INTRODUCTION: Adenocarcinoma of the exocrine pancreas is the fourth leading cause of cancer death in the USA with a five-year survival rate of less than 5%. The poor prognosis of pancreatic ductal adenocarcinoma (PDAC) can be attributed to delayed presentation, very aggressive local invasion, and the high metastatic potential combined with the lack of an early detection assay or an effective treatment. Routine imaging (CT scan or MRI) alone can neither detect PDAC at its early stage, nor differentiate benign lesions from malignant. Image-guided fine-needle aspiration (FNA) biopsy has become a relatively safe, but very specific modality used for routine cytological evaluation during the diagnosis of pancreatic adenocarcinoma (PDAC). However, there is still no effective biomarker-based strategy that could be used for the early differential detection of pancreatic cancer. Over the past few years, expression profiles of microRNA (miRNA) have been shown to be significantly altered in various human cancers. Recently, using macro-dissected frozen pancreatic tissue specimens, we identified specific miRNAs whose expression was deregulated in pancreatic adenocarcinoma. In particular, mis-regulation of miR-196a and miR-217 allowed discrimination of normal pancreas and chronic pancreatitis from pancreatic adenocarcinoma. (1) The purpose of the study herein was to evaluate the suitability of these candidate miRNA biomarkers for molecular characterization of pancreatic FNAs.

METHODS: Thirteen patients with suspected pancreatic masses underwent endoscopic ultrasound-guided FNA biopsies, which were collected and stored in RNARetain™ Pre-analytical RNA Stabilization Solution (Asuragen, Austin, TX). Normal pancreatic tissue specimens were collected ex vivo from resected
pancreas. Total RNA was extracted using an optimized mirVana™ miRNA Isolation Kit Protocol (Ambion, an Applied Biosystems Business, Austin, TX). Expression levels of miRNAs and mRNAs in FNA samples were quantified by qRT-PCR using TaqMan® miRNA and TaqMan® Gene Expression Assays (Applied Biosystems, Foster City CA) and compared to a set of frozen pancreatic tissue samples.

RESULTS: Extraction of triple-pass pancreatic FNA specimens collected in RNARetain™ yielded an average of 4.9 µg total RNA per sample. We observed that the quantitative expression analysis of selected miRNAs and mRNAs in PDAC and normal pancreas FNA samples correlated well with the changes observed in respective frozen tissue samples. In addition, we verified that altered expression of miR-196a and miR-217 enabled clear segregation of PDAC FNA specimens from non-malignant FNA samples. Noteworthy, our analysis revealed that the expression of selected mRNAs, either individually or in combination, did not offer an advantage in segregation of benign and malignant pancreatic tissue, as compared to miRNAs.

DISCUSSION: In this study, we demonstrated that total RNA could be efficiently extracted from FNA samples preserved in RNARetain™ in a sufficient quantity to allow a thorough qRT-PCR expression analysis. We confirmed that altered expression of miR-196a and miR-217, previously identified as potential candidate biomarkers of PDAC, can also successfully distinguish benign pancreatic FNA tissue samples from those that are malignant. In addition, we determined that deregulation of these two biomarkers observed in the FNA sample suspected for malignancy, was in fact consistent with a pancreatic adenocarcinoma specimen. Our study demonstrates that analysis of expression profiles of miR-196a and miR-217 could aid evaluation of cases deemed suspicious via pathological analysis, and could become a valuable asset in the definitive diagnosis of PDAC.

REFERENCES: