this segmentation is a binary image where 1 = structure and 0 = background.

The algorithm used in this study contains the blue-colored modules for image processing, green modules for

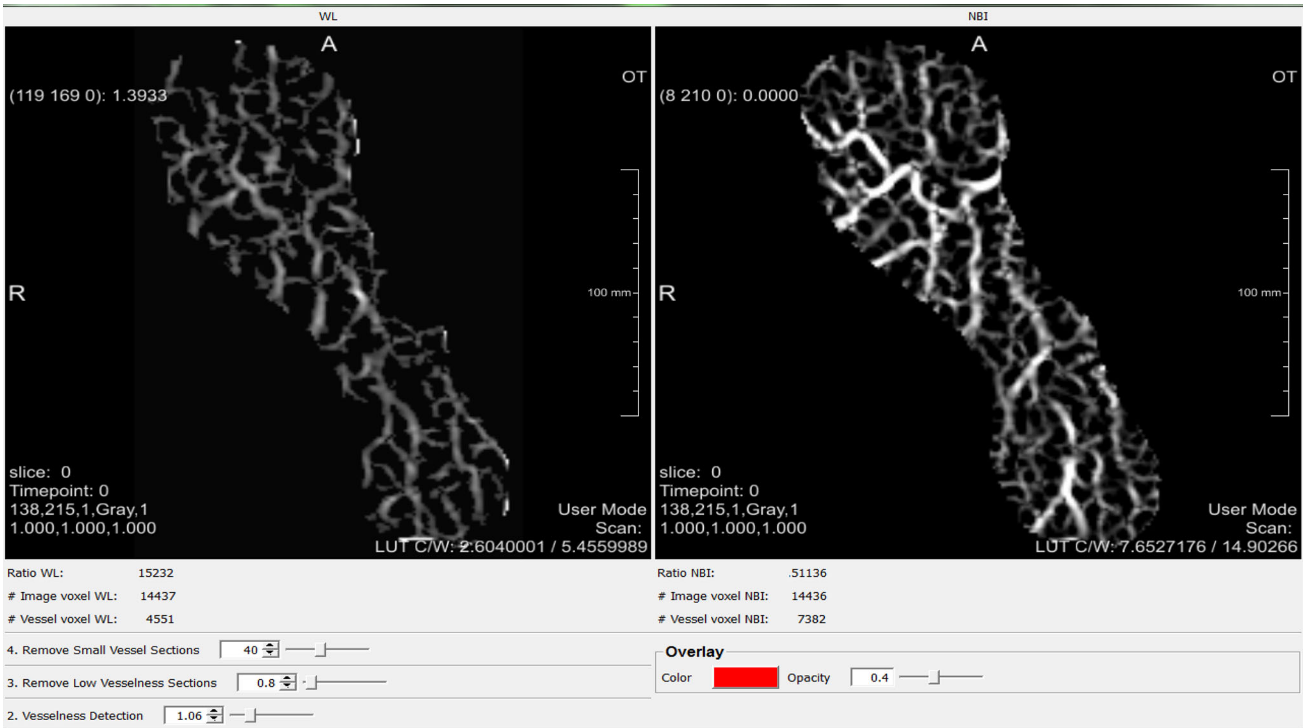

Fig. 5 Settings of the MeVisLab user interface as used for this study set at "remove small vessel sections" 40, "remove low vesselness sections" 0.8 and "vesselness detection" 1.06

visualization, and macro modules, which contain single processing and visualization modules, shown as brown modules. The first image processing module is a threshold module for discrimination between foreground and background. Afterward, the picture frame was cut out by a morphology module to avoid misinterpretation of the frame as a vessel structure. Elongated structures were detected with the "vesselness"-filter [28]. The multi-scale approach detects elongated structures of various diameters and yields a result image, where each pixel of the result image indicates the probability that this pixel belongs to a vessel. Next, a threshold module converts the result image into a binary image with 1 = vessel and 0 = background. To get rid of noise or small artifacts, the "connected components" module filters out very small objects of spatially connected pixels. A minimum number of 40 pixels were used for this study. On the user screen, i.e., the graphical user interface (Fig. 5), this function appears as a parameter next to "remove small vessel". The "calculate volume" module calculates the number of pixels that are assigned to a vessel and the total number of pixel and extracts their ratio.

A filter function improves the visible image information with minimal loss of information.

Setting with "remove small vessel sections 40," "remove lower vesselness sections 0.8," and "vesselness detection 1.06" seemed to deliver the best results for NBI and WL.

Results

The data analysis was performed with SPSS 22 for Windows (IBM, Armonk, USA) and Open Office spreadsheet for Linux, Ubuntu (Canonical Foundation).

For statistical analysis, the ratio of all voxels and vessel voxels was included. Verification of the Gaussian distribution was done by creating a histogram of the NBI and WL ratios.

To compare the ratio of NBI and WL, the mean and standard deviation were used to perform a *t* test for paired sample. The *t* test for paired samples showed a higher ratio for NBI than WL ($p = 0.000$).

The splitting of the right and left vocal folds offered the possibility to look for differences between the examinations of the individual vocal fold. This was achieved using a general linear model (ANOVA), where the two factors were side (left/right) and method (NBI/WL). Only the images of the subjects which showed both sides were included ($n = 47$) in this test.

ANOVA showed that there was a significant difference between the methods NBI and WL ($p = 0.002$), and between the sides left and right ($p = 0.033$). However, it could not be shown that there was an interaction between these two factors ($p = 0.467$).

The effect of both sides occurred independently from the method. This leads to the assumption that either one method generates a vessel/epithelium ratio in general, or one side of the vocal fold shows more vessels in general.

To get further information, a mixed model analysis was performed using all images, including the unpaired ones ($n = 101$). The fixed effects table shows that there was still a significant difference between the methods ($p = 0.000$) and the sides ($p = 0.031$); however, there was still no interaction between method and side ($p = 0.807$).

Discussion

In recent years, NBI developed from a new tool for diagnosis and examination into an integral part of clinical routine examinations in otolaryngology. It has been shown that NBI is superior to WL-endoscopy for detecting changes in vascular pattern, diagnosis of malignant, and benign lesions compared [15, 31, 32]. The advantage of NBI is its better illustration of the mucosal vessels using light with a defined wavelength that is specific for hemoglobin. In addition, this approach reduces the background noise recorded that must be filtered out and compensated for when the white light analysis is used. This simplifies the analysis significantly and frees up computing capacity that could be used, for example, maximizing the contrast of the images. Today, NBI is already used for an in vivo differentiation between malignant and benign laryngeal lesions [33].

It could not been shown objectively whether the effect of NBI is based upon a better contrast between the vessels and the mucosa, or if NBI really shows more blood vessels compared with white light. Most of the studies were influenced by the subjective rating of the examiner [34].

NBI can be used to improve the assessment of the vascular pattern and quantity of the vocal fold vessels in the diagnosis of premalignant, inflammatory, or benign lesions, which come along with changes in the vessel growth [31, 32, 35].

Therefore, the better representation of the vessels by NBI is not only an effect of the better contrast between the vessels and mucosa, but also the representation of quantitative more vessels.

NBI seems to be suitable as a screening tool in otorhinolaryngology due to its more accurate detection of changes in pattern, morphology, and distribution of the vessels, tumors, and other lesions can be diagnosed earlier [36].

The possibility of the detailed representation of the vessels by NBI could be used to create new classifications, or to expand existing ones based on the distribution pattern of the vessels like ICPL [36]. Other studies have shown the superiority of NBI in the assessment of vascular patterns. As part of the diagnosis of reflux disease, laryngeal erythema and vocal cord edema could be identified better by NBI compared with WL [37, 38].

The handedness of the examiner who performs the endoscopy seems to have an influence on the procedure. The comparison between the right and left sides of the vocal fold showed a significant difference. The assumption that one or another side is better illuminated depending on the handedness of the examiner could be confirmed. There is no interaction between the method and the side of the vocal fold.

For daily routine, it is not important if the endoscopy is performed with the right or left hand. For follow-up examinations, however, it seems to be useful if the same examiner performs the survey to get comparable results.

Conclusion

The present method is particularly suitable for finding the empirical evidence of incipient vascular changes, which have been described as ponogene vascular changes subjectively, but have yet to be verified. The ratio between vascular and epithelial tissues, semiautomatic generated using MeVisLab, could even be used for documentation of results of specific therapeutic interventions for hemorrhagic vocal cord polyps.

As the potential for automatic elimination of artefacts' is under development, MeVisLab could also be used in clinical trials. Here, it would be necessary to work without human influence (i.e., fully automatically). The challenge for the future lies in further automation, particularly the artefact elimination or segmentation.

Compliance with ethical standards

Conflict of interest All authors declare that no conflict of interest exists.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (Project Number: 107/14) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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