Review Article

Value of immunohistochemical IMP3 expression with endoscopic ultrasound-guided-fine needle aspiration (EUS-FNA) in diagnosing pancreatic lesions

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Abstract:

Pancreatic cancer (PC), a lethal condition with a poor prognosis, ranks fourth among the most common causes of cancer-related mortality as early diagnosis of PC is so tricky. Consequently, most cases at the time of initial diagnosis already harbor metastasis. PC cases' early detection and survival depend mainly on improving diagnostic approaches. This review sheds light on the role of endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) as a minimally invasive method in early PC diagnosis and differentiation

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between different pancreatic lesions. The discovery of new diagnostic and prognostic markers for PC will raise the accuracy of proper diagnosis, and in turn, patients will gain better survival and prognosis. Insulin-like growth factor II mRNA binding protein3 (IMP3) is overexpressed in several malignant tumors, including pancreatic cancer, which may raise its role in diagnosis and prognosis as well as its therapeutic benefit for PC.

Keywords: Pancreatic cancer; IMP3; EUS-FNA; Immunohistochemistry

Introduction

Cytology in diagnosis of pancreatic cancer:

Cytological sampling from the pancreas is achieved mainly by either percutaneous FNA under ultrasound guidance, EUS-FNA transduodenal for head or neck lesions, or transgastric for body and tail lesions. Percutaneous and EUS FNA have the same complication rate from 0 -5%, and the percutaneous technique is an accurate and safe method for diagnosing pancreatic lesions [1]. Both procedures have the same accuracy, and percutaneous FNA can be used as an alternative for EUS FNA as it is cheaper [2]. However, EUS-FNA replaced percutaneous FNA. It is superior in detecting smaller lesions even if they are less than 2 cm, correctly detects vascular involvement, and can stage pancreatic cancer cases, affecting the prognosis and therapeutic decisions [3]. Also, the peritoneal metastasis rate is higher in patients undergoing percutaneous FNA for PC diagnosis due to tumor seeding into the peritoneal cavity during this technique **[4].**

Indications and contraindications of EUS-FNA:

EUS-FNA has been used more frequently to improve the diagnosis of pancreatic lesions using cytopathological evaluation and constitutes a handy tool for the loco-regional staging of PC **[5].** The indications of EUS-FNA include the presence of solid or cystic mass, enlarged lymph node, intrapleural/abdominal fluid, differentiation between benign and malignant lesions, staging of cancers, and providing histopathological evidence for starting therapy.

All solid pancreatic lesions should be aspirated before surgery to rule out autoimmune/focal pancreatitis lymphoma and assess for different types of cancer other than adenocarcinoma. Contraindications that limit its use include the inability to visualize the lesion site and the presence of vessels along the target's pathway. In addition, the FNA result cannot affect the management and pseudocysts aspiration due to high complications rate unless in case of therapeutic drainage of the cyst after its aspiration **[6], [7].**

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Technique:

The diagnostic ability of EUS-FNA depends on several factors such as size, site, and properties of target tissues and procedural and technical elements (type of needle, material processing, and biopsy technique) **[8]**.

- The size of the needle used in EUS-FNA is so essential. Smaller gauge needles as 25 gauge are safer and more effective than large gauge needles like 19-gauge. They reduce the bleeding risk, especially in highly vascular tumors such as neuroendocrine neoplasms, improving diagnosis [9]. Also, the failure of a 19-gauge needle in sampling pancreatic lesions presenting in the head or uncinate process **[10],** 22 gauge is influential in both aspiration and core biopsy **[8]**. Also, a 22-gauge needle is the most commonly used, but this needle was unsuccessful in about 33% of cases with uncinate lesions. However, 25-gauge needle was more flexible and successful in EUS-FNA of head and uncinate process lesions **[11]**. Gimenco-Garcia stated no significant difference between 22- and 25-gauge needles. [12]. 22- or 25-gauge needles can be used in any FNA approach; however, a 25-gauge needle is the best choice for transdoudenal FNA **[13]**. There is no significant difference between using 19- or 25-gauge needles, and the needle choice depends only on endoscopist desire. The number of aspirates in pancreatic lesions is five or six passes. For a highly accurate diagnosis, seven passes are recommended, which is so high compared to other organs, requiring only two or three passes**[14]**. Nevertheless, this will require less frequent needle passes, such as specific cytologic diagnoses, prolonged procedure time, higher risk, and additional needles [15].

Stains:

-There are multiple types of stains used in FNA staining. Romanowsky stain, although rapid, defines cell size and stromal components so well, but its nuclear morphology is so limited. Rapid Papanicolaou stain shows a high ability to focus through overlapping cell clusters and thicker smears. Toluidine blue stain is an ultra-fast stain, but it requires constant stain for destaining and restaining. Finally, hematoxylin and eosin stain is more time-consuming, but most pathologists prefer it **[16].**

- Rapid on-site evaluation (ROSE) means the evaluation of cytological smears at the endoscopic suite point of care (Point of care in the endoscopy ward). This process is done by a pathologist using the light microscope to provide rapid feedback to the endosonographer [17]. In addition, ROSE raises the diagnostic ability of EUS-FNA to reduce the number of needles passed, reducing the time of procedure and allowing proper earlier therapeutic decisions **[9].**

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Factors affecting diagnosis:

Difficulties:

-Although EUS-FNA has many advantages, it also has some complications such as bleeding, infection, perforation, and malignant seeding. **[16]** complications happen only in 2.2% of cases in the form of pancreatitis, retroperitoneal bleeding, and bradycardia (may be caused by mechanical factors).[18]. Besides, many challenges may face the EUS-FNA, such as the fibrotic or inflammatory nature of tumors and cystic tumor aspirations being hypocellular, which may yield false results [16], so the number of aspirates from tumor should be at least five to seven for optimal results. Also, the small lesions and those far away from GIT lumen are very challenging targets **[19].**

Among the disadvantages of EUS-FNA is the defective diagnostic material in cases of cystic lesions compared to solid and solid cystic lesions. Radiological findings and cell block preparation with the help of immunohistochemical markers can give better diagnostic results and a more accurate diagnosis of cystic lesions **[20].**

Interpretation of EUS-FNA results:

-The interpretation of pancreatic cytology requires proper knowledge of the following normal cells such as acinar, ductal, and islet cells to avoid pitfalls. **[16]** Some contaminants during the FNA procedure may appear, such as benign hepatocytes, duodenal epithelium, gastric mucosa, and mesothelial cells. The pancreatic FNA includes the background pattern, which may be mucinous, bloody, clean, inflammatory, or necrotic, the type of parenchymal epithelium that may be ductal, acinar, or islet cells, the stromal elements, which may be spindle cells, fibrovascular cores, or fibrous stromal elements **[16].**

Solid cellular neoplasms:

-Highly cellular smears characterize specific solid pancreatic lesions such as pancreatic endocrine neoplasm, acinar cell carcinoma, solid-pseudopapillary neoplasm, pancreatoblastoma **[21].** Comparing these lesions, solid pseudopapillary neoplasm (SPN) shows a cribriform pattern of cells as they have cytoplasmic extensions with or without cytoplasmic vacuoles and hyaline globules **[16], [21].** Meanwhile, neuroendocrine neoplasms are characterized by plasmacytoid nuclei, salt, pepper chromatin, and cytoplasmic neurosecretory granules usually surrounded by a bloody background. Acinar cell carcinoma (ACC) is characterized by acinar or grape-like clusters, granular cytoplasm, and minimal anisonucleosis.

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SPN, ACC, and neuroendocrine neoplasms share close features of cytological smears, so depending on immunohistochemical markers could help solve this, as neuroendocrine neoplasms exhibit synaptophysin chromogranin and CD56. At the same time, ACC shows a positive expression of trypsin, chymotrypsin, and phospholipase A2. Vimentin and beta-catenin are positive in SPN [22],[23].

Pancreatoblastoma (PB) is characterized by round blast-like cells twice the size of RBCs, squamoid morules, epithelioid cells with eosinophilic cytoplasm, and syncytial arrangements can be detected on both smears and cell blocks **[24].**

Mucinous Cystic Lesions:

Pancreatic lesions characterized by their mucinous background in FNA are intraductal papillary mucinous neoplasm (IPMN) and mucinous cystic neoplasms (MCN). Although they share some features such as classification into low grade, usually with hypocellular smear and abundant mucin, and a high degree of variable cellularity of atypical ductal cells and less prominent mucin, they differ in their incidence, gross and microscopic features, presence of papillary structures in IPMN and cystic fluid analysis of amylase (clinicopathological criteria) **[21].**

The criteria that favor the diagnosis of IPMN are the occurrence in the head of the pancreas. Male gender **[27]**. besides that IPMNs, are cystic pancreatic lesions that show two characteristic features: papillary projections bulging into the pancreatic duct and mucin production and microscopically IPMN offers four distinctive morphologic types of papillae (1) intestinal pattern, which has the same appearance of colonic villous adenomas (2) pancreatobiliary pattern, their papillae are lined by cuboidal cells with prominent nucleoli (3) gastric pattern, rarely some papillae have a gastric foveolar appearance. (4) Oncocytic pattern is characterized by abundant granular eosinophilic neoplastic cells and also contains intracellular mucin **[28],[29].**

Determining the degree of dysplasia is a crucial target in FNA interpretation. Several cytological features can identify the grade of dysplasia of IPMN as the hypercellularity of the specimens in the form of crowded epithelial clusters, the presence of necrosis, the presence of papillary fragments, parachromatin clearing, open chromatin, irregular nuclear membranes and nucleoli, and background of acute inflammation, all of these features support IPMN carcinoma diagnosis or at least IPMN-CIS [21].

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Notwithstanding, MCN is more common in women in 95% of cases and distal pancreas in 97% of patients **[30].** It is predominant in premenopausal females in the body and tail. **[24].**

Grossly known as multilocular large cysts surrounded by thick fibrotic walls, while at the microscopic view, the cysts are lined by tall, columnar mucin-producing epithelium. The stroma of MCN is characterized by being similar to the ovarian stroma, which is an essential key in defining these neoplasms **[28].** Usually, it is associated with an elevated cystic fluid of CEA, while the occurrence of elevation of CEA, CA19-9 levels in cystic fluid of IPMN means the presence of invasive carcinoma **[24].** The examination of cystic fluid is beneficial, as elevated amylase levels connect with the duct and are characteristically high in IPMNs while low in other cystic lesions **[31].** Specific mutated genes are identified in the cystic fluid of cystic pancreatic lesions—K-ras gene mutation in cystic fluid diagnoses mucinous cysts [32]. GNAS gene mutation is detected in more than half of cases of IPMN **[33].**

The risk features of mucinous pancreatic cystic neoplasms (MCN and IPMN) are moderate and high-risk. Moderate risk features include more than 3 cm cystic size, sudden change in diameter of the main pancreatic duct, regional lymphadenopathy, size of main pancreatic duct ranges from 5-9 mm, mural nodules, and cystic wall thickening. High-risk features include pancreatic head lesions associated with the common bile duct obstruction, the primary pancreatic duct size of more than 10 mm, and enhanced solid components within the cyst. The detection of just one of the hazardous features and two of the moderate features of a lesion is highly dysplastic or invasive cancer [34].

-Neoplastic mucin should be differentiated from contaminating mucin by its quality and quantity, and the presence of degenerated inflammatory cells and histiocytes confirm the neoplastic changes. The presence of neoplastic mucin, thick and colloid inconsistency, is enough for diagnosing the neoplastic mucinous cyst **[21].**

Intraductal tubulopapillary neoplasm (ITPN) is differentiated from IPMN by lacking mucin and papillary structures, but the cytological smears are highly cellular and arranged in tubular patterns with no mitosis. The cytological features of ITPN reported that it resembles IPMN by being ductal in origin and ACC by some morphology features [35].

Serous cystic lesion:

-FNA hardly detects serous cystic neoplasms as serous epithelium is seen only in 20% of cases, and cytology is usually non-diagnostic due to their scarcity of cellularity even after re-aspiration **[21]**. In serous cystadenoma FNA, the sparsely cellular smear is present with

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cuboidal cells, arranged in small sheets, harboring rounded central or eccentric nuclei and scanty cytoplasm in a clean background without features of malignancy such as mitosis, nuclear enlargement, or necrosis **[16], [21].**

Solid mass lesions:

-The solid lesions of ductal origin such as chronic pancreatitis and pancreatic ductal adenocarcinoma (PDA) remain the most crucial obstacle in EUS-FNA, as the false-negative cases are very high (23%) **[36],** (15%) **[37]**. Until now, FNA could not correctly differentiate between pseudo-tumorous pancreatitis and adenocarcinoma.

- PDA is characterized by ductal cells with overlapping nuclei, chromatin clearing, mitosis, and necrosis. Nevertheless, the components of a cytological smear of pancreatitis vary according to the stage. The smear mainly contains ductal, acinar cells, and inflammatory cells early. In contrast, later stage, mostly ductal cells are due to atrophy of acinar cells with no or rare mitosis **[16],** [38]**.** Several important cytological features can diagnose Well-differentiated PDA: 1) chromatin clearing, 2) anisonucleosis, 3) nuclear overlapping 4) nuclear membrane irregularity, 5) nuclear enlargement, 6) macronuclei 7) hyperchromasia 8) necrosis, 9) mitosis, 10) gap versus confluent cell space **[38].** Supporting the diagnosis of PDA, CA19-9 is not only the most important serum marker, but it is also a valuable prognostic marker that can detect the survival and response of cases to chemotherapy with a cut-off value above 200u\mL as reported by Ballehaninna UK (2013) **[39].**

Regarding autoimmune pancreatitis, FNA is characterized by mainly plasma cells and lymphocytes, fibrous tissue fragments, a population of ductal or acinar cells, and occasionally fibroblasts **[40**]. In addition, the serum level of IgG4 is highly elevated in autoimmune pancreatitis, which helps differentiate it from other causes of pancreatitis [**41**].

Nonneoplastic cystic lesions:

-Pseudocysts represent the most common benign pancreatic cystic lesions, which happen mainly because of acute pancreatitis and auto-digestion of pancreatic tissue by the release of pancreatic enzymes. According to FNA, Pseudocysts are classified into complicated forms with mucinous background and numerous inflammatory cells or uncomplicated forms with transparent non-mucinous background, few inflammatory cells, bile, and histiocytes. No mitosis or nuclear features of malignancy occur **[21].** Very high amylase

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levels are characteristically demonstrated **[31]**. Also, Martínez-Ordaz (2016) reported elevated serum levels in 79% of cases [42].

- Lymphoepithelial cyst (LEC) is a rare lesion, but its incidence begins to rise and mimics pseudocyst clinically and radiologically. The cytological findings include anucleated squamous cells, keratin, amorphous debris, a few nucleated squamous cells, and lymphocytes **[43].**

IMP3 (Insulin-like growth factor II mRNA binding protein 3):

The structure of IMP3 is composed of six RNA-binding domains: four K-homology (KH) domains in the C-terminal region and two RNA recognition motifs (RRMs) in the N-terminal area. The six parts are arranged in three pairs (RRM1 with 2, KH1 with 2, and KH3 with 4) and separated by flexible linkers **[44], [45]**. It binds to RNA through the C-terminal of KH domains. [**46]**

IMP3, its other name is IGF2BP3 or KOC, which means K homology domain-containing protein overexpressed in cancer, is a gene detected on the chromosome 7p11.5 by fluorescence in situ hybridization (FISH) **[46].**

It plays a vital role during embryogenesis in cell migration [47]. In addition, IMP3-RNA binding protein participates in post-transcriptional gene regulation **[48].**

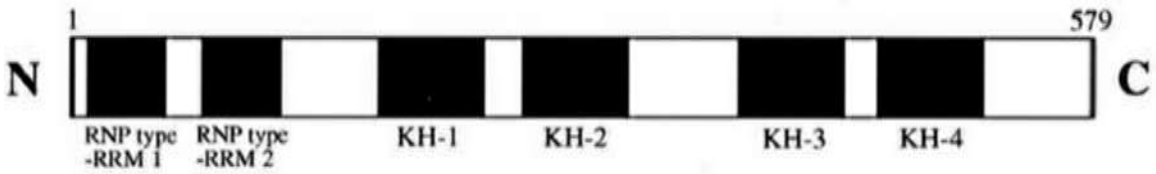

Fig1: Schematic diagram IMP3 structure, N, N- terminal, C, C-terminal, RRM, RNA recognition motifs, KH, K-homology [49].

IMP3 normal function:

IMP3 expression in normal tissues:

High IMP3 is found in pancreatic tissue during embryogenesis and ductal PC and not in adult exocrine pancreatic tissue **[50].** Typically, IMP3 is absent in various tissues, e.g.,

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pancreas, esophagus, stomach, heart, lung, kidney, and other tissues. However, it could be seen in a few tissues and particular cell types, for example, syncytiotrophoblast, cytotrophoblast, decidua, lymph follicles in lymph nodes and tonsils, absorptive cells of the ileum, crypt cells of the rectal mucosa, mucus cells of submandibular and sublingual glands, spermatogonia, ciliated cells of the bronchial mucosa and the fallopian tube, secretory cells of the endocervix, and cells of the adenohypophysis of the anterior lobe of the pituitary gland **[47].**

IMP3's role in modulating tumor cell fate:

- Heterogeneous nuclear ribonucleoprotein M (HNRNPM) manage the nuclear stabilization and transport of IMP3, as some studies confirmed its presence as nuclear not only cytoplasmic distribution relevant to its role in cyclins regulation and cancer cells proliferation, so IMP3 cytoplasmic to nuclear ratio could be used to determine the rate of cancer cells growth and might also be used as a therapeutic target **[51].**

IMP3 binds to CD44 mRNA, which acts as an adhesion molecule with extracellular matrix proteins including collagen, hyaluronan, laminin, and fibronectin, promoting tumor invasiveness **[52].**

Through a pro-metastatic behavior of pancreatic cancer cells, IMP3 shows enhanced aggressiveness of PDA by promoting the dissemination of cancer cells **[53].** In addition, IMP3 has been detected to increase the levels of nerve growth factorβ (NGFβ) and facilitate the translation of IGF-2 mRNA, which enhances the angiogenesis and lymphangiogenesis of the tumor **[50].** Also, the regulation of KIF11 mRNA, a mitotic kinesin, has been suggested to promote cancer cell proliferation tumor formation and play a vital role in coordinating cell movement **[54].**

IMP3 in malignant vs. normal pancreas:

IMP3 marker shows cytoplasmic distribution with evidence of malignant pancreatic tumors and high-grade dysplastic lesions, somehow in low-grade dysplastic lesions. Nevertheless, it is scarcely found in normal pancreatic tissue or benign pancreatic lesions. IMP3 was positive in 78.7% of PAC, 91.7% of MCN high-grade dysplasia, 100% of IPMN high-grade dysplasia. While negative nearly in all benign cases (95.8%) **[36].** Also, it was detected in 80.8%, 92% of malignant lesions Senoo (2018) and Yantiss (2008) respectively, and 0% of benign lesions in both studies [55], [56]. Evaluation of IMP3 on different pancreatic lesions and specimens (core needle biopsies and resection) showed that, in contrast to normal or inflamed pancreatic tissue, which was negative in 47 of 65 (72.3%) cases and weakly

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positive in 15 of 65 (23.1%) cases, strong IMP3 expression was found in 99 of 112 (88.4%) PDA. So, IMP3 expression sensitivity and specificity in PDA differentiation from chronic sclerosing pancreatitis on core needle biopsies were 88.4% and 94.6%, respectively **[57].**

IMP3 with high grades and stage pancreatic cancer:

Expression of IMP3 usually accompanies poor prognostic factors. Higher expression was detected in the advanced TNM stage and poorer prognosis. **[56], [58].** In urinary bladder cancers and esophageal adenocarcinomas, overexpression was correlated with high stage

and grade. Also, a significant correlation with shortened survival in gastric and lung adenocarcinomas was observed **[47].**

-IMP3 expression is positive mainly in malignant cases such as (PDA MCN with high-grade dysplasia) and harmful in benign cases (pseudocyst, serous cystadenoma, and pancreatitis). The score is directly proportional to the grade. Higher expression of IMP3 detected in advanced TNM stages and poorer prognosis. Malignant lesions with over-expression of IMP3 often suggest a poorer prognosis **[56], [58]**. Higher expression was detected with perineural, vascular, deeper, and metastasis to lymph nodes **[59].**

IMP3 diagnostic and prognostic factors in different organs

IMP3 expression in various cancers:

Expression of IMP3 was widely demonstrated in several human cancers. For example, it was seen in neuroblastoma (88%), Hodgkin's lymphoma (90%), and squamous cell carcinoma in distinct organs **[47].**

IMP3 was detected in nearly 67% of the cases in hepatocellular carcinoma and gastric cancer. Over-expression in both cancers was associated with poor outcomes **[59], [60].** Also, high expression was demonstrated in 41% of lung adenocarcinomas **[61].** IMP3 expression in colon cancer is correlated with cancer metastasis and has a high recurrence rate **[62].** IMP3 is a diagnostic and prognostic marker in renal cell carcinoma [63].

IMP3 has been studied in different organs to assess its diagnostic and prognostic value. For diagnosis of endometrial cancers and their premalignant lesions, IMP3 showed a marked and diffuse expression, mainly in endometrial serous and clear cell carcinomas, including their precursor lesions [64]. On the other hand, high expression was considered a poor prognostic predictor for duodenal papillary carcinoma. Also, an objective diagnosis based

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on IMP3 evaluation can be offered for patients with papillary tumors to determine if endoscopic papillectomy can be employed **[65].**

IMP3 was considered crucial in predicting mucoepidermoid carcinoma of salivary glands outcome as positive expression was related to age above 60 years, tumors of the submandibular gland, size more than 4 cm, higher grade, lymph node involvement, perineural invasion, surgical margins involvement, distant spread, higher stages, tumor relapse, and death. Also, as a diagnostic marker, IMP3 could distinguish between benign and malignant lesions of salivary glands, as it was negative in pleomorphic adenoma and normal salivary gland tissues and positive in 51.4% mucoepidermoid carcinoma. Increased expression in submandibular gland tumors and lymph node involvement are independent

prognostic factors of free survival **[66].** In addition to pilocytic and pilomyxoid astrocytomas, overexpression was considered a poor prognostic predictor **[67].**

By using EUS-FNA, the sensitivity and, specificity were 80.8%, 100%, and 80.3%, 92.3%, respectively **[55],** [68]. While combination of EUS-FNA and IMP3 expression raised the sensitivity reaching 89% **[57],** 87.9% **[54],** and 92%, **[56] [69].** The four studies showed specificity of 100% for this combined diagnostic tool. Also, Rashed (2021) and Ezzat et al 2016 have reported sensitivity, and specificity of 78.2%, 95.8%, and 91.2%, 86.7%, respectively [36][70].

Conclusion:

EUS-FNA is considered a flexible, safe procedure, particularly with the ROSE technique to have sufficient material for a proper diagnostic approach of pancreatic lesions. In addition, immunohistochemical expression of IMP3 on cytological smears or cell blocks obtained by EUS-FNA will add an excellent diagnostic and prognostic value if added to the diagnosis panel.

Footnotes.

Peer- Reviewers: Amr Shaban Hanafy (professor of internal medicine), Aziza E Abdelrahman (Assistant professor of pathology), Abdalla Hussein (Military hospitals, department of pathology).

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