Current and future biomarkers for pancreatic adenocarcinoma

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Abstract

Although pancreatic cancer is only the twelfth most common type of cancer in the world, it features a very unfavorable prognosis. The mortality rate almost equals the incidence rate, corroborating the very poor prognosis of pancreatic cancer. The 5-year survival rate for all stages of pancreatic ductal adenocarcinoma is only 7%. Surgical resection represents the only potentially curative treatment option for pancreatic ductal adenocarcinoma patients but is often not feasible due to the advanced stage of the disease upon diagnosis. For advanced disease, palliative chemotherapy is the treatment of choice although the regimens available to date are untargeted and have extensive side-effect profiles, making them unsuitable for patients with a low performance status. For this reason, early detection of pancreatic cancer is essential in order to provide patients with an optimal therapeutic approach. Up to the present day, carbohydrate antigen 19-9 is the only diagnostic marker approved by the U.S. Food and Drug Administration but its diagnostic potential is limited due to its restricted sensitivity and specificity, supporting the urgent need for novel biomarkers. In addition, prognostic and treatment-predictive biomarkers might provide essential information regarding personalized treatment decisions for individual patients. In this article, we aim to review current and future diagnostic, prognostic, and treatment-predictive biomarkers for pancreatic cancer.

Keywords

MicroRNAs, macrophage inhibitory cytokine 1, PAM4, gemcitabine, FOLFIRINOX, human equilibrative nucleoside transporter 1

Introduction

Pancreatic cancer is the twelfth most common cancer in the world with a global incidence of 2.4 to 8.6 cases per 100,000 people per year. The incidence is highest in the developed world and among men.1 About 85% of all pancreatic cancers are pancreatic ductal adenocarcinoma (PDAC).2 The mortality rate of 2.3 to 8.3 deaths per 100,000 people per year almost equals the incidence rate, emphasizing the very poor prognosis of the disease.1 In recent years, the 5-year survival rate for all stages of PDAC only marginally improved and reaches only 7% for all stages.3 The only potentially curative treatment option is surgical resection, partly combined with neoadjuvant and/or adjuvant chemoradiotherapy, but only 15%–20% of all patients are eligible for this treatment option due to advanced stage of disease at the time of diagnosis.2 For locally advanced and metastatic pancreatic cancer, systemic chemotherapy is the treatment of choice, whereas the importance of radiotherapy has been controversially discussed.2 Nevertheless, systemic chemotherapy for PDAC is non-targeted and features a wide range of side-effects.4

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Due to its unfavorable prognosis and the lack of effective treatment options at the later stages of disease, early diagnosis of pancreatic cancer is essential to optimize possible treatment options and to improve the outcome of patients suffering from PDAC. Furthermore, treatment-predictive and prognostic biomarkers represent a valuable tool to divide pancreatic cancer patients into different subgroups in order to provide them with an optimal personalized therapeutic approach according to their likelihood to benefit from a specific surgical, chemotherapeutic, or conservative treatment. Up to the present day, carbohydrate antigen (CA) 19-9 is the only available biomarker for PDAC approved by the U.S. Food and Drug Administration (FDA), but it features a poor diagnostic sensitivity and specificity.\(^5,6\)

For this review on currently available and potential future biomarkers for the diagnosis, prognosis, and treatment prediction of pancreatic adenocarcinoma, studies indexed in MEDLINE between 1990 and 2016 (April) were reviewed. The terms “pancreatic cancer,” “PDAC,” “biomarker,” “diagnostic,” “prognostic,” “therapeutic,” and combinations of these terms were used.

**Diagnostic biomarkers**

Due to its unfavorable prognosis, particularly at late stage of disease progression, early detection of pancreatic cancer is essential to provide patients with a curative therapeutic approach. To date, no ideal diagnostic biomarker for early detection of PDAC exists. The following section reviews the advantages and limitations of present and future diagnostic biomarkers for pancreatic cancer. Table 1 gives a brief summary on the discussed markers and shows their sensitivity and specificity if applicable.

**CA19-9, carcinoembryonic antigen, and other carbohydrate antigens**

CA19-9 is the most extensively studied biomarker for PDAC and the only one approved by the FDA.\(^5\) Nevertheless, in terms of diagnostics, it can be considered as a rather poor biomarker for pancreatic cancer as it is also elevated in other medical conditions, such as acute cholangitis, liver cirrhosis, pancreatitis, and obstructive jaundice.\(^28–31\) The median sensitivity and specificity of CA19-9 for the diagnosis of PDAC are only 75.5% and 77.6%, respectively,\(^6\) and with a low positive predictive value of 0.5%–0.9%, which does not qualify it as a useful screening parameter.\(^31\) Furthermore, patients with Lewis-null blood type do not produce CA19-9. Given the fact that approximately 5%–10% of Caucasians are of this phenotype, the diagnostic power of CA19-9 is even more inferior.\(^5,32\) The diagnostic potential of CA242 and carcinoembryonic antigen (CEA) for early detection of PDAC is also limited. Zhang et al.\(^6\) just recently described a median sensitivity/specificity of 67.8%/83% for CA242 and 39.5%/81.3% for CEA, respectively. Similar negative results were found for CA50, CA195, CA72-4, and CA125.\(^33\) Although the combination of CA19-9 and CA242 resulted in a higher sensitivity of 89% (with no impact on the specificity), none of these conventional biomarkers has proven to be a specific and reliable tool for early detection of PDAC.\(^6\)

**MicroRNAs and other non-coding RNAs**

MicroRNAs (miRNAs) are a group of small non-coding RNAs consisting of 18–25 nucleotides that are capable of regulating post-transcriptional modifications of multiple genes.\(^34,35\) In recent years, the usage of miRNA as a detection marker for different types of cancer has increasingly gained importance.\(^36,37\) As a potential biomarker for pancreatic cancer, miRNAs have been most intensively investigated in pancreatic tumor tissue, blood samples, pancreatic juice, stool, and urine.\(^38\) Among others, the most promising findings have been made for miR-21, miR-155, miR-196a, and miR-210, which were shown to be upregulated in pancreatic tissue,\(^22,25,39–41\) serum samples,\(^19,23,24,42,43\) fecal specimen,\(^20,44\) and pancreatic juice\(^21,45,46\) from PDAC patients. In contrast, miR-216 and miR-217 seemed to be consistently downregulated in pancreatic tissue, stool samples, and pancreatic juice.\(^20,25,45–47\) Since the acquisition of pancreatic tissue samples and pancreatic juice requires invasive diagnostic approaches, these methods are not suitable for a diagnostic screening test. Thus, non-invasive approaches like serum and stool samples should be preferred as a diagnostic tool. Just recently, urinary miRNA-concentrations as a further non-invasive marker for early detection of pancreatic cancer were investigated. The group showed that urine levels of miR-143, miR-223, and miR-30e were significantly increased in Stage I PDAC when compared with healthy individuals, showing a sensitivity of 83.3% and a specificity of 96.2% for the combinational use of miR-143 and miR-30e.\(^26\) Comparable results were shown for fecal specimens by Yang et al.\(^20\) who described a sensitivity and specificity of 83.3% each by combining miR-21, miR-155, and miR-216 as a potential screening tool for detection of PDAC.

Particular attention should be paid to miR-21, miR-155, and miR-196a since different studies have shown an upregulation of these miRNAs in tissue samples of intraductal papillary mucinous neoplasm (IPMN) and pancreatic intraepithelial neoplasia (PanIN), suggesting their use as promising biomarkers especially for early detection of precursor lesions with malignant potential.\(^40,48–52\) So far, no consistent data on the existence of these miRNAs in serum or stool samples of patients with precursor lesions are available, indicating the need for future studies to evaluate the role of a non-invasive way to detect miRNAs as early detection markers.
Besides microRNAs, other non-coding RNAs have been identified that might play a potential role as a detection marker for PDAC. Long non-coding RNAs (lncRNAs) are a group of RNAs that are not translated into proteins and consist of more than 200 nucleotides. Although the biological functions of these RNAs have not been fully elucidated to date, a growing body of evidence exists, suggesting significant changes in the expression of lncRNAs in pancreatic cancer tissue and cell lines. Particularly, the expression of HSATII appears to be highly upregulated in pancreatic cancer and pre-cancer tissues compared to healthy pancreatic tissue.

Currently, there is limited data on whether lncRNAs are as stable as miRNAs and could therefore function as a useful serum/plasma marker for PDAC.

Small ncRNAs are a group of RNAs that consist of up to 200 nucleotides, including small nucleolar RNA (snoRNA), small nuclear RNA (snRNA), and Piwi-interacting RNA (piRNA). Although different small ncRNAs have been shown to be highly abundant in serum and plasma samples of various cancer types and U2snRNA was shown to be highly expressed in PDAC patients, no specific small ncRNA for pancreatic cancer has been identified so far.

Macrohage inhibitory cytokine 1 and PAM4

Macrophage inhibitory cytokine 1 (MIC-1) is a distant member of the transforming growth factor beta (TGF-β) superfamily that is overexpressed in different cancer entities. Koopmann and colleagues were the first to analyze MIC-1 serum levels from 50 patients with resectable pancreatic adenocarcinoma. The receiver operating characteristic (ROC) curve analysis showed that MIC-1 was significantly better than CA19-9 in differentiating patients with pancreatic cancer from healthy controls (area under
the curve (AUC) of 0.99 and 0.78, respectively; p = 0.003), but not in distinguishing pancreatic cancer from chronic pancreatitis (AUC of 0.81 and 0.74, respectively; p = 0.63). In a recently published meta-analysis including a total of 1235 pancreatic cancer patients, Chen et al. described a pooled sensitivity of 79% with a specificity of 86% for serum measurements of MIC-1. Similar results have been observed by other groups who suggested that the lack of diagnostic specificity of MIC-1 might be enhanced using a combination of MIC-1 and CA19-9.64-66 Furthermore, MIC-1 had a sensitivity of 63.1% in detecting patients with CA19-9-negative PDAC.65

PAM4, a monoclonal antibody known as clivatuzumab, is reactive to Mucin 5AC (MUC5AC), a secretory mucin, which is expressed de novo in early pancreatic neoplasia and retained throughout disease progression.67-69 In a large study analyzing sera from 596 patients with confirmed PDAC, other types of cancer, benign diseases of the pancreas and healthy controls, Gold et al. demonstrated an overall sensitivity of 76% for PAM4 detection of PDAC with a high specificity of 85% when compared to benign pancreatic disease with a positive likelihood ratio of 4.93. In comparison to CA19-9, showing a sensitivity of 77% with a specificity of only 68% and a positive likelihood ratio of 2.85 (p = 0.026 compared to PAM4), PAM4 seems to be a biomarker of equal if not better validity for the detection of pancreatic carcinoma in their study. Again, the combined PAM4 and CA19-9 biomarker assay demonstrated the best results with a sensitivity and specificity of 84% and 82%, respectively.14

Other diagnostic biomarkers

S100A6 is a calcium-binding protein that was shown to be overexpressed in pancreatic cancer.70 Although its function in the pathogenesis of PDAC has not been fully elucidated to date, Ohuchida et al. found that S100A6 levels measured in the pancreatic juice significantly discriminated between patients with chronic pancreatitis and PDAC or IPMN (area under the ROC curve for PDAC and IPMN is 0.864 and 0.749, respectively). Similar observations were made for S100A6 expression levels in endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) specimens of PDAC patients. To date, no data on circulating S100A6 serum levels are available.

Osteopontin (OPN), a secretory protein with a variety of functions, including inflammatory reaction and apoptosis, has been found to be upregulated in PDAC and was linked to invasiveness as well as metastatic growth of pancreatic cancer cells.72,73 Moreover, serum OPN levels in PDAC patients are significantly elevated compared to healthy controls.74 In a cohort of 50 PDAC patients, Koopmann et al. showed that elevated serum levels of OPN had a sensitivity of 80% with a specificity of 97% for the detection of pancreatic cancer. Although OPN appeared to differentiate patients with PDAC from chronic pancreatitis patients and even distinguished early stage PDAC from healthy controls, the diagnostic accuracy of OPN was not superior to CA19-9 in a larger study published by Poruk et al. However, using a diagnostic algorithm that combines OPN, CA19-9, and tissue inhibitor of metalloproteinase 1 (TIMP-1), the group obtained a superior diagnostic sensitivity and specificity for PDAC of 87% and 91%, respectively, thus providing a panel that improves diagnostic accuracy compared to any of these three biomarkers individually.17

Brandt et al. attained comparable results with a serum biomarker panel consisting of osteoprotegrin (OPG), intercellular adhesion molecule 1 (ICAM-1), and CA19-9 showing a sensitivity and specificity of 88% and 90%, respectively, for diagnosis of PDAC. Nevertheless, contradictory observations were made for serum protein levels of ICAM-1 and TIMP-1. In a study comprising 100 patients with PDAC, chronic pancreatitis, and benign jaundice due to gall stones, levels of ICAM-1 and TIMP-1 were found to be upregulated in patients with jaundice due to both PDAC and gall stones but neither protein did qualify for the detection of early PDAC.75

Genetic and epigenetic markers

KRAS is the most frequently mutated oncogene in pancreatic cancer showing somatic mutations clustered in specific hotspot regions in more than 90% of cases.76-78 Other mutated genes, which are most commonly found in PDAC include TP53 (tumor protein p53), SMAD4, and CDKN2A (cyclin dependent kinase inhibitor 2A).79 A recently published meta-analysis examining the accuracy of KRAS gene mutation analysis for diagnosing PDAC revealed that a combination of KRAS mutation analysis and the cytological analysis of an EUS-FNA specimen can increase the sensitivity from 80.6% to 88.7%, compared to EUS-FNA alone, with a pooled specificity of 92%.80 Especially for cases where cytology is inconclusive, KRAS seems to be of diagnostic value.80 Considering rather low levels of cell-free circulating tumor DNA (ctDNA) in the patients’ serum, the application of KRAS mutation analysis as a non-invasive diagnostic biomarker seems to be challenging. However, Kinugasa et al. demonstrated that the overall survival of patients with KRAS mutations in ctDNA was significantly shorter compared to patients without mutations, suggesting this non-invasive method as a novel strategy for the diagnosis of PDAC as well as for predicting survival. Contrary results were published by Singh et al., who did not find a significant correlation between serum KRAS mutation status and different clinicopathological parameters or survival. Thus, additional studies are needed to investigate the potential role of KRAS mutation analysis as a diagnostic biomarker. Mutations of TP53 and the loss of SMAD4 emerge during
later stages of disease and are most commonly found in grade 3 pancreatic intraepithelial neoplasia and invasive carcinoma. For this reason, their use as a potential biomarker for early disease detection is rather limited.

Circulating tumor cells

Although hematogenous dissemination of tumor cells is generally thought to occur at an advanced stage of cancer progression, it was shown that in a genetic mouse model of PDAC pancreatic cells were detectable in the bloodstream prior to tumor formation. In line, a subsequently performed study provided evidence that circulating epithelial cells were also detectable in 73% of patients with PDAC but in none of the patients without cystic lesions or pancreatic cancer.

Treatment predictive markers

Disease specific and effective treatment options for pancreatic cancer are limited. Therefore, treatment-predictive markers might represent a possibility to improve treatment response and to individually tailor personalized treatments for patients suffering from PDAC. Different markers have been proposed to optimize the treatment of pancreatic cancer:

Gemcitabine markers

Gemcitabine can be referred to as the standard chemotherapy for patients with metastatic pancreatic cancer as well as for patients with resected pancreatic cancer in an adjuvant setting. The intracellular uptake of hydrophilic gemcitabine is mainly dependent on physiologic nucleoside transporter proteins. Both known subgroups of this protein family, equilibrative nucleoside transporters (ENTs), and concentrative nucleoside transporters (CNTs) have been suggested as biomarkers for gemcitabine treatment.

Human ENTI. There is a growing body of evidence promoting human ENTI (hENT1) as a predictive and prognostic marker for gemcitabine treatment. Using immunohistochemistry to analyze hENT1 expression in biopsies of patients treated with gemcitabine for advanced pancreatic cancer, Spratlin et al. showed a superior median survival of 13 versus 4 months (p = 0.01) for patients with uniformly detectable hENT1 immunostaining. Similar results were obtained in a cohort of 198 patients with early stage pancreatic cancer receiving gemcitabine or 5-fluorouracil (5-FU) as part of their systemic adjuvant therapy. Overall and disease-free survival in the gemcitabine arm but not in the 5-FU arm was significantly improved in patients with high hENT1 protein expression determined by immunohistochemistry assessment on tissue microarrays. These findings were recently confirmed in a group of 380 pancreatic cancer patients from the European Study Group for Pancreatic Cancer (ESPAC)-3 trial.

Besides immunohistochemical analyses, evaluation of hENT1 gene expression has also been demonstrated as a diagnostic tool. Using quantitative reverse transcription polymerase chain reaction (qPCR) for tumor tissues of patients with different stages of pancreatic cancer, Giovannetti et al. found that patients with high levels of hENT1 had a significantly longer overall survival of 25.7 months compared to 8.5 months in the lower expression subgroup. These results were corroborated by in vitro experiments, showing an increased sensitivity to gemcitabine in pancreatic cancer cell lines with high hENT1 gene expression.

Yamada et al. analyzed pre-treatment hENT1 expression levels in EUS-FNA specimens obtained from patients with different stages of PDAC and compared them to resected specimens after gemcitabine-based chemoradiotherapy. They confirmed that hENT1 expression was an independent prognostic factor in all patients and in the subgroup that underwent resection. Interestingly, hENT1-negative patients with resection at advanced disease stage had a similar prognosis to those without resection. Thus, analysis of pre-treatment hENT1 expression levels obtained by EUS-FNA might function as a potential biomarker in order to select a subgroup of patients eligible for surgical treatment.

In an ongoing interventional randomized clinical trial, the Alberta Health Service (AHS) Cancer Control aims at assessing whether hENT1 is capable of predicting a treatment response to gemcitabine in advanced pancreas cancer and whether a combination of 5-FU, leucovorin, and oxaliplatin (FOLFOX) instead of gemcitabine might be a superior treatment option for patients with low hENT1 expression (ClinicalTrials.gov Identifier: NCT01586611).

Human CNT3 and deoxycytidine kinase. Human CNTs (hCNTs) represent the second group of gemcitabine membrane transporters, using the sodium gradient to shift gemcitabine across the plasma membrane. Analyzing tumor blocks from 45 PDAC patients treated with gemcitabine-based radiochemotherapy after curative resection, Maréchal et al. showed significantly longer 3-year survival rates for patients with high hCNT3 expression compared to patients with low hCNT3 expression (54.6% vs 26.1%; p = 0.028). The combination of hCNT3 and hENT1 as treatment-predictive biomarkers seems to be even more superior, with a 3-year survival rate of 81.1% for hCNT3high/hENT1high patients, supporting the combined use of those biomarkers rather than using one alone. Deoxycytidine kinase (dCK) is the rate-limiting enzyme, which phosphorylates and thereby converts gemcitabine into its active form. In a cohort of 40 pancreatic cancer patients receiving gemcitabine-based adjuvant chemotherapy, high dCK messenger RNA (mRNA) levels significantly correlated with longer
disease-free survival.\textsuperscript{96} Given the limited data available to date, further clinical investigations are needed to assess the potential use of hCNT3 and dCK as treatment-predictive biomarkers for gemcitabine therapy.

**FOLFIRINOX markers**

FOLFIRINOX is a chemotherapy regimen for advanced stage PDAC combining leucovorin, 5-FU, irinotecan, and oxaliplatin.\textsuperscript{97} Although many clinical trials have shown a superior efficacy of FOLFIRINOX over standard gemcitabine, its use is limited to patients with good performance status (PS) due to increased systemic toxicity.\textsuperscript{97–99} Given the unfavorable side-effect profile, treatment-predictive biomarkers are essential to determine a subgroup of patients that will benefit most from FOLFIRINOX therapy.

Carboxylesterase 2 (CES2) is an enzyme that converts irinotecan, one of the components of FOLFIRINOX, into its active form SN-38.\textsuperscript{100} CES2 expression in PDAC tumor tissue has been suggested as a potential predictive biomarker.\textsuperscript{101} Capello et al.\textsuperscript{101} showed that high-expression levels of CES2 in tumor tissue were associated with longer overall and progression-free survival in resectable and borderline resectable patients treated with neoadjuvant FOLFIRINOX.

Moreover, genome analyses of pancreatic cancer might function as a predictive marker for platinum-based chemotherapy, such as oxaliplatin as part of the FOLFIRINOX regimen. Using whole-genome sequencing, Waddell et al.\textsuperscript{79} described an above-average response to platinum therapy in individuals with inactivation of DNA maintenance genes such as BRCA1, BRCA2, or PALB2. BRCA1 and PALB2 have been proposed as potential response predictors of pancreatic cancer to DNA damaging agents in the past and might therefore play a role as markers for personalized therapy decisions in future.\textsuperscript{102}

**Nab-paclitaxel markers**

Nab-paclitaxel (albumin-bound paclitaxel particles) has been shown to significantly improve overall survival, progression-free survival, and response rate when added to the standard gemcitabine treatment regimen in patients with metastatic pancreatic cancer.\textsuperscript{103} Secreted protein acidic and rich in cysteine (SPARC), also known as osteonectin, is a glycoprotein highly expressed in different human malignancies, and its deregulation has often been correlated with disease progression and a poor prognosis.\textsuperscript{104} In head and neck cancer, SPARC overexpression has been associated with tumor response to nab-paclitaxel in a small group of patients,\textsuperscript{105} suggesting that tumoral SPARC-albumin interactions facilitate an accumulation of albumin in the tumor, thereby increasing the efficacy of albumin-bound paclitaxel.\textsuperscript{105} Despite promising early data,\textsuperscript{106} Hidalgo et al.\textsuperscript{107} showed that SPARC expression in the tumor stroma, tumor epithelia, and patient’s plasma is not associated with the efficacy of nab-paclitaxel and gemcitabine in patients with metastatic pancreatic cancer. To our knowledge, no other reliable treatment-predictive markers for nab-paclitaxel therapy have been described to date.

**Prognostic biomarkers**

For a disease as lethal as pancreatic cancer, prognostic factors are indispensable to provide a personalized treatment for PDAC patients with an individualized balance between treatment efficacy and side effects.

**Eastern Cooperative Oncology Group PS**

The Eastern Cooperative Oncology Group (ECOG) PS has been described as a prognostic marker for different malignancies.\textsuperscript{108,109} For pancreatic cancer, an unfavorable PS is an independent negative prognostic marker.\textsuperscript{110,111} Especially with regard to an optimal treatment choice, ECOG PS should be considered as a valuable marker because PDAC patients with poor PS usually do not benefit from combination or intensified chemotherapy regimens such as FOLFIRINOX.\textsuperscript{112} Furthermore, patient’s age significantly influences survival in pancreatic cancer.\textsuperscript{113,114} However, the physiological age needs to be differentiated from the chronological age when it comes to treatment decisions.\textsuperscript{112,115}

**SPARC**

SPARC—already discussed as treatment-predictive biomarker for nab-paclitaxel treatment—also has a prognostic value. As already shown for a variety of other cancers,\textsuperscript{116–118} SPARC overexpression in PDAC patients correlates with a poor prognosis.\textsuperscript{119} Interestingly, in pancreatic cancer, the location of SPARC expression seems to play a decisive role. Infante et al.\textsuperscript{120} published a study demonstrating that overexpression of SPARC by peritumoral fibroblasts but not by tumor cells themselves predicts prognosis for patients with pancreatic cancer. Patients with positive tumor stroma for SPARC had a significantly shorter median survival compared to patients with SPARC negative stroma (15 vs 30 months; p < 0.001). Similar prognostic capabilities were published for SPARC mRNA levels measured in tissue samples of pancreatic adenocarcinoma patients.\textsuperscript{121}

**CA19-9**

As the most extensively studied and validated biomarker for PDAC, CA19-9 has also been examined in terms of its prognostic value. In a large meta-analysis, Ballehaninna and Chamberlain\textsuperscript{11} concluded that normal (<37 U/mL) or moderately elevated pre-operative CA19-9 serum levels (<100 U/mL) independently predict improved overall...
survival, whereas elevated CA19-9 serum levels (>100 U/mL) are associated with a poor prognosis. Furthermore, the group stated that post-operative normalization or a downward trend of CA19-9 serum levels after pancreatic resection is associated with prolonged survival, while constantly elevated CA19-9 levels following pancreatic resection reflect residual disease and thereby predict a poor survival.31

MicroRNAs
Besides the emerging role of miRNAs as a potential diagnostic biomarker for PDAC, their use as a prognostic tool has also been evaluated. A recently published meta-analysis including 1525 patients treated for PDAC demonstrated a significantly shortened overall and disease-free survival in patients with high tumoral miR-21. In addition, a poor overall survival was found for high levels of miR-155 and miR-203 as well as low miR-34a levels.122 In addition, among others, low-expression levels of miR-218 and miR-494 in tumor tissue as well as high miR-221/222 and miR-744 levels in tumor tissue and plasma, respectively, have been found to predict poor survival in PDAC patients.123–126

Prognostic indices
Combination of various prognostic markers has led to the establishment of prognostic indices. Using a combination of five parameters (PS, hemoglobin, leucocyte count, neutrophil–lymphocyte ratio, and CEA), Park et al.127 divided patients with metastatic pancreatic cancer into three risk groups and showed a median overall survival of 11.7, 6.2, and 1.3 months for the low, intermediate, and high-risk groups, respectively (p < 0.001). For patients with advanced disease receiving palliative chemotherapy, a prognostic index model of three clinical parameters (ECOG PS, CA19-9 serum levels, and C-reactive protein (CRP) serum levels) was published by Xue et al. The median overall survival for the low-risk and high-risk group was 9.9 and 5.3 months, respectively (p < 0.001) with estimated 1-year survival rates of 40.5% (low-risk group) and 5.9% (high-risk group; p < 0.05).128

Markers of disease recurrence
Soluble iC3b (siC3b), the cleavage product of complement factor C3b, has been investigated as a marker to predict disease recurrence in patients with adjuvant treatment after resection of PDAC.129 The inactivated complement component is expressed on apoptotic cells, including pancreatic cancer cells.130 Märtten and colleagues129 showed that siC3b plasma levels were increased up to 4 months before radiologically defined recurrence with a sensitivity and specificity resulting in an AUC of 0.85. The combination of siC3b and CA19-9 leads to an AUC of 0.92.

Conclusion
Pancreatic cancer remains one of the most devastating diagnoses to date. Only in case of early diagnosis, curative treatment approaches are available. Thus, diagnostic modalities allowing tumor detection at an early time-point might have a decisive role in the treatment of patients with PDAC. However, at present, no serum-based (and therefore easily accessible) biomarker has a sufficient sensitivity or specificity to be used in clinical routine. Recent research in the context of PDAC and other cancers suggest that innovative molecules such as circulating miRNAs might overcome these limitations of the present protein-based biomarkers. Besides being used in the context of diagnosis, treatment-predictive biomarkers and prognostic biomarkers might have an essential role in providing an optimal and personalized treatment to all the patients with pancreatic cancer. Despite major scientific efforts during the last decades, the ideal biomarker for these purposes has not been identified yet. There is a large number of promising biomarkers, including various tumor and serum proteins, microRNAs, as well as genetic markers that might fulfill these requirements in future. Especially the combination of different markers as diagnostic or prognostic indices appears promising. Nevertheless, further studies are needed to validate novel biomarkers and to further evaluate potential future markers. Thus, in spite of recent progresses in the therapy of patients with PDAC, serum-based biomarkers might not only help to allow an early diagnosis but also to estimate patients’ prognosis and to guide patients’ therapy through the different available therapeutic options.

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