The role of K-*ras* gene mutation analysis in EUS-guided FNA cytology specimens for the differential diagnosis of pancreatic solid masses: a meta-analysis of prospective studies CME

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Background: Differential diagnosis of pancreatic solid masses with EUS-guided FNA (EUS-FNA) is still challenging in about 15% of cases. Mutation of the K-*ras* gene is present in over 75% of pancreatic adenocarcinomas (PADC).

Objective: To assess the accuracy of K-*ras* gene mutation analysis for diagnosing PADC.

Design: We systematically searched the electronic databases for relevant studies published. Data from selected studies underwent meta-analysis by use of a bivariate model providing a pooled value for sensitivity, specificity, diagnostic odds ratio, and summary receiver operating characteristic curve.

Setting: Meta-analysis of 8 prospective studies.

Patients: Total of 931 patients undergoing EUS-FNA for diagnosis of pancreatic solid masses.

Intervention: K-*ras* mutation analysis.

Main Outcome Measurements: Diagnostic accuracy of K-*ras* mutation analysis and of combined diagnostic strategy by using EUS-FNA and K-*ras* mutation analysis in the diagnosis of PADC.

Results: The pooled sensitivity of EUS-FNA for the differential diagnosis of PADC was 80.6%, and the specificity was 97%. Estimated sensitivity and specificity were 76.8% and 93.3% for K-*ras* gene analysis, respectively, and 88.7% and 92% for combined EUS-FNA plus K-*ras* mutation analysis. Overall, K-*ras* mutation testing applied to cases that were inconclusive by EUS-FNA reduced the false-negative rate by 55.6%, with a false-positive rate of 10.7%. Not repeating EUS-FNA in cases in which mutation testing of the K-*ras* gene is inconclusive would reduce the repeat-biopsy rate from 12.5% to 6.8%.

Limitations: Small number of studies and between-study heterogeneity.

Conclusion: K-*ras* mutation analysis can be useful in the diagnostic work-up of pancreatic masses, in particular when tissue obtained by EUS-FNA is insufficient, and the diagnosis inconclusive. (Gastrointest Endosc 2013;78:596-608.)

Abbreviations: PADC, pancreatic adenocarcinoma; QUADAS, Quality Assessment of Diagnostic Accuracy Studies.

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Pancreatic cancer represents a significant health problem worldwide, with 1 of the lowest 5-year survival rates of all cancers.[1] In 2013, about 46,000 new cases of pancreatic cancer were estimated in the United States,[2,3] and the average survival time after diagnosis was 6 months.[4]

Despite advances in diagnostic imaging techniques and the pivotal role played by EUS and tissue sampling by FNA, the differential diagnosis between malignant and nonmalignant pancreatic masses is still inconclusive in about 15% of cases. A recent meta-analysis demonstrated that EUS-guided FNA (EUS-FNA) with cytology analysis is a highly accurate diagnostic test for solid pancreatic masses, with a pooled sensitivity of 85% and a specificity of 98%.[5] However, the diagnostic accuracy of EUS-FNA is influenced by several factors, such as the quantity and quality of material obtained, the size and location of the mass, and the technical skill of the endoscopist as well as the presence of a cytopathologist on site.[5-8] Cytopathologic assessment may be difficult or not feasible because the material aspirated may be bloody, with scarce or inadequate material.

Several methods, mainly based on genetic analyses, have been investigated for improving pancreatic cancer diagnosis.[9-11] Pancreatic adenocarcinoma (PADC) has a high incidence of K-*ras* gene mutations, generally reported in more than 75% of cases,[12-14] and these mutations appear to occur early during carcinogenesis.[15] For these reasons, K-*ras* gene mutation analysis has been considered a possible marker for PADC detection. In the last decade, several prospective case series studying detection of K-*ras* gene mutations in EUS-FNA have been published.[16-18] However, the potential impact of K-*ras* mutation determination on the accuracy of EUS-FNA for PADC and its role in the diagnostic algorithm for pancreatic masses are still unclear.

The aim of our study was to perform a structured meta-analysis of the available evidence on the diagnostic accuracy of K-*ras* gene mutation detection in pancreatic solid mass lesions.

Take-home message

- EUS-guided FNA (EUS-FNA) plus K-*ras* testing in cases of inconclusive results increases the overall sensitivity for diagnosis of pancreatic masses.
- K-*ras* gene mutation analysis reduces the need for repeating EUS-FNA in cases in which EUS-FNA results are inconclusive.

METHODS

Data sources and searches

A protocol was written before the meta-analysis was carried out. We identified relevant studies by searching PubMed, EMBASE, Google Scholar, Scopus, and the Cochrane Library. We searched the literature without language restriction through December 31, 2012. Search terms were K-*ras* or Kras and endoscopic ultrasound or EUS or fine-needle aspiration or FNA and pancreas or cancer. In addition, we identified relevant studies from the reference list of each selected article. We also handsearched abstracts presented through 2012 at the American Gastroenterological Association Digestive Disease Week, United European Gastroenterology Week, and at the Italian National Congress of Gastroenterology. Selection criteria were established a priori to minimize bias.[19] Inclusion and exclusion criteria are summarized in Table 1. When we found multiple articles for a single study, we used the latest publication and supplemented it, if necessary, with data from the previous publications. If any clarification of data was necessary, we contacted the authors for detailed information. Eligibility assessment was performed independently by 2 reviewers (L.F., L.L.).

Data extraction and quality assessment

Three investigators independently extracted data on the following items from the selected studies: year of publication, location of the study, number of centers involved, type of publication (full-text or abstract form), enrollment period, number of patients enrolled, sex, size of the diagnostic FNA needle (ie, 19, 22, or 25 gauge), number of needle passes (mean and/or range), the presence of an on-site cytopathologist, number of PADCs according to the final diagnosis, number of non-PADC lesions, number of PADC and non-PADC lesions diagnosed by EUS-FNA, number of inconclusive results on EUS-FNA (inconclusive defined as insufficient material, atypia, or suspicion of malignancy), number of inconclusive results on EUS-FNA in PADC and non-PADC groups, type of DNA analysis for K-*ras* gene mutation detection, number of codons analyzed (codons 12, 13, and 61), number of cases in which K-*ras* mutation analysis was successful, number of PADC and non-PADC cases in which K-*ras* was mutated, number of cases with inconclusive EUS-FNA results (total and according to PADC and non-PADC groups) in which K-*ras* was mutated. The Quality Assessment of Diagnostic Accuracy Studies (QUADAS) questionnaire was used to assess the quality of the selected studies.[20] Items were rated as yes, no, or unclear. Disagreements were resolved by discussion.

Statistical analysis

For each study, a 2 × 2 contingency table was constructed that compared the final disease diagnosis with test results. The final diagnosis was established by cytologic and/or histologic examinations, the histopathologic examination of the surgically resected specimen, and the results of other diagnostic investigations or clinical follow-up. For the purpose of this meta-analysis, cases that were

TABLE 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Prospective study	Retrospective study
Used EUS-guided FNA for diagnosis of solid pancreatic masses	Used abdominal US-guided FNA
K-*ras* mutation analysis based on the material obtained by FNA	Not pancreas specific
Used a reference standard of definitive surgical histology, unequivocal histo-cytopathology, or clinical follow-up	Review articles, case reports, editorials, and corresponding letters that did not report their own data
Data available to construct contingency tables for true-positive, false-positive, false-negative, and true-negative determinations	Insufficient data (not completed even after directly contacting first and/or corresponding authors)

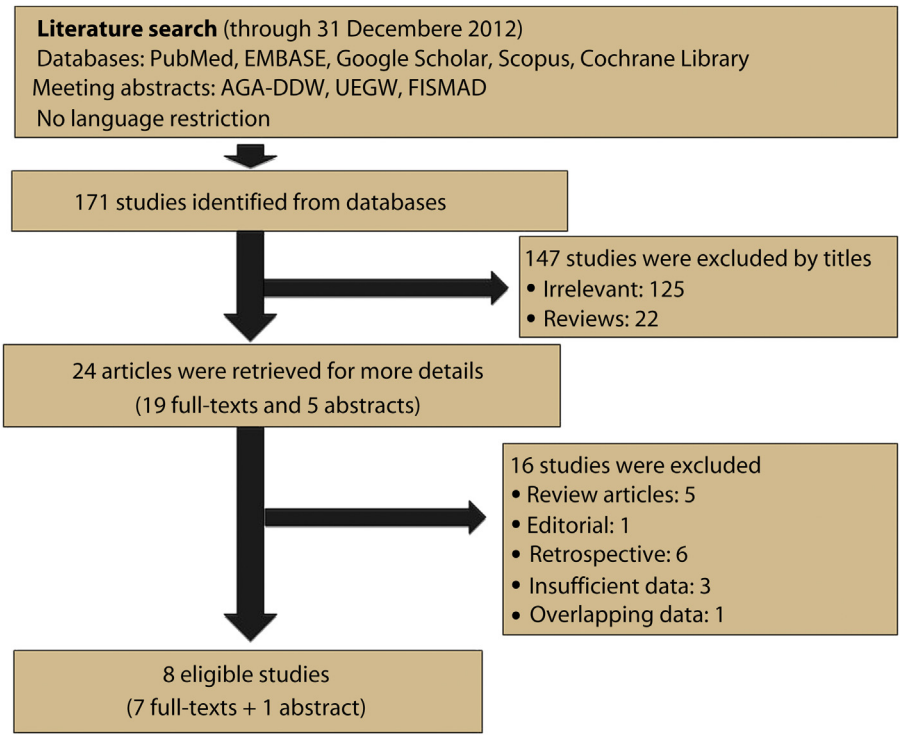

Figure 1. Flowchart demonstrating the algorithm for identifying suitable articles for inclusion. *AGA-DDW,* American Gastroenterological Association-Digestive Disease Week; *UEGW,* United European Gastroenterology Week; *FISMAD,* Italian National Congress of Gastroenterology.

inconclusive by EUS-FNA were considered as negative results, being classified as either false negative or true negative according to the final diagnosis.

Descriptive statistics for the dataset included sensitivity, specificity, and false-positive rate of the primary studies, their positive and negative likelihood ratios, and their diagnostic odds ratios (OR). The degree of variability among study results was first evaluated graphically by plotting sensitivity and specificity from each study on a forest plot. The chi-square test was performed to assess heterogeneity of studies results, the null hypothesis being in both cases that all are equal. The I^2 statistic provides an estimate of the amount of variance due to heterogeneity rather than chance and is based on the traditional measure of variance, the Cochrane Q statistic. Values of I^2 equal to 25%, 50%, and 75% were assumed to represent low, moderate, and high heterogeneity, respectively. We used a bivariate model for diagnostic meta-analysis to obtain an overall sensitivity and an overall specificity.[21]

The bivariate model uses a random-effects approach for both sensitivity and specificity, which allows for between-study variability. To graphically present the results, we plotted the individual and summary points of sensitivity and specificity in a receiver operating characteristic graph, plotting the index test's sensitivity (true-positive rate) on the y axis against 1-specificity (false-negative rate) on the x axis. In addition, we plotted a 95% prediction region around the pooled estimates to illustrate the precision

TABLE 2. Study characteristics of included studies

Study (reference)	Year	Country	No. of centers	Enrollment period	Patients	Final diagnosis*	PADC Total	Non-PADC total lesions	Non-PADC benign lesions	Other pancreatic neoplasia
Tada (22)	2002	Japan	1	Feb 1998-Mar 2001	34	1,2,3,4,5	26	8	8 (CP)	0
Pellisé (18)	2003	Spain	1	Sep 2001-Mar 2002	57	1,5	33	24	5 (CP)	19 (6 PNET, 5 IPMN, 5 CyA, 2 ME, 1 Ly)
Takahashi (23)	2005	Japan	1	Aug 1998-Apr 2003	77	1,5	62	15	15 (CP)	0
Maluf-Filho (17)	2007	Brazil	1	May 2002-Apr 2004	74	1,5	57	17	11 (CP)	6 (PNET)
Bournet (16)	2009	France	4	Jan 2005-Apr 2007	178	1,2,5	129	49	33 (27 CP, 6 BI)	16 (12 PNET, 4 ME)
Wang (10)	2011	China	1	Jan 2008-Mar 2010	82	1,2,3,5	54	28	17 (10 CP, 3 AIP, 1 BI, 1 PT, 2 PP)	11 (4 IPMN, 7 MCN)
Ogura (24)	2012	Japan	1	Mar 2004-Sep 2009	394	1,2,5	307	87	47 (24 CP, 23 AIP)	40 (20 PNET, 8 ME, 3 ACC, 3 Ly, 3 SPT, 2 SCT, 1 LC)
Visani (25)	2012	Italy	1	Nov 2010-Oct 2011	35	1,2,3,5	18	17	7 (5 PP; 2 SCT)	10 (4 PNET, 1 ME, 2 IPMN, 1 MCN, 2 SPT)

PADC, Pancreatic adenocarcinoma; *CP,* chronic pancreatitis; *PNET,* pancreatic neuroendocrine tumor; *IPMN,* intraductal papillary mucinous tumor; *CyA,* cystadenoma; *ME,* metastasis; *Ly,* lymphoma; *BI,* benign inflammation sequelae of inflammatory pancreatitis; *AIP,* autoimmune pancreatitis; *PT,* pancreatic tuberculosis; *PP,* pancreatic pseudocyst; *IPMN,* intraductal papillary mucinous tumor; *MCN,* mucinous cystic neoplasm; *ACC,* acinar cell carcinoma; *SPT,* solid-pseudopapillary tumor; *SCT,* serous cystic tumor; *LC,* lymphoepithelial cyst.
*Final diagnosis: 1 = surgery; 2 = histologic/cytologic examinations; 3 = imaging techniques; 4 = autopsy; 5 = follow-up.

with which the pooled values were estimated (confidence ellipse of a mean) and to show the amount of between-study variation (prediction ellipse; the likely range of values for a new study). Diagnostic accuracies of the different tests were compared by using a logistic mixed-effect model in which the primary studies were considered as random effects, with test as fixed effects. These analyses were undertaken by using *R* statistical software (R package version 0.5.1.mada: Meta-analysis of Diagnostic Accuracy (mada); R Core Team (2012). R Foundation for Statistical Computing, Vienna, Austria.).

RESULTS

Eligible studies and quality assessment

As shown in Figure 1, the literature search identified 8 studies published (7 in extensus[10,16-18,22-24] and 1 in abstract form[25]) that fulfilled the inclusion criteria. Their main characteristics are reported in Table 2. Overall, 931 cases were entered in the meta-analysis, ranging from 34 to 394 patients per study.

EUS-FNA was technically successful in all patients, almost all studies used the same size FNA needle (22 gauge), and the same mean number of needle passes per patient was performed across all the studies. Notably, K-*ras* gene mutation analysis was feasible in all cases independently of the adequacy of the cellularity obtained by FNA. Details on EUS-FNA and K-*ras* gene mutation analyses are reported in Table 3.

The quality of the eligible studies, as assessed according to the QUADAS criteria, is reported in Figure 2. The percentage of high-quality studies (ie, those for which a yes response applied) ranged from 66% to 75% for each of the 12 items. In most of the studies, it was unclear whether the patients received the same reference standard regardless of the index test result and whether the authors interpreted the reference standard results without knowledge of the results of the K-*ras* gene mutation analysis.

Synthesis of results

The results of the included individual studies are provided in Table 4.

EUS-FNA

In 116 cases (12%), the EUS-FNA material was deemed as inconclusive. Of these, 88 cases (76%) were false negatives (ie, PADC at the final diagnosis), and 28 were true

TABLE 3. Details on EUS-FNA (technically successful in all cases) and K-*ras* gene mutation analysis

Study (reference)	EUS-FNA sample adequate (%)	Size of needle, gauge	Mean no. needle passes/patient	On-site cytopathologist	K-*ras* gene mutation analysis	Codon	K-*ras* gene mutation analysis successful, %
Tada (22)	71	22	2.5	No	Mutation specific	12	100
Pellisé (18)	93	22	2.4	Yes	PCR	12	100
Takahashi (23)	92	22	2.3	Yes	PCR	12	100
Maluf-Filho (17)	93	22	3	Yes	PCR	12	100
Bournet (16)	84	22	At least 2/patient	No	PCR + sequencing	12, 13	100
Wang (10)	80	19, 22	2.6	No	PCR + sequencing	12, 13	100
Ogura (24)	90	22	2.3	Yes	Mutation specific	12	99.7
Visani (25)	80	19, 22, 25	2.4	Yes	Mutation specific + sequencing	12, 13, 61	100

EUS-FNA, EUS-guided FNA; *PCR,* polymerase chain reaction.

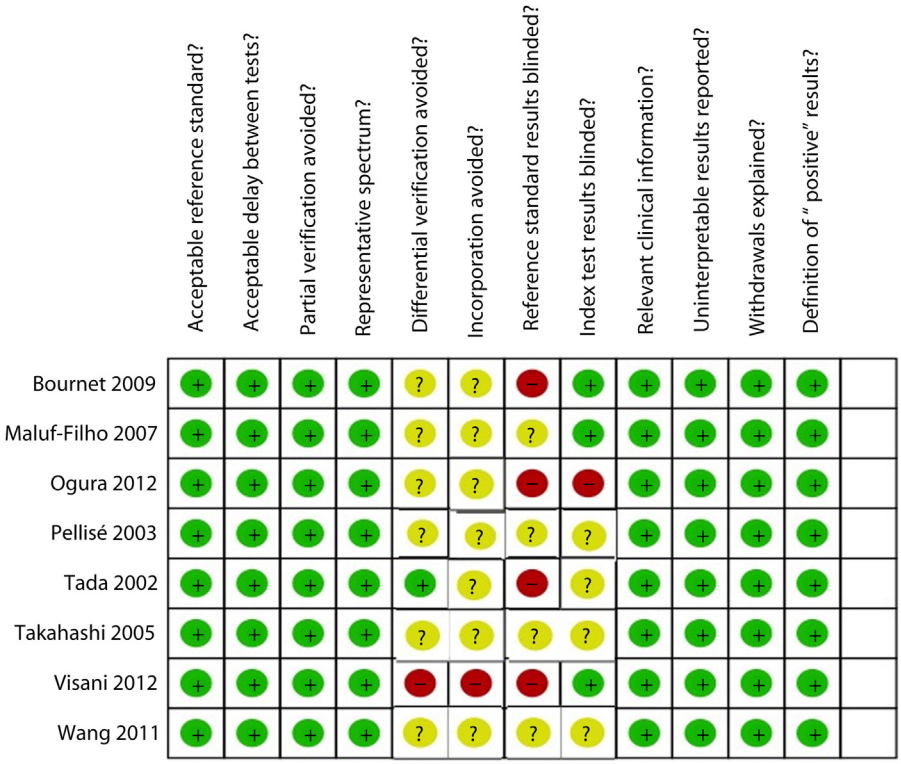

Figure 2. The quality of the eligible studies as assessed according to the 12 items included in the Quality Assessment of Diagnostic Accuracy Studies[20] (QUADAS) criteria.

negatives (ie, negative at the final diagnosis). The sensitivity for PADC detection ranged from 61% to 93%, and specificity ranged from 92% to 99% (Fig. 3). Estimated sensitivity and specificity was 80.6% (95% confidence interval [CI], 72.1-86.9) and 97.0% (95% CI, 93-99%). Between-study heterogeneity was substantial, with an I^2 of 76.5% for sensitivity ($P < .001$). The reverse was true for specificity, with an I^2 of 0% ($P = .588$). The summary receiver operating characteristic curve (SROC) graph for the diagnosis of PDAC is shown in Figure 4. The partial area under the curve (restricted to observed false-positive rates) was 73.7%. A positive correlation across studies was detected between sensitivity and specificity, not the negative correlation that would be expected.

TABLE 4. Results of individual studies for EUS-FNA and K-*ras* gene mutation analysis

	EUS-FNA				K-*ras*				EUS-FNA + K-*ras*			
Study (reference)	**TP**	**FP**	**FN**	**TN**	**TP**	**FP**	**FN**	**TN**	**TP**	**FP**	**FN**	**TN**
Tada (22)	16	0	10	8	20	0	6	8	21	0	5	8
Pellisé (18)	31	0	2	24	24	0	9	24	32	0	1	24
Takahashi (23)	52	0	10	15	46	0	16	15	58	0	4	15
Maluf-Filho (17)	47	1	10	16	38	1	19	16	48	2	9	15
Bournet (16)	108	0	21	49	79	1	50	48	115	1	14	48
Wang (10)	33	0	21	28	48	9	6	19	43	9	11	19
Ogura (24)	268	0	39	87	267	3	40	84	286	3	21	84
Visani (25)	16	0	2	17	15	1	3	16	18	1	0	16

EUS-FNA, EUS-guided FNA; *TP,* true positive; *FP,* false positive; *FN,* false negative; *TN,* true negative.

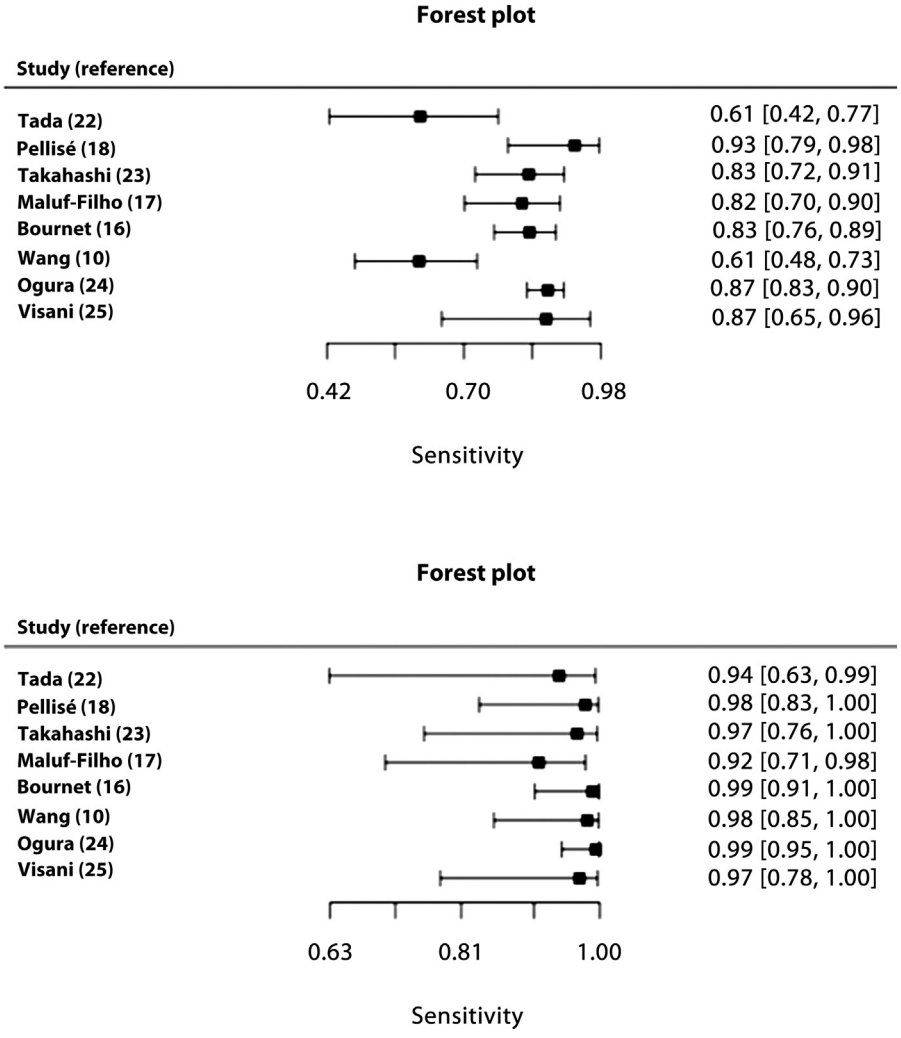

Figure 3. EUS-guided FNA. Forest plots show the sensitivity and specificity with 95% CIs for each individual study. Estimated sensitivity and specificity was 80.6% (95% CI, 72.1%-86.9%) and 97.0% (95% CI, 93%-99%). In this graph, between-study variability is also provided, showing substantial heterogeneity for sensitivity. *CI,* confidence interval.

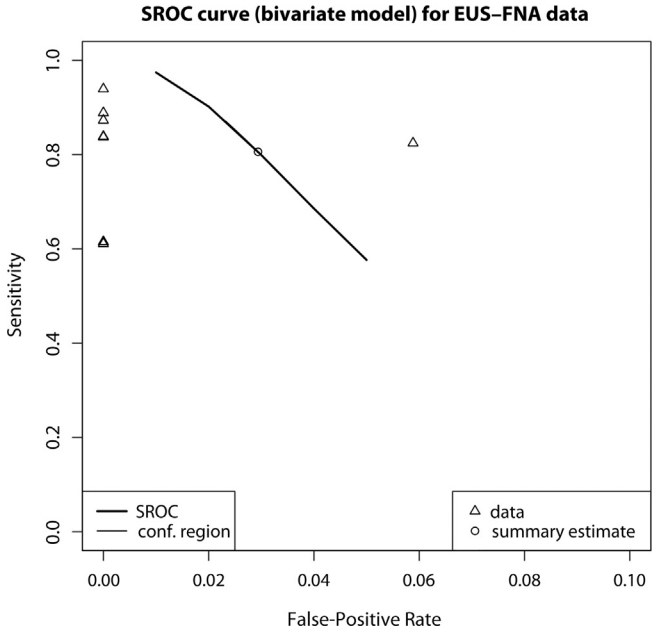

Figure 4. EUS-guided FNA. In this figure, the SROC curve is plotted. The point estimate of the pair of sensitivity (80.6%; 95% CI, 72.9%-86.9%) and false-positive rate (0.029; 95% CI, 0.012-0.071) also is plotted. *CI,* confidence interval; *SROC,* summary receiver operating characteristic; *EUS-FNA,* EUS-guided FNA.

K-*ras*

K-*ras* gene mutation analysis was feasible in all 931 patients. The pooled sensitivity (based on a bivariate approach) was 76.8% (95% CI, 68-84), and the overall specificity was 93.3% (95% CI, 84.9%-97.2%). As shown in Figure 5, there was substantial heterogeneity for both sensitivity (I^2 84.1%; $P < .001$) and specificity (I^2 =73.7%; $P < .001$). Figure 6 plots the SROC with the summary operating point for PADC diagnosis. The partial area under the curve was 79.9%.

Combination of EUS-FNA with K-*ras* mutation determination

The pooled sensitivity and specificity for PADC diagnosis, calculated by using data from all studies, was 88.7% (95% CI, 83.6%-92.4%) and 92.0% (95% CI, 83.0%-96.5%). There was significant heterogeneity across studies for both sensitivity ($I^2 = 75.1\%$; $P = .007$) and specificity ($I^2 = 64.6\%$; $P < .001$) (Fig. 7). Figure 8 plots the SROC curve with the summary operating point for PADC diagnosis. The partial area under the curve was 83.9%.

Comparison of SROC curves

K-*ras* mutation determination versus EUS-FNA plus K-*ras* mutation determination. The summary estimates of sensitivity and specificity are well-separated (Fig. 9). Furthermore, logistic regression analysis also indicated that EUS-FNA in combination with K-*ras* mutation analysis offered a better accuracy for PADC diagnosis ($P < .001$) than K-*ras* gene analysis alone. It would be safe to conclude that EUS-FNA in combination with K-*ras* mutation determination is a more reliable way for PADC diagnosis than K-*ras* mutation determination alone.

EUS-FNA versus EUS-FNA plus K-*ras* mutation determination. The summary estimates of sensitivity and specificity are well-separated (Fig. 10). Logistic regression analysis also indicated that EUS-FNA in combination with K-*ras* analysis offered a better accuracy for PADC diagnosis than EUS-FNA determination alone ($P = .008$). It would be safe to conclude that EUS-FNA in combination with K-*ras* mutation determination is a more reliable way for PADC diagnosis than EUS-FNA alone.

K-*ras* mutation determination in inconclusive EUS-FNA results

Of the 116 patients with inconclusive EUS-FNA results, 88 patients (75.9%) were eventually diagnosed with PADC. Overall, K-*ras* was positive in 52 of the inconclusive cases (45%), correctly classifying 49 of the 88 PADCs, corresponding to a pooled sensitivity and specificity of 56% and 89%, respectively. The overall absolute number of PADCs diagnosed when we passed from EUS-FNA to a sequential EUS-FNA + K-*ras* strategy increased from 572 of 931 (61%) to 604 of 931 (65%).

DISCUSSION

According to our meta-analysis, K-*ras* gene mutation analysis is an accurate technique for diagnosing PADC in patients with pancreatic solid masses, with an overall sensitivity and specificity of 76.8% and 93.3%, respectively. Importantly, our meta-analysis showed a potential synergism between K-*ras* testing and EUS-FNA, with a major role of K-*ras* mutation determination for those cases in which EUS-FNA was inconclusive. In fact, when we applied K-*ras* mutation determination to cases in which EUS-FNA was inconclusive, the false-negative rate was reduced by 55.6%, with a false-positive rate of 10.7%. However, it should be pointed out that the combination strategy EUS-FNA + K-*ras* testing, although it increases the sensitivity by 8% when compared with the EUS-FNA–only approach, decreases the specificity by 5%. Despite the substantial reduction of the number of false-negative cases, the combined technique represents an undeniable advantage, and the risk, albeit small, of false-positive results prompts a cautious integration of the combined strategy results with all the other clinical variables of the patient.

The K-*ras* gene is the most commonly mutated oncogene in pancreatic cancers (>75% of cases), generally by point mutations in codon 12.[12-14,26,27] The K-*ras* gene is located on chromosome 12p and encodes a membrane-bound guanosine triphosphate (GTP)–binding protein, which mediates various cellular functions, such as proliferation and cellular survival; once mutated, the regulated GTPase activity is abolished, which results in

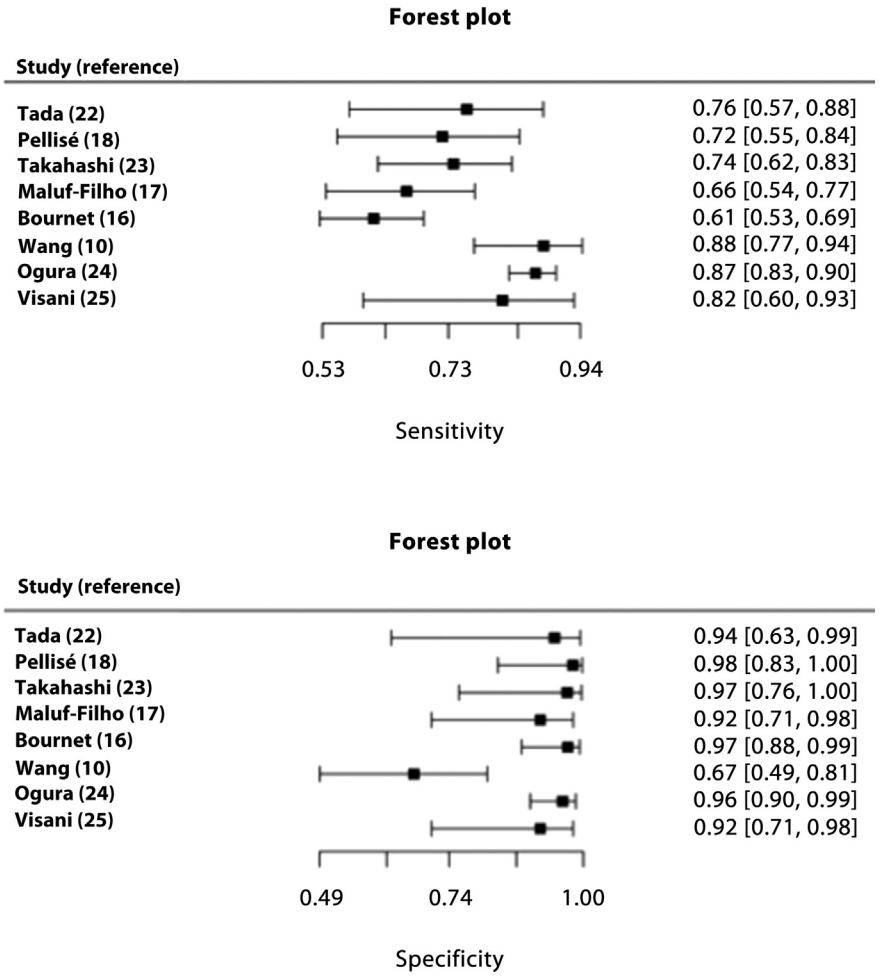

Figure 5. K-*ras* testing. Forest plots show the sensitivity and specificity with 95% CIs for each individual study. The pooled (based on a bivariate approach) sensitivity was 76.8% (95% CI, 0.68-0.84), and the overall specificity was 93.3% (95% CI, 84.9-97.2). In this graph, we can view the results of variation across studies. For both sensitivity and specificity, there was substantial heterogeneity. *CI,* confidence interval.

constitutive signalling.[28] Mutations in the K-*ras* gene are considered 1 of the earliest genetic events in pancreatic tumorigenesis.[15,29,30] Furthermore, a stepwise increase in K-*ras* mutations, with an increasing grade of dysplasia in pancreatic intraepithelial neoplasia from patients with ductal adenocarcinoma has been found.[15]

The management of patients with pancreatic solid masses and inconclusive FNA results has not been widely standardized. According to the European Society of Gastrointestinal Endoscopy clinical guidelines, in patients with inconclusive findings at initial EUS-FNA, repetition of EUS-FNA is strongly advised.[31] However, published studies on this issue have shown that the repetition of EUS-FNA may have suboptimal accuracy, yielding a correct diagnosis in about 60% to 80% of cases.[32-34] In the retrospective study of Nicaud et al,[32] based on 28 patients, repeating EUS-FNA in patients with pancreatic solid masses provided a sensitivity for the diagnosis of cancer of 35% and an overall accuracy of 61%. In a similar study including 24 patients, Eloubeidi et al[33] found a diagnostic accuracy of 84%. It should be highlighted that both studies were performed in tertiary-care referral centers by highly experienced endosonographers, thus limiting their external validity.

Based on the results of our meta-analysis, the implementation of K-*ras* gene mutation analysis for cases in which EUS-FNA was inconclusive would reduce the need for repeat EUS-FNA. It could be argued that K-*ras* sensitivity appears to be reduced substantially when K-*ras* sensitivity for PADC is assessed only in cases in which EUS-FNA results are inconclusive. This decrease in the sensitivity may in part be explained by the heterogeneous distribution of the K-*ras* gene mutation within the tumor mass and may reflect the scarcity of biologic material that usually characterized cases in which the EUS-FNA diagnosis was inconclusive. On the other hand, K-*ras* mutation analysis was feasible in all cases, independent of the adequacy of cellularity obtained by FNA, suggesting that other factors may be responsible for the decreased sensitivity observed in specimens from cases in which the EUS-FNA diagnosis was inconclusive.

The impact of such suboptimal sensitivity is worsened by the extremely high prevalence of PADC in the study

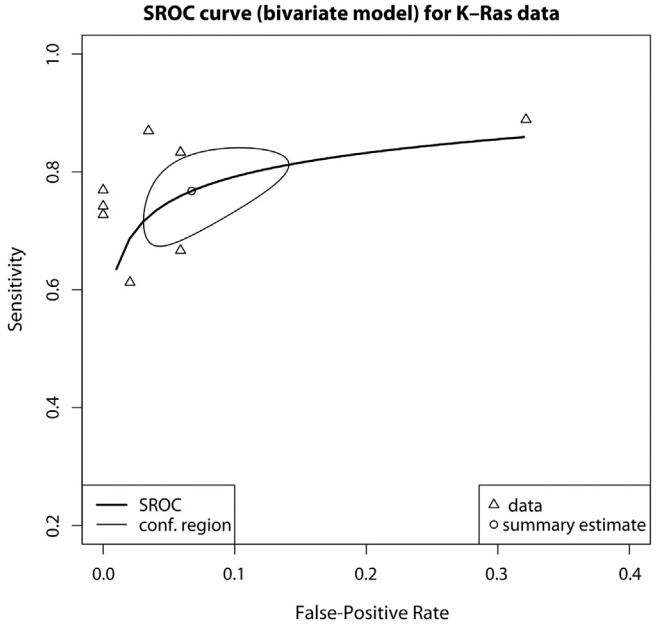

Figure 6. K-*ras* testing. In this figure, the SROC curve is plotted. The point estimate of the pair of sensitivity (76.6%; 95% CI, 67.9%-83.7%) and false-positive rate (0.067; 95% CI, 0.028-0.151) is also plotted. *SROC,* summary receiver operating characteristic.

population and especially in those cases in which the EUS-FNA findings were inconclusive, in which a PADC prevalence rate of nearly 80% was estimated by our analysis. Therefore, a possible synergism between EUS-FNA and K-*ras* gene mutation testing could contribute to limiting the repetition of EUS-FNA only to those cases that are negative at K-*ras* gene mutation analysis (ie, wild-type K-*ras*), while considering as true positive cases in which K-*ras* testing is positive after inconclusive EUS-FNA results. Indeed, not repeating EUS-FNA in those cases with inconclusive results for K-*ras* gene mutation testing would reduce the repeat-biopsy rate from 12.5% to 6.8%.

Avoiding further biopsy would lead to a substantial saving of financial and medical resources. It should be noted that the total cost for K-*ras* assay, including sequencing, is estimated 60 dollars. Furthermore, K-*ras* gene mutation analysis is not operator-dependent and is feasible in almost all cases. On the other hand, a repeat EUS-FNA with the presence of an on-site cytopathologist may cost more than 1300 to 1600 dollars, and repeat EUS-FNA has a somewhat comparable or slightly higher diagnostic accuracy.

Administration of serum CA 19-9 (carbohydrate antigen) is routinely performed in patients with pancreatic solid masses. A recently published meta-analysis, including data from 57 studies with more than 3200 patients with pancreatic cancer and 1800 with benign pancreatic disease, showed that the summary estimates for CA 19-9 (cut-off $\geq$37 U/mL) were 78.2% mean sensitivity and 82.8% mean specificity for discriminating pancreatic carcinoma from benign pancreatic disease.[35] Based on the results of our meta-analysis, the only K-*ras* testing presented a pooled sensitivity and specificity of 78.3% and 93.9%, respectively. The study by Wang et al,[10] including 82 patients, evaluated the diagnostic accuracy of the combination of both tests, K-*ras* testing and serum CA 19-9, in the diagnosis of cases in which EUS-FNA results were indeterminate and demonstrated that the sensitivity of the combination strategy was significantly higher than for serum CA 19-9 alone, but with no differences in the other diagnostic parameters. Further studies, with larger sample size, are warranted to confirm the benefit of the combination strategy.

Our meta-analysis presents some limitations. The meta-analysis was focused on the role of K-*ras* gene mutation analysis in solid pancreatic masses, therefore any inference to cystic lesions is not appropriate. However, it should be pointed out that 4 of the 8 included studies also included some cystic lesions in the non-PADC group[10,18,24,25]; in particular, a total of 36 cystic lesions of a total of 245 non-PADC lesions (14.7%) were included, as reported in Table 2. Because of a paucity of data, we were not able to exclude cystic lesions from the analyses. The prevalence of K-*ras* gene mutations has been reported to range from 0% to 42% in benign cystic lesions and from 20% to 53% in malignant lesions.[36-39] The role of K-*ras* testing in the differential diagnosis between mucinous and nonmucinous, malignant and benign cystic neoplasms is still unclear.[36,38,40] In a study of 36 patients, K-*ras* mutation combined with the loss of heterozygosity had a sensitivity of 91% and a specificity of 93% for the diagnosis of malignant cysts.[40] Subsequently, Khalid et al[38] performed a larger study, including 113 patients, and reported that the presence of the K-*ras* mutation did not differ between malignant and premalignant cysts, and the combined strategy still continued to have a high specificity (94%) but a substantially decreased sensitivity (37%). Several other studies have confirmed the low sensitivity and the high specificity of K-*ras* mutation testing in the differentiation of benign and malignant pancreatic cysts.[41] K-*ras* mutation analysis in cystic lesions may provide useful information, but published data suggest that it cannot be recommended as the only test but always should be considered in addition to other genetic analyses (ie, loss of heterozygosity) and diagnostic modalities (ie, dosage of cyst fluid carcinoembryonic antigen). K-*ras* mutation testing might also play a role as a prognostic factor; indeed, it has been shown recently that the detection of the K-*ras* mutation is an independent risk factor significantly associated with a non-benign course of cystic lesions (OR 3.4).[39]

Only prospective studies were included in our meta-analysis, therefore, although an extensive literature search was performed, only 8 studies were included: 7 were published in extensus and 1 in abstract form; in this latter case, the authors were directly contacted and provided further information. Three studies were excluded because of insufficient data to construct contingency tables: 2 published in

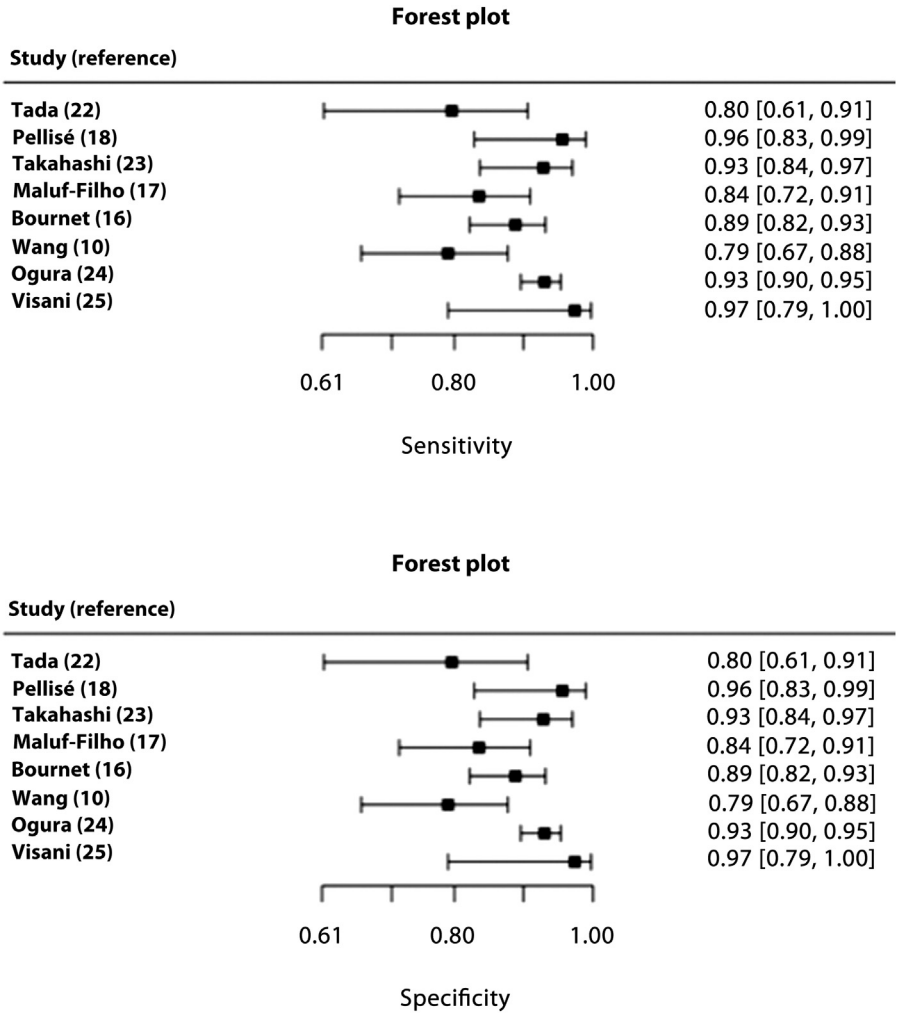

Figure 7. Combination strategy EUS-FNA plus K-*ras* testing. Forest plots show the sensitivity and specificity with 95% CIs for each individual study. The pooled sensitivity and specificity for PADC diagnosis, calculated using data from all studies, was 88.7% (95% CI, 83.6%-92.4%) and 92.0% (95% CI, 83.0%-96.5%), respectively. In this graph, we can view the results of variation across studies. For both sensitivity and specificity, there was substantial heterogeneity. *EUS-FNA,* EUS-guided FNA; *PADC,* pancreatic adenocarcinoma.

abstract form[42,43] and 1 published as full text.[11] In 2 cases, e-mails with requests for further details were sent to first and/or corresponding authors[11,42]; in 1 case we were not able to find a contact address.[43]

Potential factors of heterogeneity were evaluated. The studies did not differ according to the needle size used and the mean number of needle passes per patient; most of the studies[17,18,23-25] included an on-site cytopathologist. All the studies used the currently suggested methods for mutation analysis (polymerase chain reaction, mutation specific and direct sequencing analyses),[44] however, unavoidably, different methodologies present different diagnostic accuracy.[45] The true rate of K-*ras* mutation in carcinoma of the pancreas has been widely debated during recent years because it is directly influenced by several variables, including the methodology implemented.[46] Considering the impact that K-*ras* gene mutation detection has in clinical practice (ie, management of metastatic colon cancer), it is almost surprising that a widely accepted consensus on the methodology to use for K-*ras* gene analysis has not yet been proposed. Every mutation detection technique presents drawbacks; sequencing, for example, is a highly specific technique with a very low false-positive rate, but it is not very sensitive; mutation specific techniques depend on the original design of the assay, testing for a subset of the most common mutations and leading to false negatives when different mutations are present. Several promising detection techniques with high sensitivity and specificity have been proposed in recent years (ie, peptide nucleic acid–directed polymerase chain reaction), and validating studies are warranted. The implementation in the future of K-*ras* gene mutation testing assays with increased sensitivity and specificity might theoretically further increase the usefulness of K-*ras* mutation testing in cases in which EUS-FNA results are indeterminate. The discrepant values for

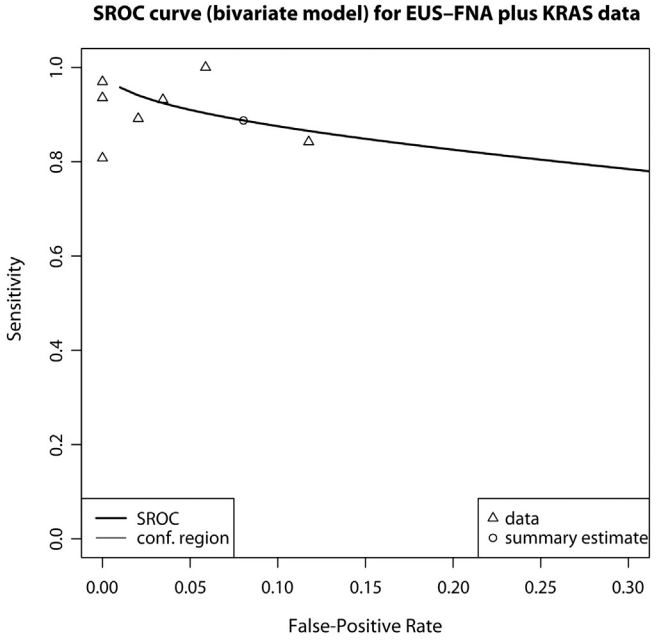

Figure 8. Combination strategy EUS-FNA plus K-*ras* testing. In this figure, the SROC curve is plotted. The point estimate of the pair of sensitivity (88.7%; 95% CI, 83.4%-92.4%) and false-positive rate (0.08; 95% CI, 0.035-0.17) is also plotted. *EUS-FNA,* EUS-guided FNA; *SROC,* summary receiver operating characteristic.

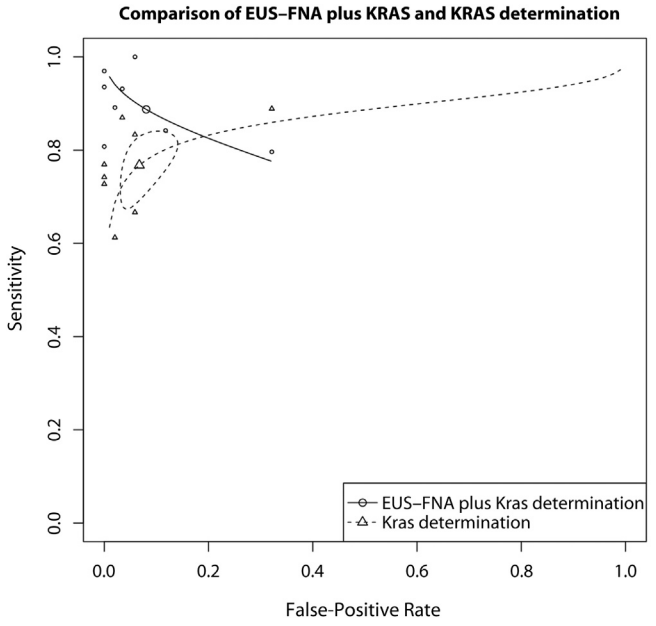

Figure 9. Comparison of SROC curves: EUS-FNA plus K-*ras* testing versus K-*ras* testing alone. *SROC,* summary receiver operating characteristic; *EUS-FNA,* EUS-guided FNA.

sensitivity and specificity associated with different assays used in the studies included in our meta-analysis might have conditioned the results of our analysis and contributed to the heterogeneity.

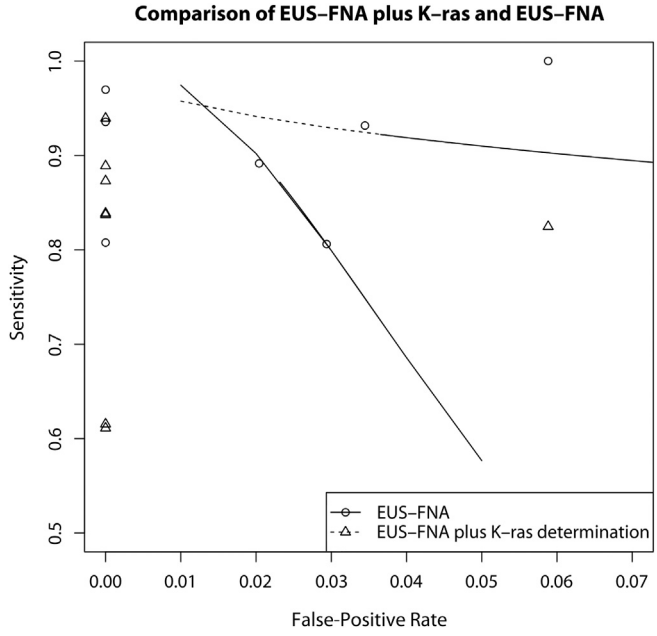

Figure 10. Comparison of SROC curves: EUS-FNA plus K-*ras* testing versus EUS-FNA alone. *SROC,* summary receiver operating characteristic; *EUS-FNA,* EUS-guided FNA.

All the included studies in our analysis evaluated mutation at codon 12; 2 studies evaluated both codons 12 and 13,[10,16] and only 1 study analyzed 3 codons, 12, 13, and 61.[25] It should be pointed out that mutations at codons 13 and 61 are generally rare.[47] Indeed, in the study of Bournet et al,[16] no mutation of the K-*ras* gene at codon 13 was detected in codon 12, K-*ras*–negative samples. In the study of Wang et al,[10] of the 57 patients with K-*ras* mutations, 56 had mutations at codon 12, and only 1 patient with pancreatic cancer had a mutation at codon 13. Similarly, in the study of Visani et al,[25] no mutations at codon 13 were detected but only at codon 12 and in 4 cases also at codon 61. Therefore, it is unlikely that the lack of analysis of codons 13 and 61 in some studies might have substantially influenced the K-*ras* gene mutation detection rates.

The quality assessment was performed according to the 12-item QUADAS questionnaire[20] (Fig. 2), and a positive response was applied for most of the items. Therefore, the included studies can be considered high-quality studies. Notably, almost all studies did not mention whether or not the reference standard was independent of the results of the index test (K-*ras* status), therefore it is not possible to exclude the presence of incorporation bias for some of the included studies. Furthermore, in 4 studies,[16,22,24,25] the results of the reference standard were interpreted with knowledge of the K-*ras* status, therefore a review bias cannot be avoided. Finally, patients without PADC and without benign pancreatic masses differed among the studies (Table 1). Subgroup analysis, however, could not be performed because of a paucity of

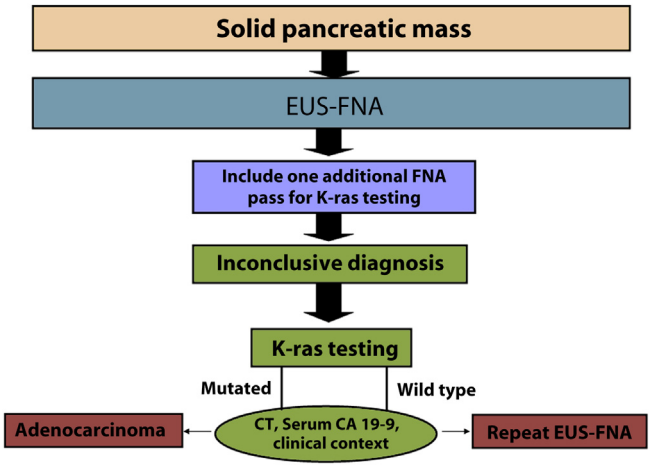

Figure 11. Algorithm for the management of solid pancreatic mass. During EUS-FNA, an additional pass should be performed for K-*ras* testing in case of inconclusive diagnosis. The result of K-*ras* gene analysis should then be considered in the clinical context and compared with all other diagnostic findings. *CA,* carbohydrate antigen.

data to construct contingency tables in most of the included studies.

In conclusion, K-*ras* mutation analysis can be useful in the diagnostic work-up of pancreatic mass lesions and may complement other diagnostic modalities, in particular when EUS-FNA cytology specimens are judged inconclusive. In these cases, discovery of a K-*ras* gene mutation can spare an unnecessary repeat EUS-FNA procedure. An additional FNA pass during EUS for solid pancreatic masses could be performed and used for K-*ras* mutation testing in case of an inconclusive diagnosis (Fig. 11). However, when adopting K-*ras* mutation analysis as an additional test in the diagnosis of solid pancreatic masses, the clinician should be aware that the substantial reduction in the false-negative rate is counterbalanced by a relatively small increase in the false-positive rate. Therefore, K-*ras* mutation testing should always—as any other similar modality—be cautiously interpreted within the clinical context. Further studies are needed to confirm this finding.

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