

Review

Circulating Tumor Cells, Circulating Tumor DNA and Other Blood-based Prognostic Scores in Pancreatic Ductal Adenocarcinoma – Mini-Review

MARIAN LIBERKO^{1,2}, KATARINA KOLOSTOVA³, ARPAD SZABO⁴, ROBERT GURLICH⁵,
MARTIN OLIVERIUS⁵ and RENATA SOUMAROVA^{1,2}

¹Department of Radiotherapy and Oncology, University Hospital Kralovske Vinohrady, Prague, Czech Republic;

²Third Faculty of Medicine, Charles University in Prague, Prague, Czech Republic;

³Department of Laboratory Genetics, Laboratory Diagnostics,
University Hospital Kralovske Vinohrady, Prague, Czech Republic;

⁴Department of Pathology, Third Faculty of Medicine,
Charles University in Prague and University Hospital Kralovske Vinohrady, Prague, Czech Republic;

⁵Department of General Surgery, University Hospital Kralovske Vinohrady, Prague, Czech Republic

Abstract. Pancreatic ductal adenocarcinoma represents a disease with increasing incidence. Its prognosis is the worst among all malignancies despite the aggressive combined multimodal treatment across all stages. In metastatic disease, median survival is approximately one year. The mainstay of treatment is chemotherapy (neoadjuvant, palliative) and in selected subgroups of patients even radiotherapy. Nevertheless, nowadays there are very few prognostic and/or predictive biomarkers available that can be used to identify and better stratify patients based on risk to tailored treatment. Potentially, promising areas of research are circulating tumor cells and circulating tumor DNA, which can be easily obtained from peripheral blood – so called liquid biopsy. They may serve as a tool to assess response to applied treatment, and to detect the emergence of treatment-resistant clones or early disease relapse. Moreover, their study may allow identification of potentially tumor-specific alterations, which may serve as target structures for targeted (tailored) therapy. Alternatively, different prognostic

indexes/scores calculated by analysis of selected parameters of blood and/or biochemistry can be used to better stratify patients based on risk and better predict prognosis. The aim of this mini-review is to provide a basic overview of the current state of the art in this area and its potential significance for clinical practice.

Pancreatic ductal adenocarcinoma represents a disease with dismal prognosis across all its stages. Its incidence and mortality is rising and it is expected that within the next decade (in year 2030) it will become the second most common cause of death among malignancies (1, 2). Pancreatic ductal adenocarcinoma may be diagnosed in 4 different stages: resectable, borderline resectable, locally advanced and metastatic disease (3). Surgery represents the only potentially curative approach for resectable pancreatic ductal adenocarcinoma. In other clinical stages surgery is/may be considered as a potentially curative treatment (patients with borderline resectable pancreatic cancer who responded well to neoadjuvant treatment), or as a palliative method (by-pass surgery, e.g. gastroenteroanastomosis) in patients with locally advanced or metastatic disease.

Due to asymptomatic or non-specific symptoms in early stages, most patients are diagnosed in advanced stages (borderline resectable, locally advanced or metastatic carcinoma). In advanced stages, oncological treatment (chemotherapy, radiotherapy, chemoradiotherapy) is preferred over surgery. Current standard of treatment for inoperable patients is chemotherapy and in selected subgroups of patients even radiotherapy. Radiotherapy can be used

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Correspondence to: Marian Liberko, Third Faculty of Medicine, Charles University in Prague, Prague, Czech Republic. E-mail: marian.liberko@fnkv.cz

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sequentially for patients without early progression after 3-6 months of so called “induction“ chemotherapy, specifically in subgroup of patients with rather indolent disease with predominance to local or locoregional progression. In that case, the aim of radiotherapy is to increase local control rate and to allow a “chemotherapy-free period“ of treatment.

Chemotherapy is nowadays still the leading modality of oncological treatment for patients across all stages of disease. In the last few years, there is a lot of improvement/research in this area, which resulted in improved survival rates. Besides standard adjuvant chemotherapy (gemcitabine), combination regimens are available, which result in increase in the response rate, prolongation of progression-free survival (PFS) and overall survival (OS). In the adjuvant setting, the new options are the combination of gemcitabine + capecitabine (4) and mFOLFIRINOX (5). For the treatment of metastatic pancreatic ductal adenocarcinoma there is, besides gemcitabine monotherapy, the option of the combination such as gemcitabine + nab-paclitaxel (6), and for patients in very good clinical condition, without limiting comorbidities, the mFOLFIRINOX regimen (7). The new regimen for second line treatment, after progression to gemcitabine-based regimen, is the nanoliposomal formulation of irinotecan in combination with 5-fluorouracil (8). Pancreatic carcinoma, in contrast to other malignancies, was not considered for treatment with targeted agents until recently. The results of a study of patients with *BRCA1/2* mutations who were treated with the targeted agent olaparib was recently published (9).

Furthermore, despite the current progress in systemic treatment of pancreatic carcinoma, there are very few prognostic and predictive biomarkers available for routine clinical use to tailor treatment with the aim to improve survival of these patients.

In this mini-review, we present the results of studies evaluating the benefits of new biomarkers [circulating tumor cells (CTC), circulating tumor DNA (ctDNA)] and prognostic scores (prognostic score/index), which may allow better stratification and individualized treatment for these patients.

Search Strategy and Selection Criteria

Our research strategy was aimed at evaluating studies on the role of circulating biomarkers in patients with pancreatic carcinoma. Scientific articles from year 2000 to 2020 were searched using the PubMed and Web of Science databases. All searches were up to April 2020. The search terms used included “circulating tumor cells”, “circulating tumor DNA”, “prognostic markers in pancreatic carcinoma”, “systemic inflammatory response in pancreatic carcinoma”, and “NLR, PLR and prognosis in pancreatic carcinoma”. Only English language papers were reviewed.

Circulating Tumor Cells in Pancreatic Ductal Adenocarcinoma

CTCs are cells shed to the blood stream either from a primary tumor or its metastases. Their isolation and subsequent quantitative and qualitative analysis across different stages of disease have proved their prognostic and predictive significance in different malignancies (10). CTCs may serve as a liquid biopsy, and provide data regarding the presence of minimal residual disease after surgery or after completion of adjuvant treatment and therefore, identify patients with high risk of relapse. Moreover, they may be used to monitor the effect of ongoing treatment, to reveal resistance to treatment, or even to detect relapse early. Their further molecular characterisation allows the study their specific molecular alterations, and subsequently it can lead to the preparation of targeted therapy, targeting alterations in these cells, which are considered to be precursors of metastatic foci. Finally, their study may result in individualised treatment, thus, leading to improvement in survival in patients with this malignancy.

There are a lot of methods to isolate and detect CTC from peripheral blood of patients with cancer. The most common methods are based on immunomagnetic separation of cells, whereas another technique relies on cell size-based filtration. Advantages and disadvantages of these methods have been reviewed elsewhere (10).

There are a lot of studies evaluating the impact of CTC presence in the peripheral blood of patients with pancreatic cancer on PFS and OS. They have proven that CTCs are independent predictors of prognosis and may allow better stratification of patients than classical parameters (TNM classification, imaging methods, Ca19-9 levels).

Effenberger *et al.* evaluated 69 patients with pancreatic carcinoma. The presence of CTCs was confirmed in 23 out of 69 patients (33.3%). CTC-positive patients had significantly shorter PFS and OS in univariate analysis ($p=0.009$ for PFS, $p=0.030$ for OS) and in multivariate analysis (HR=4,543, $p=0.006$ for PFS and HR=2,093, $p=0.028$ for OS). The prognostic significance of CTCs in this study was confirmed even in patients treated with chemotherapy. PFS was significantly shorter in CTC-positive patients in univariate ($p=0.013$) and in multivariate analysis (HR=4,203, $p=0.010$). Moreover, four patients without CTCs were alive after 36 months (11).

Besides identification of patients with worse prognosis, there are also studies showing that the presence of CTCs in the peripheral blood of patients may predict occult metastatic spread better, or even earlier than classic imaging methods (12). Court *et al.* have evaluated 126 patients (100 with pancreatic cancer and 26 with benign lesions) and assessed the association of CTC presence and occult metastatic spread and prognosis. CTCs were detected in 78% of patients and

the number of CTCs correlated with stage of disease ($p < 0.001$). Out of 53 patients, who were (based on regular imaging methods) indicated for potentially curative surgery, 13 (24.5%) were confirmed with metastatic disease during surgery. Patients with occult metastatic disease had significantly more CTCs than patients with locally advanced disease (median 7 vs. 1 CTC, $p < 0.0001$). The presence of CTC allowed the identification of patients with occult metastatic disease preoperatively ($p < 0.0001$). Moreover, CTCs were independent predictor of DFS in univariate analysis (HR=2.36, $p=0.017$) and also independent predictor of OS in multivariate analysis (HR=1.38, $p=0.040$) (12).

Similar results indicating the prognostic significance of CTCs in patients with pancreatic carcinoma had already been published in 2 large metaanalyses, comprising more than 600 patients each. They proved the association of CTC positivity and worse survival. The first of them proved clearly worse OS (HR=1.64, $p < 0.00001$) and PFS (HR=2.36, $p < 0.00001$) in CTC-positive patients. CTCs were also associated with worse prognosis across all analyzed subgroups (before, during and after treatment) and post-treatment CTCs were the most significant predictor of a poor PFS (HR=8.36, $p < 0.0001$) (13). The second metaanalysis that comprised 623 patients showed that CTC-positive patients had worse PFS (HR=1.89, $p < 0.001$) and OS (HR=1.23, $p < 0.001$) (14).

CTCs are a heterogenous population with a different potential to generate metastatic lesions. Some CTCs present signs/markers of so called tumor initiating cells, which are probably responsible for distant dissemination.

Amantini *et al.* have evaluated CTCs in the blood of 20 patients with pancreatic carcinoma during treatment. They found, that patients with a high number of CTCs (above 10 CTCs/ml) before initiation of chemotherapy had shorter OS than patients with a lower number of CTCs (below 10 CTC/ml), ($p < 0.0006$) and showed a trend for shorter PFS ($p < 0.067$). They also evaluated the gene expression profile of CTCs in patients before and after chemotherapy. They found, that during chemotherapy there were changes in the expression of 19 genes. Among them, were genes related to stemness, aggressiveness, ability to invasion and epithelial-to-mesenchymal transition (EMT) - markers of bad prognosis. Results showed, that by applying chemotherapy, mesenchymal-like CTCs were found in the peripheral blood of patients, and that they signal increased aggressiveness of disease and resistance to applied treatment. Specifically, there was an increase in the rate of patients with mesenchymal-like CTCs, from 5% before chemotherapy to 16% after chemotherapy. Their phenotype was described as having high levels of CD44 and ALCAM, markers of cancer stemm cells or tumor initiating cells (15).

Similarly, Zhao *et al.* have evaluated the presence of phenotypically different CTCs in the peripheral blood of 107 patients with pancreatic carcinoma. They identified 3 groups

of CTCs. Epithelial CTCs (E-CTC), epithelial-mesenchymal, or hybrid CTCs (E/M-CTC, H-CTC) and mesenchymal CTCs (M-CTC). E-CTC were present in 65 (60.7%), E/M CTC in 39 (36.4%) and M-CTC in 49 (45.8%) patients. The presence of M-CTC correlated positively with TNM stage ($p < 0.01$) and distant metastases ($p < 0.01$) (16).

Similar results indicating that CTCs with markers of tumor initiating cells or cancer stemm cells are responsible for bad prognosis of patients were also obtained in another study. Porouk *et al.* examined the peripheral blood of 60 patients with pancreatic carcinoma for the presence of tumor initiating cells. These cells were identified by means of immunofluorescence positivity for CD133, CD44 and ALDH. Forty-seven of patients (78%) were positive for at least one marker of tumor initiating cells. ALDH-positive and triple-positive CTCs were independent predictors of relapse and were associated with shorter survival without progression. ALDH-positive CTCs were associated with higher risk of recurrence/relapse (HR=3.37, $p=0.03$) and median DFS was 9.7 months in patients with ALDH-positive CTCs, meanwhile in patients without ALDH-negative CTC median was not reached ($p=0.03$). Moreover, patients with triple-positive CTCs had a higher risk of recurrence/relapse than patients without these CTCs (HR=6.4, $p < 0.01$). Median DFS in the triple-positive CTC group was 9.3 months compared to 19.8 months in the group without these CTCs ($p=0.001$). In this study, ALDH-positive and triple-positive CTCs were found to have prognostic significance for OS ($p < 0.01$) (17).

Finally, Bobek *et al.* examined the blood of 24 patients with pancreatic cancer who were potential candidates for surgery. They identified CTCs in 16 out of 24 patients (66.7%). CTC positivity did not reflect disease stage, tumor size or lymph node involvement. By using the size-based filtration method they were able to cultivate CTCs for further downstream analysis. They also found, that some of the CTCs displayed plasticity which enabled some cells to invade separating membrane, representing thus a more invasive fraction of CTCs (18). The main results of studies on CTCs in pancreatic ductal adenocarcinoma are summarized in Table I.

Circulating Tumor DNA (ctDNA) in Pancreatic Ductal Adenocarcinoma

With the improvement in molecular-genetic methods circulating tumor DNA (ctDNA) can be detected and isolated for further analysis. There is a growing body of evidence, that specific genetic and epigenetic alterations in ctDNA may be markers of early detection of malignancy and assessment of treatment response in real-time, and a tool to better stratify patients. Furthermore, the identification of specific mutations in the ctDNA may also allow evaluation of the efficacy of new personalized targeted agents.

Table I. Studies evaluating the impact of CTC on outcome of pancreatic carcinoma patients.

Author	Number of patients	CTC positivity	Main findings
Effenberg (11)	69	23/69 (33.3%)	Median OS 8 months in CTC+ vs. median OS 12 months in CTC-; $p=0.030$ HR=2.093 for OS in multivariate analysis; $p=0.028$ Median PFS NR in CTC+ vs. median PFS NR in CTC-; $p=0.009$ HR 4.53 for PFS in multivariate analysis; $p=0.006$
Court (12)	126	78/100 (78%)	HR=1.69 for OS in univariate analysis; $p<0.001$ HR=2.36 for PFS in univariate analysis; $p=0.017$ HR=1.38 for OS in multivariate analysis; $p=0.040$ in 13/53 (24.5%) patients, CTC+ predicted occult metastatic spread at surgery; $p<0.0001$
Ma (13)	603	NR	HR=1.64 for OS; $p<0.00001$ (whole cohort) HR=2.36 for PFS; $p<0.00001$ (whole cohort) HR=2.20 for OS; $p=0.13$ (post treatment) HR=8.36 for PFS; $p<0.0001$ (post treatment)
Han (14)	623	268/623 (43%)	HR=1.23 for OS; $p<0.001$ HR=1.89 for PFS; $p<0.001$
Amantini (15)	20	20/20 (100%)	Shorter OS in patients with over 10 CTC/ml vs. below 10 CTC/ml; $p<0.0006$ Shorter PFS in patients with over 10 CTC/ml vs. below 10 CTC/ml; $p<0.067$ Increase in CTC with aggressive phenotype (CD44+, ALCAM+) during treatment 5% → 16%
Zhao (16)	107	84/107 (78.5%)	Shorter OS in patients with over 6 CTC/ml vs. below 6 CTC/ml (11 vs. 18 months), HR=0.54; $p<0.001$ Shorter PFS in patients with over 6 CTC/ml vs. below 6 CTC/ml (8 vs. 13 months), HR=0.52; p-value NR Epithelial CTC 65/107 (60.7%) Epithelial/mesenchymal CTC 39/107 (36.4%) Mesenchymal CTC 49/107 (45.8%) - significant correlation with TNM ($p<0.01$), M1 ($p<0.01$)
Poruk (17)	60	47/60 (78%)	ALDH+, or tripple+ CTC show significant correlation with OS; $p<0.01$ Median PFS 9.7 months in ALDH+ CTC vs. not reached in ALDH- CTC, HR=3.37; $p=0.03$ Median PFS 9.3 months in tripple+ CTC vs. 19.8 months in tripple- CTC, HR=6.4; $p=0.001$
Bobek (18)	2	16/24 (66.7%)	Some of CTC were able to invade separating membrane, representing thus more invasive fraction of CTC

CTC: Circulating tumor cells; OS: overall survival; PFS: progression-free survival; HR: hazard ratio; NR: not reported.

ctDNA is usually double-stranded, consists of small fragments (70-200 base pair) and larger fragments with a molecular weight of about 211 kb, and is present in both the plasma and the serum. It has been suggested that ctDNA originates from cells undergoing apoptosis, necrosis or proliferation, and also from the lysis of CTCs. In many malignancies, there is an increase in cell turnover and apoptosis, leading to significantly higher levels of ctDNA in this group of patients compared to those in the healthy population (19).

In pancreatic cancer, 4 genes are most commonly mutated: *KRAS*, *CDKN2A*, *SMAD4* and *TP53*. Most studies that evaluated the impact of ctDNA on the prognosis of patients with pancreatic carcinoma were based on the detection of *KRAS* mutations, which occurred in about 90-95% of cases.

Kruger *et al.* have examined the presence of mt*KRAS* ctDNA in the blood of patients with pancreatic carcinoma treated with chemotherapy (gemcitabine). In their study, comprising 54 patients, ctDNA was detected in 36 (67%) of

them. The presence of ctDNA significantly correlated with shorter PFS and OS in patients treated with gemcitabine. A change in the concentration (kinetics) of ctDNA during treatment was able to predict response earlier than the markers CEA and Ca19-9. A decrease in ctDNA concentration during the first month of treatment was an indicator of early response to treatment, meanwhile the correlation between the kinetics of CEA, Ca19-9, CYFRA 21-1 and response was not observed (20).

A similar study evaluating the potential of ctDNA was performed in patients with resected pancreatic carcinoma, who were about to start adjuvant treatment. ctDNA was detected in 23 of 37 (62%) patients preoperatively, and in 13 out of 35 (37%) patients postoperatively. Preoperatively, the presence of ctDNA vs. no ctDNA was associated with a higher risk of recurrence, median PFS of 10.3 months vs. not reached (HR=4.1, $p=0.003$), and also with worse OS, 13.6 months vs. not reached (HR=4.1, $p=0.015$). By univariate and multivariate analysis, only the presence of ctDNA

showed statistically significant relation to recurrence, relapse and OS. Similarly, postoperatively, the presence of ctDNA *vs.* no ctDNA after resection was associated with shorter time to recurrence 5.4 *vs.* 17.1 months (HR=5.4, $p<0.001$) and shorter OS, 10.6 months *vs.* not reached (HR=4.0, $p=0.003$). During a median follow-up of 38.4 months, 10 out of 22 patients with negative ctDNA after resection recurred, meanwhile in the group of ctDNA-positive patients, 13 out of 13 (100%) recurred. Moreover, this prognostically unfavourable subgroup using doublet chemotherapy in the adjuvant setting, showed a trend towards prolongation of PFS to 10.1 months *vs.* 5.1 months with gemcitabine monotherapy ($p=0.15$) (21).

A recently published study evaluated the importance of ctDNA in 104 patients with metastatic pancreatic carcinoma. This study also confirmed the prognostic significance of ctDNA. Detectable ctDNA was associated with significantly shorter PFS (HR=1.93, $p=0.012$) and OS (HR=2.36, $p=0.018$) (22).

The presence of ctDNA (and mainly changes in concentration of ctDNA during treatment) and its role in the prediction of prognosis and response to treatment was evaluated in the study of Watanabe *et al.* They longitudinally evaluated ctDNA and Ca19-9 in a group of 39 patients, who underwent surgery. Twenty-two of the patients recurred and Fisher exact test indicated that the increase in Ca19-9 levels was significantly associated with recurrence or relapse ($p<0.0001$). Nevertheless, the increased levels of Ca19-9 were not associated with the prognosis of these patients ($p=1.0$). However, the appearance of ctDNA during the longitudinal follow-up was associated with prognosis ($p<0.001$) regardless of recurrence/relapse. In a subgroup of 39 patients, who did not undergo surgery, Fisher exact test indicated that detection of ctDNA during the longitudinal follow-up was associated with prognosis ($p=0.005$), meanwhile Ca19-9 levels were not ($p=0.692$). Moreover, changes in the concentration of ctDNA during the first 6 months of chemotherapy were able to predict response to treatment, while changes in Ca19-9 levels were not (23). The inverse correlation between the concentration of ctDNA and the survival of patients was also confirmed in other studies (24, 25).

Recently, the interesting study of Patel *et al.* was published. They evaluated the presence of ctDNA and its correlation with prognosis. Moreover, they also evaluated the possibility of treatment based on alterations detected in ctDNA. The presence of ctDNA was again found to be a negative prognostic factor. They showed that the most common mutations detected in the ctDNA were in *TP53* and *KRAS* genes and that patients with advanced pancreatic carcinoma had significantly more alterations present in the ctDNA than patients with resectable stages of disease (median 2 *vs.* 0.5, $p=0.04$). Moreover, in this study, treatment based on the analysis of mutations present in the ctDNA was

applied. In a patient with alterations in 3 genes affecting the MEK pathway (*GNAS*, *KRAS* and *NF1*), 6 months of response to trametinib was achieved (26). The main results of studies on ctDNA in pancreatic ductal adenocarcinoma are summarized in Table II.

Other Prognostic and Predictive Markers in Pancreatic Ductal Adenocarcinoma

Besides the role of CTCs and ctDNA as predictive and prognostic markers, there are also other, generally cheap and easy to measure parameters to obtain prognostic scores, indexes and nomograms, which can be used to better stratify patients and identify those with a good or bad prognosis. Most of these prognostic scores evaluate different values of common laboratory parameters, for example blood count and/or specific biochemical parameters. Most of them are assessing/evaluating the influence of systemic inflammatory response, which is common in cancer, in regard to different parameters of treatment, PFS and OS.

Systemic Inflammatory Response Index (SIRI)

SIRI was initially evaluated in patients with hepatocellular carcinoma following tumor resection (27). Other separate indexes, such as neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), evaluate the impact of the systemic inflammatory response observed in many malignancies on survival. There are a lot of studies showing, that platelets may protect CTC from damage in the circulation, they also stimulate EMT (28, 29), neutrophils facilitate adhesion and colonisation to distant areas (30, 31) and lymphocytes play important role in protection against tumors by inducing cellular death (32). Nevertheless, SIRI was developed to combine in one index different parameters such as platelets, neutrophils and lymphocytes, *e.g.* pro-inflammatory and immune status of patient, which could better predict prognosis of patients than different prognostic indexes separately.

Zhang *et al.* evaluated the association of SIRI with prognosis in 419 patients with advanced pancreatic carcinoma (locally advanced 27.5% and metastatic 72.5%). By univariate and multivariate analysis, SIRI was found to be an independent predictor of OS. The group of patients with SIRI-low had better OS than the group of patients with SIRI-high ($p<0.0001$). Prognostic significance of SIRI was confirmed regardless of Ca19-9 levels (33).

Similarly, Jomrich *et al.* evaluated the significance of SIRI, but in a group of patients who underwent radical resection for pancreatic carcinoma. The study comprised 321 patients. Blood samples were drawn during a period 7 days before surgery for the primary tumor. In univariate analysis, SIRI-high was significantly associated with shorter OS ($p=0.007$).

Table II. Studies evaluating impact of ctDNA on outcome of pancreatic carcinoma patients.

Author	Number of patients	ctDNA positivity	Main findings
Kruger (20)	54	36/54 (67%)	Presence of ctDNA significantly associated with shorter PFS and OS
Lee (21)	37	23/37 (62%)	Change in ctDNA concentration → earlier predictor of response than CEA, Ca19-9 Median OS 13.6 months vs. not reached, HR 4,1; $p=0.015$ → preoperatively ctDNA+ Median PFS 10.3 months vs. not reached, HR 4,1; $p=0.003$ → preoperatively ctDNA+ Median OS 10.6 months vs. not reached, HR 4,0; $p=0.003$ → postoperatively ctDNA+ Median PFS 5.4 months vs. 17,1 months, HR 5,4; $p<0.001$ → postoperatively ctDNA+
Bernard (22)	104	NR	ctDNA+ associated with shorter OS, HR 2.36; $p=0.018$
Watanabe (23)	78	NR	ctDNA+ associated with shorter PFS, HR 1.93; $p=0.012$ ctDNA+ associated with prognosis during longitudinal follow-up; $p<0.001$ (patients after surgery) ctDNA+ associated with prognosis during longitudinal follow-up; $p=0.005$ (patients with no surgery)
Perets (24)	17	5/17 (29.4%)	ctDNA+ associated with shorter OS → 8 months vs. 37.5 months; $p<0.004$
Pietrasz (25)	31 resectable 104 advanced	6/31 (19.3%) 50/104 (48%)	ctDNA+ associated with shorter OS → 19.3 months vs. 32.2 months; $p=0.027$ ctDNA+ associated with shorter PFS → 4.6 months vs. 17.6 months; $p=0.03$ ctDNA+ associated with shorter OS → 6.5 months vs. 19.0 months; $p<0.001$ in univariate analysis HR 1.96; $p=0.007$ for OS in multivariate analysis
Patel (26)	94	70/94 (75%)	ctDNA+ associated with shorter OS → 6.3 months vs. 11.7 months; $p=0.001$ in univariate analysis HR 4.35 for OS; $p=0.001$ in multivariate analysis

ctDNA: Circulating tumor DNA; OS: overall survival; PFS: progression-free survival; HR: hazard ratio; NR: not reported.

Table III. Studies evaluating systemic inflammatory response index (SIRI) and outcome of pancreatic carcinoma patients.

Author	Number of patients	Main findings
Zhang (33)	419	SIRI-low patients have better OS than SIRI-high patients; $p<0.0001$
Jomrich (34)	321	SIRI-high patients associated with shorter OS, $p=0.007$ in univariate analysis SIRI-high patients associated with shorter OS, $p=0.004$ in multivariate analysis

OS: Overall survival.

In multivariate analysis, SIRI remained an independent prognostic factor for OS ($p=0.004$). Moreover, in multivariate analysis, there was no correlation between NLR, PLR and survival of patients when evaluated as separate markers (34). The main results of studies on SIRI in pancreatic ductal adenocarcinoma are summarized in Table III.

Other Inflammation-based Prognostic Scores and Indexes

Besides SIRI, there are also different separate indexes for patients with malignancy, including pancreatic carcinoma. These include NLR (35-38), PLR (39), prognostic index (PI) (40), and the combination of albumin level and lymphocyte count in prognostic nutrition index (PNI) (41-43).

Another one is Glasgow score (GPS) and modified Glasgow score (mGPS) (44, 45). GPS was initially evaluated in patients with non-small cell lung cancer to assess relation

of elevated CRP and low albumin levels with prognosis (46). Later, because some of studies were not able to show hypoalbuminemia as an independent predictor for OS, GPS was modified to emphasize the elevated CRP levels, known as mGPS (47). mGPS is able to better predict survival of patients regardless of primary tumor site (48).

A large study by Proctor *et al.* compared the relation of these indexes and prognostic scores with the prediction of prognosis in patients with tumors. They evaluated 8,759 patients who had assessed blood samples during a 2-year period after diagnosis of their malignancy. Elevation of mGPS, NLR, PLR, PI and PNI were predictive of shorter cancer specific survival regardless to age, sex and location of tumor (all $p<0.001$). Further, subanalysis showed that all immune-based indexes and scoring systems have prognostic

significance, but especially mGPS and PI can be used for the assessment of prognosis of patients with cancer (49).

Conclusion

This short overview of studies evaluating the significance of CTCs and ctDNA, shows that they are very promising biomarkers, which could potentially lead to individualization of treatment for patients with pancreatic carcinoma. Analysis of these biomarkers provides a better assessment of the aggressiveness of disease and the prognosis of patients than the classic TNM classification and/or standard histopathologic evaluation of tumor specimens. Furthermore, CTCs and ctDNA are more sensitive in predicting metastatic spread than imaging methods (*e.g.* computed tomography). Similarly, prognostic scores and indexes may lead to individualization of treatment and a better assessment of prognosis. Their advantage is mainly the fact that they are easy to obtain by analysing selected values of blood counts and biochemistry, and that they are relatively cheap. Nevertheless, despite advances in the systemic treatment of patients with pancreatic carcinoma, there is room for improvement, which should lead to improvement in the prognosis of patients with this dismal disease.

Conflicts of Interest

The Authors state that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

Marian Liberko - Conceptualization, Writing – Original draft, Writing – Review and Editing; Katarina Kolostova - Writing – Review and Editing; Arpad Szabo - Writing – Review and Editing; Robert Gurlich - Writing – Review and Editing; Martin Oliverius - Writing – Review and Editing; Renata Soumarova - Supervision, Writing – Review and Editing.

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