REVIEW

Cell-block procedure in endoscopic ultrasound-guided-fine-needle-aspiration of gastrointestinal solid neoplastic lesions

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Abstract

In the present review we have analyzed the clinical applications of endoscopic ultrasound-guided-fine-needle-aspiration (EUS-FNA) and the methodological aspects obtained by cell-block procedure (CBP) in the diagnostic approach to the gastrointestinal neoplastic pathology. CBP showed numerous advantages in comparison to the cytologic routine smears; in particular, better preservation of cell architecture, achievement of routine haematoxylin-eosin staining equivalent to histological slides and possibility to perform immunohistochemistry or molecular analyses represented the most evident reasons to choose this method. Moreover, by this approach, the differential diagnosis of solid gastrointestinal neoplasias may be more easily achieved and the background of contaminant non-neoplastic gastrointestinal avoided. Finally, biological samples collected by EUS-FNA CBP-assisted should be investigated in order to identify and quantify further potential molecular markers.

Key words: Endoscopic ultrasound-guided-fine-needle-aspiration; Cell-block procedure; Gastrointestinal tract; Immunohistochemistry; Diagnosis

Core tip: Cell-block procedure (CBP) represents the most suitable complement in diagnostic cytopathology of many gastrointestinal lesions. Hence this method allows high quality morphological evaluation and immunocytochemical analyses. On this way, the differential diagnosis of solid gastrointestinal neoplasms may be more easily achieved and the background of contaminant non-neoplastic gastrointestinal avoided, with an evident gain compared to the traditional cytological techniques. In the present review, the application of CBP in gastrointestinal solid lesions approached by endoscopic ultrasound-guided-fine-needle-aspiration, the methodological aspects and the accuracy of this diagnostic process are analyzed and discussed.

INTRODUCTION

Endoscopic ultrasound-guided-fine-needle-aspiration (EUS-FNA) represents a useful diagnostic procedure in the field of gastrointestinal pathology[1-3]. It is performed by using a curved linear array video-echo-endoscope equipped with various needles which provide cytological samples; in this way, the ability to obtain cytologic material is greatly increased due to direct visualization, with a consequent better opportunity to perform an accurate diagnosis. Since its introduction, EUS-FNA emerged as a minimally-invasive, safe and accurate technique for the diagnosis of various luminal, submucosal and extra luminal gastrointestinal neoplasms[4].

The European Society of Gastrointestinal Endoscopy published the guidelines for EUS-guided sampling, with comments on the technical prerequisites for maximizing the diagnostic yield of this procedure[5]. However, the acquisition of diagnostic samples should be approached in different ways depending on the type of the lesion. Moreover, the actual efficacy of EUS-FNA partly depends on the site, size and characteristics of the target tissue as well as on the expertise, training and interaction between endosonographer and cytopathologist[6,7].

Cell-block procedure (CBP) is a diagnostic tool which has been carried out by using different procedural steps and protocols over the years[7-11]. This technique presents several advantages compared to the cytologic routine smear: preservation of cell architecture, achievement of routine haematoxylin-eosin staining equivalent to that of surgical samples and, finally, the possibility to perform ancillary methods, such as immunohistochemistry or molecular analyses[7,8,12,13]. In particular, CBP allows the availability of an adequate number of serial sections, with increased possibility to detect malignant cells and contaminating or reactive non-neoplastic elements[6,7,13].

Aims of the present review are to discuss the application of CBP in gastrointestinal solid lesions approached by EUS-FNA and to analyze the methodological aspects and accuracy of this diagnostic process.

Methodological aspects of EUS-FNA

One of the most debated issues on EUS-FNA relates to the number of needle passes required to provide adequate diagnostic material. The presence of a well trained cytopathologist, able to evaluate the quality of samples, is probably crucial in order to decrease the number of unsatisfactory results and to reduce the need for additional passes. Indeed, the prompt cytopathology response may be useful for the endosonographer to know whether the needle aspirate is diagnostic or not[2,4,14-19]. Although it has been repeatedly reported that on-site cytological evaluation improves the diagnostic yield and accuracy of EUS-FNA, other factors, such as the localization, nature, presentation, size and sonography characteristics of the lesion, may influence the number of needle passes[2-4,20]. In detail, the percentage of adequate specimens and sensitivity of EUS-FNA are lower in intra-parietal lesions of the gastrointestinal tract (GI) compared to those of lesions in other sites[1,21,22]. In addition, the diagnostic yield and accuracy for EUS-FNA also depend on the size of the lesion and they are significantly lower in lesions less than 10 mm in size[1,23,24]. On the whole, two to five needle passes are considered to be sufficient to obtain enough diagnostic material for a correct diagnosis by EUS-FNA[2,3,20,22,25,26]. The needle size is another relevant factor. 19-G needle seems to be the most adequate to provide higher amount of diagnostic material, especially when the cytopathologist is not present in the endoscopy room. Nonetheless the 22-G or 25-G are the most commonly used needles for the cytological sampling of gastrointestinal lesions because of their easier penetration without any further complications[2,16,27,28].

Finally, a special technical training in EUS-FNA should be mandatory, as recommended by the American Society of Gastrointestinal Endoscopy which codifies the minimum number of cases that should be analyzed depending on the site of lesion[29-31].

Needle-based confocal laser endomicroscopy (CLE) is a novel endoscopic method, in which imaging is based on tissue illumination and detection of tissue-reflected fluorescence; interestingly this technique gives high-quality images which are similar to those obtained by traditional histology[32-34]. The development of tissue specific contrast agents might further extend the application of CLE to pancreatic masses, either solid or cystic, intra-parietal or submucosal gastric and esophageal tumours, biliary tract and ampullary lesions[2,33,35].

Methodological aspects and advantages of CBP

CBP has been extensively used in cytology as a helpful tool to achieve a definitive diagnosis[8-10,36,37]. CBP may be carried out by using different protocols based on various fixatives and embedding techniques[8,10,38-40].

In the manual traditional method, following the rapid on-site evaluation of specimen adequacy and preliminary cytological diagnosis by quick stains, the needles and syringes used in the procedure are rinsed with 10 mL of 50% ethanol into a special container in order to recover further material. All content is centrifuged in a 10 mL disposable centrifuge tube at 4000 rpm for 6 min to create 1 or 2 pellets; the

supernatant fluid is decanted and the pelleted material obtained by sedimentation is immediately fixed in a freshly prepared solution of 4% neutral buffered formalin for 45 min. Then, the cell pellets are placed in a cassette and stored at 80% ethanol until they are ready for processing in an automatic tissue processor[36].

CBP may be based on thrombin or albumin methods. In the former, six drops of discarded human plasma and six drops of thromboplastin-DS are added to the cell sediment in order to form a clot, while in the latter 3-4 drops of 22% bovine albumin and 95% ethanol are added to the cell sediment to form a precipitate[9,41]. Whatever is the method, clots or/and precipitates are embedded in paraffin at 56 ℃ to realize cell blocks which are cut into 3 μm thick sections routinely stained with H and E or mounted on poly-lysine-coated glasses for immunocytochemical and molecular procedures.

A novel automated method for cell block production is the Cellient™ Automated Cell Block System. Compared to the traditional manual method, the automated one allows to achieve higher cellularity and better cellular presentation in terms of architecture and details; in addition it is faster and more reliable due to lack of operator dependency[9,39,42]. Gorman and coll. showed that Cellient cell blocks gives an adequate cellularity in all the analyzed cases, while formalin and thrombin cell blocks show a progressively decreasing adequacy[37]. The main drawback of Cellient system is methanol-based fixation, which may have negative impact on the following immunohistochemical analysis[8,9]. Indeed weaker staining intensity for ER, PR, MIB-1 and HER2 was shown by using this procedure[8,37,43,44]. However this issue may be overcome by formalin pre-fixation prior to Cellient[9]. Thirty minutes pre-fixation seems to be preferable to longer fixation to ensure good morphological quality[9].

On the whole, CBP allows the collection of higher quantity of diagnostic material. Hence it may be relevant in reducing the false negative diagnoses in EUS-FNA, which may depend, not only on erroneous interpretation of the cytological samples, but also on the availability of low cytological material. In addition it was shown that CBP greatly increases the diagnostic accuracy of EUS-FNA[7,22]. CBP also represents the most appropriate method to obtain cytological preparations for subsequent immunocytochemistry. Indeed immuno-stains on CBP sections show minimal background and appear similar to those observed in surgical pathology material. In addition, numerous serial sections may be obtained from a single cell block, allowing the evaluation of a large spectrum of antigens, also in archival samples. The number of antibodies that can be applied in routinely CBP has been expanding over the years[2,3,7-9,13,37]. The possibility to test serial sections with different antibodies may allow to identify and discriminate gastrointestinal hyperplastic or reactive contaminating cells from well differentiated tumour cells[7,13,45].
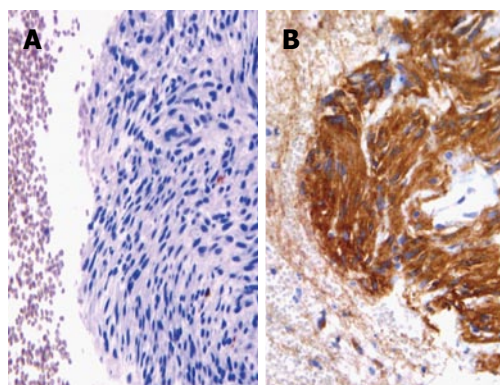

Figure 1 Cell block from gastrointestinal stromal tumour exhibiting aggregates of spindle cells with elongated nuclei (haematoxylin-eosin, × 200) (A), with an evident immunoreactivity for CD117 (immunoperoxidase, × 200, Mayer's Haemalum nuclear counterstain) (B).

CLINICAL APPLICATION OF EUS-FNA CBP-ASSISTED IN GI TRACT

Subepithelial/intramural neoplasms of the gastrointestinal wall

Although conventional endoscopy, CT scan and MRI may identify subepithelial/intramural lesions in the gastrointestinal wall, they can not reveal the nature and origin of those lesions. A wide range of subepithelial tumours, such as leiomyomas, neurinomas, granular cells tumours, gastrointestinal stromal tumours (GISTs), neuroendocrine tumours, leiomyosarcomas and lymphomas, may involve the GI tract[1,6,46] and many of those neoplastic entities exhibited over-lapping cytological features[6,46], being composed by monomorphic, uniform spindle shaped cells with eosinophilic cytoplasm, vesicular elongated nuclei characterized by finely granular chromatin, sometimes dispersed and inconspicuous nucleoli (Figure 1A). For this reason, the use of immunocytochemistry, which is easily applicable to CBP, may be helpful for the differential diagnosis. In detail, the coexistence of smooth muscle actin and desmin stains strongly supports the muscle origin of the lesion, while positivity for CD-34, CD-117 (Figure 1B) or S-100 suggests other diagnostic hypotheses, such as inflammatory fibroid polyp, GIST or schwannoma[6,46,47]. The assessment of the growth fraction by using Ki-67 labeling index (Figure 2A) may further discriminate the benign or malignant nature of intra-parietal neoplasias, and may allow distinction among leiomyoma, leiomyosarcoma, spindle cells amelanotic melanoma or undifferentiated sarcomatoid carcinoma[6,46,48].

The great efficacy of EUS-FNA associated with the higher accuracy obtained by CBP are helpful to achieve the correct preoperative diagnosis of a sub-epithelial mass which is relevant to establish the operative planning and type of surgery, and to avoid unnecessary procedures for extensive malignant lesions[6,46,49]. In addition, periodic follow-up with EUS is considered to be more acceptable to evaluate eventual changes in
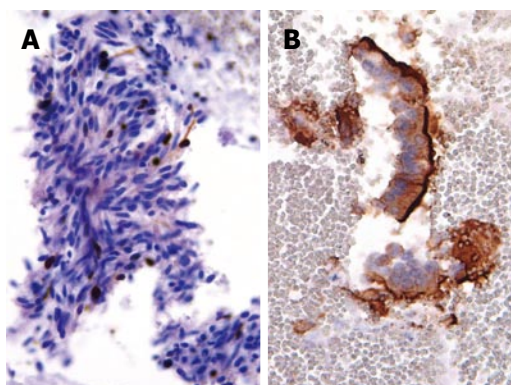
Figure 2 Spindle cells of gastric gastrointestinal stromal tumour documented only a sporadic nuclear Ki67 immunopositivity (immuno-peroxidase, × 400, Mayer's Haemalum nuclear counterstain) (A) in benign contaminant gastrointestinal cells, the apical cytoplasm showed a peculiar CD10 immunoreactivity (immunoperoxidase, × 400, Mayer's Haemalum nuclear counterstain) (B).

Figure 3 Well differentiated pancreatic carcinoma with a pseudo-glandular pattern (haematoxylin-eosin, × 400) (A), a nuclear strong p53 immunostaining was encountered in neoplastic elements (immuno-peroxidase, × 400, Mayer's Haemalum nuclear counterstain) (B).

tumour size in those patients who refused surgery[49-51].

Solid pancreatic masses

The pre-operative correct diagnosis of ductal pancreatic adenocarcinoma is crucial for patients management and prognosis, and to reduce costs due to unwarranted procedures[1,13,52,53]. The cytological detection of pancreatic ductal adenocarcinoma is usually not difficult for the experienced cytopathologist; indeed this neoplasia is characterized by distinctive cytological features, such as the presence of groups of atypical cells with irregular roundish hyperchromatic dense nuclei, evident nucleoli, mitotic figures and absence of the honeycomb benign pattern[13]. Frequently, pancreatic smears exhibited a hemorrhagic background with clusters or small aggregates of epithelial cells, occasionally arranged in glandular or pseudo-papillary structures. Nevertheless, in a subset of carcinomas the cytological diagnosis may be hard to achieve, due to the presence of extensive necrosis, associated inflammation, contaminating intestinal epithelial cells or limited sampling[7,13,54]. In those cases, again CBP appears as a significant tool for the pathologist, either the microscopic evaluation and application of immunostainings in serial sections. In fact, it has been shown that carcinoembryonic antigen was expressed in neoplastic pancreatic elements of great majority of ductal adenocarcinomas[13]. However, carbohydrate antigen (CA 19-9) represented the most widely used biomarker for pancreatic cancer, even if it showed limitations in differential diagnosis between pancreatic neoplasms, being positive also in solid pseudopapillary tumour and not only in cancer[55-61]. An intriguing challenge, even for the expert cytopathologist, is repre-sented by the distinction between well differentiated pancreatic neoplastic cells and gastrointestinal epithelial contaminating elements, sampled by EUS-FNA through the stomach or the duodenum[7,13,55-58]. Several efforts were made to solve this crucial diagnostic point[7,13,20,55-58].
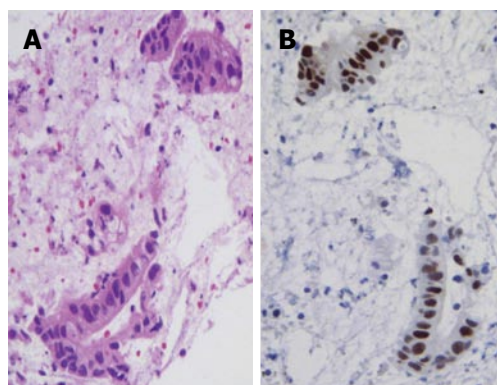
Firstly, it was reported that a mucin panel comprising four antibodies (MUC1, MUC2, MUC5AC, MUC6) may be helpful in differentiating normal/reactive gastro-duodenal cells from neoplastic pancreatic elements[55]. Successively, the utility of immunocytochemistry against CD10 was highlighted (Figure 2B); indeed this antigen is expressed at the apical membrane of the benign contaminant gastrointestinal cells, but not in the neoplastic elements of well differentiated pancreatic adenocarcinoma[7,13,59,60]. The absence of CD10 stain in pancreatic adenocarcinomas has been also documented in surgical histological samples[59,60]. However, CD10 expression has been evidenced in 100% of solid pseudo-papillary pancreatic neoplasms[61-63] and in 30% of pancreatic endocrine tumours with focal staining[7,63,64]. As a consequence, CD10 immunostaining alone cannot be used for the differential diagnosis of pancreatic lesions[7]. An immunohistochemical panel against CK7, CDX2, chromogranin A and synap-tophysin is useful for the differential diagnosis among invasive ductal carcinomas, endocrine tumours and acinar cell tumours of the pancreas[12,20,65]. Finally, a further analysis of p53 immunoreactivity may be of diagnostic help in pancreatic pathology (Figure 3); indeed immunocytochemical positivity for mutant p53 protein with long half-life has been recorded in 50%-70% of pancreatic carcinomas, but not in chronic pancreatitis[66-68].

Solid hepatic lesions

A variety of hepatocellular nodules (hyperplastic, benign, dysplastic and malignant) and secondary tumors can be detected in the liver and subjected to EUS-FNA, especially when they were confined to left hepatic lobe[3,69,70]. In particular, while a significant rate of lesions smaller than 1 cm in diameter is missed by CT and MRI, EUS shows excellent diagnostic accuracy in the identification of hepatic lesions less than 0.5 cm in size[69-72]. It is noteworthy that most of < 1 cm hepatic lesions are non-malignant, whereas the large majority of lesions exceeding 2 cm are represented
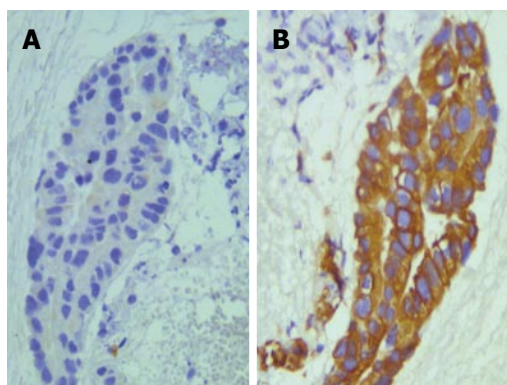
Figure 4 Peripheral cholangiocarcinoma with a papillary pattern (haematoxylin-eosin, × 400) (A), in a serial section obtained from CBP neoplastic elements exhibited an evident cytokeratin 7 immunoreaction (B) (immunoperoxidase, × 400, Mayer's Haemalum nuclear counterstain).

by hepatocellular carcinomas (HCCs); hence in the group of lesions greater than 2 cm a diagnosis of non-malignancy should induce the suspicion of a diagnostic error[73]. Although nodular precursors such as liver regenerative (LRN) or low-grade dysplastic (LGDN) and high grade dysplastic (HGDN) nodules are related to hepatocarcinogenesis, they should be discriminated from adenomas and differentiated HCCs. LGDN category also includes the so-called LRN and it shows mild increase in cell density with a monotonous pattern and bland cytological atypia[73]. On the other hand, HGDN always exhibit more marked cytological atypia and irregular trabecular pattern[73]. Discrimination of well differentiated and hypovascular HCCs from dysplastic nodules may be particularly challenging; in those cases, CBP associated with EUS-FNA or EUS-guided biopsy are warranted, as recently acknowledged[74]. Several immunomarkers were proposed for the distinction between well differentiated HCC and non-malignant lesions[75]. Specifically, Glypican 3 appeared as a good tissue marker with 77% sensitivity and 96% specificity for HCC[74]. In addition, Heat Shock Protein 70 was reported as the most abundantly up-regulated gene in early HCC, and the protein for which it encodes can be detected by immunocytochemistry in up to 78% of the cases with 95% specificity[74]. Finally, Glutamine Synthetase is overexpressed in malignant hepatocytes with diffuse and strong pattern in 50% of HCCs[74,76]. The combined use of the aforementioned was proposed in order to increase the diagnostic accuracy in cases with dubious morphology[76], and so the availability of serial consecutive sections obtained from CBP applied to EUS-FNA could represent the gold standard. With regards to cytokeratins (CK) profile, CK8 and 18 are expressed in both normal and neoplastic hepatocytes, while about 70% of HCC are negative for CK7, CK19, and CK20[73,77]. Furthermore, the combined use of CK7 and CK20 may help to identify the origin of adenocarcinomas occurring in GI tract; in particular, CK7 and CK20 expression in cholangiocarcinomas (CC) varies along the biliary tract, with higher sensitivity of CK7$^+$/CK20$^-$ profile in peripheral CC compared to non-peripheral ones (Figure 3)[73,77]. On the other hand, CK7$^+$/CK20$^+$ profile supports the diagnosis of pancreatic adenocarcinoma, while CK7$^-$/CK20$^+$ is the typical pattern of colonic cancer[73,77].

Gallbladder and biliary tract lesions

Approach by EUS-FNA of the lesions of biliary tract, and mainly of the hilar ones, may avoid the risk of unnecessary extensive surgery[78,79]. Indeed the sensitivity and specificity of obtaining diagnostic samples in biliary neoplasms is variable by endoscopic-retrograde cholangiography[3]. Moreover, the endoscopic retrograde cholangiopancreaticography (ERCP), used at times for hilar cholangiocarcinomas, has frequently inconclusive diagnosis[80]. Consequently, a morphological diagnosis on cytological samples provided by EUS-FNA and submitted to CBP may allow to recognize the nature of malignant biliary lesions (Figure 4) and to change the preplanned surgical approach. Generally, tumour cells appear in loosely structured groups or disorder flat sheets exhibiting as cytologic atypia that varies depending upon tumour grade; occasionally, tumour cells may exhibit cytoplasmic vacuolization and focal mucin secretion. What's more, regional lymph nodes may be evaluated for metastasis by EUS-FNA in patients with unresectable hilar carcinomas[81,82].

A sensitivity and accuracy of 95% have been recorded for EUS-FNA in distal biliary malignancies[7,83] and similar values have been reported in patients with obstructive jaundice due to nodular lesion such as epithelial and non-epithelial tumours, lymphomas and metastases[84-86].

In gallbladder masses, the CBP-assisted EUS-FNA procedure has been used either for diagnostic and staging purposes, with rates of sensitivity and specificity ranging between 80% and 100%, especially in lesions of the gallbladder wall[87-91].

In ampullary tumours, EUS-FNA has higher diagnostic accuracy in the distinction between benign and malignant tumours compared to other operative procedures such as biopsy or brushing cytology during ERCP[92,93]. In addition, it is of help in the identification of patients with low or high grade dysplasia or affected by adenocarcinomas[93].

In this anatomical district, some very severe complications such as bile peritonitis and cholangitis have been described[1]; they probably represent a consequence of inadvertent needle penetration inside intrahepatic or common bile ducts as well as gallbladder. By contrast, bleeding is mild and self-limited, even when patients were taken aspirin or anti-inflammatory drugs, in absence of portal hypertension[1].

CONCLUSION

The clinical applications of EUS-FNA and the methodological advantages obtained by CBP in the diagnosis of solid neoplasms of the GI tract were reviewed.

Although on-site cytological evaluation during the ultra-sonographic needle aspirative procedure may increase the diagnostic yield of EUS-FNA, in our opinion CBP represents its most appropriate diagnostic complement. Indeed this method allows high quality morphological microscopic evaluation and multiple immunocytochemical analyses. By this approach, the differential diagnosis of neoplasms may be more easily achieved, and the background of contaminant non-neoplastic gastrointestinal avoided, which represent evident advantages compared to the traditional cytological techniques. Finally, the identification and quantification of potential molecular markers may represent a promising field to be further investigated on the same biological samples collected by EUS-FNA CBP-assisted.

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