Review Article

Endoscopic ultrasound in pancreatic cancer: innovative applications beyond the basics

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Contributions: (I) Conception and design: All authors; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: None; (V) Data analysis and interpretation: None; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Abstract: Endoscopic ultrasound (EUS) has become a mainstay in assisting in the diagnosis and staging of pancreatic cancer. In addition, EUS provides a modality to treat chronic pain through celiac plexus neurolysis. Currently, there is growing data and utilization of EUS in more diverse and innovative applications aimed at providing more sophisticated diagnostic, prognostic and therapeutic options for patients with pancreatic cancer. EUS delivery of chemotherapy, viral and biological vectors and fiducial markers may eventually revolutionize the way clinicians approach the care of a patient with pancreatic cancer.

Keywords: Endoscopic ultrasound (EUS); pancreatic cancer; fine needle injection (FNI); fiducial markers; immunotherapy

Submitted May 30, 2016. Accepted for publication Jul 06, 2016.
doi: 10.21037/jgo.2016.08.07
View this article at: http://dx.doi.org/10.21037/jgo.2016.08.07

Introduction

Pancreatic ductal adenocarcinoma (PDA) is rarely curable at the time of diagnosis as most patients present with either locally advanced or metastatic disease. It has been estimated that less than 20% of patients with newly diagnosed pancreatic cancer have surgically resectable disease, and approximately 30% of patients present with locally advanced disease (1). Locally advanced disease is defined as unresectable pancreatic cancer without evidence of distant metastatic disease. Of the patients who are eligible for surgical resection, most will relapse and experience a median survival of 23 months (2). Even in patients with margin-negative resection, the risk of both local and systemic recurrence is high, and in the cases without adjuvant therapy, the 5-year survival is 10–13% (3,4).

Endoscopic ultrasound (EUS) represents one of the most innovative gastrointestinal procedures that has been developed in recent years with respect to the diagnosis and treatment of patients with pancreatic cancer. EUS is routinely used to assist in the diagnosis and staging of pancreatic cancer along with providing a modality for pain control during celiac plexus neurolysis. The role of EUS in pancreatic cancer is rapidly expanding with new prognostic and therapeutic modalities becoming more common. This review will aim to summarize these innovative applications of EUS in pancreatic cancer outside its more traditional uses.

Targeted EUS guided delivery of chemotherapy

EUS has the potential to revolutionize the delivery of chemotherapy by improving selectivity of treatment and reducing undesirable side effects in surrounding healthy tissue (5). Currently, one of the main limiting factors of systemic chemotherapy is its side effects. The common chemotherapy agents used for pancreatic cancer include 5-flurouracil and gemcitabine, each of which has significant clinical toxicity. EUS enables access to the pancreas in a minimally invasive manner. EUS allows for a less invasive way to apply localized chemotherapy to the

pancreatic tumor, thus preventing side effects of systemic chemotherapy. It also allows for a more comprehensive real-time image, a shorter puncture pathway, and a lower risk of complications when compared to via computed tomography (CT) or abdominal ultrasound (US)-guided procedures.

EUS-guided fine needle injections (EUS-FNIs) were initially studied in a porcine model where they injected paclitaxel into the pancreas; clinically detectable concentrations of the drug could not be detected beyond a distance of 30–50 mm from the injection site (6). Levy *et al*. studied EUS-FNI of gemcitabine in patients with unresectable cancer and demonstrated that several patients were able to be down staged and undergo subsequent resection (7). EUS-FNI of chemotherapy can be limited by the high density of fibrosis in pancreatic cancer, making it difficult to pierce the needle into the pancreatic tumor, and make it challenging to inject adequate amounts of an injected solution into the mass (7). Although interventional EUS has not been shown to significantly improve the survival rate and prolong the survival time in patients with pancreatic cancer, it can effectively induce tumor cell death. Additional studies are needed to further explore this therapeutic application in the future.

EUS in predicting prognosis and response to chemotherapy

In addition to the potential for directly administering chemotherapy, assessing the prognosis and response to therapy is another developing role for EUS. Currently, many academic institutions and industry trials have adopted the response evaluation criteria in solid tumors (RECIST) criteria to help standardize the assessment of prognosis and response to therapies (8). RECIST criteria are largely based on radiographic cross-sectional imaging. It has been proposed that tumor response to neoadjuvant chemotherapy (defined by the RECIST criteria) would be required prior to surgery for borderline resectable pancreatic tumors. However, in a study by Katz *et al*. only 12% of cases had radiographic changes associated with neoadjuvant chemotherapy that met the RECIST criteria (9). Furthermore, only one patient out of 129 patients had enough of a reduction in tumor size to be reclassified as resectable via radiographic criteria, and yet 60% of those patients underwent surgical resection, suggesting that surgical resection in patients with borderline resectable cancer should not be based only on these radiographic changes. The current literature suggests that patients with borderline resectable pancreatic adenocarcinoma undergoing neoadjuvant therapy should undergo resection unless they develop metastatic disease, local progression that would prohibit resection, or a decline in performance status. In patients with locally advanced disease, such as those with tumors encasing or obliterating celiac or superior mesenteric vessels, it is extremely uncommon to be able to downstage their tumors with current neoadjuvant therapies (10).

Imaging modalities such as contrast-enhanced endoscopic ultrasound (CE-EUS) could also be used to help select patients for chemotherapy that are predicted to have an improved survival with chemotherapy. Pancreatic cancer usually has a hypovascular nature and appears as such on CE-EUS. The hypovascular nature of pancreatic cancer typically results in poor drug delivery, and gemcitabine, one of the current chemotherapies of choice for unresectable pancreatic cancer, is not always effective (11,12). In the study by Sofuni *et al*., the authors indicated that CE-EUS could be utilized to identify patients who have more intratumor blood flow, since these patients have a significantly better response to chemotherapy (13). They suggested that when there is greater intratumor blood flow, more of the chemotherapeutic agent can enter the tumor, which may provide better drug delivery. Recent studies have also shown that patients with large intratumoral vessels also have significantly longer progression-free survival and overall survival, and that a positive vessel sign was an independent factor associated with longer survival (14).

CE-EUS has also been shown to be an effective method by which to demonstrate response to chemotherapy. Early studies in pigs have suggested that CE-EUS could be utilized to visualize pancreatic perfusion after tissue ablation, and how it could aid in post-treatment follow-up (15). Sofuni *et al*. demonstrated that the before and after treatment imaging patterns of CT and CE-EUS did not always correlate, as the rate of concordance before treatment was 92% and only 76% after treatment (13). In this study, CT imaging after treatment with gemcitabine often failed to show significant changes despite the fact that CH-EUS often did reveal an increase of intratumor blood flow (13). Furthermore, increasing intratumor blood flow was found to correlate with decreasing CA19-9 serum levels and better outcomes. Additional studies evaluating CE-EUS as a means to follow the response of pancreatic cancer to chemotherapy could establish it as a safe, highly accurate, and cost-effective alternative to CT and PET imaging (16).

EUS may therefore become indispensable in diagnosing and prognosticating pancreatic adenocarcinoma, monitoring tumor response to chemotherapy, and delivering chemotherapy in patients with pancreatic cancer in the

near future. Large prospective, randomized controlled trials are still needed to prove that CE-EUS monitoring and interventional EUS are effective in pancreatic adenocarcinoma treatment. However, considering the variety of chemotherapeutic options, it is possible that survival for patients with pancreatic cancer could be significantly improved, and the goal of qualifying for surgery with a curative intent may be achieved more frequently.

EUS delivery of viral and biologic vectors

Introduction

In pancreatic cancer, the pathophysiology leading to the development of a pancreatic tumor has been shown to have three precursor lesions that proceed in a multistep progression to become pancreatic adenocarcinoma. These include pancreatic intraepithelial neoplasia, intraductal papillary neoplasia, and mucinous cystic neoplasms (17). Typically, the initial mutation is an activation mutation in the *KRAS* gene, followed by a mutation in one or more tumor suppressor genes (18). Progression to metastatic pancreatic adenocarcinoma has been found to require both activating mutations and the loss of a tumor suppressor gene in murine models. The difficulty in treating pancreatic cancer is thought to be in part due to the anatomic and histologic features of the involved tissue. The dense extracellular matrix in pancreatic cancer distorts the normal architecture of tissue and causes an abnormal configuration of blood and lymphatic vessels, resulting in a hypoxic tumor mass. The resulting tumor often has poor perfusion, and is thought to be one reason why systemic chemotherapy has not been more effective in treating pancreatic cancer (19).

The application of utilizing viruses to deliver oncotherapy, in part due to their tumor selectivity and ability to cause lysis in cancer cells, remains an emerging topic in the treatment of pancreatic cancer. Such viruses are genetically engineered to target genes on malignant cells, while avoiding the binding to, viral replication of, and eventual destruction of normal cells (20). In addition, these viruses have been engineered to replicate throughout tumor cells in order to more effectively attack them (21). Viral vectors have previously been administered intravenously, intraoperatively when tumors have been found to be unresectable, and percutaneously via CT guidance. All of these methods, however, have been found to carry significant side effects and morbidity. As a result, the administration of viral vectors via EUS has gained popularity, and EUS as a method to deliver multiple types of viruses has been studied in both animal models and clinical trials. It has also been suggested that EUS administration can provide a more diffuse viral infectivity of the tumor due to the ability to perform multiple FNIs (22).

Adenovirus

Adenovirus is a double stranded DNA virus that incorporates itself into its host genome for replication and binds to cells with higher affinity than other viruses (23). It also subsequently infects nearby cells after cellular lysis, making it a desirable vector for oncolytic therapy. Two types of adenovirus, Gendicine and Oncorine, are already currently approved for treatment of multiple types of cancer in China (24).

Enabling adenoviruses to be specifically active towards malignant cells involves the deletion of essential viral genes needed for replication in normal cells, rendering the virus only functional in tumor cells not requiring these genes. An example of this is ONYX-015, an adenovirus engineered to lack the *E1B* gene, which in normal cells binds to tumor suppressor *p53* and causes progression of the cell cycle and viral replication. E1B-deleted viruses do not typically replicate in normal cells. Pancreatic tumors, however, lack *p53* in 50–75% of tumor cells, allowing E1B-deleted viruses to replicate and spread to nearby malignant cells. ONYX-015 was shown to be effective, and increased survival when intratumorally injected in murine models (25). Although prior administration of ONYX-015 has been performed via intravenous route and CT-guided injection, administration via EUS poses to be an alternative delivery method. This is in part due to the lack of systemic effects that intravenous administration can carry, as well as the less cumbersome nature and shorter injection pathway of EUS as compared with CT-guided injection, and the ability to perform multiple injections, to diffusely spread virus throughout the entire tumor (22). ONYX-015 was the first replication-competent virus used in a clinical trial, and when administered via EUS in phase I/II clinical trials for patients with pancreatic cancer, it was found to be a well-tolerated therapy (22). Unfortunately, no significant responses (i.e., decrease in tumor size or prolonged survival) were seen when ONYX-015 was used as a single agent, and only 2/21 patients showed mild responses when EUS injection was combined with gemcitabine (22). Similar adenoviruses with other deletions have also proven effective in treating pancreatic cancer, including Oncorine, another adenovirus with a larger deletion of the *E1B* gene, and

adenoviruses with deletion of the *E1A* gene, which binds to retinoblastoma protein (pRb). These viruses remain to be tested via EUS administration, but given the successful administration of ONYX-015, they may show promise as another EUS-delivered therapy. Adenoviruses are also being developed that incorporate multiple gene deletions, further increasing the selectivity towards cancerous cells. Importantly, these viruses have been shown to remain equally efficacious in addition to having increased selectivity with multiple deletions (26,27).

Though the use of adenoviruses has shown significant promise, there are disadvantages to their use as well. One disadvantage is that they are not very infective towards malignant pancreatic tumor cells. This is due in part to the primary type of adenovirus used in oncolytic models, which uses a receptor to bind to cells that is typically expressed very little in pancreatic cancer cells. The attempt to overcome this involves using new adenovirus mutants, which have a different binding site, increasing their infectivity towards pancreatic tumor cells (28). Another technique devised to improve efficacy of adenoviruses involves equipping these viruses with therapeutic genes which prime the immune system to improve destruction of cancerous cells. An example of this involves interleukin 24 (IL-24), which has been shown to improve the immune system's recognition of pancreatic cancer, but has severe side effects, which limits its use in systemic administration. When an adenovirus was engineered to manufacture IL-24 locally within tumor cells *in vitro*, there was a significant decrease in tumor growth and a strong immune response to pancreatic cancer (29). Thus, the administration of adenovirus equipped with IL-24 via EUS may have significant therapeutic effects while avoiding systemic side effects.

Herpes virus

Herpes simplex 1 virus (HSV-1) is a double stranded DNA virus that has shown promise against pancreatic cancer. The HSV genome is larger than most viruses, and as a result can have many therapeutic genes inserted to replace many of the nonessential genes, while not integrating itself into host DNA (30). Most importantly, HSV has a strong T-cell mediated tumor reactivity, and it can indirectly cause an immune response to cancer, causing local killing and destruction of the tumor by the body's own defense cells. Like adenoviruses, HSV viruses use two major strategies for improving selectivity towards cancer cells. These include the deletion of viral genes for replication and the deletion of genes that regulate the protein kinase response pathway. One particularly encouraging HSV oncolytic virus is FusOn-H2, which has a deletion of the *ICP10* gene, which is involved in the Ras-mitogen activated protein kinase pathway. Intratumoral injections showed complete eradications of pancreatic xenografts in mouse models. Intravenous administration showed significant antitumor effects, and intraperitoneal administration showed eradication of 75% of tumors and prevention of metastasis (31).

Although other types of HSV viruses have been used in intraoperative injection of pancreatic tumors (32), HSV has also showed promise when injected into tumors via EUS. An example of this is OncoVex GM-CSF, which has a deletion that makes it selective to tumor cells. In addition, it is hypothesized that the ability of this virus to express human GM-CSF will potentiate the recruitment and activation of dendritic T cells to the location of the tumor, and promote tumor destruction (33,34). The OncoVex GM-CSF virus has been shown to be well-tolerated in clinical trials in other solid tumors, including head and neck, squamous cell, and breast cancer, and is currently being used as an EUS-guided therapy in a phase I trial for pancreatic cancer, the results of which have not yet been published (34).

Other viruses

In addition to the above-mentioned viruses, there remain other viruses that may show benefit in the treatment of pancreatic cancer in the future, particularly by EUS administration. Among these are poxviruses, which have been shown to be equally infective under hypoxic conditions, which as mentioned, is a feature of pancreatic cancer thought to make it so resistant to systemic chemotherapy. A number of poxviruses have been studied both *in vitro* and *in vivo*, and have shown a benefit to oncolysis when combined with gemcitabine (35,36). Similar in nature to poxviruses, parvoviruses have direct oncolysis and immunomodulatory effects. Parvovirus has been shown to reduce tumor growth *in vivo*, and improve animal survival and decrease metastases when given with gemcitabine (37). Measles viruses have also been shown to have oncolytic activity in pancreatic tumor xenografts in mice, and improve survival (38). Another type of measles virus, which was engineered to target prostate stem cell antigen (PSCA), a protein expressed in pancreatic cancer, has been shown to have beneficial effects particularly in gemcitabine resistant

pancreatic adenocarcinoma (39). Reovirus, a virus whose replication is dependent on KRAS, a frequently found mutation in pancreatic cancer, has been shown to decrease tumor mass both locally and in the liver when administered intraperitoneally (40,41). Although there have not been clinical trials using EUS for these viruses, they have shown promise in treating pancreatic cancer, and delivery via EUS should strongly be considered in the future.

Immunotherapy

In addition to viral therapies, other forms of endoscopically-administered immunotherapy have been used in pancreatic cancer with promising results. These include local administration of immunologic agents, in an attempt to boost the local immune response to the malignant cells of pancreatic adenocarcinoma. The first example of this involved administration of cytoimplant, which was an EUS-administered injection of mixed lymphocytic tissue, derived from both healthy donors and the patient's own peripheral blood lymphocytes (42). Of the eight patients with advanced pancreatic cancer in whom cytoimplant was used, only minimal side effects occurred, including low grade fevers, gastrointestinal toxicity, and hyperbilirubinemia. The median survival of the eight patients was 13 months, with two partial responses and one minor response. A second form of administration, involving EUS-guided administration of dendritic cells, was reported by Nonogaki *et al*. in 2007. Of the five patients with unresectable pancreatic cancer, one patient showed a partial response, with two others showing stable disease for over 6 months (43). Multiple other small studies were subsequently performed, which did not demonstrate complications with dendritic cell injection, and showed stable disease in some patients receiving therapy (44,45).

In addition to adenovirus being used as previously discussed to infect and lyse pancreatic cancer cells, it has also been used as a vector to carry human tumor necrosis factor-alpha genes into pancreatic cancer cells. This therapy, named TNFerade, was shown by Hecht *et al*. to have benefit when locally injected into advanced pancreatic cancers using both EUS and percutaneous administration (46). In the phase I/II study performed, patients also received concomitant radiation therapy and chemotherapy. Of the 50 patients who received therapy, one showed a complete response, 3 patients showed a partial response, and 12 patients showed stable disease. Seven patients were able to undergo surgery, with three surviving over 2 years. There was no difference in outcomes from the different method of delivery (EUS versus percutaneous route). Another randomized phase III multi-institutional study, however, showed that injected TNFerade, either by EUS or percutaneous transabdominal injection, when combined with fluorouracil and radiotherapy, was not effective for prolonging survival in patients with locally advanced pancreatic cancer (47). It was found, however, to be a safe treatment alternative. As in the prior study, responses appeared similar by EUS and percutaneous administration.

Another novel treatment of pancreatic cancer involves plasmids, double stranded DNA molecules independent of the host cell's DNA. Intratumoral injection by both EUS and CT guided percutaneous injection of BC-819, a double stranded DNA plasmid, has been studied in a recent phase I/II clinical trial (48). BC-819 carries the diphtheria toxin-A gene, which is activated by an H19 promoter, which is overexpressed in multiple malignancies, including pancreatic cancer. In a phase I/II trial performed by Hanna *et al*., BC-819 was injected via both EUS and percutaneously. Although this study involved only nine subjects, it was found to be well-tolerated with only asymptomatic elevation of lipase in one patient, three patients were found to have a partial response 3 months after injection, with two patients being able to have their tumors downgraded to surgically resectable. More success was seen with patients with a higher dose of BC-819 (48).

Although much more research needs to be performed on novel therapies of treating pancreatic cancer, the above-mentioned local treatments have shown promise, particularly by EUS, and may be able to improve the currently bleak survival in locally advanced pancreatic cancer.

EUS guided implantation of fiducial markers

Introduction

Stereotactic body radiation therapy (SBRT), also known as image guided radiation therapy (IGRT), is a technique that allows for the delivery of high doses of radiation to a precise target area within the body. The technique involves directing beams of radiation in three separate planes to converge on a specific locus, allowing for concentrated high doses of radiation to be delivered while limiting radiation exposure to surrounding areas. SBRT is modeled after stereotactic radiosurgery (SRS), which was first introduced in 1951 by the Swedish neurosurgeon Lars Leksell (49,50).

Using the fixed anatomical structures of the bony skull as fixed landmarks to guide beams of radiation, SRS was initially developed for the treatment of intracranial tumors. SRS was effective because of its ability to deliver high doses of radiation therapy within the frame of a fixed space. SBRT is the extension of SRS to lesions outside the skull, and is now being applied to the treatment of locally advanced pancreatic adenocarcinoma (50).

One of the main challenges of applying SBRT to pancreatic tumors is the need to account for movement. Unlike intracranial tumors, pancreatic tumors do not exist within a fixed space such as the bony skull, and are estimated to move as much as 2–3 cm during the respiratory cycle (51). Radiographic markers (i.e., fiducials) implanted into pancreatic tumors help to overcome the challenge of a moving target by acting as fixed reference points within the tumor. Tracking fiducials as surrogates of the tumor allows for real time targeting of radiation beams (50). As the use of fiducials in the treatment of pancreatic tumors continues to evolve, one of the key questions that arise is determining what is the safest and most effective method to implant fiducials. This section will review the current literature as it pertains to the placement of fiducials using EUS. It will include sections dedicated to the methods, materials, and outcomes of fiducial markers placed by EUS.

Placement of fiducial markers by EUS versus traditional methods

Traditionally, fiducial markers have been implanted either surgically or percutaneously via CT or US guidance. Percutaneous placement of fiducial markers under CT or US guidance is often feasible when lesions are relatively superficial or have a clear window (52). There are, however, risks associated with percutaneous placement. One notable risk is of vascular damage or puncture. In a retrospective review by Kothary *et al*. of 61 cases of CT guided percutaneous fiducial marker implantation for pancreatic cancer performed between 2003 and 2008, 3.3% were complicated by minor hemorrhage (53). Authors such as Park *et al*. have hypothesized that the placement of fiducial markers using EUS reduces the risk of bleeding secondary to damage or puncture of vascular structures due to the ability to use real time Doppler imaging (54). In his own prospective case series of 57 patients who underwent fiducial marker implantation for locally advanced pancreatic adenocarcinoma, one case was reported to be complicated by "*minor bleeding…with no significant decrease in hemoglobin*," ultimately limiting the number of fiducials able to be placed (54). An additional risk with the percutaneous approach, though uncommon, is tumor seeding of the peritoneum. Tumor seeding has been estimated to occur between 0.005% and 0.009% during percutaneous FNA under CT or US guidance (52). Multiple authors have stated that this risk, similarly, seems to be lower with EUS compared to a percutaneous approach, as the puncture path is considerably shorter (52,54,55). To date, only three cases of tumor seeding as a result of EUS-FNA have been reported (56-58).

Fiducial markers can also be implanted during surgery. This method typically involves tying sutures into the periphery of the tumor, then tying fiducials into the sutures (59). Despite clearly being a more invasive technique compared to EUS, there have been advantages reported with surgical implantation, namely, the ability to achieve ideal fiducial geometry (IFG) when multiple fiducials are implanted. Parameters of IFG have been specified by systems such as the Cyberknife System (Accuray, Sunnyvale, California, USA) to ensure fiducial tracking during IGRT. For example, the Cyberknife System recommends that at least three fiducials are placed with a minimum interfiducial distance of greater than 2 cm and minimum interfiducial angle of 15 degrees, with noncollinear placement in the imaging plane (60). According to a study by Majumder *et al*., IFG for this system was achieved at a higher rate with surgical placement compared to EUS, with rates of 47.4% during surgery and 17.9% by EUS (P<0.005) (59). Interestingly, however, fiducial tracking and subsequent successful delivery of IGRT was achieved in 90% of cases placed by EUS, compared to 82% of cases placed by surgery (95% CI, 67–92%) (59). Based on the results of this study, it would seem that IFG is not a necessity for successful tracking and delivery of IGRT. Furthermore, in contrast to the Cyberknife System, other systems currently exist which only require one fiducial to be placed, making the importance of IFG even less relevant.

Fiducial markers

Fiducial markers come in a variety of lengths and diameters, though in terms of design, fiducials are typically either traditional or coiled. Unlike traditional fiducial markers, coiled fiducials are flexible, which theoretically helps to decrease the rate of fiducial migration once implanted. In a study by Khashab *et al*., a total of 103 fiducials were placed in 39 patients, 77 of which were traditional and 26 coiled. The

results of the study revealed that there was no significant difference in the rate of fiducial migration between the two groups. Additionally, there was no significant difference in the number of fiducials able to be placed, indicating similar degrees of technical difficulty between the two groups. Notably, however, visibility was significantly better for traditional fiducials compared to the coiled fiducials used in the study (61).

Technique of EUS-guided fiducial implantation

Several techniques have been published describing ways to implant fiducial markers by EUS, and currently, no singular technique exists as the standard method. All the techniques described have utilized linear-array EUS, however, variations exist in the gauge of needle used, how fiducials are loaded within the needle, and how the fiducial is ultimately advanced and deployed. Many of the early techniques include the use of a 19-gauge needle, in order to accommodate the commercially available fiducials at the time (62). More recently, however, fiducial markers have been designed that are compatible with 22-gauge needles. Authors such as Ammar *et al*. advocate for the use of 22-gauge as opposed to 19-gauge needles and state that smaller gauge needles allow for greater flexibility, and therefore, easier passage of the needle through the endoscope even in the setting of acute endoscope angulations (63). Additionally, it has been suggested that smaller caliber needles carry less risk of mechanical complications, such as puncturing the channel of the endoscope (64). In a study of 13 patients referred for EUS-guided placement of fiducial markers, Ammar *et al*. found that fiducial markers were successfully implanted in all 13 patients using a 22-gauge needle, 9 by transgastric approach and 4 by transduodenal approach (63).

The two main approaches to loading fiducials into the lumen of a needle are the back-loading technique and the push-stylet technique. In the back-loading technique, as described by Owens *et al*., the stylet is drawn back from the tip of a 19-gauge needle in order to leave adequate space for a fiducial to be loaded directly into the hollow needle tip. Once loaded, bone wax is then pressed onto the needle tip to seal the fiducial inside (65). The needle with the back-loaded fiducial is then passed through the working channel of the endoscope. When ready for deployment, the fiducial is advanced by pushing the stylet to the end of the needle.

In the push-stylet technique, the needle is first inserted into the target lesion. The stylet is then fully retracted, and the fiducial is loaded into the hollow needle through the handle, and advanced forward by reinserting the stylet and pushing the fiducial forward. In the description of the procedure by Ammar *et al*., the stylet is advanced until approximately 10 mm of the stylet remains exposed, so as to avoid pushing the fiducial forward into the lesion, and potentially coiling the fiducial. Instead, the fiducial is deployed by withdrawing the needle the remaining 10 mm, while keeping the stylet in place, leaving the fiducial within the target lesion (63).

Advantages and disadvantages have been described with both techniques. One notable disadvantage reported with the push-stylet technique is the inability to advance the fiducial with the stylet due to resistance or kinking, especially when the tip of the endoscope is angulated (63,65). This complication, however, has primarily been reported to occur when using a 19-gauge needle, and both Ammar *et al*. and Ghassemi *et al*. have reported success using the push-stylet technique with a 22-gauge needle, without meeting resistance due to kinking (63,66). Ammar *et al*. goes on to point out that, compared to the back-loading technique, other potential advantages of the push-stylet technique include less risk of injury related to manually back-loading a fiducial into the tip of a needle, decreased risk of fiducial loss while advancing the needle down the accessory channel of the endoscope and accessing the target lesion, and the ability to implant multiple fiducials without completely removing the needle in systems that require more than one fiducial to be implanted (63,67).

Another disadvantage pertaining more to the push-stylet technique is that once the fiducial has been loaded, air is often introduced into the tumor during deployment as the stylet is advanced, thereby obscuring EUS visualization (65). While the back-loading technique does overcome this disadvantage to a degree, a hydrostatic deployment technique, used in conjunction with back-loading, has been described. In the hydrostatic technique, described by Park *et al*., the stylet is completely removed and the needle channel is then flushed with sterile water. Multiple fiducials can then be back-loaded into the tip of the needle and sealed with bone wax. The needle is then inserted into the tumor under EUS guidance, and 1–2 mL of sterile water is then injected through the needle channel to deploy the fiducials. This technique, according to Park *et al*., decreased the amount of air artifact and also overcame difficulties related to angulations of the endoscope encountered during push-stylet technique (54).

Challenges and complications

Multiple studies have shown EUS to be a safe and effective technique capable of implanting fiducial markers for SBRT in the treatment of pancreatic cancer (52,54,62,65-67). Reasons for failed implantation of fiducials seem to stem primarily from mechanical and technical factors, such as difficulty inserting fiducial markers through an acutely angled endoscope tip, early deployment of fiducials before the use of sterile bone wax, and needle malfunction (54,62). Other reasons for failure involve characteristics of the pancreatic tumor itself, such as a very hard or fibrotic pancreatic head tumor preventing deployment, or inability to position the endoscope in alignment with the tumor due to gastric outlet obstruction or difficult tumor location (62).

Generally, the safety profile of fiducial placement by EUS is similar to that of diagnostic and interventional EUS (62). There have not been many reports of significant early post-procedure complications thus far other than minor bleeding during the procedure, with no significant decrease in hemoglobin, some complaints of post-procedural abdominal pain or nausea, and mild pancreatitis (54,59). Pishvaian *et al.* reported one case of cholangitis in a patient 25 days post-procedure, though stated that it was unclear if infection was related to the procedure (62). In all subsequent patients, Pishvaian *et al.* used prophylactic antibiotics at the time of procedure and for 3 days afterward (62). The practice of prophylactic antibiotics has been adopted and applied by others as well (54,63,67).

Migration of fiducials after deployment by as much as several millimeters has been reported to occur, occasionally resulting in inability to proceed with SBRT (54,59). Migration is thought to occur secondary to resolution of procedurally-related inflammation, or due to movement of fiducial markers within the tumor. Notably, however, there has not been shown to be a significant difference in the rate of fiducial migration when placed by EUS versus surgery. Additionally, as previously discussed, Ammar *et al.* demonstrated a higher rate of successful fiducial tracking and delivery of IGRT with EUS compared to surgery despite fiducial migration (54,59).

Conclusions

The role of EUS in pancreatic cancer is rapidly evolving and its current and potential applications are limitless. As its role continues to expand, it will hopefully maintain an important role in revolutionizing the diagnosis and treatment in patients with pancreatic cancer.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Yoo J, Kistler CA, Yan L, Dargan A, Siddiqui AA. Endoscopic ultrasound in pancreatic cancer: innovative applications beyond the basics. J Gastrointest Oncol 2016;7(6):1019-1029. doi: 10.21037/jgo.2016.08.07

