FAP expression in alpha cells of Langherhans insulae – implications for FAPI radiopharmaceuticals' use

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Short Report

Keywords:

Posted Date: March 2nd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2631118/v1

Version of Record: A version of this preprint was published at European Journal of Nuclear Medicine and Molecular Imaging on May 4th, 2023. See the published version at https://doi.org/10.1007/s00259-023-06246-9.

Abstract

Purpose

Radiopharmaceuticals targeting fibroblast activation protein alpha (FAP) are increasingly studied for diagnostic and therapeutic applications. We discovered FAP expression at immunohistochemistry (IHC) in the alpha cells of the Langerhans insulae of few patients. Therefore, we planned an investigation aimed at describing FAP expression in the pancreas and discussing the implications for radioligand applications.

Methods

We retrospectively included 40 patients from 2 institutions (20 pts each) according to the following inclusion/exclusion criteria: i) pathology proven pancreatic ductal adenocarcinoma and neuroendocrine tumors (NET), 10 pts per each group at each center; ii) and availability of paraffin-embedded tissue and iii) clinical-pathological records. We performed IHC analysis and applied a semiquantitative visual scoring system (0, negative staining; 1, present in less than 30%; 2, present in more than 30% of the area). FAP expression was assessed according to histology – NET(n = 20) vs ductal adenocarcinoma(n = 20) - and to previous treatments within the adenocarcinoma group. Local ethics committee approved the study (No. INT 21/16, 28 January 2016).

Results

The population consisted of 24 males, 16 females, median age 68, range (14–84) years; 8/20 adenocarcinoma patients received chemotherapy. In all the Langerhans insulae (40/40) pancreatic alpha cells were found to express FAP, with a score of 2. No difference was found among NET(20/20) and adenocarcinoma(20/20); nor according to neoadjuvant chemotherapy in the adenocarcinoma cohort (received or not received).

Conclusion

Pancreatic Langerhans islets alpha cells normally express FAP. This is not expected to influence the diagnostic accuracy of FAP-targeting tracers. In the therapeutic setting, our results suggest the need to better elucidate FAPI radioligands' effects on the Langerhans insulae function.

Introduction

The fibroblast activation protein alpha (FAPα) is a type II transmembrane proteolytic enzyme[1]. FAPα has both dipeptidyl peptidase activity and a collagenolytic activity thus it is capable of degrading gelatin and type I collagen[2]. It has been described to be underexpressed in healthy tissues while it is overexpressed

in disease states such as wound-healing granulation tissue and neoplasms[3]. FAP positivity has been found in 50–100% of cancer patients. By producing a variety of structural and regulatory molecules that contribute to local immunosuppression, extracellular matrix remodeling, and stimulation of angiogenesis, FAP-positive stromal cells generate a protumorigenic microenvironment. Indeed, a higher FAP expression is associated with higher local invasion and increased risk of nodal metastases. Additionally, in FAP positive cancer patients decreased survival has been reported[4].

In recent years, FAP-targeted diagnostics and therapy research gained a lot of interest. By exerting effects on the tumor microenvironment, FAP-targeting approaches (e.g. low molecular weight FAP inhibitors, FAP antibodies and their conjugates, FAP vaccines and FAP-targeting genetically engineered chimeric antigen receptor T cells - FAP-CAR T) have been tested to disrupt several hallmarks of cancer[4].

Aiming at nuclear medicine theragnostic approach, diagnostic (both PET and SPECT) and therapeutic radiopharmaceuticals targeting FAP have recently been investigated[5, 6]. Small-molecule and peptide-based tracers are currently the most promising FAP-targeting compounds[7]. According to ClincialTrials.gov and EU Clinical Trials registers, currently, numerous clinical trials are ongoing with the long-term goal of translating these compounds into the clinical practice.

However, physiological role of FAP has not been widely investigated. Incidentally, we found FAP expression at immunohistochemistry (IHC) in the alpha cells of the Langerhans insulae in a patient affected by neuroendocrine tumour (NET). Therefore, we planned an investigation aimed at describing FAP expression in the pancreas.

Materials And Methods

Patients

For the present retrospective multicenter study, in 2 institutions (Institution 1 – Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy and Institution 2 - IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy) we applied the following inclusion/exclusion workflow. We randomly selected i) 10 patients affected by a pathology proven pancreatic NET and 10 patients by a pathology proven pancreatic adenocarcinoma (total n = 20 per each institution), ii) for whom formalin-fixed paraffin-embedded healthy tissue and iii) clinical-pathological records were available. For all patients we collected: age, sex, tumor grading, pathological TNM staging, and treatment before surgery.

Local ethics committee approved the study; informed consent was waived due to the retrospective nature of the investigation (No. INT 21/16, 28 January 2016).

Immunohistochemistry

We performed immunohistochemical analysis of the pancreas using FAP monoclonal antibody similarly to Mona et al [8]. Briefly, sections 2.5/3 micron-thick were cut from paraffin blocks, dried, de-waxed, rehydrated, and unmasked with Dako PT-link, EnVision™ FLEX Target Retrieval Solution (High pH – 96°C

– 15min). Rabbit monoclonal anti-FAP alpha (Clone EPR20021 – Abcam - diluition 1:250) was incubated with a commercially available detection kit (EnVision™ FLEX+, Dako, Agilent) in an automated Immunostainer (Dako Autostainer Link 48 - Agilent). An experienced surgical pathologist (M.M.) confirmed the histologic diagnoses and performed the immunohistochemistry analysis using a semiquantitative visual scoring system (0, negative staining; 1, present in less than 30% of the area; 2, present in more than 30% of the area).

Statistical analysis

Frequency tables and descriptive statistics were used to summarize baseline study cohort data and to assess FAP expression according to histology (NET vs ductal adenocarcinoma) and previous treatments.

Results

Patient characteristics are summarized in Table 1.

Table 1
Patient characteristics (n = 40).

	Institution 1		Institution 2	
	NET (n = 10)	Adenocarcinoma (n = 10)	NET (n = 10)	Adenocarcinoma (n = 10)
Sex (M/F)	9/1	6/4	2/8	6/4
Age (median, range) years	64.5 (14–81)	73.5 (55–84)	60.5 (44–81)	67.5 (58–83)
Grading				
G1	5	1	4	0
G2	3	7	6	5
G3	2	2	0	3
NA				2
Stage				
T1N0	4	0	3	4*
T1N2	0	0	0	1°
T2N0	2	3	2	3§
T2N1	1	3	1	1
T2N2	0	1	0	1°
T3N0	1	0	2	0
T3N1	0	2§	2	0
T4N1	0	1	0	0
T3N0M1	2	0	0	0
Neoadiuvant chemotherapy				
Yes	0	1	0	7
FOLFIRINOX	0	1	0	2
gemcitabine + nab-paclitaxel	0	0	0	5
No	10	9	10	3

F-female; M-male; NET-neuroendocrine tumour; ° – 1 patient received neoadiuvant chemotherapy; §- 2 patients received neoadiuvant chemotherapy; *-3 patients received neoadiuvant chemotherapy.

FAP expression

In all the patients from both institutions (40/40) FAP expression was demonstrated in the human endocrine pancreas. In particular, in all the specimens (40/40) we found a strong FAP expression (score 2) in the pancreatic alpha cells (Fig. 1).

Additionally, in all specimens FAP expression was found in fibrous tissue close to pancreatic primitive tumors and in large pancreatic ducts. Interestingly, intense FAP expression was reported in hyperplastic Langerhans insulae. Specifically, in 2/20 NET patients with hyperplastic Langerhans insulae FAP expression resulted to involve the whole insulae (Fig. 2).

In one patient affected by adenocarcinoma intense FAP expression was found in the fibrotic tissue, while epithelial pancreatic cells resulted negative (Fig. 3).

FAP expression according to histotype and chemotherapy

Using a semiquantitative visual scoring system, no differences in FAP expression in pancreatic alpha cells among NET and adenocarcinoma patients were reported (Fig. 4 – panel A).

Eight out of 20 patients affected by pancreatic adenocarcinoma received neoadjuvant chemotherapy (Table 1). We did not observe differences in terms of location and intensity score between the two groups (Fig. 4 – panel B).

Discussion

Our study demonstrated that pancreatic alpha cells normally express FAP. Indeed, all the patients (40/40) resulted positive at immunohistochemistry. Additionally, no difference in FAP expression was found according to different pancreatic cancer types (NET vs adenocarcinoma) nor according to neoadjuvant chemotherapy in the adenocarcinoma cohort (received or not received).

Our results are in line with data from Busek et al. [9], who demonstrated the co-expression of FAP and dipeptidyl peptidase-IV (DPP-IV) in pancreatic alpha cells in adult humans, potentially implying modulation of the paracrine signaling in the human Langerhans islets.

The implications for FAPI-PET/CT imaging are expected to be limited since Langerhans insulae are definitely smaller (mean islet diameter of 108.92 μm (± 6.27 μm) [10] than the resolution limit of a PET scanner (2.36 mm FWHM)[11]. According to biodistribution studies, Mona et al. found average [^{68}Ga]Ga-FAPi-46 SUVmean at the pancreas to be < 2.5 [12]. Additionally, in the head-to-head comparison, Giesel et al. found that mean SUVmax in the pancreas was significantly lower for [^{68}Ga]Ga-FAPI than [^{18}F]FDG (1.82 vs. 1.99; p = 0.027)[13]. Figure 5 shows no significantly increased uptake in the pancreas as compared to background in the rectal cancer patient scanned by our group at Department of Nuclear

Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology in Wuhan, China.

Consequently, alpha cells uptake does not affect primary pancreas neoplasms detection. According to the recent paper from Hirmas et al., mean primary exocrine pancreatic cancer tumor-to-background ratio was prominent, and significantly higher for [^{68}Ga]Ga-FAPI than [^{18}F]FDG (14.7 vs. 3.0, $p < 0.001$) [14].

Indeed, FAP expression by cancer lesions confirms the opportunity to detect and stage malignant lesions, both NET and adenocarcinoma lesions. FAPI uptake has been demonstrated by initial investigations [15, 16] in NET patients. Kratochwil et al. reported NET lesions to display an average SUVmax ranging between 6 and 12 [16]. A case of a NET patient, scanned by our group at Department of Nuclear Medicine and Minnan PET Center, Xiamen University, is illustrated in Fig. 6.

Moreover, Kreppel suggested FAPI PET/CT-derived parameters for patient risk-stratification. Indeed, they found ratio between volumes of liver metastases positive on FAPI and DOTATOC scans to be significantly and strongly correlated with Ki-67 (rho = 0.808, $p < 0.01$)[17]. In pancreatic ductal carcinoma Kratochwil et al. reported that average SUV_{max} ranged between 6 and 12, while the tumor-to-background ratios were more than 3 [18]. Hirmas et al. found mean primary tumor and metastatic lesions SUV_{max} to be 13.2 and 9.4, respectively[14]. These high uptake values result in high image contrast and excellent tumor delineation. Moreover, in the study by Röhrich et al. the authors found FAPI PET/CT changed TNM staging in 10/19 patients compared to the contrast-enhanced CT [19].

The implications for FAPI-targeted treatments on glucose metabolism have not been elucidated yet. The initial reports in a variety of cancer types have not reported increased glucose levels interpreted as radioligand treatment related. Ballal et al. in a cohort of 15 radioiodine-refractory differentiated thyroid cancer found that none of the patients experienced grade 3 or 4 hematological, renal, or liver toxicity after [^{177}Lu]Lu-DOTAGA.$(SA.FAPi)_2$[20]. In the study by Fendler et al. [^{90}Y]-FAPI-46 treatment-related grade 3 or 4 adverse events were observed in 38% of patients being thrombocytopenia and anemia the most prevalent [21].

As for dosimetric assessments, in a recent study dose-limiting organ with [^{177}Lu]Lu-DOTA.SA.FAPi was found to be the kidney, followed by colon. While the highest estimated absorbed radiation dose by [^{177}Lu]Lu-DOTAGA.$(SA.FAPi)_2$ was observed in the colon, followed by gall bladder, pancreas, and kidneys[22]. The dosimetric assessments in the study be Fendler et al. focused on bone marrow, liver, lung and kidneys[21]. In view of the peculiar Langerhans insulae anatomy, future microdosimetry studies might elucidate properly the absorbed dose to the endocrine component of the pancreas and eventually establish with radionuclide holds the best properties for FAPI-targeted treatments.

We acknowledged some limitations in our study. Firstly, the retrospective design; however, multicenter data availability provided robustness to the results. Some of the included patients received different chemotherapy regiments before surgery which might have influenced the results; however,

immunohistochemistry demonstrated score 2 expression of FAP in both treatment naïve and treated groups, suggesting expression is not significantly related to treatment. We have not performed PET/CT imaging since it was out of scope of the present study.

In conclusion, pancreatic Langerhans islets alpha cells normally express FAP, in both chemotherapy treated and treatment naïve patients. In view of promising anticancer activity using FAP-targeting radioligands as reported in recent literature these radiopharmaceuticals are expected to be increasingly used and early translated to clinics. Our results suggest the need to better elucidate FAPI radioligand therapy effects on the Langerhans insulae function and establish the limit absorbed dose.

Declarations

Acknowledgments

We acknowledge the support from the AIRC (Italian Association for Cancer Research) MFAG 2020 grant n. 24952.

Funding

We acknowledge the support from the AIRC (Italian Association for Cancer Research) MFAG 2020 grant n. 24952.

Competing Interests

All the authors declare no conflict of interest.

Author Contributions

Margarita Kirienko, Giovanni Centonze, Ettore Seregni, Massimo Milione contributed to the study conception and design. Material preparation, data collection and analysis were performed by Giovanni Centonze, Giovanna Sabella, Mauro Sollai, Martina Sollini, and Luigi Terracciano. PET/CT clinical cases were curated by Xiaoli Lan and Haojun Chen. The first draft of the manuscript was written by Margarita Kirienko and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Fondazione IRCCS Istituto Nazionale dei Tumori, Milan (No. INT 21/16, 28

January 2016).

Consent to participate

In view of the retrospective study design focused on pathology slides, informed consent was waived.

Patients, who's data and images are displayed in Figure 5 and 6, gave their consent for publication.

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Figures

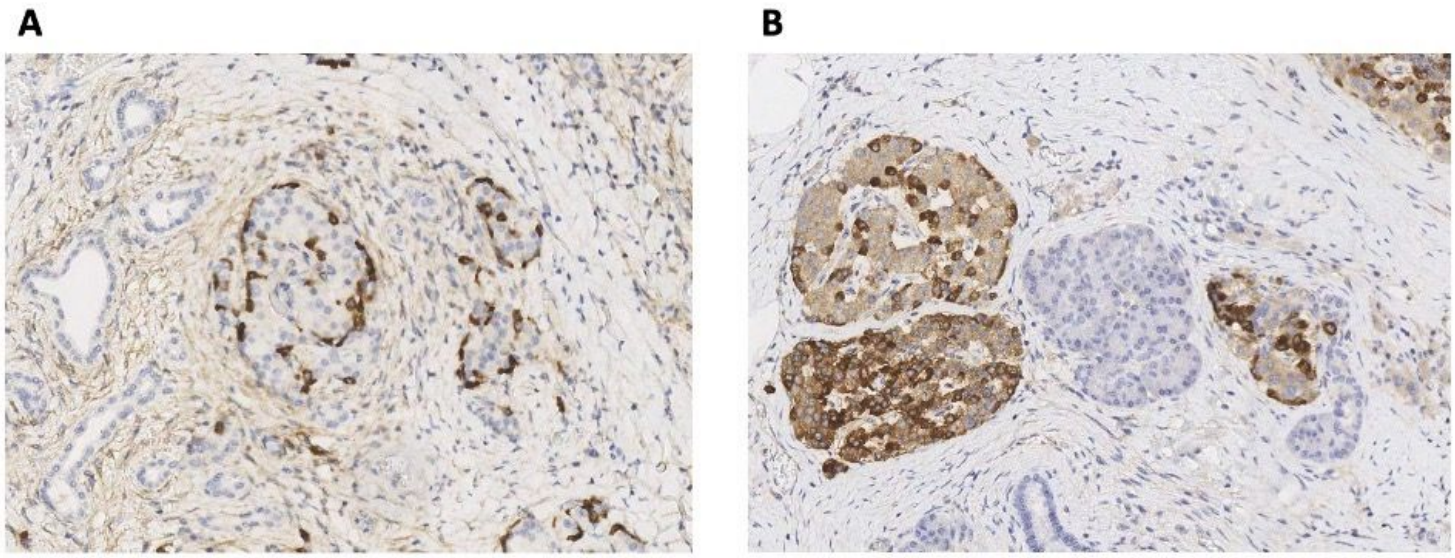

Figure 1

Panel A – FAP Immunohistochemistry sample (200x magnification) of a male patient, affected by NET G2.

Panel B – FAP Immunohistochemistry sample (200x magnification) of a male patient, affected ductal adenocarcinoma.

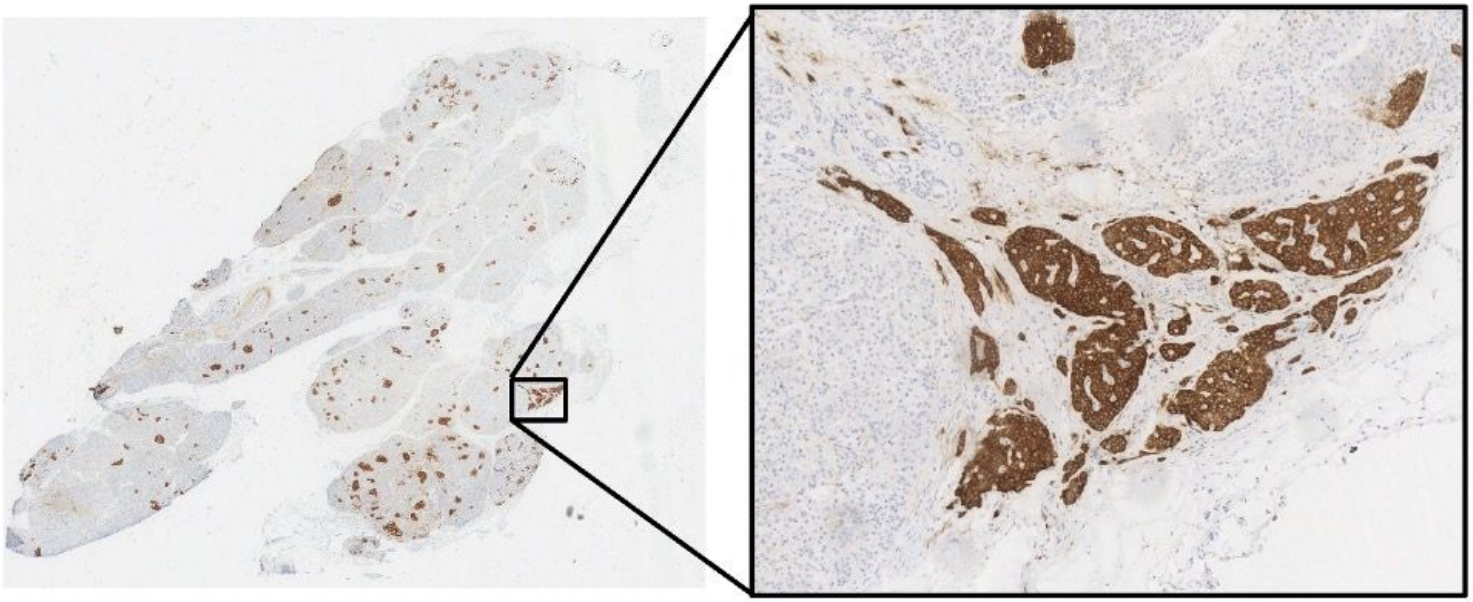

Figure 2

FAP Immunohistochemistry sample (whole slide 10x, insert at 100x magnification) of a male patient, affected by NET G1.

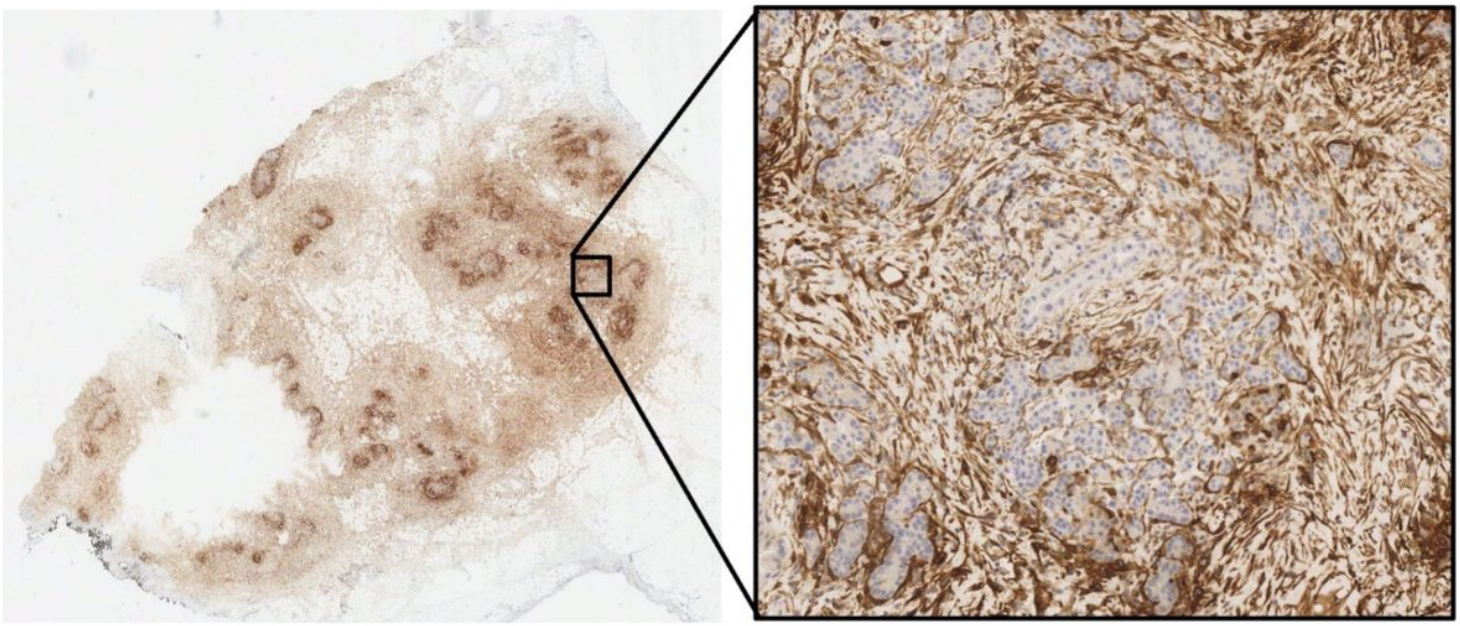

Figure 3

FAP Immunohistochemistry sample (whole slide 10x, insert at 200x magnification) of a female patient, affected by ductal adenocarcinoma.

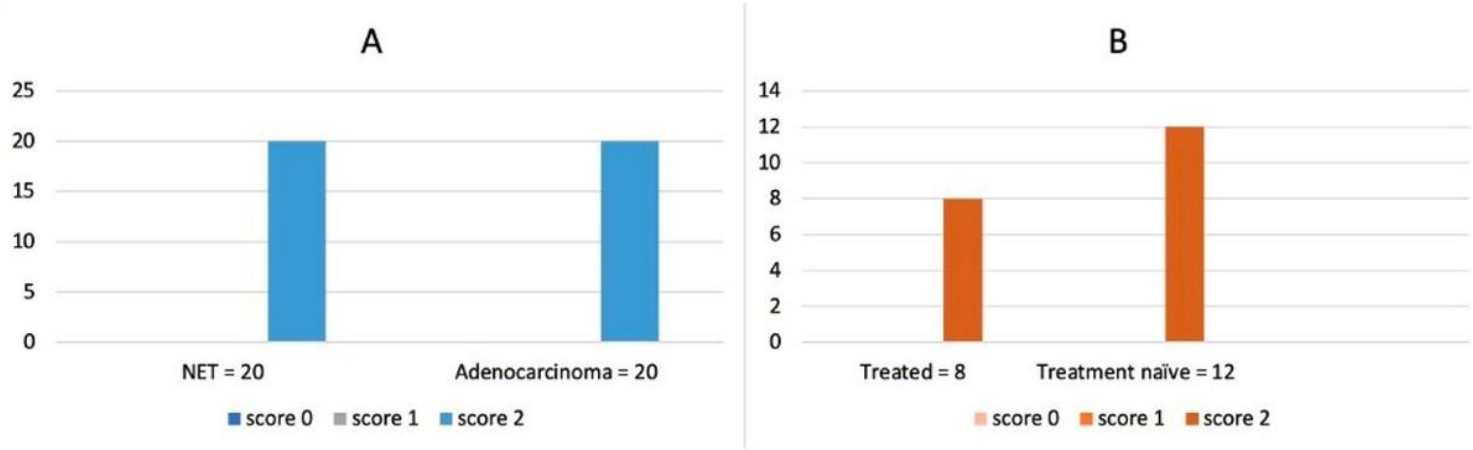

Figure 4

FAP expression score results.

Panel A – score results according to cancer histology (n=40). Panel B – score results in adenocarcinoma patients according to neoadiuvant chemotherapy (n=20).

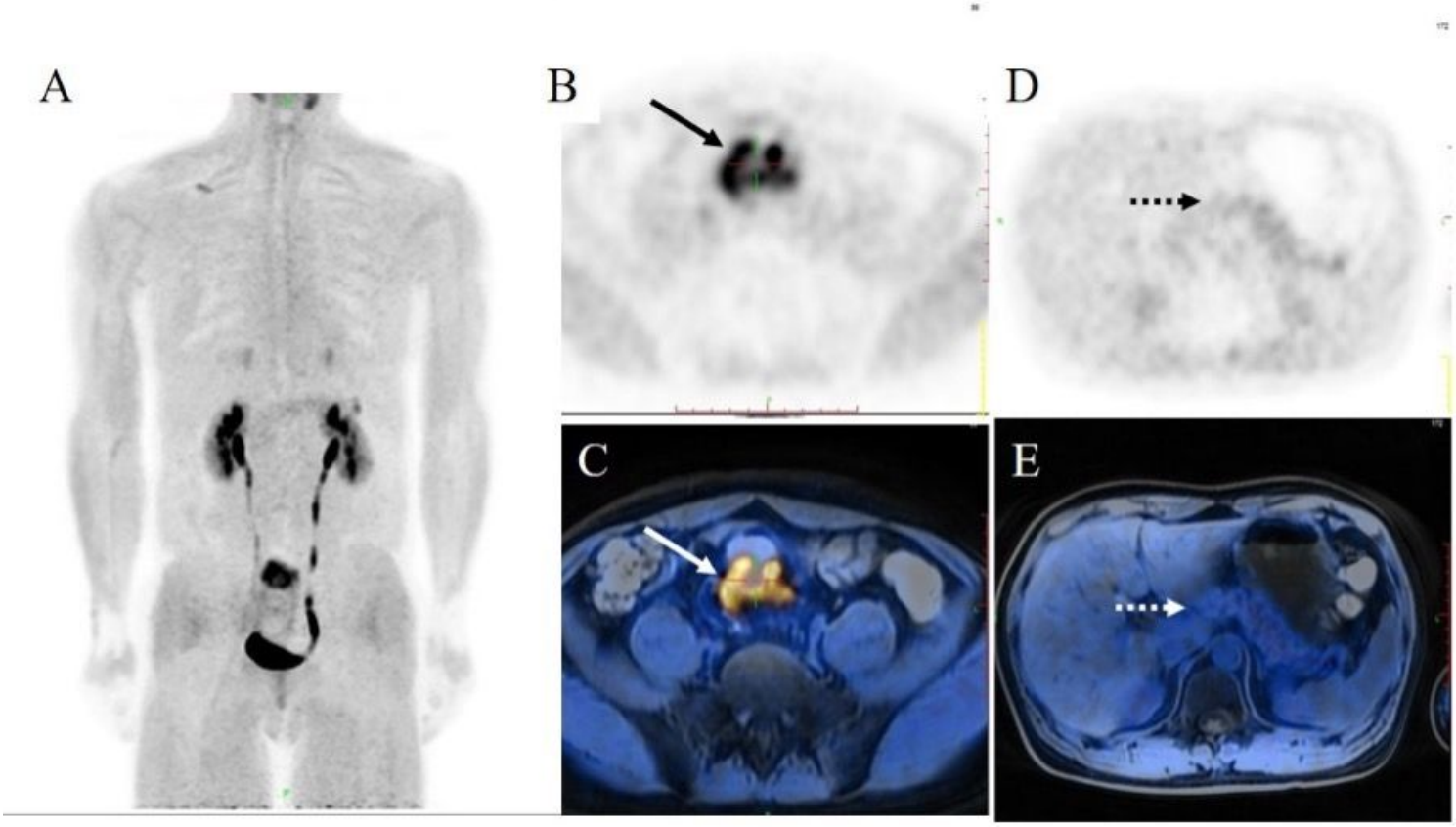

Figure 5

Male patient, 43 years-old, affected by rectum adenocarcinoma. Panel A – [^{68}Ga]Ga-FAPI-04 PET maximal intensity projection image; panels B (PET only) and C (fused PET/CT) show intense uptake (SUVmax 10.3) of rectum tumor (solid arrows point to the tumor); panels D (PET only) and E (fused PET/CT), show mild uptake of pancreas (dotted arrows).

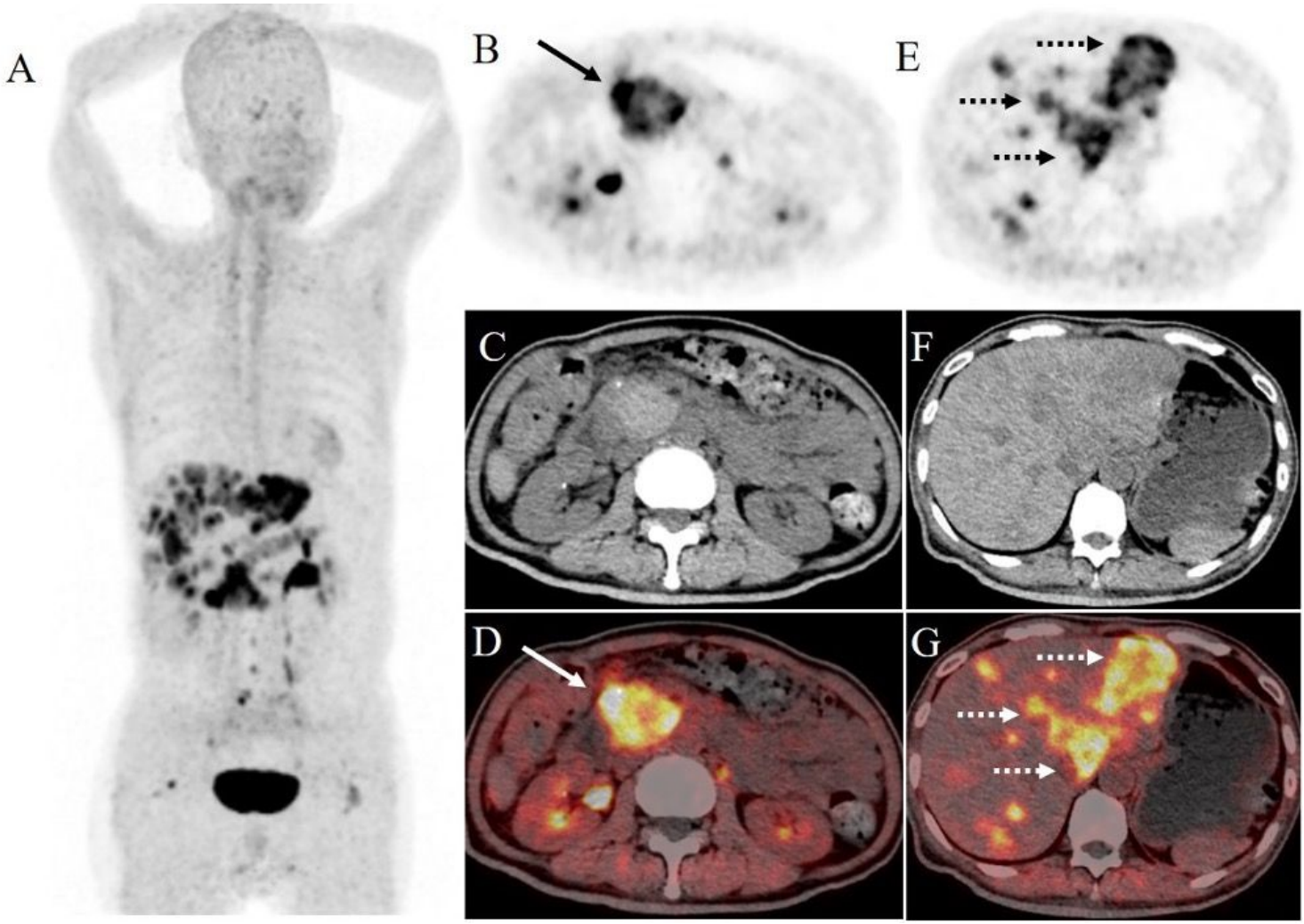

Figure 6

Male patient, 61 years-old, affected by pancreatic neuroendocrine tumor (G2) with numerous liver metastases. Panel A, [^{68}Ga]Ga-FAPI-46 PET maximal intensity projection image; B (PET only) – C (CT only) – D (fused PET/CT), the lesion shows intense uptake (SUVmax 15.2) of primary tumor on head of pancreas (solid arrows point to the tumor); E (PET only) – F (CT only) – G (fused PET/CT), multiple liver metastases (dotted arrows) with high uptake (SUVmax 10.9-19.1).