Do molecular tests really differentiate malignant IPMNS from benign?

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submitted 19. October 2016
accepted after revision
25. October 2016

Bibliography
DOI http://dx.doi.org/10.1055/s-0042-121004
Endoscopy International Open 2016; 04: E1236–E1237

E-ISSN 2196-9736

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Bournet et al. have questioned the role of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) plus *KRAS* and *GNAS* mutations in malignant intraductal papillary mucinous neoplasms (IPMNs) of the pancreas in this issue of *Endoscopy International Open* [1]. Bournet et al. claimed that testing for *KRAS* mutations in cystic fluid improved the accuracy of results for cytopathologic diagnosis of malignancy whereas *GNAS* mutation testing did not improve the results. How should clinicians interpret these outcomes and do these results help to detect and treat an IPMN before it progresses to a pancreatic adenocarcinoma?

IPMNs are the most frequently detected type of mucin-producing neoplasm and the exact rate of progression to malignancy has not yet been defined clearly (ranging from 38 to 68% for Main Duct-IPMNs [MD-IPMNs] and 12 to 47% for Branch Duct-IPMNs [BD-IPMNs] in surgical series of symptomatic patients) [2]. The goal of any diagnostic test for a pancreatic cystic neoplasm is accurate detection of its malignant potential. Recent guidelines on pancreatic cysts recommend a multimodal diagnostic approach including cross-sectional imaging, EUS-FNA and cyst fluid analysis (such as biochemistry, cytology and molecular analysis) to overcome this complex assessment. Although cross-sectional imaging provides detailed images of the high-risk lesions, use of EUS-FNA has increased the accuracy of diagnosis of advanced neoplasia. Cytology is highly specific but approximately 50% sensitive for diagnosis of a malignancy arising from IPMN, due to inadequate cellularity in most cases. On the other hand, elevated cyst fluid carcinoembryonic antigen (CEA) level is considered the most accurate test to distinguish a mucinous cyst from non-mucinous. However, CEA alone can be used neither to differentiate IPMN from a mucinous cystic neoplasm nor a malignant IPMN from a noninvasive IPMN.

Several molecular techniques have been designed for further evaluation of pancreatic cystic neoplasms; however, DNA-based assays on aspirated cyst fluid have emerged as the most useful and reproducible tool. Recent studies on DNA sequencing have not only shown the genetic alterations specific for pancreatic cystic neoplasms, but also may help diagnose and differentiate these neoplasms. The most commonly found genetic alteration in IPMNs is *KRAS* mutation (found in over 80% of cases). It occurs predominantly in codon 12, but it may also occur in codons 13 and 61. *KRAS* mutations are associated with BD-IPMNs and more often present in pancreatobiliary and gastric type IPMNs. Moreover, *GNAS* mutation is a unique mutation for IPMNs with a frequency of 58% to 65%, occurring in codon 201 or 227. *GNAS* mutation is mostly found in MD-IPMNs rather than BD-IPMNs and mainly present in intestinal subtype. A mutation in *KRAS* and/or *GNAS* is found in over 90% of IPMNs.

Bournet et al. enrolled 37 IPMN patients with clinical and/or imaging predictors in a 4-year study [1]. The final diagnosis of IPMNs (n=10 were benign and n=27 were malignant) was obtained from pancreatic resections (n=18), biopsies during laparotomy, EUS-FNA analysis and follow-ups (n=19). Aspirated cyst fluid was evaluated for cytology. *KRAS* (codon 12) and *GNAS* (codon 201) mutation assays were performed using the TaqMan® allelic discrimination on EUS-FNA fluid. *KRAS* and *GNAS* assays were successful in all but one sample. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) and accuracy of cytology alone to diagnose malignancy in IPMN were 55%, 100%, 100%, 45% and 66%, respectively. When *KRAS* mutation analysis was combined with cytology, these values were 92%, 50%, 83%, 71% and 81%, respectively. *GNAS* analysis improved performance of neither cytology alone, nor cytology combined with *KRAS*. The authors concluded that using the

TaqMan® allelic discrimination assay was feasible in IPMN-associated malignancy evaluation. Further, although performing *GNAS* mutation did not add value to diagnosis of malignant IPMNs, the combination of a KRAS mutation with cytology increased the performance of cytology alone for sensitivity, specificity, NPV, accuracy and predicting malignancy to 80%.

The first study evaluating the role of *KRAS* mutations for malignancy assessment in preoperative pancreatic cyst fluid reported that *KRAS* mutations were present in 10 of 11 malignant cysts and the sensitivity and specificity of *KRAS* mutations followed by allelic loss to predict a malignant cyst were 91% and 93%, respectively [3]. Later a multicenter prospective study (the PANDA study) included 113 patients and reported a higher number of DNA mutations in malignant cysts. However, presence of a *KRAS* mutation was similar between malignant and premalignant cysts. In the PANDA study, the high-amplitude *KRAS* mutation followed by allelic loss had 96% specificity but 37% sensitivity for malignancy [4]. Another study by the same group revealed long-term follow-up results in 63 patients and found that presence of KRAS mutation was associated with progression of malignancy [5]. In a study including 618 patients, KRAS mutation was found to be 54% sensitive and 100% specific for a mucinous cyst and combining KRAS mutation with an elevated cyst fluid CEA level increased sensitivity to 83% with specificity unchanged at 85% [6]. On the other hand, CEA alone was found to be 63% sensitive and 88% specific for differentiation of a mucinous cyst from non-mucinous in a meta-analysis of 12 studies [7].

In our investigation of pancreatic cysts, we examined 943 patients with *KRAS* results and found 48% sensitivity, 100% specificity, 100% positive predictive value, and 47% negative predictive value (NPV) for *KRAS* mutation in a mucinous cyst. In the same cohort of patients, sensitivity improved to 75% and NPV to 60% when a *KRAS* mutation was combined with CEA elevation. Moreover, 56 patients in this cohort had a malignant cyst (34 adenocarcinoma and 22 high-grade dysplasia [HGD]) and *KRAS* mutation was more frequent in malignant mucinous cysts than in benign tumors (73.2% – 37.3%). The NPVs of *KRAS* mutation alone and together with CEA elevation for a malignant cyst were 77.6% and 83.3%, respectively. In our study, we suggested that although the diagnostic value of KRAS mutation positivity for malignant cysts remains limited, because it lacks specificity for malignant and non-malignant differentiation, the high NPV might help to exclude a malignant cyst in clinical practice [8].

With the addition of *GNAS* mutation tests, the sensitivity of molecular analysis for detection of a mucinous cyst has increased. Detection of *KRAS* and/or *GNAS* mutation had 65% sensitivity and 100% specificity for mucinous cyst detection in a study [9]. Interestingly, although almost all of the studies suggest that a *GNAS* mutation is unique for IPMNs, a recent study found *GNAS* mutation in 2 patients with serous cystadenoma [10] and we found one in a patient with pseudocyst [11]. A recent study included 38 patients with resected malignant IPMNs that had sufficient tissue for micro-dissection and showed that *KRAS* and *GNAS* mutations did not differ according to the degree of neoplasia (*KRAS*: invasive IPMN 71%, HGD 62%, low-grade dysplasia 74%; *GNAS*: invasive IPMN 61%, HGD 59%, low-grade dysplasia 53%) [12]. A recent meta-analysis of 36 studies revealed that *KRAS* and *GNAS* mutations could be diagnostic markers for IPMN, however, neither *KRAS* nor *GNAS* mutations were associated with the malignant potential or prognosis in patients with IPMN [13].

DNA-based assays have improved in recent years. When compared with conventional Sanger sequencing, next-generation sequencing is highly specific and sensitive for detection of pancreatic cysts that have malignant potential. Besides, the other advantages of next-generation sequencing are that it requires smaller amounts of DNA for analysis and it can simultaneously assay multiple genes. Although *KRAS* and/or *GNAS* mutation tests alone may help diagnose IPMNs, it is difficult to say if these mutations can replace classification and prognostication with multimodal diagnostic methods. Further studies (combination with *TP53*, *PTEN* and *PICK3CA*) are needed to establish the concordance of these tests with diagnosis and prognosis of pancreatic cystic neoplasms.

Competing interests: None

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