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Detection of Cancerous Endobronchial Lesions by Autofluorescence Bronchoscopy Combined with Mutation Analysis of p53

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Abstract: Early lung cancer screening failed to reduce lung cancer mortality. New techniques such as autofluorescence bronchoscopy (AF) and the identification of specific genetic alteration might change future outcomes of lung cancer screening.

It was the aim of our study to combine p53 analysis with white-light bronchoscopy (WL) or WL and AF to improve the diagnostic yield in a series of 36 patients with histologically proven lung cancer, pulmonary metastasis or suspected lung cancer.

Endobronchial sites were analysed by WL (n = 71), AF (Storz) (n = 34), histopathology (n = 71) and p53 mutations were examined by SSCP analysis on additional biopsies (n = 69). The overall frequency of cancerous lesions was 19, of which 14 were macroscopically visible lesions. The addition of p53 and autofluorescence improved the yield to 17 of 19 cases. In 7 preinvasive lesions (dysplasia/metaplasia) 4 were identified macroscopically and 5 of 7 lesions by all 3 methods. In the WL/p53 group the diagnostic yield was 7 of 9 cancerous lesions compared to 10 of 10 cancerous lesions in the AF group.

It should be noted that all methods were associated with false positive results. However, the combination of conventional with autofluorescence bronchoscopy and mutation analysis is a promising approach which is applicable to clinical routine and may be further enhanced by the inclusion of a panel of markers of tumour progression.

Key words: p53, autofluorescence, lung cancer

Introduction

Bronchial carcinoma is not only one of the most frequent malignant tumours worldwide, but is also associated with a distressingly high mortality [20]. In spite of vast therapeutic progress in recent decades cure rates are low unless radical resection can be achieved in the very early stage Ia. Early identification of lung cancer has therefore received much attention in the hope that it would allow to improve the cure rate by early tumour resection or chemoprevention. While several early randomized controlled studies have not shown a reduction in lung cancer mortality as a result of screening [1, 11, 19], new techniques might change future outcomes of screening for lung cancer and randomized trials are underway to evaluate their efficacy [4-6, 14, 17, 22].

Many efforts to develop suitable screening methods for risk patients for the early detection of lung cancer could not fulfil the hopes put into them. Among the methods believed to have the greatest potential are low-dose CT scan, improved endoscopic procedures, improved sputum collection and analysis and the identification of specific genetic alterations [16-18, 23, 25-27].

Autofluorescence bronchoscopy was developed to improve the sensitivity and specificity of conventional endoscopy [3]. In large Canadian and US studies the addition of fluorescence systems increased the detection of dysplasia or CIS up to a detection rate of more than 80% while specificity decreased at the same time [5, 12-14].

Among the candidate genes for molecular screening p53 mutations are a suitable target because they occur early in tumour development, they can be detected by well established methods and are known to be induced by tobacco consumption [7, 10].

In our study we investigated 36 patients with known, pretreated bronchial carcinoma or with suspected lung carcinoma and compared white-light with a combination of white light and autofluorescence bronchoscopy for the detection of malignant changes. In addition p53 mutation analysis was carried out to see whether this marker of early tumour progression could improve the diagnostic yield.

Patients, Methods and Statistics

The study population comprised 36 adults (11 females and 25 males, mean age 61 ±10 years) with histologically proven lung cancer (n = 26), pulmonary metastasis of other solid tumours (n = 3) or suspected lung cancer (n = 7) undergoing diagnostic bronchoscopy at the University Hospital of Munich, Innenstadt, Germany. Patients were selected from participants of the ongoing multicentre "European study for early detection of central lung cancer" and they agreed to have additional biopsies taken for molecular analysis. The main criteria for inclusion in the study were a smoking

history of at least 20 pack years (1 pack a day for one year = 1 pack year), chronic obstructive pulmonary disease or a history of workplace exposure to carcinogens. Exclusion criteria were poor health status (Karnofsky Index < 60), pregnancy, psychiatric disease, severe mucus production, significant bronchial bleeding, exacerbation of bronchitis, general contraindications for bronchoscopy and chemotherapy within the preceding 6 months.

The study protocol was approved by the ethics committee of the University of Munich. Written informed consent was obtained from all study participants.

Treatment Assignments

Consecutively enrolled patients were participants of the multicentre "European study for early detection of central lung cancer" and were randomized by the central study centre to receive either conventional white-light or autofluorescence and conventional bronchoscopy.

Bronchoscopic Procedures

The bronchoscopies were carried out with a flexible bronchoscope with D-Light/AF mode (Storz, Tuttlingen, Germany). D-light was used for conventional white-light bronchoscopy and the AF mode for autofluorescence (wavelengths of the blue-light between 380 and 480 nm) [2]. Multiple biopsies were taken and sent to the pathologist for histological examination of suspicious lesions. For the molecular analysis two additional biopsies were taken each from macroscopically suspicious tissue and from the contralateral normal appearing mucosa. These biopsies were stored frozen at -20°C.

DNA analysis

For DNA extraction a DNA isolation kit (nucleon BACC 1 extraction kit from Amersham Life Science, Braunschweig, Germany) was used and the DNA yield was determined photometrically at 260 nm for quantity and at 280 nm for purity. Exon 5–8 of the p53 gene were amplified by PCR using the protocol published previously [15]. All primers were from MWG biotech (Munich, Germany).

Mutations of the p53 gene were analysed by single strand conformation polymorphism (SSCP) analysis of amplified DNA. After non-denaturing polyacrylamide gel electrophoresis, DNA bands were visualised with a 0.1% silver stain and band size was assessed by comparison with known standard DNA (123 bp DNA ladder; Gibco BRL, Bethesda, Maryland, USA). A preparation of wild type DNA from peripheral leucocytes of healthy volunteers served as negative control.

Statistical Analysis

Data were analysed using SPSS software (release 10.0; SPSS, Inc. Chicago, IL, USA). Results are expressed as mean (SD). Statistical comparisons between the groups were performed using the Pearson chi-square test. Significance was accepted at a $p<0.05$ level.

Results

18 patients were randomized to receive conventional white-light bronchoscopy and 18 patients had white-light and autofluorescence bronchoscopy. The study groups were comparable in age, gender distribution and tumour stage (Table 1). In the autofluorescence group, there were more squamous carcinomas, the white light group had more adenocarcinomas.

Table 1. Patient characteristics in the white-light (WL) and autofluorescence (AF) group.

Study group	**WL**	**AF**
N	18	18
Age (years)	62 (10)	60 (11)
Gender (f/m)	(6/12)	(5/13)
Apparent tumour		
stage I	0	0
stage II	0	1
stage IIIA	1	0
stage IIIB	3	4
stage IV	11	9
Histological type		
Adenocarcinoma	5	2
Small cell carcinoma	2	2
Large cell carcinoma	2	1
Squamous carcinoma	3	8
Other histologies	3	1
No apparent tumour	3	4

Data are given as mean ± SD

Biopsies taken from 71 sites were sent for histological assessment. 69 additional biopsies were processed for DNA analysis and interpretable results could be obtained in 61 sites. There was no difference in the average number of biopsies taken between the white-light and the autofluorescence group. The histological results of the 71 bioptic sites and their correlation with white-light, autofluorescence bronchoscopy and p53 analysis are given in tables 2 and 3. In the white-light group histological assessment showed 9 carcinomas and 5 preinvasive histologies (dysplasia or metaplasia). 6 of 9 biopsies with carcinoma and 2 biopsies from premalignant lesions were from a visible tumour or from a suspicious lesion. With the addition of p53 analysis one more carcinoma and one more metaplastic lesion could be identified. In the autofluorescence group carcinoma was present in 10 cases, dysplasia or metaplasia in 1 case each. Macroscopically visible tumor or suspicious lesions were present in 8 of 10 cancerous sites and two additional sites had suspicious autofluorescence. Both preinvasive lesions were detected by macroscopical assessment alone.

It should be noted that autofluorescence, conventional bronchoscopy and p53 analysis were also associated with false positive results. From 23 sites with inflammation, scar tissue or normal mucosa 6 were mis-

Table 2. Detection of tumour tissues by white-light and autofluorescence bronchoscopy.

Histology			Invasive carcinoma	Dysplasia	Metaplasia	Inflammation	Normal or scar
Number of bioptic sites			**19**	**2**	**5**	**20**	**25**
Macroscopic finding (N = 71 from 37 x WL + 34 x AF)	Visible tumor (N = 15)	WL	**4**	**0**	**1**	**1**	**1**
		AF	**6**	**1**	**1**	**0**	**0**
	Suspicious site (N = 11)	WL	**2**	**1**	**0**	**3**	**1**
		AF	**2**	**0**	**0**	**1**	**1**
	Abnormal site (N = 8)	WL	**0**	**0**	**2**	**1**	**2**
		AF	**1**	**0**	**0**	**1**	**1**
	Unsuspicious site (N = 37)	WL	**3**	**0**	**1**	**4**	**10**
		AF	**1**	**0**	**0**	**9**	**9**
Fluorescence (N = 34)	Suspicious site (N = 11)	AF	**7**	**1**	**0**	**2**	**1**
	abnormal site (N = 6)	AF	**1**	**0**	**1**	**3**	**1**
	unsuspicious site (N = 16)	AF	**1**	**0**	**0**	**6**	**9**
	Not diagnostic (N = 1)	AF	**1**	**0**	**0**	**0**	**0**

Table 3. Detection of tumour tissues by p53 analysis.

Histology			Invasive carcinoma	Dysplasia	metaplasia	inflammation	Normal or scar
P53	Mutation	WL	**4**	**1**	**2**	**0**	**1**
		AF	**6**	**0**	**0**	**1**	**1**
	wildtype	WL	**4**	**0**	**2**	**6**	**11**
		AF	**4**	**1**	**1**	**9**	**7**

judged as suspicious (n = 4) or tumour (n = 2) in the white light group and 2 of 22 sites in the autofluorescence group (Table 2).

Altered P53 was detected in 10 of 19 carcinomas (4 in the white-light group and 6 in the autofluorescence group) and in 3 of 7 preinvasive lesions. 3 mutations were found in histologically inflamed or normal appearing tissues (Table 3).

The addition of autofluorescence or p53 mutational analysis added further diagnostic information because the histological diagnosis was concordant with the macroscopic impression gained by conventional bronchoscopy only in 49 of 71 biopsy sites. With the addition of fluorescence and p53 mutational analysis only 10 biopsies had histological results which would have been missed by all 3 modalities used.

Discussion

Endoscopic screening for early lung cancer with different modes of autofluorescence has been shown to work in risk populations [2, 5, 8, 9, 10, 13, 21]. The detection rate of preinvasive or early invasive lesions could be improved by the use of autofluorescence from 27% up to 71% in the USA and Canada [12, 13] and from 47% up to 83% in Europe [10]. Even higher rates were reported from Japan (51% up to 88%) [23]. The differences reported may be due to selection criteria such as presence of atypical cells in sputum as inclusion criterion. The worldwide experience shows detection rates of approx. 40% of preinvasive lesions by conventional bronchoscopy. The addition of autofluorescence can double this detection rate [2, 5].

However, in clinical practice screening not only encompasses risk populations but more often patients with known treated carcinoma with a high risk of recurrence or metachronous lesions. Conventional endoscopy often faces the problem how pretreated lesions should be interpreted. While the addition of autofluorescence does not substantially prolong the endoscopic procedure and is associated with virtually no extra risk for the patient, our data show that the addition of autofluorescence and mutation analysis may add further diagnostic information.

In our series of mostly pretreated lung cancer patients the diagnostic yield was 7 of 9 cancerous lesions in the white light/p53 group compared to 10 of 10 cancerous lesions in the group with all modalities combined. In 7 preinvasive lesions (dysplasia/metaplasia) 4 were identified macroscopically and 5 of 7 lesions by all 3 methods. It was not only the diagnostic yield of malignant or preinvasive lesions but also the diagnostic accuracy which was supported by a high number of concordant findings in our series of patients.

While it is still not clear what kind of genetic alteration is essential for the development of lesions to cancer, it is known that somatic molecular genetic changes underlie and drive tumour progression. P53 mutations are typically first seen in dysplasia and are thought to be indicative of a high risk to develop lung cancer. On the other hand, p53 mutations do not occur in all bronchial tumours and negative results do not necessarily imply the absence of tumour [16, 22, 24].

The detection of early genetic alterations as well as suspicious autofluorescence may be additional tools in our armamentarium to diagnose endobronchial lesions. One of the drawbacks of the endoscopic procedures is the fact that autofluorescence improves sensitivity but not specificity. Therefore additional information such as the presence of abnormal p53 or better a combination of molecular markers of tumour progression may be useful to interpret these lesions.

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