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Cell-free tumour DNA as a diagnostic and prognostic biomarker in non-small cell lung carcinoma

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Abstract

More than 1 million people dies worldwide due to lung cancer which is the first most incident cancer in males and the third in females. Only early diagnosis makes possible to achieve long-lasting remission or even cure the disease. Unfortunately, no tumour marker to achieve this goal has been identified, yet. One of putative lung cancer markers is free circulating tumor DNA. Its concentration seems to be related to cancer burden. Moreover, it can be subjected to mutational status analysis allowing for introduction of targeted treatment. This led to the idea of liquid biopsy which can substitute for a standard biopsy not feasible in certain clinical circumstances. Assessment of cell free tumour DNA can also inform about progression/recurrence of cancer and may have a prognostic value. Therefore, the aim of this article is to review on free circulating DNA as a potential marker in lung cancer.

Key words: free circulating DNA, liquid biopsy, lung cancer, biomarker

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Biomarkers of malignancy

Incidence of malignant tumours worldwide is growing, making them a serious epidemiological hazard. The data obtained from the National Cancer Registry over the last three decades confirm that the incidence of malignant tumours in Poland has increased twice (from 64 700 in 1980 to above 140 500 cases in 2010); in 2013, this number reached almost 156 500 cases. Lung cancer occupies the first place in males (18.7%) and the third place in females (8.8%) as the most frequently diagnosed malignancy [1]. Early detection allows to implement an optimal treatment on time, and consequently to obtain a long overall or progression-free survival. To this end, screening diagnostic tests have been pursued, and recently a low-dose CT scan has been proposed as a screening tool for lung cancer in a population at risk, i.e. heavy smokers [2, 3].

Apart from imaging studies, a different approach to screening have been based on biomarkers of cancer, which ideally, should be a sensitive (but also relatively specific) substance present in body fluids, e.g. blood, urine or sputum, definitely easier to collect than cancer tissue [4]. Moreover, despite diagnostic potential, biomarkers can also exhibit predictive (of a response to treatment) or prognostic (overall survival or progression-free survival) attributes [5].

From the historic perspective, the first cancer biomarker was an immunoglobulin present in the urine of patients with multiple myeloma, which was identified as early as in 1848 [6]. The twentieth century witnessed discoveries of numerous hormones, proteins and enzymes, marking patients with a particular malignancy. In the 1960s, an alpha-fetoprotein and CEA (carcinoembryonic antigen) were identified in blood. Subsequently,

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other proteins, such as CA 15.2 (cancer antigen), CA 19.9, CA 125 and PSA (prostate specific antigen) were discovered and validated. All these substances have been widely used in monitoring therapy, evaluation of radical surgical treatment and in some cases, allowed for early detection of cancer. However, their major drawbacks, limiting clinical merit, have been insufficient sensitivity or specificity, and consequently not sufficiently high predictive values. Moreover, as a potential tool to monitor cancer progression, their concentration in body fluids weakly correlated with a clinical stage of the tumour [5]. Technological progress which took place at the end of the twentieth century contributed to the development of molecular diagnostics. In effect, circulating cell-free tumour DNA (ct-DNA) was identified in blood and proposed as a plausible biomarker of malignancy [7, 8].

Cell-free circulating DNA

Circulating blood contains natural cell-free DNA (cf-DNA) that comes mostly from apoptotic cells. Rapid turnover of tumour cells during growth, metastatic disease or cancer therapy, results in a release of a number of molecular biomarkers into the peripheral blood. One of these is ct-DNA that differs from its physiological counterpart by more variable helix length and by the presence of specific mutations. Apoptotic cf-DNA length is a multiple of 180–200 base pairs from nucleosome derived fragments while ct-DNA reveals variable and usually shorter or longer strand length with fragments over 10kb considered characteristic of necrosis [9, 10]. Obviously, in subjects with no malignancy, only physiological cf-DNA is found in blood. In case of cancer, concentration of its neoplastic counterpart is highly variable and may constitute from less than 1 up to 90% of total cf-DNA, which probably depends on tumour type and stage [11, 12]. For instance, ct-DNA was present in almost all patients with colorectal or pancreatic carcinoma, while in minority of cases with prostate or renal cell carcinoma [13]. Theoretically, to detect circulating ct-DNA in blood, the cancer must consist of at least 50 million cells, which is a small number, considering the fact that a tumour 7–10 mm in diameter that can be detected by imaging studies consists of at least 1 billion cells [5]. In effect, detecting ct-DNA may be more sensitive than other diagnostic modalities and may plausibly allow for early diagnosis.

Moreover, ct-DNA may be subjected to qualitative analysis of a mutational status of a tumour [12, 14]. Some of the mutations detectable in ct

-DNA are believed to be responsible for malignant transformation, leading to uncontrollable growth and cell divisions, hence, they are called driver or primary mutations and, of note, they can be a target of specific drugs, e.g. inhibitors of receptor kinases. Other mutations are new mutations occurring in the course of cancer (secondary ones) which may be responsible for the emergence of resistance to cancer therapy [15].

Cell-free circulating DNA as a source of material to assess mutational status of the tumour

The assessment of mutational status of the tumour is paramount for introduction of specific treatment, e.g. inhibitors of receptor kinases. Still, an analysis of material from biopsy of a tumour or metastases is considered the gold standard. Nevertheless, this standard has been lately challenged by some discoveries implying heterogeneity of mutational status within tumour or between tumour and metastases. For instance, we have recently found that the material from separate parts of the tumour specimen harboured different KRAS mutation at codon 12, or some parts of the tumour harboured a wild gene while the others a mutated one [16]. Therefore, finding no mutation on a standard biopsy may not always be a true negative result, meaning the lack of sensitivity, and in effect low negative predictive value. So, the analysis of ct-DNA may plausibly be a better source of material as it presumably derives from all parts of the tumour, with cells harbouring different mutations or a wild gene simultaneously. Therefore, an isolation of ct-DNA from a small amount of blood for analysis of mutations was called a “liquid biopsy” [12].

What is more important, in some patients, it is not feasible to obtain a suitable tissue sample for assessment of mutations relevant to introduction of a second line treatment. This may be due to insufficient amount of the biopsy material or safety, ethical or technical issues entailed to re-biopsy. Detection of ct-DNA in the peripheral blood might be an alternative procedure. A small sample of blood contains enough cf-DNA (including ct-DNA), to be used for rapid genotyping in order to detect mutations. An analysis of ct-DNA could be repeated any time as needed and should cause neglectable discomfort to patients [5, 17]. Moreover, as a half time of ct-DNA is only about 2 hours, it can reflect changing mutational status of a tumour [12]. Genetic material obtained in this way is biologically rich enough to enable detections of mutations within ct-DNA and its

quantitative evaluation. For instance, Sacher *et al.* found that analysis of common KRAS and EGFR mutations in ct-DNA among NSCLC patients revealed high positive predictive value potentially informing an optimal treatment [18]. Further, recently published meta-analysis showed lower survival in NSCLC patients with KRAS mutation detected in ct-DNA [19]. Another approach to ct-DNA analysis was to look for methylation of promoters of tumour suppressor genes. Disappointingly, methylation analysis of various promoters of genes implicated in NSCLC pathogenesis (e.g. p16, APC, RARb) was plagued by low sensitivity [20].

Finally, regulatory bodies in Europe and the USA have recently approved some diagnostic tests detecting EGFR receptor mutations in ct-DNA to inform specific treatment in case no NSCLC tissue can be harvested directly from the tumour or metastases [21–24]. Nevertheless, the problem highlighted by the ASSESS study was too low sensitivity of only about 50% leading to many false negative results, thus leaving many patients harbouring this driver mutation without optimal treatment [25].

Concentration of cell-free circulating DNA as a predictive biomarker

The idea that the heavier tumour burden is reflected by the higher concentration of ct-DNA and by this means it can be a predictor of survival is quite attractive. However, the published data on the value of a ct-DNA as a predictor of survival in solid tumours are somewhat contradictory. In one of the first studies, Gautschi *et al.* found that elevated concentration of ct-DNA correlated with poor survival and tumour progression after chemotherapy [26]. Likewise, Sozzi *et al.* [27] reported overall high median ct-DNA in patients relapsing in the course of NSCLC, but clinical usefulness of this finding was weakened by some relapsing patients with low level of ct-DNA, i.e. false negatives. Nygaard *et al.* [28] conducted a study on a small group of 53 patients and observed significantly shorter overall survival and progression-free survival in a subgroup of patients with the upper highest quartile of ct-DNA concentration. Similar results were obtained by Tissot *et al.* [29], namely ct-DNA at the highest tertile of concentration spectrum was associated with shorter overall survival and progression-free survival. Extracellular DNA can also be detected in other easily obtainable body fluids, e.g. exhaled breath condensate. In NSCLC patients with confirmed KRAS mutation at codon 12 after a tumour

resection, a ratio of mutated to wild type KRAS in exhaled breath condensate decreased over one month of observation [30]. This phenomenon may possibly be used in monitoring patients for completeness of resection or recurrence of a tumour. In line with this assumption are results published by Kumar *et al.* [31], namely high basal ct-DNA concentration, and smaller decreases of its level observed in the course of chemotherapy were related to worse outcome.

Quite the contrary, a study published by Li *et al.* [32] on a group of 103 patients showed no relation between concentrations of ct-DNA before any systemic treatment and the survival. Also ct-DNA changes did not correlate with the RECIST (Response Evaluation Criteria in Solid Tumours) criteria of response to treatment. Similarly, ct-DNA concentration did not predict survival in patients treated with tyrosine kinase inhibitors [33]. Nevertheless, a meta-analysis of 16 studies showed that a high ct-DNA concentration increased the risk of death of lung cancer patients but did not affect progression-free survival [34].

Concentration of cell-free circulating DNA as a diagnostic biomarker

Another way to assess clinical value of ct-DNA is quantitative analysis with presumption that in malignancy, higher concentration can have discriminatory value and allow for an early, non-invasive cancer detection. Szpechcinski *et al.* [35] found that ct-DNA level was higher in NSCLC patients compared to healthy controls and subjects suffering from chronic inflammatory lung diseases. Similarly, in another work by this group, ct-DNA concentration was markedly elevated in NSCLC vs. patients with benign lung nodules [36]. Nevertheless, at the discriminatory concentration levels proposed by the authors calculated predictive values were below 90%, not allowing for ruling in or out NSCL diagnosis. Further, Leng *et al.* [37] showed that in NSCLC patients higher and differential ct-DNA concentrations were observed compared to tuberculosis group. Moreover, the authors emphasised a greater sensitivity of ct-DNA compared to traditional biomarkers, such as CA125, NSE and CEA. Unfortunately, calculating predictive values from data provided by the authors yields mediocre results, not fully supporting the overoptimistic conclusions.

Conclusions

A small sample of blood is sufficient to determine genetic changes in the tumour by analysis

of ct-DNA. Determination of new mutations and assessment of concentration of ct-DNA seem to increasingly have place in non-invasive cancer screening. Nevertheless, the lack of standardisation of the methods used to assess ct-DNA is a major drawback and hinders wider clinical application. Therefore, standardisation of research on ct-DNA regarding its reference plasma concentration and in effect cut-off values for patients with NSCLC is of paramount importance. Moreover, clinical evaluation on larger groups of patients in prospective studies in respect to NSCLC diagnosis and prognosis may lead to wide implementation of liquid biopsy. Perhaps in the near future, this can be an robust alternative to a traditional tumour biopsy.

Conflict of interest

The authors declare no conflict of interest.

References:

- Didkowska J, Wojciechowska U, Mańczuk M, et al. Lung cancer epidemiology: contemporary and future challenges worldwide. *Ann Transl Med.* 2016; 4(8): 150, doi: [10.21037/atm.2016.03.11](#), indexed in Pubmed: [27195268](#).
- Tanoue LT, Tanner NT, Gould MK, et al. Lung cancer screening. *Am J Respir Crit Care Med.* 2015; 191(1): 19–33, doi: [10.1164/rccm.201410-1777CI](#), indexed in Pubmed: [25369325](#).
- Aberle DR, Adams AM, Berg CD, et al. National Lung Screening Trial Research Team. Reduced lung-cancer mortality with low-dose computed tomographic screening. *N Engl J Med.* 2011; 365(5): 395–409, doi: [10.1056/NEJMoa1102873](#), indexed in Pubmed: [21714641](#).
- Siravegna G, Marsoni S, Siena S, et al. Integrating liquid biopsies into the management of cancer. *Nat Rev Clin Oncol.* 2017; 14(9): 531–548, doi: [10.1038/nrclinonc.2017.14](#), indexed in Pubmed: [28252003](#).
- Francis G, Stein S. Circulating Cell-Free Tumour DNA in the Management of Cancer. *Int J Mol Sci.* 2015; 16(6): 14122–14142, doi: [10.3390/ijms160614122](#), indexed in Pubmed: [26101870](#).
- Jones HB. Some Account of a New Animal Substance Occurring in the Urine of a Patient Labouring under Mollities Ossium. *Edinb Med Surg J.* 1850; 74(185): 357–368, indexed in Pubmed: [30331059](#).
- Leon SA, Shapiro B, Sklaroff DM, et al. Free DNA in the serum of cancer patients and the effect of therapy. *Cancer Res.* 1977; 37(3): 646–650, indexed in Pubmed: [837366](#).
- Sorenson GD, Pribish DM, Valone FH, et al. Soluble normal and mutated DNA sequences from single-copy genes in human blood. *Cancer Epidemiol Biomarkers Prev.* 1994; 3(1): 67–71, indexed in Pubmed: [8118388](#).
- Jahr S, Hentze H, Englisch S, et al. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. *Cancer Res.* 2001; 61(4): 1659–1665, indexed in Pubmed: [11245480](#).
- Wang BG, Huang HY, Chen YC, et al. Increased plasma DNA integrity in cancer patients. *Cancer Res.* 2003; 63(14): 3966–3968, indexed in Pubmed: [12873992](#).
- Schwarzenbach H, Stoecklacher J, Pantel K, et al. Detection and monitoring of cell-free DNA in blood of patients with colorectal cancer. *Ann N Y Acad Sci.* 2008; 1137: 190–196, doi: [10.1196/annals.1448.025](#), indexed in Pubmed: [18837946](#).
- Diaz LA, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *J Clin Oncol.* 2014; 32(6): 579–586, doi: [10.1200/JCO.2012.45.2011](#), indexed in Pubmed: [24449238](#).
- Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med.* 2014; 6(224): 224ra24, doi: [10.1126/scitranslmed.3007094](#), indexed in Pubmed: [24553385](#).
- Pathak AK, Bhutani M, Kumar S, et al. Circulating cell-free DNA in plasma/serum of lung cancer patients as a potential screening and prognostic tool. *Clin Chem.* 2006; 52(10): 1833–1842, doi: [10.1373/clinchem.2005.062893](#), indexed in Pubmed: [16423903](#).
- Newman AM, Bratman SV, To J, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nat Med.* 2014; 20(5): 548–554, doi: [10.1038/nm.3519](#), indexed in Pubmed: [24705333](#).
- Kordiak J, Szmraj J, Grabska-Kobylecka I, et al. Intratumor heterogeneity and tissue distribution of KRAS mutation in non-small cell lung cancer: implications for detection of mutated KRAS oncogene in exhaled breath condensate. *J Cancer Res Clin Oncol.* 2019; 145(1): 241–251, doi: [10.1007/s00432-018-2779-1](#), indexed in Pubmed: [30368666](#).
- Remon J, Gorham J, Besse B, et al. Circulating free DNA, new dynamic marker in nonsmall cell lung cancer patients? *Eur Respir J.* 2015; 46(6): 1548–1550, doi: [10.1183/13993003.01482-2015](#), indexed in Pubmed: [26621882](#).
- Sacher AG, Paweletz C, Dahlberg SE, et al. Prospective Validation of Rapid Plasma Genotyping for the Detection of EGFR and KRAS Mutations in Advanced Lung Cancer. *JAMA Oncol.* 2016; 2(8): 1014–1022, doi: [10.1001/jamaoncol.2016.0173](#), indexed in Pubmed: [27055085](#).
- Zhuang R, Li S, Li Q, et al. The prognostic value of KRAS mutation by cell-free DNA in cancer patients: A systematic review and meta-analysis. *PLoS One.* 2017; 12(8): e0182562, doi: [10.1371/journal.pone.0182562](#), indexed in Pubmed: [28796802](#).
- Warton K, Samimi G. Methylation of cell-free circulating DNA in the diagnosis of cancer. *Front Mol Biosci.* 2015; 2: 13, doi: [10.3389/fmolb.2015.00013](#), indexed in Pubmed: [25988180](#).
- Douillard JY, Ostoros G, Cobo M, et al. Gefitinib treatment in EGFR mutated caucasian NSCLC: circulating-free tumor DNA as a surrogate for determination of EGFR status. *J Thorac Oncol.* 2014; 9(9): 1345–1353, doi: [10.1097/JTO.0000000000000263](#), indexed in Pubmed: [25122430](#).
- Goss G, Tsai CM, Shepherd F, et al. Osimertinib for pretreated EGFR Thr790Met-positive advanced non-small-cell lung cancer (AURA2): a multicentre, open-label, single-arm, phase 2 study. *The Lancet Oncology.* 2016; 17(12): 1643–1652, doi: [10.1016/s1470-2045\(16\)30508-3](#).
- Oxnard GR, Thress KS, Alden RS, et al. Association Between Plasma Genotyping and Outcomes of Treatment With Osimertinib (AZD9291) in Advanced Non-Small-Cell Lung Cancer. *J Clin Oncol.* 2016; 34(28): 3375–3382, doi: [10.1200/JCO.2016.66.7162](#), indexed in Pubmed: [27354477](#).
- Malapelle U, Sirera R, Jantus-Lewintre E, et al. Profile of the Roche cobas® EGFR mutation test v2 for non-small cell lung cancer. *Expert Rev Mol Diagn.* 2017; 17(3): 209–215, doi: [10.1080/14737159.2017.1288568](#), indexed in Pubmed: [28129709](#).
- Reck M, Hagiwara K, Han B, et al. ctDNA Determination of EGFR Mutation Status in European and Japanese Patients with Advanced NSCLC: The ASSESS Study. *J Thorac Oncol.* 2016; 11(10): 1682–1689, doi: [10.1016/j.jtho.2016.05.036](#), indexed in Pubmed: [27468938](#).
- Gautschi O, Bigosch C, Huegli B, et al. Circulating deoxyribonucleic Acid as prognostic marker in non-small-cell lung cancer patients undergoing chemotherapy. *J Clin Oncol.* 2004; 22(20): 4157–4164, doi: [10.1200/JCO.2004.11.123](#), indexed in Pubmed: [15483026](#).
- Sozzi G, Roz L, Conte D, et al. Plasma DNA quantification in lung cancer computed tomography screening: five-year results of a prospective study. *Am J Respir Crit Care Med.* 2009; 179(1): 69–74, doi: [10.1164/rccm.200807-1068OC](#), indexed in Pubmed: [18787214](#).
- Dowler Nygaard A, Spindler KLG, Pallisgaard N, et al. Levels of cell-free DNA and plasma KRAS during treatment of advanced NSCLC. *Oncol Rep.* 2014; 31(2): 969–974, doi: [10.3892/or.2013.2906](#), indexed in Pubmed: [24316734](#).

29. Tissot C, Toffart AC, Villar S, et al. Circulating free DNA concentration is an independent prognostic biomarker in lung cancer. *Eur Respir J*. 2015; 46(6): 1773–1780, doi: [10.1183/13993003.00676-2015](https://doi.org/10.1183/13993003.00676-2015), indexed in Pubmed: [26493785](https://pubmed.ncbi.nlm.nih.gov/26493785/).
30. Kordiak J, Szymraj J, Hamara K, et al. Complete surgical resection of lung tumor decreases exhalation of mutated KRAS oncogene. *Respir Med*. 2012; 106(9): 1293–1300, doi: [10.1016/j.rmed.2012.06.019](https://doi.org/10.1016/j.rmed.2012.06.019), indexed in Pubmed: [22795503](https://pubmed.ncbi.nlm.nih.gov/22795503/).
31. Kumar S, Guleria R, Singh V, et al. Plasma DNA level in predicting therapeutic efficacy in advanced nonsmall cell lung cancer. *Eur Respir J*. 2010; 36(4): 885–892, doi: [10.1183/09031936.00187909](https://doi.org/10.1183/09031936.00187909), indexed in Pubmed: [20185420](https://pubmed.ncbi.nlm.nih.gov/20185420/).
32. Li BT, Drilon A, Johnson ML, et al. A prospective study of total plasma cell-free DNA as a predictive biomarker for response to systemic therapy in patients with advanced non-small-cell lung cancers. *Ann Oncol*. 2016; 27(1): 154–159, doi: [10.1093/annonc/mdv498](https://doi.org/10.1093/annonc/mdv498), indexed in Pubmed: [26487589](https://pubmed.ncbi.nlm.nih.gov/26487589/).
33. Wang S, Han X, Hu X, et al. Clinical significance of pretreatment plasma biomarkers in advanced non-small cell lung cancer patients. *Clin Chim Acta*. 2014; 430: 63–70, doi: [10.1016/j.cca.2013.12.026](https://doi.org/10.1016/j.cca.2013.12.026), indexed in Pubmed: [24378285](https://pubmed.ncbi.nlm.nih.gov/24378285/).
34. Cargnin S, Canonico PL, Genazzani AA, et al. Quantitative Analysis of Circulating Cell-Free DNA for Correlation with Lung Cancer Survival: A Systematic Review and Meta-Analysis. *J Thorac Oncol*. 2017; 12(1): 43–53, doi: [10.1016/j.jtho.2016.08.002](https://doi.org/10.1016/j.jtho.2016.08.002), indexed in Pubmed: [27543256](https://pubmed.ncbi.nlm.nih.gov/27543256/).
35. Szpechcinski A, Chorostowska-Wynimko J, Struniawski R, et al. Cell-free DNA levels in plasma of patients with non-small-cell lung cancer and inflammatory lung disease. *Br J Cancer*. 2015; 113(3): 476–483, doi: [10.1038/bjc.2015.225](https://doi.org/10.1038/bjc.2015.225), indexed in Pubmed: [26125447](https://pubmed.ncbi.nlm.nih.gov/26125447/).
36. Szpechcinski A, Rudzinski P, Kupis W, et al. Plasma cell-free DNA levels and integrity in patients with chest radiological findings: NSCLC versus benign lung nodules. *Cancer Lett*. 2016; 374(2): 202–207, doi: [10.1016/j.canlet.2016.02.002](https://doi.org/10.1016/j.canlet.2016.02.002), indexed in Pubmed: [26854716](https://pubmed.ncbi.nlm.nih.gov/26854716/).
37. Leng S, Zheng J, Jin Y, et al. Plasma cell-free DNA level and its integrity as biomarkers to distinguish non-small cell lung cancer from tuberculosis. *Clin Chim Acta*. 2018; 477: 160–165, doi: [10.1016/j.cca.2017.11.003](https://doi.org/10.1016/j.cca.2017.11.003), indexed in Pubmed: [29113814](https://pubmed.ncbi.nlm.nih.gov/29113814/).