Imaging and Cytopathological Criteria Indicating Malignancy in Mucin-Producing Pancreatic Neoplasms

A Series of 68 Histopathologically Confirmed Cases

Charitini Salla, MD, Eleni Karvouni, MD, PhD,† Ilias Nikas, MD,‡ Aristidis Ikonomakis, MD,§ Panagiotis Konstantinou, MD,|| Ioannis Karoumpalis, MD, PhD,¶ Athanasia Sepsa, MD,# Kleio Papaparaskeva, MD, PhD,** Maria Tsopanomichalou, PhD,†† Despoina Georgiadou, MD, PhD,‡‡ Akrivi Kostopoulou, MD, PhD,§§ Gregory Tsiotos, MD, PhD,|||| Stamatios Theocharis, MD, PhD,¶¶ Theodoros N. Sergentanis, MD, PhD,## and Ekaterini Politi, MD, PhD****

Objectives: This study aims to evaluate the performance of clinical, imaging, and cytopathological criteria in the identification of high-grade dysplasia/carcinoma (HGD/Ca) in pancreatic mucin-producing cystic neoplasms.

Methods: Sixty-eight consecutive, histopathologically confirmed mucin-producing cystic neoplasms, evaluated by endoscopic ultrasound-guided fine-needle aspiration, were enrolled; specifically, 39 branch duct intraductal papillary mucinous neoplasms (BD-IPMNs), 21 main duct IPMNs, and 8 mucinous cystic neoplasms. The associations between HGD/Ca in histopathology and findings of endoscopic ultrasound and cytology, demographic, lifestyle, and clinical parameters were evaluated, separately in IPMNs and mucinous cystic neoplasms.

Results: Age 65 years or more was associated with HGD/Ca in IPMNs. In BD-IPMNs, cyst diameter 3 cm or greater (sensitivity, 68.8%; specificity, 65.2%), a mural nodule (sensitivity, 56.3%; specificity, 78.3%), main pancreatic duct diameter 5 to 9 mm (sensitivity, 50.0%; specificity, 87.0%), and suspicious cytology (sensitivity, 81.3%; specificity, 100%) signaled the presence of HGD/Ca. Similarly, in main duct IPMNs, suspicious cytology predicted HGD/Ca with high sensitivity (88.9%) and excellent specificity (100%). Regarding cytopathological criteria, in BD-IPMNs, HGD/Ca was associated with a high nuclear/cytoplasmic ratio, background necrosis, presence of papillary structures, hypochromatic nuclei, hyperchromatic nuclei, and major nuclear membrane irregularities (thickening and/or indentations).

Conclusions: Clinical, imaging, and cytopathological criteria are useful in the identification of HGD/Ca in IPMNs.

From the *Department of Cytopathology, Hygeia-Mitera Hospital; †Department of Pathology, Aretaieion Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece; ‡School of Medicine, European University of Cyprus, Nicosia, Cyprus; §Department of Gastroenterology and ||Department of Pathology and Cytopathology, 251 Air Force Hospital; ¶Department of Gastroenterology, "G. Gennimatas" General Hospital; #Department of Pathology, Metropolitan Hospital; **Department of Pathology, Konstantopoulio Agia Olga General Hospital, Athens, Greece; ††Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; ‡‡Institute of Liver Studies, King's College Hospital, London, UK; §§Department of Pathology, "G. Gennimatas" General Hospital; ||||First Department of Surgery, Mitera Hospital; ¶¶First Department of Pathology, ##Department of Hygiene, Epidemiology and Medical Statistics, and ***Department of Cytopathology, Aretaieion Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece.

Received for publication September 23, 2017; accepted September 4, 2018.

Address correspondence to: Charitini Salla, MD, Department of Cytopathology, Hygeia-Mitera Hospital, 6 Erythrou Stavrou Street, Maroussi, Athens 15123, Greece (e-mail: charitinisalla@yahoo.com).

The authors declare no conflict of interest.

Supplemental digital contents are available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pancreasjournal.com).

DOI: 10.1097/MPA.0000000000001182

Key Words: intraductal papillary mucinous neoplasm, mucinous cystic neoplasm, grade, cytopathology, EUS-FNA

(*Pancreas* 2018;47: 1283–1289)

Mucin-producing cystic neoplasms of the pancreas include intraductal papillary neoplasms [intraductal papillary mucinous neoplasms (IPMNs) and intraductal tubulopapillary mucinous neoplasms (ITPNs)], which represent cystic dilatations of the main and/or branch pancreatic ducts due to neoplastic epithelial proliferation, as well as mucinous cystic neoplasms (MCNs), which are multilocular cysts with thick fibrous capsule and ovarian-type stroma.[1] Endoscopic ultrasound–guided fine-needle aspiration (EUS-FNA) plays a crucial role in the differential diagnosis between mucin-producing cystic neoplasms and other types of neoplastic or nonneoplastic pancreatic cysts.[2] Regarding IPMNs, a principal distinction pertains to the affected pancreatic duct; they are subdivided into main duct (MD-IPMNs), branch duct (BD-IPMNs), and mixed-type IPMNs. According to the 2012 International Association of Pancreatology (IAP) consensus guidelines (Fukuoka guidelines), surgical resection is recommended for MD-IPMNs with diameter of the main pancreatic duct (MPD) 10 mm or greater and MCNs, whereas the decision about surgery or follow-up in BD-IPMNs and mixed-type IPMNs is based on an algorithm integrating a set of "high-risk stigmata" and "worrisome features"[3]; most recently, a revision of the Fukuoka guidelines has been published.[4] The presence of high-risk stigmata prompts surgery without further evaluation, whereas worrisome features are followed by EUS-FNA, as high-risk stigmata have been associated with a 10-fold higher risk of an IPMN-induced death compared with worrisome features.[3–5] Recent meta-analyses have examined factors distinguishing between malignant and benign BD-IPMNs and pointed to useful imaging findings, such as cyst size larger than 3 cm, presence of a mural nodule, dilatation of the MPD.[6–8]

Regarding the degree of cytopathological atypia, low-grade (LG) atypia of mucinous epithelium includes low-grade dysplasia (LGD) and intermediate-grade (IG) dysplasia. High-grade atypia of mucinous epithelium includes invasive adenocarcinoma and high-grade dysplasia (HGD), when the quality and quantity of atypia are not adequate for the diagnosis of adenocarcinoma.[9] This subgrouping is important in the context of BD-IPMNs, because "suspicious cytology" [presence of high-grade (HG) atypia] prompts the decision for resection.[3,4] Strict cytopathological criteria for the designation of HG atypia have been proposed, including small cell size (<12 μm duodenal enterocyte), which corresponds to high nuclear/cytoplasmic ratio (N/C ratio), abnormal chromatin pattern (hyper- or hypochromatic nuclei), and necrotic

background.[9,10] Intermediate-grade dysplasia is a diagnostic challenge and is often followed up conservatively, similarly to the LG lesions.[9]

In view of the above, this study evaluates the performance of a compendium of clinical, imaging, and cytopathological criteria in the identification of histopathologically confirmed HGD/carcinoma (Ca) in pancreatic mucin-producing cystic neoplasms, in an attempt to assist the selection of cases who will undergo resection; to this end, a series of 68 consecutive, histopathologically confirmed mucin-producing cystic neoplasms was analyzed.

MATERIALS AND METHODS

Selection of Patients and Data Collection

In this prospective study, 68 consecutive patients with histopathologically confirmed mucin-producing cystic neoplasms were included during the period March 2010 to March 2013 in the Department of Gastroenterology, "G. Gennimatas" General Hospital, Athens, Greece. Patients were referred to EUS-FNA for the evaluation of pancreatic lesions detected by other imaging procedures (ultrasonography, computed tomography, magnetic resonance imaging, and magnetic resonance cholangiopancreatography). This was the first EUS procedure in all cases. Patients included in this study were treated following the 2006[11] (for those diagnosed before the publication of the 2012 Guidelines) or the 2012 International Consensus Guidelines.[3]

Demographic, lifestyle, and clinical data were collected from patients' hospital records. Written informed consent was obtained from all participants for the inclusion in this study. The study is in accordance with the Helsinki Declaration and has been approved by the local Institutional Review Board.

Endoscopic Ultrasound–Guided Fine-Needle Aspiration

Endoscopic ultrasound–guided FNA was performed by an experienced endoscopist (I.K.) with the use of the Pentax FG-3830UT linear ultrasound endoscope (Pentax ion Instruments, Tokyo, Japan), the Hitachi EUB-6000 processor (Hitachi Ltd, Tokyo, Japan), and a 22-gauge ultrasonic FNA needle system (SONOTIP MediGlobe, Achenmühle, Germany). There was no report of sampling failure in terms of adequacy.

On the basis of the EUS findings, the endoscopist suggested the presence of a mucin-producing cystic neoplasm. In BD-IPMN, the endoscopist evaluated the diameter of the cyst, the presence of a mural nodule (defined as a nodule with lack of mobility, presence of Doppler flow, and subsequent verification of tumor presence by the FNA), the diameter of the MPD, and multiplicity and multilocularity of the lesion. In MD-IPMN, the endoscopist measured the diameter of the MPD and assessed the presence of a mural nodule.

Thereafter, the cytopathologist evaluated the appearance of the aspirated fluid (quantity, color, and, mainly, viscosity). In cases of low or moderate viscosity of the aspirated fluid, 1 mL of the sample was sent for measurement of carcinoembryonic antigen and amylase levels, either diluted with saline or undiluted, depending on the quantity of the fluid; the remaining liquid was collected in a tube and proceeded immediately to the cytology laboratory for centrifugation from which cytology slides (conventional and liquid-based ones) or rarely cell block material was obtained.[12] In cases of high-viscosity aspirated fluid, the material was smeared onto 2 or 3 slides for conventional and liquid-based cytology smears and sometimes also for cell block. In the latter cases, the difficulty in the aspiration and expression of the fluid onto the slides because of high viscosity, the "string sign,"[13] or the "drop sign"[14] confirmed the presence of a mucin-producing neoplasm.

Liquid-based cytology smears were prepared with Thin Prep (Hologic, Marlborough, Mass) and stained with routine Papanicolaou stain. Air-dried conventional cytology smears were stained with Giemsa (Merck KGaA, Darmstadt, Germany), and alcohol-fixed smears were stained with Papanicolaou stain. Cell blocks were fixed in 10% formalin solution and routinely processed with hematoxylin-eosin.

Two expert cytopathologists (C.S. and P.K.), blind to each other's results, reviewed all EUS-FNA specimens, assessing both the diagnosis and the grade of atypia, based on the 2015 Papanicolaou Society of Cytopathology System for Reporting Pancreatobiliary Cytology[9]; suspicious cytology was defined as the presence of HG atypia. The following cytopathological criteria were assessed: N/C ratio, chromatin pattern (euchromatic nuclei; hypochromatic nuclei or with parachromatin clearing; hyperchromatic nuclei/coarsely granular chromatin; 2 types of chromatin, namely, hyperchromatic coexisting with hypochromatic nuclei in the same case), background necrosis, nucleoli, thick extracellular mucin, presence of papillary structures, papillary architecture morphology [length at least twice the width ($l \geq 2d$) of the papilla in the absence of other distinct papillary characteristics; $l \geq 2d$ in the presence of other distinct papillary characteristics (namely, branching papillae and fibrovascular cores); no papilla with $l \geq 2d$], lesional mucinous epithelium (ie, not derived from gastrointestinal contamination), presence of major nuclear membrane irregularities (thickening and/or indentations), and nuclear elongation.

Histopathological Assessment

The type (BD-IPMN, MD-IPMN, and MCN) and grade of lesions were evaluated according to the 2010 World Health Organization classification by 2 expert pathologists (K.P. and A.K.).[15] Following the Baltimore Consensus Meeting for Neoplastic Precursor Lesions, cases were subsequently grouped as "LGD" (low-grade dysplasia, including LG or IG dysplasia)[16,17] and "HGD/Ca," including HG dysplasia and carcinomas.[18,19] Mixed-type IPMNs were grouped together with BD-IPMNs, when the MPD diameter was between 5 and 9 mm; when the diameter of the MPD was 10 mm or more, the lesion was considered MD-IPMN.[6]

Statistical Analysis

Descriptive statistics of the study sample were calculated, separately by type of lesion (MD-IPMN, BD-IPMN, and MCN); the differences were evaluated with Fisher's exact test (for categorical variables) or Kruskal-Wallis test (for continuous variables). The associations between the presence of a HGD/Ca lesion (BD-IPMNs and MD-IPMNs grouped together) and demographic characteristics, lifestyle habits, personal/family history, and symptoms were evaluated by Pearson χ^2 test or Fisher's exact test, as appropriate.

The associations between EUS/cytology findings and HGD/Ca were separately evaluated for BD-IPMNs (cyst ≥3 cm, mural nodule ≥5 mm, diameter of the MPD, multiplicity, multilocularity, and suspicious cytology) and MD-IPMNs (diameter of the MPD, mural nodule, and suspicious cytology) in view of the distinct features of the 2 entities. Pearson χ^2 test or Fisher's exact test was performed, as appropriate; the sensitivity and specificity were also calculated.

Regarding cytopathological criteria, an analysis was presented only for BD-IPMNs; Pearson χ^2 test or Fisher's exact test was implemented. In MD-IPMNs, no *P* values were presented because of the small number of cases. Statistical analysis was performed using STATA/SE version 13 statistical software (Stata Corp, College Station, Tex).

RESULTS

Description of the Study Sample

The study included 39 BD-IPMNs, 21 MD-IPMNs, and 8 MCN cases. Table 1 presents the description of the study sample. mucinous cystic neoplasm was associated with female sex ($P = 0.002$, Fisher's exact test), compared with BD-IPMN and MD-IPMN, where males represented 51.3% and 71.4% of cases, respectively. Although MCN cases were approximately 10 years younger than IPMNs, the difference did not reach statistical significance ($P = 0.127$, Kruskal-Wallis test). There was no difference in the frequency of smoking ($P = 0.935$), obesity ($P = 0.389$), diabetes mellitus ($P = 0.326$), history of pancreatitis ($P = 0.274$), family history of cancer ($P = 0.454$), abdominal and/or back pain ($P = 0.879$), weight loss ($P = 0.236$), and dyspepsia ($P > 0.999$). Jaundice was a symptom noted only in MD-IPMN cases (38.1% vs 0% in BD-IPMNs and MCNs; $P < 0.001$).

Of the 39 BD-IPMN cases, 23 (59.0%) were histologically LGD (18 LG and 5 IG cases), whereas 16 cases (41.0%) were HGD/Ca (8 HG and 8 carcinomas). Of the 21 MD-IPMNs, most (85.7%, 18 cases) were HGD/Ca (4 HG and 14 carcinomas); the remaining 3 lesions (14.3%) were LGD (2 LG and 1 IG case).

Demographic, Lifestyle, and Clinical Parameters in Association With HGD/Ca

Table 2 presents the associations between demographic, lifestyle, and clinical parameters and HGD/Ca (BD-IPMNs and MD-IPMNs grouped together). Among demographic characteristics and lifestyle habits, age 65 years or more was associated with the likelihood of HGD/Ca (76.5% of HGD/Ca were ≥65 years vs 46.2% of LGD, $P = 0.016$); on the other hand, sex ($P = 0.538$), ever smoking ($P = 0.251$), and obesity at diagnosis ($P = 0.967$) were not significantly associated with HGD/Ca. Concerning personal and family history, no significant associations were noted; specifically, the prevalence of diabetes mellitus ($P = 0.816$), history of pancreatitis ($P = 0.875$), and family history of cancer ($P = 0.689$) did not differ between LGD and HGD/Ca. Jaundice emerged as a symptom alerting for HGD/Ca, as 23.5% of HGD/Ca presented with jaundice versus 0% in LGD ($P = 0.008$). Abdominal and/or back pain ($P = 0.202$), weight loss ($P = 0.302$), and dyspepsia ($P > 0.999$) were not associated with HGD/Ca.

Endoscopic Ultrasound Parameters, Cytology, and HGD/Ca

The associations between HGD/Ca and EUS parameters as well as cytology are summarized in Table 3, separately for BD-IPMNs and MD-IPMNs. In BD-IPMNs, a cyst with diameter of 3 cm or greater (sensitivity, 68.8%; specificity, 65.2%; $P = 0.037$), a mural nodule (sensitivity, 56.3%; specificity, 78.3%; $P = 0.027$), MPD diameter 5 to 9 mm (sensitivity, 50.0%; specificity, 87.0%; $P = 0.012$), and suspicious cytology (sensitivity, 81.3%; specificity, 100%; $P < 0.001$) signaled the presence of HGD/Ca. On the other hand, multiplicity ($P = 0.593$) and multilocularity ($P = 0.709$) were not significantly associated with HGD/Ca.

In MD-IPMNs, similarly to BD-IPMNs, suspicious cytology predicted HGD/Ca ($P < 0.001$) with high sensitivity (88.9%) and excellent specificity (100%). Main pancreatic duct diameter and presence of a mural nodule were not significantly associated with HGD/Ca, but this analysis should be deemed explorative due to the small number of LGD MD-IPMNs (only 3 cases).

Cytopathological Criteria in Association With HGD/Ca

Table 4 presents the associations between the cytopathological criteria and HGD/Ca in BD-IPMNs and MD-IPMNs. In BD-IPMNs, HGD/Ca was associated with high N/C ratio ($P = 0.001$), background necrosis ($P = 0.002$), presence of papillary structures ($P = 0.012$), presence of hypochromatic nuclei or parachromatin clearing ($P = 0.001$), hyperchromatic nuclei/coarsely granular chromatin ($P = 0.025$), and presence of major nuclear membrane irregularities (namely, thickening and/or indentations of the nuclear membrane; $P < 0.001$). Details regarding the possible combinations of nuclear membrane irregularities are provided in Supplemental Table 1 (http://links.lww.com/MPA/A685). Trends were noted regarding the frequent presence of nucleoli ($P = 0.066$), papillary architecture with $l \geq 2d$, branching papillae and fibrovascular cores ($P = 0.089$), and 2 coexisting types of chromatin (simultaneous presence of hyper- and hypochromatic nuclei; $P = 0.071$) in

TABLE 1. Description of the Study Sample

	BD-IPMN (n = 39)	MD-IPMN (n = 21)	MCN (n = 8)	*P*
Demographic features				
Sex, male, n (%)	20 (51.3)	15 (71.4)	0 (0)	**0.002**
Age, mean (SD), y	64.0 (11.2)	64.9 (13.8)	53.8 (14.4)	0.127*
Lifestyle habits, n (%)				
Smoking, ever	26 (66.7)	13 (61.9)	5 (62.5)	0.935
Obesity at diagnosis (BMI ≥ 30 kg/m^2)	9 (23.1)	5 (23.8)	0 (0)	0.389
Clinical symptoms, n (%)				
Diabetes mellitus	8 (20.5)	2 (9.5)	0 (0)	0.326
History of pancreatitis	9 (23.1)	2 (9.5)	0 (0)	0.274
Family history of cancer	8 (20.5)	5 (23.8)	0 (0)	0.454
Abdominal and/or back pain	16 (41.0)	8 (38.1)	4 (50.0)	0.879
Weight loss	6 (15.4)	7 (33.3)	1 (12.5)	0.236
Jaundice	0 (0)	8 (38.1)	0 (0)	**<0.001**
Dyspepsia	4 (10.3)	2 (9.5)	0 (0)	>0.999

Bold cells denote statistically significant differences.

**P* values were derived from Fisher's exact test, except for those marked with an asterisk (the latter were derived from Kruskal-Wallis test).

BMI indicates body mass index.

TABLE 2. Associations Between Demographic, Lifestyle, Clinical Parameters, and Histological Diagnosis of HGD/Ca in IPMNs (BD-IPMNs and MD-IPMNs grouped together)

	LGD Lesions (n = 26)	HGD/Ca Lesions (n = 34)	*P*
Demographic characteristics and lifestyle habits, n (%)			
Sex			0.538
Male	14 (53.9)	21 (61.8)	
Female	24 (46.1)	13 (38.2)	
Age, y			**0.016**
≥65	12 (46.2)	26 (76.5)	
<65	14 (53.8)	8 (23.5)	
Ever smoking			0.251
Yes	19 (73.1)	20 (58.8)	
No	7 (26.9)	14 (41.2)	
Obesity at diagnosis			0.967
Yes	6 (23.1)	8 (23.5)	
No	20 (76.9)	26 (76.5)	
Personal and family history, n (%)			
Diabetes mellitus			0.816
Yes	4 (15.4)	6 (17.6)	
No	22 (84.6)	28 (82.4)	
History of pancreatitis			0.875
Yes	5 (19.2)	6 (17.6)	
No	21 (80.8)	28 (82.4)	
Family history of cancer			0.689
Yes	5 (19.2)	8 (23.5)	
No	21 (80.8)	26 (76.5)	
Symptoms, n (%)			
Abdominal and/or back pain			0.202
Yes	8 (30.8)	16 (47.1)	
No	18 (69.2)	18 (52.9)	
Weight loss			
Yes	4 (15.4)	9 (26.5)	0.302
No	22 (84.6)	25 (73.5)	
Jaundice			**0.008***
Yes	0 (0)	8 (23.5)	
No	6 (100)	26 (76.5)	
Dyspepsia			>0.999*
Yes	3 (11.5)	3 (8.8)	
No	23 (88.5)	31 (91.2)	

Bold cells denote statistically significant associations.

*P values were derived from Pearson χ^2 test except for those marked with an asterisk (in the latter, Fisher's exact test was performed due to small numbers).

HGD/Ca BD-IPMNs, but did not reach statistical significance. Thick extracellular mucin (P = 0.209), lesional mucinous epithelium (P = 0.169), and nuclear elongation (P = 0.174) were not associated with HGD/Ca in BD-IPMN.

Similar trends were noted in MD-IPMNs, but no statistical analysis was presented in view of the small numbers of LGD MD-IPMNs. The evaluation of cytopathological criteria in MCNs was presented in Supplemental Table 2 (http://links.lww.com/MPA/A685); no statistical analysis was conducted given the small number of included MCNs.

DISCUSSION

This study highlighted the use of clinical, imaging, and cytopathological criteria in the identification of HGD/Ca in IPMNs. In BD-IPMNs, the imaging criteria included cyst size larger than 3 cm, presence of a mural nodule 5 mm or greater, and dilatation of the MPD over 5 mm; on the other hand, multiplicity and multilocularity did not seem capable of predicting malignancy. Excellent specificity and high sensitivity were observed regarding suspicious cytology; cytopathological criteria encompassed those recommended by the Papanicolaou Society in 2015, namely, high N/C ratio, hyper/hypochromasia, and background necrosis.[9,10] In addition, the presence of papillary structures and major nuclear membrane irregularities (thickening and/or indentations) seemed

TABLE 3. Associations Between EUS Parameters, Cytology, and Histological Diagnosis of HGD/Ca in IPMNs, Separately in BD-IPMNs (Upper Panels) and MD-IPMNs (Lower Panels)

Parameters Evaluated in BD-IPMN, n (%)	LGD Lesions (n = 23)	HGD/Ca Lesions (n = 16)	*P*
Cyst ≥3 cm			**0.037**
Yes	8 (34.8)	11 (68.8)*	
No	15 (65.2)†	5 (31.2)	
Mural nodule ≥5 mm			**0.027**
Yes	5 (21.7)	9 (56.3)*	
No	18 (78.3)†	7 (43.7)	
MPD diameter, mm			**0.012**
5–9	3 (13.0)	8 (50.0)*	
<5	20 (87.0)†	8 (50.0)	
Multiplicity			0.593
Yes	6 (26.1)	3 (18.7)*	
No	17 (73.9)†	13 (81.3)	
Multilocularity			0.709
Yes	13 (56.5)	10 (62.5)*	
No	10 (43.5)†	6 (37.5)	
Cytology			**<0.001**‡
Suspicious	0 (0)	13 (81.3)*	
Not suspicious	23 (100)†	3 (18.7)	
Parameters Evaluated in MD-IPMN, n (%)	**LG Lesions (n = 3)**	**HG Lesions (n = 18)**	***P***
MPD diameter, mm			>0.999‡
≥10	1 (33.3)	11 (61.1)*	
5–9	2 (66.7)†	7 (38.9)	
Mural nodule			>0.999‡
Yes	1 (33.3)	8 (44.4)*	
No	2 (66.7)†	10 (55.6)	
Cytology			**<0.001**‡
Suspicious	0 (0)	16 (88.9)*	
Not suspicious	3 (100)†	2 (11.1)	

Bold cells denote statistically significant associations.

*This percentage is equal to the sensitivity of criterion.

†This percentage is equal to the specificity of the criterion.

‡P values were derived from Pearson χ^2 test except for those marked with a double dagger (in the latter, Fisher's exact test was performed due to small numbers).

TABLE 4. Evaluation of Cytopathological Criteria in Relation to Histological Diagnosis of HGD/Ca in IPMNs

	BD-IPMN (n = 39)					MD-IPMN (n = 21)			
	LGD Lesions		HGD/Ca Lesions			LGD Lesions		HGD/Ca Lesions	
Criteria, n (%)	LG (n = 18)	IG (n = 5)	HG (n = 8)	Invasive (n = 8)	*P* (LG vs HG)	LG (n = 2)	IG (n = 1)	HG (n = 4)	Invasive (n = 14)
Nucleoli					0.066				
Yes	13 (72.2)	5 (100)	8 (100)	8 (100)		1 (50.0)	1 (100)	3 (75.0)	14 (100)
No	5 (27.8)	0 (0)	0 (0)	0 (0)		1 (50.0)	0 (0)	1 (25.0)	0 (0)
High N/C ratio					**0.001**				
Yes	0 (0)	2 (40.0)	4 (50.0)	6 (75.0)		0 (0)	0 (0)	4 (100.0)	10 (71.4)
No/not estimable	18 (100)	3 (60.0)	4 (50.0)	2 (25.0)		2 (100)	1 (100)	0 (0)	4 (28.6)
Background necrosis					**0.002**				
Yes	0 (0)	0 (0)	0 (0)	6 (75.0)		0 (0)	0 (0)	0 (0)	10 (71.4)
No	18 (100)	5 (100)	8 (100)	2 (25.0)		2 (100)	1 (100)	4 (100.0)	4 (28.6)
Thick extracellular mucin					0.209				
Yes	12 (66.7)	3 (60.0)	3 (37.5)	4 (50.0)		1 (50.0)	0 (0)	1 (25.0)	9 (64.2)
No	6 (33.3)	2 (40.0)	5 (62.5)	4 (50.0)		1 (50.0)	1 (100)	3 (75.0)	5 (35.7)
Presence of papillary structures					**0.012**				
Yes	9 (50.0)	3 (60.0)	8 (100)	7 (87.5)		0 (0)	0 (0)	2 (50.0)	14 (100)
No	9 (50.0)	2 (40.0)	0 (0)	1 (12.5)		2 (100)	1 (100)	2 (50.0)	0 (0)
Papillary architecture morphology									
No $l \geq 2d$ architecture*	9 (100)	0 (0)	3 (37.5)	2 (28.6)	Ref.	No papillary structures	No papillary structures	0 (0)	3 (21.4)
Papillary architecture with $l \geq 2d$ (in the absence of other distinct papillary characteristics)*	0 (0)	1 (33.3)	2 (25.0)	1 (14.3)	0.275	No papillary structures	No papillary structures	1 (50.0)	0 (0)
Papillary architecture with $l \geq 2d$ (in the presence of other distinct papillary characteristics)*	0 (0)	2 (66.7)	3 (37.5)	4 (57.1)	0.089	No papillary structures	No papillary structures	1 (50.0)	11 (78.6)
Lesional mucinous epithelium					0.169				
Yes	8 (44.4)	5 (100)	6 (75.0)	7 (87.5)		1 (50.0)	1 (100)	2 (50.0)	11 (78.6)
No	10 (55.6)	0 (0)	2 (25.0)	1 (12.5)		1 (50.0)	0 (0)	2 (50.0)	3 (21.4)
Chromatin pattern									
Euchromasia	12 (66.7)	1 (20.0)	0 (0)	0 (0)	Ref.	1 (50.0)	1 (100)	0 (0)	0 (0)
Hypochromatic nuclei or parachromatin clearing	6 (33.3)	3 (60.0)	7 (87.5)	6 (75.0)	**0.001**	1 (50.0)	0 (0)	1 (25.0)	8 (57.1)
Hyperchromatic nuclei/coarsely granular chromatin	0 (0)	1 (20.0)	0 (0)	2 (25.0)	**0.025**	0 (0)	0 (0)	3 (75.0)	3 (21.4)
Two types of chromatin (hypercoexisting with hypochromatic nuclei)	0 (0)	0 (0)	1 (12.5)	0 (0)	0.071	0 (0)	0 (0)	0 (0)	3 (21.4)
Presence of nuclear membrane irregularities					**<0.001**				
Major	1 (5.6)	2 (40.0)	7 (87.5)	8 (100)		0 (0)	0 (0)	3 (75.0)	14 (100)
No or minor	17 (94.4)	3 (60.0)	1 (12.5)	0 (0)		2 (100)	1 (100)	1 (25.0)	0 (0)
Nuclear elongation					0.174				
Yes	2 (11.1)	3 (60.0)	2 (25.0)	5 (62.5)		0 (0)	0 (0)	4 (100.0)	10 (71.4)
No	16 (88.9)	2 (40.0)	6 (75.0)	3 (37.5)		2 (100)	1 (100)	0 (0)	4 (28.6)

P values were derived from Fisher's exact test; bold cells denote statistically significant associations. *P* values were not presented in MD-IPMNs due to small numbers.

*Evaluable only in cases where papillary structures were present (27 BD-IPMN, 16 MD-IPMN); the distinct papillary characteristics were branching papillae and fibrovascular cores.

helpful in tracing HGD/Ca. Cytology had an excellent performance also in assessing HGD/Ca in MD-IPMNs.

The ultimate aim of the evaluation with radiology and/or EUS-FNA was to recognize the subgroup of mucin-producing pancreatic cystic neoplasms that carry a high risk of harboring HGD or invasive carcinoma in histology.[4,20–22] Main duct IPMNs have a 62% risk of malignancy (range, 36%–100%), whereas the respective percentage is 31.1% (range, 14%–48%) in BD-IPMNs and 10% to 17% in MCNs[4,22]; these figures are compatible with the distribution of grading in our cases.

Cyst size 3 cm or greater, although part of the high-risk stigmata in a previous edition,[11] is classified as a worrisome feature in the latest IAP guidelines[3,4] due to its reported poor positive predictive value for the presence of HGD/Ca in histology.[4] As shown in a study by Genevay et al,[23] cyst size larger than 3 cm had a sensitivity and specificity of 37% and 70%, whereas the presence of mural nodule had a sensitivity and specificity of 39% and 93%, respectively; however, cyst size was not an independent predictor of HGD/Ca, in contrast to cytology, mural nodule, and dilated MPD greater than 6 mm. In a recent evidence-based review of the American Gastroenterological Association, a cyst size greater than 3 cm was significantly associated with an almost 3-fold (odds ratio, 2.97) and a solid cyst component with an almost 8-fold (odds ratio, 7.73) elevated odds of malignancy, respectively.[24] Of interest, a significant percentage of cysts less than 3 cm might harbor malignant lesions,[25,26] and this has led the IAP to acknowledge cytology value in the evaluation of small BD-IPMNs not accompanied by worrisome features, when performed in medical centers with expertise, which handle a high volume of pancreatic cases.[4] A recent meta-analysis by Kim et al[6] reported a pooled sensitivity of 59% and specificity of 64% for cyst size greater than 3 cm; in our study, the respective percentages were 68.8% and 65.2%. In the same meta-analysis, the presence of a mural nodule larger than 5 mm predicted malignancy with a sensitivity of 59% and a specificity of 83%; in our study, the respective values were 56.3% and 78.3%. Our study is also in accordance with the aforementioned meta-analysis[6] regarding the role of MPD dilatation.

The latest IAP consensus guidelines recommend the use of EUS-FNA cytology in the assessment of suspected BD-IPMN with worrisome features.[3,4] Previous IAP guidelines[11] ignored cytopathological atypia of any grade and required only the presence of "positive cytology" in their BD-IPMN algorithm; however, this had resulted in suboptimal malignancy detection rates.[27] Thus, both the IAP and American Gastroenterological Association have recently established suspicious cytology as a detection threshold in their guidelines for the management of BD-IPMNs,[3,4,20] taking also into account the fact that invasion has an adverse impact on patient prognosis.[28] This threshold change from positive cytology to suspicious cytology has been reported to increase sensitivity and accuracy of cytology.[19,27] Patients with HGD, where invasion has not appeared yet, are the ones to mostly benefit from a potential surgical procedure; in cases of lower-grade dysplasia, the significant morbidity issues of surgery—especially in older patients, where the majority of these cysts are identified—have to be seriously taken into account, given the fact that the life expectancy of these patients could probably exceed dysplasia time to progress all the way to malignancy.[4,20,22,28]

Cytology performed better than symptomatology, showing higher sensitivity and specificity, a fact that agreed with other studies.[29] However, its sensitivity is still not regarded as optimal; reasons include poor cellularity, undersampling of the HG/malignant portion of the lesion due to the heterogeneity of mucin-producing neoplasms, and imperfect interobserver reproducibility concerning grading of atypia in cytology.[4,19,27,30,31] Cytology has a very good specificity for both HG IPMNs and MCNs,[19,27,32] even reaching 100% in some series.[18,23,33] In addition, negative predictive value reaches 99% in IPMN cases that lack high-risk stigmata and worrisome features.[34]

Regarding histology, we based it on the 4-tiered system (low; intermediate; high grade; carcinoma) of the latest World Health Organization Classification[15]; subsequently, however, we proceeded to a 2-tiered system (LGD and HGD/Ca), setting our cutoff in HGD and not carcinoma, which is in complete accordance with the latest IAP guidelines[3,4] and also with the Baltimore Consensus concerning the reporting of precursor neoplastic lesions of the pancreas.[16] This new scheme highlights the necessity to clearly separate IG dysplasia from in situ–type lesions (HGD) to align pathology reporting with clinical management.[16]

As far as cytopathological criteria are concerned, high N/C ratio was a hallmark of HGD/Ca, in agreement with other studies[10]; there are, however, difficulties in its assessment in cases where the cell cluster is crowded. Moreover, in our study, significant associations between HGD/Ca and hyperchromasia as well as hypochromasia were noticed in BD-IPMNs. Parachromatin clearing, namely, extreme hypochromasia, corresponding to "washed-out" nuclei and sharply demarcated nuclear membrane due to peripheral margination of chromatin, is a reliable sign of well-differentiated invasive IPMN that has similar morphological features to well-differentiated conventional pancreatic ductal adenocarcinoma.[1] Furthermore, background necrosis is an indication of invasion[9]; notably, there was no necrosis present in cases of LGD IPMNs.

In a previously published study,[10] prominent nucleoli were present in 7.7% of LG atypia and in 19.2% of HG atypia. We evaluated the presence of nucleoli regardless of size, and therefore the prevalence of recorded nucleoli seemed higher; only a borderline association between HGD/Ca and nucleoli was noted in our study. The presence of papillary structures indicated HGD/Ca in BD-IPMNs; the effort to subclassify papillary structures on the basis of architectural criteria revealed only a borderline trend suggesting HGD/Ca in the presence of branching papillae with a distinct fibrovascular core.

Recent research has evaluated the role of next-generation sequencing (NGS) as an adjunct of cytology and/or clinical/radiological features in grading pancreatic mucin-producing neoplasms; Rosenbaum et al[33] reported that sensitivity was increased from 75% to 79%, whereas specificity remained 100%, when NGS was considered along with cytopathological analysis. In a similar mode, Springer et al[35] showed an increased accuracy when a combination of clinical and molecular markers was used.

Among the limitations of this study, the relatively limited sample size should be declared; some associations did not reach statistical significance and were therefore reported only as trends. Some null associations may in fact be due to type II statistical error; for example, recent evidence has shown that diabetes in patients with IPMNs is significantly associated with HGD and invasive carcinoma,[36] but this association was not reproduced in our study. Importantly, in view of the small number of MCNs and LGD MD-IPMNs (8 and 3, respectively), no statistical analysis was presented for these lesions. No separate analysis was performed in the small number of mixed-type IPMNs; the latter were analyzed according to a recent systematic review and meta-analysis of the literature.[6] Specifically, when the MPD diameter was between 5 and 9 mm, mixed-type lesions were grouped with BD-IPMNs; when the diameter of the MPD was 10 mm or more, the lesion was considered MD-IPMN.[6] Accordingly, the predictive performance of combined preoperative variables was not analyzed in view of the limited sample size. Last, no molecular analysis was performed.

In conclusion, well-established and reproducible morphological criteria are crucial in the performance of cytology to detect HG/invasive IPMNs alongside the existing clinical and imaging features. Further studies that will encompass clinical, radiologic, endoscopic, cytopathological, and molecular data (NGS) in a multiparameter approach could reveal the best strategy concerning the management of mucinous pancreatic neoplasms.

ACKNOWLEDGMENT

The authors would like to thank Professor Petros Karakitsos, MD, PhD, who died on June 26, 2017, for his helpful remarks during this study.

REFERENCES

1. Campbell F, Verbeke CS. *Pathology of the Pancreas*. London, UK: Springer; 2013.
2. Gray W, Kocjan G. *Diagnostic Cytopathology*. 3rd ed. London, UK: Churchill Livingstone; 2010.
3. Tanaka M, Fernández-del Castillo C, Adsay V, et al. International consensus guidelines 2012 for the management of IPMN and MCN of the pancreas. *Pancreatology*. 2012;12:183–197.
4. Tanaka M, Fernández-Del Castillo C, Kamisawa T, et al. Revisions of international consensus Fukuoka guidelines for the management of IPMN of the pancreas. *Pancreatology*. 2017;17:738–753.
5. Crippa S, Bassi C, Salvia R, et al. Low progression of intraductal papillary mucinous neoplasms with worrisome features and high-risk stigmata undergoing non-operative management: a mid-term follow-up analysis. *Gut*. 2017;66:495–506.
6. Kim KW, Park SH, Pyo J, et al. Imaging features to distinguish malignant and benign branch-duct type intraductal papillary mucinous neoplasms of the pancreas: a meta-analysis. *Ann Surg*. 2014;259:72–81.
7. Heckler M, Michalski CW, Schaefle S, et al. The Sendai and Fukuoka consensus criteria for the management of branch duct IPMN—a meta-analysis on their accuracy. *Pancreatology*. 2017;17:255–262.
8. Anand N, Sampath K, Wu BU. Cyst features and risk of malignancy in intraductal papillary mucinous neoplasms of the pancreas: a meta-analysis. *Clin Gastroenterol Hepatol*. 2013;11:913–921; quiz e59–e60.
9. Pitman MB, Layfield LJ. *The Papanicolaou Society of Cytopathology System for Reporting Pancreatobiliary Cytology*. New York, NY: Springer; 2015.
10. Pitman MB, Centeno BA, Daglilar ES, et al. Cytological criteria of high-grade epithelial atypia in the cyst fluid of pancreatic intraductal papillary mucinous neoplasms. *Cancer Cytopathol*. 2014;122:40–47.
11. Tanaka M, Chari S, Adsay V, et al. International consensus guidelines for management of intraductal papillary mucinous neoplasms and mucinous cystic neoplasms of the pancreas. *Pancreatology*. 2006;6:17–32.
12. Centeno BA, Cowden DJ. Chapter 7: fine needle aspiration biopsy cytology of pancreas: a diagnostic approach based on pattern recognition. In: Field A, Zarka M, eds. *Practical Cytopathology: A Diagnostic Approach to Fine Needle Aspiration Biopsy*. St. Louis, MO: Elsevier; 2016.
13. Bick BL, Enders FT, Levy MJ, et al. The string sign for diagnosis of mucinous pancreatic cysts. *Endoscopy*. 2015;47:626–631.
14. Karoumpalis I, Christodoulou DK. Cystic lesions of the pancreas. *Ann Gastroenterol*. 2016;29:155–161.
15. Bosman FT, Carneiro F, Hruban RH, et al. *WHO Classification of Tumours of the Digestive System*. Geneva, Switzerland: WHO; 2010.
16. Basturk O, Hong SM, Wood LD, et al. A revised classification system and recommendations from the baltimore consensus meeting for neoplastic precursor lesions in the pancreas. *Am J Surg Pathol*. 2015;39:1730–1741.
17. Reid MD, Lewis MM, Willingham FF, et al. The evolving role of pathology in new developments, classification, terminology, and diagnosis of pancreatobiliary neoplasms. *Arch Pathol Lab Med*. 2017;141:366–380.
18. Scourtas A, Dudley JC, Brugge WR, et al. Preoperative characteristics and cytological features of 136 histologically confirmed pancreatic mucinous cystic neoplasms. *Cancer Cytopathol*. 2017;125:169–177.
19. Pitman MB, Yaeger KA, Brugge WR, et al. Prospective analysis of atypical epithelial cells as a high-risk cytologic feature for malignancy in pancreatic cysts. *Cancer Cytopathol*. 2013;121:29–36.
20. Vege SS, Ziring B, Jain R, et al. American Gastroenterological Association institute guideline on the diagnosis and management of asymptomatic neoplastic pancreatic cysts. *Gastroenterology*. 2015;148:819–822; quize12–e13.
21. Lee KS, Sekhar A, Rofsky NM, et al. Prevalence of incidental pancreatic cysts in the adult population on MR imaging. *Am J Gastroenterol*. 2010; 105:2079–2084.
22. Stark A, Donahue TR, Reber HA, et al. Pancreatic cyst disease: a review. *JAMA*. 2016;315:1882–1893.
23. Genevay M, Mino-Kenudson M, Yaeger K, et al. Cytology adds value to imaging studies for risk assessment of malignancy in pancreatic mucinous cysts. *Ann Surg*. 2011;254:977–983.
24. Scheiman JM, Hwang JH, Moayyedi P. American Gastroenterological Association technical review on the diagnosis and management of asymptomatic neoplastic pancreatic cysts. *Gastroenterology*. 2015;148: 824–848.e22.
25. Wong J, Weber J, Centeno BA, et al. High-grade dysplasia and adenocarcinoma are frequent in side-branch intraductal papillary mucinous neoplasm measuring less than 3 cm on endoscopic ultrasound. *J Gastrointest Surg*. 2013;17:78–84; discussion p.84–p.85.
26. Fritz S, Klauss M, Bergmann F, et al. Pancreatic main-duct involvement in branch-duct IPMNs: an underestimated risk. *Ann Surg*. 2014;260:848–855; discussion 855–856.
27. Pitman MB, Genevay M, Yaeger K, et al. High-grade atypical epithelial cells in pancreatic mucinous cysts are a more accurate predictor of malignancy than "positive" cytology. *Cancer Cytopathol*. 2010;118: 434–440.
28. Pitman MB. Revised international consensus guidelines for the management of patients with mucinous cysts. *Cancer Cytopathol*. 2012; 120:361–365.
29. Ono J, Yaeger KA, Genevay M, et al. Cytological analysis of small branch-duct intraductal papillary mucinous neoplasms provides a more accurate risk assessment of malignancy than symptoms. *Cytojournal*. 2011;8:21.
30. Michaels PJ, Brachtel EF, Bounds BC, et al. Intraductal papillary mucinous neoplasm of the pancreas: cytologic features predict histologic grade. *Cancer*. 2006;108:163–173.
31. Pitman MB, Centeno BA, Genevay M, et al. Grading epithelial atypia in endoscopic ultrasound-guided fine-needle aspiration of intraductal papillary mucinous neoplasms: an international interobserver concordance study. *Cancer Cytopathol*. 2013;121:729–736.
32. Cizginer S, Turner BG, Bilge AR, et al. Cyst fluid carcinoembryonic antigen is an accurate diagnostic marker of pancreatic mucinous cysts. *Pancreas*. 2011;40:1024–1028.
33. Rosenbaum MW, Jones M, Dudley JC, et al. Next-generation sequencing adds value to the preoperative diagnosis of pancreatic cysts. *Cancer Cytopathol*. 2017;125:41–47.
34. Wu RI, Yoon WJ, Brugge WR, et al. Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) contributes to a triple-negative test in preoperative screening of pancreatic cysts. *Cancer Cytopathol*. 2014;122: 412–419.
35. Springer S, Wang Y, Dal Molin M, et al. A combination of molecular markers and clinical features improve the classification of pancreatic cysts. *Gastroenterology*. 2015;149:1501–1510.
36. Morales-Oyarvide V, Mino-Kenudson M, Ferrone CR, et al. Diabetes mellitus in intraductal papillary mucinous neoplasm of the pancreas is associated with high-grade dysplasia and invasive carcinoma. *Pancreatology*. 2017;17:920–926.