Size and Predictive Factors of Microscopic Tumor Extension in Locally Advanced Non-Small Cell Lung Cancer

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Abstract

Purpose : Radiation therapy for locally advanced non-small cell lung cancer (NSCLC) should treat the whole tumor, including its microscopic extensions, while protecting adjacent organs at risk as much as possible. The aim of our study is to evaluate the size of microscopic tumor extension (MEmax) in NSCLC, and search for potential predictive factors.

Methods and Materials : We retrospectively selected 70 patients treated with post-operative radiation therapy for a NSCLC with N2 nodal status, then 34 additional patients operated for a squamous cell lung cancer with N1 or N2 nodal status. On the digitized slides originating from the resected tumors of these 104 patients, we outlined the border of the tumor, as seen with the naked eye. We then searched for microscopic tumor extension outside of these borders, with a magnification as high as x 40, and measured the maximum size of MEmax.

Results : The median MEmax in the whole cohort was 0.85 mm [0-9.95]. The MEmax was less than 5.3 mm in 95% of adenocarcinomas (6.5 mm in the subgroup without neo-adjuvant chemotherapy) and less than 3.5 mm in 95% of squamous cell carcinomas (3.7 mm in the subgroup without neo-adjuvant chemotherapy). After multivariate analysis, the factors associated with the size of MEmax were vascular invasion (p=0.0002), histologic type, with a wider MEmax for adenocarcinomas in

comparison with squamous cell carcinomas (p=0.002), tumor size, which was inversely related with the size of MEmax (p=0.024), and high blood pressure (p=0.03). Macroscopic histologic tumor size was well correlated with both radiologic tumor size on a mediastinal setting CT (correlation coefficient of 0.845) and on a parenchymal setting CT (correlation coefficient of 0.836).

Conclusions : The Clinical Target Volume margin, accounting for microscopic tumoral extension, could be reduced to 7 mm for adenocarcinomas and 4 mm for squamous cell carcinomas.

Key words : Non-small cell lung cancer, Conformal radiation therapy, Clinical Target Volume, Microscopic tumoral extension, Computed Tomography.

Introduction

Non-small-cell lung cancer (NSCLC) is the leading cause of cancer death worldwide. The standard treatment for non-operable locally advanced NSCLC is concurrent chemoradiotherapy, followed by maintenance immunotherapy. Nevertheless, high rates of progression are still observed, with a five-year progression-free survival approximating only 30%. (1). One of the challenges of radiation therapy is to treat the whole tumoral disease, including its potential microscopic extensions, while preserving the healthy surrounding organs as much as possible. Radiation therapy techniques have been rapidly evolving in the last decades, notably with the emergence of Intensity Modulated Radiation Therapy (IMRT), and now allow us to precisely adapt the dose to the geometry of the target volume. Even though no prospective trial proved the superiority of IMRT over conventional three-dimensional conformal radiation therapy (3DRT) in lung cancer, IMRT has clearly demonstrated its dosimetric benefit, with a reduction of the dose received by the heart, lung and œsophagus ; and its benefit in terms of toxicity, with lower rates of treatment-related pneumonitis (2–5). For all these reasons, IMRT is now widely used in the treatment of locally advanced lung cancers. This possibility to spare increasingly more lung parenchyma might be associated with a risk of missing some of the tumoral disease, in case of microscopic tumoral extensions that would not be seen on imaging. It is therefore of the utmost importance to evaluate the size of microscopic tumoral extensions, so that it is properly included in the target volumes.

The International Commission on Radiation Units and Measurements (ICRU) defined three major target volumes in its reports 50, 62 and 83 (6–8) : the Gross Tumor Volume (GTV), corresponding to the macroscopic tumor, as seen on imaging or physical examination ; the Clinical Target Volume (CTV) which also encompasses the potential microscopic tumoral disease ; and the Planning Target Volume (PTV), built with a safety margin, which takes into account all potential sources of uncertainties and errors in the treatment.

A few studies already evaluated the size of microscopic tumoral extension in non-small-cell lung cancer (9–16). The studied population (stages I to III), number of patients (ranging from 5 to 70), and methods of definition of the macroscopic tumor differed among the studies, resulting in sometimes contrasting results. The first and most conclusive of these studies, by Giraud et al (9), led to the French and European guidelines for the definition of clinical target volumes : the CTV is built by adding a 6 millimeter margin around the GTV for squamous cell carcinomas, and an 8 millimeter margin around the GTV for adenocarcinomas (17,18). The difference of margin size between adenocarcinomas and squamous cell carcinomas is explained by a larger microscopic tumoral extension that was found in lung adenocarcinomas in comparison with squamous cell lung carcinomas (9,12,16). A better knowledge of the factors associated with the size of microscopic tumoral extension would allow us to personalize the size of the margin between the GTV and the CTV more finely, depending on the patient's and the tumor's characteristics.

The aims of this study were to update the evaluation of the size of microscopic tumoral extension in locally advanced NSCLC, with modern pathology techniques ; and to search for clinical, histopathological, and radiological factors, that could be associated with the size of microscopic tumoral extension.

Materials and Methods

Patients

We retrospectively selected all consecutive patients treated between 2002 and 2016 at academic center A by post-operative radiation therapy for a locally advanced NSCLC. We included patients operated with a curative intent, and with mediastinal lymph node involvement (N2). Only patients with available pre-operative thoracic imaging (computerized-tomography (CT) scanner or positron emission tomography (PET)-CT) and histological material of the resection specimen were included.

We thus included 70 NSCLC patients, most of them with adenocarcinomas (N=44). We enriched the data with a second cohort of patients, operated for a locally advanced squamous cell lung carcinoma, in order to increase the number of squamous cell carcinomas in our population. For these patients, with N1 or N2 nodal status, and available pre-operative thoracic imaging and histological material, surgery was performed at academic center B between 2015 and 2016. This second cohort consisted of 34 patients, for a total of 104 patients.

The study was approved by the local ethics committee (CPP Ile de France II, No. 2008-133, No. 201206-12 and No. 2018 MS1).

Treatment

Patients' treatment was decided according to our institutional guidelines in the setting of a multidisciplinary team discussion. All the patients in our study underwent surgical resection with ~~a~~ curative intent, either by pneumonectomy, lobectomy, segmentectomy or wedge resection, depending on the tumor size, and patient's respiratory function ; with a systematic mediastinal lymph node dissection. Depending on the tumor's stage, patients could receive neo-adjuvant chemotherapy, or adjuvant chemotherapy (platinum-based chemotherapy), and/or adjuvant mediastinal radiation therapy for patients with N2 nodal status (54 Gy in 27 fractions, as in the Lung Art trial IFCT 0503).

Pathological material and data

Formalin-fixed paraffin-embedded tumor samples were collected and reviewed by two thoracic expert pathologists and classified according to the latest World Health Organization classification of 2015 (19). The following data were collected from the initial pathological reports : macroscopic tumor size, presence or absence of vascular involvement, and pathological TNM classification (8^{th} edition, 2017).

Microscopic extension

The hematoxylin-eosin stained slides from the resected specimens were all reviewed. We selected for each patient the slides containing both tumoral disease and healthy lung parenchyma. Among these slides, we selected those with the largest microscopic extension (1 to 4 slides per tumor) after an evaluation on conventional microscopy. These slides were scanned with Nanozoomer (Hamamatsu®). For each slide, the macroscopic tumor limits were assessed with the naked eye (without magnification) and marked on its digitized version. We then used a magnification of x 10 to x 40, to search for tumor cells outside the macroscopic tumor limits. For each tumor, the maximal microscopic extension (MEmax) was defined as the longest distance between macroscopic tumor limits and tumor cells outside of these limits, on all the examined slides for this tumor.

Radiological data

For all patients, the radiological macroscopic tumor size was determined as the longest axis of the tumor in all three directions, measured by a radiation oncologist blinded with respect to the macroscopic tumor sizes ; on both the CT scanner with a mediastinal setting (level +20 HU ; window 400 HU) and with a parenchymal setting (level -600 HU ; window 1600 HU) (20).

Depending on their location in the lung, tumors were classified as central if they were situated less than 2 cm around the bronchial tree, close to the chest wall if they were situated less than 2 cm from the chest wall, and peripheral if they were situated in none of the two previous locations.

Statistical methods and analyses

Demographical and clinical characteristics of the patients, as well as tumor characteristics, were described by median, minimal, and maximal values for continuous features, and frequency (proportion in percentage) for categorical features. Radio-histological agreement was performed by calculating a Lin's concordant correlation coefficient between the radiological tumor size and the macroscopic histological tumor size. Microscopic extension was described with median, minimal, and maximal values, and 90^{th} and 95^{th} percentiles observed in the distribution.

For univariate analyses, the statistical significance of the association between two qualitative variables was tested with a $\chi 2$ test or Fisher's exact test if conditions for $\chi 2$ test were not fulfilled ; between a quantitative variable and a qualitative variable with a Student's test or Mann-Whitney's test in case of non-Gaussian distribution of the quantitative variable ; and between two quantitative variables with Pearson correlation coefficient or Spearman correlation coefficient in case of non-Gaussian distribution of at least one of the variables.

For multivariate analysis, variables that were associated with the size of microscopic tumoral extension in univariate analyses, with a p-value $\leq 20\%$, were filtered. In a second step, independent factors associated with the size of microscopic tumoral extension were selected thanks to a stepwise procedure implemented during final modelling. Due to a zero-inflated and skewed to the right distribution, modelling consisted of fitting a log-normal regression model, for determination of the final independent factors associated with the size of microscopic tumoral extension. Results are given by log-normal regression coefficients and their 95% confidence intervals.

Statistical analyses were performed using R software (R Studio Version 1.1.383 and R.Version 3.6.1) and SAS 9.4 software (SAS Institute Inc., Cary, NC, USA).

Results

Population

Ninety-one patients were treated with post-operative radiation therapy, between 2002 and 2016, at academic center A, after a surgery with a curative intent for a NSCLC, with N2 nodal status. For 70 of these patients, pre-operative thoracic imaging and histological material were available. These patients form the first set of patients, including 44 adenocarcinoma patients, and 17 squamous cell carcinoma patients. Additionally, 49 patients were operated at academic center B between 2015 and 2016 for a squamous cell lung carcinoma with N1 or N2 nodal status. For 34 of these patients, pre-operative

thoracic imaging and histological material were available. These patients form the second set of patients. Therefore, we studied a total of 104 patients.

The median age was 64 years old [43-84]. Forty-four tumors (42.3%) were adenocarcinomas, and 51 (49%) were squamous cell carcinomas. Tumor status was mainly T2 (42 patients, 40.3%) or T3 (35 patients, 33.7 patients), and tumor stage was mostly IIIA (63 patients, 60.6%). Twenty-eight patients (27%) received two to six cycles of neo-adjuvant chemotherapy (median, two). All these patients received a platinum-based doublet chemotherapy (with docetaxel for eight patients, pemetrexed for seven patients, gemcitabine for five patients, paclitaxel for four patients, vinorelbine for three patients, and missing data for one patient). The patients' and tumors' characteristics are detailed in Table 1. Squamous cell carcinomas were larger than adenocarcinomas (median size 44 mm [10 – 109] vs 29 mm [10 -100]). The distribution of disease stages was different between patients with adenocarcinomas and patients with squamous cell carcinomas, with no stage II adenocarcinomas and 23.5% of stages IIB squamous cell carcinomas, due to the two different types of recruitment between our two cohorts. The median time between pre-operative imaging and surgery was 25 days [1-139]. One to four slides (median, two) were analyzed after digitizing for each tumor ; a total of 194 digitized slides were analyzed, including 84 slides from adenocarcinomas, 94 slides from squamous cell carcinomas, and 16 slides from other histologic types.

Radio-histological correlation

The mean macroscopic tumoral size was 42 mm (standard deviation, 21.5 mm) while the median macroscopic tumoral size 36 mm [10-109]. The mean radiological size measured with a mediastinal setting was 44.9 mm (standard deviation, 26 mm) and the median 37.5 mm [3.4 – 128.2] ; the mean radiological size measured with a parenchymal setting was 46.3 mm (standard deviation, 25 mm) and the median 39.6 mm [9 – 129.6]. Lin's concordant correlation coefficient between radiological tumor size with a mediastinal setting and macroscopic histological tumor size was 0.845 (95% CI [0.784 –

0.890]) (Figure 1). It was 0.836 (95% CI [0.772 – 0.884]) between radiological tumor size with a parenchymal setting and macroscopic histological tumor size (data not shown).

Microscopic tumoral extension

The analysis of the slides revealed three main situations concerning microscopic tumoral extension :

- The tumor has clear-cut limits, with no microscopic tumor extension above the macroscopic limits, as it is often the case for squamous cell carcinomas.

- The tumor is poorly limited with the presence of tumor cells outside the macroscopic tumor limits, due to direct contiguous extension of tumor cells, or vascular extension, or distant tumor islets (Figure 2).

- There is an inflammatory reaction around the tumor, which cannot be distinguished from the tumor itself at macroscopy. In this case, the macroscopic tumor limits overestimate the actual tumor, and there is no microscopic tumor extension.

Overall, among all patients, the MEmax ranged from 0 to 9.95 mm, with a median size of 0.85 mm. It was less than 4.5 mm in 95% of cases, and less than 3.6 mm in 90% of cases. We observed a difference in the size of MEmax between adenocarcinomas and squamous cell carcinomas. Among adenocarcinomas, the MEmax ranged from 0 to 9.95 mm, with a median size of 1.22 mm. It was less than 5.3 mm in 95% of cases, and less than 4.4 mm in 90% of cases. Among squamous cell carcinomas, the MEmax ranged from 0 to 4.64 mm, with a median of 0.38 mm. It was less than 3.5 mm in 95% of cases, and less than 3.1 mm in 90% of cases (Figure 3).

In the subgroup of patients who did not receive neo-adjuvant chemotherapy (30 adenocarcinomas, and 39 squamous cell carcinomas), the MEmax ranged from 0 to 9.95 mm for adenocarcinomas (median 2.27 mm, less than 6.5 mm in 95% of cases, less than 4.3 mm in 90% of cases) ; and from 0 to

4.64 mm for squamous cell carcinomas (median 0.46 mm, less than 3.7 mm in 95% of cases, and less than 3.4 mm in 90% of cases).

Predictive factors of the size of microscopic tumor extension

We searched for clinical, histopathological, and radiological factors that could be associated with the size of MEmax. Results of the univariate analysis are presented in Table 2. Factors that were significantly associated with the size of MEmax were the macroscopic histological tumor size, which was inversely related with the size of microscopic extension (p=0.02), vascular invasion (p=0.045), histological type, with a wider microscopic extension for adenocarcinomas compared with squamous cell carcinomas (p=0.04), and nodal status, with a wider microscopic extension for N2 compared with N1 tumors (p=0.02). We found almost statistically significant associations between the location of the tumor and the size of MEmax (with a wider microscopic extension for tumors close to the chest wall ; p=0.058), and between the completion of a neo-adjuvant chemotherapy and a smaller microscopic tumoral extension (p=0.097). Results of the multivariate analysis, among patients with adenocarcinomas or squamous cell carcinomas only, are presented in Table 3. Factors that were independently associated with the size of MEmax are the macroscopic histological tumor size, with a smaller microscopic extension for larger macroscopic tumors (p=0.024) ; histological type, with a smaller microscopic extension for squamous cell carcinomas in comparison with adenocarcinomas (p=0.002) ; the existence of vascular invasion, associated with a larger microscopic extension (p=0.0002) ; and a high blood pressure, associated with a larger microscopic extension (p=0.03).

We also performed the multivariate analysis of factors associated with the size of MEmax in the subgroup of patients who did not receive neo-adjuvant chemotherapy, and whose tumor was either an adenocarcinoma or a squamous cell carcinoma (the two main histological types studied). Factors independently associated with the size of MEmax in this subgroup were, in the same way as in the whole population : histological type, with a smaller microscopic extension for squamous cell carcinomas in comparison with adenocarcinomas (p=0.002) ; the existence of vascular invasion,

associated with a larger microscopic extension (p=0.0013) ; and a high blood pressure, associated with a larger microscopic extension (p=0.026). The macroscopic histological tumor size was not independently associated with the size of MEmax in this subgroup (p=0,16).

Discussion

Radiation therapy is an important part of the treatment of locally advanced NSCLC. The CTV is commonly built by adding an 8 millimeter margin for adenocarcinomas, and a 6 millimeter margin for squamous cell carcinomas, based on the results by Giraud et al published in 2000 (9). In our study, we updated the evaluation of the size of microscopic tumor extension in locally advanced NSCLC, with modern pathology techniques. In our cohort, constituted by a total of 104 patients, including 44 adenocarcinomas, and 51 squamous cell carcinomas, we found that the size of MEmax was less than 5.3 mm in 95% of cases for adenocarcinomas, and less than 3.5 mm in 95% of cases for squamous cell carcinomas. In the subgroup of patients who did not receive neo-adjuvant chemotherapy, the size of MEmax was less than 6.5 mm in 95% of cases for adenocarcinomas, and less than 3.7 mm in 95% of cases for squamous cell carcinomas. Based on our results, we propose to reduce the GTV to CTV margin to 7 mm for adenocarcinomas, and 4 mm for squamous cell carcinomas. This would still ensure an adequate coverage of the whole tumoral disease, including its microscopic extensions, in more than 95% of cases. Concerning other histologic types, they were insufficiently represented in our cohort to draw reliable conclusions.

These margins are smaller than that proposed by Giraud et al (9) (7 mm vs 8 mm for adenocarcinomas, and 4 mm vs 6 mm for squamous cell carcinomas). The analysis of all the other studies published regarding the size of microscopic extension in lung cancer reveals quite discordant results (9–16). They are summarized in Table 4. The median size of microscopic extension ranges from 0 to 8 mm depending on the studies, and the maximal size of microscopic extension from 9.95 mm to 60 mm. These

differences can be explained by several factors. First of all, the disease stages studied are not comparable in all studies, with stages I only in some studies, whereas others included more or exclusively advanced stages (II and III). Secondly, and most importantly, the techniques used to define the macroscopic tumor were different among the different studies, which necessarily results in differences in the size of microscopic extension observed. The delimitation of the macroscopic tumor was performed directly on the resected specimen before it was cut in slides in one study (14), on the tissue blocks before paraffin-embedding and hematoxylin-eosin staining in some studies (10,11,13), and on the slides after paraffin-embedding and hematoxylin-eosin staining in other studies (9,12,15). In our study, the delimitation of the macroscopic tumor was performed on the slides, after paraffin-embedding and hematoxylin-eosin staining, that were digitized. Finally, the possible deformations of the resected specimen after surgery were not taken into account in the same way among the different studies. In that respect, Stroom et al (15), who found the largest size of microscopic extension, actually applied to their results a corrective factor supposed to take into account these deformations. Despite these discordant results, the difference between adenocarcinomas and squamous cell carcinomas in terms of microscopic extension, with larger microscopic extension for adenocarcinomas compared with squamous cell carcinomas, was found in all studies comparing these two histologic types.

Our study highlighted the existence of some factors that are predictive of the size of MEmax. Among these, histological type and vascular invasion are logically explained by histological observations. Indeed, squamous cell carcinomas, that are associated with less extensive microscopic extension, are often very well limited tumors with clear-cut edges ; and vascular invasion, that is associated with a larger microscopic extension, is one of the mechanisms of tumor spread (we considered vascular emboli that were on the tumor extension front as part of microscopic tumor extension). The macroscopic tumor size, which is inversely related with the size of microscopic extension, makes less intuitive sense. In their study, Van Loon et al (13) observed opposite results, with a wider microscopic tumor extension for higher volume tumors. The fact that squamous cell carcinomas, that are associated with less important microscopic extension, were larger tumors in our cohort, might

contribute to explain this surprising result, even though we found macroscopic tumor size and histologic type as two independent factors associated with the size of microscopic extension. Another hypothesis to explain this result is the prospect that larger tumors might be more often associated with a peri-tumoral inflammatory reaction, which is generally associated with an absence of microscopic extension. The last factor that we found to be associated with the size of microscopic extension was blood pressure. We have no direct explanation for this result. It might be due to a link, that seems to exist, between blood pressure and immunity (21,22). In univariate analysis, a trend for an association was found regarding the size of MEmax between the location of the tumor, and the completion of neo-adjuvant chemotherapy. These trends were not confirmed in multivariate analysis, possibly due to a lack of power, but are interesting findings. Twenty-seven patients in our cohort received neo-adjuvant chemotherapy, and tended to present with a less important microscopic extension. This comforts us in the validity of the construction of the primary tumor CTV by adding a margin around the post-chemotherapy primary tumor GTV, as it is usually done in practice. If these results were confirmed, it could even lead to a reduction of the GTV to CTV margin size in case of the completion of a neo-adjuvant chemotherapy. In contrast, if a wider microscopic tumor extension were confirmed in case of the proximity of the tumor to the chest wall (possibly with a pleural involvement), it could lead to an increase of the GTV to CTV margin size in these cases.

One of the main strengths of our study is the important number of patients – 104 - in comparison with other studies published on the same subject. Furthermore, even though our population is by definition constituted only by patients with operable tumors, it is quite representative of patients who are treated with chemoradiation therapy, with an important proportion of stages IIIA and even IIIB. An innovation in our study is the fact that the histological analysis of microscopic extension was performed on digitized slides, allowing for a more precise delimitation of the tumors. Digital images are indeed particularly suitable for measurements because they are much more precise than conventional microscopy, allowing more easily a precision of about 1 μm more easily.

However, our study has some limits. Firstly, a selection bias was introduced with the different selection criteria for our two cohorts. This bias could affect mostly the results concerning survival, which was not the purpose of our study. Secondly, a relatively low number of slides was analyzed for each tumor (1 to 4 slides per tumor). This is responsible for a less exhaustive analysis of the tumors. We did however select the most relevant slides for the analysis of microscopic tumor extension (the ones with the largest microscopic extension after an evaluation of all slides with conventional microscopy). Thus, even if in typical pathologic analysis, a more exhaustive analysis of all slides would be performed, we only needed the slides with the largest tumor microscopic extension. In the case of well limited tumors such as squamous cell carcinomas, with very small to no microscopic extension, one slide could be representative of all slides in terms of microscopic extension. Thirdly, an important proportion of our cohort (27%) received neo-adjuvant chemotherapy, which is responsible for modifications of tumor characteristics (23). However, this is representative of patients treated with chemoradiation therapy for a locally advanced NSCLC, as they very often receive one or two cycles of chemotherapy before the start of radiation therapy, essentially for organizational reasons. In order to address this potential limit of our study, we performed separate statistical analyses in the subgroup of patients who did not receive neo-adjuvant chemotherapy. The results were quite comparable to those observed in the whole population. Only the microscopic tumoral extension observed for adenocarcinomas without neo-adjuvant chemotherapy was slightly increased compared to the microscopic tumoral extension observed among all adenocarcinomas. Finally, one of the main limits of our study, which is inherent to this type of clinicopathological study, is that we deduced the adequate GTV to CTV margin size from histological analyses, whereas the margin will be applied to a radiological GTV. This reasoning requires, to be valid, that the radiological tumor reflects the histological tumor. In the same way as other authors before us (9,24), we did find in our study a good correlation between radiological size of the tumor, measured on CT, and macroscopic histological size.

Another approach for evaluating the relevance of the GTV to CTV margin size is to evaluate the local control after radiation therapy, depending on the margin size that was used. Liang et al (25)

retrospectively compared locoregional control for patients with stage III NSCLC treated with an association of chemotherapy and IMRT, with a GTV to CTV margin of 6 to 8 mm for 50 patients, and 0 mm for 55 patients. The three year local control, metastasis free survival, and overall survival were not significantly different between the two groups. Similarly, in a retrospective study concerning 82 patients treated with 3DRT or IMRT, Yegya-Raman et al (26) found no difference in loco-regional control depending on the GTV to CTV margin size. The analysis of the patterns of failures after radiation therapy also comforts us in the possibility to reduce our GTV to CTV margin. In a retrospective study, Kilburn et al (27) observed only two marginal failures (defined as failures situated in the 5 mm area around the PTV) among 22 locoregional failures for 110 patients treated with 3DRT or IMRT, with no margin between GTV and CTV. Dosimetric studies can also provide data to answer the question of the relevance of the GTV to CTV margin size. In their dosimetric study, concerning 13 patients treated with IMRT, Xia et al (28) found that even in the absence of a GTV to CTV margin, the 50 Gy isodose line, which is considered as sufficient for controlling microscopic tumoral disease (29), appropriately covers a 10 mm expansion outside of the PTV.

Conclusion

Based on the results of our pathological analysis of 104 locally advanced NSCLC resected specimens (including 44 adenocarcinomas and 51 squamous cell carcinomas), we suggest that the GTV to CTV margin size could be reduced to 7 mm for adenocarcinomas and 4 mm for squamous cell carcinomas, still ensuring an appropriate coverage of the whole microscopic tumoral disease in more than 95% of cases. The reduction of the GTV to CTV margin size would allow us to reduce the treated volume of healthy lung, which has been shown to be associated with the risk of treatment-related pneumonitis (30,31). It might also in some cases allow a reduction of the cardiac radiation dose, which has been shown to be associated with all-cause mortality (32). We highlighted the existence of clinical and histological factors associated with the size of microscopic tumoral extension, notably the histologic

type (with a wider microscopic extension for adenocarcinomas in comparison with squamous cell carcinomas) and vascular extension (which is associated with a wider microscopic extension). A perspective of our study would be to search for other predictive factors of the size of microscopic tumor extension, such as characteristics of the immune tumoral micro-environment, and radiomic features extracted from pre-operative radiological data that we collected. This would allow us to personalize more finely the GTV to CTV margin size, depending on the tumor's characteristics.

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Figure 1 : Correlation between radiological tumoral size measured on CT with a mediastinal setting and macroscopic histological size.

Figure 2 : Example of a slide with a tumor presenting with microscopic tumor extension.

Figure 3 : Cumulative distribution of microscopic tumoral extension in the adenocarcinoma (ADC) and in the squamous cell carcinoma (SCC) groups.

Table 1 : Patients' and tumors' characteristics

Table 2 : Results of the univariate analysis of factors associated with the size of microscopic tumoral extension.

Table 3 : Results of the multivariate analysis of factors associated with the size of microscopic tumor extension

Table 4 : Literature review of the analysis of microscopic tumor extension in lung cancer

Figure 1

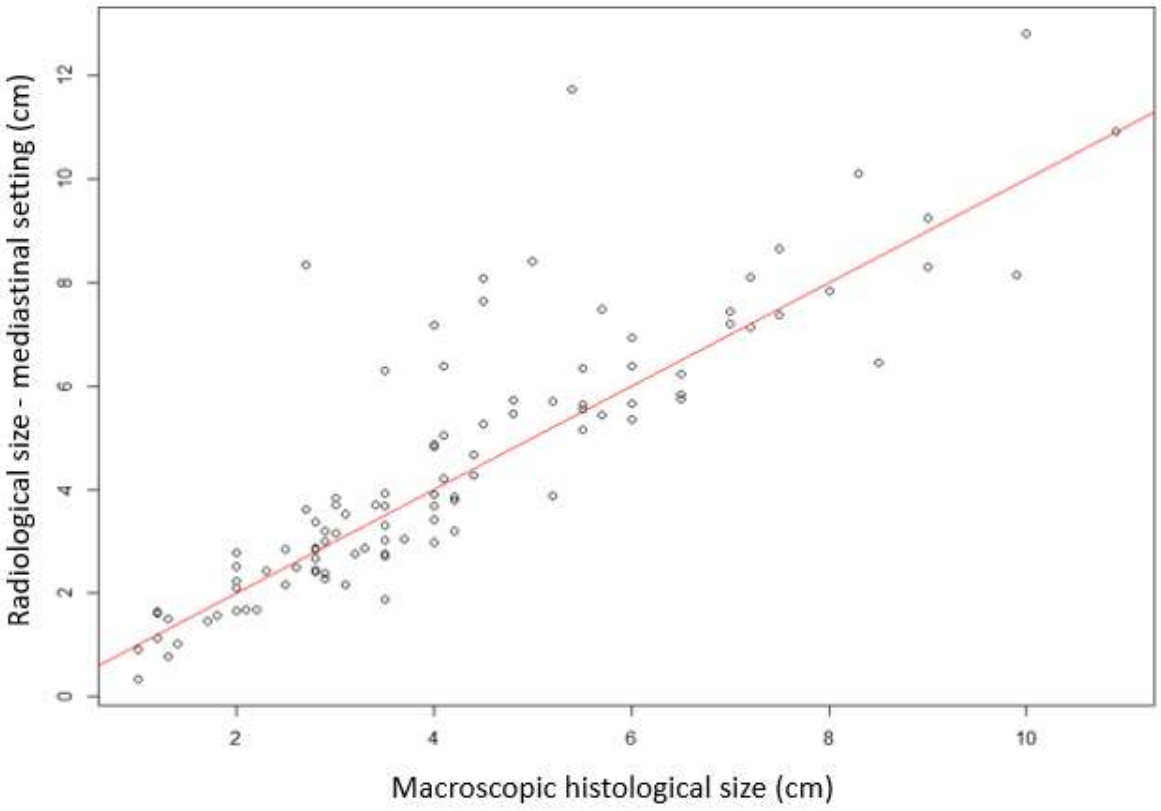

Figure 1 : Correlation between radiological tumoral size measured on CT with a mediastinal setting and macroscopic histological size.

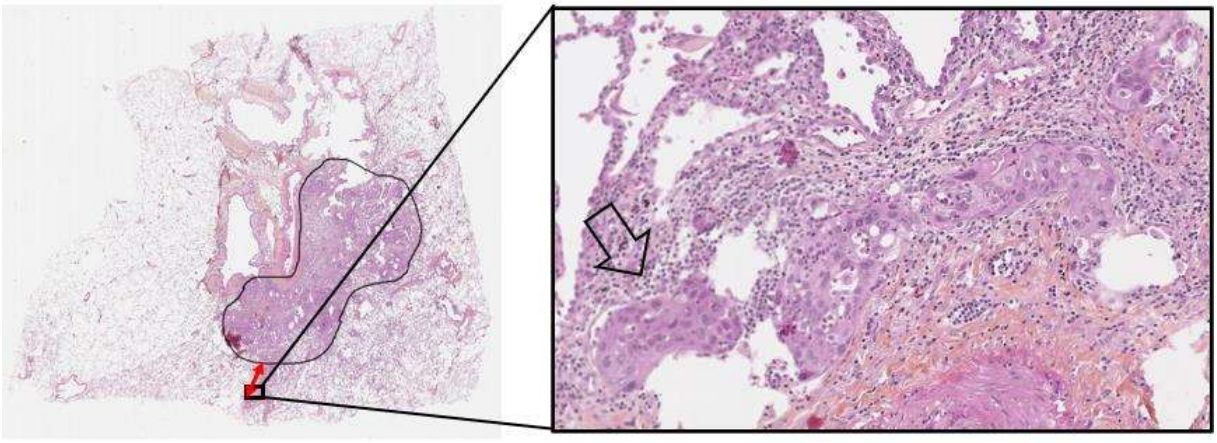

Figure 2 : Example of a slide with a tumor presenting with microscopic tumor extension.

On the left : image of the slide without magnification. The black freehand line represents the macroscopic limits of the tumor. The red arrow represents the size of the microscopic tumoral extension.
On the right : Magnification of a part of the slide on the left (in the square). The black arrow points at tumor cells.

Figure 3

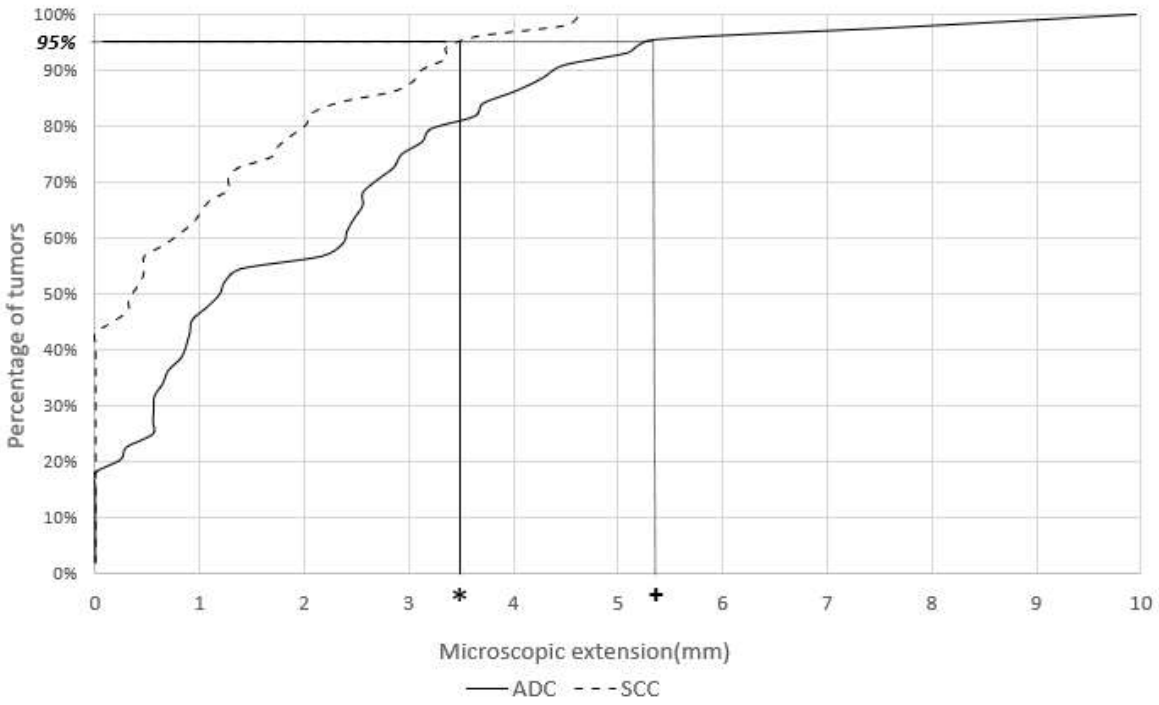

Figure 3 : Cumulative distribution of microscopic tumoral extension in the adenocarcinoma (ADC) and in the squamous cell carcinoma (SCC) groups.
ADC : 95th percentile = 5.3 mm (+)
SCC : 95th percentile = 3.5 mm (*)

Table 1

	Total population (N=104)	Adenocarcinomas (N = 44)	Squamous cell carcinomas (N= 51)	p-value
Median age [min-max]	64 yo [43 – 84]	63 yo [43 – 81]	65 yo [45 – 84]	**0.05**
Sex	Male : 66 (63%) Female : 38 (37%)	Male : 19 (43.1%) Female : 25 (56.8%)	Male : 41 (80.3%) Female : 10 (19.6%)	**0.0002**
Smoking	Yes : 92 (88 %) No : 12 (12%)	Yes : 36 (81.8%) No : 8 (18.2%)	Yes : 49 (96.1%) No : 2 (3.9%)	**0.024**
Neo-adjuvant chemotherapy	Yes : 28 (27%) No : 76 (73%)	Yes : 14 (31.1%) No : 30 (68.9%)	Yes : 12 (23.5%) No : 39 (76.5%)	0.37
Surgery	Wedge resection : 2 (1.9%) Segmentectomy : 3 (2.9%) Lobectomy : 62 (59.6%) Bilobectomy : 8 (7.7%) Pneumonectomy : 29 (27.9%)	Wedge resection : 2 (4.4%) Segmentectomy : 1 (2.2%) Lobectomy : 31 (71.1%) Bilobectomy : 3 (6.7%) Pneumonectomy : 7 (15.6%)	Wedge resection : 0 (0%) Segmentectomy : 1 (2%) Lobectomy : 28 (54.9%) Bilobectomy : 5 (9.8%) Pneumonectomy : 17 (33.3%)	0.18
Histological types and subtypes	Adenocarcinomas : 44 (43%) Squamous cell carcinomas : 51 (49%) Other : 9 (8.7%) Large cell euro-endocrine carcinomas : 7 (6.7%) Sarcomatoïd carcinoma : 1 (0.9%) Adenosquamous carcinoma : 1 (0.9%)	Acinar : 23 (52.3%) Solid : 12 (27.3%) Papillary : 5 (11.4%) Mucinous : 2 (4.5%) Mixed mucinous and non mucinous : 2 (4.5%)	Keratinizing : 31 (60.8%) Non keratinizing : 18 (35.3%) Basaloïd : 2 (3.9%)	-
T status	pT1a : 3 (2.9%) ypT1a : 2 (1.9%) pT1b : 4 (3.8%) ypT1b : 2 (1.9%) pT1c : 4 (3.8%) ypT1c : 4 (3.8%) pT2a : 27 (26%) ypT2a : 6 (5.8%) pT2b : 7 (6.7%) ypT2b : 2 (1.9%) pT3 : 27 (26%) ypT3 : 8 (7.7%) pT4 : 7 (6.7%) ypT4 : 1 (0.9%)	pT1a : 1 (2.3%) ypT1a : 1 (2.3%) pT1b : 3 (6.8%) ypT1b : 1 (2.3%) pT1c : 2 (4.5%) ypT1c : 4 (9.1%) pT2a : 13 (29.5%) ypT2a : 3 (6.8%) pT2b : 2 (4.5%) ypT2b : 1 (2.3%) pT3 : 8 (18.2%) ypT3 : 2 (4.5%) pT4 : 3 (6.8%) ypT4 : 0 (0 %)	pT1a : 1 (2%) ypT1a : 1 (2%) pT1b : 1 (2%) ypT1b : 1 (2%) pT1c : 1 (2%) ypT1c : 0 (0%) pT2a : 11 (21.6%) ypT2a : 2 (3.9%) pT2b : 5 (9.8%) ypT2b : 1 (2%) pT3 : 17 (33.3%) ypT3 : 5 (9.8%) pT4 : 4 (7.8%) ypT4 : 1 (2 %)	0.51
N status	ypN0 : 1 (0.96%) pN1 : 21 (20.2%) ypN1 : 6 (5.8%) pN2 : 58 (55.8%) ypN2 : 18 (17.3%) Single-site N2 : 52 (50%)	ypN0 : 0 (0%) pN1 : 0 (0%) ypN1 : 1 (2.2%) pN2 : 32 (73.3%) ypN2 : 11 (24.4%) Single-site N2 : 28 (62.2%)	ypN0 : 1 (2%) pN1 : 21 (41.2%) ypN1 : 5 (9.8%) pN2 : 19 (37.3%) ypN2 : 5 (9.8%) Single-site N2 : 20 (39.2%)	**<0.0001**

	Multiple-sites N2 : 24 (23.1%)	Multiple-sites N2 : 15 (35.6%)	Multiple-sites N2 : 4 (7.8%)	
Stage	II B : 12 (11.5%) III A : 63 (60.6%) III B : 29 (27.9%)	II B : 0 (0%) III A : 31 (71.1%) III B : 13 (28.9%)	II B : 12 (23.5%) III A : 26 (51%) III B : 13 (25.4%)	**0.0025**
Tumor location	Central : 36 (34.6%) Peripheral : 9 (8.7%) Close to chest wall : 39 (37.5%) Central and close to chest wall : 20 (19.2%)	Central : 9 (20%) Peripheral : 5 (11.1%) Close to chest wall : 24 (55.6%) Central and close to chest wall : 6 (13.3%)	Central : 27 (53%) Peripheral : 3 (5.9%) Close to chest wall : 9 (17.6%) Central and close to chest wall : 12(23.5%)	**0.0005**
Median macroscopic tumor size [min-max]	36 mm [10 – 109]	29 mm [10 – 100]	44 mm [10 – 109]	**0.0014**

Table 1 : Patients' and tumors' characteristics

Table 2

Quantitative variables	Spearman's correlation coefficient with size of microscopic tumoral extension	p-value
Radiological tumor size (mediastinal setting)	-0.168	0.088
Radiological tumor size (parenchymal setting)	-0.136	0.169
Macroscopic histological tumor size	-0.227	**0.02**
Age	-0.038	0.7
Qualitative variables	**Median size of microscopic tumoral extension**	**p-value**
Vascular invasion	Yes : 2.3 mm (0-9.95) No : 0.7 mm (0-5.3)	**0.045**
Disease stage	IIB : 0.6 mm (0-4.5) IIIA : 1.1 mm (0-9.95) IIIB : 0.6 mm (0-7.7)	0.65
Histologic type	Adenocarcinomas : 1.2 mm (0-9.95) Squamous cell carcinomas : 0.4 mm (0-4.6) Other types : 0.9 mm (0-3)	**0.04**
Nodal status	N1 : 0.2 mm (0-4.5) Single-site N2 : 1.2 mm (0-9.95) Multiple-sites N2 : 0.9 mm (0-4.6)	**0.02**
Location	Central : 0.3 mm (0-4,6) Peripheral : 0.6 mm (0-5.1) Close to the chest wall : 1.5 mm (0-9.95) Central and close to the chest wall contact : 0.6 mm (0-7.7)	0.058
Sex	Female : 0.6 mm (0-9.95) Male : 0.9 mm (0-7.7)	0.65
Smoking	Yes : 0.7 mm (0-9.95) No : 2 mm (0-4.3)	0.12
Blood Pressure	High : 1.3 mm (0-9.95) Normal : 0.6 mm (0-5.3)	0.11
Diabetes	Yes : 0.9 mm (0-7.7) No : 0.8 (0-9.95)	0.49
COPD	Yes : 0.6 mm (0-3.7) No : 0.9 mm (0-9.95)	0.97
Neo-adjuvant chemotherapy	Yes : 0.5 mm (0-5.3) No : 0.9 mm (0-9.95)	0.097

Abbreviations : COPD = Chronic Obstructive Pulmonary Disease.

Table 2 : Results of the univariate analysis of factors associated with the size of microscopic tumoral extension.

	Log-normal regression coefficient [95% CI]	p-value
Macroscopic histological tumor size	-0.006 [-0.01 – -0.001]	**0.024**
Histological type (SCC, ref : ADC)	-0.38 [-0.61 – -0.15]	**0.002**
Lympho-vascular invasion (yes, ref : no)	0.45 [0.23 – 0.67]	**0.0002**
Neo-adjuvant chemotherapy (yes, ref : no)	-0.19 [-0.45 – 0.07]	0.16
High blood pressure (yes, ref : no)	0.26 [0.02 – 0.49]	**0.03**

Abbreviations : ADC = adenocarcinomas ; SCC = squamous cell carcinomas; ref = reference.

Table 3 : Results of the multivariate analysis of factors associated with the size of microscopic tumor extension (histological types other than adenocarcinomas and squamous cell carcinomas excluded).

Interpretation of the log-normal regression coefficients - Example with macroscopic histological tumor size :
Other things being equal a difference in the macroscopic histological tumor size of -10 mm between two patients yields to a size of microscopic tumor extension multiplied by exponential (-0.006)(-10)=+1.062, which corresponds to a 6.2% increase.*

Table 4 final

Authors	Publication date	Number of patients	Disease stage	Histological types	Mean size of microscopic tumoral extension (mm) [min-max]	Median size of microscopic tumoral extension (mm)	GTV to CTV margin covering all microscopic extension in 95% of cases	GTV to CTV margin covering all microscopic extension in 90% of cases
Giraud et al (9)	2000	42	I : 46% II : 25% IIIa : 27% IIIb : 1% IV : 1%	ADC : 23 SCC : 19	ADC : 2.69 [0-12] SCC : 1.48 [0-13]	ADC : 1.6 SCC : 0	ADC : 8 mm SCC : 6 mm	ADC : 7 mm SCC : 5 mm
Kara et al (14)	2000	70		ADC : 15 SCC : 38 Other : 17	ADC : 0 [0-30] SCC : 0 [0-20]			
Goldstein et al (10)	2003	31	I (T1N0)	ADC : 31	7.4 [3-14]	7		
Li et al (16)	2003	43			ADC : 2.18 SCC : 1.33			ADC : 7 mm SCC : 5 mm
Grills et al (11)	2007	35	I (T1N0)	ADC : 35	7.2 [2-16]	8	13 mm	12.5 mm
Stroom et al (15)	2007	5	I to III	SCC : 3 Other : 2	[0-9]			
Meng et al (12)	2010	39	I to IIIa	ADC : 22 SCC : 17	ADC : 3.77[0,6-13] SCC : 2.87 [0-10]	ADC : 2.9 SCC : 2.4	ADC : 10 mm SCC : 5 mm	ADC : 6.5 mm SCC : 4.8 mm
Van Loon et al (13)	2012	34		ADC : 18 SCC : 6 Other : 10	[0-60]	0		26 mm
Current study	2020	104	II : 11.5% III :88.5%	ADC : 44 SCC : 51 Other : 9	ADC : 2.05[0-9.95] SCC : 1.01 [0-4.64]	ADC : 1.22 SCC : 0.38	ADC : 5.3 mm SCC : 3.5 mm	ADC : 4.4 mm SCC : 3.1 mm

Abbreviations : ADC = Adenocarcinomas ; SCC = Squamous Cell Carcinomas.

Table 4 : Literature review of the analysis of microscopic tumor extension in lung cancer