Endoscopic narrow-band imaging—quantitative assessment of airway vascularity after lung transplantation

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Abstract. In lung transplant recipients, the submucosal vascular plexus of the airway wall potentially represents one of the key structures of graft injury. Narrow band imaging is a novel endoscope technique that allows visual enhancement of the mucosa vasculature. It was our aim to investigate the ability of narrow-band imaging in combination with computerized image analysis to quantitatively assess airway vascularity in lung transplant recipients. In consecutive lung transplant recipients, in addition to the routine procedures, optical analysis of the main carina (autologous tissue) and the upper lobe carina (allogeneic tissue) were performed. From every site, three representative pictures were chosen. A total of 63 bronchoscopies were analyzed. The intraclass correlation coefficient (measure for test–retest reliability) of the three measurements were 0.69 and 0.74 for the main carina and the upper lobe carina, respectively. A mixed linear regression revealed increased vascularity in autologous tissue of patients with cystic fibrosis ($p=0.06$) and decreased vascularity in allogeneic tissue with time after transplantation ($p=0.09$). Endoscopic narrow-band imaging (NBI) in combination with computerized image analysis allows consistent assessment of airway vascularity *in vivo*. In lung transplant recipients, there might be differences in airway vascularity in both autologous and allogeneic large airways.
 [DOI: 10.1117/1.3076189]

Keywords: airway vascularity; allogeneic; autologous; lung transplantation; narrow-band imaging (NBI).

Paper 08027RRR received Jan. 22, 2008; revised manuscript received Nov. 22, 2008; accepted for publication Nov. 25, 2008; published online Feb. 4, 2009.

1 Introduction

Chronic allograft dysfunction in the form of histological bronchiolitis obliterans (BO) or its clinical correlate bronchiolitis obliterans syndrome (BOS) remains the most important reason for morbidity and mortality in long-term survivors after lung transplantation, affecting up to 50 to 60% of patients who are still alive five years after surgery.[1,2] Inflammatory airway injuries might occur at every stage after lung transplantation and are thought to represent major risk factors in the development of chronic organ dysfunction.[3] Inflammation patterns in both small[4] and large[5,6] airways have been investigated and characterized. Recently, we were able to show that inflammatory responses in large airways are not limited to the donor parts of airways.[7] Despite its scientific and clinical relevance, the pathogenesis of airway inflammation in lung transplant recipients remains incompletely understood. Both alloimmune[8] and nonalloimmune[9,10] underlying factors are discussed. Particularly, early vascular changes associated with ischemic injuries of the transplanted lung and the role of airway vessels in the consecutive development of BO are matters of current evaluation.[11]

Narrow-band imaging (NBI) is a novel endoscope technique that uses optical filters to narrow the bandwidth of the illuminating light during endoscopy. (In the current study, 415 and 540 nm were utilized.) Due to the differential optical absorption of light by hemoglobin, this technique allows visual enhancement of the mucosa vascularity. In combination with magnification endoscopy, this system was evaluated mainly in gastrointestinal endoscopy to improve diagnostic accuracy of premalignant and malignant lesions by better recognition of pathological vascular structures.[12] To a lesser extent, data considering NBI in combination with magnification bronchoscopy were published recently.[13]

It was the rationale of the present study to investigate the ability of an NBI bronchovideoscopy system in combination with computerized image analysis to quantitatively assess airway vascularity in lung transplant recipients. The comparison of these findings with clinical parameters was a further purpose of this study.

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1083-3668/2009/14(1)/014010/6/$25.00 © 2009 SPIE

2 Materials and Methods

Consecutive bilateral lung transplant recipients scheduled for routine surveillance bronchoscopy or indication bronchoscopy in case of lung function deterioration were included into the study. In our program, surveillance bronchoscopies are performed monthly during the first six months after discharge after transplantation. Before the routine procedures were performed [in most cases, bronchoalveolar lavage (BAL) and transbronchial biopsies], an NBI examination of the main carina (autologous tissue, proximal of the anastomosis) and the upper lobe carina (allogeneic tissue, distal of the anastomosis) were performed. Single lung transplant recipients were excluded from the study. The study was approved by the Ethics Committee of our hospital, and written informed consent from each patient was obtained.

2.1 *Pulmonary Function Testing*

Multiple serial pulmonary function tests (spirometry and body plethysmography, Sensor Medics Autobox plethysmograph, Yorba Linda, California) were available; the latest test was performed one day prior to bronchoscopy. The term BOS was used as defined elsewhere.[1]

2.2 *NBI Examination and Image Analysis*

We used an Olympus BF-Q180 EXERA II bronchoscope in combination with a CV-180 EXERA II processor and a CLV-180 Xenon EXERA II light source. This system uses a xenon light source and an interposed rotating narrow-band filter. With this device, wavelengths in the visible spectrum are filtered with the exclusion of narrow bands in the blue (415 nm) and green (540 nm) spectra. The size of the wavelength bands are 30 nm and 20 nm (half value width), respectively. The wavelengths of the narrow-band filters are selected on the basis of earlier studies, where compared to broadband illumination, an enhanced contrast for vascular patterns were shown.[14] This phenomenon is due to the fact that hemoglobin and its compounds have their peak absorption spectra near the respective wavelengths. The B 3 structure enhancement function of the processor was chosen for all examinations. This is an enhancement algorithm that electronically increases the sharpness of an endoscopic image by band enhancement processing. With this technique, the fine components in an image are enhanced more sharply than other parts of the image, and therefore, observation of finer mucosal structure changes are facilitated (precise algorithm is not yet disclosed).

The entire NBI examination was recorded by means of an analog/digital converter system (Pinnacle MovieBox DV, Pinnacle Systems, Braunschweig, Germany) on a laptop computer. Further analysis of the images was performed in a second step outside the endoscopy unit. Step by step (Pinnacle Studio 8, Version 8.6), the digitized film was screened in a standardized fashion by one investigator for three representative views from the anterior portion of the main carina and the upper lobe carina of the right lung. Effort was made to avoid overlapping of the particular images. The selection of the representative images was performed on the day of the investigation when no results from the bronchoalveolar lavage or the biopsy were yet available. The files were then blinded and saved in TIFF format. In a following step, the images were processed and measured on a computerized basis (analySIS software, version 5.0, Soft Imaging System, Olympus, Munster, Germany). First, in order to obtain homogenously exposed images, a shading correction procedure was applied (multiplicative option, N×N average filter iteration 2, size 45 pixels). Second, the pictures were binarized (color thresholds R 0/200, G 0/200, B 0/90–110) so as to reveal the number of white pixels (blood vessels) and black pixels (nonvessels). An example of this procedure is shown in Fig. 1. Relative vascularity was defined as white pixels / (white pixels+black pixels). All three images of every site were analyzed in the same way. For statistical analysis, the mean values of the three measurements were used. The entire digital processing of the images and all statistical analyses were performed after the last study bronchoscopy was completed.

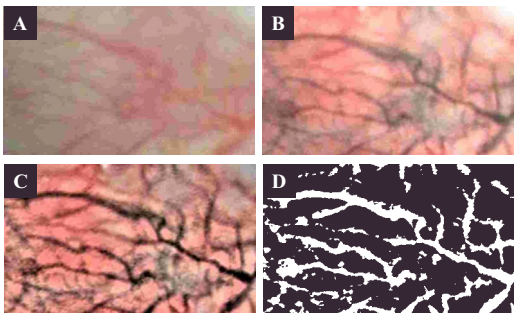

Fig. 1 Representative endoscopic image of the main carina with conventional white light (a) and narrow-band imaging technique, before (b) and after (c) shading correction. In a next step, binarization of the image is performed (d).

2.3 *Bronchoscopy and Transbronchial Biopsies*

Bronchoscopies and transbronchial biopsy staining were carried out as described elsewhere.[7] The assessment of acute and chronic rejection was performed according to standard criteria.[4]

2.4 *Statistical Analysis*

Statistica software (release 6.0, StatSoft, Inc., Tulsa, Oklahoma) and SPSS 13.0 (SPSS, Inc., Chicago) were used for statistical analyses. Descriptive data were expressed in absolute numbers and median and interquartile range of the raw data. The nonparametric Mann-Whitney U test and Fisher's exact test were used where appropriate. To estimate the reliability of the assessment algorithm of the relative vascularity, we calculated the intraclass correlation coefficients (ICC) for the three measurements of both the main carina and the upper lobe carina.[15] The ICC is appropriate when the three images are analyzed in arbitrary order.[16] It is the part of the variability of measurements explained by the variability between patients. Thus, an ICC of zero means that all variability reflects only measurement variation, whereas an ICC of one stands for a measurement without any error. To address correlations of measurements within the same patient, a mixed analysis of covariance (ANCOVA) was performed with relative vascularity as the dependent variable. Diagnosis of cystic fibrosis (yes/no) and time after transplantation (days) were the categorical factors and continuous predictors, respectively. The individual code of each patient was used as random factor in this model.

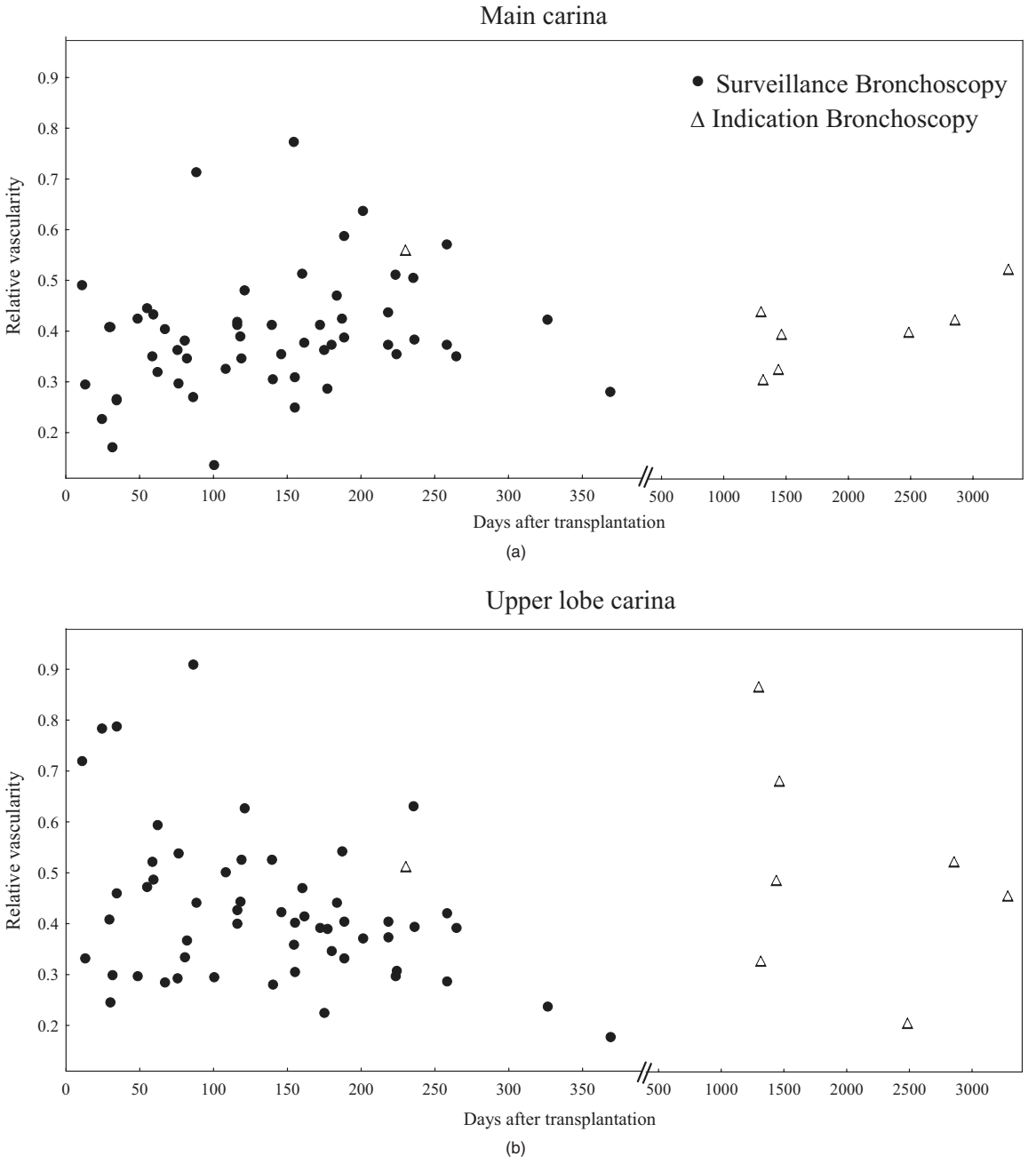

Fig. 2 Relative vascularity of the main carina (a) and upper lobe carina (b) assessed in surveillance bronchoscopies (circles) and indication bronchoscopies (triangles) in lung transplant recipients.

As the diagnosis of cystic fibrosis is patient-related, it was considered nested in the patient. p values less than 0.05 were considered statistically significant.

3 Results

A total of 63 bronchoscopies performed in 24 patients were analyzed. Of these, 55 were scheduled surveillance procedures, and 8 were indication bronchoscopies, respectively. The baseline characteristics of the examinations are summarized in Table 1. All procedures were performed by the same investigator (S.I.) without complications.

The intraclass correlation coefficient values of the measurements of the relative vascularity of the three different images of all 63 examinations were 0.69 and 0.74 for the main carina and the upper lobe carina, respectively. The size of the images we have analyzed were 6.3×10^4 (4.6 to 8.6×10^4) and 5.6×10^4 (4.1 to 7.9×10^4) for the main carina and the upper lobe carina, respectively (median, interquartile range).

The results of the measurements of the relative vascularity of the main carina and upper lobe carina, respectively, in relation to time after transplantation are shown in Fig. 2. The case profiles of the corresponding relative vascularity of the main carina and the upper lobe carina, respectively, are shown

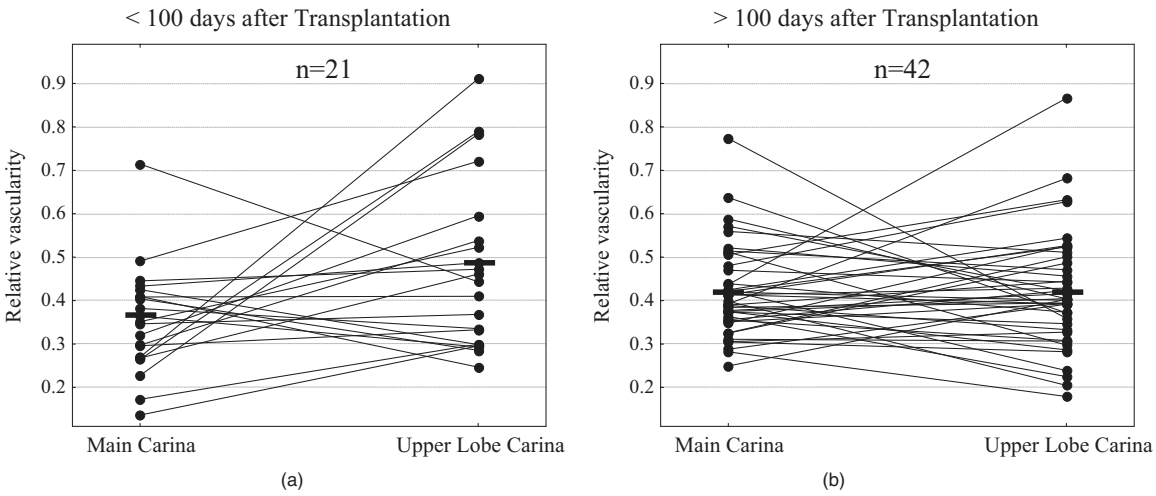

Fig. 3 Relative vascularity of the main carina (left column) and upper lobe carina (right column) assessed during 63 bronchoscopies performed in 24 lung transplant recipients. To show the particular profile more distinctly, arbitrary time periods of less than 100 days (a) and more than 100 days (b) after transplantation were chosen, respectively.

in Fig. 3. To make the plot more distinctive, arbitrary time periods of more than and less than 100 days after transplantation were chosen, respectively. Since this plot shows a certain trend toward decreasing vascularity of the upper lobe carina with time after transplantation, we plotted the consecutive measurements of the relative vascularity of nine patients separately in whom three or more surveillance bronchoscopies were available (Fig. 4). The relative vascularity of the upper lobe carina was consistently decreasing with time in seven of these nine patients, whereas the relative vascularity of the main carina remained more stable over time on an individually different level. Due to these observations, we fitted a regression model with each patient as a random factor. In surveillance bronchoscopies, the vascularity of the main carina showed no association with the time after transplantation (Table 2). In contrast, the upper lobe carina showed a trend ($p=0.09$) toward decreasing vascularity with time after transplantation. A representative example of the changes of bronchial vascularity during the time course after transplantation is shown in Fig. 5. Our mixed ANCOVA revealed cystic fibrosis as a factor that was associated with increased relative vascularity of the main carina ($p=0.06$).

Table 1 Baseline characteristics of 63 bronchoscopies performed in lung transplant recipients.

	Surveillance bronchoscopies ($n=55$)	Indication bronchoscopies ($n=8$)	p
Number of individual Pts	16	8	
Cystic fibrosis y/n	18/37	4/4	0.28
COPD y/n	10/45	2/6	0.47
Time after Tx (days)	139 (67–188)	1449 (1306–2669)	<0.001
Acute rejection (≥A1) y/n	9/25[b]	0/2[b]	0.57
Infection[a] y/n	9/37[b]	1/4[b]	0.98
BOS y/n	3/52	6/2	<0.001
BAL lymphocytes (%)	3 (1–6)	4 (3–9)	0.24
BAL neutrophils (%)	2 (1–5)	4 (3–4)	0.4

Data are presented as absolute numbers and median (interquartile range). Pts: patients, COPD: chronic obstructive pulmonary disease, Tx: transplantation, BOS: bronchiolitis obliterans syndrome, BAL: bronchoalveolar lavage.

[a]For definition, see text.

[b]BAL and biopsy, respectively, were not performed during every bronchoscopy.

4 Discussion

In lung transplant recipients, the submucosal vascular plexus of the airway wall potentially represents one of the key structures of graft injury and consecutive graft failure. Both ischemic and inflammatory changes might influence airway vascularity. Despite its probable impact on the pathology of post-transplant airway complications, data concerning airway vascularity after lung transplantation are scarce,[11,17,18] and studies addressing airway vascularity *in vivo* in this population are almost completely lacking.[19] It is the main intention of NBI to significantly enhance visualization of vascular structures during endoscopy. So far, this minimally invasive *in vivo* technique has been applied mostly to cancer research. It was the aim of the current study to investigate the ability of the combination of NBI with computerized image analysis to quantitatively assess airway vascularity. Furthermore, a comparison of the achieved results with clinical parameters was attempted. To our knowledge, this is the first study investigating NBI for quantitative assessment of airway vascularity, particularly in lung transplant recipients.

Using a computerized image analysis system (analySIS), we were able to quantitatively assess the relative area of vascularity of the main carina (autologous tissue) and upper lobe

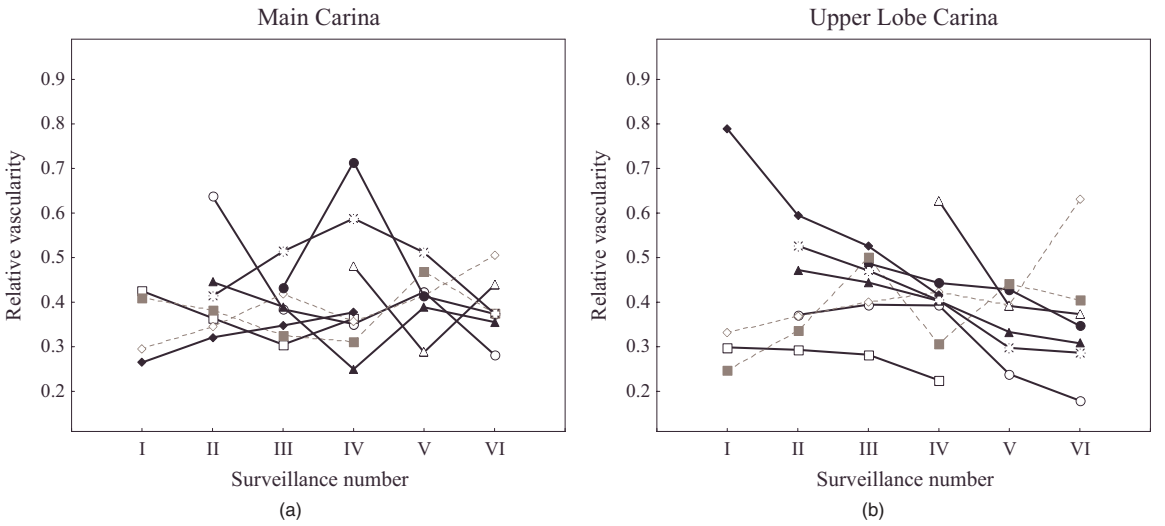

Fig. 4 Relative vascularity of the main carina (a) and upper lobe carina (b) of nine individual lung transplant recipients in whom three or more surveillance bronchoscopies were available (black: patients with decreasing vascularity of the upper lobe carina; gray: patients with increasing vascularity of the upper lobe).

carina (allogeneic tissue) in blinded images of airways of lung transplant recipients in a consistent manner. The ICC calculations of 378 different images confirmed objective and reproducible measurements in both autologous and allogeneic parts of airways. Due to these results, further quantitative analyses of the measurements were justified.

Considering the intra-individual course of the airway vascularity after transplantation, in most cases, the vascularity of the allogeneic part was decreasing in a surprisingly consistent manner. Although not statistically significant, applying a conservative statistical model on the entire population of surveillance bronchoscopies, a trend of decreasing vascularity could be shown in allogeneic central airways. In previous studies,[11,17] histological assessment of endobronchial biopsies revealed no association between time after transplantation and vascularity. In contrast, in our study, we were able to show that mucosal vascularity of the central graft airways decreases in most cases during the first months after transplantation. Since the bronchial arterial supply to the graft is not reanastomosed at the time of transplantation, blood flow in the submucosal vascular plexus of the graft should be decreased in the early period after transplantation. However, it seems that this can be compensated with collateral perfusion soon after transplantation. Therefore, airway ischemia might have been overestimated as a significant factor of tissue injury in the setting of lung allo-engraftment in the early post-transplant period. The pathogenesis of this time-dependent change of airway vascularity of lung allografts remains speculative. Since so far, repeated assessment of non-artificially processed tissue was not possible, little is known about time-dependent alteration of airway vascularity. Considering the fact of non-reanastomosis of the bronchial circulation during lung transplant surgery, alloimmune or other inflammatory mechanisms rather than simple mechanical factors might be responsible for this observation.

When we compared the results of the autologous part of airways, we found cystic fibrosis to be an independent factor for increased airway vascularity. This result fits well with earlier studies that have demonstrated that increased airway vascularity can be the result of chronic inflammatory processes under various circumstances.[20] Particularly in patients suffering from cystic fibrosis, airway inflammation has been described in detail.[21] Nevertheless, to our knowledge, changes of vascularity of the large airways of cystic fibrosis patients

Table 2 Mixed analysis of covariance of the relative vascularity of the main carina and upper lobe carina, respectively, in surveillance bronchoscopies after lung transplantation ($n=55$)

Covariates[a]	Relative vascularity[b] main carina	Relative vascularity[b] upper lobe carina
Time after Tx (days)	−0.008 (−0.02; 0.009) p=0.32	−0.015 (−0.03; 0.002) p=0.09
Cystic fibrosis	0.21 (−0.01; 0.42) p=0.06	−0.072 (−0.15; 0.29) p=0.52

Numbers represent the regression coefficient and 95% confidence interval, respectively. Tx: transplantation.

[a]Identification number of the patient is a random factor in this model, and cystic fibrosis is corrected for time after transplantation.
[b]For definition, see text.

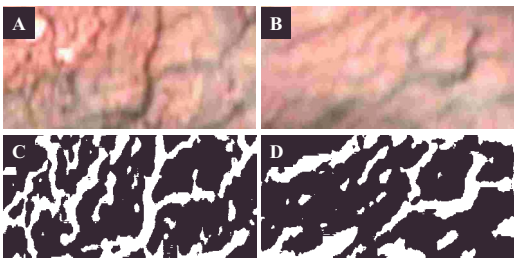

Fig. 5 Representative endoscopic image of the identical part of the right upper lobe carina with narrow-band imaging technique in a lung transplant recipient two (a) and six (b) months after transplantation, respectively. After binarization, relative vascularity was measured by computerized image analysis [(c) relative vascularity at two months: 0.45; (d) relative vascularity at six months: 0.28].

have not been described before, although there is evidence of elevated serum vascular endothelial growth factor in cystic fibrosis patients related to airway infection.[22]

NBI technique in combination with computerized image analysis provides multiple advantages over histological tissue examination. It is a minimally invasive procedure, and therefore, large amounts of visible airway structures can be assessed repeatedly. Furthermore, due to its *in vivo* applicability, this technique offers thrilling opportunities, particularly for issues of vascular research.

This study is limited by the relatively small number of patients, which made it impossible to assess the influence of graft infection, graft rejection, and BOS on airway vascularity in a statistically adequate fashion. Furthermore, a direct comparison between macroscopically assessed vessels and the finding of histological examinations of endobronchial biopsies would provide more detailed information about airway vascular changes in lung transplant recipients. We plan further investigations with these two complementary methods. Since we attempted to avoid any artifacts as rigorously as possible, in the current study, we did not take endobronchial biopsies from the location that we have investigated using NBI. Another drawback is the fact that the distance between the endoscope and the tissue surface is not completely stable during sequential endoscopic investigations *in vivo*. As a consequence, absolute calibration of the images cannot be obtained, and therefore, rather than absolute sizes, relative measurements were used in the current study.

In summary, for the first time, investigations of airway vessels have been performed with the aid of NBI in combination with computerized image analysis. We showed distinct airway vascular structures and described methods to quantitatively assess airway vascularity in a consistent manner. Differences were found between cystic fibrosis and non–cystic fibrosis patients (autologous airways). According to our findings, airway vascularity of the allogeneic central airways decreases during the first months after transplantation.

These preliminary results show that (1) NBI is a safe and feasible procedure in lung transplant recipients, that (2) NBI in combination with computerized image analysis allows consistent assessment of airway vascularity, that (3) there might be differences in airway vascularity in both autologous and allogeneic large airways under certain clinical circumstances, and (4) that additional studies are warranted to further define airway vascular patterns in lung transplant recipients with the aid of this new endoscopy tool.

Acknowledgment

The authors gratefully acknowledge the contribution of equipment by Olympus® Switzerland.

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