The Natural Course of Preneoplastic Lesions in Bronchial Epithelium

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ABSTRACT

Purpose: **To study the natural history of preneoplastic lesions in the bronchial mucosa of the individuals at risk.**

Patients and Methods: **White light and autofluorescence bronchoscopy examinations have been done in 52 individuals harboring 134 preneoplastic lesions (WHO criteria). End points were the development of carcinoma *in situ* (CIS) or squamous cell cancer (SCC) or the highest category of dysplasia up until March 1, 2003 for the remaining preneoplastic lesions.**

Results: **Distribution and outcome of preneoplastic lesions have been found to be unrelated to various risk factors such as smoking history, past history of cancer, or chronic obstructive pulmonary disease. Nonstepwise changes of preneoplastic lesions are seen. Regression rate has been 54%. Progression to CIS/SCC has been 13.4% (18 of 134) and was for severe dysplasia, significantly higher ($P < 0.003$) than preneoplastic lesions showing lower-grade dysplasia (squamous metaplasia, mild and moderate dysplasia). Time to progression was not significantly different. However, when analyzed per individual, no significant difference of progression rate between individuals with or without severe dysplasia was seen (39% versus 26%; $P = 0.36$).**

Conclusions: **The 54% regression rate of all preneoplastic lesions, 26% to 39% progression rate to CIS/SCC of individuals with lower-grade dysplasia or severe dysplasia with no significant difference in progression rate and time to progression combined with nonstepwise histologic changes unrelated to the initial histologic grading indicate that one cannot differentiate the potentially more malignant preneoplastic lesions among the many preneoplastic lesions present in the bronchial mucosa. The initial WHO classification of any preneoplastic lesion cannot be reliably used for accurate risk assessment of field carcinogenesis.**

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INTRODUCTION

It is generally assumed that squamous cell cancer (SCC) develops in a gradual and stepwise fashion according to the WHO grading of preneoplatic lesions from normal epithelium, hyperplasia, squamous metaplasia, mild, moderate, severe dysplasia towards carcinoma *in situ* (CIS), and microinvasive SCC (1–3). Currently, the finding of any histologic class in any preneoplastic lesion has been considered predictive for the malignant development of SCC (i.e., a higher grade of dysplasia indicates a higher risk for becoming malignant; ref. 4). Data from studies of sputum cytology suggest that ~11% of moderate dysplasia and 19% to 46% of severe dysplasia will progress to SCC (5, 6). The updated CIS data in our study population showed that all lesions have become SCC (7). Recent data by Bota et al. (8) showed up to 87.5 % CIS to persist or progressed during follow up, whereas the progression of low grade dysplasia was 2.5%, which increased to 6.1% in the presence of a high grade dysplastic lesion. We have analyzed the changes over time of various preneoplastic lesions (i.e., severe dysplasia or less, excluding CIS) in a cohort population at risk to develop (metachronous) SCC.

PATIENTS AND METHODS

Individuals at risk for developing lung cancer based on various risk factors (e.g., hemoptysis, positive sputum cytology, suspicious chest X-ray, or follow-up after curative resection of cancer in the upper respiratory tract) that harbored bronchial preneoplastic lesions were included in this analysis. At baseline, all subjects were free of overt cancer based on HRCT, autofluorescence bronchoscopy (AFB), and histology findings. Patient's characteristics are shown in Table 1.

Bronchoscopic Examination. White light bronchoscopy (Olympus BF-20D, Tokyo, Japan) and AFB (Xillix-LIFE, Richmond, British Columbia, Canada) have been done at regular interval as has been previously reported (7, 9, 10) following a rigid protocol. All subjects gave their consent for repeat bronchoscopic interventional procedures according to the various existing protocols approved by the medical ethical committee of our hospital and in accordance with the national and international guidelines for interventional bronchoscopy (11). Biopsies were taken from all suspicious areas and from at least one additional normal site as indicated by both white light bronchoscopy and AFB (7, 9, 10). To construct the natural history of bronchial preneoplastic lesions, longitudinal and accurate sampling, if necessary under AFB imaging, of specimens have been done using one pair of flexible biopsy forceps for each site to prevent contamination.

Histologic Examination. H&E-stained slides were microscopically examined and categorized according to the WHO criteria: normal epithelium, hyperplasia, squamous metaplasia, mild, moderate, severe dysplasia, CIS, and SCC (2). Illustrations regarding a representative example of histology of each preneoplastic lesions are shown in Fig. 1. Repeat bronchoscopy and

deep biopsies have been repeated in case specimens lack the basement membrane for proper classification of CIS.

After routine histologic classification by a staff pathologist and a pathologist trainee, all slides were reviewed by another staff pathologist with special interest in bronchial carcinogenesis (E.J.R., $\kappa = 0.70$; ref. 12).

Per Lesion Analysis. In analysis per lesion, all preneoplastic lesions were divided in cohorts according to the initial histologic grade according to the WHO classification criteria. Progression and regression at the exact spot of the initial biopsy site were scored to show the evolution of preneoplastic lesions between the different histologic grades during follow-up. Progression towards CIS/SCC is shown separately (Table 2; Fig. 2).

Per Individual-Based Analysis

Due to the recognition of individual's susceptibility for lung cancer, the intraindividual and interindividual variability of histologic classification (12, 13) and recent data showing the relative more importance of high-grade lesions (HGD) in term of malignant progression (8), analysis of outcome has also been determined per cohort of individuals based on the premise of possible higher progression rate to CIS/SCC in case a HGD (severe dysplasia) lesion is found at baseline (see Fig. 3).

A division in two cohorts of individuals at risk who harbored either ≥1 HGD (thus severe dysplasia) versus those harboring ≥1 lower-grade dysplasia (LGD: either squamous metaplasia, mild dysplasia, or moderate dysplasia) at baseline has been made. For example, if an individual harbors a squamous metaplasia, two mild dysplastic lesion and two severe dysplastic lesions, assignment to the HGD cohort is made and the development of any severe dysplasia determined the outcome. Progression is considered more important than regression or stable lesion. If another individual harbors two squamous metaplasias, and one mild dysplasia, assignment to the LGD cohort based on the absence of severe dysplasia is made and outcome would be determined by the progression to the highest histology grade of any preneoplastic lesion during follow-up. Progression is considered more important than stable lesion or regression. Progression to CIS/SCC for a HGD, progression to either HGD or CIS/SCC for a LGD, regression to either LGD or normal/inflammation for a HGD, and regression to normal/inflammation for a LGD have all been separately analyzed. preneoplastic lesions were regarded stable over time when they remain in their (HGD or LGD) histologic categories at the time point closest to March 1, 2003. Outcome of the LGD cohort, individuals harboring squamous metaplasia, mild and moderate dysplasia, versus the HGD cohort harboring severe dysplasia has been separately analyzed. Note that in comparison to per lesion analysis (Fig. 2) numbers may not match as individuals at risk may harbor more preneoplastic lesions per patient.

Influence of Gender, Chronic Obstructive Pulmonary Disease, Past History of Cancer(s), and Smoking Status

Correlation of gender, chronic obstructive pulmonary disease (COPD), medical history and tobacco use to the distribution and outcome of preneoplastic lesions have also been analyzed (see Table 2). Smoking history was obtained through a questionnaire. Subjects were divided into three groups: current smokers, ex-smokers (divided in two groups: who stopped <5 years before the bronchoscopic examination or who stopped >5 years), and never-smokers. Age at smoking initiation and cessation, number of pack-years, and duration of smoking cessation before baseline examinations have been documented.

Outcome Analysis

The development of CIS or SCC for each particular site is considered the end point of its natural history. Outcome of the lesion(s) that have thus far not become CIS/SCC was determined by comparing the baseline histologic grade of any lesion with the last histologic grade closest to the March 1, 2003 time point.

Clinical Management

Primary or synchronous cancer lesions were given appropriate treatment immediately after confirmation of the diagnosis of CIS by the reviewer pathologist (10). Severe dysplasia has been followed more closely every 3 to 4 months (7, 10). If the pathologic grade progressed to CIS, IBT was applied in case tumor is occult, under AFB assistance to treat tumor margins more precisely (9).

Table 1 Characterisitics of 52 individuals at risk harboring preneoplastic lesions (preneoplastic lesions: squamous metaplasia, mild, moderate, and severe dysplasia) in the central airways at baseline bronchoscopic examinations

Total number of individuals	52
Gender:	
Male	44
Female	8
Median age (y), (range)	64 (42-78)
COPD	31
Non-COPD	21
Smoking history	
Current smokers	12
Ex-smokers	38
Never-smokers	2
Median no. (range) of pack-years smoked	39.5 (0-120)
Age (range) at smoking initiation	19 (8-27)
Median no. years (range) after smoking cessation	3.5 (1-39)
Medical history	
Group 1: at risk for lung cancer primary	21
Group 2: at risk for subsequent primary	
Past ENT cancer	8
Past lung cancer treated surgically	11
Past lung cancer treated bronchoscopically	5
≥2 primaries: ENT-Lung; Lung-Lung	7
Total no. of bronchoscopies	237
Median no. (range) of bronchoscopies per subject	4 (2-19)
Total no. of lesions (preneoplastic lesions) sampled longitudinally	483
Median no. lesions (preneoplastic lesions) sampled per subject	5 (2-61)

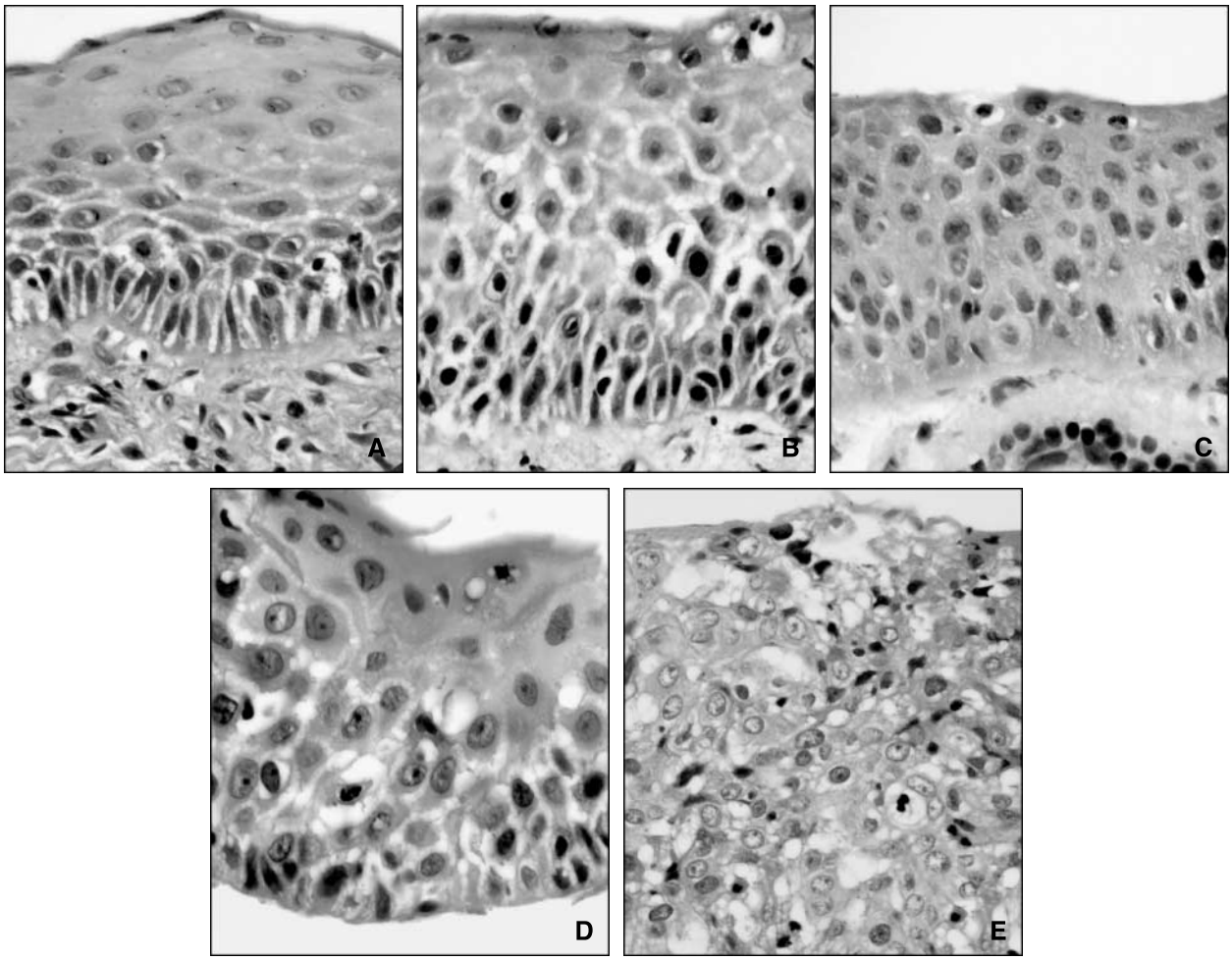

Fig. 1 A representative example of histology of each preneoplastic lesions (HE 400×). *A,* squamous metaplasia; *B,* mild dysplasia; *C,* moderate dysplasia; *D,* severe dysplasia; *E,* CIS.

Statistics

Analyses were done using SPSS software version 11. A Mann-Whitney *U* test was used to compare the follow-up times of the various preneoplastic lesions' pathologic classes and its outcome. The *z* test for two independent proportions with Yates continuity correction was applied to test the influence of gender, COPD status, past history of cancer in the upper respiratory tract, and smoking history on LGD, HGD, and CIS/SCC in Table 2. A χ^2 test was used to test whether there is an association between row and column variables in contingency tables associate with Figs. 2 and 3. In all cases but one, the number of expected observations was >5. In that single case, Kendal's τ B categorical association test was also done. For all tests, a difference with a $P < 0.05$ was considered statistically significant.

RESULTS

From November 1995 until March 1, 2003, 52 subjects have been monitored because of the presence of preneoplastic lesions at baseline bronchoscopic examinations. In total, 237 white light bronchoscopy and AFB have been done and 483 biopsy specimens with preneoplastic lesions have been sampled longitudinally. Sixty percent of the individuals had COPD. Thirty-one out of 52 (60%) individuals had past history of cancer primaries of the upper respiratory tract. Twelve patients (23%) were current smokers, 2 never-smokers, and 38 ex-smokers. Characteristics of the population cohorts are shown in Table 1.

Outcome of Per Lesion Analysis

The natural history of 134 preneoplastic lesions based on their WHO classification at baseline is shown in details together with the absolute and relative numbers of progression, regression and for preneoplastic lesions that remained stable as well as the progression rate to CIS/SCC (Fig. 2).

Nonstepwise changes of preneoplastic lesions are seen. Regression rate has been 54%. In total, 18 of 134 (13.4%) preneoplastic lesions have progressed to CIS or SCC with a median time to progression of 17.5 months (range, 1-59). Significantly more severe dysplasia (8 of 25; 32%) have progressed versus mild/moderate dysplasia (6 of 64; 9%) and

Table 2 The distribution of preneoplastic lesions at baseline bronchosocpic examinations in 52 individuals, tabulated according to factors such as gender, COPD, etc

	No. patients	Total no. lesions	Average no. lesions	*P*	LGD	*P*	HGD	*P*	Progression to CIS/SCC	*P*
Gender	52	134		0.5		1.0				0.23
Male	44 (85%)	115 (85%)	2.6		29 (66%)		15 (34%)	1.0	12/44 (27%)	
Female	8 (15%)	19 (15%)	2.4		5 (62%)		3 (38%)		4/8 (50%)	
COPD	31 (60%)	85 (63%)	2.7	0.4	20 (65%)	1.0	11 (35%)	1.0	11/31 (35%)	0.54
Non-COPD	21 (40%)	49 (37%)	2.3		14 (67%)		7 (33%)		5/21 (24%)	
Medical history										
At risk	21 (40%)	56 (42%)	2.7	0.90	12 (57%)	0.21	9 (43%)	0.38	7/21 (33%)	0.77
Previous primaries	31 (60%)	78 (58%)	2.5		22 (71%)		9 (29%)		9/31 (29%)	
Current smokers (average pack years 47)	12 (23%)	36 (27%)	3.0	0.37	8 (67%)	1.0	4 (33%)	1.0		0.30
Ex-smokers+ (average pack-years = 42)	40* (77%)	98 (73%)	2.5		26 (65%)		14 (35%)		14/40 (35%)	
Ex-smokers	38	96		0.85		0.74				0.18
Stop <5 y	20 (53%)	57 (59%)	2.8		12 (60%)		8 (40%)	0.74	9/20 (45%)	
Stop ≥5 y	18 (47%)	39 (41%)	2.2		12 (67%)		6 (33%)		4/18 (22%)	
Pack-years	52			0.16		0.21			16	0.51
0-25	15* (29%)	32 (24%)	2.1		12 (80%)		3 (20%)	0.21	6/15 (40%)	
>26	37 (71%)	102 (76%)	2.7		22 (59%)		15 (41%)		10/37 (27%)	

NOTE. LGD cohort of individuals (harboring ≥1 squamous metaplasia, mild, or moderate dysplasia) is compared with HGD cohort of individuals (harboring ≥1 severe dysplasia whether or not accompanied by ≥1 LGD lesion). Progression rate to CIS or SCC and *P*s are also shown.

*Including two never smokers.

squamous metaplasia (4 of 45; 9%) with $P = 0.020$ and $P = 0.021$, respectively.

Severe dysplasia categorized as high grade dysplastic lesions (HGD 8 of 25 = 32%; median time to progression, 16.5 months; range, 1-32) progressed significantly more frequent ($P < 0.01$) to CIS/SCC compared with all other preneoplastic lesions together, categorized as LGD (10 of 109 = 9%; median time to progression, 21.5 months; range, 4-59). Kendal's τ B categorical association test was done being 0.26 with a still significant $P < 0.003$. No significant differences were found in follow-up times and median time to progressions between the different pathologic grades of preneoplastic lesions.

Outcome Per Individual Analysis

Per cohort analysis of individuals with severe dysplastic lesion at baseline (HGD cohort) versus individuals with mainly LGD preneoplastic lesions (LGD cohort) showed insignificant $P = 0.36$ ($P = 0.54$ using Yates continuity correction). Thus, the cohort of individuals at risk harboring severe dysplasia, showed relatively higher progression rate of 39% versus 26% for LGD cohort, but this difference is not significant (Fig. 3).

Gender, COPD, Past History of Cancer(s), Smoking Status, and Pack-Years

Male and female harbored proportionally equal average number of preneoplastic lesions lesions at baseline, with LGD around 60% in both groups and no significant difference in the rate of progression to CIS or SCC (27% versus 50% respectively; $P = 0.23$; Table 2).

Patients with COPD had an average of 2.7 preneoplastic lesions versus non-COPD patients 2.3 preneoplastic lesions, the difference was not statistically significantly ($P = 0.40$) In both groups, 65% to 67% of lesions were LGD. No significant difference in rate of progression to CIS or SCC was found between COPD versus non-COPD ($P = 0.54$)

With regard to smoking status, the average number of baseline preneoplastic lesions was not significantly different ($P = 0.16$), neither the distribution of LGD versus HGD, nor the rate of progression to CIS/SCC were statistically significantly different. No significant differences were found regarding several factors related to smoking history.

Many sites ($n = 41$) have been found to show nonstepwise and erratic fluctuations between the different histologic grades over time (see Fig. 4).

DISCUSSION

Despite many advances in molecular biology, it is yet unsettled whether a set of biomarkers can be used prospectively to accurately predict individual or a cohort population at highest risk to develop SCC (14, 15). To elucidate squamous cell carcinogenesis, longitudinal sampling of bronchial mucosa specimens containing preneoplastic lesions is currently the only feasible way to pursue.

The use of AFB for collecting representative preneoplastic specimens has ultimately been shown to be more sensitive than using conventional white light bronchoscopy alone (16). In our study protocol, sampling error can be ruled out by using AFB to exactly locate and delineate the margins of all preneoplastic lesions during specimens' sampling.

Unfortunately, biopsy in itself may introduce changes that influence the natural history. Biopsy was reported to radically remove the entire clonal cells' group, precluding study of its natural history, as ~50% of dysplastic lesions have been shown to be smaller than the size of the biopsy forceps (17). This will be theoretically more important concerning preneoplastic lesions showing LGD. However, the regression rates of LGD (55% versus 52% HGD) did not seem to differ greatly between the initial sites that contained moderate dysplasia

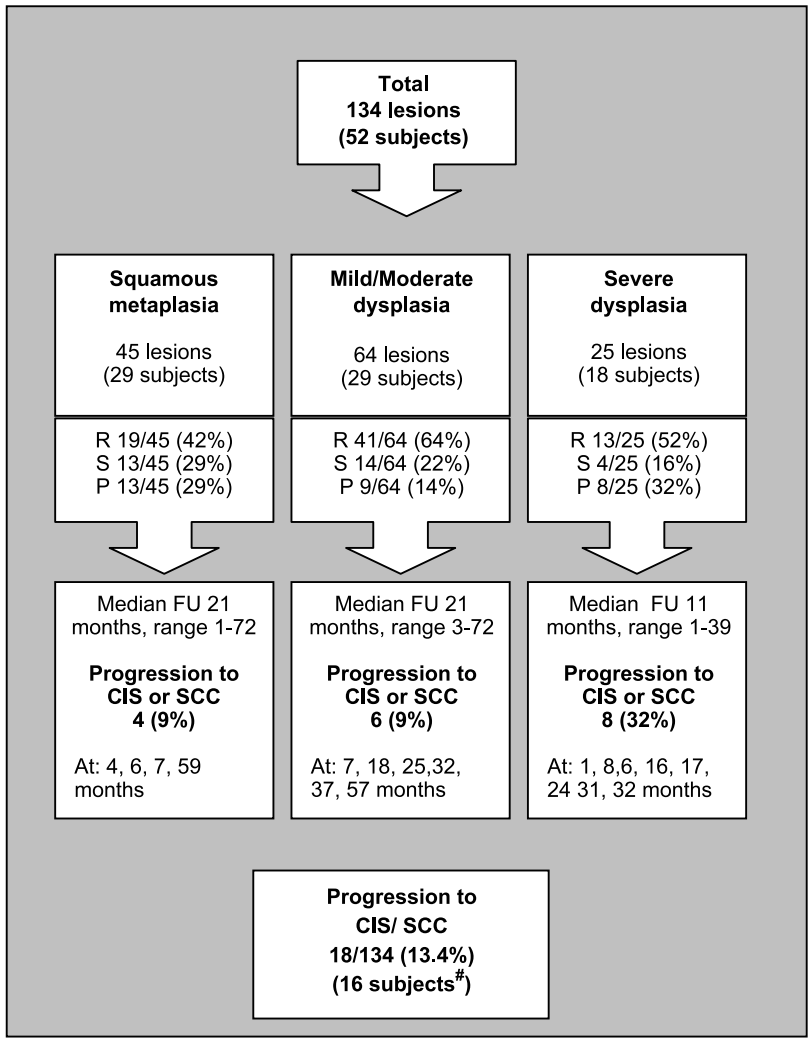

Fig. 2 Distribution of preneoplastic lesions and outcome of a longitudinal study in 52 individuals at risk harboring squamous metaplasia, mild, moderate, and severe dysplasia at baseline examination. Preneoplastic lesions progressing to CIS or SCC and its corresponding time to progression. Note that in comparison with Fig. 3, individuals at risk may initially harbor and/or develop multiple progressive lesions during follow-up. Outcome is determined by the highest grade of each preneoplastic lesion at the last analysis closest to the March 1, 2003 time point, (*R,* regression; *S,* stable; *P,* progression).

versus the remaining sites showing severe dysplasia. It is peculiar by looking at the cohort of moderate dysplasia, that especially this cohort has thus far showed the highest regression rate. The reason for this is unclear, however, variability of histologic reporting with the low κ value for moderate dysplasia of 0.02 may be of influence (12). Nevertheless, data show that even the lowest grades of preneoplastic lesions such as squamous metaplasia also progressed to CIS/SCC.

Surprisingly, many changes were found to fluctuate between the different pathologic grades over time and did not follow the expected stepwise changes. All factors may be of influence, the interindividual and intraindividual variability of histology classification (12, 13), influence of biopsy taking, and possible dynamism in the clonal behavior of preneoplastic lesions.

A significantly higher rate of progression to CIS/SCC has been found for severe dysplasia (32%) with a trend of shorter median time to progression; however, even lesions classified as squamous metaplasia at baseline has progressed to CIS/SCC at a 9% rate. This is also true in LGD cohorts of lesion and individual despite the absence of severe dysplasia.

Both follow-up time and time to progression of these preneoplastic lesions did not suggest a significant trend of acceleration by the presence of severe dysplasia at baseline. Time to progression of each potentially malignant lesion seemed an expression of an individual time clock. This is more apparent when looking at the range of time to malignant progression in Fig. 2 and the separate analysis of outcome of LGD versus HGD cohort in Fig. 3. The presence of HGD or severe dysplasia does not seem to accelerate malignant progression of the remaining lesions other than the expression of possible lead time of severe dysplasia by itself.

In contrast to the lower rate of progression of 2.5% in the study of Bota et al., the 9% progression rate to CIS/SCC of squamous metaplasia and mild dysplasia as LGD (with or without severe dysplasia, data not shown), the range of regression and progression of all preneoplastic lesions into the different histology grades during follow-up suggest a time constant for cumulative $\geq$4% per year progression rate reported previously in the

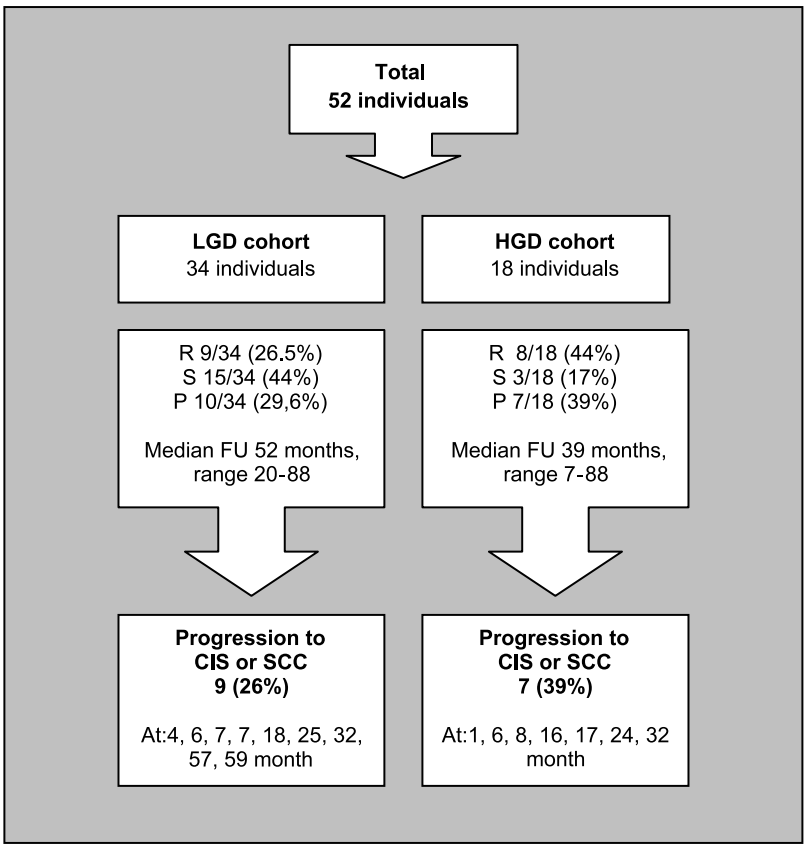

Fig. 3 Outcome of preneoplastic lesions per individual at risk. HGD cohort consists of 18 individuals with ≥1 severe dysplasia at baseline whether or not accompanied by ≥1 LGD. LGD group consists of 34 individuals with ≥1 squamous metaplasia, mild dysplasia, or moderate dysplasia preneoplastic lesion at baseline (thus without severe dysplasia). Outcome is determined by the highest grade of any preneoplastic lesion at the last analysis closest to the March 1, 2003 time point in each individual during follow-up (*R,* regression; *S,* stable preneoplastic lesion; *P,* progression).

population at risk (5, 6, 18). Thus, the trends found in our data suggest a relatively constant rate of progression over time, irrespective of the initial histologic class of preneoplastic lesions, of each potentially malignant clones in the bronchial mucosa of the individual at risk. This is concordant with data recently published by Jeanmart et al. (19). The lack of correlation between the evolution in different histology grades and factors regarding smoking habits and the relatively high number of preneoplastic lesions at baseline in the various subcategories seem to indicate the overrepresentation of individuals already being genetically susceptible (previous cancer primaries) harboring potentially malignant clones with irreversible molecular genetic damages among the many nonmalignant clones that may spontaneously regressed in a relatively late time sequence of field cancerization. Also due to the fluctuations in a nonstepwise manner of preneoplastic lesions over time, our longitudinal data cannot support the use of the initial finding of histologic grade of any preneoplastic lesion to reliably predict the chance for malignant progression. This is in accordance with our recent findings about the value of the higher number of suspicious lesions seen with

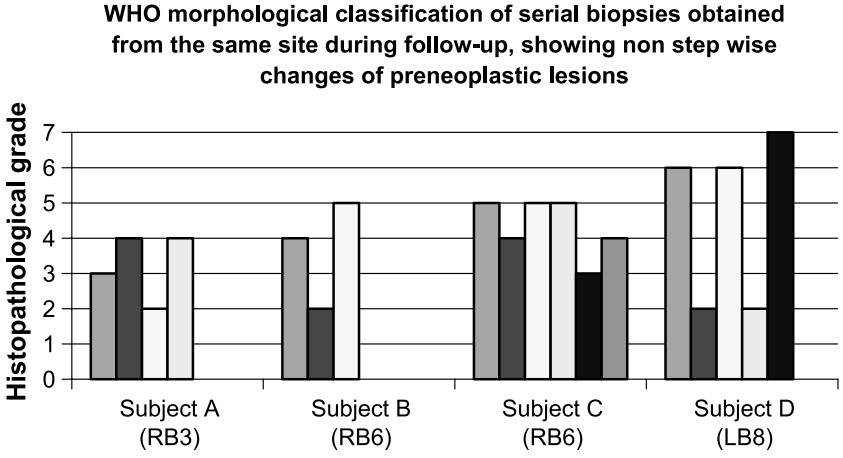

Fig. 4 Examples of the nonstepwise changes of preneoplastic lesions in four patients (*A-D*) according to the WHO morphological classification. *1,* Normal; *2,* hyperplasia; *3,* squamous metaplasia; *4,* mild dysplasia; *5,* moderate dysplasia; *6,* severe dysplasia; *7,* CIS/micro-invasive SCC. *RB3,* segmental carina anterior right upper lobe; *RB6,* segmental carina superior right lower lobe; *LB6,* segmental carina superior left lower lobe.

autofluorescence bronchoscopy, which significantly predicts malignant outcome better than histology per se (20). Caution is necessary to rely heavily on the initial classification of any preneoplastic lesion as a variable for carcinogenesis such as in many chemoprevention studies.

In summary, a 9% to 32% rate of malignant development has been found for squamous metaplasia–severe dysplastic lesions. Progression rate to CIS/SCC of low-grade dysplasia is not negligible, even in the absence of severe dysplasia. This suggests the presence of malignant clones in the bronchial mucosa of the individuals at risk. Smoking cessation at this relatively late stage of carcinogenesis does not seem to influence the outcome of potentially malignant preneoplastic lesions. Severe dysplasia indicates a high chance for becoming malignant of that particular lesion at the later stage of carcinogenesis. Each preneoplastic lesion must be seen as containing potentially malignant clonal cells, regardless of the initial histologic category at baseline biopsy. Thus, histologic classification per se cannot be reliably and accurately used as a variable. Fluctuations of preneoplastic lesions between the different grades of dysplasia are common, making accurate predictions of outcome of preneoplastic lesions based on WHO classification rather obscure.

REFERENCES

1. Auerbach O, Hammond EC, Garfinkel L. Changes in bronchial epithelium in relation to cigarette smoking, 1955-1960 vs. 1970-1977. N Engl J Med 1979;300:381–5.

2. Brambilla E, Travis WD, Colby TV, Corrin B, Shimosato Y. The new WHO classification of lung tumours. Eur Respir J 2001;18:1059–68.

3. Saccomanno G, Archer VE, Auerbach O, Saunders RP, Brennan LM. Development of carcinoma of the lung as reflected in exfoliated cells. Cancer 1974;33:256–70.

4. Kerr KM. Pulmonary preinvasive neoplasia. J Clin Pathol 2001;54: 257–71.

5. Frost JK, Ball WCJ, Levin ML, et al. Sputum cytopathology: use and potential in monitoring the workplace environment by screening for biological effects of exposure. Int J Occup Med Environ Health 1986; 28:692–703.

6. Risse EK, Vooijs GP, van't Hof MA. Diagnostic significance of "severe dysplasia" in sputum cytology. Acta Cytol 1988;32:629–34.

7. Venmans BJ, van Boxem TJ, Smit EF, Postmus PE, Sutedja TG. Outcome of bronchial carcinoma *in situ*. Chest 2000;117:1572–6.

8. Bota S, Auliac JB, Paris C, et al. Follow-up of bronchial precancerous lesions and carcinoma *in situ* using fluorescence endoscopy. Am J Respir Crit Care Med 2001;164:1688–93.

9. Sutedja TG, Codrington H, Risse EK, et al. Autofluorescence bronchoscopy improves staging of radiographically occult lung cancer and has an impact on therapeutic strategy. Chest 2001;120: 1327–32.

10. Venmans BJ, Linden van der JC, Boxem van AJ M, Postmus PE, Smit EF, Sutedja TG. Early detection of preinvasive lesions in high-risk patients: a comparison of conventional flexible and fluorescence bronchoscopy. Journal of Bronchology 1998;5:280–3.

11. Bolliger CT, Mathur PN, Beamis JF, et al. ERS/ATS statement on interventional pulmonology. European Respiratory Society/American Thoracic Society. Eur Respir J 2002;19:356–73.

12. Venmans BJ, Linden van der HC, Elbers HR. Observer variability in histopathologic reporting of bronchial biopsy specimens. Influence on the results of autofluorescence bronchoscopy in detection of preinvasive bronchial neoplasia. Journal of Bronchology 2000; 7:210–4.

13. Nicholson AG, Perry LJ, Cury PM, et al. Reproducibility of the WHO/IASLC grading system for pre-invasive squamous lesions of the bronchus: a study of inter-observer and intra-observer variation. Histopathology 2001;38:202–8.

14. Hirsch FR, Franklin WA, Gazdar AF, Bunn PAJ. Early detection of lung cancer: clinical perspectives of recent advances in biology and radiology. Clin Cancer Res 2001;7:5–22.

15. Brambilla C, Fievet F, Jeanmart M, et al. Early detection of lung cancer: role of biomarkers. Eur Respir J Suppl 2003;39:36–44s.

16. Hirsch FR, Prindiville SA, Miller YE, et al. Fluorescence versus white-light bronchoscopy for detection of preneoplastic lesions: a randomized study. J Natl Cancer Inst 2001;93:1385–91.

17. Gazdar AF, Park IW, Sood IS. Clonal patches of molecular changes in smoking damaged respiratory epithelium. Lung Cancer 2000;29:S7.

18. Woolner LB, Fontana RS, Cortese DA, et al. Roentgenographically occult lung cancer: pathologic findings and frequency of multicentricity during a 10-year period. Mayo Clin Proc 1984;59:453–66.

19. Jeanmart M, Lantuejoul S, Fievet F, et al. Value of immunohistochemical markers in preinvasive bronchial lesions in risk assessment of lung cancer. Clin Cancer Res 2003;9:2195–203.

20. Pasic A, Vonk-Noordegraaf A, Risse EK, Postmus PE, Sutedja TG. Multiple suspicious lesions detected by autofluorescence bronchoscopy predict malignant development in the bronchial mucosa in high risk patients. Lung Cancer 2003;41:295–301.