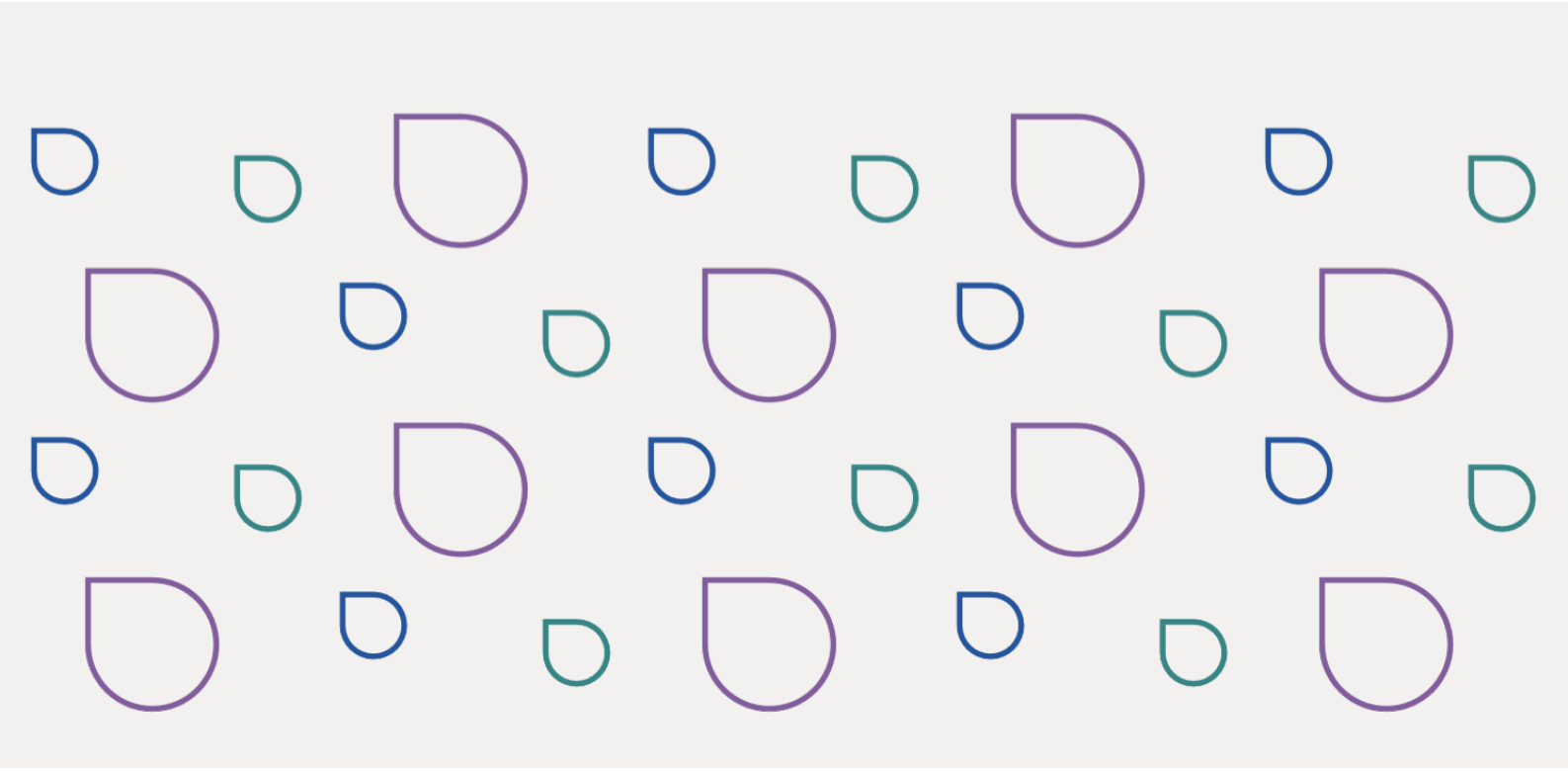
White Paper

Prof. Roland Andersson

March 2024



RECCAN

Blood-based pancreatic cancer diagnostics:

Reccan™ Immunoassay

A breakthrough in the detection of pancreatic cancer

The challenge of pancreatic cancer: global health concern and economic burden
Pancreatic cancer is one of the major global healthcare challenges today because of its aggressive nature, poor prognosis, late diagnosis and limited response to conventional chemotherapies.

Pancreatic cancer ranks as the EU's 8th most prevalent cancer, representing 3.7% of all cancer cases, and stands as the 3rd leading cause of cancer-related deaths at 7.5% (1). Notably, pancreatic cancer has the lowest survival rate among all cancers in Europe (2). In 2020, Europe experienced 140,116 new cases of pancreatic cancer and 132,134 deaths attributed to the disease, reflecting the stark reality behind the statistics. Globally, 495,773 pancreatic cancer diagnoses and approximately 470,000 deaths occurred in 2020 making pancreatic cancer the 12th most common cancer and 7th leading cause of cancer-related deaths (3). Consequently, pancreatic cancer imposes a significant burden on healthcare systems, with Germany alone incurring direct pancreatic cancer-related costs of approximately €721M in 2015 (4). In Sweden, the societal costs of pancreatic cancer were approximated to €125 million in 2018 (5). Patients with pancreatic cancer experience monthly medical expenses 15 times higher than those without, with costs escalating as the disease progresses (6).

Growing incidence and mortality
In contrast to decreasing death rates in most cancer types, pancreatic cancer incidence and mortality have risen over the past decade (7). In the EU, pancreatic cancer deaths surged by 62% between 1992 and 2016 (8).
Despite significant scientific progress in understanding pancreatic cancer mechanisms, survival rates have stagnated for nearly four decades (7), primarily due to late-stage diagnoses and the lack of new effective treatment options. Once pancreatic cancer metastasizes, it becomes practically incurable, underscoring the pivotal role of early diagnosis in altering the trajectory of cancer mortality (9). Pancreatic cancer mortality is projected to surpass colorectal cancer before 2030, becoming the second leading cause of cancer-related death in the US (10).

Diagnosis and survival rates
The 5-year overall survival rate of pancreatic cancer is less than 10% and depends heavily on the stage at diagnosis. Early-stage pancreatic cancer presents minimal symptoms, often overlapping with other conditions (e.g., weight loss, appetite loss, new-onset diabetes), leading to misdiagnosis. Consequently, 52% of pancreatic cancer cases are diagnosed at an advanced, metastatic stage with minimal prospects for cure (11). For these patients, the 5-year survival rate is less than 3%. Metastatic pancreatic cancer diagnosis carries an average life expectancy of

merely three to six months (12). Approx. 85% of pancreatic cancer cases are diagnosed at non-resectable stages, rendering them incurable (13) as surgical resection remains the sole curative option. However, early detection of localised pancreatic cancer (currently only 12% of cases) allows for surgical intervention, significantly elevating the 5-year survival rate to around 44% (14).

Challenges and opportunities in pancreatic cancer screening

Cancer of the pancreas remains difficult to diagnose and treat. Early detection of localised pancreatic cancer enables the identification of curable lesions and may have a profound effect on the course of the disease. Several high-risk cohorts have been identified where screening is warranted, such as individuals with familial/genetical risk, new-onset diabetes at >50 years of age or patients with diffuse symptoms suggestive of pancreatic cancer where the clinicians consider ruling out pancreatic cancer (15) (16) (17). However, there is currently and historically a lack of simple, accurate and cost-effective screening tests to select patients for image-based diagnostic confirmation.

Today's standard of care

The current gold standard for pancreatic cancer diagnosis and staging is CT with a pancreas protocol (Figure 1). CT provides high temporal and spatial resolution and wide anatomic coverage but is inappropriate for surveillance due to ionizing radiation and limited diagnostic performance in small lesions (18). CT studies reported varied results with sensitivity values ranging from 78% to 96.8% and specificity values between 40% and 100% (19). If pancreatic cancer tumours of less than 2 cm in diameter were included in the CT detection, the sensitivity drops to 63–77% (20).

MRI can complement CT in the diagnosis of pancreatic cancer with an average sensitivity of 93% and specificity of 89% (19). PET/CT provide complementary information, while Endoscopic US may enable tumour biopsy. Ultrasound examination is not optimal for accurately capturing small pancreatic cancer lesions due to the interference of gases in the gastrointestinal tract (21).

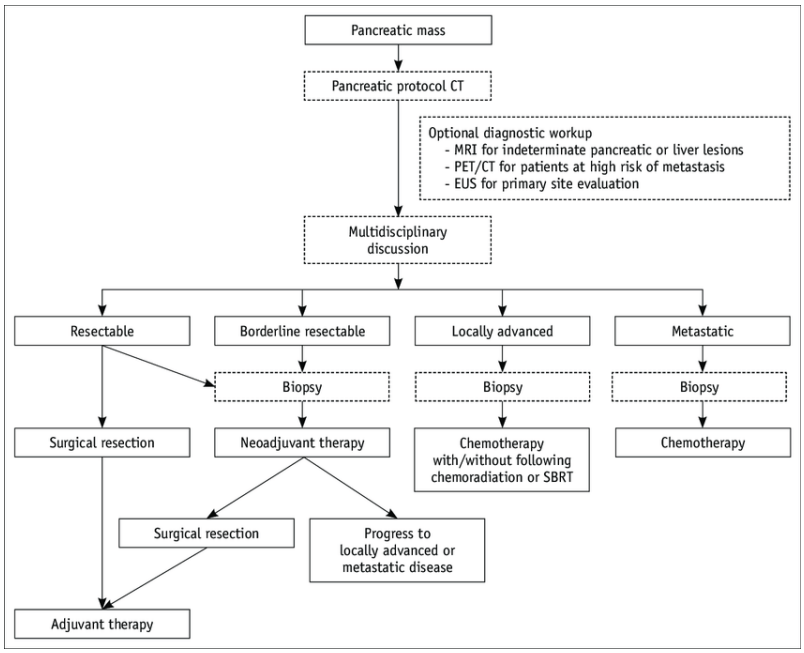


Figure 1. Diagnostic work-up of pancreatic cancer (22)

	Strengths	Weaknesses	Sensitivity	Specificity
Ultrasound	Accessibility	Operator dependent	88%	94%
CT	High spatial and temporal resolution; anatomical coverage	Ionizing radiation; limited diagnostic performance of small lesions	63-96%	40-100%
MRI	Excellent soft-tissue contrast; superior visualization of pancreatic and biliary anatomy; small lesions	Low spatial resolution; motion artifacts	93%	89%
EUS	Excellent spatial resolution; enables tissue biopsy	Invasive; operator dependent	91%	86%
PET	Most pancreatic cancers have increased 18FDG uptake; useful for evaluation of metastatic spread	Difficult to differentiate pancreatic cancer and pancreatitis	89%	70%

Table 1. The characteristics of different imaging modalities (19)

Current blood-based biomarkers available

Carbohydrate antigen (CA19-9) is the only established serum biomarker for pancreatic cancer. It may be used in the diagnostic work-up of pancreatic cancer in conjunction with imaging or for treatment monitoring (23), but not for screening purposes due to limited performance, with an inadequate sensitivity of 79-81% and a specificity of 82-90% (24). Although multiple serum markers for pancreatic cancer have been investigated (25) (26), none have been validated for routine clinical use.

Limitations of CA19-9 (27)*:*

- Not tumour type-specific, with elevated levels also observed in other malignancies (colorectal cancer, cholangiocarcinoma, hepatocarcinoma, gastric cancer) and benign diseases (pancreatitis, obstructive jaundice, cirrhosis, cholangitis, and other gastrointestinal disease), limiting its diagnostic accuracy (28).
- Various types of adenocarcinomas may lead to elevated CA19-9 levels.
- Approximately 5-10% of the Caucasian population are Lewis negative and thus unsuitable for widespread screening purposes (29).
- Poor sensitivity for early, small pancreatic tumors. Only around 50% of small pancreatic tumors <3 cm has elevated CA19-9 levels. (30).
- Low sensitivity and specificity render it inadequate as a standalone diagnostic tool for early pancreatic cancer detection.

Multi-biomarker assays

Pancreatic cancer is notoriously difficult to detect in its early stages due to nonspecific symptoms and lack of effective screening methods. Single biomarkers are insufficient since not all pancreatic cancer tumours (e.g., low-stage or low-grade) exhibit a single molecular change. Single biomarkers show ineffectiveness not only for pancreatic cancer but also for other cancers. Accordingly, the concept that the presence or absence of one molecular biomarker will aid clinical evaluation has not proved to be the case.

During recent years there has been a growing interest in combining various biomarkers. Multiplex testing enables the identification of biomarker patterns associated with pre-cancerous lesions or early-stage pancreatic cancer, facilitating early intervention and potentially improving patient outcomes.

One example is Gu YL et al. (31) investigating a biomarker combination of CA19-9, CEA, CA125, and CA242 that showed high sensitivity and specificity for pancreatic cancer diagnosis, with up to 90.4% and 93.8%, respectively, these figures are significantly higher than the accuracy of a single serum marker. Another example is the IMMray PanCan-d test that combined an 8-plex biomarker signature with CA19-9 in a proprietary algorithm to detect pancreatic cancer in serum samples. Before the withdrawal from the market, the test distinguished pancreatic cancer stages I–IV vs familial/hereditary high-risk individuals with 98% specificity and 87% sensitivity (29).

Urine proteins have also been established as a means through which pancreatic cancer can be detected, with previous proof-of-concept studies demonstrating that protein signatures associated with pancreatic cancer can be detected in the urine. Radon et al (32) reported that three proteins, lymphatic vessel endothelial hyaluronan receptor 1, REG1A and trefoil factor 1, when combined in a biomarker panel, were able to detect patients with pancreatic cancer with sensitivity and specificity of 80% and 76.9%, compared to healthy controls.

Reccan is developing a multi-biomarker blood test for early detection of high-risk pancreatic cancer patients.

The **Reccan Immunoassay** is detecting blood tumour-derived biomarkers specific to pancreatic cancer. Each biomarker plays a clear role in pancreatic cancer biology and disease progression: during tumour growth, invasion, and metastasis; being present on the surface of tumour cells, in tumour angiogenesis, affecting tumour progression or in protection of tumour microenvironment.

Reccan Immunoassay is an immunoassay test to be run in the hospital laboratory on installed base analysers. The data will be analysed using Reccan Clinical Decision Support Software, enabling biomarker data analysis. This software processes biomarker concentrations from the Reccan Immunoassay, employing Reccan Risk-Indicating Algorithm to generate a pancreatic cancer risk score. The system's compatibility with current laboratory information systems streamlines data analysis, offering a holistic diagnostic solution that improves early detection and treatment of pancreatic cancer.

Multiplexed Immunoassay technique

The biomarker detection is based on standard enzyme-linked immunosorbent assay (ELISA) coupling technique, but in a multiplexed manner. Multiplex techniques have mainly been used for research, but in recent years, advancements in biomarker technology for diagnostics have occurred. The technology utilized is a multiplexed immunoassay system in a microplate format that simultaneously detect many biomarkers in a single sample. For biomarkers that are present in the sample, an immuno-sandwich is generated.

Phased approach to the development of Reccan Immunoassay
The methodological approach deployed to identify a diagnostic pancreatic cancer signature is depicted in **Figure 2.**

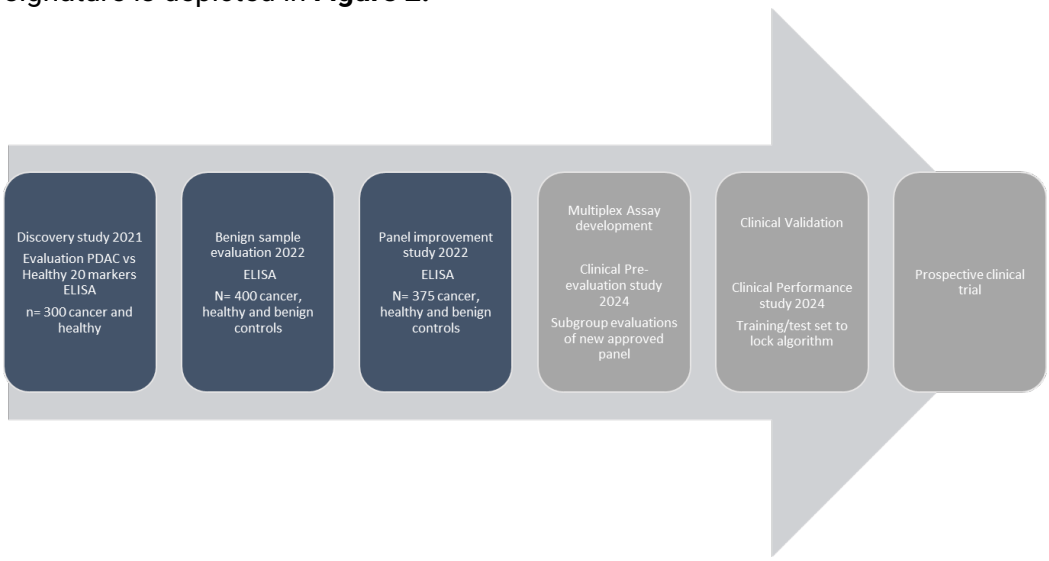


Figure 2. Phased approach for development of Reccan Immunoassay

Initially a group of 20 candidate biomarkers including CA19-9 were evaluated for their contribution in distinguishing patients with pancreatic cancer from healthy subjects. The potential clinical utility of the candidate protein biomarkers was assessed from serum samples using commercial ELISA kits. In a cohort of 300 patients (100 with pancreatic cancer), a 5-protein biomarker signature achieved a diagnostic sensitivity of 95% and specificity of 97% and an AUC of 99.6% in the test set [data on file, 2021]. (80% of the data was used as a training set, to build the models, and the remaining 20% of the data was used as a blind set, to test the performance of the model on un-seen data).

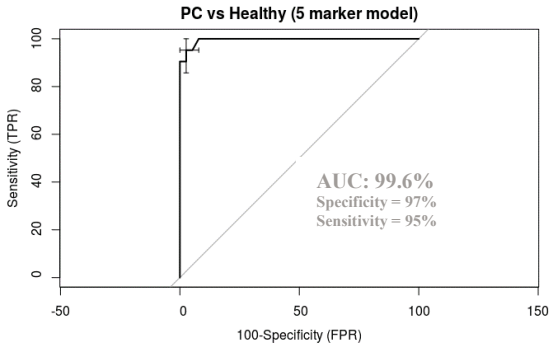


Figure 3. Diagnostic work-up of pancreatic cancer (22)

Appreciating that benign conditions can adversely affect the performance of biomarker signature the top biomarker proteins were further evaluated in an extended cohort comprising of an additional 100 acute benign controls with non-tumorous liver, biliary and pancreatic disease. 388 samples were used in the analysis, due to missing data for 12 samples.
A new model was trained, to separate pancreatic cancer from healthy in the benign sample evaluation. This model gave an AUC of 95.6% in separating pancreatic

cancer from healthy and an expected lower sensitivity and specificity with an AUC of 82.9% in separating pancreatic cancer from all controls.

A final round of analysis was conducted, in which previously measured 5-protein biomarkers were revalidated, and 18 new biomarkers were evaluated, to assess if better performance could be achieved in separating PC from a cohort of healthy and acute benign controls. The final dataset consisted of N=375 samples (N=100 acute benign, N=100 PC and N=175 healthy). 70% of the data was used as a training set, and 30% as a test set. Based on individual AUC and ranking of the biomarker's predictive performance several different biomarker combinations were assessed for its clinical utility.

Each combination was trained to stratify 'pancreatic cancer vs healthy' and 'pancreatic cancer vs acute benign' separately, all showing similar performance with an AUC between 98.4%-98.8% in separating pancreatic cancer vs healthy and an expected lower AUC of 83.2%-90.3% in distinguishing between pancreatic cancer and acute benign disease. This methodology was chosen, as it was deduced from the previous analyses that the same panel of markers could be trained to stratify each group separately, to provide a better understanding of the biomarker signatures in each subgroup than if they were trained on one group.

The most promising biomarkers have been selected for further optimization and validation on the preferred platform. This process aims to assess potential cross-reactivity, collinearity, and antibody characteristics.

Summary

The quest for early detection methods of pancreatic cancer is imperative and pressing, underscoring the urgency to uncover effective solutions.
Reccan is in the process of developing a compelling test for early detection, poised to make a global impact for patients, paving the way for improved health and lives saved.

References

1. European Cancer Information System. *European Commission.* [Online] 2018.
2. UEG Pancreatic Cancer Across Europe. [Online] 2018.
3. GLOBOCAN 2020. [Online]
4. www.destatis.de. *Destatis SB.* [Online] 2020.
5. *Model-based screening for pancreatic cancer in Sweden.* Tomasz Draus, Daniel Ansari and Roland Andersson. 2022, SCANDINAVIAN JOURNAL OF GASTROENTEROLOGY.
6. *Scott, et al. 2019, Am J Manag Care, Vol. 25.*
7. *Wu et al. 2018, Clnical Epidemomiology.*
8. *Roser et al. 2015, Cancer.*
9. *The early detection of pancreatic cancer: what will it take to diagnose and treat curable pancreatic neoplasia? Lennon AM et al. 2014, Cancer Research.*
10. *Rahib et al. 2021, JAMA Netw Open.*
11. *Sung et al. 2021, CA Cancer J Clin.*
12. *Ferlay et al. 2016 , Acta Oncology.*
13. *Kommalapati et al. 2018, Cancers (Basel) .*
14. *al., Siegel et. 2024, CA Cancer J Clin, Vol. 74, pp. 12-49.*

15. Screening for Pancreatic Cancer: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. Henrikson NB, Aiello Bowles EJ, Blasi PR, Morrison CC, Nguyen M, Pillarisetty VG, Lin JS. 2019, JAMA.
16. Pancreatic Cancer Surveillance in Carriers of a Germline CDKN2A Pathogenic Variant: Yield and Outcomes of a 20-Year Prospective Follow-Up. Klatte DCF, Boekestijn B, Wasser M, Feshtali Shahbazi S, Ibrahim IS, Mieog JSD, Luelmo SAC, Morreau H, Potjer TP, Inderson A, et al. 2022, J Clin Oncology, pp. 40:3267-3277.
17. Early detection of pancreatic cancer. . Pereira SP, Oldfield L, Ney A, Hart PA, Keane MG, Pandol SJ, Li D, Greenhalf W, Jeon CY, Koay EJ, et al. 2020, Lancet Gastroenterol Hepatology, pp. 5:698-710.
18. The Role of Imaging in Current Treatment Strategies for Pancreatic Adenocarcinoma. Rhee H, Park MS. s.l. : Korean J Radiology, 2021, Korean J Radiology, Vols. 22:23-40.
19. Imaging modalities in the diagnosis of pancreatic adenocarcinoma: A systematic review and meta-analysis of sensitivity, specificity and diagnostic accuracy. Toft et al. 2017, Eur J Radiol, pp. ;92:17-23.
20. Francis IR. 2007, Cancer Imaging.
21. Journal Ultrasound. Gandolfi L, et al. 2003.
22. The Role of Imaging in Current Treatment Strategies for Pancreatic Adenocarcinoma. Rhee H, Park MS. 2021, Korean J Radiology, pp. 22:23-40.
23. Diagnostic value of serum carbohydrate antigen 19-9 in pancreatic cancer: a systematic review and meta-analysis. Zhao et al. 2022, Eur J Gastroenterol Hepatol, pp. 1;34(9):891-904.
24. The clinical utility of serum CA 19-9 in the diagnosis, prognosis and management of pancreatic adenocarcinoma: An evidence based appraisal. Ballehaninna UK, Chamberlain RS. 2012, J Gastrointest Oncol, pp. 3:105-119.
25. Diagnostic accuracy and added value of blood-based protein biomarkers for pancreatic cancer: A meta-analysis of aggregate and individual participant data. Boyd LNC, Ali M, Leeflang MMG, Treglia G, de Vries R, Le Large TYS, Besselink MG, Giovannetti E, van Laarhoven HWM, Kazemier G. 2023, E Clinical Medicine , p. 55:101747.
26. Diagnostic Accuracy of Blood-based Biomarkers for Pancreatic Cancer: A Systematic Review and Meta-analysis. Kane LE, Mellotte GS, Mylod E, O'Brien RM, O'Connell F, Buckley CE, Arlow J, Nguyen K, Mockler D, Meade AD, et al. 2022, Cancer Research Communications, pp. 2:1229-1243.
27. Tumor markers in pancreatic cancer: a European Group on Tumor Markers (EGTM) status report. . Duffy MJ, Sturgeon C, Lamerz R, Haglund C, Holubec VL, Klapdor R, Nicolini A, Topolcan O, Heinemann V. 2010, Ann Oncology, pp. 21:441-447.
28. Scara S et al. 2015, Adv Exp Med Biology.
29. Detection of Early-Stage Pancreatic Ductal Adenocarcinoma From Blood Samples: Results of a Multiplex Biomarker Signature Validation Study. Brand et al. 2022, Clin Transl Gastroenterol.
30. CA 19-9 and pancreatic cancer. Wu Z, et al.,. 2013, Clin Adv Hematol Oncology.
31. Gu YL et al. 2015, Asian Pac J Cancer Prev, pp. 16(15):6569–73.
32. Identification of a Three-Biomarker Panel in Urine for Early Detection of Pancreatic Adenocarcinoma. . Radon TP, Massat NJ, Jones R, Alrawashdeh W, Dumartin L, Ennis D, Duffy SW, Kocher HM, Pereira SP, Guarner posthumous L, Murta-Nascimento C, Real FX, Malats N, Neoptolemos J, Costello E, Greenhalf W, Lemoine NR, Crnogorac-Jurcevic T. 2015, Clin Cancer Res. , pp. 21:3512–3521.
33. 2022, Clinical and Translational Gastroenterology 13(3), p. march.
34. Plasma biomarker for detection of early stage pancreatic cancer and risk factors for pancreatic malignancy using antibodies for apolipoprotein-AII isoforms. Honda K, Kobayashi M, Okusaka T, Rinaudo JA, Huang Y, Marsh T, et al. 2015, Sci Rep., p. 5:15921.
35. Evaluation of circulating ADH and MIC-1 as diagnostic markers in Egyptian patients with pancreatic cancer. Mohamed AA, Soliman H, Ismail M, Ziada D, Farid TM, Aref AM, et al. 2015, Pancreatology. , pp. 15:34–39.