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Circulating Tumor Cells and cfDNA: Key Predictive Biomarkers in Non - Small Cell Lung Cancer Progression and Treatment

Alexandre Tavartkiladze[1, 2], Revaz Turmanidze[3], Gaiane Simonia[1, 2], Margalita Gogoladze[3], Pati Revazishvili[2], George Dundua[2], Irine Andronikashvili[1, 2]

[1]Tbilisi State Medical University

[2]Institute for Personalized Medicine

[3]New Vision University

Abstract: ***Background/ Introduction: With the evolution of translational research in the realm of liquid biopsy, the relevance of circulating tumor cells (CTCs) and cfDNA or ctDNA is emerging. cfDNA is a fragment of DNA released into the plasma after cell apoptosis by lysis, carrying genome - wide DNA information. In contrast, ctDNA, a part of cfDNA, can be derived from primary tumors, metastases, and even CTCs. This novel approach has the potential for earlier lung cancer detection and monitoring of minimal residual disease (MRD) and resistance development, consequently paving the way for effective therapeutic decisions aimed at curing lung cancer. Materials and Methods: This article lays a comprehensive focus on CTCs as an advanced and validated marker in liquid biopsy. The ongoing research and discussions are targeted at attaining the new milestone of personalized (precision) medicine as a common standard of care for lung cancer. Results: The research findings indicate that CTCs, with their potential to serve as an effective biomarker in cancer, hold promise in recent advancements of cancer diagnosis and treatment. These cells could revolutionize the early detection and close monitoring of lung cancer, further informing appropriate therapeutic choices. Conclusion: The emerging utility of liquid biopsy, particularly CTCs and ctDNA, introduces a new dimension to personalized medicine in lung cancer treatment. The advancements summarized in this article highlight the promising prospects of this novel approach in achieving a standard of care in lung cancer, tailored to each patient's unique genetic profile.***

Keywords: Non - Small Cell Lung Cancer, Circulating Tumor Cells, Cell - Free DNA, Early Diagnostic of Cancer, Personalized Treatment of Cancer

1. Background/ Introduction

The global statistical analysis of malignant tumors today suggests that there are about 35 million registered patients at different stages of the disease. Each year, 11 million new cases of this pathology are revealed, which translates to about 27, 000 new patients diagnosed with malignant tumors per day. Treatment is provided to 14 million patients diagnosed with malignant tumors, of whom sadly, 8.2 million are considered incurable.

The World Health Organization reports 11 million new cases of malignant tumors every year. Lung cancer makes up 12.8% of these detected pathologies. In developed countries, lung cancer is found in 58% of patients with malignant tumors, leading to 17.8% of deaths caused by this pathology.

Despite the general ratio of pathologies and death rate incidents increasing, there is some positivity for specific forms of the disease. This can be attributed to improvements in medical diagnosis and treatment methods.

When considering the distribution of malignant diseases by countries: in the United States, among male patients, lung cancer is most prevalent, followed by stomach and prostate cancer. Among women, the leading types are breast, colon, and cervical cancers. The mortality rate varies from 77–217 men and 100–140 women per 100, 000 people. The situation is similar in Europe, with lung, colon, and stomach cancers leading among men and breast, colon, and lung cancers among women. Notably, lung cancer claims the lives of three times more men than prostate cancer and three times more women than breast cancer.

In terms of lung cancer incidence, the United States tops the list among developed countries, followed by the United Kingdom. Patients aged 70 are 7–10 times more likely to be affected by it than 50 - year - olds, and 50 - year - old patients are 20 times more likely to be affected than 40 - year - old patients.

Evidently, the prevalence of malignant tumors, particularly lung cancer, is a pressing global issue in the 21st century. As for the methods of its early detection and diagnosis, they still rely heavily on active participation in screening. An important biomarker is the cell research method of CTC - related cfDNA (4), which involves the detection and analysis of tumor cells (CTC, cfDNA) circulating in the blood and bone marrow.

Circulating tumor cells (CTCs) are a principal cause of the development of malignant tumors and metastases within the body. The aforementioned test is applicable to the detection of both early and advanced stages of cancer. It also aids in treatment planning and follow - up, representing an important cellular research technology and a crucial tool for treatment management (7).

CTCs, first discovered by Ashworth 150 years ago, are tumor cells identical to those in metastatic lesions. Stephen Paget's "seed and soil" theory (1889) asserts these cells can spread via the bloodstream, growing secondary tumors. Today, CTC presence signifies tumor progression and metastasis risk. Detection methods have vastly improved over the last two decades (5).

In 2004, Allard's team successfully detected CTCs in various cancer patients. That year, Cristofanilli's team also found that higher pre - treatment CTC count in metastatic breast cancer patients predicted poorer prognosis, impacting both progression - free and overall survival. Furthermore, molecular profiling of CTC DNA in non - small cell lung cancer patients enabled monitoring of tumor evolution, including therapy - related and unrelated mutations in the EGFR gene (5, 8.10).

The method of researching circulating tumor cells (CTCs) and cell - free DNA (cfDNA) in non - small cell lung cancer permits us to determine the cellular histocytogenesis of the cancer. Following this, personalised or precision polychemotherapy, targeted, or immunotherapeutic treatment regimens are selected. This is based on the immunocytochemical and immunogenetic studies of the CTCs, facilitating dynamic observation of cancerous processes within the body. Additionally, this allows for some degree of prediction of the anticipated mutations and development of resistance, as well as an evaluation of treatment effectiveness and the prediction of expected outcomes for the related disease (5, 7).

The method of studying CTCs and cfDNA has become a fundamental principle in oncology treatment, aiding in the early detection of minimal residual disease and personalised medicine.

In the specified study, the CellSearch CTC test method utilises a blood sample to detect free circulating non - cellular DNA, allowing for an accurate assessment and determination of the clinical status of cancer patients.

The CellSearch system is a unique technology that captures CTCs in the blood, separates them from other cells and counts them. The process is as follows: A 7.5 mL blood sample is placed in a special test tube. The blood cells are separated from plasma through centrifugation and then placed in the CELLTRACKS® AUTOPREP® system. Ferrofluid nanoparticles, which bind to epithelial cells, are used. The CTC cells are then magnetically separated.

The CTC cells are stained with monoclonal antibodies specific to cytokeratin, an epithelial cell marker. To identify leukocytes in a blood sample, monoclonal antibodies are used as a marker for CD45, which may contaminate the sample. The nuclei of the CTCs and leukocytes are then highlighted using DAPI staining solution.

These cells are placed in a cartridge subjected to a magnetic force. The CELLTRACKS ANALYZER II® system scans the stained CTC cells, and after scanning, displays the expected tumor cells that are positive for cytokeratin and DAPI. Finally, the selected tumor cells are re - examined using fluorescence microscopy.

The advantages of the CellSearch CTC test are that it allows for early assessment of a patient's condition and facilitates regular monitoring of changes. In the case of solid tumor cancer, there should ideally be five circulating immune cells per 7.5 ml of blood.

2. Materials and Methods

Patients were successfully diagnosed at the Institute for Personalised Medicine in Georgia using the CellSearch system and fluorescence microscopy control through the CTC testing method. The study comprised 18 patients with stage II - III non - small cell lung cancer, including 13 men and 5 women. The stages were distributed as follows: stage II included 4 patients (2 women and 2 men), and stage III included 14 patients (3 women and 11 men).

The types of surgical treatment were distributed as follows: 8 patients (6 men and 2 women) underwent lobectomy; 4 men underwent right - sided pneumonectomy; and 6 patients (3 women and 3 men) underwent tumourectomy. The morphological verifications were as follows: 12 patients (4 women and 8 men) were diagnosed with adenocarcinoma, while 6 patients (1 woman and 5 men) had squamous cell carcinoma.

All patients underwent radiation therapy with a dose range of 54–62 Gy. All eighteen patients were in remission for 5 years, after which they all underwent blood tests for CTCs and cfDNA.

The outlined observation of patients (standard therapy plus blood analysis study for CTCs) was reviewed and approved by the Local Ethical Committee (LEC) of the Institute of Personalised Medicine (Georgia, Tbilisi), LEC document Ref. Number 29/2019 - 17b. Furthermore, informed consent for additional blood analysis studies was obtained from all patients, verified by their signatures.

3. Results

The 18 patients were categorised into two groups, labelled as A and B. Group A consisted of four patients with stage II cancer (3 women and one man), and Group B comprised fourteen patients with stage III cancer (3 women and 11 men). Upon collating the findings of the CTC and cfDNA study, it was observed that two patients, one each from groups A and B, had a surplus of CTC tumour cells, with 37 (Group A) and 56 (Group B) CTCs in 7.5 mL of blood. All 18 participants in the study underwent whole - body magnetic resonance imaging using a contrast agent in 3 Tesla mode.

4. Discussion and Conclusions

A patient in Group A, with 37 circulating tumour cells, was diagnosed with a metastatic breast lesion. Meanwhile, a patient in Group B, with 56 circulating tumour cells, was diagnosed with a metastatic lesion in the 9th and 11th

thoracic vertebrae. No radiological damage was observed in the other patients. Both patients with metastatic spread received appropriate treatment, including radiation and systemic drug therapy.

The patient in the first group, diagnosed with stage II, underwent a course of radiation therapy on the brisket with a single dose of 4 Gy in 5 fractions, totalling 20 Gy. The second patient, diagnosed with stage III, received radiation therapy to the 9th and 11th thoracic vertebrae with a single dose of 3 Gy in 5 fractions each, totalling 30 Gray. Both patients underwent chemotherapy, with the regimen comprising vinorelbine 25 mg/m2 (on days 1 and 8) and gemcitabine 1000 mg/m2 (on days 1 and 8), across 6 courses in total.

After 3 courses of drug therapy, a control analysis of the cfDNA of CTC was conducted. In the first patient, who initially had 37 circulating tumour cells, only 11 circulating tumour cells were detected, while the second patient had just 4 circulating tumour cells (down from 56). Following six courses of chemotherapy, neither of the patients had any circulating tumour cells.

This study and its results highlighted the predictive significance of circulating tumour cells in non - small cell lung cancer. Research into CTCs and cfDNA allowed for a comprehensive investigation into metastasis processes and related changes, monitoring the effectiveness of non - small cell lung cancer treatment and determining long - term results. The ultimate goals of these are to halt the tumour process and its spread, and achieve timely and effective treatment of any existing pathology.

CTCs and cfDNA cells were confirmed as the fastest and most prognostically valuable carriers, serving as a true biomarker for assessing the progression and emergence of metastases in non - small cell lung cancer. This study, conducted in the Louis Pasteur laboratory of the Institute for Personalised Medicine, solidifies the method of studying CTC and cfDNA as an innovative approach to lung cancer diagnosis and treatment. Its active application enables us to undertake the following in relation to non - small cell lung cancer, taking into account its genetic characteristics:

- Early (preclinical) diagnosis
- Monitoring the course of the disease
- Evaluating the effectiveness of the initiated treatment
- Timely adjustment of treatment regimens
- Monitoring the process of metastasis and disease complications

Dependent on the type of tumour and treatment options, circulating tumour DNA (ctDNA) has been demonstrated to be a valid and independent biomarker for confirming and regularly monitoring cancer. With its high specificity, ctDNA can be utilised to predict the early recurrence of a wide variety of cancers following initial therapy. This enables the guidance towards earlier and more effective treatment options, including those with curative intent, while also protecting patients from unnecessary treatments when there's no indication for minimal residual disease (MRD) or early detection of resistance development. Numerous larger and ongoing clinical studies with ctDNA as a marker for monitoring MRD or treatment response will enhance our understanding of how best to apply these strategies (1, 2, 3, 5, 9).

This study further corroborates that routine determination of circulating tumour cells (CTCs) (via CellSearch with controlled fluorescent microscopy) in patients with non - small cell lung cancer in remission has predictive value for early detection and prevention of disease recurrence and progression (6, 10).

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Data Availability: All relevant data are within the paper and its Supporting Information files.

Author Contributions:
Alexandre Tavartkiladze[2] (AT) conceived and designed the experiments.
Alexandre Tavartkiladze[2] (AT) and **Revaz Turmanidze**[1] (RT) performed the experiments, analyzed the data, and wrote the manuscript.
Alexandre Tavartkiladze[2] (AT) and **George Dundua**[2] (GD) contributed to data collection and manuscript revision.
Alexandre Tavartkiladze[2] (AT) and **Margalita Gogoladze**[3] (MG) provided technical support and assisted in the design of the experiments.

All authors contributed to manuscript revision, read, and approved the submitted version.

Conflict of Interest: The authors declare that they have no conflict of interest.
https: //orcid. org/0000 - 0002 - 9589 - 7417

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Author Profile

Tavartkiladze is corresponding author: e - mail: alexandre. tavartkiladze[at]gmail.com
or a. tavartkiladze[at]tsmu. edu
mobile/portable: +995577090977 or +995595150215