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Clinical prognostic indicators of high-grade pre-invasive bronchial lesions

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Clinical prognostic indicators of high-grade pre-invasive bronchial lesions. D. Moro-Sibilot, F. Fievet, M. Jeanmart, S. Lantuejoul, F. Arbib, M.H. Laverrière, E. Brambilla, C. Brambilla. ©ERS Journals Ltd 2004.

ABSTRACT: Lung cancer arises from multistep genetic damage of bronchial epithelium, driving multifocal progressive dysplastic lesions. However, the risk of progression of high-grade pre-invasive bronchial lesions to cancer is poorly assessed. The purpose of this study was to better define the parameters that predict the outcome of these lesions.

The current authors prospectively studied 27 patients with 31 histologically proven severe dysplasia (SD) and carcinoma *in situ* (CIS), with repeated bronchoscopy and endobronchial treatment. The influence of respiratory-cancer history, histopathological classification, tobacco consumption, and number of biopsies on the progression rate into cancer was studied.

The actuarial progression rate to cancer was 17% at 1 yr and 63% at 3 yrs. A total of 11 cases of CIS progressed to invasive cancer, 17 were stable or regressed during the study, two with SD regressed and one progressed to invasive cancer. Progression of CIS appeared more frequent in lesions diagnosed as "questionable CIS". Persistence of smoking did not influence high-grade lesion outcome. The existence of synchronous lung cancer did not seem to impact on progression. The number of biopsies did not influence the outcome.

In conclusion, the current study suggests that the outcome of high-grade pre-invasive lesions is not modified by the number of biopsies performed on these lesions. Careful pathological examination of these lesions and pathological revision seem necessary, since questionable cases have the worse progression rate.

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Lung carcinoma arises through a stepwise process of morphological and/or genetic alterations, leading to progression from normal bronchial epithelium to invasive squamous cell carcinoma [1–5]. The morphological changes are thought to progress from hyperplasia to metaplasia, which are rather common reactive lesions, to dysplasia of progressive severity (mild, moderate and severe) and carcinoma *in situ* (CIS), considered as true premalignant lesions with a high-risk of cancer development [6–9]. However, all these lesions are able to regress, including CIS, at least in animal studies of experimental carcinogenesis [10, 11].

Bronchial carcinogenesis is characterised by accumulation of successive molecular genetic and epigenetic abnormalities, resulting in selection of clonal cells with uncontrolled growth capacities throughout the whole respiratory tract. Genetic lesions long precede the morphological transformation of these pre-invasive lesions [2, 4], and genetic and molecular abnormalities increase with their histological grade [4, 5, 12–14]. These pre-invasive lesions are often multiple, reflecting the fact that the carcinogenic process may randomly affect any site in the bronchial tree, where concomitant lesions are not of the same age and may progress at different rates toward invasion [9]. This typically represents the "field cancerisation" process. There is no definite prediction of delay of progression to invasion identifiable from the morphological grade, since even CIS may progress or regress. The current hypothesis is that molecular characteristics of any individual lesion, with regards to deregulation of cell cycle or apoptosis, may reflect their potential for progression [15]. However, most high-grade lesions are now treated locally, which prevents their natural history from being followed and therefore clearly understood.

Advances in endoscopic technology, such as autofluorescence bronchoscopy, have recently improved the detection of pre-invasive bronchial lesions [9]. Nevertheless, the outcome, treatment and follow-up methods for these lesions are still open questions. Concerning the management of early superficial bronchogenic carcinoma, recommendations vary and only a few reports are based on prospective follow-up [16–18]. Traditionally, the only treatment available for these cancers was surgical resection, which may be possible, but at the expense of a wide excision and loss of significant healthy pulmonary parenchyma. However, many patients have reduced cardiopulmonary reserve due to chronic obstructive pulmonary disease and/or previous surgical resection and are not candidates for surgery.

Endobronchial therapies, such as photodynamic therapy, electrocautery or cryotherapy, that preserve lung function have been developed and show encouraging results after being applied to CIS.

In this context, the aim of this prospective study was to determine the outcome and the predictive factors of progression of bronchial high-grade pre-invasive lesions (severe dysplasia (SD) and CIS) in a high-risk, lung-cancer population.

Patients and methods

From September 1998–November 2002, 31 bronchial high-grade pre-invasive lesions were diagnosed in 27 patients and were followed in a prospective endoscopic study (fig. 1).

Bronchoscopic examination, endobronchial treatment and follow-up procedures

White-light bronchoscopy (WLB) was carried out using an Olympus BF20D bronchoscope (Rungis, France) and followed by autofluorescence bronchoscopy, using the Xillix Lung imaging fluorescence endoscopy-Lung System (Xillix Technologies Corp. Richmond, BC, Canada), under topical anaesthesia. Areas without any visual abnormality were classified as normal. Under WLB examination, areas with nonspecific erythema, swelling or thickening, or irregularities of the bronchial mucosa were classified as abnormal. Those which appeared as a definite brown or brownish-red colour under fluorescence examination were classified as abnormal [9]. During the procedure, abnormal bronchial areas under WLB or fluorescence examination were biopsied and sampled separately for pathological examination.

Patients were followed according to the initial histological diagnosis with both WLB and autofluorescence bronchoscopy (fig. 1), *i.e.* a 3-month follow-up for SD, immediate treatment for CIS and then a 3-month follow-up. Each bronchoscopy was preceded by a high resolution computed tomography to assess mediastinal lymph nodes, bronchial wall involvement or extra luminal tumour growth.

CIS and persistent SD (fig. 1) were treated either by photodynamic therapy (PDT), cryotherapy or electrocautery. Patients were given different endobronchial treatments because not all possible endobronchial treatments were available during the study period. Cryotherapy with flexible probes has been available since 1989 in Hôpital Albert Michallon, Grenoble, France and has been widely used in the current study.

PDT with *i.v.* injection of Photofrin® (AXCAN Pharma Inc, Houdan, France) at 2 $mg \cdot kg^{-1}$ and illumination with laser light 40–50 h following injection has been available since 1998. Electrocautery (Erbotom ICC 350 with endobronchial electrodes; ERBE Electromedizin GmbH, Tübingen, Germany) was introduced in the Hôpital Albert Michallon in 1999.

Endobronchial treatment was chosen according to the size and number of lesions, WLB and autofluorescence bronchoscopy were used to assist treatment by delineating the tumour margins more accurately.

PDT was selected as the first treatment option in patients with multiple pre-invasive bronchial lesions or with an extensive superficial lesion (>1 cm in size). Patients with a single lesion <1 cm in size were treated with cryotherapy or thermocoagulation, according to availability or the clinical experience of the bronchoscopist.

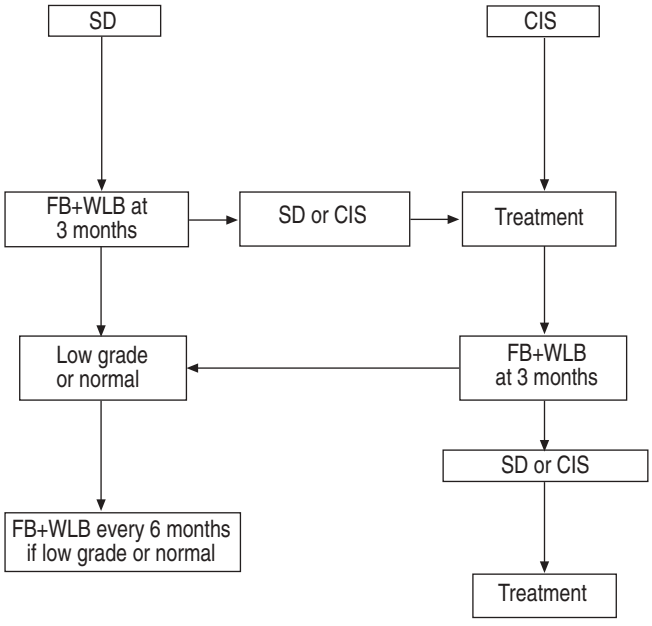

Fig. 1. – Details of the follow-up bronchoscopy procedure. A severe dysplasia (SD) that persists at the second endoscopy was treated. A carcinoma *in situ* (CIS) was treated as soon as the diagnosis was performed. FB: autofluorescence bronchoscopy; WLB: white-light bronchoscopy.

Definition of progression/regression

Progression status was assessed by comparison of the initial, baseline biopsy *versus* the last follow-up biopsy performed on the same site [19, 20]. Regression was defined as the change in a given lesion from a high grade to a lower grade lesion or to normal histology. Progression was defined as the change from SD to CIS or an invasive carcinoma, or from CIS to an invasive lesion. The persistence of the same high-grade pre-invasive lesion was considered as "stable disease".

Pathological examination

Pre-invasive lesions were classified according to classical criteria, recently emphasised in the revised WHO classification [21]. Subgrouping of lesions was performed as follows. Squamous metaplasia and mild dysplasia were considered as low-grade lesions. Moderate dysplasia is now considered as a high-grade lesion [22, 23], but they were not included in this study since they are not usually treated. SD and CIS were considered high-grade lesions and were included in the present study. All the lesions were interpreted for an initial diagnosis with the use of $\geqslant 10$ serial sections. Serial sections were performed and examined due to the high probability of a high-grade lesion being adjacent to a microinvasive area at a 300 μm distance. Patients were further treated or followed, according to this initial diagnosis. All pathology specimens were reviewed after the end of the current study. Serial sections were blindly reviewed by expert pathologists in the field (E. Brambilla, S. Lantuejoul). This pathology review has been justified by the change in the 1999 WHO classification [21], referring to pre-invasive lesions since the beginning of the current study. In this classification, preneoplastic and early cancers have been subclassified on the basis of objective criteria by a panel of expert pathologists, including one of the current authors (E. Brambilla).

In case of a discrepancy of diagnosis between two pathologists, pathological specimens were reviewed by a third pathologist (M.H. Laverrière).

Cases initially considered as SD or CIS were classified into two groups after pathological review. One group consisted of

cases where the three pathologists agreed on the morphological classification (consensual pre-invasive group), whereas the other consisted of cases where there was some degree of disagreement between the pathologists, with regards to either pre-invasive or microinvasive lesions (questionable pre-invasive group).

End-points of the study

The primary end-point was the local progression of CIS to invasive cancer, or the local progression of SD to CIS or invasive cancer. Other end-points included survival characteristics and the identification of prognostic factors associated with progression.

Statistical analysis

Median follow-up was calculated by the method of SCHEMPER and SMITH [24].

Overall survival was calculated from the date of diagnosis to the last day of follow-up or death. Progression-free interval was calculated from the date of diagnosis to the date of progression, the last day of follow-up or death. The method of KAPLAN and MEIER [25] was used.

Comparison was performed using Chi-squared tests and Fisher′s exact test. A p-value <0.05 was considered statistically significant.

Results

Clinical and pathological features

A total of 27 patients with 31 high-grade pre-invasive lesions were included in the current study. Clinical characteristics are shown in table 1. In total, 20 patients had a synchronous or a previously cured lung or head and neck cancer.

There were 19 squamous carcinomas and one adenosquamous carcinoma. Four patients had two synchronous pre-invasive lesions; all four of these patients had a current or past history of lung cancer. Therapeutic modalities are summarised in table 2.

In total, 128 autofluorescence bronchoscopies were performed; each patient had a mean of 4 and a median of 5 (interquartile range (IQR): 3–5). The mean time interval between the first and second autofluorescence bronchoscopy was 132 days (median (IQR) 110 days (91–156)). The mean time interval that elapsed between autofluorescence bronchoscopy procedures was 162 days (median (IQR) 141 days (98–183)). The mean number of biopsies (range) on each lesion was 3.3 (1–5).

A total of 28 cases of CIS and three of SD were diagnosed after pathological revision (table 3). In 14 cases, initially considered as CIS, the diagnosis was confirmed after revision (fig. 2). In six other cases of CIS, there was disagreement between the pathologists, with one pathologist giving a diagnosis of a microinvasive lesion (fig. 3). These six cases were considered as CIS. However, in the statistical analysis, these cases were studied separately in a questionable pre-invasive group. Two SD were confirmed, one CIS was reclassified as SD, and eight SD were reclassified as CIS.

Pre-invasive lesions and synchronous or previous lung cancer were in distinct locations in all cases.

Patients' follow-up and outcome

At the time of statistical analysis (November 2002), the median (IQR) follow-up for the 27 patients included in the study was 102 weeks (60–176).

The 4-yr actuarial survival was 84.7% (median not reached). Three patients had died; one from a massive stroke, one from post-operative complications after pneumonectomy for lung cancer and one from metastatic progression of lung cancer.

Table 1. – Main characteristics of the patients studied

Characteristics	Study patients
Patients n	27
Males/females	24/3
Age yrs	63.3 (49–84)
Current or past history of lung or head and neck cancer	20
Synchronous lung cancer	4
Previous lung cancer	14
Previous head and neck cancer	2
Previous high-grade pre-invasive lesion	6
Smoking history	
Lifelong nonsmokers	2
Active smokers	4
Former smokers	21
Pack-yrs	50 (15–100)
Age at smoking initiation yrs	19 (11–27)
Duration of smoking yrs	38 (26–56)
Age at smoking cessation yrs	57 (46–74)
Time since tobacco cessation yrs	7.5 (1–23)

Data are presented as n or mean (range).

Table 2. – Outcome of high-grade lesions according to the initial endobronchial treatment during follow-up

	Regression or stabilisation		Progression to invasive cancer	
Endobronchial treatment	CIS	SD	CIS	SD
Cryotherapy	7	1	4	1
PDT	3		3	
Electrocautery	2		2	
None	5	1	2	

Data are presented as n. Cryotherapy was used to treat 13 lesions (carcinoma *in situ* (CIS) n=11, severe dysplasia (SD) n=2); photodynamic therapy (PDT) was used to treat six lesions (all CIS); electrocautery was used to treat four lesions (all CIS); no treatment was used for eight lesions (CIS n=7, SD n=1).

Table 3. – Histological diagnosis of the 31 lesions and median progression-free survival

Histology	Cases n	Median progression-free survival weeks
CIS	28	144
CIS confirmed after revision	22	not reached
Questionable CIS	6	92
SD	3	86

CIS: carcinoma *in situ*; SD: severe dysplasia.

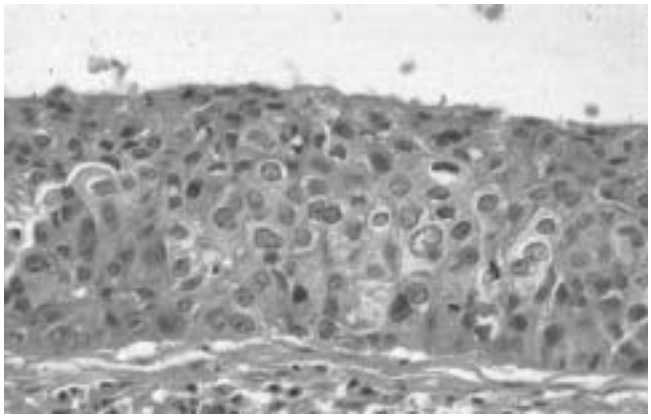

Fig. 2. – Carcinoma *in situ* with respect to the basal lamina.

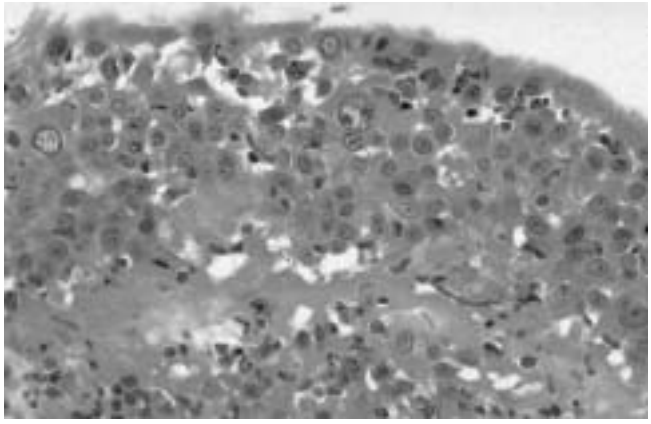

Fig. 3. – Carcinoma *in situ* with questionable continuity of the basal lamina.

Outcome of pre-invasive lesions

The actuarial progression-free survival curve is shown in figure 4. The median progression-free interval was 144 weeks for the 31 lesions, the actuarial progression rate was 17% at 1 yr and 63% at 3 yrs. Only eight lesions were untreated; six were initially considered as SD, and secondary pathological examination identified a small area of CIS. According to the current authors' protocol, they were not initially treated (fig. 1). One patient with CIS refused treatment, and one patient had a confirmed SD and confirmed regression after follow-up. Among the eight untreated lesions, two progressed (invasive carcinoma), two CIS remained stable, and four lesions regressed to a lower-grade lesion or to normal histology (three CIS and one SD). Of the 23 treated lesions, nine CIS and one SD progressed to an invasive carcinoma, one CIS remained stable, and 11 CIS and one SD regressed to normal histology (tables 2 and 4).

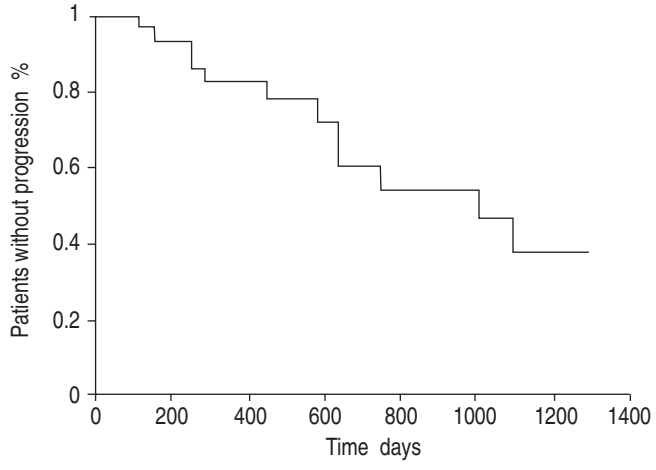

Fig. 4. – Progression-free interval of the 31 lesions.

Table 4. – Evolution of the 31 lesions

	Regression	Stabilisation	Progression to	
			CIS	Invasive cancer
SD untreated	1	0	0	0
SD treated	1	0	0	1
CIS untreated	3	2		2
CIS treated	11	1		9

Data are presented as n. SD: severe dysplasia; CIS: carcinoma *in situ*.

Parameters influencing outcome

All six questionable cases of CIS progressed to an invasive cancer. In contrast, only five of 22 confirmed cases of CIS progressed to an invasive cancer (p=0.0012). The number of biopsies performed on each lesion did not seem to influence the regression of these lesions (p=0.20).

Table 5 shows the proportion of SD and CIS lesions that progressed according to the presence or absence of a synchronous lung cancer, or history of lung or head and neck cancer. No significant difference could be found in the number who progressed in one group as compared with the others (p=0.39).

No difference in progression rate for SD or CIS lesions could be observed between current smokers or exsmokers (p=0.9; table 6).

Discussion

The current bronchial carcinogenesis concept is that preneoplastic or pre-invasive lesions eventually progress to squamous cell carcinoma, although the natural history of these lesions and their timing towards progression are not certain. Longitudinal follow-up, sputum-cytology studies have led to an estimation of the time-to-progression from a low-grade premalignant lesion to an invasive cancer to be several years [26]. NASIELL *et al.* [27] analysed the spontaneous evolution of five early occult lesions, characterised by the presence of malignant squamous cells in the sputum. The interval between cytological, radiological, bronchoscopic and/or histological demonstration of the tumour varied between 2 months and 9 yrs. A recent study [17] of nine patients with CIS showed that about half of these lesions evolved into invasive lung cancer during 6 months of follow-up. Nevertheless, not all patients had received endobronchial treatment, no detail was given as to the number of biopsies taken at each pathological site, and there was no actuarial survival description of this progression. Conversely, BOTA *et al.* [16] showed, more recently, that none of 32 untreated-CIS lesions had progressed to invasive cancer after 3 months. After

Table 5. – Outcome of 31 high-grade lesions in 27 patients according to previous history of cancer

Patients	High-grade lesions		Evolution of lesions	
	SD	CIS	Progression	Stable or regression
Synchronous lung cancer	1	4	3 CIS/0 SD	1 CIS/1 SD
Previous lung cancer	0	17	6 CIS	11 CIS
Previous H&N cancer	0	2	1 CIS	1 CIS
No previous history of cancer	2	5	1 CIS/1 SD	4 CIS/1 SD

Data are presented as n. Synchronous lung cancer: n=4; previous lung cancer: n=14; previous head and neck (H&N) cancer: n=2; no previous history of cancer: n=7. SD: severe dysplasia; CIS: carcinoma *in situ*.

Table 6. – Outcome of 31 high-grade lesions in 27 patients according to tobacco status

Patients	High-grade lesions		Evolution of lesions	
	SD	CIS	Progression	Stable or regression
Active smokers		4	1 (1 CIS)	3 (3 CIS)
Former smokers	2	23	11 (1 SD, 10 CIS)	4 (1 SD, 3 CIS)
Never smokers	1	1		2 (1 SD, 1 CIS)

Data are presented as n. Active smokers: n=4; former smokers: n=21; never smokers: n=2. SD: severe dysplasia; CIS: carcinoma *in situ*.

endoscopic treatment, there was no progression for at least 24 months.

Another study [18] reported on 35 CIS lesions treated with cryotherapy. The complete response rate at 1 yr was 91%. Local recurrence was observed within 4 yrs in 28% of patients. In this study, the progression-free interval at 5 yrs was close to 50%. It should be stressed that this multicentre study recruited patients with small lesions eligible for cryotherapy and, furthermore, only 42% had a current or past history of lung or aerodigestive cancer. In contrast with the current authors' study, most of these lesions were also treated with rigid cryoprobes and this may explain the better therapeutic results.

The current authors' data were obtained in a cohort of 27 patients (31 lesions). The median progression-free survival time was 144 weeks, the actuarial progression rate was 17% at 1 yr and 63% at 3 yrs. Compared to DEYGAS *et al.* [18], the current authors' data show a worse outcome. This may be partly explained by differences in cryotherapy techniques, by more extensive CIS (treated with PDT or electrocautery) and by the fact that eight lesions were untreated. Furthermore, the current authors' study included more higher-risk patients with a current or past history of lung or head and neck cancer (71%).

The pathological and bronchoscopic diagnosis of pre-invasive lesions remains difficult. Large biopsies are often difficult to perform; moreover, it is often difficult to repeat the bronchoscopy to improve the quality and size of biopsy specimens. These technical problems explain, to some extent, the existence of questionable cases where the absence of the penetration of subepithelial basement membrane is not fully assessable. It is difficult to formally exclude that some early invasive lesions may have been considered as pre-invasive lesions, which may explain the highest progression rate and the shorter progression-free interval of this group. Lung-cancer pathologists have experienced the frequency with which a microinvasive carcinoma with overt invasion of submucosa is observed in the vicinity (200–500 μm) of high-grade lesions. It is striking to see that high-grade lesions share most of the molecular alterations of invasive cancer [23]. According to previous studies, the size of clonal patches increases with worsening grade, implying that a given high-grade lesion might sit within the patch of an invasive one [28]. All previous studies are also hampered by this crucial fact.

It has been suggested [16], but never demonstrated, that multiple biopsies of a pre-invasive lesion may alter their natural history and evolution. This possible effect on the natural evolution may be explained, either by mechanical removal of the lesion or by secondary induced-mucosal inflammation. In the current study, the present authors found no impact of the number of biopsies on the rate of progression or regression of high-grade pre-invasive lesions.

It has been shown that pre-invasive lesions develop more frequently in patients with prior squamous carcinomas [29]. All the patients with a current or prior cancer, included in this study, had a tumour with a squamous component. The proportion of SD or CIS that progressed was not influenced by the presence of a synchronous cancer. It should be stressed that similar findings were reported by BOTA *et al.* [16] with high-grade lesions.

Since high-grade pre-invasive lesions may occur at multiple sites of the bronchial tree, conservative endobronchial therapy should be the first-line treatment, sparing the pulmonary parenchyma and still allowing surgical salvage when necessary. However, these treatments require careful follow-up with repeated bronchoscopies to detect recurrence or other lesions at new locations. The current authors' results, although not intended to evaluate therapeutic efficacy of individual treatment modalities, show that, despite endobronchial treatment, nine of 21 CIS and one persistent SD progressed to invasive cancer.

Therapeutic options for carcinoma *in situ* and severe dysplasia remain uncertain. Only a few medical centres regularly treat and follow up high-grade pre-invasive lesions, and the final selection of treatment remains a matter of experience, skill, equipment and cost. In this context, the current authors believe that it is important to define prognostic factors associated with progression. The current authors' study allows better discrimination of this high-risk population, which must be closely followed and treated. The current authors' study emphasises the clear need of an immediate histological review by trained pathologists before therapeutic decisions, and the need of a multicentre study to overcome the limitations of small subgroups of patients.

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