

Three Different Techniques for KRAS Mutational Analysis in EUS-FNA Pancreatic Lesions: Sanger Sequencing, Next Generation Sequencing and Real-Time PCR

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Context *KRAS* is an oncogene frequently mutated in pancreatic carcinomas. 90% of the mutations are localized at the level of codons 12, 13 and 61. Detecting a *KRAS* mutation could help to distinguish pancreatic cancer from pseudotumoral chronic pancreatitis. Identifying such mutations in preoperative endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) material is an important diagnostic aid. For this reason it is necessary to have highly sensitive and specific molecular techniques. **Patients and Methods** In 60 consecutive patients, who underwent EUS-FNA for typing of focal lesions of the pancreas, FNA material was processed for routine cytological analysis and an aliquot collected in ethanol for *KRAS* analysis. The material was used for the analysis of *KRAS* through: Sanger sequencing (Beckman CEQ2000 platform), Allele specific LNA qPCR (ASLNAqPCR) e Roche 454 GS-Junior sequencer (Next Generation Sequencing; NGS). **Results** At

microscopic examination the cytological diagnosis ranged from malignant to non-neoplastic lesions. The analysis with the Sanger sequencing revealed *KRAS* mutations in 17/60 samples, using ASLNAqPCR *KRAS* mutations were detected in 25/60 patients while NGS allowed to detect mutations in 30/60 samples. With regard to cases with cytological diagnosis of primary adenocarcinoma, Sanger sequencing showed *KRAS* mutations in 6/15 (40%) cases, ASLNAqPCR in 10/15 (66.7%) and NGS in 12/15 (80%). **Conclusions** ASLNAqPCR and NGS techniques were found to be more sensitive than Sanger sequencing. The NGS allows to detect infrequent mutations and ASLNAqPCR provides data on the fraction of mutated cells in the analyzed sample. The use of one of these two techniques (or both of them) allows to characterize in a very precise way the *KRAS* molecular status of a pancreatic lesion starting from material obtained with EUS-FNA.