Original article

A multidisciplinary approach for the differential diagnosis between multiple primary lung adenocarcinomas and intrapulmonary metastases

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Abstract

***Purpose*:** The distinction between multiple primary lung cancers (MPLCs) and intrapulmonary metastases has a significant impact on tumor staging and therapeutic choices. Several criteria have been proposed to solve this diagnostic issue, but a definitive consensus is still missing. We tested the efficacy of a combined clinical, histopathological and molecular ("real world") approach for the correct classification of multiple lung tumors in a selected cohort of patients.

***Methods*:** 24 multiple lung tumors with a diagnosis of adenocarcinoma from 10 patients were retrospectively reviewed. Radiological, pathological and clinical information, including follow-up, were integrated with molecular profiling via a routine multigene panel sequencing.

***Results*:** Comprehensive histologic assessment revealed readily distinguishable histologic patterns between multiple tumors suggesting unrelated lesions in 7 cases, in agreement with clinical, radiological and molecular data, thus leading to final diagnosis of MPLCs. In the remaining 3 cases, the differential diagnosis between MPLCs and intrapulmonary metastases was challenging, since the histologic features of the lesions were similar or identical. The final interpretation (2 MPLCs and 1 most likely intrapulmonary metastases) was reached thanks to the integration of all available data, and was confirmed by follow-up.

***Conclusions*:** A multidisciplinary approach including a routinely affordable multigene panel sequencing is a useful tool to discriminate MPLCs from intrapulmonary metastases in multiple lung nodules sharing the adenocarcinoma histotype.

Keywords: Multiple primary lung cancers; Synchronous; Metachronous; Intrapulmonary metastasis; NGS

1 Introduction

In recent years, the continuous improvement of imaging technologies is allowing identification of an increasing number of multiple synchronous or metachronous lung cancers, which to date account for about 0.2–20% of the lung cancer diagnoses [1–3].

A crucial issue in these cases is the differential diagnosis between multiple primary lung cancer (MPLC) and intrapulmonary metastases (IPM) especially if the tumors show the same histotype. This is extremely important for clinical management of lung cancer patients, since it affects staging, prognostication and therapeutic choices [4]. Indeed, MPLCs confer lower staging and a better prognosis compared to IPMs, with more room available for surgery in the former and need for aggressive chemo or targeted therapies for the latter [4–6].

Pathologic evaluation plays an important role in distinguishing IPM from MPLCs. To this end, diagnostic criteria were firstly published by Martini and Melamed in 1975. In their classification anatomic location, presence/absence of lymphatics involvement and convergent/divergent histotype of the multiple lesions were the most important parameters. Since then, these criteria were updated several times [7–10] but their application in the clinical setting is not always easy and/or may lead to conflicting results, as recently focused by Chen et al. [11]. Of course, the issue becomes more critical when multiple lesions show the same histotype. A comprehensive histological assessment (CHA), including the percentage of various histologic subtypes, nuclear features and stromal characteristics has been shown to improve discrimination between MPLCs and IPMs [12]. While this approach provides remarkable advantages, it remains partially limited by the subjective evaluation of the operator. Indeed, analysis of interobserver variation among expert lung pathologists has shown a good, although far from perfect agreement in using CHA to distinguish MPLCs from IPMs [13].

Searching for molecular aberrations of specific driver genes, such as EGFR mutations or ALK and ROS rearrangements became a routine in the management of non-small cell lung cancer (NSCLC) patients since they can predict response to specific targeted therapies [14,15]. While mutations in driver genes are most often "truncal" and thus shared between primary and metastatic lesions, tumor heterogeneity and/or methodological issues may sometimes be responsible for discordant molecular findings between primary and metastatic sites. In addition, the most commonly searched molecular aberrations, such as EGFR mutations and ALK or ROS rearrangements, are not very frequent (especially in western countries) eventually being informative only for a minority of cases. Despite single gene molecular determinations have been also used to address clonal relationship among multiple lung adenocarcinoma [16,17] their utility for this purpose appears scant, in light of the above mentioned limitations.

Whole genome sequencing or large multigene panel sequencing were shown to provide objective information to assess clonal relationship between different lesions within the same patients uniquely based on their molecular profiling [18–20]. However, these wide-ranging approaches are not readily compatible with the clinical setting because they are expensive and not widely available in diagnostic laboratories. Targeted Next Generation Sequencing (NGS) multigene panels are routinely used in the clinical practice to assess eligibility for targeted therapies of cancer patients [21–27].

Due to the expanding number of molecular aberrations relevant for predictive purposes (which now include characterization of at least BRAF, STK11, and KRAS^{G12C} mutations in addition to EGFR, ALK and ROS aberrations) multigene panel sequencing was implemented in our routine for NSCLC molecular profiling [22].

Here, we report on the efficacy of a combined clinical (including follow-up data), radiological, histopathological and molecular approach based on multigene panel sequencing to support the differential diagnosis between MPLCs and IPM in a cohort of patients with lung adenocarcinoma (ADC).

2 Materials and ~~M~~methods

2.1 Patients characteristics and specimen collection

From January 2015 to December 2019, 225 patients underwent surgery for ADC at our Institution. Ten patients (4.5 %) had multiple tumors, accounting for a total of 24 lesions (Table 1). The histologic diagnosis, including tumor grading, was based on morphologic and immunohistochemical findings according to the 2015 WHO classification [26]. Clinical information, including history of other neoplasia, follow-up and disease progression were collected for each patient. Radiological features of all tumors were also reviewed (Table 1). Patients with potentially resectable lung cancer had been preoperatively staged with total body TC, brain MRI, and bone scintigraphy. The histological slides were reviewed and the mutational profile was assessed for each lesion by NGS, using a 22 genes panel on an IT-PGM platform. Samples used for molecular analysis were selected for neoplastic cell count at least > 30 %. A single lymph node metastasis was characterized by 5~~–10~~–10 % neoplastic cell count. All information regarding human material included in the study was managed using anonymous numerical codes, and all samples were handled in compliance with the principles outlined in the declaration of Helsinki.

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Table 1

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Clinical, radiological and pathological features of the study population.

N#	Sex	Age	Sy/Me	N° of tumors	Radiologic appearance	Type of surgery	Size (cm)	Location	Node stage	Histologic subtypes (%)	Stromal features and presence of mucin	TTF1	G	N	LVI	STAS	FU (months)
1	F	65	Sy	T1	Solid	W	1.2	LUL	N0	S (50) / C (30) / A (20)	Necrosis, Mucin	+/-	3	D	–	–	–15
				T2	Part-solid	L	1.7	LLL		A (60) / L (40)	Desmoplasia	++	2		–	–	
–2	F	77	Sy	T1	Part-Solid	W	1.0	LUL	N0	P (70) / A (30)	Inflammation	+	2	D	–	–	–12
				T2	Solid	W	2.2	RLL		A (40) / P (30) / S (30)	Desmoplasia	++	3		–	–	
–3	M	72	Sy	T1	GGN	W	1.4	RUL	N0	L (40) / P (30) / A (30)	Desmoplasia	+++	2	S	–	–	–12
				T2	GGN	W	0.7	RML		L (60) / P (30) / A (10)	Desmoplasia	+++	2		–	–	
				–T1	GGN	W	1.0	RUL		L (60) / P (40)	Inflammation	+++	2		–	–	
–4	M	77	Sy	T1	Solid	W	2.0	LUL	N0	S (70) / A (30)	Necrosis	+++	3	D	–	–	–24
				T2	Part-solid	W	1.6	RUL		A (40) / L (60)	Desmoplasia, Mucin	+/-	2		–	–	
–5	M	77	Sy	T1	Solid	L	2.5	RML	N1	S (90) / A (10)	Necrosis	++	3	D	+	–	-23
				T2	Part-solid	W	3.0	RUL		A (60) / L (40)	Desmoplasia, Mucin	+/-	2		–	–	
–6	F	64	Sy	T1	GGN	L	1.3	RLL	N0	L (60) / P (40)	Desmoplasia	+++	2		–	-+	55
			Sy	T2	GGN	L	1.5	RLL		L (70) / A (30)	Desmoplasia	+++	2		–	-+	
			Sy	T3	GGN	L	0.7	RLL		L (50) / A (30) / P (20)	Inflammation	+++	2	S	–	-+	
			Me	T4	Part-solid	L	1.2	RML		A (70) / L (30)	Lymphoid hyperplasia	++	2	D	+	–	
-7	F	63	Me	T1	Solid	W	2.6	RUL	N0	S (80) / A (20)	Lymphoid hyperplasia	+++	3	S	+	–	-46
				T2	Solid	L	2.5	LLL		S (80) / A (20)	Lymphoid hyperplasia	+++	3		+	–	
-8	M	72	Me	T1	GGN	W	2	RUL	N0	L (80) / A (20)	Desmoplasia	+++	2	S	–	–	–52
				T2	Part-solid	W	1,5	RUL		L (50) / A (50)	Lymphoid hyperplasia	++	2		–	–	
–9	M	66	Me	T1	Part-solid	W	3.5	LUL	N0	L (50) / A (50)	Desmoplasia	+	2	D	–	–	–29
				T2	Solid	W	1.2	RUL		S (90) / Sarc (10)	Necrosis	+++	3		–	–	
-10	M	64	Me	T1	Part-solid	W	0.8	LUL	N0	A (50) / L (30) / MP (20)	Desmoplasia	++	3		–	–	–37
				T2	Part-solid	W	1.5	RLL		A (60) / L (40)	Inflammation	+++	2	S	–	–	
				–T3	GGN	W	0.8	RUL		L (60) / A (30) / P (10)	Desmoplasia	++	2		–	–	

~~-~~Sy: synchronous; Me: metachronous; GGN: ground glass nodule; W: wedge resection; L: lobectomy; RUL: right upper lobe; RLL: right lower lobe; LUL: left upper lobe; LLL: left lower lobe; RML: right median lobe; A: acinar; P: papillary; L: lepidic; S: solid; C: cribriform; MP: micropapillary; Sarc: sarcomatoid component; G: grading; N: Nuclei : Ssimilar; D: different; LVI: lymph-vascular invasion; FU: follow up.

2.2 Pathological examination

For each tumor sample, hematoxylin and eosin (H&E) stained slides were blindly reviewed by two pathologists (AP and GdA); based on the dimension of each tumor, a variable number of slides (from three to six) were available. A CHA of each lesion was performed, which included: i) semiquantitative assessment of the solid, cribriform, micropapillary, acinar, papillary, lepidic subtypes (in 10 % increments); ii) nuclear atypia; iii) mucin content and iv) stromal features such as desmoplasia, inflammation, lymphoid hyperplasia and/or necrosis [12]. The expression of tumor transcription factor 1 (clone 8G7G3/1, Abcam, Cambridge, United Kingdom) was also evaluated for each case, according to a recently proposed algorithm for the differential diagnosis between MPLCs and IPMs [28]. Finally, the presence of lymph vascular invasion and spread through airspaces (STAS) was recorded for each lesion.

2.3 Molecular analysis

2.3.1 DNA extraction

Histological slides from formalin-fixed and paraffin-embedded (FFPE) tumor tissue were reviewed to select for neoplastic cell content at least > 30 %. DNA was extracted by 24 lung tumor biopsies plus 2 metastatic sites (lymph node and adrenal gland) as previously described [21,22].

~~2.3.2.~~ 2.3.2 IT-PGM sequencing and variant calling

~~-~~The mutational profile of each lesion was assessed by NGS, using the Ion AmpliSeq Colon and Lung Cancer Research Panel V2 (CLV2, Thermo Fisher Scientific, Guilford, CT 06437, USA) on an IT-PGM platform, as described [21,24,25]. Briefly, 10 ng of DNA samples was required to construct barcoded and adaptor-ligated libraries using the Ion AmpliSeq Library kit 2.0 (Thermo Fisher Scientific, Van Allen Way, Carlsbad, CA 92008 USA). Templated spheres were prepared using the Ion One Touch 2.0 machine (Thermo Fisher Scientific, Van Allen Way, Carlsbad, CA 92008 USA) and sequenced by IT-PGM machine (Thermo Fisher Scientific, Van Allen Way, Carlsbad, CA 92008 USA). Sequencing data were analyzed with the Ion Torrent Suite (Thermo Fisher Scientific, http://github.com/iontorrent/TS) and visualized with the Integrative Genomics Viewer. Variants with a quality <30 were filtered out. For variants with low allele frequency (<5%) a minimum coverage of 500 reads was required. Germline variants (identified on the bases of allele frequency, ClinVar database and literature review) were excluded from the analysis.

3 Results

3.1 Clinical-pathological features and molecular characterization of the study population

In this study we analyzed 24 tumors (plus two metastatic lesions) from 10 patients with multiple pulmonary nodules. Patients characteristics, including the radiological features of the nodules, are reported in Table 1. Seven patients had two lesions, two had three tumors and one had four. One patient also had metastatic lesions in a mediastinal lymph node and in the adrenal gland. In 5/10 cases tumors were syncronous or metacronous, with the second lesion occurring from 2 to 4 years from the first. Surgery consisted in lobectomy or wedge resection. All the patients are alive at the present time, with follow-up ranging from 12 to 53 months. All nodules were classified as adenocarcinomas, and characterized in more details for histopathological features. Routine molecular profiling was performed by multigene panel sequencing. We found at least one somatic mutation in 20 (83 %) and at least two somatic mutations in 9 (37 %) of the 24 lung tumors. As expected, high mutation frequency was detected in TP53 and KRAS genes.

3.2 Multidisciplinary assessment of the multiple lesions

In 7 out of 10 cases (1, 2, 4, 5, 8-10) the diagnosis of MPLCs was based on the concordance between radiologic, pathologic and molecular features of the multiple tumors (Table 1 and 2). Comprehensive histologic assessment showed stiking differences in stromal features, percentages of histologic subtypes, presence of mucin and nuclear pleomorphism in the majority of these cases (Fig. 1 A-D, Table 1). The paired tumors from patient 8 and the three from patient 10, all occurring metachronously, showed less marked differences in the subtype percentage, with the same degree of nuclear pleomorphism and similar stromal features (Fig. 3 ~~C~~ C–F). The expression of TTF-1 was different between the lesions, in line with the hypothesis of MPLCs. The radiological appearance of the lesions was also consistent with multiple primaries (Table 1, Fig. 3 A, B). Multigene panel sequencing (Fig. 2) detected somatic mutations in different genes in the multiple tumors from patients 1, 4, 9 and 10. Tumors from patients 5 and 8 showed different mutations of the same genes (KRAS and TP53). Finally, in patient 2 molecular analysis detected the activating $EGFR^{L858R}$ mutation with high allele frequency in one lesion (T1), while the other showed no somatic mutations

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Table 2

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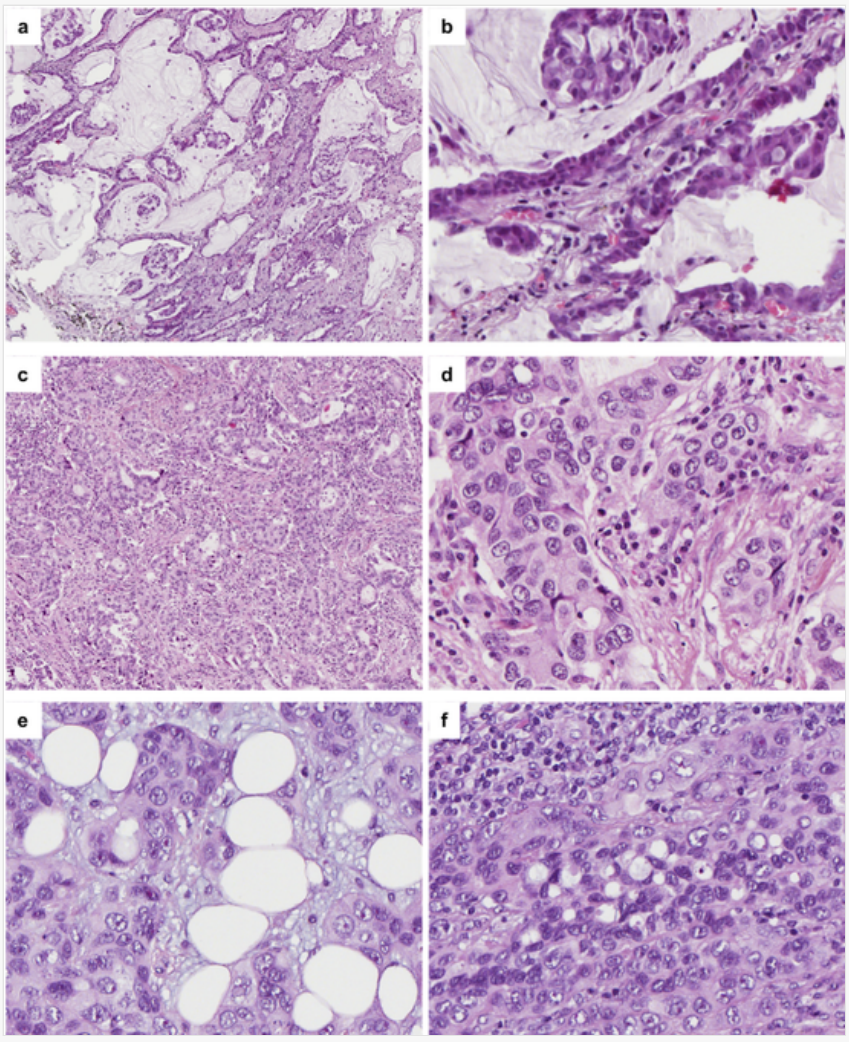
Classification of lesions as MPLCs vs IPMs based on radiologic, histologic and molecular features.

Patient n.	Radiology	Histology	Molecular analysis
1	MPLCs	MPLCs	MPLCs
2	MPLCs	MPLCs	MPLCs
3	Multifocal GGN	MPLCs	Indeterminate
4	MPLCs	MPLCs	MPLCs
5	MPLCs	MPLCs	MPLCs
6	Multifocal GGN (T1-T3), MPLC (T4)	MPLCs (T1-T3) MPLC (T4)	IPMs (T1-T3) MPLC (T4)
7	IPMs	IPMs	MPLCs
8	MPLCs	MPLCs	MPLCs
9	MPLCs	MPLCs	MPLCs
10	MPLCs	MPLCs	MPLCs

alt-text: Fig. 1

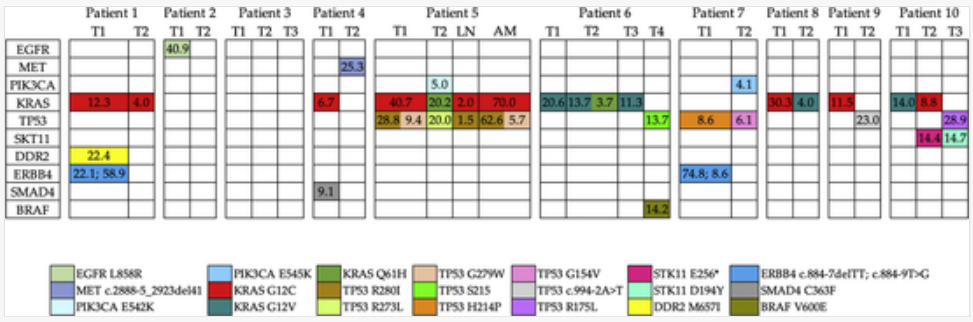
Fig. 1



a (4x) Pt 1 (T2) Abundant mucin production and lepidic pattern; **b** (20x) medium size polarized cells with hyperchromatic nuclei and focal intracytoplasmic mucin vacuoles; **c** (4x) Pt 1 (T1) solid and acinar subtypes; **d** (20x) cells characterized by pleomorphic nuclei with vesicular chromatin and eosinophilic cytoplasm; **e, f** (20x) both adrenal gland and lymph node metastases from Pt 1 show the same growth pattern and degree of nuclear pleomorphism of T1 (H&E).

alt-text: Fig. 2

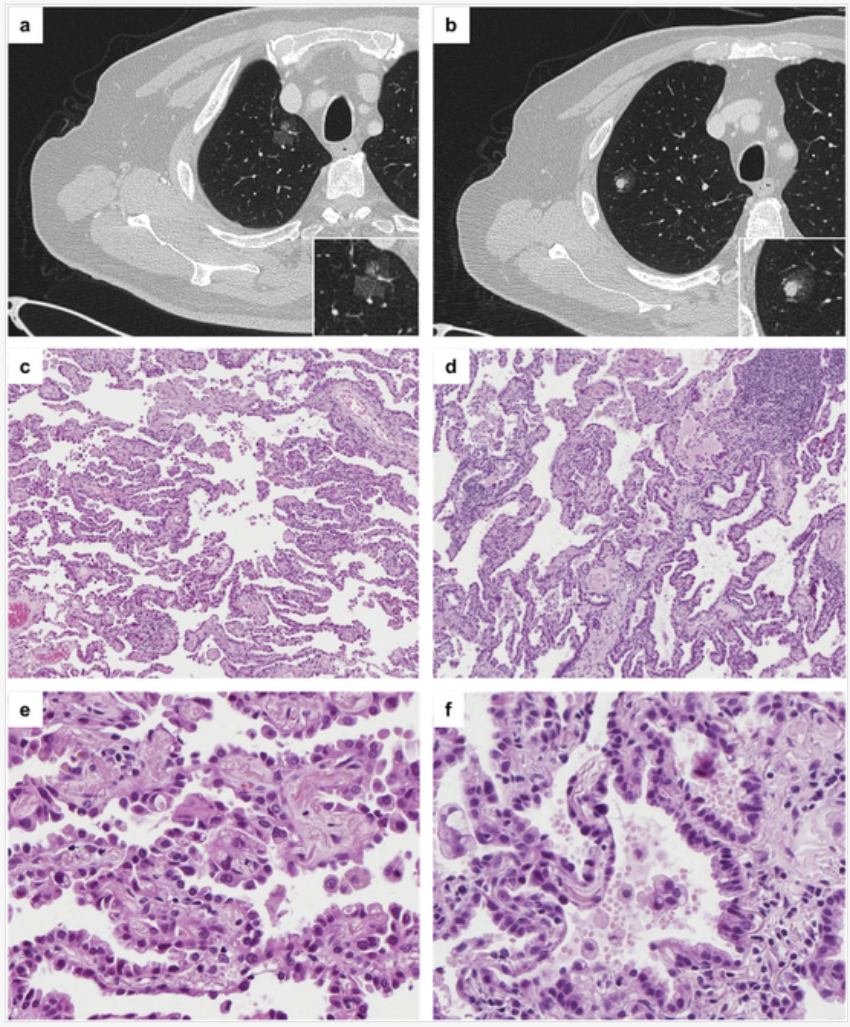
Fig. 2



Somatic molecular changes and allele frequencies (reported as percentage) in the 24 lung tumors.

alt-text: Fig. 3

Fig. 3



Pt 8: High resolution CT scans performed on T1 (**a)** and on T2 (**b)** respectively show ground glass lesion and a part-solid lesion; **c** (4x) lepidic growth pattern of T1 and **d** (4x) prevalent lepidic growth pattern of T2 with lymphoid stromal hyperplasia; **e** (20x) and **f** (20x) the neoplastic cells of T1 and T2 have similar degree of nuclear atypia.

Patients 1, 2, 4, 8, 9 and 10 are alive and disease free respectively at 15, 12, 24, 52, 29 and 37 months (Table 1). Lymph nodes and adrenal metastases developed in patient 5. Interestingly, they showed the same histologic features of one of the paired lesions (T1), characterized by a solid pattern of growth, high nuclear grade and extensive necrosis, consistent with an aggressive behaviour (Fig. 1 E, F). The molecular pattern of the metastases also aligned with that of the T1 lesion (Fig. 2).

In three cases (patients 3, 6 and 7) the achievement of the final diagnosis was challenging, since the radiological, pathological and molecular results were discordant (Tables 1 and 2).

All the three synchronous ipsilateral lesions of patient 3 were small (< cm 2), with a radiological ground glass appearance. On CHA they showed a predominant lepidic growth pattern and only a slight difference in the subtype percentage, with the same degree of nuclear pleomorphism and similar stromal features (Table 1); immunoistochemical expression of TTF-1 was similar. All three lesions shared a no-mutation profile at the 22 gene panel analysis (Fig. 2). The lesions were temptatively classified as MPLCs based on radiological appearance and the prevalent lepidic growth patten on histology. The patient is free from disease 24 months after surgery, in line with this diagnostic hypothesis.

Patient n. 6 had four tumours, of which three occurring synchronously in the same lobe with a radiologic ground glass nodule and one, with a part-solid radiological pattern, presenting after four years in a different ipsilateral lobe. The three synchronous lesions showed a diffuse lepidic growth pattern, similar degree of nuclear atypia and partially different stromal features (Table 1). Their molecular pattern was characterized by a shared $KRAS^{G12V}$ mutation. An additional $KRAS^{Q61H}$ mutation occurred at very low frequency (3.7%) in T2, possibly suggesting development of a subclonal population. Based on their close spatial relationship, their small size (ranging from 0.7 to 1.5 cm), their ground glass pattern and the prevalent lepidic component, these tumours were staged as multifocal lesions (33). Interestingly, the three synchronous tumors showed diffuse spread through airspaces (STAS) which could explain the local dissemination. The fourth lesion, occurring 4 years after lobectomy, displayed distinctive molecular features ($BRAF^{V600E}$ and $TP53^{S215I}$, Fig. 2) with a prevalent acinar growth pattern, marked nuclear pleomorphism, and extensive vascular invasion, suggestive of hematogenous spread. This lesion was classified as an independent primary. After a few months, the patient developed further neoplastic nodules, involving both lungs.

The two paired tumours of patient 7, occurring metachronously with a time interval of 24 months, both had a solid radiologic appearance (Fig. 4 A, B). On CHA they showed identical percentages of subtypes (Fig. 4 C, D), same degree of nuclear pleomorphism, analogous immunistochemical pattern of positivity for TTF-1, and similar stromal features (Fig. 4 E, F), favouring the hypothesis of metastatic disease. However, the mutational pattern of the two lesions was remarkably different, with T1 showing mutations in TP53 and ERBB4 and T2 showing a different mutation in TP53 and one in PIK3CA, rather supporting the hypothesis of MPLCs (Fig. 1).

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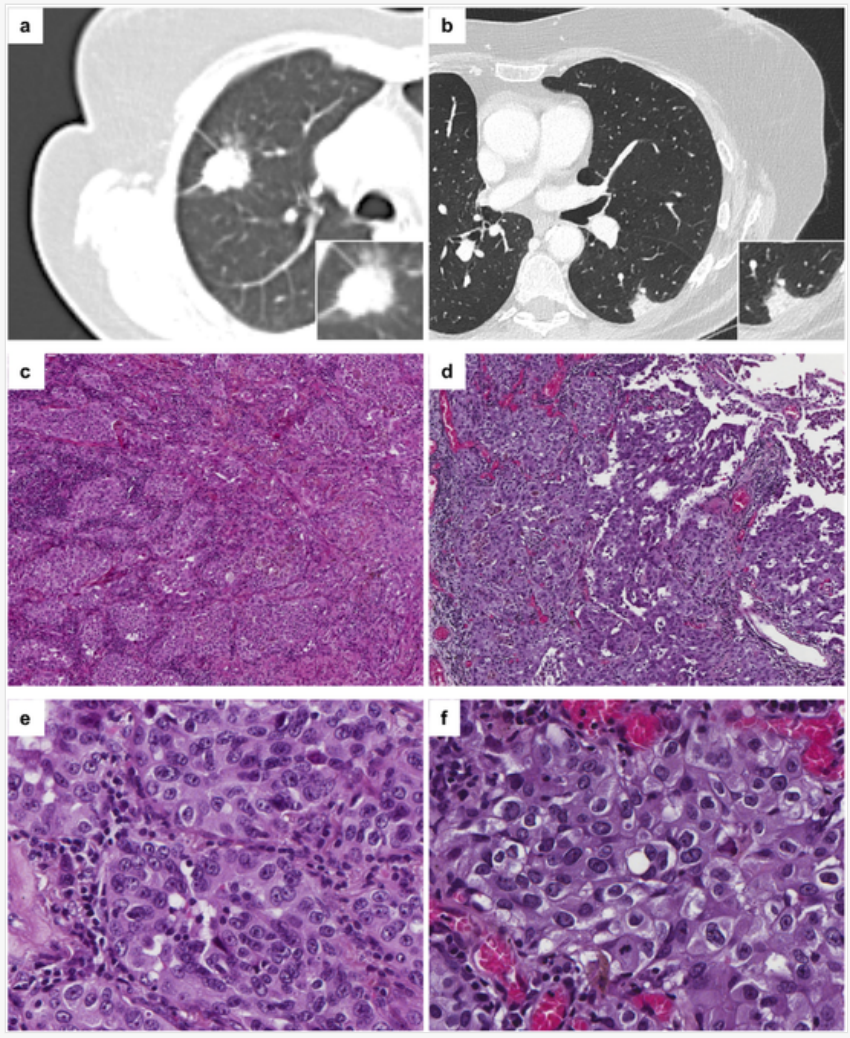
Fig. 4



Pt 7: High resolution CT scans performed on T1 (**a)** and on T2 (**b)** respectively show solid nodular lesion; **c, d** (4x) both T1 and T2 show the same solid growth pattern and identical stromal.

4 Discussion

Lung cancers can present with multiple lesions, occurring synchronously or metachronously [1,2,29]. A critical issue is the correct classification of the lesions as separate or related entities. In fact, the classification of multiple lung nodules as distinct primaries or intrapulmonary metastases may impact on therapeutic choices, determining if the patient undergoes surgical therapy instead of chemotherapy and/or radiotherapy.

In the last decades, several criteria have been proposed to solve this diagnostic dilemma, based on the difference in tumor histotypes, extent of nodal involvement and/or molecular profile of the lesions [10,30,31]. Despite these efforts, the matter is still debated and a definitive consensus is not reached.

The objective of our study was to investigate whether the application of a recently proposed multidisciplinary approach [29], acknowledged in the current AJCC classification [32], including diagnostic imaging, comprehensive histologic assessment and molecular analysis via multigene panel sequencing could efficiently allow discrimination between MPLCs and IPMs in cases with multiple lung tumors of the same histotype, in our case adenocarcinoma.

According to our results, in the majority of cases (7 out of 10 patients) there was a concordance between radiological, pathological and molecular results, allowing the diagnosis of MPLCs. In five of these cases (1, 2, 4, 5, 9) there were striking differences in the histologic pattern of paired adenocarcinomas as assessed by CHA and quite different TTF-1 expression (~~Mansuet-Lupo A, + ref 40~~), that have proven themselves to be a reliable criterium to predict that multiple tumors are unrelated. Detection of the KRASG12C mutation in both lesion of patient 1 did not challenge the diagnosis of MPLCs, since this is a very common mutation in lung cancer [33]. In patient 5, the histological and molecular features of the secondary lesions occurring in the mediastinal lymph nodes and the adrenal gland, were identical to those of the T1 primary lesion, bearing morphologic traits suggestive of an aggressive biological behavior. In patient 8 and 10, despite multiple adenocarcinomas showed similar degree of nuclear pleomorphism and histologic subtypes, the slight differences in percentage of patterns based on CHA and the pattern of TTF 1 expression led to the diagnosis of MPLCs, as confirmed by multidisciplinary evaluation. The good clinical outcome of these patients confirmed the diagnosis of MPLCs.

In three patients radiological, pathological and molecular results were discordant. In these cases, CHA was very similar (patients 3, 6) or identical (patient 7), thus assessing whether the lesions are multiple primaries or metastasis based only on pathologic evaluation was more difficult.

We finally classified the lesions of patient 3 as MPLCs, even though molecular results were not informative, based on radiological and pathological features (especially in consideration of the predominant lepidic growth pattern). The appropriateness of this approach for the categorization of was further supported by follow up.

Regarding patient 6, interestingly, the three synchronous lesions showed very similar histologic subtypes and an identical mutational profile. These lesions were more properly classified as multifocal, based on their close proximity, their ground glass appearance on CT scan and on the pathological finding of a prevalent lepidic pattern [34]. The presence of diffuse STAS, as in our case, suggests that local spread could be the mechanism underlying the rare occurrence of multifocal lesions with the same genomic profile [35]. We could hypothesize that bad clinical outcome of the patient was related to T4, the metachronous lesion that showed features of aggressiveness on both pathological examination and molecular analysis.

In one case (n. 7) there was a discrepancy between radiological and histological data, consistent with IPMs, and the mutational profile of the lesions, suggesting the diagnosis of MPLCs. The rapid progression of the disease in this case is more likely to be in line with a IPM diagnosis [29,36]. Indeed, discordant mutational profile between primary and metastatic sites was reported even for driver mutations, although this is more likely observed when single target gene analyses rather than multigene analysis are performed [17,19,29,37–41]. Large gene panel analysis may reverse the pathologic diagnosis, when indicated by distinct complex molecular patterns [18,19]. This case remains controversial.

The described comprehensive multidisciplinary and multiparametric evaluation led to a final consensus on the diagnosis for 9 out of 10 patients affected by multiple lung cancers. It is important to emphasize that a 22 multigene panel sequencing routinely affordable in clinical settings for predictive purposes because of low costs and turn-around-time supported the reliability of the differential diagnosis between MPLCs and IPMs for 8 out of 10 patients. This approach, based on a limited gene panel, has been recently proposed as an useful tool in this specific setting [42,43].

Current literature indicates that only whole genome or multigene analysis (~50–100 genes) may afford unambiguous clonality evaluation in lung cancer [18,19,41,44,45]. Unfortunately, these approaches are not affordable in the “real world” clinical setting, yet.

Finally, it is worth mentioning that “real world” multigene panel determination not only contributes to the management of cases with multiple lung nodules by helping with the differential diagnosis, but also provides indication on the correct target therapy to be administered. Indeed, when progression occurs in a MPLC case, determining the molecular profile of the metastatic lesion(s) becomes essential for the choice of the most appropriate therapy. This is best exemplified by case 6, where progression occurs with $BRAF^{V600E}$ positive metastasis, which may indicate the use of anti-BRAF targeted therapy.

5 Conclusions

In conclusion, our experience supports the concept that categorization of multiple lung ADC should rely on the collective judgment of a multidisciplinary team, after taking into account all of available information. We observed a high rate of concordance between radiological, pathological and molecular data, as demonstrated by previous results [28,35,43]. In agreement with clonality studies in a limited number of reports, in our series the most frequent diagnosis was MPLCs [12,29,46]. This finding is also in line with the results of the extensive literature review by Detterbeck et al., suggesting that in doubtful cases it could be more appropriate to consider the tumors as separate entities, since intrapulmonary metastases seem to be rarer [35].

A limitation of our study is the relatively low number of cases collected that prevents any statistical analysis. However, we provide a complete and careful description of the characteristics of this limited cohort of patients, underling the importance of the multidisciplinary approach, including radiologic features, and proposing possible etiopathology explanation in challenging cases.

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CRediT authorship contribution statement

Francesca Belardinilli: Conceptualization, Investigation, Formal analysis, Writing - original draft. **Angelina Pernazza:** Conceptualization, Investigation, Formal analysis, Writing - original draft. **Yasaman Mahdavian:** Investigation, Formal analysis, Methodology. **Bruna Cerbelli:** Formal analysis, Methodology. **Massimiliano Bassi:** Writing - review & editing. **Angela Gradilone:** Writing - review & editing. **Anna Coppa:** Writing - review & editing. **Maria Gemma Pignataro:** Writing - review & editing. **Marco Anile:** Writing - review & editing. **Federico Venuta:** Writing - review & editing. **Carlo Della Rocca:** Supervision, Writing - review & editing. **Giuseppe Giannini:** Project administration, Supervision, Writing - review & editing. **Giulia d’Amati:** Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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