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Circulating Plasma Cell-free DNA (cfDNA) as a Predictive Biomarker for Radiotherapy: Results from a Prospective Trial in Head and Neck Cancer

MICHAEL I. KOUKOURAKIS[1], ERASMIA XANTHOPOULOU[1], IOANNIS M. KOUKOURAKIS[2], SOTIRIOS P. FORTIS[3], NIKOLAOS KESESIDIS[4], IOANNIS KARAKASILIOTIS[4] and CONSTANTIN N. BAXEVANIS[3]

[1]*Department of Radiotherapy – Oncology, Medical School, Democritus University of Thrace, Alexandroupolis, Greece;*
[2]*Radiation Oncology Unit, 1st Department of Radiology, Aretaieion University Hospital, Athens, Greece;*
[3]*Cancer Immunology and Immunotherapy Center, Cancer Research Center, Saint Savas Cancer Hospital, Athens, Greece;*
[4]*Laboratory of Biology, Medical School, Democritus University of Thrace, Alexandroupolis, Greece*

Abstract. *Background/Aim: The plasma levels of cell-free DNA (cfDNA) in cancer patients increase due to rapid cancer cell proliferation and death. Therefore, cfDNA can be used to study specific tumor-DNA features. In addition, the non-specific cfDNA concentration may be an important biomarker of cancer prognosis. Patients and Methods: We prospectively examined the predictive role of cfDNA levels and the kinetics in the outcome of chemo-radiotherapy (CRT) in a cohort of 47 patients with locally advanced squamous cell head-neck cancer (SCHNC) treated with definitive chemo-radiotherapy. Results: Increased cfDNA levels after therapy completion (after/before treatment ratio; A/B-ratio >1) were found in 26/47 patients (55.3%). Locally advanced T4-stage was significantly associated with higher cfDNA levels after CRT (3.3 ng/μl in T4-stage vs. 1.3 ng/μl in T1-3 stages, p=0.007). Patients who responded to CRT (partial/complete response) had significantly lower cfDNA levels before therapy (mean values 1.2 ng/μl vs. 2.7 ng/μl, p=0.03). A significantly worse locoregional progression-free survival in patients with an A/B-ratio >1 was documented (p=0.01; hazard ratio 3.5, 95%CI=1.2-9.7). This was also confirmed in multivariate analysis, where the A/B-ratio was an independent predictive variable of locoregional relapse (p=0.03, hazard ratio 3.9, 95%CI=1.2-13). Conclusion: High post-CRT cfDNA levels could be an early biomarker for the immediate recruitment of patients with SCHNC in consolidation chemo-immunotherapy protocols.*

Correspondence to: Prof. Michael I. Koukourakis, MD, Ph.D., Department of Radiotherapy/Oncology, Democritus University of Thrace, Alexandroupolis 68100, Greece. Tel: +30 2551351628, Fax: +30 2551030349, e-mail: targ@her.forthnet.gr

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Circulating cell-free DNA (cfDNA) is fragmented DNA released by tissues; its size can range from 100 to 800 bp (1). cfDNA is of genomic, mitochondrial, or viral origin and is released through various cellular processes, including apoptosis, necrosis, and other cell death pathways, or vesicle release (2). The clinical role of cfDNA detection and analysis is evolving, and is expected to be used in several diseases, including prenatal testing, rheumatic diseases, organ transplantation, and cancer.

Because cancer tissues are characterized by rapid cancer cell proliferation and high rates of programmed cell death and necrosis due to impaired blood flow and hypoxic/acidic tumoral microenvironmental conditions, a significant proportion of the cfDNA in patients with cancer is of tumoral origin (named as circulating tumor DNA; ctDNA). Plasma cfDNA can be assessed using reverse transcription polymerase chain reaction (RT-PCR) or next-generation sequencing (NGS) techniques. Quantitative analysis of cfDNA and ctDNA, as well as analysis of methylation or gene mutations using NGS, provide important tools for cancer diagnosis and screening, and can also be used to develop prognostic and predictive biomarkers (3).

In healthy individuals, the cfDNA concentration in the blood ranges from 0-100 ng/ml (4, 5). Concentration increases as the size of the primary tumor and the extent of lymph node involvement advances (6-8). Metastatic disease is also linked to higher cfDNA levels (9).

The eventual role of cfDNA quantification in predicting therapeutic efficacy has been suggested as early as 1977 (10). Surgical resection of the tumor and chemotherapy have been shown to reduce the cfDNA concentration (11, 12). Several studies noted that the reduction of cfDNA in the plasma is associated with a better and sustained response to chemotherapy in gastric, pancreatic, and colorectal cancers (13, 14). Conflicting results have been reported regarding the pre-chemotherapy prognostic value of cfDNA levels in NSCLC (15, 16).

In the current report, we provide the results of a prospective study focusing on the role of plasma cfDNA concentration in the outcome of radiotherapy (RT) for squamous cell head neck cancer (SCHNC).

Patients and Methods

Ethical considerations. The study was approved by the local Ethics and Research Committee (ES1 23-01-2019 and ES2 22-02-2019) and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent to enter the trial was obtained from all patients before therapy. Patient consent included permission to anonymously publish their clinical and laboratory data for research and educational purposes.

Patients. Between 2020 and 2022, 47 patients with histologically diagnosed SCHNC treated with RT combined with chemotherapy were prospectively enrolled. Table I shows the patient and disease characteristics. The median follow-up of patients was 16 months (range=1-36 months). For patients alive at the last follow-up, this was 18 months (range=6-36 months).

Treatment details. Volumetric Modulated Arc Therapy (VMAT) was applied using a 6 MV ELEKTA Infinity™ Linear Accelerator (Elekta, Stockholm, Sweden) endowed with an Agility™ head (Elekta). Image-guided radiotherapy (IGRT), using daily cone-beam CT, was applied. All patients were treated with a simultaneous integrated boost (SIB) technique delivering 22 daily fractions within 30 days. For prophylactic neck irradiation, a daily dose of 2.15 Gy was applied, while the primary tumor received fractions of 2.6-2.7 Gy. Enlarged nodes received 2.3-2.5 Gy per fraction. The inversed plans were produced using Monaco TPS version 5.11.03 (Elekta CMS, Maryland Heights, MO, USA), and each planning target volume (PTV) received at least 95% of the prescribed dose to 98% of its volume.

Patients received concurrent chemo-RT (CRT) with cisplatin (35-40 mg/m^2 per week; 12 patients) without or with cetuximab (250 mg/m^2/week; 28 patients).

Response evaluation and follow-up. Tumor response was assessed two months after RT completion with a computed tomography (CT-scan) or magnetic resonance imaging (MRI) as appropriate. CT evaluation was repeated at four months and every six months thereafter. We used the WHO criteria (17) for response assessment with in-house modifications as follows: Complete response (CR) was defined as a 95-100% reduction of 2D dimensions of all measurable lesions. Any residual scar measuring less than 5% of the initial tumor volume that does not progress for at least four months following response documentation was considered to indicate a complete response. Partial (PR) and minimal response (MR) refers to 50-95% and 25-49% reduction of tumor 2D dimensions, respectively. Tumor reductions between 0-24% that lasted at least two months were considered stable disease. All other cases were considered progressive disease (PgD), regardless of the initial response.

Plasma collection. Blood was collected in in vials containing ethylenediaminetetraacetic acid (EDTA) (BD Biosciences, Franklin Lakes, NJ, USA). Plasma and peripheral blood mononuclear cell (PBMCs) isolation was performed with density gradient centrifugation using a 1,077 g/ml synthetic epichlorohydrin sucrose polymer Lymphosep (Lymphocyte Separation Media-500 ml; density 1,077 g/ml; Cat no: LM-T1702/500; Biosera, Cholet, France), at a 1:1 ratio of the total volume. Centrifugation of blood samples was

Table I. *Patient and disease characteristics*.

Patients No	47
Age	
Range	32-88
Median	66
Performance status	
Median	0-1
Range	0
Sex	
Male	42
Female	5
Location	
Larynx	19
Oropharynx	6
Oral cavity	8
Hypopharynx	3
Nasopharynx	6
Parotids	3
Neck	2
Histology	
Squamous grade 1	6
Squamous grade 2	29
Squamous grade 3	10
Undifferentiated	2
T-stage	
T0	2
T2	6
T3	19
T4	20
N-stage	
N0	18
N1	7
N2	10
N3	12

performed at 1,500 rpm (400 × *g*), for 30 min at 25˚C. The plasma layer was transferred in new sterile Eppendorf tubes, and then gradually frozen at –20˚C and then at –80˚C for long-term storage.

Plasma-cfDNA quantification. The MagMAX Cell-Free (Cat. no: A36716; Thermo Fisher Scientific, Waltham, MA, USA) isolation kit, specifically designed for nucleic acid enrichment in liquid biopsies such as the plasma, was used to isolate total cell-free nucleic acids. The isolation was based on MagMAX magnetic bead technology to recover high-quality nucleic acids suitable for NGS. Isolation was performed using 4 ml of plasma from each patient, before and at the end of treatment. Quantification of cfDNA concentration was performed using the Qubit fluorometer and the Qubit 1X dsDNA HS (Cat. no: Q33231; Thermo Fisher Scientific), for specific and selective quantification of double-stranded DNA (dsDNA).

Statistical analysis. The GraphPad Prism 7.0 package (Boston, MA, USA) was used for statistical analysis and graph presentation. The Wilcoxon matched pairs non-parametric two-tailed test was used for testing relationships between paired continuous variables. The Mann–Whitney non-parametric test was used to compare non-paired continuous variables. Locoregional progression free survival (LRFS), distant metastasis-free survival (DMFS), and disease-specific overall survival (OS) curves were plotted using the method of Kaplan–Meier. A Cox-regression analysis model was used to assess the independent significance of variables in the treatment outcome. A *p*-value of less than 0.05 was considered statistically significant.

Results

cfDNA levels. The plasma cfDNA levels before CRT ranged between 0.09-5.2 ng/μl [median 0.91, 95% confidence interval (95%CI)=1.06-1.75], while after CRT ranged between 0.18-19.40 (median 1.01, 95%CI=1.2-3.1) (Figure 1). The overall increase did not reach significance (p=0.09). The ratio between cfDNA levels after/before CRT (A/B-ratio) ranged from 0.08 to 9.2 (median 1.1; 95%CI=1.2-2.4). Twenty-six out of 47 patients (55.3%) had an A/B-ratio >1 (median 1.8, range=1.1-9.2, 95%CI=1.7-3.7), thus increased cfDNA after CRT (Figure 1A).

cfDNA levels and histopathological variables. Comparison of T4-stage *vs*. all other stages showed a significant increase in cfDNA levels after therapy (3.3 ng/μl *vs*. 1.3 ng/μl, respectively, p=0.007; Figure 1B). Analysis according to N-stage (N0 *vs*. N1 *vs*. N2 *vs*. N3) did not demonstrate any significant association (p>0.56) (data not shown). Regarding the histopathological grade, a marginally higher cfDNA A/B-ratio was noted after therapy in grade 3 tumors compared to grade 1,2 (median value 1.2 ng/μl *vs*. 0.68 ng/μl, respectively, p=0.08).

cfDNA and response to CRT. Patients with MR or PgD after therapy had significantly higher cfDNA levels before therapy compared to patients with PR or CR (mean value 2.7 ng/μl

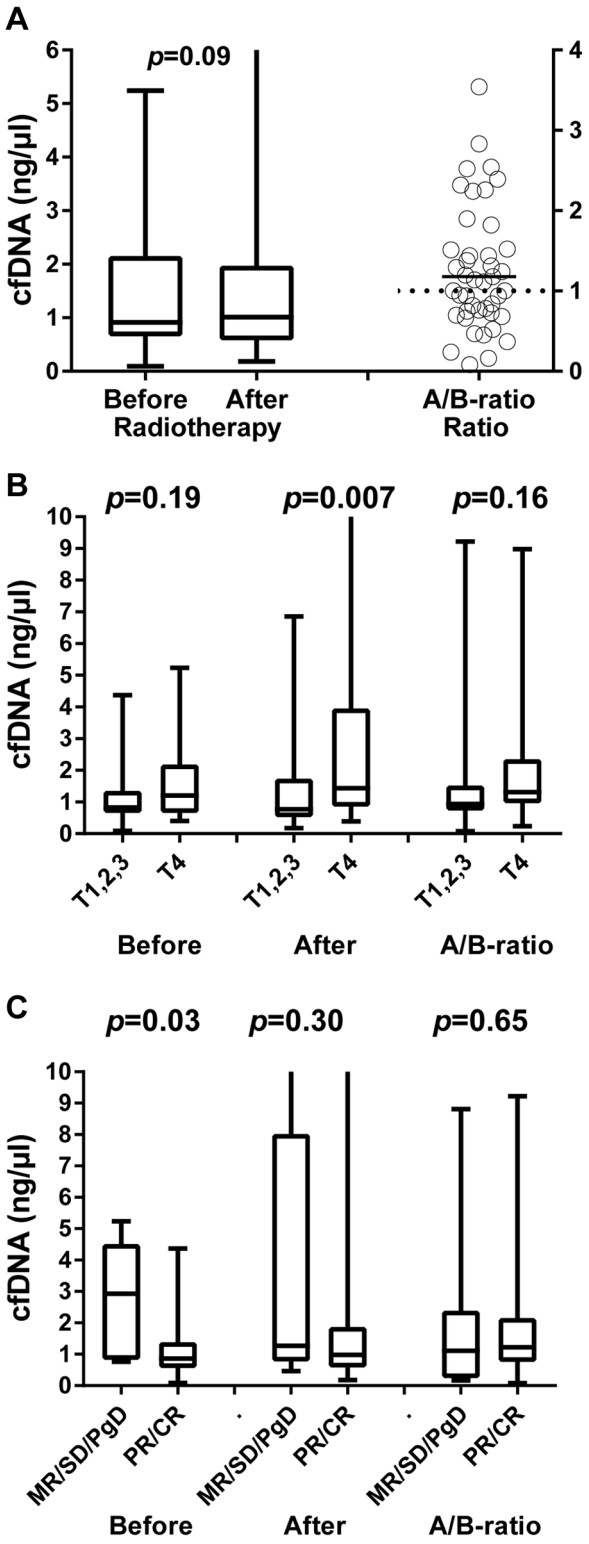

Figure 1. *Cell-free DNA (cfDNA) levels before and after radiotherapy, and after/before ratio (A/B-ratio): (A) overall cfDNA levels and A/B-ratio distribution, (B) grouping according to T-stage, (C) grouping according to response to radiotherapy. CR: Complete response; PR: partial response; SD: stable disease; MR: minimal response; PgD: progressive disease.*

vs. 1.2 ng/μl, respectively, p=0.03; Figure 1C). No significant difference was noted for the cfDNA levels after therapy or for A/B-ratio (p=0.30 and 0.65, respectively).

cfDNA and survival. Kaplan–Meier survival curves showed no statistically significant impact of an A/B-ratio >1 on OS (p=0.12; Figure 2A). A significantly worse LRFS was recorded in patients with an A/B-ratio >1 (p=0.01; hazard ratio 3.5, 95%CI=1.2-9.7; Figure 2B). No association was noted for the DMFS (p=0.53; Figure 2C). A multivariate Cox-regression analysis model including the AB/ratio (<1 *vs*. >1), T-stage (1, 2, 3 *vs*. 4), N-stage (0, 1, 2 *vs*. 3), and histopathological grade (1, 2 *vs*. 3), showed that the A/B-ratio was the only independent prognostic variable predicting locoregional relapse (p=0.03, hazard ratio 3.9, 95%CI=1.2-13).

Taking the median cfDNA value as a cut-off point, survival analysis was performed for the levels of cfDNA before and after CRT. No association of cfDNA levels (either before or after therapy) with OS and LRFS was noted (Figure 3A-F). A non-significant trend for worse DMFS was observed in patients with higher than the median cfDNA levels before and after CRT.

Discussion

cfDNA is readily detectable in the plasma of patients. Overall higher levels of cfDNA are present in cancer patients compared to healthy controls. In the current prospective trial, we found that the plasma cfDNA in patients with SCHNC ranged from 0.09 to 5.2 ng/μl. In our study, higher pretreatment levels were significantly associated with advanced T-stage, which is in accordance with a previous study by Muhanna *et al*. (8). This could be a result of increased tumor burden, frequently accompanying T4-stage tumors, which eventually is associated with increased release of fragmented DNA from a higher number of dying cancer cells, and, eventually, from necrotic tissue areas existing in large tumors.

In 2016, Mazurek *et al*. reported a study on 200 patients with HNC where plasma cfDNA was measured using a technique based on telomerase reverse transcriptase amplification (18). Patients with oropharyngeal cancer had significantly higher levels compared to other tumor types. Moreover, stage IV cases and lymph node involvement were linked with higher cfDNA plasma levels. The study, however, did not report an analysis of treatment outcomes. As the incidence of distant metastasis is overall low in SCHNCs, in the range of 10% (19), the association of high cfDNA levels with metastatic disease is interesting. Such a biomarker could be useful for identifying patients at a higher risk of metastasis. Our study found an association between cfDNA levels with DMFS and LRFS, though with no statistical significance. This discrepancy could be due to the short clinical follow-up, the small number of patients, and the low incidence of distant metastasis.

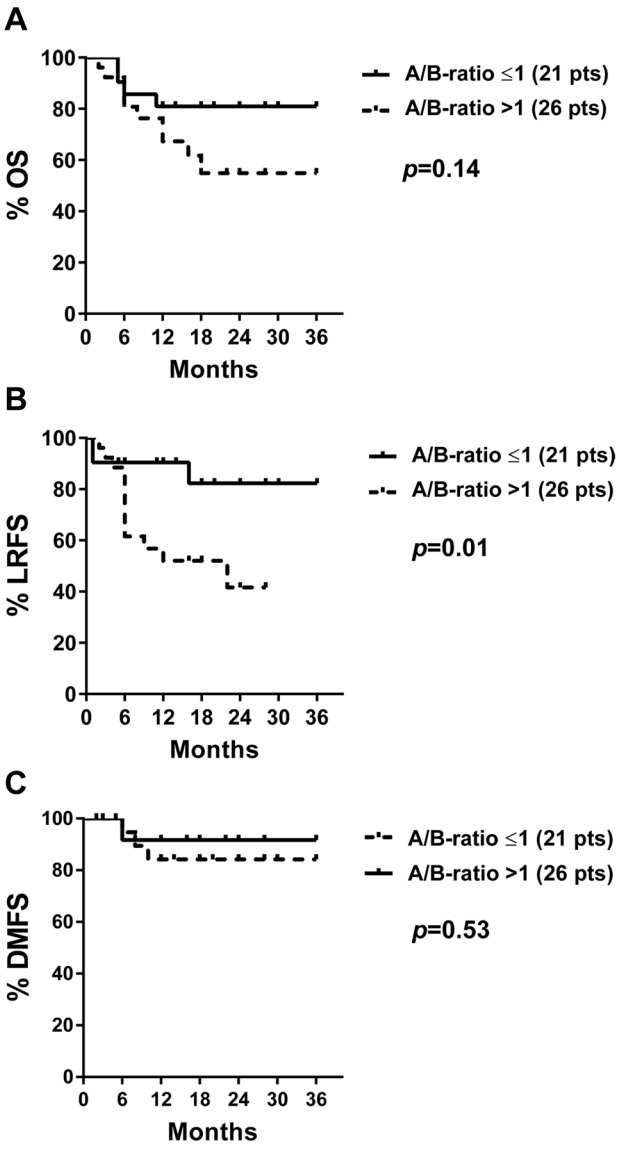

Figure 2. *Kaplan–Meier survival curves according to A/B-ratio ≤1 vs. >1: (A) Disease-specific overall survival (OS), (B) locoregional progression-free survival (LRFS), (C) distant metastasis-free survival (DMFS).*

Monitoring the kinetics of cfDNA levels in patients with SCHNC undergoing CRT, we observed that levels increased at the end of therapy in 55.3% of patients. In a study by Verma *et al*., 24 patients with HNC were studied by monitoring the cfDNA levels before, during, and after CRT (20). The authors found decreasing cfDNA levels in responders three months after the end of therapy. However, increasing overall levels were noted in patients with persistent disease. In our study, patients with minimal response or progressive disease after CRT had more than 2-fold higher cfDNA levels before therapy than responders. We suggest that high cfDNA levels before treatment

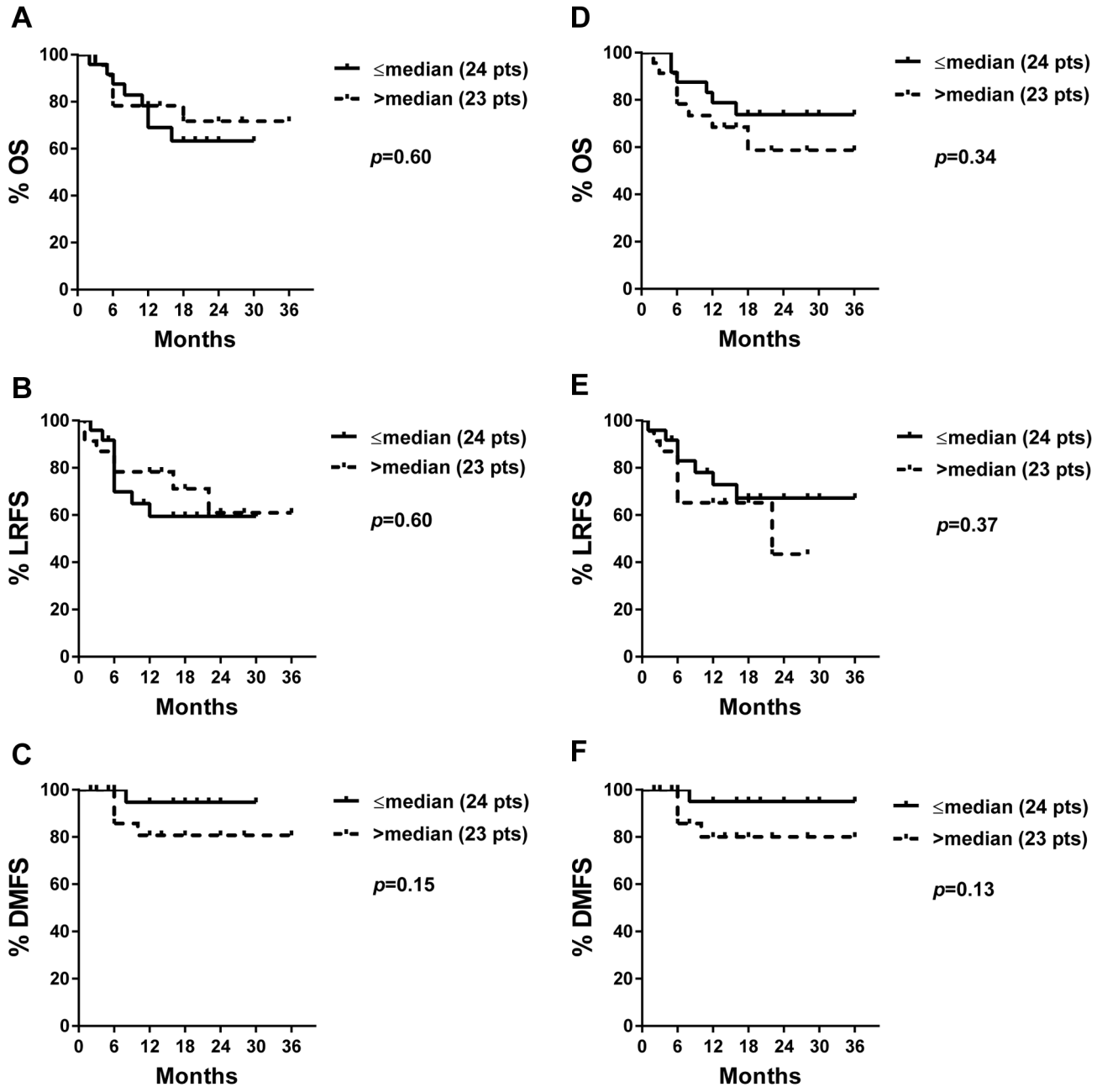

Figure 3. *Kaplan–Meier disease specific overall survival (OS), locoregional progression free survival (LRFS) and distant metastasis free survival (DMFS) curves according to cfDNA levels before CRT (A, B, C, respectively) and after CRT (D, E, F, respectively).*

reflect radioresistant tumors with high proliferating activity and necrosis that lead to an intense release of DNA fragments in the peripheral blood. Kaplan-Meier analysis showed that the subgroup of patients with increasing cfDNA levels after CRT had a significantly worse LRFS. Although no similar analysis is available in the literature regarding patients with SCHNC, studies in patients with lung and hepatocellular cancer shoed better disease control rates in patients with low cfDNA after CRT (21, 22). Whether this finding represents a high intrinsic radio-resistance or a rapid cancer proliferation activated during radiotherapy, both conditions leading to radiotherapy failure, are questions that require further investigation.

The role of the kinetics of cfDNA during RT in predicting locoregional recurrence is clinically relevant. As the

assessment of response of SCHNC and other carcinomas after CRT is usually performed two months after the end of therapy, the use of cfDNA levels can be important to identify high-risk patients immediately after treatment. Such patients could be candidates for immediate consolidation chemotherapy or, even better, be recruited in immunotherapy protocols to enhance the eradication of the remnant disease by taking advantage of the postulated radio-vaccination effect of otherwise ineffective CRT (23, 24).

Conclusion

The assessment of cfDNA levels in the plasma of patients with SCHNC before and after CRT provides useful information regarding the locoregional control and recurrence probability. A high after/before CRT cfDNA levels ratio indicates incomplete tumor eradication and poor LRFS. This information becomes available earlier than the routine CT-assessment, which is performed several weeks after treatment completion. Whether high levels of cfDNA before and after therapy are markers of high risk for the development of metastasis requires further investigation in larger cohorts of patients. cfDNA indexes may prove important in stratifying patients with SCHNC at a high risk of local and distant relapse into trials of consolidation chemo-immunotherapy.

Conflicts of Interest

There are no conflicts of interest to report in relation to this study.

Authors' Contributions

Conceptualization, M.I.K., C.N.B.; methodology, I.K., E.X, N.K.; validation, S.P.F., I.M.K.; M.I.K., E.X.; investigation, M.I.K., I.M.K.; writing – original draft preparation, M.I.K., E.X.; writing – review and editing, I.M.K., S.P.F., N.K., I.K., C.N.B.; supervision, M.I.K., C.N.B.; All Authors have read and agreed to the published version of the manuscript.

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References

1 Rumore PM, Steinman CR: Endogenous circulating DNA in systemic lupus erythematosus. Occurrence as multimeric complexes bound to histone. J Clin Invest 86(1): 69-74, 1990. DOI: 10.1172/JCI114716

2 Grabuschnig S, Bronkhorst AJ, Holdenrieder S, Rosales Rodriguez I, Schliep KP, Schwendenwein D, Ungerer V, Sensen CW: Putative origins of cell-free DNA in humans: A review of active and passive nucleic acid release mechanisms. Int J Mol Sci 21(21): 8062, 2020. DOI: 10.3390/ijms21218062

3 Yan YY, Guo QR, Wang FH, Adhikari R, Zhu ZY, Zhang HY, Zhou WM, Yu H, Li JQ, Zhang JY: Cell-free DNA: Hope and potential application in cancer. Front Cell Dev Biol 9: 639233, 2021. DOI: 10.3389/fcell.2021.639233

4 Chen E, Cario CL, Leong L, Lopez K, Márquez CP, Chu C, Li PS, Oropeza E, Tenggara I, Cowan J, Simko JP, Chan JM, Friedlander T, Wyatt AW, Aggarwal R, Paris PL, Carroll PR, Feng F, Witte JS: Cell-free DNA concentration and fragment size as a biomarker for prostate cancer. Sci Rep 11(1): 5040, 2021. DOI: 10.1038/s41598-021-84507-z

5 Szpechcinski A, Chorostowska-Wynimko J, Struniawski R, Kupis W, Rudzinski P, Langfort R, Puscinska E, Bielen P, Sliwinski P, Orlowski T: Cell-free DNA levels in plasma of patients with non-small-cell lung cancer and inflammatory lung disease. Br J Cancer 113(3): 476-483, 2015. DOI: 10.1038/bjc.2015.225

6 Park S, Lee EJ, Rim CH, Seong J: Plasma cell-free DNA as a predictive marker after radiotherapy for hepatocellular carcinoma. Yonsei Med J 59(4): 470-479, 2018. DOI: 10.3349/ymj.2018.59.4.470

7 Muhanna N, Eu D, Chan HHL, Douglas C, Townson JL, Di Grappa MA, Mohamadi RM, Kelley SO, Bratman SV, Irish JC: Cell-free DNA and circulating tumor cell kinetics in a pre-clinical head and neck Cancer model undergoing radiation therapy. BMC Cancer 21(1): 1075, 2021. DOI: 10.1186/s12885-021-08791-8

8 Małusecka E, Giglok M, Suwiński R, Rutkowski TW, Mazurek AM: Quantitative analysis of plasma DNA in anal cancer patients. Contemp Oncol (Pozn) 26(2): 128-132, 2022. DOI: 10.5114/wo.2022.118132

9 Sun Z, Zhong J, Lu J, Si L, Pan X, He K, Wang Q, Zhang Y, Bao J: The kinetics of circulating cell-free DNA to predict the clinical efficacy of first-line chemotherapy in patients with metastatic colorectal cancer. Research Square, 2022. DOI: 10.21203/rs.3.rs-2018900/v1

10 Leon SA, Shapiro B, Sklaroff DM, Yaros MJ: Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res 37: 646-650, 1997.

11 Adusei E, Ahenkorah J, Adu-Aryee NA, Adutwum-Ofosu KK, Tagoe EA, Koney NK, Nkansah E, Aryee NA, Blay RM, Hottor BA, Clegg-Lamptey JN, Arko-Boham B: Reduced serum circulation of cell-free DNA following chemotherapy in breast cancer patients. Med Sci (Basel) 9(2): 37, 2021. DOI: 10.3390/medsci9020037

12 Peled M, Agassi R, Czeiger D, Ariad S, Riff R, Rosenthal M, Lazarev I, Novack V, Yarza S, Mizrakli Y, Douvdevani A: Cell-free DNA concentration in patients with clinical or mammographic suspicion of breast cancer. Sci Rep 10(1): 14601, 2020. DOI: 10.1038/s41598-020-71357-4

13 Zhong Y, Fan Q, Zhou Z, Wang Y, He K, Lu J: Plasma cfDNA as a potential biomarker to evaluate the efficacy of chemotherapy in gastric cancer. Cancer Manag Res 12: 3099-3106, 2020. DOI: 10.2147/CMAR.S243320

14 Christenson ES, Lim SJ, Durham J, De Jesus-Acosta A, Bever K, Laheru D, Ryan A, Agarwal P, Scharpf RB, Le DT, Wang H: Cell-free DNA predicts prolonged response to multi-agent

chemotherapy in pancreatic ductal adenocarcinoma. Cancer Res Commun 2(11): 1418-1425, 2022. DOI: 10.1158/2767-9764.CRC-22-0343

15 Tissot C, Toffart A, Villar S, Souquet P, Merle P, Moro-sibilot D, Pérol M, Zavadil J, Brambilla C, Olivier M, Couraud S: Circulating free DNA concentration is an independent prognostic biomarker in lung cancer. Eur Respir J 46(6): 1773-1780, 2015. DOI: 10.1183/13993003.00676-2015

16 Li BT, Drilon A, Johnson ML, Hsu M, Sima CS, McGinn C, Sugita H, Kris MG, Azzoli CG: 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 27(1): 154-159, 2016. DOI: 10.1093/annonc/mdv498

17 Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, Van Oosterom AT, Christian MC, Gwyther SG: New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 92(3): 205-216, 2000. DOI: 10.1093/jnci/92.3.205

18 Mazurek AM, Rutkowski T, Fiszer-kierzkowska A, Małusecka E, Składowski K: Assessment of the total cfDNA and HPV16/18 detection in plasma samples of head and neck squamous cell carcinoma patients. Oral Oncol 54: 36-41, 2016. DOI: 10.1016/j.oraloncology.2015.12.002

19 Garavello W, Ciardo A, Spreafico R, Gaini RM: Risk factors for distant metastases in head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 132(7): 762, 2006. DOI: 10.1001/archotol.132.7.762

20 Husain N, Verma T, Kumari S, Mishra S, Rastogi M, Tiwari V, Agarwal G, Anand N: Circulating free DNA as a marker of response to chemoradiation in locally advanced head and neck squamous cell carcinoma. Indian J Pathol Microbiol 63(4): 521, 2020. DOI: 10.4103/IJPM.IJPM_28_20

21 Bortolin MT, Tedeschi R, Bidoli E, Furlan C, Basaglia G, Minatel E, Gobitti C, Franchin G, Trovò M, De Paoli P: Cell-free DNA as a prognostic marker in stage I non-small-cell lung cancer patients undergoing stereotactic body radiotherapy. Biomarkers 20(6-7): 422-428, 2015. DOI: 10.3109/1354750X.2015.1094139

22 Park S, Lee EJ, Rim CH, Seong J: Plasma Cell-Free DNA as a Predictive Marker after Radiotherapy for Hepatocellular Carcinoma. Yonsei Med J 59(4): 470-479, 2018. DOI: 10.3349/ymj.2018.59.4.470

23 Koukourakis IM, Papadimitriou M, Desse D, Zygogianni A, Papadimitriou C, Koukourakis MI: Is anti-PD-1 immunotherapy a means for post-irradiation tumor clearance in head and neck cancer? Med Oncol 39(12): 186, 2022. DOI: 10.1007/s12032-022-01778-w

24 Koukourakis MI, Giatromanolaki A: Tumor microenvironment, immune response and post-radiotherapy tumor clearance. Clin Transl Oncol 22(12): 2196-2205, 2020. DOI: 10.1007/s12094-020-02378-8

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