Glypican-1 in exosomes as biomarker for early detection of pancreatic cancer

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Abstract: On June 24, 2015 *Nature* published an article entitle "Glypican-1 identifies cancer exosomes and detects early pancreatic cancer", which demonstrates that exosomes positives for the proteoglycan glypican-1 (GPC1) are expressed in serum of patients with pancreatic cancer since very early stages but not in benign pancreatic disease. Additionally, these GPC1^{+} circulating exosomes correlate with tumor burden and could be used as prognostic biomarker in pre and post-surgical patients. The study is pioneer since GPC1 biomarker in exosomes offers better sensitivity and specificity than any other under evaluation or used in clinical practice. However, methodology for exosomes isolation still remains at investigational phase. Further studies are need to translate this technology to a practicable clinical method. Further research is also required to validate this biomarker in larger prospective cohort including more cases of premalignant lesions and then replicated results could possible guide changes in clinical practice.

Keywords: Biomarker; early detection; exosomes; glypican-1 (GPC1); pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) has a dismal prognosis, with 5-year survival <5%, making mortality and incidence rates almost identical (1). The majority of patients with pancreatic cancer progress to either metastatic or locally advanced disease in the asymptomatic phase, as many as 80% presents late with metastasis at diagnosis (2). PDAC is usually diagnosed when patient present clinical symptoms and subsequent blood and image tests are performed [computed tomography (CT) or magnetic resonance imaging (MRI)]. Often, confirmed diagnostic and staging is performed by endoscopic ultrasound (EUS) with biopsy or surgery. For clinical decisions, surgery or pharmacological treatment, histologic confirmation of the mass is needed but frequently cells or tissue are inconclusive to provide a precise diagnostic. Sampling diminutive lesion is technically challenging and negatively impacts diagnostic sensitivity.

So far, CA19-9 is the only biomarker in routine use for the management of pancreatic cancer (3) and has important limitations including lack of expression in ~5% of the population and elevation in related diseases including chronic pancreatitis and obstructive jaundice (4,5). CA19-9 has a variable sensitivity of ~85% and specificity of ~85% for the detection of pancreatic cancer (6) but the low prevalence of the disease makes this biomarker not applicable for screening and not relevant to confirm diagnosis in many cases where similar nonspecific symptoms presented. It is clear then that diagnostic biomarkers are need to provide precise diagnostic since the opportunity to detect pancreatic cancer, while it remains curable, depends on the ability to identify reliable biomarkers for diagnostic at early stage.

For decades, both clinicians and investigators have tried to face the retroperitoneal position of the pancreas ant the lack of specific serum biomarkers challenging the early detection of pancreatic cancer. Multiple research studies have explored biomarkers in biofluids-blood, urine, stool, saliva or pancreatic juice, but diagnostic performance has not been further validated. These include overexpressed

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and underexpressed RNAs (7), mutations and other genetic alterations, epigenetic changes such as methylation (8,9), modification of secreted proteins such as mucins (10) or most recently the detection of free nucleid acids (11), circulating pancreatic cells (CPCs) (12) or cancer stem cells (CSCs) (13). However, recent development in science and technology have not resulted in improved survival for patients. Consequently, experts around the world try to fill the gaps in collaborator studies, meetings, conferences and work groups in order to find reliable biomarkers for early detection of pancreatic cancer (14).

The results recently published in *Nature* by Melo *et al*. (15) offer a new insight of a biomarker potentially enabling early detection of pancreatic cancer helping in precise diagnosis and the design of curative surgical or treatment options.

Exosomes are extracellular vesicles produced during endosomes formation and have been demonstrated as source of DNA in circulation (16). Glypican-1 (GPC1) is a membrane anchored protein that is overexpressed in several types of tumors including glioma, breast and pancreatic as described by several authors (15,17-19).

The study presented by Melo *et al*. includes 251 pancreatic cancer patients from two different cohorts (discovery and validation group), where it is demonstrated that exosomes from cancer patients express higher levels of GPC1 than healthy subjects. Moreover, the percentage of $GPC1^+$ increases proportionally with tumor size and correlates with tumor burden. Overall, the study provides good evidence of GCPC1 is a good candidate for detection and isolation of exosomes in the circulation of screening of patients where pancreatic cancer is suspected. Additional data are supported by mice experiments.

However, this interesting and pioneer study must be taken as a preliminary discovery where further studies are needed to validate GPC1 as discriminatory biomarker for early detection of pancreatic cancer. Overall GPC1 could not be cancer type specific since in addition to other publications (17,19), the same authors show that 75% (24 out of 32) of breast cancer patients also possess $GCP1^+$ exosomes in serum. In order to demonstrate type tumor specificity in pancreatic cancer, it would be worthwhile to explore GPC1 expression in exosomes isolated from patients suffering from related diseases presenting similar symptoms such as hepatobiliary pathologies, obstructive disease-cholelithiasis, ampullary cancers, cholangiocarcinomas and pancreatic neuroendocrine tumors. In this referred study, only 21 chronic pancreatitis were included and further studies with higher number of patients should be analyzed for strong conclusions as well as more information about severity of chronic pancreatitis and reasons to perform surgery in all of them is required.

Furthermore, based on their results it is difficult to asseverate that GPC1 could be a real early stage biomarker because of two reasons. First, 70-89% of patients were advanced stages-IIb, III and IV whereas only 11-30% was early stage (I, IIa). Second, only five intraductal papillary mucinous neoplasm (IPMN), a precursors pancreatic cancer lesions was included in the study and it is well-known that IPMN lesions does not always evolve to malignant tumor. On another hand, only eight serous cystadenomas (benign lesions) while no mucinous cists were assessed in the study. Although evolutionary concept of PanIN is still controversial in humans but not in mice, higher and variated PanIN lesions should be included in further validation studies. Ideally, good biomarker should be universally present in advanced preinvasive cancer and curable-stage PC, while absent in individuals who are cancer-free or who have PanIN-1.

At the same time, it would be also interesting to elucidate whether high-risk population (including familial pancreatic cancer, Peutz-Jeghers syndrome, hereditary pancreatitis and familial-atypical multiple mole melanoma) have $GPC1^+$ exosomes and what is the changing profile in a longitudinal study. Therefore, this biomarker could also useful for screening purposes. A longitudinally collected cohort using also MRN and EUS is essential now.

Comparing GPC1 with the current biomarker CA19-9, whereas most of the publications demonstrate this biomarker as a prognostic marker for survival after resection (20,21), Melo *et al*. did not find significant decrease in 10 out of 29 PDAC resected patients after surgery. Since CA19-9 is a well-established but controversial marker that correlates with clinical evolution of pancreatic cancer, prognostic value must be reviewed in further studies. Moreover, combination of CA19-9 values together with GPC1 should be explored in an algorithm to improve sensitivity and specificity.

Regarding to technology, Melo *et al*. used ultracentrifugation at high speed and overnight to isolate exosomes from serum samples and then electron microscopy for GPC1 detection. Although high resolutive technology for a preclinical exploratory phase, it is far from being clinically operative. Method optimization is necessary to end with an accurate and inexpensive biomarker available in a simple blood test performed in clinical laboratories. Improvements in ELISA, Flow cytometry, immunochemistry or some feasible assay with same values

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of predictive value and AUC would ensure a successful biomarker validation in prospective studies.

Indeed, the progress toward blood test for early detection of early detection of pancreatic cancer have been hampered by the lack of available specimens for proper validation due in part to the lack of feasible technologies and the nature of the cancer itself. From a validation design perspective, it is recommendable to adjust studies to the prospective-specimen-collection, retrospective-blinded-evaluation (PRoBE) criteria set forth by Pepe *et al*. (22) for the early detection research network (EDRN) should be followed, with the notable exception of assays that cannot utilize banked specimens.

Once this pioneer article has established GPC1 as a more sensitive and specific biomarker than any other in the market, a prospective and longitudinal study aimed at the early detection of pancreatic cancer is mandatory. Recognized as costly and long-lasting clinical research, these type of studies are necessary to translate discovery through validation and market phases. Enrolling a large number of clinically asymptomatic and symptomatic patients will allow approved biomarkers which could finally improve pancreatic cancer survival.

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Footnote

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