Applying comprehensive histologic assessment and genetic testing to synchronous multifocal lung adenocarcinomas and further survival analysis

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To the Editor: The differentiation of multiple primary lung adenocarcinomas (MPLAs) from metastases is important to allow for proper clinical management and prognostic prediction. The current revised diagnostic criteria for MPLAs proposed by the American College of Chest Physicians (ACCP) have been commonly applied since 2003.[1] However, it remains technically challenging to determine whether 2 or more lung adenocarcinomas from the same patient are homologous. Various approaches and algorithms including gene mutation analysis addressing this problem have been described.[2,3] Here, we present a cohort of 45 patients with synchronous multifocal lung adenocarcinomas.

All of the 45 patients were diagnosed through surgical resection from October 2012 to December 2017. Patients with multiple lung adenocarcinomas in the same lobe were excluded from the study because of their limited impact on treatment strategy. Of the 45 patients, 39 had double lung adenocarcinomas, 5 had triple lung adenocarcinomas, and 1 had 4 lung adenocarcinomas. All patients underwent bronchoscopy before surgery. Cranial computed tomography (CT)/magnetic resonance imaging (MRI), abdominal ultrasonography/CT, and nuclide bone scanning were adopted to rule out extrapulmonary metastasis before positron emission tomography (PET)-CT was widely adopted. PET-CT was performed in 36 patients, and mediastinoscopy or endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) was performed in 14 patients preoperatively to exclude suspicious N2 cases. Pulmonary function test (PFT) and general condition assessment were routinely performed before surgery. The detailed clinical and pathological characteristics of the patients are shown in Supplementary Table 1, http://links.lww.com/CM9/A6.

All 97 lung adenocarcinomas from the 45 patients were surgically removed. Different surgical procedures are illustrated in Supplementary Table 2, http://links.lww.com/CM9/A6. Thirty-seven patients underwent completely unilateral or bilateral video assisted thoracic surgery (VATS), while 8 patients received or were converted to open thoracotomy. Of the 14 patients with bilateral disease, 6 received synchronous bilateral procedures and 8 received operations within a 6- to 12-week interval. The patients generally recovered well. Two patients (4.4%) suffered postoperative complications, one developed sustained pulmonary air leakage but recovered after the application of prolonged intermittent negative pressure suction, and the other developed postoperative atrial fibrillation but recovered after being given antiarrhythmic treatment. There were no perioperative deaths.

Comprehensive histologic assessment was used to determine whether the adenocarcinomas were metastatic carcinomas or synchronous MPLAs. According to the updated diagnostic criteria proposed by ACCP[1] and the international multidisciplinary classification,[4] lung adenocarcinoma was classified as invasive adenocarcinoma and preinvasive lesion including atypical adenomatous hyperplasia (AAH), adenocarcinoma *in situ* (AIS) and minimally invasive adenocarcinoma (MIA). For invasive adenocarcinoma, the relative percentage of each histologic subtype was semiquantitatively evaluated in 10% increments, including lepidic, acinar, papillary, micropapillary, and solid components; variants of invasive adenocarcinomas were also included and evaluated. Two cases were diagnosed as multiple AIS/MIA and were considered synchronous MPLAs. The largest or main lesions of the other 43 patients were all identified as invasive adenocar-

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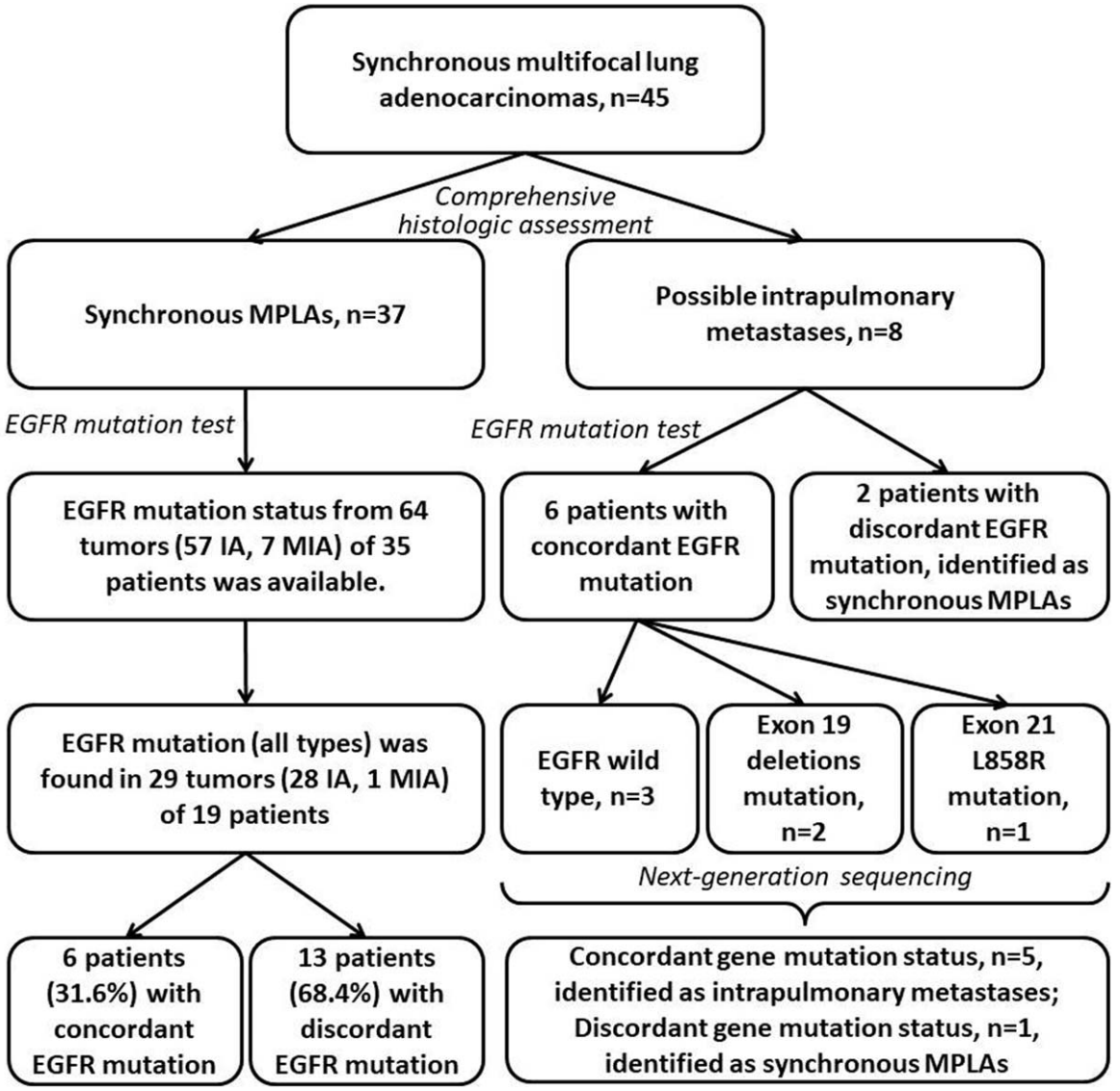

Figure 1: Diagnostic flow chart of the 45 patients with synchronous multifocal lung adenocarcinomas. MPLA: multiple primary lung adenocarcinoma; MIA: minimally invasive adenocarcinoma.

cinoma. Among these 43 patients, 18 were identified as having only 1 invasive lung adenocarcinoma, while the other 25 proved to have multiple invasive lung adenocarcinomas. The tumor pairs from 8 of the 25 patients had similar histologic subtypes and were considered possible intrapulmonary metastases. The remaining 35 patients with at least 1 invasive lung adenocarcinoma had different histologic subtypes or different cytological features and were considered synchronous MPLAs [Figure 1].

The mutational status of *EGFR* in exon 18 to 21 was obtained using kits from San Valley Diagnostics (Beijing, China) based on amplification refractory mutation system real-time polymerase chain reaction technology. Next-generation gene sequencing (NGS) was applied to patients with similar *EGFR* mutation types for further differentiation from intrapulmonary metastases. Semiconductor sequencing based on the Ion Personal Genome Machine (PGM^{TM}) System (Thermo Fisher Scientific, USA) was performed using the Ion AmpliSeq Cancer Hotspot Panel v2 (Thermo Fisher Scientific) to sequence more than 2800 loci from 50 oncogenes and tumor suppressor genes in the tumor DNA.[5] Among the 37 synchronous MPLA patients, the *EGFR* mutation status of 64 tumors of 35 patients was available. *EGFR* mutation was found in 29 tumors (28 invasive lung adenocarcinoma, 1 MIA) of 19 patients, including 15 tumors with Exon 19 deletion mutation, 9 tumors with Exon 21 L858R mutation, 3 tumors with Exon 20 insertion mutation, 1 tumor with Exon 21 L861Q mutation and 1 tumor with Exon 20 S768I mutation. The concordant frequency rate of the *EGFR* mutation distribution was 31.6% (6/19). For the 8 possible intrapulmonary metastasis patients, *EGFR* mutation status was available for all 16 tumors. Eight tumors from 5 patients were *EGFR* mutation-positive, 2 patients had both tumors presenting Exon 19 deletion mutation, and 1 patient had both tumors presenting Exon 21 L858R mutation. The remaining 2 patients each had 1 tumor with

Table 1: NGS results for the 6 patients with similar histologic subtypes and concordant *EGFR* mutation status

Patient No.	Location	Pathological subtypes	Gene mutation status	
			Tumor 1	Tumor 2
1	RUL + RML	Lepidic 80% + Papillary 20%	-	-
2	RUL + RLL	Lepidic 60% + Papillary 40%	*TP53*/ C277F	*TP53*/ C277F
3	LUL + LLL	Papillary 70% + Micropapillary 20% + Acinar 10%	*KRAS*/ G12C *PTEN*/ R130G	*KRAS*/ G12C *PTEN*/ R130G
4	LUL + RLL	Acinar 70% + Papillary 30%	*EGFR*/Exon 19 deletion *KRAS*/ G12V	*EGFR*/Exon 19 deletion *PIK3CA*/ E545K
5	LUL + LLL	Acinar 70% + Micropapillary 30%	*EGFR*/Exon 19 deletion *TP53*/ D281H *BRAF*/ V600E	*EGFR*/Exon 19 deletion *TP53*/ D281H *BRAF*/ V600E
6	RML + RLL	Papillary 90% + Solid 10%	*EGFR*/ L858R	*EGFR*/ L858R

LLL: left lower lobe; LUL: left upper lobe; NGS: Next-generation gene sequencing; RLL: right lower lobe; RML: right middle lobe; RUL: right upper lobe.

an Exon 19 deletion mutation showing a discordant *EGFR* mutation status and the tumors were identified as synchronous MPLAs. To further differentiate synchronous MPLAs from intrapulmonary metastases, NGS was applied to the 12 tumors of the 6 patients with similar histologic subtypes and concordant *EGFR* mutation status. One patient (Patient 4) was detected as having discordant gene mutation status and was identified as having synchronous MPLAs [Figure 1, Table 1].

According to the 8th edition of TNM staging for NSCLC, the tumor pairs from 40 synchronous MPLA patients were staged separately. If the pT stage of the largest tumor or main lesion was defined as the highest pT stage, 27 cases would be classified as pT1, and 11 and 2 cases would be classified as pT2 and pT3, respectively. Similarly, 29 of the 40 synchronous MPLA patients were confirmed as pN0, while 11 patients were confirmed as having at least 1 pN1 lesion. The 5 patients with intrapulmonary metastases were all staged as pT4N1M0 because of their location in different lobes of the same side and being identified with positive hilar, interlobular or intrapulmonary lymph nodes. No positive N2 lymph node was identified in the entire cohort.

Postoperative adjuvant chemotherapy was applied to 2 of 4 synchronous MPLA patients with one pT2aN0M0, IB stage lung adenocarcinoma; to all 11 synchronous MPLA patients with at least 1 pN1 lesion; and to the 5 patients with intrapulmonary metastases. A combination chemotherapy regimen with pemetrexed and platinum was regularly adopted. All patients received regular follow-up at the clinic. The median follow-up time was 45 months (10–83 months). Chest CT was performed every 3 months for the first year, every 6 months for the second year, and annually thereafter. Serum tumor markers, cranial CT/MRI, abdominal ultrasonography/CT, and nuclide bone scans were performed annually or whenever necessary as positive symptoms arose.

SPSS 19.0 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA) was used for the statistical analysis. The Kaplan–Meier estimator was used to draw survival curves. Univariate analysis was performed to compare the progression-free survival (PFS) and overall survival (OS) between groups according to age, sex, smoking history, tumor distribution, the largest tumor's dimension on high-resolution CT, the preoperative percentage of forced expiratory volume in the first second (FEV1%, defined as actual FEV1/predicted FEV1 × 100%), Charlson comorbidity index, Eastern Cooperative Oncology Group (ECOG) performance status, the use of a minimally invasive approach and different operational options, highest pT stage, pN stage, the number of invasive adenocarcinomas and the use of postoperative adjuvant chemotherapy using the log-rank test. Based on the univariate analysis results, candidate variables ($P < 0.15$) were selected for inclusion into the Cox proportional hazards model for multivariable analysis and to identify the independent risk factors. The odds ratio and 95% confidence interval were identified for multivariate predictors, and significance was defined as $P < 0.05$.

Kaplan–Meier survival curves of the PFS and overall survival OS time of the 40 synchronous MPLA patients and 5 patients with intrapulmonary metastases are shown in Figure 2. The postoperative median PFS time of the 40 synchronous MPLA patients was 51 months. The 3-year and 5-year progression-free survival rates were 72.8% and 42.4%, respectively, which were significantly better than those of the 5 patients who were diagnosed as having intrapulmonary metastases ($P = 0.001$). The postoperative median OS time of the 40 synchronous MPLA patients was 64 months. The 3-year and 5-year overall survival rates were 86.7% and 52.7%, respectively, which were significantly better than those of the intrapulmonary metastasis patients ($P < 0.001$).

For further identify the risk factors of PFS and OS for the synchronous MPLA patients, univariate analysis was first adopted to exclude potentially meaningless variables (Supplementary Table 3, http://links.lww.com/CM9/A6). Candidate variables ($P < 0.150$) that were selected for the multivariable analysis of PFS risk factors included larger maximal tumor dimension ($P < 0.001$), ECOG performance status ($P = 0.033$), the use of a minimally invasive approach ($P < 0.001$), operational option ($P = 0.132$), highest pT stage ($P < 0.001$), pN stage ($P < 0.001$), the number of invasive adenocarcinomas ($P = 0.048$) and the use of postoperative chemotherapy ($P < 0.001$). Candidate

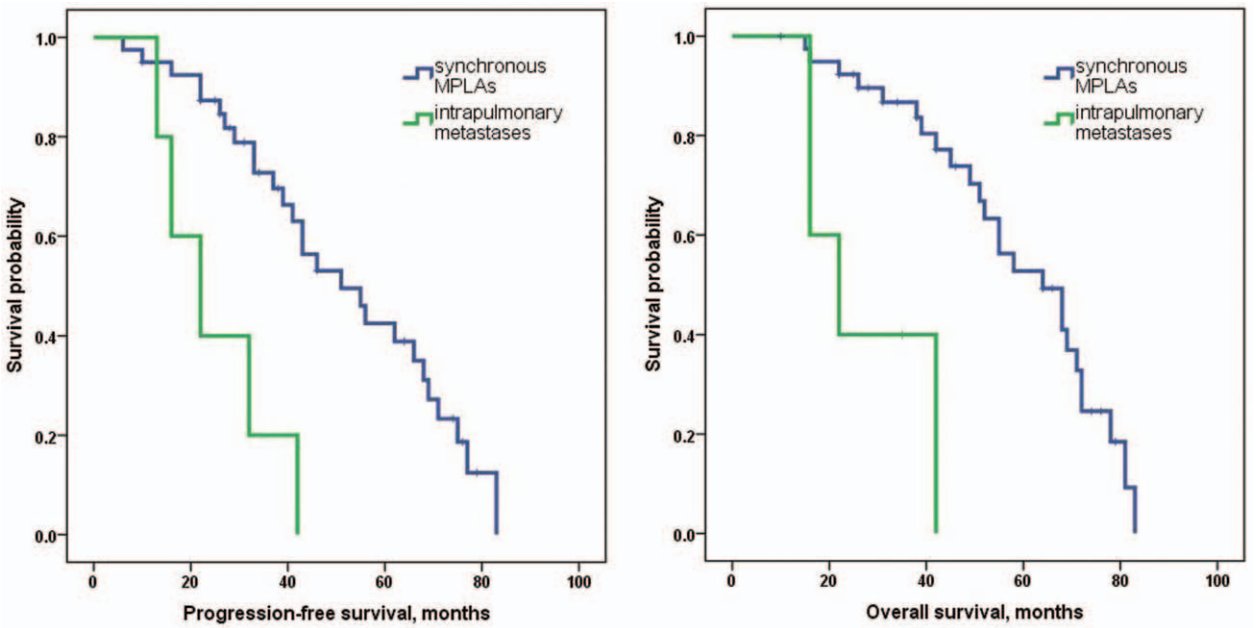

Figure 2: Kaplan–Meier PFS and OS curves of the 45 patients diagnosed as synchronous MPLAs and intrapulmonary metastases. PFS: Progression-free survival, OS: Overall survival; MPLA: Multiple primary lung adenocarcinoma.

Table 2: Multivariate analysis result of the independent risk factors of PFS and OS for the 40 patients with synchronous MPLAs

Characteristics	Reference	*OR* (95% CI)	*P*
Progression-free survival			
ECOG performance status	0-1	7.97 (2.15–29.60)	0.002
Highest pT stage	pT1	4.80 (1.71–13.45)	0.003
pN stage	pN0	4.79 (1.34–17.12)	0.016
Overall survival			
ECOG performance status	0-1	5.27 (1.46–19.08)	0.011
Minimally invasive approach	VATS	4.12 (1.08–15.72)	0.038
Highest pT stage	pT1	2.70 (1.05–6.94)	0.040

CI: confidence interval; MPLAs: Multiple primary lung adenocarcinomas; OS: Overall survival; PFS: Progression-free survival.

variables ($P<0.15$) selected for multivariable analysis of OS risk factors included larger maximal tumor dimension ($P=0.006$), ECOG performance status ($P=0.029$), the use of a minimally invasive approach ($P=0.001$), highest pT stage ($P=0.004$), pN stage ($P=0.003$), the number of invasive adenocarcinomas ($P=0.144$) and the use of postoperative chemotherapy ($P=0.001$).

The results of the multivariate analysis are shown in Table 2. For progression-free survival rate, ECOG performance status ($P=0.002$), highest pT stage ($P=0.003$) and pN stage ($P=0.016$) were identified as independent risk factors. Larger maximal tumor dimension ($P=0.848$), the use of a minimally invasive approach ($P=0.137$), operational option ($P=0.348$), the number of invasive adenocarcinomas ($P=0.254$) and the use of postoperative chemotherapy ($P=0.252$) were not independent predictors. For overall survival rate, ECOG performance status ($P=0.011$), the use of a minimally invasive approach ($P=0.038$) and highest pT stage ($P=0.040$) were identified as independent risk factors, while larger maximal tumor dimension ($P=0.941$), pN stage ($P=0.261$), the number of invasive adenocarcinomas ($P=0.669$) and the use of postoperative chemotherapy ($P=0.147$) had no effect on overall survival rate in our cohort. As far as we know, the diagnosis, staging, and treatment of multiple primary lung cancer has always been a subject of intense interest in thoracic surgery.

Comprehensive histologic assessment has proven an ideal method for the identification of synchronous MPLAs.[6] However, a proportion of patients with 2 or more lung adenocarcinomas of similar pathological subtypes do exist. In our cohort, this rate was as high as 8/45, and 3 of the 8 patients were finally confirmed to have synchronous MPLAs on this occasion.

The *EGFR* mutation status of 80 tumors in 43 patients was available (80/97, 82.5%) in our cohort. *EGFR* mutations were identified in 37 tumors of 24 patients (37/80, 46.3%). Two of the 8 patients with similar pathological subtypes were identified as having discordant *EGFR* mutation status and were diagnosed as having synchronous MPLAs.

In theory, the simultaneous identification of multiple tumor drive genes should provide better identification.[7] A diagnostic lineage test based on genomic rearrangements from mate-pair sequencing had previously been applied for distinguishing independent primary from metastatic lung cancers.[8] We chose to apply NGS due to the awareness of genetic heterogeneity in non-small-cell lung cancers. In our cohort, 1 of the 6 patients with a similar comprehensive histologic assessment result and *EGFR* mutation type was identified as having different gene mutation types by NGS, and the patient was diagnosed as having synchronous MPLAs.

Timely surgical treatment might be helpful for the prognosis of synchronous MPLA patients, especially for those with multiple GGO-like tumors.[9] Although there were high numbers of pT2 and pN1 lung adenocarcinoma patients in our cohort, the 3-year PFS and OS rates still reached 72.8% and 86.7%, values that were significantly higher than those for the intrapulmonary metastasis patients.

Our study proved that ECOG performance status can affect both the postoperative PFS and OS of synchronous MPLA patients, which again emphasizes the importance of preoperative evaluation and patient selection due to the complexity of the surgical procedures involved and the particulars of the perioperative management. The highest pT stage was undoubtedly a direct influential factor for the prognosis of the synchronous MPLA patients. Interestingly, pN stage was only an independent risk factor for PFS rate and did not affect OS. A possible explanation for this is that pN stage is strongly related to tumor progression, but the progress in chemotherapy and target therapy effectively prolongs the interval time from tumor progression to death. The use of a minimally invasive approach did not affect the PFS but had a positive impact on OS, indicating that VATS would bring overall benefit to the synchronous MPLA patients and should be applied to suitable cases.

Some limitations exist, mainly due to the retrospective nature of this study. In addition, the limited number of cases enrolled and the specific characteristics of the synchronous MPLAs could have led to selection bias. Future well-designed, multicenter studies with larger sample sizes are needed to validate these results and clarify the related issues.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Conflicts of interest

None.

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