Case series

Five Case Reports of Surgical Lung Cancer Patients Who Presented Molecular Progression before Imagiological Diagnosis

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Introduction

With the dynamic development of molecular medicine, we can aspire to personalize medicine. Our aim is not only survival and response rates, but also best quality of life, lowest toxicity and more cost-effective choices for each individual patient. The use of liquid biopsies, processed by next generation sequencing (NGS), has clinical utility and validity in the metastatic scenario. Evaluating its importance in early stage lung cancer is a challenging issue. We report five different cases submitted to lobectomy and lymph node dissection, where circulating free tumor DNA (cfDNA) plays an important role in the diagnosis of residual disease, progression or relapse. Blood samples analyzed were obtained before surgery (Pre-Operatory), from pulmonary vein (Intra-Operatory), before discharge (Post-Operatory) and during follow-up (F-UP) as can be observed in the workflow plan (Figure 1).
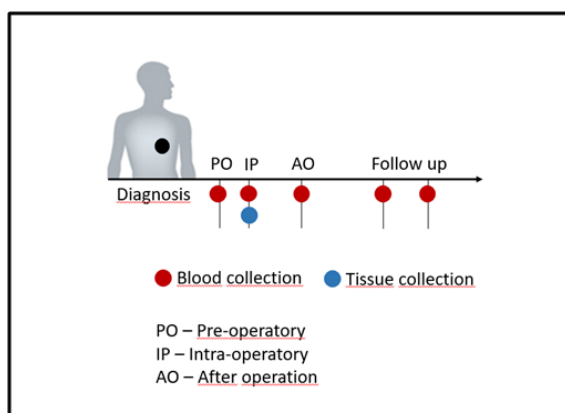

Figure 1. Workflow plan

First Case Report

A 69-year-old women, non-smoker, with history of pulmonary tuberculosis was referenced with a thoracic computerized tomography (CT) scan with an opacity on the medium lobo. A bronchoscopy revealed an adenocarcinoma. A Positron Emission Tomography-CT (PET-CT) scan was negative. She was staged as cT1aN0M0. She was submitted to surgery and was staged as pT1aN0V0L0PN0PV0M0R0–IA1. The patient stayed in surveillance.

Tissue tumor DNA (tDNA) revealed an EGFR mutation with an allele frequency (AF) of 27% and cfDNA concentration was 9.8ng/ml before surgery. cfDNA from the pulmonary vein was 44.0ng/ml. On the fourth day post-surgery, plasma cfDNA concentration was 48.9ng/ml. Twenty-three days later, at her first appointment, plasma cfDNA had decreased to 14.9ng/ml. Twelve months after discharge plasma cfDNA had decreased to 10.3ng/ml.

Sixteen months after the first follow-up appointment, the value of cfDNA increased to 57.1ng/ml. CT scan performed four months later showed no progression. Only the CT scan 11 months after cfDNA increase or 27 months after the first follow-up appointment showed progression of the disease as can be observed on the Figure 2 (Table 1).
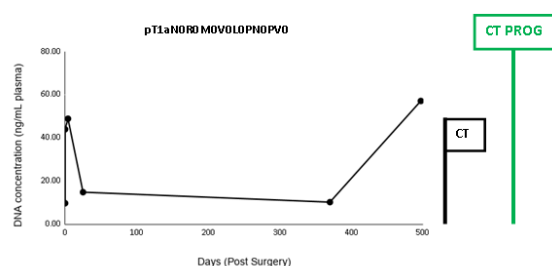

Figure 2. First case report Progression of the disease

Second Case Report

A 51-year-old man, smoker (30UPY), was referenced with a thoracic CT scan showing a nodule in the inferior right lobe with 41x34mm. Bronchoalveolar washing showed a squamous lung carcinoma. A PET-CT showed the same lesion with a SUVmax of 4.6. He was staged as cT2bN0M0. He was submitted to surgery and was staged as pT2aN0V0L0P-N0PV0M0R0 – IB. The patient stayed in surveillance.

tDNA revealed an TP53 mutation with an AF of 61.5% and plasma cfDNA concentration was 11.8ng/ml before surgery. cfDNA from the pulmonary vein was 15.2ng/ml. On the fourth day post-surgery, plasma cfDNA concentration was 40.4ng/ml. Twenty-four days later, plasma cfDNA had decreased to 6.4ng/ml. One year after discharge plasma cfDNA had 9.0ng/ml. The CT scan at that time was negative for disease progression. Seventeen months after the first follow-up appointment cfDNA increased to 296ng/ml. The patient died five months after the last plasma withdrawal from disease progression (Figure 3, Table 1).
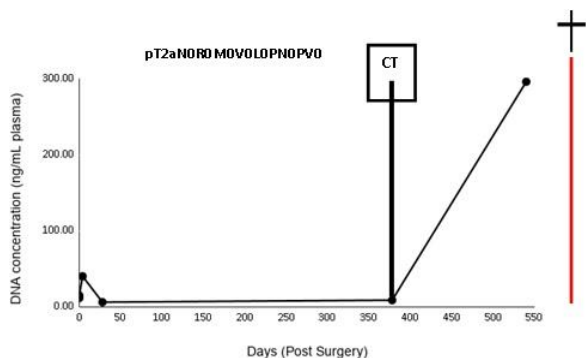

Figure 3. Second case report disease progression

Third Case Report

A 52-year-old man, smoker (36UPY), was referenced with a thoracic CT scan which showed a nodule in the medium lobe with 30x22mm. A lung biopsy revealed a squamous lung carcinoma. A PET-CT showed the same lesion with a SUVmax of 5.7. He was staged as a cT1cN0MO.

tDNA revealed a TP53 mutation with an AF of 52% and plasma cfDNA concentration was 3.09ng/ml before surgery. He was submitted to surgery and cfDNA concentration from the pulmonary vein was 13.08 ng/ml. On the fifth day post-surgery cfDNA concentration was 21.84ng/ml. Histology revealed a squamous carcinoma with invasion of the visceral pleura, pT2aN1M0V0L0PN0PV1R0, stage IIIB. One month after surgery, plasma cfDNA concentration was 2.95ng/ml. Adjuvant chemotherapy was performed. Six months after first follow-up appointment CT scan

showed progression and 11 months after, plasma cfDNA concentration showed an increase of 45.02ng/ml, as well as a gradual increase during chemotherapy (Figure 4, Table 1). The patient died one month later.
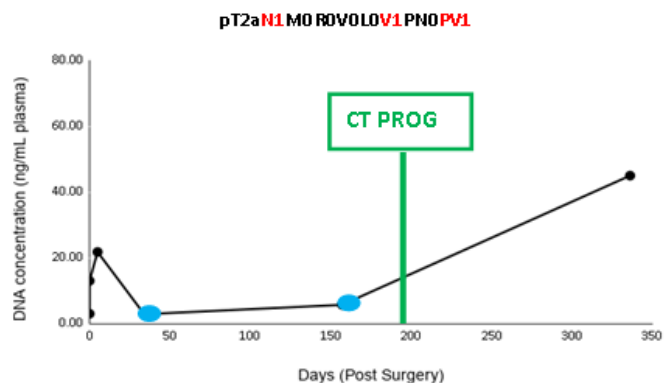

Figure 4. Third case report progression of the disease

Fourth Case Report

A 68-year-old man, smoker (100UPY), was referenced with a thoracic X-Ray with a suspicious image on the medium lobe. A CT scan showed a 16mm nodule on the medium lobe. The PET-CT showed the same lesion. A lung biopsy revealed an adenocarcinoma. He was staged as cT1bN0M0. He was submitted to surgery and was staged as pT1cN0M0R0V0L1P N0PV0 – IA. The patient performed adjuvant chemotherapy.

tDNA revealed an KRAS mutation with an AF of 34.0% and plasma cfDNA concentration was 1.66ng/ml before surgery. cfDNA from the pulmonary vein was 7.26ng/ml. On the fourth day post-surgery, plasma cfDNA concentration was 17.4ng/ml. Twenty-four days later, plasma cfDNA had decreased to 2.09ng/ml. One month after first follow-up appointment plasma cfDNA had 3.55ng/ml.

Ten months after first follow-up appointment the value of cfDNA increase to 44.9ng/ml. The CT scan performed twenty-three months after the first follow-up appointment showed progression of the disease as can be observed in the figure 5 (Table 1).
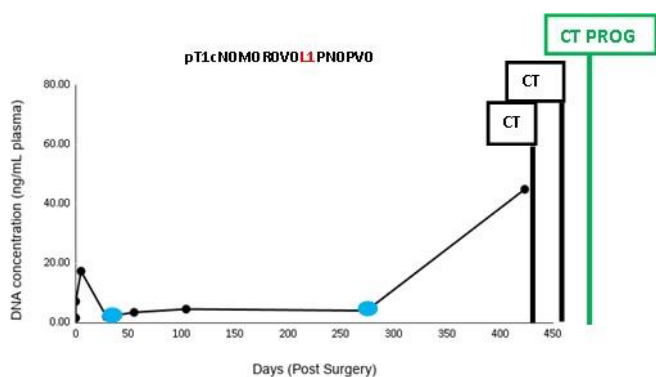

Figure 5. Fourth case report progression of the disease

Table 1. Timeline of cfDNAs and CT scans of Five Case Reports.

	H	pTNM	Pre-Op	Intra-Op	Post-Op	F-UP	F-UP	F-UP	F-UP	F-UP	CT scanProg.
1	A	pT1aN0M0R0 V0L0PN0PV0	13.07.17 9.8	13.07.17 44.0	17.07.17 48.9	09.08.17 14.9	19.07.18 10.3	23.11.18 57.1			18.10.19
2	S	pT2aN0M0R0 V0L0PN0PV0	12.10.17 11.8	12.10.17 15.2	16.10.17 40,4	9.11.17 6.4	25.10.18 9.0	04.04.19 296.0			October 2018 - normal
3	S	pT2N1M0R0 V1L0PN0PV1	06.07.17 3.09	06.07.17 13.08	11.07.17 21.84	9.8.17 2.95	12.12.17 5.79	08.06.18 45.02			16.01.18
4	A	pT1cN0M0R0 V0L1PN0PV0	28.09.17 1.66	28.09.17 7.26	02.10.17 17.40	26.10.17 2.09	22.11.17 3.55	10.01.18 4.65	04.04.18 4.12	31.08.18 44.9	09.09.19
5	A	pT2aN1M0R1 V1L1PN0PV0	05.03.18 0.00	05.03.18 6.9	08.03.18 29.9	28.03.18 8.3	06.06.18 41.10	04.09.18 68.80	25.01.19 58.29		31.08.19

H: Histology; A: Adenocarcinoma; S: Squamous carcinoma; Pre-Op: Pre-Operation; Intra-Op: Intra-Operatory (pulmonary vein); Post-Op: Post-Operative; F-UP: follow-up; BLUE: chemotherapy; Green: cfDNA increase; Prog: Progression

**Table 2.** Timeline to Molecular and Imagiological Progression after first follow-up appointment.

	Histology	cTNM	PET-CT (Suv-max)	pTNM	MDT	Time to MP (months)	Time to IP (months)
1	Adenocarcinoma	cT1aN0M0	-	pT1aN0M0R0V0L0PN0PV0	S	16	27
2	Squamous Carcinoma	cT2bN0M0	4.6	pT2aN0M0R0V0L0PN0PV0	S	17	-
3	Squamous Carcinoma	cT1cN0M0	5.7	pT2N1M0R0V1L0PN0PV1	C	11	6
4	Adenocarcinoma	cT1bN0M0	+	pT1cN0M0R0V0L1PN0PV0	C	10	23
5	Adenocarcinoma	cT2N1M0	5.4	pT2aN1M0R1V1L1PN0PV0	C	2	5

MDT: Multi-Disciplinary Team; Time to MP (months): Time to Molecular Progression; Time to IP (months): Time to Imagiological Progression; S: surveillance; C: chemotherapy.

She was staged as cT2N1M0. She was surgery and the disease was staged as pT2aN1V1L1PN0PV0M0R1, with surgical intersection of an adenopathy.

tDNA revealed no mutation due to bad quality. cfDNA concentration was 0.0ng/ml before surgery. cfDNA from the pulmonary vein was 6.9ng/ml. She was discharged on the third day post-surgery, with a plasma cfDNA concentration of 29.9ng/ml. Twenty days after surgery, plasma cfDNA concentration a decreased (8.3ng/ml) (Figure 5). Adjuvant chemotherapy was performed.

Two months after first follow-up appointment and during chemotherapy cfDNA concentration was 41.10ng/ml and increased progressively during the next six months. CT scan showed progression at this time and tissue biopsy revealed an adenocarcinoma, TTF1+; tissue NGS revealed an epidermal growth factor receptor (EGFR) mutation on exon 19, p(Glu746_Ala750del) (AF39%) (Table 1). cfDNA before therapy was 68.80ng/ml. The patient started EGFR tyrosine kinase inhibitor (green dot in Figure 6). The patient maintains therapy with a clinical, imagiological stable disease and a gradual decrease cfDNA concentration.
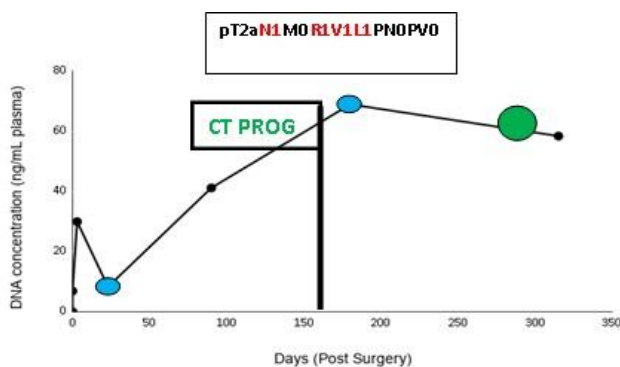
Figure 6. Fifth case report progression of the disease

Discussion

We report two cases who stayed in surveillance according to pathological staging (first and second case reports). Liquid biopsies showed to be crucial, to evaluate residual disease, stratify risk of relapse and identify the patients that showed molecular progression (increase in cfDNA) prior to imagiological diagnosis (Table 1 and 2). In the second case the patient unfortunately died before imagiological confirmation of disease progression. In both cases, once cfDNA increase was detected, 16 and 17 months after first follow-up appointment respectively, imagiological imaging should have been performed as soon as possible, in order to initiate appropriate treatment. In the last three cases adjuvant chemotherapy was performed. In the third and fourth case reports, the time from first follow-

up appointment until cfDNA progression was ten months. Being the fifth case an R1 resection, two months was the time to identify molecular progression in the same timing.

In all cases the difference of time to progression considering molecular and imagiological progression, observed in Table 1 and 2, show significant disparities in time (months). This difference is greatest in the patients who stayed in surveillance due to a less aggressive disease. While in the third and fourth cases the difference in time was smaller and in the fifth case even more (Table 1).

Conclusions

Lung Cancer is one leading cause of death worldwide, and patients would greatly benefit from an early diagnosis. Tissue biopsy is still considered the gold standard for diagnosis and the is a main potential to identify biomarkers. However, it is still an invasive and inadequate technique to diagnose tumor heterogeneity [1]. Through liquid biopsies real-time information on the molecular profile of the tumor can be obtained. cfDNA is the only procedure which identifies with high precision tumor biomarkers in order to target the disease with high precision.

A few active studies are ongoing with the aim of exploring the feasibility and utility of liquid biopsy in early stage NSCLC [2]. Results are yet to be awaited. Recent papers are favoring a molecular profile besides the classical TNM staging system may be used to better classify early lung cancer patients submitted to surgery. TNMB classification might be nearer than expected and with clinical implications soon [3,4]. In other words, liquid biopsies taken after surgery have shown to be a strong promising tool, biomarker, to identify the patients who will most benefit from adjuvant treatment or who are most likely to relapse when cfDNA is positive after surgery in a phenotypical negative local disease [5-7].

It seems that molecular profiling by using liquid biopsies may help us understand the aggressiveness of each tumor at a particular moment in time, besides the classical T (tumor size) and N (lymph nodal involvement) and the presence of other negative prognostic factors such as a positive involvement of lymphovascular, perineural, visceral pleura and margins resection [8]. An up or downstage of these character-istics can be evaluated by incorporating high or low molecular risk factors or genomic features such an increase of cfDNA [9]. In other words, cfDNA could supplement the re-staging approaches after a curative surgery in lung cancer [10]. This is the main observation that can be withdrawn from these five case reports. Further trials need to be performed to change standard

of care of surveillance and diagnosis in lung cancer patients submitted to a curative surgery, with higher precision and efficiency.

References

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