

Methylation-Based Liquid Biopsy Markers for Early Detection of Malignant Transformation in Patients with Pancreatic Cystic Neoplasms: A Review

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Abstract

Pancreatic cysts are found incidentally in a large number of older adults, most will never become cancer, and the criteria used to decide who needs surgery are not accurate enough to separate the two groups. Methylation markers have been proposed as a solution. Whether they amount to a liquid biopsy depends on which fluid is being sampled, and that distinction is often lost.

Objectives: To review the published evidence on methylation markers for identifying advanced neoplasia in pancreatic cystic lesions: what has been measured in cyst fluid, in pancreatic juice and in plasma, how each performs against current criteria, and how far the evidence supports the phrase liquid biopsy as applied to this setting.

Material and Methods: Narrative review of marker discovery and validation studies, diagnostic accuracy studies and management cohorts indexed in PubMed and the major gastroenterology and oncology journals. Studies were eligible if they reported the performance of DNA methylation markers for detecting advanced neoplasia or pancreatic cancer in a defined population, or compared molecular testing with conventional criteria. No new patient data were generated; every accuracy estimate quoted is one published by the original investigators.

Results: A two-marker cyst fluid panel of methylated TBX15 and BMP3 separated cysts with high-grade dysplasia or cancer from those with low-grade or no dysplasia with sensitivity of 90 per cent and specificity of 92 per cent, at an area under the curve of 0.93, outperforming both mutant KRAS and carcinoembryonic antigen. The Fukuoka high-risk criteria, against which this is measured, have reported sensitivity of 56 to 81 per cent and specificity of 69 to 73 per cent. A three-marker panel assayed in pancreatic juice detected all-stage pancreatic cancer with 83 per cent sensitivity at 86 per cent specificity. In plasma, a two-gene panel of methylated ADAMTS1 and BNC1 reached 97.3 per cent sensitivity and 91.6 per cent specificity for pancreatic cancer, at an area under the curve of 0.95 against 0.571 for CA 19-9.

Conclusion: The strongest evidence in cystic neoplasms comes from cyst fluid, which requires endoscopic ultrasound-guided aspiration and is therefore not a liquid biopsy in the sense the term usually carries. The plasma data are genuinely non-invasive but were generated in patients with established pancreatic cancer rather than in cysts under surveillance. No published study has tested a plasma methylation panel prospectively in a cyst surveillance cohort, which is the study the field needs.

Abbreviations: PCL – Pancreatic Cystic Lesion, IPMN – Intraductal Papillary Mucinous Neoplasm, MCN – Mucinous Cystic Neoplasm, HGD – High-Grade Dysplasia, LGD – Low-Grade Dysplasia, PDAC – Pancreatic Ductal Adenocarcinoma, MDM – Methylated DNA Marker, CF-MDM – Cyst Fluid Methylated DNA Marker, cfDNA – Cell-Free DNA, EUS-FNA – Endoscopic Ultrasound-Guided Fine Needle Aspiration, CEA – Carcinoembryonic Antigen, AUC – Area Under the Receiver Operating Characteristic Curve.

Keywords: DNA Methylation; Pancreatic Cystic Neoplasm; Liquid Biopsy; Intraductal Papillary Mucinous Neoplasm; Early Detection

Introduction

Cross-sectional imaging finds pancreatic cysts in a substantial minority of older adults, and the number detected has risen with imaging use rather than with any change in disease. Most of these lesions will never progress. A minority, principally intraductal papillary mucinous neoplasms and mucinous cystic neoplasms, are

genuine precursors, and a meta-analysis has put the cumulative incidence of pancreatic cancer in unresected IPMNs at ten years somewhere between 8 and 25 per cent [1].

The clinical problem is therefore one of triage rather than detection. Consensus criteria classify cysts by worrisome and high-risk features drawn from imaging, symptoms and cyst fluid chemistry, and those criteria carry the decision about whether a

patient undergoes pancreatic resection, an operation with real mortality. Their reported accuracy is modest: for identifying cancer in cystic lesions, high-risk criteria have shown sensitivity between 56 and 81 per cent and specificity between 69 and 73 per cent [1]. The consequence in practice is both directions of error, with cancers missed on surveillance and benign cysts resected.

Molecular testing has been proposed to close that gap, and methylation markers are an attractive candidate because aberrant methylation occurs early in pancreatic tumorigenesis, before invasive disease is established [2,3]. What complicates the picture is where the marker is measured. The phrase liquid biopsy is used across this literature for three quite different specimens: cyst fluid obtained by endoscopic ultrasound-guided aspiration, pancreatic juice collected endoscopically, and plasma. Only the last is non-invasive in the ordinary sense.

The aim of this review is to set out what methylation markers have achieved in each of those compartments, how that compares with the criteria currently used to make the surgical decision, and what the evidence does and does not yet support.

Material and Methods

Search strategy: PubMed and the archives of the major gastroenterology, oncology and epigenetics journals were searched for studies of DNA methylation markers in pancreatic cystic lesions and pancreatic cancer. Search terms combined DNA methylation, methylated DNA marker and cell-free DNA with pancreatic cyst, intraductal papillary mucinous neoplasm, advanced neoplasia, high-grade dysplasia, cyst fluid, pancreatic juice, plasma and early detection. Reference lists of retrieved studies and reviews were screened by hand.

Eligibility: Studies were included if they reported the diagnostic performance of one or more DNA methylation markers for advanced neoplasia in pancreatic cystic lesions or for pancreatic cancer, in a defined population with a stated comparator, or if they compared molecular testing against conventional clinical and imaging criteria for cyst management. Marker discovery studies without independent validation were excluded from the performance synthesis. Studies of non-ductal pancreatic neoplasms are cited only where they bear on assay methodology.

Data extraction and handling: Design, sample size, specimen type, assay platform, comparator and reported accuracy estimates were extracted as published. Accuracy figures are reported together with the specimen and the population studied, because the two are not separable in this literature. No pooling or re-analysis was performed and no patient-level data were obtained. Figures 1 to 4 and Tables 1 and 2 reproduce published values only. This is a narrative synthesis and was not registered as a systematic review.

Results

Cyst fluid methylation against current criteria: The most direct evidence in cystic neoplasms comes from a study that moved from whole-methylome discovery through tissue validation to cyst fluid assay. A tree-based algorithm using two cyst fluid methylated DNA markers, TBX15 and BMP3, separated lesions with high-grade dysplasia or cancer from those with low-grade or no dysplasia with sensitivity and specificity both above 90 per cent, at an area under the curve of 0.93. Discrimination was significantly better than that achieved by mutant KRAS or by carcinoembryonic antigen in the same samples [1].

Set against the criteria that currently drive the surgical decision, the difference is substantial rather than marginal. High-risk

consensus criteria have reported sensitivity of 56 to 81 per cent and specificity of 69 to 73 per cent for cancer in cystic lesions (Figure 1) [1]. A test that holds both measures above 90 per cent would change how many operations are performed and on whom, if it replicates.

DETECTING ADVANCED NEOPLASIA IN PANCREATIC CYSTS

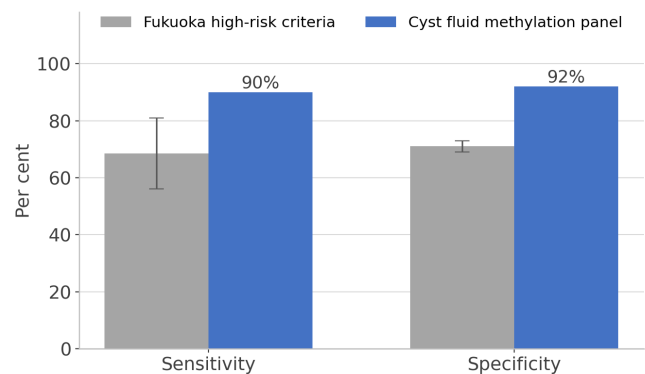


Figure 1: Reported performance of high-risk consensus criteria, with the range across studies, against the two-marker cyst fluid methylation panel [1]

The same markers in less accessible fluids: Methylation signal has also been sought in pancreatic juice, collected endoscopically from the duodenum after secretin stimulation. A three-marker panel assayed in pancreatic juice detected all-stage pancreatic cancer with 83 per cent sensitivity at a predefined specificity cutoff of 86 per cent [4]. That study established proof of concept but included a limited number of early-stage cases.

In plasma, a two-gene panel of methylated ADAMTS1 and BNC1 measured in cell-free DNA reached 97.3 per cent sensitivity and 91.6 per cent specificity for pancreatic cancer, with an area under the curve of 0.95. Adding preoperative CA 19-9 to the panel did not improve sensitivity [5]. Set against these three compartments side by side, the reported figures are broadly comparable, which is itself the finding worth pausing on (Figure 2) [1,4,5].

METHYLATION MARKER PERFORMANCE BY COMPARTMENT SAMPLED

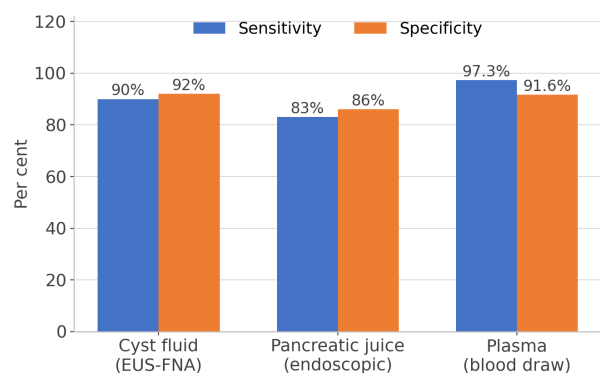


Figure 2: Reported sensitivity and specificity of methylation marker panels by the compartment sampled [1,4,5]

Study	Specimen	Target	Principal reported finding
Majumder et al. 2019 [1]	Cyst fluid	HGD or cancer	TBX15 and BMP3 panel, sensitivity and specificity above 90%
Majumder et al. 2020 [4]	Pancreatic juice	All-stage PDAC	Three-marker panel, 83% sensitivity at 86% specificity
Eissa et al. 2019 [5]	Plasma	PDAC	ADAMTS1 and BNC1 panel, AUC 0.95 against 0.571 for CA 19-9
Springer et al. 2019 [6]	Cyst fluid	Management class	CompCyst would have spared surgery in over half of unnecessary resections
Liu et al. 2020 [7]	Plasma	Multi-cancer	Methylation signatures detect and localise multiple cancers

Table 1: Principal studies reviewed, with specimen type and the finding as reported by the original investigators

Panels outperform their components: The plasma study is instructive on why single markers have not been sufficient. Measured alone, methylated ADAMTS1 gave 87.2 per cent sensitivity and 95.8 per cent specificity (AUC 0.91), while BNC1 gave 64.1 per cent and 93.7 per cent (AUC 0.79). Combining the two as a panel raised sensitivity to 97.3 per cent while specificity fell modestly to 91.6 per cent (Figure 3) [5]. The pattern is the same one seen in cyst fluid, where a two-marker algorithm outperformed either marker considered on its own [1].

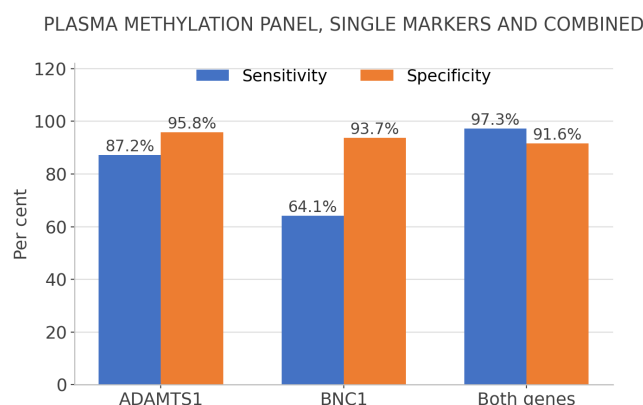


Figure 3: Plasma methylation markers measured individually and as a two-gene panel in patients with pancreatic cancer [5]

Comparison with the established serum marker: CA 19-9 remains the only widely used blood marker in this disease, and it performs poorly as a detection test. In the same plasma cohort, CA 19-9 alone produced an area under the curve of 0.571 against 0.95 for the two-gene methylation panel (Figure 4) [5]. Methylation markers in cyst fluid similarly outperformed carcinoembryonic antigen, the corresponding established cyst fluid marker [1].

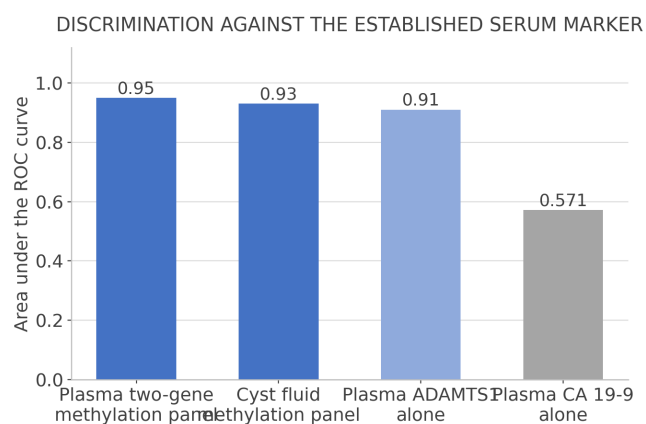


Figure 4: Discrimination reported for methylation panels against CA 19-9 measured in the same plasma cohort [1,5]

Molecular testing in the management decision: The question of whether molecular data change management, rather than merely correlating with pathology, has been addressed directly. A machine-learning classifier built from clinical features, imaging characteristics and cyst fluid genetic and biochemical markers was trained on 436 patients and tested on an independent cohort of 426, with histopathology as the reference standard. Management informed by the classifier was more accurate than management dictated by conventional clinical and imaging criteria alone, and applying it would have spared surgery in more than half of the patients who underwent unnecessary resection [6]. That model did not include methylation markers, which is precisely the gap the cyst fluid methylation data suggest could be filled.

Compartment	How obtained	What it has been shown to detect
Cyst fluid	EUS-guided aspiration	Advanced neoplasia within the cyst; strongest evidence in this setting [1]
Pancreatic juice	Endoscopic collection	All-stage pancreatic cancer; proof of concept only [4]
Plasma	Venous blood draw	Established pancreatic cancer; not tested in cyst surveillance [5,7]

Table 2: The three compartments described as liquid biopsy in this literature, and what has actually been demonstrated in each

Discussion

Two conclusions follow, and the second qualifies the first. Methylation markers discriminate advanced neoplasia in pancreatic cysts better than the criteria and the fluid markers currently in use. Sensitivity and specificity both above 90 per cent, against consensus criteria whose sensitivity may be as low as 56 per cent, is a meaningful margin in a setting where both types of error carry consequences [1].

The qualification concerns the word liquid. The cyst fluid evidence, which is by some distance the strongest, requires endoscopic ultrasound-guided aspiration of the lesion. That is a procedure with its own risks and access constraints, and it is not what most readers understand by liquid biopsy. The plasma evidence is genuinely non-invasive, but it was generated in patients who already had pancreatic cancer, tested against healthy controls [5]. Those are different questions, and a panel that separates cancer from health may not separate a cyst harbouring high-grade dysplasia from a cyst that does not.

What would settle the question

The study the field lacks is straightforward to describe. It would enrol patients with pancreatic cystic lesions under established surveillance, collect plasma at fixed intervals with prespecified marker panels and cutoffs, and follow them to a histological or clinical endpoint. Until that exists, plasma methylation in this setting remains an extrapolation from cancer detection studies rather than a demonstrated surveillance tool [5,7].

In the meantime the practical implication runs the other way from what the framing suggests. Where a patient is already undergoing aspiration for cytology and fluid chemistry, adding methylation markers to that sample carries no extra procedural risk and would use the compartment with the best evidence behind it [1,6].

Study limitations

The limitations belong to the underlying literature. The cyst fluid marker data derive from case-control designs in which cases and controls were defined by resection histology, so the population is enriched for lesions that were operated on and does not represent the surveillance population in which the test would be used [1]. Cutoffs were derived and applied within related cohorts, which inflates apparent accuracy. Plasma panel performance was measured against healthy controls rather than against patients with benign cysts or chronic pancreatitis, which is the harder and more relevant comparison [5]. Assay platforms differ substantially in input requirements and analytical sensitivity, and figures generated on one platform should not be assumed to transfer to another [3,8].

Two further gaps deserve naming. Reported performance for high-risk consensus criteria varies widely across the studies from which the 56 to 81 per cent sensitivity range is drawn, so the comparator itself is imprecise [1]. And no study reviewed here reports the interval between marker positivity and histological progression, which is the quantity that would determine how often surveillance sampling needs to be repeated.

Conclusion

Methylation markers outperform the imaging criteria and the fluid markers presently used to decide which pancreatic cysts require resection, and the margin is large enough to matter clinically. The strongest evidence comes from cyst fluid, which is obtained by aspiration rather than by blood draw, so the description of this work as liquid biopsy is accurate only in a loose sense. Plasma methylation panels perform well for detecting established pancreatic cancer and comfortably outperform CA 19-9, but they have not been tested in patients under cyst surveillance, which is the population the title of this field implies. Prospective evaluation in that population, with prespecified panels and cutoffs, is the necessary next step.

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Conflict of Interest

No competing interest attaches to any of the authors.

Ethics Statement

Every source used here was in print before this article was drafted. No patients were recruited and no new clinical data were collected, so ethical approval was not needed.

References

1. Majumder S, Taylor WR, Yab TC, Berger CK, Dukek BA, et al. "Novel methylated DNA markers discriminate advanced neoplasia in pancreatic cysts: marker discovery, tissue validation, and cyst fluid testing". *Am J Gastroenterol* 114(2019):1539-1549.
2. Tan MC, Basturk O, Brannon AR, Bhanot U, Scott SN, et al. "GNAS and KRAS mutations define separate progression pathways in intraductal papillary mucinous neoplasm-associated carcinoma". *J Am Coll Surg* 220(2015):845-854.
3. Verschuur AVD, Hackeng WM, Westerbeke F, et al. "DNA methylation profiling enables accurate classification of nonductal primary pancreatic neoplasms". *Clin Gastroenterol Hepatol* 22(2024):1245-1254.
4. Majumder S, Raimondo M, Taylor WR, et al. "Methylated DNA in pancreatic juice distinguishes patients with pancreatic cancer from controls". *Clin Gastroenterol Hepatol* 18(2020):676-683.
5. Eissa MAL, Lerner L, Abdelfatah E, Shankar N, Canner JK, et al. "Promoter methylation of ADAMTS1 and BNC1 as potential biomarkers for early detection of pancreatic cancer in blood". *Clin Epigenetics* 11(2019):59.
6. Springer S, Masica DL, Dal Molin M, Douville C, Thoburn CJ, et al. "A multimodality test to guide the management of patients with a pancreatic cyst". *Sci Transl Med* 11(2019):eaav4772.
7. Liu MC, Oxnard GR, Klein EA, Swanton C, Seiden MV. "Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA". *Ann Oncol* 31(2020):745-759.
8. Sun Z, Baheti S, Middha S, et al. "SAAP-RRBS: streamlined analysis and annotation pipeline for reduced representation bisulfite sequencing". *Bioinformatics* 28(2012):2180-2181.

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