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Research Article

Keywords: Pancreatic ductal adenocarcinoma (PDAC), [18F]FDG PET, Hemorrhage, Tumor metabolism

Posted Date: September 17th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10932499/v1>

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Abstract

Background

Pancreatic ductal adenocarcinoma (PDAC) is associated with high mortality and poor prognosis. Most patients present with unresectable PDAC, underscoring the need for reliable non-invasive imaging biomarkers. [^{18}F]fluorodeoxyglucose positron emission tomography or [^{18}F]FDG PET provides metabolic information but lacks specificity for viable tumor tissue, as tracer uptake can be affected by inflammatory processes, potentially confounding tumor characterization or treatment-response assessment. This study explores the impact of hemorrhage and associated inflammatory changes on [^{18}F]FDG uptake in PDAC.

Methods

[^{18}F]FDG PET findings were correlated with MRI and histology in orthotopic PDAC models representing quasimesenchymal (PSN1) (n = 8; 4 hemorrhagic) and classical (HPAC) (n = 9; 6 hemorrhagic) subtypes in mice, and a clinical test cohort (n = 6; 4 hemorrhagic).

Results

In non-hemorrhagic murine tumors, [^{18}F]FDG PET reliably identified hypometabolic necrotic regions, allowing exclusion from analysis. In contrast, necrotic regions containing multifocal hemorrhage showed significantly higher [^{18}F]FDG uptake (HPAC SUV_{mean} 1.42 ± 0.19 vs 1.10 ± 0.10 , $P=0.036$, PSN1 SUV_{mean} 1.72 ± 0.15 vs 1.20 ± 0.14 , $P=0.002$). In patients, tumors with hemorrhage demonstrated higher SUV_{max} than non-hemorrhagic tumors (7.57 ± 1.4 vs 3.41 ± 0.2). Reference-tissue normalization preserved these differences. Murine tumors exhibiting hemorrhage also showed increased macrophage infiltration.

Conclusions

Hemorrhage and associated inflammatory changes can confound [^{18}F]FDG PET interpretation, leading to overestimation of viable tumor tissue. Incorporating complementary structural imaging with careful region-of-interest definition improves the accuracy of [^{18}F]FDG PET-based PDAC characterization and treatment response assessment.

Keywords: Pancreatic ductal adenocarcinoma (PDAC), [^{18}F]FDG PET, Hemorrhage, Tumor metabolism

List of abbreviations

PDAC: Pancreatic ductal adenocarcinoma

[¹⁸F]FDG: 2-[¹⁸F]fluoro-2-deoxy-D-glucose

PET: positron emission tomography

MRI: magnetic resonance imaging

SUV: standardized uptake value

FOLFIRINOX: folinic acid, fluorouracil, irinotecan, and oxaliplatin

ESMO: European Society for Medical Oncology

FBS: Fetal bovine serum

NEAA: non essential amino acids

CT: computed tomography

RARE: rapid acquisition with relaxation enhancement

EANM: European Association of Nuclear Medicine

H&E: hematoxylin and eosin

IBA1: ionized calcium-binding adaptor molecule 1

MPO: myeloperoxidase

EDTA: ethylenediaminetetraacetic acid

DAB: 3,3'-diaminobenzidine

pH: potential of hydrogen

OSEM: ordered-subsets expectation maximization

ROI: Region of interest

T2w: T2-weighted

T/M: Tumor-to-muscle ratio

T/B: Tumor-to-blood ratio

MTV: metabolic tumor volume

FET: O-(2-[^{18}F]fluoroethyl)-L-tyrosine

PERCIST: positron emission tomography response criteria in solid tumors

SW: susceptibility-weighted

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) stands as one of the most aggressive malignancies, and remains one of the leading causes of cancer-related mortality worldwide [1]. Although notable progress has been made in understanding the molecular and biological mechanisms underlying PDAC, these advances have translated into only limited improvements in clinical outcomes [2]. The poor prognosis of PDAC is driven by aggressive tumor biology, late clinical presentation, and limited efficacy of current therapeutic strategies [3].

A hallmark of PDAC is its prominent biological and metabolic heterogeneity, reflected in molecular subtypes such as classical and quasimesenchymal tumors, which differ in gene expression, metabolic phenotype, therapeutic response, and clinical outcome [4–6]. Notably, these subtypes also exhibit distinct metabolic features, with classical tumors showing greater dependence on oxidative metabolism, whereas quasimesenchymal tumors show a more glycolytic phenotype [5–7]. These biological differences are clinically relevant, as molecular subtypes may respond differently to the same therapeutic regimen; for example, basal-like PDAC has demonstrated greater resistance to FOLFIRINOX than classical tumors [8,9]. Therefore, reliable non-invasive imaging biomarkers that characterize tumor phenotype before and during treatment could support patient stratification, treatment selection, and monitoring. This emphasizes the importance of factors such as development of necrosis that may confound imaging biomarkers to ensure accurate tumor classification and treatment interpretation.

Metabolic imaging using [^{18}F]fluorodeoxyglucose positron emission tomography ([^{18}F]-FDG PET) provides a non-invasive assessment of tumor glucose uptake and has been investigated for tumor characterization, prognosis, treatment response monitoring, and detection of recurrences across different cancers. [10,11]. However, in PDAC, its routine clinical use remains limited.

Current European Society for Medical Oncology (ESMO) clinical practice guidelines recommend [^{18}F]FDG PET/CT only for selected patients with non-metastatic PDAC [12]. Nevertheless, [^{18}F]FDG PET has demonstrated prognostic value for assessing treatment response in selected PDAC patients [13–15].

Despite its clinical utility, [^{18}F]FDG PET is subject to biological confounding factors that may affect tracer uptake independently of viable tumor cells [16]. For instance, inflammatory processes and activated immune cells exhibit increased glucose metabolism and may result in elevated [^{18}F]-FDG uptake despite low metabolic activity in the surrounding tumor tissue, leading to false-positive [^{18}F]FDG PET findings [17]. These effects suggest that increased tracer uptake does not necessarily represent viable tumor tissue and may therefore lead to overestimation of the residual metabolically active tumor burden, potentially resulting in the misclassification of responding tumors as non-responders during PET-based treatment assessment [18].

Tumor necrosis is a form of irreversible cell death caused by inadequate blood supply, hypoxia, or anticancer treatments such as immunotherapy, chemotherapy, or radiotherapy [19,20]. A fundamental principle in oncologic PET imaging is that necrotic tumor regions exhibit little or no glucose metabolism and consequently low FDG uptake values [21]. Hypometabolic necrotic regions are commonly excluded from quantitative PET analysis and tumor segmentation [21]. However, necrotic lesions may also undergo multifocal hemorrhage, resulting in distinct imaging characteristics, such as hypointense lesions on T1-weighted images and hyperdense lesions on T2-weighted images [22]. While hemorrhage is a recognized pathological finding in PDAC [23], its influence on [^{18}F]FDG PET signal and quantitative PET assessment has not been systematically investigated.

During multimodal imaging analysis of orthotopic PDAC xenografts, we observed that not all necrotic regions exhibited the expected hypometabolic appearance on [^{18}F]FDG PET. While conventional necrosis corresponded to areas of reduced tracer uptake, necrotic regions containing intratumoral hemorrhage frequently demonstrated preserved or even increased FDG signal. We therefore hypothesized that hemorrhage may represent an unrecognized biological confounder of [^{18}F]FDG PET quantification. To test this hypothesis, we evaluated the association between hemorrhage, FDG uptake, histopathological, and immunohistochemical findings in two orthotopic PDAC models (classical and qm) and a small clinical test cohort comprising six patients.

2. Materials and Methods

2.1. Tumor Model: Orthotopic PDAC tumors were established in 8-week-old female CRL:NU(NCr)-Foxn1nu mice (Charles River Laboratories) by implantation of a single-cell suspension of 5000 human PDAC cells into the tail of the pancreas. Two PDAC cell lines representing distinguishable molecular subtypes were selected: PSN1 (quasimesenchymal, glycolytic) and HPAC (classical, lipogenic, with low glucose metabolism) [5]. The cell lines were obtained from ATCC and regularly underwent external authentication. The cell lines were cultured in RPMI Medium 1640 (1x) +GlutaMAXTM (Gibco, Thermo Fisher Scientific, Waltham, USA), adjusted to final concentrations of 5% Fetal Bovine Serum (FBS, Thermo Fisher Scientific, Waltham, USA), 1% v/v penicillin/streptomycin (P/S, Thermo Fisher Scientific, Waltham, USA) and 1% non-essential amino acids (NEAA, Thermo Fisher Scientific, Waltham, USA).

2.2 Imaging: Animals were fasted for 4 hours prior to imaging. During imaging, mice were under anesthesia with Isoflurane 1.5-2% (v/v) in an oxygen flow rate of 2-2.5 L/min. Respiratory rate and body temperature were observed and maintained at 55–65 bpm and 37–38.5 °C, respectively. [¹⁸F]FDG (9–12 MBq) was administered intravenously, followed by a 2-hour uptake period. A 15 min static PET acquisition was subsequently performed using a small-animal PET/CT scanner (Siemens Inveon Micro PET-CT, Siemens, Texas, USA). To provide anatomical reference and identify necrotic regions coronal T2-weighted MRI (T2w rapid acquisition with relaxation enhancement (RARE), 8 slices, $0.175 \times 0.175 \times 1.75 \text{ mm}^3$, echo time TE = 48 ms) was performed at a 7 Tesla preclinical scanner (Discovery MR901 magnet and gradient system, Agilent, Santa Clara, CA, USA; AVANCE III HD electronics, Bruker, Billerica, MA, USA) with a 31 mm ¹H/¹³C dual-tuned volume resonator (RAPID Biomedical, Rimpar, Germany) as described [24]. Following completion of imaging, animals were euthanized under deep Isoflurane anesthesia with cervical dislocation.

Patients with PDAC underwent hybrid molecular imaging (n=6), including PET/CT and/or PET/MRI. Image acquisition was conducted according to the European Association of Nuclear Medicine (EANM) guidelines for [¹⁸F]FDG PET imaging. PET acquisition was performed at a mean uptake time of 86 ± 19 min after intravenous injection of 338 ± 75 MBq [¹⁸F]FDG. Tumor segmentation was performed on PET images with anatomical guidance from the corresponding MRI datasets, including axial native and contrast-enhanced T1-weighted and T2-weighted (T2w) sequences, to optimize lesion delineation.

2.3. Histological Analysis: tumors were fixed for 72 hours in 4% neutral-buffered formalin, dehydrated under standard conditions, paraffin-embedded, and sectioned at 3μm. For H&E staining, sections were deparaffinized, rehydrated, and stained according to standard protocols.

Immunohistochemical staining for IBA1 and MPO was performed using a Bond RX automated staining system (Leica, Wetzlar, Germany). Following deparaffinization and rehydration, heat-induced epitope retrieval was performed using Citrate buffer (pH 6) for 20 min for IBA1 and EDTA buffer (pH 8) for 30 min for MPO. Endogenous peroxidase activity was blocked for 5 min, followed by incubation with primary antibodies against IBA1 (polyclonal, Wako, cat. No. 019-19741, 1:500) and MPO (polyclonal, Fischer Scientific, cat. No. RB-373-A, 1:50) at room temperature. Antibody detection was performed using a polymer-based detection system with an 8-min polymer incubation, followed by visualization with 3,3'-diaminobenzidine (DAB) for 10 min. Afterward, sections were counterstained with hematoxylin for 5 min, followed by treatment with bluing reagent for 10 min, dehydrated, and mounted using Pertex medium (Histolab, Gothenburg, Sweden). Stained sections were digitized using a Leica Aperio AT2 scanner at 40x magnification.

2.4. Analysis: PET images were reconstructed with OSEM 3D; and analyzed using Inveon Workplace (Siemens Inveon, Siemens, Texas, USA). Regions of interest (ROIs) were defined using a 50% hottest voxel threshold on T2w anatomical MRI images. Standardized uptake values, SUV_{max} , SUV_{mean} , tumor-to-muscle, and tumor-to-aorta ratios were calculated for tumors. To account for intratumoral heterogeneity, three spatially separated H&E-stained sections per tumor were analyzed, including one central section and two additional sections obtained at 100 μ m intervals. Tumor regions were histopathologically classified as with hemorrhage or without hemorrhage based on the presence or absence of hemorrhage, defined as free erythrocytes within tissue. Histological and imaging findings were manually compared. Histological analyses were performed using QuPath (version 0.6.0). Positive and negative cells were identified and classified using the positive cell detection algorithm. Quantitative and statistical analyses were

conducted using GraphPad Prism (version 8.0). Data were considered for normality using the Shapiro–Wilk test. For normally distributed data, differences between two independent groups were assessed utilizing an unpaired two-tailed t-test; otherwise, the Mann-Whitney U test was used. P-values <0.05 were considered statistically significant.

3. Results

3.1. [¹⁸F]FDG Uptake Patterns in PDAC Tumors With and Without Multifocal Hemorrhage

Seventeen orthotopic murine tumors (9 HPAC, 8 PSN1) exhibited histologically confirmed necrosis, including multifocal hemorrhage within necrosis in 6 HPAC and 4 PSN1 tumors. Tumor volumes did not differ significantly between tumors with and without hemorrhage in either subtype (PSN1: 232.6 ± 173.6 vs. 337.3 ± 234.5 mm³; HPAC: 226.4 ± 116.0 vs. 241.8 ± 104.4 mm³; with hemorrhage vs. without hemorrhage, respectively; $p > 0.05$). In addition, six patients with histologically confirmed PDAC were included in the clinical test cohort, comprising four tumors with multifocal hemorrhage and inflammatory cell infiltration and two tumors lacking both histological features. Given the small sample size, the clinical cohort was analyzed descriptively, and no inferential statistical comparisons were performed.

In murine tumors without hemorrhage, PET imaging demonstrated hypometabolic regions corresponding to necrotic areas identified on histology (Fig. 1a,b). These regions were successfully excluded from ROIs using threshold-based segmentation (Fig. 1b, dotted line, determined by ‘n’ as necrotic region). On the other hand, a subset of tumors demonstrated multifocal hemorrhage within the necrotic area, identified by histology (Fig. 1c), which were not distinguishable on PET imaging (Fig. 1d). Instead, these regions exhibited elevated [¹⁸F]FDG

uptake and appeared indistinguishable from viable tumor tissue. A similar pattern was observed in the clinical test cohort. PDAC lesions without histological evidence of intratumoral hemorrhage or inflammatory cell infiltration exhibited lower SUV_{max} values (Fig. 1e,f) than tumors with multifocal hemorrhage and accompanying inflammatory cell infiltration (Fig. 1g,h). Histopathological examination of tumor tissue confirmed that areas of hemorrhage and inflammatory cell infiltration corresponded to regions of increased [^{18}F]FDG uptake, mirroring the observations in the murine models. These findings support the high translational value of the preclinical data and indicate that intratumoral hemorrhage may be an important confounding factor for the interpretation of [^{18}F]FDG PET in PDAC.

3.2. Intratumoral hemorrhage influences quantitative [^{18}F]FDG PET measurements

Quantitative PET analysis revealed significantly higher [^{18}F]FDG uptake in murine tumors with multifocal hemorrhage than in tumors without hemorrhage (Fig. 2a-d), regardless of molecular subtypes. Data are illustrated as mean $\pm$ standard deviation (SD), unless otherwise indicated. In classical PDAC, tumors with multifocal hemorrhage within necrotic regions demonstrated significantly higher SUV_{mean} (1.42 ± 0.19 vs 1.10 ± 0.10 , $p=0.036$) and SUV_{max} (1.91 ± 0.29 vs 1.47 ± 0.15 , $p=0.047$) than tumors without hemorrhage. Similar differences were observed in quasimesenchymal tumors for both SUV_{mean} (1.72 ± 0.15 vs 1.20 ± 0.14 , $p=0.002$) and SUV_{max} (2.45 ± 0.24 vs 1.67 ± 0.15 , $p=0.029$). Muscle [^{18}F]FDG uptake remained consistently low in all groups. Consistent with the preclinical observations, the clinical test cohort demonstrated higher SUV_{max} values (Fig. 2e) in tumors with multifocal hemorrhage than in tumors without hemorrhage (7.57 ± 1.4 vs 3.41 ± 0.2).

3.3. Reference tissue normalization preserves differences in [¹⁸F]FDG uptake between hemorrhagic and non-hemorrhagic tumors

To determine whether the observed differences in [¹⁸F]FDG uptake were influenced by systemic tracer distribution, tumor uptake was normalized to reference tissues. In the murine cohort, normalization to muscle (tumor-to-muscle ratio) did not alter the observed differences between tumors with and without multifocal hemorrhage (Fig. 3a,b). In classical PDAC xenografts, multifocal hemorrhage demonstrated significantly higher Tumor to Muscle Ratio_{max} (3.99 ± 1.061 vs 2.21 ± 0.43 , $p = 0.0299$) than tumors without hemorrhage. Comparable results were observed in quasimesenchymal tumors (4.42 ± 0.70 vs 3.02 ± 0.67 , $p = 0.0279$). Likewise, in the clinical test cohort, normalization to both muscle (8.75 ± 3.98 vs 3.94 ± 0.09) and aorta (3.77 ± 0.73 vs 2.04 ± 0.38) values preserved the higher [¹⁸F]FDG uptake observed in tumors with hemorrhage (Fig. 3c,d). These findings suggest that the association between the presence of hemorrhage and increased [¹⁸F]FDG uptake is independent of the choice of reference tissue for normalization.

3.4. Tumor necrosis with hemorrhage is associated with increased inflammatory cell infiltration.

Immunohistochemical analysis demonstrated increased Immune cell infiltration in tumors with multifocal hemorrhage compare to those without hemorrhage. Quantification of pan macrophages staining IBA1 revealed a higher proportion of macrophages in hemorrhagic tumors in both orthotopic PDAC models (Fig 4a-c). This difference was more evident in QM (PSN1) tumors, in which the percentage of IBA1-positive cells was significantly higher in tumors with hemorrhage compared to those without hemorrhage (28.13 ± 4.34 vs 13.33 ± 2.69 ; $P_{\text{PSN1}} = 0.0068$). A similar trend was observed in classical (HPAC) tumors (22.07 ± 4.04 vs 18.66 ± 1.55), although

no statistically significant difference was detected (Fig. 4b,c). In contrast, myeloperoxidase (MPO) staining revealed no significant difference in neutrophil infiltration between tumors with and without hemorrhage in either molecular subtype (HPAC: 0.70 ± 0.63 vs 0.48 ± 0.23 , PSN1: 0.85 ± 0.84 vs 0.39 ± 0.19) despite a trend toward higher MPO-positive cell counts in tumors with multifocal hemorrhage.

4. Discussion

Our study demonstrates that multifocal intratumoral hemorrhage significantly affects [^{18}F]FDG PET-derived uptake values in PDAC. Consistent with previous reports, tumors showing necrosis without hemorrhage exhibited reduced tracer accumulation, supporting the exclusion of necrotic regions from quantitative PET analyses[21,25]. In contrast, necrotic regions containing multifocal hemorrhage showed preserved or increased [^{18}F]FDG uptake, resulting in values comparable to those of viable tumor tissue. These findings challenge the hypothesis that necrosis is invariably associated with low metabolic activity and suggest that hemorrhagic transformation may artificially elevate PET-derived metrics, including metabolically active tumor volume (MTV) and SUVs. To our knowledge, this is the first study to systematically assess the impact of intratumoral hemorrhage on [^{18}F]FDG PET quantification in PDAC.

Our findings further support the growing body of evidence that [^{18}F]FDG uptake reflects not only the presence of viable tumor cells but also contributions from non-neoplastic components within the tumor microenvironment. Rahman et al. highlighted that inflammatory lesions are a common source of false-positive findings in oncologic PET imaging [26]. In addition, Kubota et al. demonstrated that activated inflammatory cells exhibit increased glucose metabolism and accumulate [^{18}F]FDG through similar mechanisms to those of malignant cells, providing a

biological explanation for elevated uptake patterns in inflammatory regions [17]. Molecular studies have shown that the presence of intratumoral hemorrhage initiates a local inflammatory response characterized by the recruitment of macrophages and neutrophils, which facilitate the clearance of extravasated erythrocytes and their degradation products [27,28]. Consistent with these observations, in our study, tumors with hemorrhage exhibited increased macrophage infiltration along with preserved [^{18}F]FDG uptake. Interestingly, neutrophil infiltration did not differ significantly between tumors with and without hemorrhage. This finding suggests that macrophage recruitment may contribute to the altered [^{18}F]FDG PET signal associated with hemorrhage within the tumor.

The preserved metabolic signal in necrotic regions with multifocal hemorrhage leads to important implications for quantitative [^{18}F]FDG PET analysis. Consequently, PET-derived metrics such as SUV_{max} , SUV_{mean} , MTV, and total lesion glycolysis (TLG) can be influenced by hemorrhagic components rather than exclusively reflecting viable tumor burden. This effect is particularly relevant in longitudinal imaging studies and treatment response assessment, where changes in [^{18}F]FDG uptake are commonly interpreted as indicators of therapeutic efficacy or disease progression [29,30]. Galldiks et al, also reported that, in brain tumors, treatment-induced coagulation necrosis exhibited increased *O*-(2-[^{18}F]fluoroethyl)- L-tyrosine (FET) PET tracer uptake despite the absence of viable tumor tissue, leading to a false-positive interpretation of tumor progression[18]. Our findings identify intratumoral hemorrhage as a previously underrecognized confounding factor in [^{18}F]FDG PET quantification and possibly studies of response assessment. By increasing [^{18}F]FDG uptake within necrotic tumor regions, hemorrhage may be facilitating more accurate PET interpretation and quantitative tumor assessment.

Several limitations of this study should be acknowledged. First, the human cohort was small and primarily served to confirm the presence of hemorrhage and its associated imaging characteristics rather than to assess its prevalence or prognostic relevance. Second, although the orthotopic xenograft model reproduced key imaging and histopathological features, it does not fully recapitulate the stromal complexity and immune microenvironment of human PDAC. Consequently, the extent and composition of necrosis and hemorrhage may differ from those observed in patients. Third, while increased macrophage infiltration was associated with preserved [^{18}F]FDG uptake in tumors with hemorrhagic regions, the underlying molecular mechanisms remain incompletely understood. In addition, the accurate delineation and exclusion of hemorrhagic regions from PET quantification remains challenging, particularly in small tumors, owing to the limited spatial resolution of [^{18}F]FDG PET and associated partial-volume effects. Future studies in larger clinical cohorts and more representative preclinical models, such as genetically engineered mouse models, are needed to validate these findings. Furthermore, prospective studies combining susceptibility-weighted MRI and [^{18}F]FDG PET should determine whether identifying hemorrhagic necrosis can improve PET quantification and treatment response assessment in PDAC tumors interpreted as false-positive metabolic activity and potentially affect the interpretation of PET-based response assessment, including positron emission tomography response criteria in solid tumors or PERCIST [30].

It is well established that [^{18}F]FDG PET is limited by its spatial resolution, resulting in partial-volume effects that can impair the accurate characterization and quantification of intratumoral tracer uptake. [10]. These effects appear to be particularly pronounced at the interface between viable tumor tissue, hemorrhage, and necrosis, potentially obscuring the underlying metabolic heterogeneity of the lesion. In contrast, MRI provides superior soft-tissue

contrast and enables the detection of hemorrhagic components through dedicated structural imaging techniques. In particular, susceptibility-weighted (SW)-MRI is highly sensitive to the detection and characterization of hemorrhagic lesions, owing to its ability to visualize susceptibility effects caused by blood degradation products. [31]. These findings highlight the complementary value of multimodal imaging and suggest that integrating susceptibility-sensitive MRI with [^{18}F]FDG PET may improve the detection of hemorrhage within the tumor, thereby increasing the reliability of PET-based tumor characterization and treatment-response assessment.

5. Conclusion

In conclusion, hemorrhages associated with necrosis areas within the tumor represent a previously unrecognized biological confounder of [^{18}F]FDG PET quantification in PDAC. By preserving glucose uptake within necrotic regions, hemorrhage may lead to overestimation of viable tumor burden and inaccurate interpretation of quantitative [^{18}F]FDG PET parameters. Our findings provide a biological explanation for part of the metabolic heterogeneity observed in PDAC and emphasize the importance of multimodal imaging and PET/MRI integration to improve tumor characterization and treatment response monitoring. Further validation in larger prospective clinical cohorts is warranted.

Declarations

Ethics approval and consent to participate

All animal experiments were performed in compliance with the European guidelines for the care and use of laboratory animals. The animal study was approved by Regierung von Oberbayern, Munich, Germany (license 55.2-2532.Vet_02-18-91).

The human study was approved by the Ethics Committee of the Faculty of Medicine, Technical University of Munich (protocol no. 181 17 S). Clinical trial number: not applicable.

Consent for publication

The human study was approved by the Ethics Committee of the Faculty of Medicine, Technical University of Munich (protocol no. 181 17 S). Clinical trial number: not applicable.

Availability of data and materials

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

Competing interests

All authors declare they have no financial interests, except F.S., who serves on the scientific advisory board of NVision Imaging Technologies GmbH, and A.B., who serves as a regulatory affairs representative of Gimmi GmbH, both unrelated to the presented work.

Funding statement

This work was supported by funding from the German Federal Ministry of Education and Research (grant number 13N16450 QuE-MRT) to F.S., R.B., and I.H., and Wilhelm Sander Foundation (grant number 2023.083.1) to R.B. and I.H.

Author's contributions

Conception and design: R.K., I.H., and R.B. Development of methodology: R.K., J.L., A.B., F.S., K.S., R.B., L.S., and I.H. Acquisition of data: R.K., J.L., A.B., L.S., and I.H. Analysis and interpretation of data: R.K., I.H., S.W., and L.S. Writing and/or revision of the manuscript: R.K., L.S., K.S., R.B., and I.H. Administrative, technical, or material support: F.S., R.B., and I.H. Study supervision: R.B., and I.H. All authors reviewed and approved the final manuscript.

Acknowledgements

We would like to thank Irina Skuratovska and Sybille Reder for excellent experimental and technical support.

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Figures

Figure 1

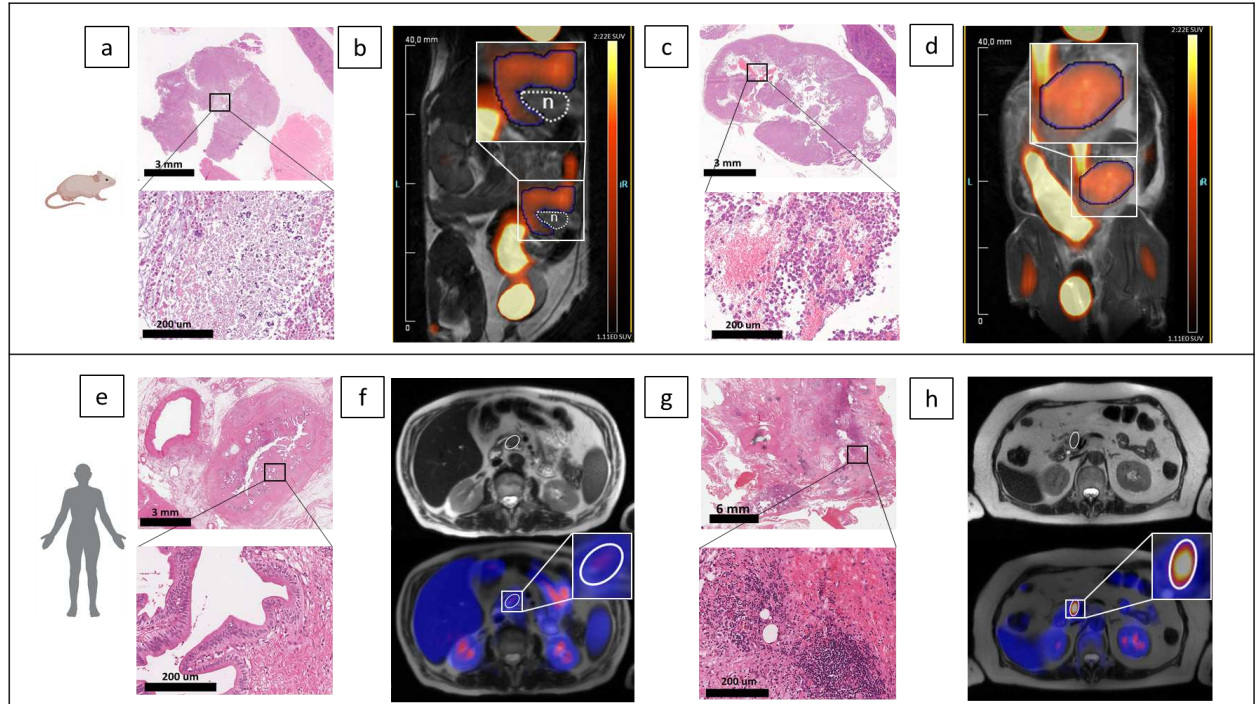


Figure 1. Multifocal hemorrhage masks the expected hypometabolic signature of necrosis on $[^{18}\text{F}]\text{FDG}$ PET in PDAC. (a, b) Representative orthotopic PDAC tumor exhibiting necrosis without hemorrhage. $[^{18}\text{F}]\text{FDG}$ PET demonstrates a hypometabolic intratumoral region (dotted line, marked by letter n as necrotic region) corresponding to histologically confirmed necrosis, allowing exclusion of the necrotic area from the PET region of interest (ROI) using threshold-based segmentation. (c, d) Representative orthotopic PDAC necrotic tumor with multifocal hemorrhage. Histology demonstrates intratumoral hemorrhage within necrotic regions that is not distinguishable on $[^{18}\text{F}]\text{FDG}$ PET images. (e–h) Representative clinical test cases. (e, f) PDAC without hemorrhage or inflammatory cell infiltration, demonstrating relatively low $[^{18}\text{F}]\text{FDG}$ uptake. (g, h) PDAC with multifocal hemorrhage and associated inflammatory cell infiltration demonstrating higher $[^{18}\text{F}]\text{FDG}$ uptake.

Figure 2

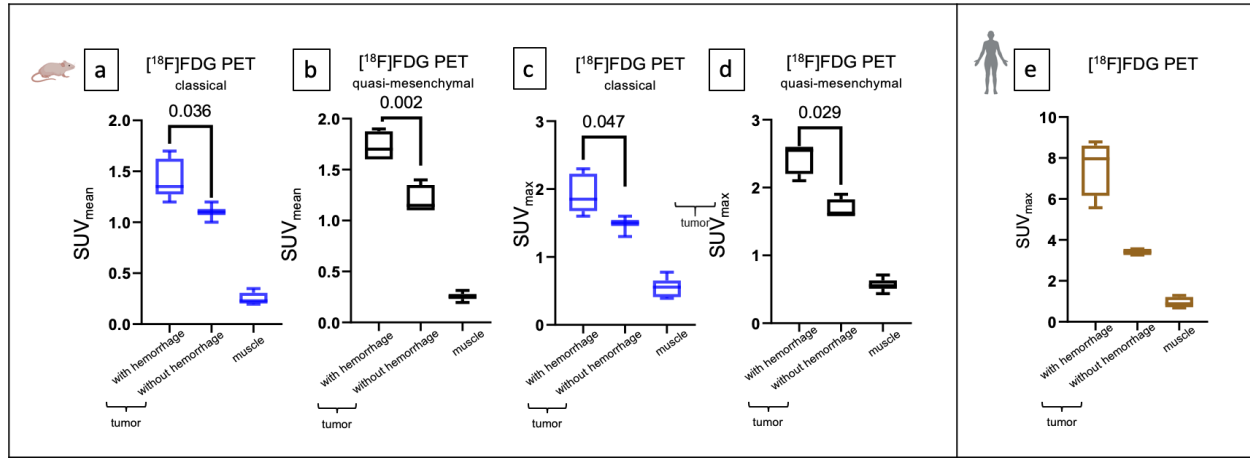


Figure 2. Quantitative $[^{18}\text{F}]$ FDG PET analysis demonstrates increased tracer uptake in tumors with multifocal hemorrhage. (a-d) SUV_{mean} in orthotopic classical (a) and quasi-mesenchymal (b) PDAC tumors with multifocal hemorrhage compared with tumors without hemorrhage and muscle tissue. SUV_{max} in classical (c) and quasi-mesenchymal (d) PDAC tumors and muscle tissue. Tumors with multifocal hemorrhage exhibited significantly higher SUV values than tumors without hemorrhage in both subtypes. (e) SUV_{max} in the clinical cohort showing higher $[^{18}\text{F}]$ FDG uptake in tumors with multifocal hemorrhage compared with tumors without hemorrhage. Statistical comparison was performed using Student's t-test for panels (a-c) and the Mann-Whitney U test for panel (d).

Figure 3

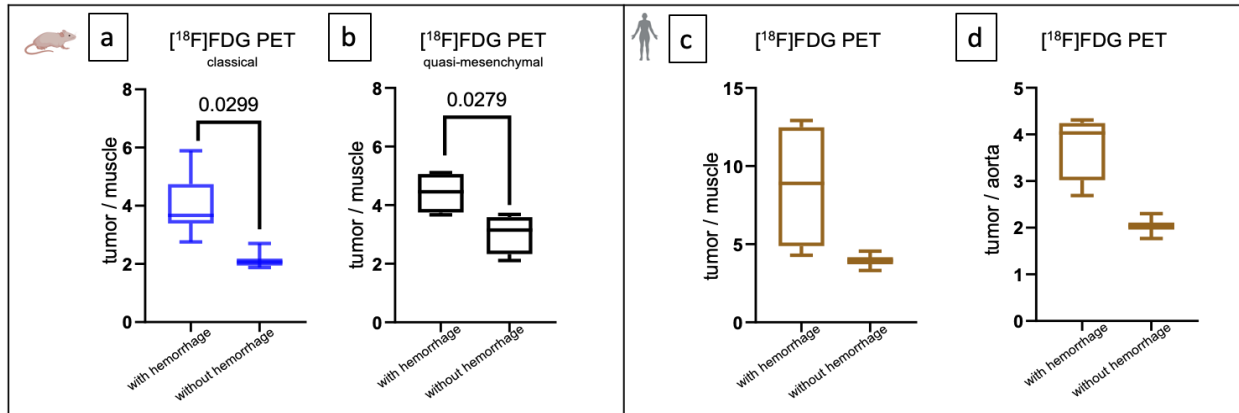


Fig3. Reference tissue normalization preserves differences in $[^{18}\text{F}]\text{FDG}$ uptake between tumors with and without hemorrhage. Tumor-to-muscle ratio (T/M_{max}) in classical (a) and quasi-mesenchymal (b) orthotopic PDAC tumors with and without multifocal hemorrhage. (c, d) Clinical cohort showing tumor-to-muscle (T/M_{max}) and tumor-to-aorta (blood) (T/B_{max}) ratios. Statistical analysis was conducted using an unpaired two-tailed Student's t-test for panels (a–b)

Figure 4

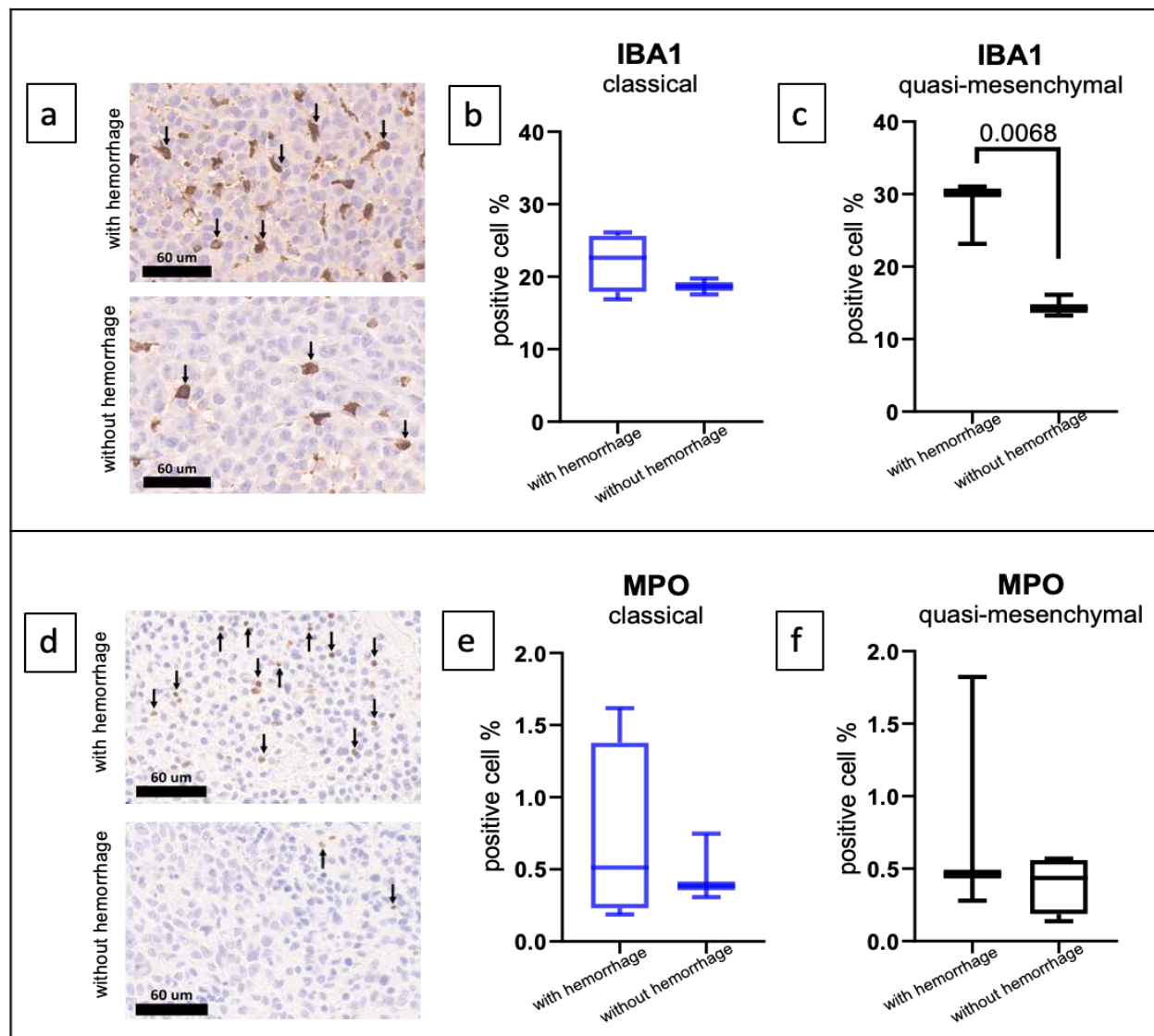


Figure 4. Immunological characterization of tumors with and without hemorrhage in orthotopic PDAC. (a) Representative IBA1 immunohistochemistry demonstrates high and low macrophage infiltration in tumors with multifocal hemorrhage compare to tumors without hemorrhage. Quantification of IBA1-positive cells in classical (b) and quasi-mesenchymal (c) orthotopic PDAC tumors with and without hemorrhage. (d) Representative immunohistochemical staining for myeloperoxidase (MPO) in tumors with and without hemorrhage. (e, f) Quantification of MPO-positive cells in classical (e) and quasi-mesenchymal (f) tumors showed no significant

differences in neutrophil infiltration between tumors with and without multifocal hemorrhage. Statistical comparisons were conducted using an unpaired two-tailed Student's t-test for panels b, c, e, and f.