Predictors of adverse events after endoscopic ultrasound-guided through-The-needle biopsy of pancreatic cysts: A recursive partitioning analysis

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ABSTRACT

Background and study aims

Endoscopic ultrasound-guided through-the-needle biopsy (TTNB) of pancreatic cystic lesions (PCLs) is associated with a non-negligible risk for adverse events (AEs). We aimed to identify the hierarchic interaction among independent predictors for TTNB-related AEs and to generate a prognostic model using recursive partitioning analysis (RPA).

Patients and methods

Multicenter retrospective analysis of 506 patients with PCLs who underwent TTNB. RPA of predictors for AEs was performed and the model was validated by means of bootstrap resampling.

Results

Mean cysts size was 36.7 mm. Most common diagnoses were intraductal papillary mucinous neoplasm (IPMN, 45%), serous cystadenoma (18.8%), and mucinous cystadenoma (12.8%). Fifty-eight (11.5%) AEs were observed. At multivariate analysis, age (odds ratio [OR] 1.32, 1.09–2.14; p=0.05), number of TTNB passes (OR from 2.17, 1.32–4.34 to OR 3.16, 2.03–6.34 with the increase of the number of passes), complete aspiration of the cyst (OR 0.56, 0.31–0.95; p=0.02), and diagnosis of IPMN (OR 4.16, 2.27–7.69; p<0.001) were found to be independent predictors of AEs, as confirmed by logistic regression and random forest analyses. RPA identified three risk classes: high-risk (IPMN sampled with multiple microforceps passes, 28% AEs rate), low-risk (1.4% AE rate, including patients <64 years with other-than-IPMN diagnosis sampled with ≤2 microforceps passes and with complete aspiration of the cyst) and middle-risk class (6.1% AEs rate, including the remaining patients).

Conclusion

TTNB should be selectively used in the evaluation of patients with IPMN. The present model could be applied during patient selection as to optimize the benefit/risk of TTNB.

INTRODUCTION

Preoperative accurate assessment of pancreatic cystic lesions (PCLs) is of paramount importance for adequate patient management [1]. Recently, given the disappointing diagnostic performance of endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) in accurately discriminating the histological type of PCL and establishing the risk of malignancy, a microforceps able to pass through a standard 19-gauge EUS-FNA needle (Moray Microforceps; Steris Endoscopy, Mentor, Ohio, USA) has been developed to perform a through-the-needle biopsy (TTNB) of the cyst wall [2].

Several studies and meta-analyses have shown TTNB to be associated with a higher diagnostic sensitivity and accuracy in the evaluation of PCLs when compared to EUS-FNA [3-8]. However, some safety concerns have recently been raised because a non-negligible rate of moderate-to-severe adverse events (AEs) has been observed. Particularly, in a recent single-center prospective study, an AE rate of 10% was registered, including a death due to post-TTNB pancreatitis [5]. Therefore, AE risk stratification is mandatory to optimize the risk/benefit ratio of this technique [9]. Potential predictors for TTNB-related AEs were analyzed in the abovementioned study, but the relatively small sample size did not allow the identification of any significant association [5]. Moreover, classical logistic regression may not be able to identify the potential interaction between different risk factors.
In the current study, we aimed to generate a prognostic model for post-TTNB AEs using recursive partitioning analysis (RPA). The final goal of this approach is to determine an easily interpretable method for classifying patients that could help clinicians in their decision-making.

METHODS

Patients

Data from consecutive patients who underwent TTNB at 10 centers from January 2016 to June 2021 were retrieved. In this retrospective analysis, we also re-evaluated those patients that had been included in previous retrospective and prospective studies with specific inclusion and exclusion criteria. In particular, 244 patients were previously included in other studies (61/117 patients from the Verona center, 132/172 from the Copenhagen center, 25/68 from the Gainesville center, 26/26 from the Rome-Campus Bio-Medico center).
Generally, the indications for TTNB were one or more of the following: (i) presence of a pancreatic cyst of greater than 3 cm; (ii) a unilocular/oligocystic lesion without communication with the main pancreatic duct (MPD) of unknown nature; (iii) MPD diameter ≥5 mm; (iv) size increase/changes in morphology during follow-up; (v) thickened walls/mural nodules; (vi) increased serum CA19.9 level [10-12]. Moreover, patients included in a previous prospective study with the inclusion criteria "cystic lesion more than 15 mm in size" [5] were also included in this retrospective analysis.
Exclusion criteria were: (i) age <18 years; (ii) extrapancreatic lesion; (iii) pregnancy or lactation; (iv) the presence of uncorrected coagulopathy (international normalized ratio >1.5 or platelet count $<50\times10^9$/L); (v) the use of concomitant anticoagulant/antiplatelet/aspirin (not to be discontinued); (vi) a history of recent pancreatitis (within the last 3 months); (vii) follow-up of <1 month.
All procedures were performed by board-certified endoscopists with at least 5 years' experience with EUS-guided tissue sampling. Procedural details are given in Appendix 1s. Institutional Review Board approval for this retrospective report was obtained (N. 3373CESC, 2021/07/12).

Outcomes

The primary outcome was to determine the hierarchy of clinical and procedural prognostic factors able to predict the occurrence of AEs to define risk groups that could help clinicians in their decision-making. The severity of AEs was classified according to the American Society for Gastrointestinal Endoscopy (ASGE) lexicon [13] and AEs were defined as

follows: (i) perforation (presence of air or luminal content outside the gastrointestinal tract); (ii) intracystic bleeding (visible echoic fluid coming from the cystic wall and filling the cystic cavity); (iii) clinically relevant bleeding (hematemesis, melena, or drop in hemoglobin levels >2 g/dL compared with preprocedural levels); (iv) acute pancreatitis (abdominal pain associated with at least three-fold increases in serum amylase/lipase and/or pancreatitis according to imaging); (v) infection (fever >38 °C for >24 hours); and (vi) abdominal pain (not caused by pancreatitis or perforation) requiring medical intervention. Events classified as "incidents" according to the ASGE lexicon [13] were not included in the analysis. The timing of AE occurrence was classified as intraprocedural, ≤14 days, or >14 days.
AEs were re-evaluated at the time of the present study in all cases, graded by the investigators at the participating centers, and double checked by the principal investigator (S.F.C.) according to the information provided. Follow-up data were obtained from electronic charts/follow-up visits and from telephone contact performed at the time of data collection.
The secondary end points were: diagnostic yield, defined as the percentage of the lesions sampled for which a tissue diagnosis was obtained; and diagnostic accuracy, defined as the percentage of lesions that corresponded to the final diagnosis [14] (intention-to-treat analysis). Additionally, subgroup analysis of diagnostic accuracy, including patients who underwent surgical resection, was performed. The gold standard for diagnosis was considered surgical histology whenever this was available. In nonsurgical patients, the final diagnosis was established by a combination of cross-sectional imaging/EUS findings, and TTNB histology/cyst fluid cytology as previously defined [6] [15-17] and summarized in Table 1s.

Statistical analysis

Patient characteristics were summarized with conventional statistics, using mean and SD for continuous variables and absolute frequencies and percentages for categorical data. Baseline factors with a potential prognostic effect on the AE rate were initially analyzed by means of uni- and multivariate logistic regression analysis. To further assess and rank the relevance of each predictor variable, we estimated random-forest variable importance measures through permutation of variable values. Because the variable importance measures based on Gini importance selection of variables are not reliable in situations where potential predictor variables vary in their scale of measurement or their number of categories, we used conditional inference trees ("cforest" function of the R package "party") with subsampling without replacement, which provide unbiased variable selection in the individual classification trees [18].
The predictor variables were then tested by RPA, a statistical method that classifies patients into risk groups by means of trees aiming to correctly classify members of the population based on several independent variables [19]. In order to select the splitting variable and its cutoff point, the model assesses all possible dichotomizations of all predictor variables to find the one dichotomization that produces the largest likelihood ratio test statistic.
The method then repeats this assessment and in each of the two daughter nodes the variable most strongly associated with the response outcome (i.e. that produces the lowest *P* value) at the optimal cutoff point is selected for the next split. In this way, splitting continues recursively until some stop condition is reached, namely identification of less than 15 patients per node [20]. Finally, the terminal nodes are grouped according to their response outcome occurrence, and the results are presented as the final set of prognostic groups that are consequently based on a data-driven approach and not predefined [19] [20].

The risk groups identified in the model were then compared to determine whether sufficient divergences in terms of AE rate and severe AE rate were present across the identified risk groups using the chi-squared test.
The model was then internally validated using bootstrap resampling. Bootstrap samples were random samples drawn with replacement from the original sample. We repeatedly fitted the model in 1000 bootstrap samples using two different seeds and evaluated its performance on the original sample [21]. The model performance before and after bootstrapping validation was expressed through the area under the receiver operating characteristic curve (AUC; c-index) [21].
Calibration was assessed by means of a plot showing the correlation between the mean predicted AE probability versus the mean observed AE rate in deciles of patients with increasing values of predicted probability. Differences between predicted probability and the observed AE rate were assessed by the Hosmer–Lemeshow test [22].
All statistical tests were two-tailed, and differences were considered significant at a *P* value of <0.05. The statistical analysis was run using the "party" and "performance" packages in R Statistical Software 3.0.2 (Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patients

Overall, 575 patients underwent TTNB during the study period. With 69 patients being excluded (47 extrapancreatic lesions and 22 lost to follow-up), the data from 506 patients were analyzed. The baseline characteristics of the whole study population are shown in [Table 1]. The mean (SD) patient age was 62.2 (14.0) years and 191 patients (37.7%) were male. The mean (SD) Charlson comorbidity index and body mass index were 4.8 (2.6) and 24.8 (7.6) kg/m^2, respectively. Most patients were asymptomatic (70.2%), although 106 patients (20.9%) reported upper abdominal pain.
The commonest indication for the procedure was PCL size >3 cm (42.3%), followed by suspected mural nodule or thickened wall (20.6%), and increasing cyst size (14.2%). Most of the lesions (40.9%) were in the pancreatic head, whereas 33.6% and 25.5% of patients presented with lesions in the body and tail of the pancreas, respectively. The mean (SD) lesion size was 36.7 (18.0) mm; 239 PCLs (47.2%) were unilocular whereas 267 (52.8%) were oligocystic. The wall of the cyst appeared thin in 392 patients (77.5%) and the majority of the lesions (64.4%) were sampled from the stomach.
The mean (SD) number of needle passes (defined as the number of punctures performed with the needle) was 1.2 (0.5) and mean (SD) number of microforceps passes (defined as the number of attempts to collect a gross specimen) was 2.5 (0.9). The cyst was aspirated entirely (i.e. until complete collapse of the cyst walls onto the needle [23]), in 367 patients (72.5%). Prophylaxis with rectal indomethacin/diclofenac and antibiotics were used in 140 patients (27.7%) and 487 (96.2%), respectively. The mean (SD) length of follow-up was 17.8 (14.2) months and 118 patients (23.3%) underwent surgical resection.

Primary outcome

A detailed list of AEs is presented in [Table 2]. Overall, the AE rate was 58/506 (11.5%), of which 15 (3.0%), 9 (1.8%), and 3 events (0.6%) were classified as moderate, severe, and fatal, respectively. There were 21 events (20 intracystic bleeds and one pericystic hematoma

without clinical consequences) that were classified as “incidents” according to the ASGE lexicon [13] and not included in the analysis. Of the three fatal events, two, which occurred in 72– and 85-year-old men, were due to acute pancreatitis that evolved into multiorgan failure. The third patient, a 75-year-old man, was admitted to intensive care unit a few days after TTNB with septic shock that developed into multiorgan failure.
The most frequent AEs were acute pancreatitis (29; 5.7%), fever/infection (10; 2.0%), and intracystic bleeding (10; 2.0%). In terms of timing, 11 AEs (18.9%) were classified as intraprocedural, 44 (75.9%) were registered within 14 days of the procedure, and three (5.2%) occurred after 14 days. The rate of AEs ranged between 9.5% and 13.2% among the centers, without significant differences.

Recursive partitioning analysis

As reported in [Table3], in univariate logistic regression analysis, patient age over 64 years (odds ratio [OR] 1.13 [95%CI 1.08–1.24]; P=0.04), number of microforceps passes (OR from 1.66 [95%CI 1.22–3.84] to 2.23 [95%CI 1.66–4.80] with increasing number of passes), complete aspiration of the cyst (OR 0.47 [95%CI 0.29–0.83]; P=0.02), use of indomethacin/diclofenac (OR 0.45 [95%CI 0.24–0.84]; P=0.01), and diagnosis of an intraductal papillary mucinous neoplasm (IPMN; OR 2.85 [95%CI 1.72–4.76]; P<0.001) were found to be significant predictors of AEs. All these variables, except the use of indomethacin/diclofenac, were also confirmed to be significant predictive factors for AEs in multivariate analysis. The importance of these variables in predicting AE occurrence was confirmed by a random forest model, in which the greater permutation accuracy importance (i.e. the higher classification error rate when permuting randomly the value of the variable) was observed in order with PCL diagnosis (whether an IPMN or other lesions), number of microforceps passes, complete aspiration of the cyst, and patient age ([Fig.1]). RPA of the prognostic factors demonstrated that the risk of AE occurrence was stratified based on diagnosis of an IPMN, complete aspiration of the cyst, number of microforceps passes, and age, namely those variables presenting the higher importance in the random forest model (Fig.1s). Aggregating the terminal nodes with similar AE rates, the final tree is shown in [Fig.2]. The presence of these factors split the whole patient series into three groups: low, medium, and high risk.
The analysis demonstrated that, in patients with an IPMN, the risk of AE occurrence depends on the number of microforceps passes performed, which split this subset of patients into a high risk group, with an AE rate of 28.1% (39 events among 139 patients) where more than one pass is performed, and a medium risk group (AE rate, 6.1%; 18 events among 294 patients) when only a single microforceps pass is performed.
Other patients falling into this medium risk group include those with a diagnosis other than IPMN whose cysts are not completely aspirated or are fully aspirated but sampled with more than two passes, and those patients who underwent complete aspiration of the cyst with no more than two microforceps passes but are aged >64 years.
At the other extreme, the low risk group (AE rate, 1.4%; 1 event among 73 patients) included only patients <64 years with a diagnosis of “other than IPMN” who underwent complete aspiration of the cyst and tissue sampling in no more than two microforceps passes ([Fig.2]). As showed in [Table4], not only the AE rate (P<0.001) but also the severity of the events differed significantly among these three risk groups. In fact, all three fatal events and 21 moderate/severe AEs occurred among the high risk group, whereas the rates of moderate/severe AEs were 1.7% (3/294) and 0% in the medium and low risk groups, respectively (P<0.001).

Performance of the model and validation

The model showed a c-index of 75.2% (95%CI 72.4%–77.9%; Fig. 2s). The model also showed proper calibration (Hosmer–Lemeshow, P=0.64), as shown by the calibration plot (Wald test for calibration slope, P=0.71; Fig. 3s).
The internal validation of the model based on a bootstrap method (1000 repetitions) showed a c-index value of 73.8% (95%CI 71.2%–76.1%) (Fig. 2s).

Secondary outcomes

With 409/506 specimens suitable to define a diagnosis, the diagnostic yield of TTNB was 80.8%. TTNB diagnosis corresponded to the final diagnosis in 395/506 cases (78.1%; intention-to-treat analysis). The diagnostic accuracy, calculated in 118 patients with final surgical pathology, was 83.3%. No differences in diagnostic yield and the accuracy of TTNB were observed when comparing patients in each of the three risk groups (Table 2s).

Discussion

To the best of our knowledge, our study represents the largest series ever published on the use of TTNB, and the first attempt to build a prognostic model for AE occurrence by means of RPA. In fact, while in traditional regression the information from different predictive variables is combined linearly, the RPA performed in the current study may capture all possible interactions of the prognostic factors, including multiple splits in the same variable [24]. The result of the combination of such interactions is shown in the final classification tree ([Fig. 2]).
RPA demonstrated that the main predictor for AE occurrence was the diagnosis of IPMN and when an IPMN was sampled with more than one microforceps pass, as is usual when sampling PCLs by TTNB, the risk of AE is particularly high (AE rate 28.1%). On the other hand, a single microforceps pass in a patient with an IPMN is associated with an intermediate risk of AEs (6.1%), although it is well known that a single pass is usually not sufficient to achieve adequate samples for histological diagnosis [6]. Therefore, our results go against TTNB sampling in IPMN patients because of the high risk of severe AEs.
In clinical practice, before a TTNB is performed, a presumptive diagnosis of IPMN can be made on the basis of cross-sectional imaging and EUS features. Indeed, as stated in the 2017 International Association of Pancreatology guidelines: “reliable distinguishing features of branch duct IPMN include multiplicity and visualization of a connection to the MPD” [11]. IPMNs are multifocal in 25%–41% of cases [25] [26], and a connection with the pancreatic ducts is visible in up to 73% of cases on magnetic resonance imaging [27]. Moreover, EUS has demonstrated a sensitivity of 88.5% and a specificity of 92.3% for the detection of cyst communication with the pancreatic ducts [28]. Therefore, patients with multifocal lesions and/or evidence of cyst communication with the MPD should be carefully selected before TTNB.
In addition to this safety concern, even the clinical impact of TTNB on IPMN management may be questionable. Indeed, the main clinical value of TTNB is in defining the nature of a cyst, which is already presumed when these typical morphological features of IPMN have been observed on imaging/EUS. Of course, we are aware that sometimes IPMNs are unifocal

and the communication between the cyst and the pancreatic ducts is difficult to identify, thereby making the diagnosis of IPMN before a TTNB is performed challenging.
Another important advantage of TTNB is the opportunity it offers to define the grade of dysplasia [6] [7]; however, the grade of dysplasia on TTNB samples could be underestimated because the specimen may not be representative of the whole cyst. Moreover, IPMN risk stratification can be accurately predicted on the basis of clinical and radiological features (i.e. jaundice, presence of worrisome features/high risk stigmata) [29] [30]. Therefore, given the higher risk of AEs in patients with presumed IPMN, TTNB should be avoided.
In contrast, when an IPMN is not suspected before TTNB, and when exact identification of the nature of a cyst is crucial to define the appropriate management strategy, TTNB can be of help in achieving a specific diagnosis, given its ability to define the specific nature of the lesion [6]. This aspect of clinical care is particularly salient in patients with large unilocular/oligocystic PCLs that lack well-defined connections to the pancreatic ducts, as the management strategies are radically different depending on the PCL histological type. Indeed, surgical intervention is indicated for most mucinous cystic neoplasms and cystic neuroendocrine tumors, while a serous cystadenoma would not require further follow-up, nor any additional testing.
Interestingly, the present study demonstrated that patients with a diagnosis other than IPMN also have a lower risk of an AE occurring. However, further trials are needed to explore the safety and accuracy of TTNB in this setting. Moreover, in the future, the role of TTNB in the diagnosis of pancreatic cysts should be redefined in the context of other available tools, such as confocal laser endomicroscopy [31] and molecular diagnostics [32].
Finally, the clinical impact of the diagnosis must be balanced in each case with the operative risk. Indeed, one of the deaths in this study occurred in an 85-year-old man in whom there would probably not have been any advantage in resection.
Of note, our study demonstrates a lack of significant correlation between antibiotic prophylaxis and the prevention of AEs, as has already been described with standard EUS-FNA for PCLs [33]. Interestingly, the use of rectal NSAIDs was a significant protective factor for AEs on univariate analysis and, despite not being significant on multivariate analysis, further specifically designed studies are needed to draw definitive conclusions on their use in this clinical setting.
On the other hand, increasing the number of microforceps passes leads to a higher risk of AEs, even in patients with a diagnosis other than an IPMN, as performing more than two passes increases the AE risk from 1.4% to 6.1% (risk group migration from low to medium risk). Therefore, considering the higher risk of AEs and the lack of diagnostic benefit with three or more specimens, as demonstrated in a previous Italian series [6], two microforceps passes seem to show the best risk–effectiveness compromise.
Another variable of significant importance in our model was complete aspiration of the cyst. Potential reasons underlying this result could be related to the higher risk of infection when complete aspiration of the cystic fluid is not possible. Interestingly, the likelihood of aspirating the cyst fluid can be checked immediately after needle puncture and, if the fluid is too thick to be aspirated, a subsequent TTNB can be avoided. On the other hand, this subgroup of patients could benefit from antibiotic prophylaxis.
There are some weaknesses to our study. First, the retrospective nature of the report and the inclusion of patients enrolled in previous studies with different inclusion/exclusion criteria may have introduced some outcome biases and our model should be validated in a prospective study. However, all patients in the study were re-evaluated with a common definition of the primary outcome and all the patients were followed up according to current guidelines.

Second, the lack of external validation could be seen as a limitation; however, internal validation by means of 1000 bootstrap samplings randomly drawn with replacement from the original population was performed. This way, both the model-building process and its performance were simultaneously validated in a broad range of random samples, all different from the original one, thereby obviating the lack of an external cohort, as recently confirmed by simulation studies [12].
Third, the final diagnosis remained undefined in 5.5% of cases; in approximately 70% of the other cases, it was established based on a combination of imaging and cyto/histological features, so some mistakes cannot be excluded. However, the data come from tertiary referral centers with experienced pancreatic multidisciplinary units, where the presumptive preoperative diagnosis is more likely to be confirmed on surgical pathology. Fourth, we were not able to collect other data potentially related to the onset of AEs (e.g. the procedural time, immune competency, or type of anticoagulant/antiplatelet agent before suspension).
In conclusion, patients with PCLs sampled by TTNB may be classified into three groups according to the risk of AE occurrence. TTNB should be avoided in patients with a presumed IPMN, whereas it seems safe in patients <64 years with PCLs not in communication with the MPD that are sampled with two microforceps passes and completely aspirated. Older patients have an intermediate risk level, therefore the risks and benefits of the procedure should be carefully discussed in a multidisciplinary setting.

Competing interests

S.F. Crinò has received speaker's fees from Steris Endoscopy. A. Larghi has provided consultancy to Pentax and Boston Scientific, and has received teaching fees from Medtronic and Boston Scientific. D. Yang has provided consultancy to Olympus, Boston Scientific, Lumendi, and Steris Endoscopy. P.V. Draganov has provided consultancy to Olympus, Boston Scientific, Cook Medical, Merit, Fujifilm, Microtech, Lumendi, and Steris. J.R. Aparicio has provided consultancy to Boston Scientific. C. Robles-Medranda has provided consultancy to Pentax Medical, Boston Scientific, Steris, Medtronic, Motus, Micro-tech, G-Tech Medical Supply, CREO Medical, and Mediconsgroup. A. Repici has provided consultancy to Boston Scientific and Medtronic, and has received grant support from Fujifilm. The remaining authors declare that they have no conflict of interest.

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Table 1
Baseline characteristics of the 506 patients with pancreatic cystic lesions included in the study and details of the through-the-needle biopsy procedures they underwent.

Patient and lesion characteristics	
Age, mean (SD), years	62.2 (14.0)
Sex, male, n (%)	191 (37.7%)
Charlson comorbidity index, mean (SD)	4.8 (2.6)
Body mass index, mean (SD), kg/m^2	24.8 (7.6)
Symptoms, n (%)	
• Asymptomatic	355 (70.2%)
• Upper abdominal pain	106 (20.9%)
• Acute pancreatitis	23 (4.5%)
• Jaundice	20 (3.9%)
• Other	2 (0.4%)
Indications for procedure, n (%)	
• Cystic lesion >30 mm in size	214 (42.3%)
• Cystic lesion >15 mm in size[*]	62 (12.3%)
• Unilocular/oligocystic lesion without communication with the main pancreatic duct	31 (6.1%)
• Increasing dimensions	72 (14.2%)
• Suspected mural nodules	63 (12.5%)
• Thickened walls	41 (8.1%)
• Main pancreatic duct diameter >5 mm	16 (3.2%)
• Increased CA19.9	2 (0.4%)

Table 1
Baseline characteristics of the 506 patients with pancreatic cystic lesions included in the study and details of the through-the-needle biopsy procedures they underwent.

Patient and lesion characteristics	
• Other	5 (0.9%)
Lesion location, n (%)	
• Head	207 (40.9%)
• Body	170 (33.6%)
• Tail	129 (25.5%)
Lesion size, mean (SD), mm	36.7 (18.0)
Cyst type, n (%)	
• Unilocular	239 (47.2%)
• Oligocystic	267 (52.8%)
Cyst wall, n (%)	
• Thin	392 (77.5%)
• Thick	69 (13.6%)
• Nodules	45 (8.9%)
Procedure details and final diagnosis	
Puncture site, n (%)	
• Stomach	326 (64.4%)
• Duodenal bulb	139 (27.5%)
• Descending duodenum	41 (8.1%)
Number of needle passes, mean (SD)	1.2 (0.5)

Table 1
Baseline characteristics of the 506 patients with pancreatic cystic lesions included in the study and details of the through-the-needle biopsy procedures they underwent.

Patient and lesion characteristics	
Number of microforceps passes, mean (SD)	2.5 (0.9)
Complete aspiration of the cyst, n (%)	367 (72.5%)
Use of rectal indomethacin/diclofenac, n (%)	140 (27.7%)
Use of antibiotics, n (%)	487 (96.2%)
Length of follow-up, mean (SD), months	17.8 (14.2)
Final diagnosis, n (%)	
• Intraductal papillary mucinous neoplasm	228 (45%)
• Serous cystadenoma	95 (18.8%)
• Mucinous cystadenoma	65 (12.8%)
• Pseudocyst	33 (6.5%)
• Neuroendocrine tumor	16 (3.2%)
• Pancreatic ductal adenocarcinoma	16 (3.2%)
• Lymphoepithelial	6 (1.2%)
• Mucinous nonneoplastic cyst	6 (1.2%)
• Squamoid cyst	5 (1.0%)
• Solid pseudopapillary tumor	4 (0.8%)
• Acinar	2 (0.4%)
• Renal cancer metastasis	2 (0.4%
• Undefined	28 (5.5%)

[*] *This indication was an inclusion criterion of the study by Kovacevic et al. [5].*

▶ **Table 2** Details of the adverse events observed.

Type of adverse event, n (%)	Severity[1]				Timing[1], days			Management/outcome
	Mild	Moderate	Severe	Fatal	Intra-procedural	≤14	>14	
Intracystic bleeding (N = 10; 2.0 %)	10 (100 %)	0	0	0	10 (100 %)	0	0	Intracystic adrenaline (n = 1), brief hospital admission with conservative management (n = 9)
Acute pancreatitis (N = 29; 5.7 %)	9 (31.0 %)	10 (34.5 %)	8 (27.6 %)	2 (6.9 %)	0	29 (100 %)	0	Hospital admission with conservative management (n = 24), EUS drainage of pancreatic collection (n = 1), ICU admission (n = 3), ICU admission + surgical necrosectomy (n = 1)
Fever/infection (N = 10; 2.0 %)	5 (50.0 %)	3 (30.0 %)	1 (10.0 %)	1 (10.0 %)	0	10 (100 %)	0	Medical treatment (n = 5), hospital admission with conservative management (n = 3), EUS drainage of pancreatic abscess (n = 1), ICU admission (n = 1)
Abdominal pain (N = 6; 1.2 %)	6 (100 %)	0	0	0	0	5 (83.3 %)	1 (16.7 %)	Hospital admission with conservative management (n = 3), emergency room consultation (n = 2), medical treatment (n = 1)
Peripancreatic collection[2] (N = 1; 0.2 %)	0	1 (100 %)	0	0	0	0	1 (100 %)	EUS drainage (n = 1)
Hypotension (N = 1; 0.2 %)	1 (100 %)	0	0	0	1 (100 %)	0	0	Medical treatment (n = 1)
Xanthogranuloma mass forming (N = 1; 0.2 %)	0	1 (100 %)	0	0	0	0	1 (100 %)	Surgical resection of the cyst requiring gastric wall resection (n = 1)
Total (N = 58; 11.5 %)	31	15	9	3	11	44	3	

EUS, endoscopic ultrasound; ICU, intensive care unit.
[1] According to the American Society for Gastrointestinal Endoscopy lexicon [13].
[2] Without evidence of pancreatitis.

▶ **Table 3** Univariate and multivariate logistic regression analysis for the prediction of adverse events.

Variables		Univariate analysis		Multivariate analysis	
		Odds ratio (95%CI)	*P* value	Odds ratio (95%CI)	*P* value
Age over 64 years		1.13 (1.08–1.24)	**0.04**	1.32 (1.09–2.14)	**0.05**
Sex (reference female)		1.02 (0.81–1.30)	0.91		
Charlson comorbidity index		0.89 (0.78–1.12)	0.58		
Body mass index		1.03 (0.97–1.22)	0.12		
Symptoms	No pain	Reference			
	Pain	0.86 (0.45–1.6)	0.60		
	Acute pancreatitis	1.96 (0.73–5.14)	0.21		
	Jaundice	1.84 (0.68–5.32)	0.26		
Indication	Unilocular/oligocystic lesion	Reference			
	Size < 3 cm	1.06 (0.58–1.94)	0.84		
	Increasing size	0.99 (0.46–2.14)	0.99		
	MPD > 5 mm	0.96 (0.34–2.73)	0.95		
	Thickened walls	0.77 (0.28–2.17)	0.63		
	Mural nodule	2.13 (0.85–5.34)	0.10		
	Increased CA19–9	5.61 (0.34–7.62)	0.22		
Site	Head	Reference			
	Body	1.28 (0.68–2.31)	0.48		
	Tail	1.32 (0.75–2.42)	0.33		
	Multifocal	1.06 (0.14–8.81)	0.78		
Size		1.04 (0.96–1.12)	0.17		
Type	Unilocular	Reference			
	Oligocystic	0.96 (0.61–1.68)	0.92		
Walls	Thin	Reference			
	Thick	1.38 (0.72–2.64)	0.32		
	Nodules	0.68 (0.25–1.79)	0.43		
Puncture site	Stomach	Reference			
	Bulb	0.64 (0.35–1.18)	0.15		
	Duodenum part II	1.62 (0.75–3.51)	0.21		
Number of needle passes	1	Reference			
	2	1.38 (0.24–3.58)	0.47		
	3	0.56 (0.15–2.83)	0.79		
	4	0.74 (0.35–1.56)	0.82		

▶ **Table 3** (Continuation)

Variables		Univariate analysis		Multivariate analysis	
		Odds ratio (95%CI)	*P* value	Odds ratio (95%CI)	*P* value
Number TTNB passes	1	Reference			
	2	1.39 (0.36–2.16)	0.79	1.43 (0.68–2.23)	0.27
	3	1.66 (1.22–3.84)	**0.02**	2.17 (1.32–4.34)	**0.02**
	4	2.16 (1.72–6.18)	**0.01**	2.63 (1.68–7.21)	**0.01**
	5	2.20 (1.53–3.18)	**0.01**	2.43 (1.63–3.68)	**0.01**
	>5	2.23 (1.66–4.80)	**0.01**	3.16 (2.03–6.34)	**0.01**
Complete aspiration (reference "No")		0.47 (0.29–0.83)	**0.02**	0.56 (0.31–0.95)	**0.02**
Indomethacin use (reference "No")		0.45 (0.24–0.84)	**0.01**	0.58 (0.30–1.13)	0.11
Antibiotic use (reference "No")		1.47 (0.45–4.42)	0.22		
Final diagnosis	Serous cystadenoma	Reference			
	IPMN	2.85 (1.72–4.76)	**<0.001**	4.16 (2.27–7.69)	**<0.001**
	MCN	1.00 (0.47–2.16)	0.98		
	Other	1.64 (0.74–3.64)	0.21		
	Pancreatic ductal adenocarcinoma	0.45 (0.05–3.59)	0.45		
	Pseudocyst	0.38 (0.08–1.70)	0.21		

MPD, main pancreatic duct; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm.

▶ **Table 4** Primary outcome stratified among the three risk groups.

	Low risk (n=73)	Intermediate risk (n=294)	High risk (n=139)	*P* value
Adverse event rate	1 (1.4%)	18 (6.1%)	39 (28.1%)	**<0.001**
Type of adverse event				**<0.001**
• Intracystic bleeding	0 (0%)	0 (0%)	10 (7.2%)	
• Acute pancreatitis	0 (0%)	8 (2.7%)	21 (15.1%)	
• Fever/Infection	1 (1.4%)	3 (1%)	6 (4.3%)	
• Abdominal pain	0 (0%)	5 (1.7%)	1 (0.7%)	
• Peripancreatic collection	0 (0%)	0 (0%)	1 (0.7%)	
• Hypotension	0 (0%)	1 (0.3%)	0 (0%)	
• Xanthogranuloma mass forming	0 (0%)	1 (0.3%)	0 (0%)	
Severity of adverse event				**<0.001**
• Mild	1 (1.4%)	15 (5.1%)	15 (10.8%)	
• Moderate	0 (0%)	2 (0.7%)	13 (9.4%)	
• Severe	0 (0%)	1 (1%)	8 (5.7%)	
• Fatal	0 (0%)	0 (0%)	3 (2.2%)	
Timing of adverse event occurrence				**<0.001**
• Intraprocedural	0 (0%)	0 (0%)	11 (7.9%)	
• ≤14 days	1 (1.4%)	18 (6.1%)	25 (17.9%)	
• >14 days	0 (0%)	0 (0%)	3 (2.2%)	

Fig. 1 *Variable importance estimated by mean decrease in permutation-based accuracy considering the adverse event rate as the outcome. Higher values of mean decrease in permutation-based accuracy indicate variables that are more important to the classification. TTNB, through-the-needle biopsy.*

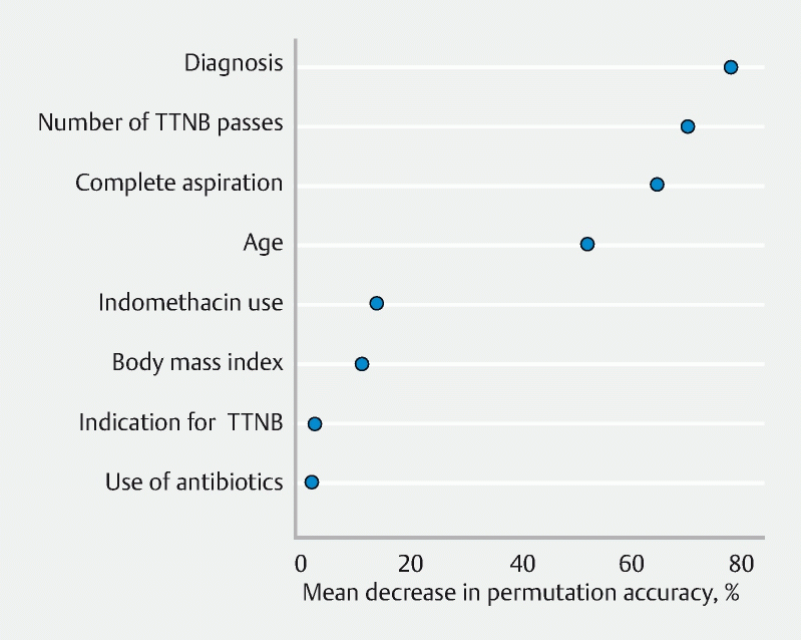

Fig. 2 *Recursive partitioning classification tree for the occurrence of adverse events. The terminal nodes categorized the study sample into three prognostic groups, with the low, medium, and high risks groups having significantly different rates of adverse events (P < 0.001).*
IPMN, intraductal papillary mucinous neoplasm; AE, adverse event.