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Clinical validity of detecting *K-ras* mutations for the diagnosis of exocrine pancreatic cancer: a prospective study in a clinically-relevant spectrum of patients

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Abbreviations BPD, benign pancreatic disease; CEBS, cancer of the extra-hepatic biliary system; CI, Confidence Interval; CP, chronic pancreatitis; EPC, exocrine pancreatic cancer; ERCP, endoscopic retrograde cholangio-pancreatography; IQR, interquartile range; OBD, other benign disease; OM, other malignancy; OR, Odds Ratio.

Running title / ‘Running head’

Role of *K-ras* mutations to diagnose exocrine pancreatic cancer

Keywords KRAS oncogene; genes, ras; oncogene protein p21 ras; pancreatic neoplasms / diagnosis; research / methods; diagnosis / diagnostic use; diagnosis / analysis; diagnosis / methods; epidemiology / diagnosis.

Abstract The diagnostic utility of detecting *K-ras* mutations for the diagnosis of exocrine pancreatic cancer (EPC) has not been properly studied, and few reports have analysed a clinically relevant spectrum of patients. The objective was to evaluate the clinical validity of detecting *K-ras* mutations in the diagnosis of EPC in a large sample of clinically relevant patients. We prospectively identified 374 patients in whom one of the following diagnoses was suspected at hospital admission: EPC, chronic pancreatitis, pancreatic cysts, and cancer of the extrahepatic biliary system. Mutations in the *K-ras* oncogene were analysed by PCR and artificial RFLP in 212 patients. The sensitivity and specificity of the *K-ras* mutational status for the diagnosis of EPC were 77.7% (95% CI: 69.2 – 84.8) and 78.0% (68.1 – 86.0), respectively. The diagnostic accuracy was hardly modified by sex and age. In patients with either mutated *K-ras* or CEA >5 ng/mL, the sensitivity and specificity were 81.0% (72.9 – 87.6) and 62.6% (72.9 – 87.6), respectively. In patients with mutated *K-ras* and CEA >5 ng/mL the sensitivity was markedly reduced. In comparisons with a variety of non-EPC patient groups sensitivity and specificity were both always greater than 75%. In this clinically relevant sample of patients the sensitivity and specificity of *K-ras* mutations were not sufficiently high for independent diagnostic use. However, it seems premature to rule out the utility of *K-ras* analysis in conjunction with other genetic and ‘omics’ technologies.

Introduction

The diagnosis of exocrine pancreatic cancer (EPC) remains a difficult challenge in clinical care. Early symptoms and laboratory findings are usually non-specific [1-4], and few tumour markers are widely accepted for routine use [2,3, 55]. When pancreatic cancer is clinically suspected, imaging techniques offer good diagnostic efficiency. Computed tomography (CT), the most sensitive procedure, non-invasively identifies approximately 90% of cases [66]. However, since CT has less than perfect specificity (approximately 54%), cytological confirmation of radiology findings is usually required. The only curative treatment for EPC is surgical resection, but it is feasible in as few as 20% of cases [1,2], and is not without problems [7-11]. More rapid and accurate methods to diagnose EPC are hence vital to identify patients who would benefit from surgical resection.

Activating point mutations at codon 12 of the *K-ras* gene are the most frequent oncogene alteration in EPC; at diagnosis they are detected in 75-90% of patients with EPC [12,13]. Therefore, it has long been argued that detecting *K-ras* mutations could be clinically useful in the differential diagnosis of EPC and related disorders [14]. In blood or stools of pancreatic patients *K-ras* mutations are detected at a much lower frequency than in tumour tissue [14-16]; thus, detection in such samples has not been proven effective in EPC diagnosis. Similarly, detecting mutated *K-ras* in pancreatic juice or bile has limited diagnostic usefulness [17]. By contrast, *K-ras* analysis can feasibly be performed in cytohistological specimens, which are often obtained through pancreatic duct brushing, fine needle aspiration, percutaneous biopsy or at surgery. Whether detecting *K-ras* mutations in these specimens has diagnostic utility is not established, with few reports of sufficient methodological quality, and conflicting results [14]: studies recommend detecting mutated *K-ras* for diagnosis of EPC as an independent test [18,19], as an adjunct

to cytology [20,21], only in certain situations such as atypical cytology [22,23], or not at all [7,24,25].

The diagnostic validity of a potential test should be evaluated in relevant populations [26-30]. It is particularly important that the ultimate stage of validation be carried out in a population as close as possible to that in which the test would be used in practice.

Therefore, reports demonstrating the utility of *K-ras* mutations for distinguishing between patients diagnosed with pancreatic cancer and a group of controls (either healthy or with a distinct diagnosis) do not reflect the relevant clinical practice, where the test would be used to detect EPC among symptomatic patients with apparently related and competing diagnoses. Furthermore, to allow for biological variation, it is important to assess the test in a large number patients. None of the above mentioned studies on *K-ras* and EPC meets these requirements: most studies had a limited patient spectrum, reduced study size, or inadequate description of inclusion criteria.

The aim of this study was to evaluate the clinical validity of detecting *K-ras* mutations in the diagnosis of EPC in a sample of clinically relevant patients.

Patients and methods

Selection of patients

Detailed methods of the PANKRAS II study have been previously described [4,31-43]. Briefly, subject recruitment took place between 1992 and 1995 at five general hospitals in the eastern Mediterranean part of Spain. Aided by the study research physicians, trained monitors prospectively identified hospital patients in whom one of the following diagnoses was suspected at admission: cancer of the exocrine pancreas, chronic pancreatitis, pancreatic cysts and pseudocysts (including those secondary to acute pancreatitis), and cancer of the extrahepatic biliary system [32,33]. The broad eligibility criteria are due to the main objective of the PANKRAS II study: to assess the clinical usefulness of detecting mutations in the *K-ras* for the diagnosis of cancers of the exocrine pancreas and the extrahepatic biliary system. A total of 602 subjects were selected. The ethics committees of the participating hospitals approved the study protocol, and all patients gave consent to be included in the study.

The present report is based on patients in whom a diagnosis of EPC was suspected at hospital intake, or who were definitively diagnosed with EPC during the study. Furthermore, the analysis is limited to individuals for whom cytological or histological material was available for *K-ras* analysis and in whom mutational status was determined (Figure 1). Table 1 presents the socio-demographic and clinical characteristics of the study population and compares the final study population with the individuals who met the inclusion criteria but in whom *K-ras* mutational status was not established [44].

Data collection

A structured form was used to collect clinico-pathological information from medical records, including details on presenting symptoms, past medical history, the physical examination at admission, ancillary diagnostic procedures, laboratory results and follow-up care [32,33,41,45]. Diagnostic tests included ultrasound scan, computerized axial tomography, fibrogastroscopy, endoscopic retrograde cholangio-pancreatography (ERCP), transparietohepatic cholangiography (TPHC), gammagraphy, laparoscopy and exploratory laparotomy [46]. We classified as ‘invasive’ diagnostic tests fibrogastroscopy, ERCP, TPHC, gammagraphy, laparoscopy and laparotomy. When a diagnostic procedure was performed more than once, the physician who extracted the data chose the more informative result. Socio-demographic information and additional information regarding symptoms and medical history were obtained directly from patients during their hospital stay by interview with trained monitors [4,44].

Definitive diagnosis

A panel of experts in gastrointestinal diseases, comprising surgeons and gastroenterologists with extensive experience in pancreatic and biliary cancer, a medical oncologist and several physician epidemiologists, reviewed the hospital discharge diagnosis and all pathological and clinical information available, including follow-up, to achieve a consensual definitive diagnosis [32,33,44]. This process was carried out blinded to the results of *K-ras* analysis and has also been described elsewhere in detail [33]. The definitive diagnosis was categorised into five groups: EPC, cancer of the extra-hepatic biliary system, other malignancy, benign pancreatic disease, and other benign disease. For the purpose of this study, these groups were used for the reference standard. The diagnostic index was calculated comparing patients definitively diagnosed with EPC with all other patients in the remaining four categories.

Detection of *K-ras* mutations

Details of laboratory protocols have also been described elsewhere [31,36-44]. Briefly, mutations in codon 12 of *K-ras* oncogene were studied using DNA extracted from paraffin-embedded tumor tissue. Amplifications were done in two steps by nested PCR; an artificial BstNI restriction endonuclease site was introduced to discriminate between wild-type and mutated *K-ras* codon 12 sequences. Products were analyzed by acrylamide gel electrophoresis and ethidium bromide staining. This technique was able to detect one homozygous mutated cell in the presence of 10^2 normal cells. To characterize the nucleotide substitution in codon 12, all mutated samples were further analyzed using a similar RFLP-based approach. Interpretation of digestion products' electrophoresis was performed independently by two investigators to confirm the results.

Statistical analysis

Univariate statistics were computed as customary [47]. For comparisons between continuous variables, Mann-Whitney's *U* test and Student's *t* test were used. In contingency tables, Fisher's exact test for homogeneity or independence was applied to assess the relationship between two categorical variables. Diagnostic accuracy of detecting *K-ras* mutation was expressed in terms of sensitivity and specificity with their corresponding 95% confidence intervals (CI) [26,28]. Data analysis was performed with SPSS 15.0 and Stata 8.0.

Results

Representativeness of the sample of patients

In this study, 212 patients contributed to the analysis of the diagnostic potential of detecting *K-ras* mutations. Although we prospectively attempted to include all individuals with suspected EPC or a definitive diagnosis of EPC, we were unable to include 162 patients due, first, to unavailability of a sample suitable for *K-ras* analysis and, to a lesser extent, to failure to establish the mutational status of the oncogene. Table 1 shows that excluded individuals were older than patients included in the analyses, and were less likely to be diagnosed definitively with EPC. Differences were observed in the diagnostic techniques performed in these two patient groups and in the recruitment hospital; diagnostic procedures vary by hospital and are linked to availability of tissue sample, the primary factor relating to failure to determine *K-ras*, and the diagnostic work-up is influenced by a number of factors, including patient characteristics as age (older patients are less likely to undergo invasive tests).

Definitive diagnoses and *K-ras* mutations

Among patients included in the study, the most frequent pathologies were EPC (n = 121 patients, 57.1%) and cancer of the extrahepatic biliary system (35, 16.5%). *K-ras* codon 12 was mutated in approximately half of all patients (114, 53.8%). While *K-ras* mutations were observed in all diagnostic groups (Table 2), the frequency was highest in patients definitively diagnosed with EPC (94, 77.7%), followed by cancer of the extra-hepatic biliary system (11, 31.4%), and other malignant diseases (6, 24.0%). Thus, 27 of the 121 subjects with EPC (22.3%) had wild-type *K-ras*. Tumours of patients with EPC had the following stage distribution: 25(20.7%) were in stage I, 21 (17.4%) in stage II, 15 (12.4%) in stage III, and 60 (48.6%) in stage IV. There was no significant difference in the

prevalence of mutated *K-ras* across stages, with 18 (72%) of stage I cancers harbouring the mutation, 19 (90.5%) of stage II, 12 (80.0%) of stage III, and 45 (75%) of stage IV cancers ($p=0.441$).

Diagnostic validity of *K-ras* mutations for exocrine pancreatic cancer

The sensitivity and specificity of the *K-ras* mutational status for the diagnosis of EPC in our patient sample were 77.7% (95% CI: 69.2 – 84.8) and 78.0% (68.1 – 86.0), respectively. If we limit the analysis to the 196 patients who entered the study with a suspected diagnosis of EPC, thus excluding 16 patients with a definitive diagnosis of EPC but missing information on the suspected diagnosis, the values of sensitivity and specificity were almost identical (77.1% and 78.0%, respectively). The diagnostic accuracy was unmodified by the sex or age of the patient (Table 3). We also evaluated the diagnostic validity of *K-ras* in certain clinical subgroups related with results from blood analyses and imaging tests, and found that the observed values for both sensitivity and specificity did not change significantly (Supplementary data, Tables S1, S2, and S3). We further assessed the potential clinical validity of detecting *K-ras* mutations in combination with other clinical information. Thus, in patients with either mutated *K-ras* or CEA >5 ng/mL, the sensitivity and specificity were 81.0% (72.9 – 87.6) and 62.6% (72.9 – 87.6), respectively. When we considered only patients with mutated *K-ras* and CEA >5 ng/mL the sensitivity was markedly reduced. This pattern was seen with all other analytes considered (Supplementary data, Tables S4 and S5). Results on the diagnostic accuracy of *K-ras* according to different comparisons and patient populations are shown in Table S6. Sensitivity was below 60% when the target disease were all malignant disorders, which is an uncommon clinical scenario. For all other comparisons, sensitivity and specificity were both greater than 75% (Supplementary data, Table S6).

Discussion

In this clinically relevant large sample of patients the sensitivity and specificity of *K-ras* mutations were less than ideal, 77.7% (95% CI: 69.2-84.8) and 78.0% (68.1-86.0), respectively. The clinical validity of mutated *K-ras* as an independent form of identifying EPC is hence limited, because more than 20% of the cases would erroneously be classified as non-pancreatic cancer (false negatives), and at least 20% of patients classified as EPC due to mutated *K-ras* would actually suffer from a distinct disease (false positives).

Diagnostic accuracy values remained remarkably stable when socio-demographic or clinical variables were considered. In this study, *K-ras* analysis was performed in histological and cytological samples, thus taking advantage of the two common procedures. *K-ras* mutational analysis could be used as an adjunct to cytology, potentially increasing the sensitivity, or it could be ordered only in cases where cytology is negative or inconclusive. In this study, we were unable to consider these scenarios, whose feasibility in a research study is uncertain.

As expected on clinical grounds, it was not possible to perform *K-ras* analysis in all patients who entered the PANKRAS II study with a suspicion of EPC. Nevertheless, selection criteria are among the widest on this field, and the pragmatic allowance of different methods of tissue collection made it possible to include a patient spectrum rather greater than in most previous studies [14]. The prompt identification of cases is another strength of the study [48]. We included 19 patients who were definitively diagnosed with EPC during the study but in whom information regarding suspected diagnosis at hospital admission was missing. The decision to include these patients did not take into account their *K-ras* mutational status and, therefore, the diagnostic sensitivity we calculated was

not influenced. In our population the prevalence of EPC may be different than in other clinical contexts; since positive and negative predictive values are highly influenced by disease prevalence, the predictive values of *K-ras* mutations will differ in other settings from values estimated in this report [27-30].

Although fieldwork for PANKRAS II study was carried out more than a decade ago, in our view the results presented here are pertinent and relevant today. During this time little progress has been made in the development of valid, evidence-based diagnostic procedures, including new molecular and ‘omics’ techniques [14]. Also regrettably, patients continue to be diagnosed late, often precluding any chance of curative treatment. On a population basis, survival rates for this aggressive tumour have remained stable in many regions worldwide [2,49].

In many cases, cytological or histological samples were collected during exploratory laparotomy, a relatively common procedure when EPC is suspected. However, *K-ras* analysis was also common in cytological samples obtained through less invasive measures as fine needle aspiration. Nevertheless, the sensitivity and specificity of *K-ras* did not depend on the method of tissue collection.

In conclusion, we have calculated the sensitivity and specificity of *K-ras* mutation for detecting EPC in a wide spectrum of patients with a variety of related pathologies common in many clinical settings. While the diagnostic index for detecting *K-ras* mutations in this context was not sufficiently high for independent diagnostic use, it seems premature to rule out the analysis of *K-ras* in conjunction with other technologies, especially in light of the advances in the ‘omics’ fields.

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Fig. 1 Flow of participants through the recruitment process

¹ At least one of the following diagnoses was suspected at hospital admission: exocrine pancreatic cancer (EPC), chronic pancreatitis, pancreatic cysts and pseudocysts, cancer of the extra-hepatic biliary system.

² Percentage calculated with respect to the box immediately above (e.g., 37.9% = 228 / 602 * 100).

³ 43 (37.1%) cases were definitively diagnosed as EPC.

⁴ 21 (45.7%) cases were definitively diagnosed as EPC.

Table 1 Comparison of 212 patients included in analysis with those who met inclusion criteria but were excluded (n = 162) due to lack of the index test

	Total (n=374) N (%)	K-ras determined		P value
		Yes (n=212) N (%)	No (n=162) N (%)	
Age				
Mean ± SD	66.6 ± 13.5	64.8 ± 13.3	68.9 ± 13.4	0.003 ¹
Median	68.0	65.8	70.8	0.003 ²
Minimum	26.0	26.0	36.7	
Maximum	93.6	91.9	93.6	
Sex				0.364 ³
Male	221 (59.1)	121 (57.1)	100 (61.7)	
Female	153 (40.9)	91 (42.9)	62 (38.3)	
Definitive diagnosis				<0.001 ³
Exocrine pancreatic cancer	185 (49.5)	121 (57.1)	64 (39.5)	
Cancer extra-hepatic biliary system	63 (16.8)	35 (16.5)	28 (17.3)	
Benign pancreatic diseases	51 (13.6)	21 (9.9)	30 (18.5)	
Other benign diseases	39 (10.4)	10 (4.7)	29 (17.9)	
Other malignancies	36 (9.6)	25 (11.8)	11 (6.8)	
Diagnostic work-up				
Cytology	174 (46.6)	118 (55.7)	56 (34.8)	<0.001 ³
Histology	235 (62.8)	165 (77.8)	70 (43.2)	<0.001 ³
Ultrasound scan	342 (91.4)	195 (92.0)	147 (90.7)	0.671 ³
Fibrogastroscopy	124 (33.2)	69 (32.5)	55 (34.2)	0.743 ³
Computed tomography	290 (77.5)	169 (79.7)	121 (74.7)	0.248 ³
ERCP	163 (43.6)	80 (37.7)	83 (51.2)	0.009 ³
Exploratory laparotomy	198 (52.9)	137 (64.6)	61 (37.7)	<0.001 ³
Study hospitals				0.004 ³
Hospital General de Elche	67 (17.9)	45 (21.1)	22 (13.6)	
Hospital del Mar	68 (18.2)	37 (17.5)	31 (19.1)	
Hospital de Son Dureta	78 (20.9)	51 (24.1)	27 (16.7)	
Hospital Valld'Hebron	112 (29.9)	48 (22.6)	64 (39.5)	
Hospital Mutua de Terrassa	49 (13.1)	31 (14.6)	18 (11.1)	

¹ Student's *t* test (two-tailed)

² Kruskal-Wallis' test

³ Pearson's chi-squared test (two-tail)

ERCP: endoscopic retrograde cholangio-pancreatography.

Table 2 Prevalence of *K-ras* mutations by definitive diagnosis

	<i>K-ras</i>			P value ¹
	Total	Mutated	Wild-type	
	(n=212)	(n=114)	(n=98)	
	N (%)	N (%)	N (%)	
Definitive diagnosis				<0.001
Cancer of the exocrine pancreas	121 (57.1)	94 (82.5)	27 (27.6)	
Cancer of the extrahepatic biliary system	35 (16.5)	11 (9.6)	24 (24.5)	
Benign pancreatic diseases	21 (9.9)	1 (0.9)	20 (20.4)	
Other benign diseases	10 (4.7)	2 (1.8)	8 (8.2)	
Other malignancies	25 (11.8)	6 (5.3)	19 (19.4)	

¹ Pearson's chi-squared test

Table 3 Sensitivity and specificity of *K-ras* mutations for the diagnosis of exocrine pancreatic cancer (EPC), overall and stratified by age and sex

	Total	EPC (n=121)		P ¹	Sensitivity	Not EPC (n=91)		P ¹	Specificity
		<i>K-ras</i> mutated	<i>K-ras</i> wild-type		% (95% CI)	<i>K-ras</i> mutated	<i>K-ras</i> wild-type		% (95% CI)
Total	212	94	27		77.7 (69.2 – 84.8)	20	71		78.0 (68.1 – 86.0)
Sex									
Male	121	55	16	0.944	77.5 (66.0 - 86.5)	11	39	0.996	78.0 (64.0 - 88.5)
Female	91	39	11		78.0 (64.0 - 88.5)	9	32		78.0 (62.4 - 89.4)
Age									
≤65 years	102	46	16	0.344	74.2 (61.5 - 84.5)	9	31	0.915	77.5 (61.5 - 89.2)
>65 years	110	48	11		81.4 (69.1 - 90.3)	11	40		78.4 (64.7 - 88.7)

¹ Pearson's chi-squared test.

References

1. Stathis A, Moore MJ. Advanced pancreatic carcinoma: current treatment and future challenges. *Nat Rev Clin Oncol*. 2010;7:163–72.
2. Hidalgo M. Pancreatic cancer. *N Engl J Med*. 2010;362:1605–17.
3. Takhar AS, Palaniappan P, Dhingsa R, Lobo DN. Recent developments in diagnosis of pancreatic cancer. *BMJ*. 2004;329:668–73.
4. Porta M, Fabregat X, Malats N, Guarner L, Carrato A, de Miguel A, et al. Exocrine pancreatic cancer: symptoms at presentation and their relation to tumour site and stage. *Clin Transl Oncol*. 2005;7:189–97.
5. Duffy MJ, Sturgeon C, Lamerz R, Haglund C, Holubec VL, Klapdor R, et al. Tumor markers in pancreatic cancer: a European Group on Tumor Markers (EGTM) status report. *Ann Oncol* 2010;21:441–7.
6. Freelove R, Walling A. Pancreatic cancer: diagnosis and management. *Am Fam Physician*. 2006;73:485–92.
7. Beger HG, Matsuno S, Cameron JL. Diseases of the pancreas: current surgical therapy. Berlin: Springer, 2008.
8. von Hoff DD, Evans DB, Hruban RH, editors. Pancreatic cancer. Boston: Jones & Bartlett; 2005.
9. Howard JM, Idezuki Y, Ihse I, Prinz RA, eds. Surgical diseases of the pancreas. 3rd. ed. Baltimore: Williams and Wilkins, 1998.
10. Beger HG, Warshaw AL, Büchler MW, Carr-Locke DL, Neoptolemos JP, Russell C, Sarr MG, eds. The pancreas. Vol. 2. Oxford: Blackwell, 1998.
11. Abraham SC, Wilentz RE, Yeo CJ, Sohn TA, Cameron JL, Boitnott JK, et al. Pancreaticoduodenectomy (Whipple resections) in patients without malignancy: are they all ‘chronic pancreatitis’? *Am J Surg Pathol*. 2003;27:110–20.
12. Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, Perucho M. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. *Cell*. 1998;53:549–54.
13. Tada M, Omata M, Ohto M. Clinical application of ras gene mutation for diagnosis of pancreatic adenocarcinoma. *Gastroenterology*. 1991;100:233–8.
14. Parker LA, Lumbreras B, Hernández-Aguado I, Porta M. How useful is it clinically to analyse the K-ras mutational status for the diagnosis of exocrine pancreatic cancer? A systematic review and meta-analysis. *Eur J Clin Invest*. 2010 (in press).
15. Marchese R, Muleti A, Pasqualetti P, Bucci B, Stigliano A, Brunetti E, et al. Low correspondance between K-ras mutations in pancreatic câncer tissue and detection of K-ras mutations in circulating DNA. *Pancreas*. 2006;32:171–7.
16. Wenger FA, Zieren J, Peter FJ, Jacobi CA, Müller JM. K-ras mutations in tissue and stool samples from patients with pancreatic cancer and chronic pancreatitis. *Langenbecks Arch Surg*. 1999;384:181–6.
17. Trümper L, Menges M, Daus H, Köhler D, Reinhard JO, Sackmann M, et al. Low sensitivity of the ki-ras polymerase chain reaction for diagnosing pancreatic cancer from pancreatic juice and bile: a multicenter prospective trial. *J Clin Oncol*. 2002;20:4331–7.
18. Liu TH, Wang ZY, Cui QC. Significance of the detection of Ki-ras codon 12 mutation in the diagnosis of pancreatic carcinoma. *Int J Surgical Path*. 1995;3:93–100.
19. Van Laethem JL, Vertongen P, Deviere J, Van Rangelbergh J, Rickaert F, Cremer M, Robberecht P. Detection of c-Ki-ras gene codon 12 mutations from pancreatic duct brushings in the diagnosis of pancreatic tumours. *Gut*. 1995;36:781–7.

20. Zheng M, Liu LX, Zhu AL, Qi SY, Jiang HC, Xiao ZY. K-ras gene mutation in the diagnosis of ultrasound guided fine-needle biopsy of pancreatic masses. *World J Gastroenterol*. 2003;9:188–91.
21. Mora J, Puig P, Boadas J, Urgell E, Montserrat E, Lerma E, et al. K-ras gene mutations in the diagnosis of fine-needle aspirates of pancreatic masses: prospective study using two techniques with different detection limits. *Clin Chem*. 1998;44:2243–8.
22. Shibata D, Almoguera C, Forrester K, Dunitz J, Martin SE, Cosgrove MM, et al. Detection of c-K-ras mutations in fine needle aspirates from human pancreatic adenocarcinomas. *Cancer Res*. 1990;50:1279–83.
23. Villanueva A, Reyes G, Cuatrecasas M, Martínez A, Erill N, Lerma E, et al. Diagnostic utility of K-ras mutations in fine-needle aspirates of pancreatic masses. *Gastroenterology*. 1996;110:1587–94.
24. Pugliese V, Pujic N, Saccomanno S, Gatteschi B, Pera C, Aste H, Ferrara GB, Nicolò G. Pancreatic intraductal sampling during ERCP in patients with chronic pancreatitis and pancreatic cancer: cytologic studies and k-ras-2 codon 12 molecular analysis in 47 cases. *Gastrointest Endosc*. 2001;54:595–9.
25. Van Laethem JL, Bourgeois V, Parma J, Delhay M, Cochaux P, Velu T, et al. Relative contribution of Ki-ras gene analysis and brush cytology during ERCP for the diagnosis of biliary and pancreatic diseases. *Gastrointest Endosc*. 1998;47:479–85.
26. Parker LA, Gómez Saez N, Lumbreras B, Porta M, Hernández-Aguado I. Methodological deficits in diagnostic research using ‘-omics’ technologies: Evaluation of the QUADOMICS tool and quality of recently published studies. *PLoS One* 2010;5(7):e11419.
27. Fletcher RH, Fletcher SW. *Clinical epidemiology. The essentials*. 4th. edition. Philadelphia: Lippincott, Williams & Wilkins; 2005.
28. Haynes RB, Sackett DL, Guyatt GH, Tugwell P. *Clinical epidemiology. How to do clinical practice research*. 3rd. edition. Philadelphia: Lippincott, Williams & Wilkins; 2005.
29. Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, et al. Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst*. 2001;93:1054–61.
30. Porta M, ed. *A dictionary of epidemiology*. 5th. edition. New York: Oxford University Press, 2008. p. 66, 69, 191, 201, 227, 233–6, 255, 258.
31. Porta M, Malats N, Jarrod M, Grimalt JO, Rifà J, Carrato A, et al. Serum concentrations of organochlorine compounds and K-ras mutations in exocrine pancreatic cancer. *Lancet*. 1999;354: 2125–9.
32. Soler M, Malats N, Porta M, Fernandez E, Guarner L, Maguire A, et al. Medical conditions in patients with pancreatic and biliary diseases: validity and agreement between data from questionnaires and medical records. *Dig Dis Sci*. 1999;44:2469–77.
33. Porta M, Costafreda S, Malats N, Guarner L, Soler M, Gubern JM, et al. Validity of the hospital discharge diagnosis in epidemiologic studies of biliopancreatic pathology. *Eur J Epidemiol*. 2000;16:533–41.
34. Porta M, Malats N, Alguacil J, Ruiz L, Jarrod M, Carrato A, et al. Coffee, pancreatic cancer, and K-ras mutations: updating the research agenda. *J Epidemiol Community Health*. 2000;54:656–9.
35. Real FX, Malats N, Lesca G, Porta M, Chopin S, Lenoir GM, et al. Family history of cancer and germline BRCA2 mutations in sporadic exocrine pancreas cancer. *Gut*. 2002;50:653–7.
36. Alguacil J, Porta M, Malats N, Kauppinen T, Kogevinas M, Benavides FG, et al. Occupational exposure to organic solvents and K-ras mutations in exocrine pancreatic cancer. *Carcinogenesis*. 2002;23:101–6.
37. Morales E, Porta M, Vioque J, López T, Mendez MA, Pumarega JA, et al. Food and nutrient intakes and K-ras mutations in exocrine pancreatic cancer. *J Epidemiol Community Health*. 2007;61:641–9.

38. Crous-Bou M, De Vivo I, Porta M, Pumarega JA, López T, Alguacil J, et al. CYP1B1 polymorphisms and *K-ras* mutations in patients with pancreatic ductal adenocarcinoma. *Dig Dis Sci*. 2008;53:1417–21.
39. Crous-Bou M, Porta M, López T, Jariod M, Malats N, Alguacil J, et al. Lifetime history of tobacco consumption and *K-ras* mutations in exocrine pancreatic cancer. *Pancreas*. 2007;35:135–41.
40. Crous-Bou M, Porta M, López T, Jariod M, Malats N, Morales E, et al. Lifetime history of alcohol consumption and *K-ras* mutations in pancreatic ductal adenocarcinoma. *Environ Mol Mutagen*. 2009;50:421–30.
41. Crous-Bou M, Porta M, Morales E, López T, Carrato A, Puigdomènech E, et al. Past medical conditions and *K-ras* mutations in pancreatic ductal adenocarcinoma: a hypothesis-generating study. *Cancer Causes Control*. 2009;20:591–9.
42. Porta M, López T, Pumarega J, Jariod M, Crous-Bou M, Marco E, et al. In pancreatic ductal adenocarcinoma blood concentrations of some organochlorine compounds and coffee intake are independently associated with KRAS mutations. *Mutagenesis*. 2009;24:513–21.
43. Gasull M, Porta M, Pumarega J, Vioque J, Bosch de Basea M, Puigdomènech E, et al. The relative influence of diet and serum concentrations of organochlorine compounds on *K-ras* mutations in exocrine pancreatic cancer. *Chemosphere*. 2010;79:686–97.
44. Crous-Bou M. Clinical and environmental influences on the prevalence of mutations in the *K-ras* oncogene in patients with pancreatic ductal adenocarcinoma. Doctoral dissertation [Dir.: Porta M], Universitat Autònoma de Barcelona, Barcelona, Spain; 2009. (http://www.imim.es/programesrecerca/epidemiologia/en_documentsgreem.html [accessed 23 Nov. 2010])
45. Porta M, Pumarega J, López T, Jariod M, Marco E, Grimalt J.O. Influence of tumor stage, symptoms and time of blood draw on serum concentrations of organochlorine compounds in exocrine pancreatic cancer. *Cancer Causes Control*. 2009;20:1893–906.
46. Porta M, Ferrer-Armengou O, Pumarega J, López T, Crous-Bou M, Alguacil A et al. Exocrine pancreatic cancer clinical factors were related to timing of blood extraction and influenced serum concentrations of lipids. *J Clin Epidemiol*. 2008;61:695–704.
47. Armitage P, Berry G, Matthews JNS. *Statistical Methods in Medical Research*. 4th edition. Blackwell: Oxford, 2002.
48. Porta M, Pumarega J, Ferrer-Armengou O, López T, Alguacil J, Malats N, et al. Timing of blood extraction in epidemiologic and proteomic studies: results and proposals from the PANKRAS II Study. *Eur J Epidemiol*. 2007;22:577–88.
49. Gudjonsson B. Pancreatic cancer: survival, errors and evidence. *Eur J Gastroenterol Hepatol*. 2009;21:1379–82.