

UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE DIN CRAIOVA
ȘCOALA DOCTORALĂ



The emerging role of endoscopic ultrasound-guided
interventions in the management of patients with
pancreatic cancer

SUMMARY OF THE PhD THESIS

Phd Coordinator

PROF. UNIV. DR. ADRIAN SĂFTOIU

PhD Student:

IRINA MIHAELA CAZACU

CRAIOVA 2019

Table of Contents

INTRODUCTION	3
CURRENT STATE OF KNOWLEDGE	3
PERSONAL CONTRIBUTIONS.....	4
Study 1. Quality of life impact of endoscopic ultrasound in patients at risk for developing pancreatic cancer	4
Study 2. Biomarkers Performed on EUS-guided FNA Samples for Improving the Diagnosis of Pancreatic Ductal Adenocarcinoma: A Systematic Review and Meta-Analysis	5
Study 3. Diagnostic value of digital droplet PCR and digital multiplexed detection of single nucleotide variants in pancreatic cytology specimens collected by endoscopic ultrasound fine-needle aspiration	7
Study 4. EUS-guided fiducial placement for gastrointestinal malignancies: a systematic review and meta-analysis.	9
Study 5. Endoscopic Ultrasound-Guided Fiducial Placement for Stereotactic Body Radiation Therapy in Patients with Pancreatic Adenocarcinoma	10
GENERAL CONCLUSIONS AND NOVELTY	12
SELECTIVE REFERENCES	13

Keywords: *pancreatic ductal adenocarcinoma, endoscopic ultrasound, EUS, fine needle aspiration, screening, quality of life, molecular analysis, biomarkers, stereotactic radiotherapy, fiducial markers*

INTRODUCTION

The diagnosis of pancreatic ductal adenocarcinoma (PDAC) bears a disheartening prognosis with a 5-year survival rate less than 10%, and there is a significant need for better early detection and treatment options to improve the survival and quality of life of our patients. The mere severity of this disease can be exemplified by the fact that the incidence rate and mortality rate have been increasing and PDAC is slated to become the second leading cause of cancer-related death by 2030[1]. This is because the progress in diagnosing, treating and affecting the mortality of other cancers has far outpaced PDAC. Pancreatic cancer incidence and mortality rates have increased significantly over the last 50 years, but this increase could be largely attributed to improvement in the diagnosis of the disease, and to less extent to changes in coding practice. Its accurate diagnosis relies on modern imaging such as endoscopic ultrasound (EUS) or multi-detector CT angiography performed using a dual-phase pancreatic protocol. Without these diagnostic tools, many pancreatic cancer cases in the past or even at present in low-income countries, might have been improperly diagnosed and registered.

This PhD thesis aims to investigate the diagnostic and therapeutic role of endoscopic ultrasound-guided interventions in the management of patients with pancreatic cancer. Since the first practical applications of EUS were developed 30 years ago, the technique has evolved in concurrence with the general trend in endoscopy from being merely diagnostic tools to full-fledged therapeutic procedures.

This thesis focuses on three aspects: the role of EUS in pancreatic cancer screening, with emphasis on the psychological impact of EUS in individuals at high risk for developing pancreatic cancer; the role of molecular analysis of the EUS-FNA sample to aid the diagnosis of pancreatic cancer; and the feasibility and safety of EUS-guided fiducial markers placement for image-guided radiotherapy in pancreatic cancer patients.

CURRENT STATE OF KNOWLEDGE

The first section of the PhD thesis, entitled *Current state of knowledge*, offers an updated overview of the three main aspects of the current research: screening, diagnosis and treatment of PDAC. Specifically, the first chapter presents new data regarding pancreatic cancer epidemiology, with emphasis on the poor prognosis and the urgent need to improve early detection and treatment strategies. In the second chapter, new data about risk factors and genetic

predisposition of pancreatic cancer is reviewed. The third chapter has the purpose of introducing the reader in the sector of EUS, highlighting the tremendous progress of this procedure in the last 30 years. The fourth chapter describes current screening strategies for pancreatic cancer and preliminary results. The fifth chapter presents recent data on EUS-FNA, with focus on technical details of the procedure and current challenges in pancreatic cancer diagnosis. The last chapter highlights the evolution of EUS from a diagnostic to a therapeutic tool. Novel EUS based techniques that have emerged as a safe minimally invasive alternative to the surgical or radiological approaches are presented. By allowing better pain control, delivering antitumor therapies and fiducial markers for radiotherapy guidance or draining obstructed bile ducts, such techniques hold a big promise to improve the quality of life of patients with pancreatic cancer

PERSONAL CONTRIBUTIONS

Study 1. Quality of life impact of endoscopic ultrasound in patients at risk for developing pancreatic cancer

The poor prognosis of PDAC is attributed to the aggressive biology, ineffective therapies, and advanced stages at the time of diagnosis. Thus, detection of precursor lesions or early-stage PC may be an effective approach to improve survival. There is an increasing global interest in screening programs aimed to detect precursor lesions or pancreatic cancer in an early and potentially curable stage. A recent study showed improved short-term outcomes including increased resectability and improved survival rates for patients with screening-detected pancreatic cancer [2]. Although the impact of EUS screening on survival is not yet fully known, participating in a screening program may yield positive outcomes including reductions in cancer fear or increase in feelings of reassurance, well-being as well as improved quality of life (QOL) [3].

The current study aimed to clarify the psychological impact of EUS in patients with cystic lesions and individuals at risk for developing pancreatic cancer. We hypothesized that a benign EUS exam in these patients may result in less distress and improved QOL.

Participants were administered the Brief Profile of Mood States (POMS) and the single-item Linear Analog Scale Assessment (LASA) Quality-of-Life (QOL). The questionnaires were chosen based on their known psychometric properties and clinical usefulness in evaluating distress and overall QOL. Participants were contacted by phone and invited to take part in the study. After giving consent, they were asked to complete the above mentioned measures regarding their pre and post EUS status for distress and QOL.

Forty patients were included in the study: 17 patients underwent EUS for evaluation of a known PCL and 23 patients were at high risk for developing PC based on their familial and/or genetic background and they underwent EUS as part of a PC screening program. There was a significant difference in patients' overall QOL assessed by the LASA QOL scale before and after the EUS procedure (mean difference 0.73, SD 1.76, $p < 0.01$). Similarly, a significant difference in the Brief POMS score was found before and after the EUS procedure (mean difference -5.46, SD -6.72, $p < 0.01$).

However, statistically significant results may not be clinically significant. In order to assess clinically meaningful changes in QOL scores, we have used the effect size (ES) method that expresses the magnitude of effect in terms of the distributional standard deviation (SD). It has been shown that a change of 0.5 of the SD of any health-related QOL tool can be considered clinically significant. The ES was 0.50 for the single item QOL scale and 0.70 for Brief-POMS ($p = 0.04$) in the group of patients who underwent EUS as part of a PC screening program. Thus, even though the impact of EUS screening on survival is not known yet, there is a psychological benefit that clinicians should be aware of when considering EUS for these patients.

This study found clinically significant changes in the QOL scores before and after the EUS-FNA for patients with pancreatic cystic lesions as well, indicating that negative EUS-FNA results have led to an improved QOL and less distress for these patients. This is likely due to the widespread belief in the general and lay community that biopsy is the gold standard to rule out or diagnose malignancy when a lesion is detected.

Study 2. Biomarkers Performed on EUS-guided FNA Samples for Improving the Diagnosis of Pancreatic Ductal Adenocarcinoma: A Systematic Review and Meta-Analysis

Despite tremendous advance in imaging techniques, the diagnosis of PDAC is still a clinical challenge. In recent years, EUS-FNA has become the most promising method of diagnosing pancreatic tumors. However, the diagnosis accuracy of EUS-FNA varies widely and the procedure has several limitations. In such a scenario, performing biomarkers on the limited EUS-FNA samples to aid the diagnosis of PDAC would be useful, with immediate clinical impact. Several genetic markers have been investigated to support diagnosis of PDAC in cytological specimens, but none has entered routine clinical practice. Numerous studies have been carried out to determine the diagnostic utility of these biomarkers, but the results are heterogeneous and conflicting.

The aim of this study was to conduct a systematic review of the literature evaluating several biomarkers as diagnostic markers for PDAC performed on EUS-FNA samples and to conduct a subsequent meta-analysis to quantify their diagnostic accuracy and identify superior candidate biomarkers. The study protocol was developed and reported following the principles of the PRISMA-P (Preferred Reporting Items for Systematic Review and Meta-analysis Protocol) statements[4]. Based on the available evidence, the study focused on the following molecular markers that can be performed on EUS-FNA samples: *KRAS*, *p53*, *p16*, and *SMAD4*. The pooled sensitivity and specificity, positive and negative likelihood ratios (PLR, NLR) and the diagnostic odds ratio (DOR) of each biomarker, along with the respective 95% confidence intervals (CIs) were calculated, using a bivariate meta-analysis model.

Regarding the role of *KRAS* analysis in PDAC diagnosis, a total of 18 articles, comprising 1753 patients (ranging from 21 to 394 patients per study) were enrolled in the meta-analysis. The pooled sensitivity and specificity were 76% (95% CI 69–82%) and 98% (95% CI, 94–99%), respectively. When *KRAS* analysis was combined with the cytological examination, the diagnostic accuracy was significantly improved with a pooled sensitivity of 89% (95% CI, 83-93%) and specificity 96% (95% CI, 92-98).

Regarding the role of *p53* analysis in PDAC diagnosis, 10 articles fulfilled the inclusion criteria and 598 cases were included in the analysis, ranging from 15 to 101 patients per study. The pooled sensitivity and specificity of *p53* for PDAC detection was 46% (95% CI: 33-59%) and 95% (95% CI, 90-97%), respectively. The diagnostic value of cytology examination in combination with *p53* analysis in detecting PDAC was reported in 8 studies, with a pooled sensitivity of 78% (95% CI, 65-87%) and specificity 94% (95% CI, 82-98).

According to our systematic review, *CDKN2A* and *SMAD4* genes were also evaluated as tumor markers for PDAC on EUS-FNA samples. *CDKN2A* mutations in EUS-FNA specimens revealed sensitivity and specificity of 13% and 100%, respectively, for PDAC diagnosis [5]. The allelic loss of the *SMAD4* gene analyzed in EUS-FNA specimens by LOH at 18q revealed a sensitivity and specificity of 78% and 57% respectively [6].

This meta-analysis assessed the diagnostic performance of different biomarkers for PDAC in cytological specimens. None of the markers was better than cytologic diagnosis and therefore, if there is enough cytomorphological evidence for malignancy, these biomarkers should not be used. However, a molecular panel may clarify the diagnosis in patients with inconclusive EUS-FNA results, and its use may lead to earlier and less invasive diagnosis.

Study 3. Diagnostic value of digital droplet PCR and digital multiplexed detection of single nucleotide variants in pancreatic cytology specimens collected by endoscopic ultrasound fine-needle aspiration

Endoscopic ultrasound (EUS)-guided sampling is recommended as the first line procedure when a histopathological diagnosis is required [7]. Molecular analysis of the limited EUS-FNA samples to aid the diagnosis of PDAC may be extremely useful. The rapidly expanding knowledge of the molecular alterations in pancreatic neoplasms is providing new targets for disease characterization and early diagnosis.

In the context of PDAC, where >90% of cases harbor a “hotspot” oncogenic mutation in *KRAS*, ddPCR can serve as an ideal first pass screening test to document the presence of mutant cell clones. At the same time, ddPCR is restricted to one or a few “hotspots” (for example, the multiplex assay we used only detected *KRAS* codon 12/13 mutations but not codon 61). Thus, there may be a value in expanding the panel of mutations that can be detected on cytology specimens, from both a diagnostic standpoint, as well as from the context of identifying potentially actionable mutations. Accordingly, to be able to detect multiple single-nucleotide variants (SNVs) simultaneously would be of pivotal importance for the molecular diagnostics of PDAC. NanoString’s optical barcoding technology now enables detection of small genomic sequence changes such as SNVs and insertion-deletions (InDels).

The current study aimed to evaluate, and for the first time, to conduct a head to head comparison, of the respective diagnostic performances of the widely used ddPCR multiplex assay versus a novel multi-gene SNV assay by NanoString technology, performed on pancreatic specimens obtained through EUS-FNA.

Thirty-one patients underwent EUS-FNA for the evaluation of a solid pancreatic lesion. After cytological examination, 14 (45%) FNA samples were classified as positive for malignancy and the final diagnosis was PDAC for all cases. Six samples (19%) were classified as negative for malignancy and 11 (35%) were considered inconclusive: 7 (22%) suspicious and 4 (13%) atypical results. The sensitivity of the cytological examination was 63.6% (95% CI: 40.8-81.9%) and the specificity was 100% (95% CI: 62.6-100%). The overall accuracy, positive predictive value (PPV), and negative predictive value (NPV) were 74.2 % (95% CI:55.4-88.1%), 100% (95%CI: 73-100 %), and 52.9% (95 % CI: 28.5-76.1 %), respectively.

***KRAS* Mutation Analysis using ddPCR**

KRAS mutations were found in 79% (11/14) of samples diagnosed as positive for malignancy, in 71.4% (5/7) of the cases suspicious for malignancy, in 25% (1/4) of atypical results, and in 16% (1/6) of cases considered negative for malignancy.

According to the final diagnosis, a KRAS mutation was detected in 77% (17/22) of PDAC cases and in 14% (1/7) cases of CP, while no mutation was observed in the pNET cases.

The diagnostic value of KRAS mutation in combination with cytological examination of the EUS-FNA samples was further analyzed. As long as the cytopathologic results were definite and sufficient for evaluation, we did not consider the results of KRAS mutation analysis to determine the final diagnosis. Using this approach, the diagnostic parameters were as follows: sensitivity 90.48% (95% CI: 69.6-98.8%), specificity 87.5% (95% CI: 47.3-99.6%), PPV 95% (95% CI: 75.1-99.1%), NPV 77.7% (95% CI: 47.7-93%), overall accuracy 89.6% (95% CI: 72.6-97.8%).

Digital Multiplexed Detection of Single Nucleotide Variants (SNV) by Nanostring Technology

Mutational status was evaluated using the NanoString Vantage 3D™ DNA SNV Solid Tumor Panel, in a cohort of 28 EUS-FNA samples including 19 PDAC, 5 CP and 4 normal pancreas specimens. Only samples with good-quality DNA were selected for analysis. Among the 19 PDAC samples, KRAS variants were detected in 17 (90%) cases; of these, 7 (41%) had G12V, 6 (35%) had G12D, 2 (12%) had G12R, 1 (6%) had Q61H and 1 (6%) had Q61L mutations. One PDAC case harbored three SNVs (EGFR, E746_A750delELREA; KRAS, G12D; PIK3CA). All KRAS G12/G13 mutations were also detected by ddPCR.

The SNV assay detected at least one variant in all FNA samples interpreted as suspicious or atypical and had a final diagnosis of PDAC; no variant was detected in the atypical specimens with a final diagnosis of CP. The diagnostic performance of cytology examination combined with the SNV assay results (considered only for the negative and inconclusive samples) was further calculated. The sensitivity was 94.7% (95% CI: 73.9-99.8%), specificity 88.9% (95% CI: 51.7-99.7%) and the overall accuracy was 92.8% (95% CI: 76.5- 99.1%)

Prognostic value of KRAS mutational status evaluated on EUS-guided fine-needle aspiration specimens in patients with unresectable pancreatic cancer

An analysis of OS showed a trend toward a longer OS in the wild type group (mutant KRAS, calculated median OS of 188 days vs. wild type KRAS, median OS of 304 days). All patients received first-line gemcitabine-based chemotherapy. No significant difference was noticed in response to therapy between the KRAS wild type and mutant patients.

EUS-FNA has greatly improved the preoperative diagnosis of PDAC, however, in a subset of cases, the diagnosis remains inconclusive. The current study illustrated that, integration of the analysis of genetic markers with EUS-FNA and cytologic evaluation, especially in inconclusive cases, can increase the diagnostic accuracy of pancreatic lesions, thus

preventing repeat biopsies, unnecessary resections for benign disease or delay in PDAC patients care.

Moreover, efforts are needed to obtain a more complete diagnostic profile for each patient in order to exploit all potential therapeutic opportunities in this lethal disease. Clinicians should bear in mind that tumor biopsies represent not only the standard for cancer diagnosis but also the primary method for molecular testing to guide the selection of personalized therapies. The gene panel we have used in this study includes some actionable mutations that could lead to a treatment that is not currently a standard of care chemotherapy for pancreatic cancer (BRCA1/2-platinum chemotherapy, PARP inhibitor; PIK3CA-PICK3CA inhibitor, STK11-mTOR inhibitor).

Study 4. EUS-guided fiducial placement for gastrointestinal malignancies: a systematic review and meta-analysis.

Technical advances in radiotherapy and the development of stereotactic body radiotherapy (SBRT) allow the accurate delivery of radiation to a target lesion. The safe delivery of higher doses of radiation for gastrointestinal tumors requires accurate assessment of tumor size and location during respiration. Image-guided radiotherapy (IGRT) uses advanced imaging technology to verify the target lesion setting immediately before, and sometimes during radiotherapy to decrease the target size and thus toxicity to surrounding tissues. IGRT uses fiducial markers to target and track the tumor in real time to assure that radiation is delivered to the target lesion with high accuracy. Fiducials are radiopaque markers (spheres, coils or seeds), usually made of gold, that are inserted into the lesion to facilitate accurate and reproducible targeting of the tumor. These markers serve as reference points for planning with computed tomography (CT), sometimes with 4D assessment, allowing simultaneous correction of lesion motion

Although increasing evidence supports EUS-guided fiducial placement in gastrointestinal malignancies, the safety and feasibility of this approach have not been studied systematically in large, prospective, randomized trials. The aim of this study was to perform a systematic review and meta-analysis of studies evaluating the safety, efficacy and technical aspects of EUS fiducial placement for IGRT in GI malignancies. The present study was conducted following the principles of the MOOSE (Meta-analyses Of Observational Studies in Epidemiology) statements[8]. A random effects model was used to determine pooled proportions of technical success, migration and complication rates.

The cohort studies enrolled in the analysis were conducted from 2009 to 2017 and included 1155 patients with confirmed gastrointestinal malignancies who underwent EUS for fiducial placement. Fourteen studies comprising 1035 patients reported on the technical success of EUS-guided fiducial placement. The overall, pooled rate of technical success was 98% (95% CI 96-99%). Five studies comprising 680 patients reported on fiducial migration. The meta-analysis yielded a pooled rate for fiducial migration of 3% (95% CI 1.0-8.0%). Fourteen studies addressed the immediate and delayed adverse events related to fiducial placement. Reported adverse events included mild acute pancreatitis, mediastinitis, pneumothorax, minor bleeding, fever, vomiting, abdominal pain, rectal pain, arterial hypotension and elevated liver enzymes. The overall rate of post procedure adverse events was 4% (95% CI 3-7%)

This is the first meta-analysis evaluating the technical success and safety of EUS fiducial placement for IGRT in gastrointestinal malignancies [9]. According to our results, the procedure is technically feasible with an overall success rate of 98% suggesting that EUS-guided fiducial placement in various gastrointestinal malignancies can be performed routinely with high success rates. Although spontaneous migration can occur, the overall rate of fiducial migration according to our results was low and no migration-related complications were reported. Our meta-analysis yielded an adverse events rate of 4%, indicating that EUS-guided fiducial placement is a safe procedure. No major adverse events such as life-threatening bleeding or death were reported in any of the included studies, but care must be employed when performing EUS-guided fiducial placement to avoid intervening vessels and to ensure placement into the proper target tissue.

This comprehensive meta-analysis showed that EUS-guided insertion of gold fiducials for IGRT is safe and feasible with high technical success rates. It should be included into any controlled, randomized trials that evaluate the effectiveness of radiotherapy with or without chemotherapy for treatment of GI malignancies.

Study 5. Endoscopic Ultrasound-Guided Fiducial Placement for Stereotactic Body Radiation Therapy in Patients with Pancreatic Adenocarcinoma

Stereotactic body radiotherapy (SBRT) has emerged as a novel therapeutic option in pancreatic cancer care. It enables the delivery of a high-radiation dose to a limited target volume and it can be concluded in a few days in contrast to 4 or more weeks required to complete conventional RT, thus preventing a delay in the course of chemotherapy. Various studies have shown encouraging outcomes for SBRT with promising rates of local control[10]. In the

absence of diagnostic quality CT on rails or on-board MRI, accurate delivery of SBRT relies on fiducial markers.

The aim of this study was to report the technical feasibility and safety of EUS-guided fiducial placement and to evaluate the technical benefit and SBRT outcomes in a cohort of patients with pancreatic cancer.

The study group consisted of patients with borderline resectable and locally advanced (unresectable) pancreatic cancer who were referred for EUS-guided fiducial placement for SBRT at MD Anderson Cancer Center between 2017 and 2019. A multidisciplinary team consisting of surgical, medical, and radiation oncologists as well as experienced endosonographers determined the candidacy for SBRT and feasibility of EUS-guided fiducial placement based on review of abdominal cross-sectional imaging with pancreas protocol CT.

During the study period, 67 pancreatic cancer patients underwent placement of 174 fiducial markers under EUS guidance. Technical success rate of fiducial placement was 97%. Technical difficulty due to intervening blood vessels was noted in 2 (3%) patients. EUS evaluation showed duodenal invasion in one case and fiducial placement was canceled. All patients received periprocedural antibiotics and no immediate or delayed adverse events were reported. The average time to planning CT after marker placement for 4.6 days (range, 1-53). 64 (96%) patients received SBRT after fiducial placement. Of the total 174 fiducials placed, 165 (95%) fiducial markers were clearly visible during both CT simulation and on the cone beam CT scan from the last day of SBRT delivery. 9 (5%) fiducial were not useful for SBRT delivery most likely due to migration or poor visibility. No major SBRT-related toxicity was reported. The most common reported AE (grade 1 or 2 according to CTCAE 4.4) were nausea, abdominal pain, fatigue, diarrhea and constipation.

The major limitation to successful placement appears to be the intervening vasculature. Compared with percutaneous and intraoperative approaches, EUS-guided placement may be less invasive and provide easier access to deep anatomic structures within the mediastinum, abdomen, pelvis, and retroperitoneum, although comparative studies are lacking. An advantage to EUS-guided fiducial placement is the ability to perform simultaneous procedures, such as FNA for tissue diagnosis, CPN for pain relief, and ERCP for palliative biliary decompression. Fiducial placement represents another application for interventional EUS and potentially expands the indications for SBRT by accessing anatomic structures that may have been otherwise inaccessible.

GENERAL CONCLUSIONS AND NOVELTY

This Phd thesis offers new perspectives regarding the emerging role of endoscopic ultrasound in pancreatic cancer care. The present studies have several elements of innovation.

Study 1 showed that, although the impact of EUS screening on survival is not yet fully known, the participants' quality of life and general distress improved after the procedure. This study was the first in the literature to assess the psychological benefit of EUS-FNA in patients with pancreatic cystic lesions. These lesions can generate patients' anxiety because of the potential risk of developing pancreatic cancer, perceived as a lethal condition in the general population

Study 2 was the first meta-analysis in the literature evaluating the diagnostic accuracy of several biomarkers tested on pancreatic EUS-FNA samples in order to identify superior candidate biomarkers. The meta-analysis has provided a better understanding of the overall performance of each biomarkers. However, no single biomarker is 100% perfect; therefore these biomarkers should be investigated in various combinations, to select an optimum panel for potential clinical application.

Study 3 was the first study in the literature comparing the diagnostic performances of the widely used ddPCR multiplex assay versus a novel multi-gene SNV assay by NanoString technology, performed on pancreatic specimens obtained through EUS-FNA. ddPCR can be easily implemented in the routine assessment of pancreatic EUS-FNA samples to quickly provide information on *KRAS* mutational status that can supplement cytological evaluation. The results of this study suggest that the detection of *KRAS* gene mutations could supplement cytopathology by improving the positive rate in indeterminate cases

PDAC is a molecularly heterogeneous disease and the use of wide gene panels can significantly improve the clinical sensitivity and specificity, minimizing the risk of false-positive results. The SNV assay by NanoString can detect single nucleotide variants from as little as 5 ng of DNA. Given the low DNA input, digital data output and rapid turn-around time, Nanostring technology may be instrumental for the preoperative molecular diagnosis of pancreatic lesions.

As our molecular understanding of PDAC continues to improve, it will become increasingly useful to obtain more information about the tumors we are treating. PDAC is a molecularly heterogeneous disease which makes therapeutic options based on a “one-size-fits

all” approach ineffective. More emphasis has to be placed on upfront molecular profiling of pancreatic cancer patients at the time of diagnosis. It is an idea whose time has come!

Study 4 was the first meta-analysis evaluating the technical success and safety of EUS fiducial placement for IGRT in gastrointestinal malignancies. This comprehensive meta-analysis showed that EUS-guided insertion of gold fiducials for IGRT is safe and feasible with high technical success rates.

Study 5 reported the technical feasibility and safety of EUS-guided fiducial placement and the technical benefit and SBRT outcomes in a cohort of patients with pancreatic cancer. The results demonstrated that EUS-guided fiducial placement is safe and effective in target volume delineation, facilitating SBRT delivery in pancreatic cancer patients.

Originality of the thesis is also given by the multidisciplinary nature of the research.

SELECTIVE REFERENCES

1. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer research*. 2014;74(11):2913-21.
2. Canto MI, Almario JA, Schulick RD, Yeo CJ, Klein A, Blackford A, et al. Risk of neoplastic progression in individuals at high risk for pancreatic cancer undergoing long-term surveillance. *Gastroenterology*. 2018;155(3):740-51. e2.
3. Cazacu IM, Chavez AAL, Saftoiu A, Bhutani MS. Psychological impact of pancreatic cancer screening by EUS or magnetic resonance imaging in high-risk individuals: A systematic review. *Endoscopic ultrasound*. 2019;8(1):17.
4. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic reviews*. 2015;4(1):1.
5. Kato K, Kamada H, Fujimori T, Aritomo Y, Ono M, Masaki T. Molecular biologic approach to the diagnosis of pancreatic carcinoma using specimens obtained by EUS-guided fine needle aspiration. *Gastroenterology research and practice*. 2012;2012.
6. Salek C, Benesova L, Zavoral M, Nosek V, Kasperova L, Ryska M, et al. Evaluation of clinical relevance of examining K-ras, p16 and p53 mutations along with allelic losses at 9p and 18q in EUS-guided fine needle aspiration samples of patients with chronic pancreatitis and pancreatic cancer. *World journal of gastroenterology: WJG*. 2007;13(27):3714.
7. Tempero MA. NCCN Guidelines Updates: Pancreatic Cancer. *Journal of the National Comprehensive Cancer Network*. 2019;17(5.5):603-5.
8. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA*. 2000;283(15):2008-12.
9. Coronel E, Cazacu IM, Sakuraba A, Luzuriaga Chavez AA, Uberoi A, Geng Y, et al. EUS-guided fiducial placement for GI malignancies: a systematic review and meta-analysis. *Gastrointestinal endoscopy*. 2018.
10. Herman JM, Chang DT, Goodman KA, Dholakia AS, Raman SP, Hacker-Prietz A, et al. Phase 2 multi-institutional trial evaluating gemcitabine and stereotactic body radiotherapy for patients with locally advanced unresectable pancreatic adenocarcinoma. *Cancer*. 2015;121(7):1128-37.