Liquid biopsy in lung cancer: tertiary prevention potential

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May 5, 2020

Abstract

Abstract Aims In the era of personalized therapy liquid biopsy is considered an important diagnostic tool in the clinical management of cancer patients. Tissue specimen represents "gold standard" for molecular evaluation of specific gene targets alterations that lead cancer patients to benefit of a "tailed therapy" based on molecular features of the tumor. This innovative source of nucleic acids was introduced in clinical setting only for NSCLC patients to test Epidermal Grow Factor Receptor (EGFR) mutations when tissue is not available or to monitor acquired resistance mutation after a first line of treatment. The study aimed at assessing the diagnostic potential of liquid biopsy in balanced tertiary screening modeling. Methods From 2014 to 2019 molecular diagnostics activity performed on liquid biopsy specimens in the Predictive Diagnostic laboratory of AOU Federico II were reviewed. Laboratory data were collected in SPSS. Non parametric analysis were performed in order to test the differences between patients WILD TYPE or not. A multivariate logistic model was performed in order to assess the effect of mutation, age and sex, on the tumor progression. The results of the revision concern 515 total cases (almost of all plasma or peripheral blood) allowed to evaluate the liquid biopsies for women and men. The average age of the Patients is 66.3 years, and the 25 percentile is 59 years. Results The cases are 221 basal and 294 by progression after first line TKIs treatment. The cases with mutation, as expected, have an OR 4,15 compared to the basal to have a tumor progression (95% IC: 2,7 - 6,3) regardless of sex and age. The mutations detected were 131 from different types of lung carcinomas.Conclusions Working on case data, specifying the characteristics of the Patients with mutations will drive a further estimate in tertiary prevention screening designs

1. Introduction

Generally, the term "biomarker" refers to any biological indicator whose variations are related to the definition of the pathological condition, to the definition of prognosis, to the follow-up or to the definition of the results of the therapeutic response. The recent discovery of genetic alterations that cause, in a not neglected part of tumor patients, neoplastic disease encouraged the formulation of molecular target therapies that arise from the evaluation of precise molecular targets. In the context of predictive molecular pathology, liquid biopsy is a venous blood sampling on which molecular analyzes can be performed to search for clinically relevant mutations in the *EGFR* gene in patients with non-small cell lung cancer (NSCLC). This is usually performed when it is not possible to have a tissue sample representative of the neoplastic lesion at baseline or to monitor any resistance indicator or mutations acquired after a first line of treatment with tyrosine kinase inhibitors (TKI) .[1] In particular, when it is not possible to perform the analysis molecular on tissue sample (difficult access to the lesion, comorbidity , especially in elderly patients, refusal by the patient, etc.) liquid biopsy can be a valid diagnostic solution. The liquid biopsy cannot replace the tissue biopsy because,

to our knowledge, finding a certain mutation in the blood does not necessarily imply that the mutation is expressed at a structured clinically relevant frequency within the selected tumor (passenger mutation). Even the limited amount of circulating tumor DNA can be a boundary that can lead to false negative results.[2]

In the last five years, the liquid biopsy has been used for different purposes in oncology. Today the new challenge in lung cancer is represented by the implementation of some screening tests o, although this requires a phase of test harmonization and sensitivity assessment.[3,4]

The molecules that can be tested in a blood sample in the context of predictive molecular pathology are different: circulating tumor DNA (ctDNA) which represents a fraction of the total free DNA (cfDNA), cells released into circulation by the tumor through active mechanisms and passive and exosomes which also represent a precious source of nucleic acids of tumor origin.[5] The cfDNA represents, to date, the specimen of greatest interest because of its immediate availability with respect to tumor DNA extracted from tissue samples; however due to the low concentration and the scarce bioavailability in blood torrent, new approaches are being optimized in order to recover tumor nucleic acids starting from the lysosomal cargo.[2,6] Clinical response data have shown that in addition to the sensitivity mutations, there is a 'wide range of different mutations that fall within exon 18, 19, 20 and 21 leading the patient to benefit from the target therapy.[8]

Thus they have a predictive potential and for this reason, new-generation gene sequencing (NGS) represents a valid analytical platform for the analysis of the mutations present in the cfDNA.[9] However, the analysis spectrum for NSCLC patients does not only exhaust the EGFR gene; the search for oncogenic activating genomic translocations such as ALK, ROS, RET has become clinical practice, a routine that makes the patient eligible for treatment with TKIs starting from tissue samples.[10]This possibility represents the direction towards which scientific research is moving to optimize patient clinical management.[11]

Tumors never reach complete molecular stability and this issue means that, over time, they can develop resistance to target therapies.[12], so it is a crucial decision-making step to identify what it is happening in the tumor. The presented study is aimed at assessing the diagnostic and predictive potential of liquid biopsy into management of lung cancer path of care.

2. Methods

From 2014-2019 liquid biopsy specimens were analyzed for EGFR sensitive and resistance mutations in Predictive Molecular Pathology (Department of public Health, University of Naples Federico II). For each patient 5 ml of plasma and serum sample were withdrawn and collected in Vacutainer tubes (BD, Plymouth, UK) by a dedicated nurse. Plasma and serum were immediately separated by two centrifugation steps at 2300 r.p.m. for 10 min, then supernatant (where nucleic acids were carried out) was stored at -20C® . Cell-free DNA (cfDNA) was purified by using the QIAsymphony robot (Qiagen, *Hilden, Germany*) and the QIAsymphony DSPVirus/ Pathogen Midi Kit, according to the manufacturer's instructions, finally cfDNA was eluted in a final volume of 35 µl of*not DEPC* water *(Ambion).* Libraries were produced on the Ion Chef (Thermofisher, *Waltham, Massachusetts*) by using DL-8 kit and serum and plasma of 4 patients were processed for a total of eight specimens for run. After library amplification step performed by 22 cycles for cfDNA amplification and 6 cycles for library reamplification after barcoding, purified libraries derived from eight cfDNA samples were diluted to 60 pM and combined with eight additional cfDNA-derived libraries to obtain a 16 Ion Code pooled library. The two-pooled libraries were re-loaded into the Ion Chef instrument, and templates were prepared using the Ion S5 520-530 chef kit (Thermofisher, *Waltham, Massachusetts*). Finally, templates were loaded into the 520 chip and sequenced on S5 platform. Signal processing and base calling were evaluated by adopting the default base-caller parameters on Torrent Suite [v.5.0.2]. Results were visually inspected with the Golden Helix Genome Browser v.3.0. (Bozeman,MT, USA). Statistical analysis were carried out by adopting a binary logistic regression model on SPSS and IBM software system. Age and sex parameters were added as covariates.

Sample related to 5 years of activity regarding the molecular diagnostics performed with liquid biopsy in the predictive diagnostic laboratory of the AOU Federico II were analyzed. These patients came from the oncological network pathways related to lung cancer and refer to the HUB center of the AOU Federico II.

The samples of 900 patients enrolled in the pathway were collected. The availability and epidemiological interest was verified.

After database review, 515 plasma or blood samples were included in the analysis (Fig 1). Descriptive analyzes were performed (Tab1). To verify the differences with respect to the report of liquid biopsy between patients with basal or progressing tumors, non-parametric analyzes were performed for 2 independent samples. Furthermore, the effect of the presence of mutations on the probability of tumor progression, taking into account age and sex, was evaluated through a multivariate logistic regression model (Tab2)

3. Results

Both tissue samples and liquid biopsies can provide complementary information about the risk of disease progression.[1]The results collected concern 515 total cases 241 women and 274 men. The average age of the patients is 66.3 years, while the 25th percentile is 59 years. Non-parametric tests (ANOVA and Kruskall Wallis Test), showed a significant difference between the basal group and the one with mutations. Cases with mutations as expected have an OR of 4.15, compared to wild types, of progression profile (95% CI: 2.7 - 6.3) regardless of age and gender. (Tab 2).

4. Discussion

In the future, liquid biopsy will be used either as a surrogate or alternative to tissue biopsy or as valid tool to decide among therapeutic issues.

Observational data suggest that liquid biopsy is able to highlight predictive progression signals. This potential may be developed on a technical level in facilities characterized by strong methodological experience and a high availability of technologies; these centers are often configured as HUBs. In oncological networks, having a predictive diagnostic HUB means to have the capability of strengthening the appropriateness of the diagnostic and therapeutic path. Figure 2 shows the resolution capability of the methodology used in the study; that could integrate the overall risk assessment. The research is also focusing on other research protocols for innovative molecular tests.9,13 When, in fact, patients respond to treatment with EGFR inhibitor drugs a reduction in blood mutation levels is observed and the liquid biopsy could confirm this process quickly and effectively.[7]integrating prognosis information. In fact, where there is a residual disease liquid biopsy may establish the risk of recurrence.[14.]

To obtain a greater prognostic accuracy, especially in the minimal residual disease evaluation, it may be appropriate to proceed to an analysis of the prognostic and predictive weight for both either the single mutations ,or their modular combinations.

A significant limit, to reach this goal, is the lack of complete digital health file where all the information related to the treatment path may be connected. Nevertheless, the data presented evidenced the potential of liquid biopsy, as a predictive measure of tissue progression and therefore of tertiary prevention. The value of targeted screening is crucial for identifying decision steps and directing the treatment pathway (Fig 1). In the context of lung cancer, but also in other similar pathologies the definition of screening markers can foresee the following steps of care.

Conclusions

The analysis of data presented, even within the boundaries of observational finding, provides a trend indication directly from the operational context. As also current evidence, suggest, the data presented drive investment of expertise and analysis toward liquid biopsy testing in the context of tertiary prevention screening designs

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