# 18 F-FDG uptake heterogeneity predicts KRAS/NRAS/BRAF mutation status in patients with liver metastases of colorectal cancer

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**Research article**

**Keywords:** 18F-FDG, predict, KRAS/NRAS/BRAF, Mutation, liver metastases of colorectal cancer

**Posted Date:** May 27th, 2020

**DOI:** https://doi.org/10.21203/rs.3.rs-23443/v1

## Abstract

# Background

Cetuximab and Panitumumab,serve as monoclonal antibodies against the epidermal growth factor receptor (EGFR), have been used in the treatment of metastatic colorectal cancer. However, mutations in KRAS, NRAS, BRAF indicate a lack of response to treatment for EGFR. The identification of KRAS/NRAS/BRAF mutation status is important to improve the success rate of this therapy. PET/CT imaging is a biomolecular imaging technique that can supply various information about malignant tumors. Hence, we investigated whether KRAS/NRAS/BRAF mutation status is related to $^{18}$F-FDG uptake heterogeneity in liver metastases of colorectal cancer, and whether $^{18}$F-FDG PET / CT imaging can predict the status of KRAS / NRAS / BRAF mutation and guide treatment of liver metastases of colorectal cancer in this study.

# Methods

60 patients with liver metastases of colorectal cancer (lmCRC) were analysed retrospectively who had received $^{18}$F-FDG PET / CT before surgical operation. Heterogeneity index (HI) was defined by divided SUVmax by SUVmean. HI were assessed for patient (-P), colorectal cancer (-T) and liver metastatic lesions (-L).

# Results

HI-L was significantly higher in NRAS/ KRAS/BRAF mutation group than in NRAS/ KRAS/BRAF wild-type group (2.48 ± 0.69 and 2.05 ± 0.31, respectively; $p = 0.01$). The groups of the number of liver metastatic sites were significantly different ($p = 0.02$). In multivariate analysis, HI-L was only significantly associated with KRAS/NRAS/BRAF mutation status ($p = 0.008$). Using a HI-L cut-off of 2.12, the sensitivity and specificity of predicting KRAS/NRAS/BRAF mutation were 72.7% and 70.4%, respectively.

# Conclusions

Higher HI-L is associated with elevated NRAS/KRAS/BRAF mutation status in patients with lmCRC. $^{18}$F-FDG PET/CT can be used to predict the KRAS/NRAS/BRAF mutation status of lmCRC and to select the optimal therapeutic strategy.

## Background

In the world, colorectal cancer(CRC) is one of the most common cancer, causing nearly 700000 deaths every year(1). Liver metastasis is one of the main death factors of colorectal cancer, and approximately

20% of colorectal cancer patients have liver metastasis at the same time(2). Cetuximab and Panitumumab, serve as monoclonal antibodies against the epidermal growth factor receptor (EGFR), have been used in the treatment of metastatic colorectal cancer(3). Several studies have shown that mutation in RAS, including KRAS and NRAS, indicates a lack of response to targeted therapy to EGFR, and HRAS mutation is a negligible event because of the low mutation rate(4, 5). It is well known that BRAF mutation has a negative effect on prognosis(6–9), and some evidences have shown that anti EGFR therapy is ineffective in BRAF mutant tumors(10–12). Identifying KRAS, NRAS and BRAF mutation is now standard practice to select patients for targeted therapy to EGFR(3). Therefore, it is very important to predict KRAS/NRAS/BRAF mutation status, including KRAS/NRAS/BRAF mutation (at least one of the KRAS, NRAS, BRAF is mutant), KRAS/NRAS/BRAF wild-type (each of the KRAS, NRAS, BRAF is a wild-type).

$^{18}$F-FDG PET/CT is being widely applied to the diagnosis, the prognosis and predictors for response to treatment in the various types of cancer. Meanwhile, as molecular imaging, PET/CT has ability to offer an important noninvasive approach to biologically characterize(13). Researcher tried to study the relativity between PET parameter and KRAS mutation in patients with CRC(14). However, the latest meta-analysis showed the common PET parameters might not be useful for prediction of KRAS mutation(15). Recently, the vigorous development of intratumor heterogeneity has profound implications to have been demonstrated on biologically characterize and treatment responses. Common approaches include fractal analysis, texture analysis, and so on(13). Previous texture analysis might exhibit a good performance for the prediction of the KRAS mutation status in CRC by CT imaging(16). Therefore, we supposed that intratumor heterogeneity might predict KRAS/NRAS/BRAF mutation status in patients with lmCRC by PET/CT.

In this study, we investigated whether KRAS/NRAS/BRAF mutation status is related to $^{18}$F-FDG uptake heterogeneity in liver metastases of colorectal cancer, and whether $^{18}$F-FDG PET / CT imaging can predict the status of KRAS / NRAS / BRAF mutation and guide treatment of liver metastases of colorectal cancer.

## Methods

# Study population

The study group comprised 60 patients with lmCRC (33 men and 27 women; age range 29–86 years 62y). All of them had received $^{18}$F-FDG PET/CT scan before surgery operation or pathology at the Shanghai cancer center (affiliated with Fudan University) between 2018.1.4 and 2019.8.30. All patients of colorectal cancer and liver metastasis were confirmed by pathology. Imaging data, mutation information were obtained from department of nuclear medicine and department of pathology, respectively. All patients were given written and informed consent for PET/CT procedures. The institutional review board has approved this study. The need for written informed consent was waived as it is a retrospective study.

# $^{18}$ F-FDG PET/CT imaging

$^{18}$F-FDG was made out automatically by the cyclotron (CTIRDS Eclipse ST, Siemens, Knoxville, TN). Over 95% of the radiochemical purity was to meet the using standard,. Blood glucose levels of all patients were under 140 mg/dL. All patients fasted for at least 6 h before received an intravenous injection of $^{18}$F-FDG at a dose of 7.4 MBq/kg. The mean uptake time was 1 h. All patients underwent a whole body scanner of $^{18}$F-FDG PET/CT, ranging from groin to skull base.

# Imaging Interpretation

Images were processed by a multimodal computer platform (syngo; Siemens). For each primary tumor and liver metastasis lesion, SUV values were measured by (radioactivity) / (injected dose / body weight). The maximum SUV (SUVmax) and the mean SUV (SUVmean) were measured by manually placing an individual volume of interest on fused transaxial PET/CT images. SUVmax of liver metastasis lesion was defined as the highest SUVmax value among all liver metastasis lesions, and SUVmax of patient was defined as the highest SUVmax value among all lesions, including primary and metastatic lesions. SUVmean was measured using margin threshold of SUVmax ≥ 2.5. The analysis software measured automatically SUVmax and SUVmean value. Heterogeneity index (HI) was defined as SUVmax divided by SUVmean. Heterogeneity index were assessed for patient (-P), colorectal cancer (-T) and liver metastatic lesions (-L).

# Kras/nras/braf Mutation Status

Gene mutation analysis were completed in Shanghai cancer center, China. Genomic data was obtained from tumors according to standard protocols (RNeasy Mini Kit, and QiAamp DNA Mini Kit, Qiagen, Hilden, Germany). Single-stranded cDNA was reverse transcribed from RNA by RevertAid First Strand cDNA Synthesis Kit (Fermentas, St Leon-Rot, Germany). Either genomic data, including DNA or cDNA, were performed by polymerase chain reaction (PCR) amplification and sequencing. KRAS (exons 2 to 4), NRAS (exons 2 to 4), and BRAF (exons 15) were PCR amplified using cDNA for further sequencing.

# Statistical Analysis

Statistical analyses were performed using SPSS, version 19.0 (SPSS Inc., Chicago, IL, USA). All data are shown as means ± SD. T test, Mann–Whitney U test and chi-squared test estimated the significance of differences between groups, where applicable. A $p$ value < 0.05 was statistically significant in this study.

## Results

# Patient And Tumor Characteristics

Clinical characteristics are shown in Table 1. A total of 60 patients with lmCRC had undergone $^{18}$F-FDG PET/CT imaging in Shanghai cancer center before treatment. Median age of these patients was 62 years (range 29–86). Of the 60 primary tumours, 47 (78.33%) were colon cancer and 13 (21.67%) were rectal cancer. 13 (21.67%) of the patients had ≥ 5 liver metastatic sites (group by colorectal liver oligometastases criteria(17)). 30 were KRAS/NRAS wild-type, 56 were BRAF wild-type, and 27 were KRAS/NRAS/BRAF wild-type.

Table 1
Patients and tumor characteristics.

| Characteristic | Patients (n = 60) |
|---|---|
| Age(y) | |
| Median (range) | 62(29–86) |
| Sex | |
| Male | 33 |
| Female | 27 |
| Blood glucose | |
| Mean ± SD | 5.48 ± 0.78 |
| Range | 4.2–8.2 |
| CEA | |
| < 5.0 | 9 |
| ≥ 5.0 | 51 |
| Location | |
| colon | 47 |
| rectum | 13 |
| NO. of liver Metastatic sites | |
| ≤ 5 | 47 |
| > 5 | 13 |
| KRAS/NRAS | |
| wild-type | 30 |
| mutation | 30 |
| BRAF | |
| wild-type | 56 |
| mutation | 4 |
| KRAS/NRAS/BRAF | |
| wild-type | 27 |
| mutation | 33 |

# Correlations Between Kras/nras/braf Mutation Status And Patient Characteristics

Two groups were divided among patients according to the mutation status of the KRAS/NRAS/BRAF. In the 60 patients with lmCRC, correlations between the KRAS/NRAS/BRAF mutation status and clinical characteristics were evaluated by univariate analysis. Because all patient have liver metastasis, the relationship between mutation of KRAS/NRAS/BRAF and TNM stage was not evaluated. There was no significant difference in age, gender, location, CEA between the KRAS/NRAS/BRAF mutation group and the wild-type group. However, the two groups were significantly different in terms of the number of liver metastatic sites ($p = 0.02$; Table 2).

Table 2
Univariate analysis of factors associated with KRAS/NRAS/BRAF mutation status.

| Factor | KRAS/NRAS/BRAF mutation (n = 33) | KRAS/NRAS/BRAF wild-type (n = 27) | *p* value |
|---|---|---|---|
| Mean age ± SD (y) | 58.18 ± 13.49 | 58.96 ± 10.24 | 0.98 |
| Sex | | | |
| Male | 16 | 17 | 0.26 |
| Female | 17 | 10 | |
| Location | | | 0.59 |
| Colon | 25 | 22 | |
| Rectum | 8 | 5 | |
| CEA | | | 0.49 |
| < 5.0 | 4 | 5 | |
| ≥ 5.0 | 29 | 22 | |
| NO. of liver metastatic sites | | | 0.02[a] |
| < 5 | 22 | 25 | |
| ≥ 5 | 11 | 2 | |
| SUVmax - P | 18.65 ± 9.27 | 14.74 ± 5.88 | 0.13 |
| MTV - P | 80.26 ± 165.55 | 49.12 ± 55.26 | 0.35 |
| TLG - P | 564.04 ± 1135.22 | 279.49 ± 315.73 | 0.21 |
| SUVmax - T | 18.35 ± 9.48 | 14.55 ± 6.04 | 0.12 |
| MTV - T | 46.89 ± 40.98 | 33.54 ± 23.85 | 0.14 |
| TLG - T | 358.67 ± 436.62 | 189.69 ± 130.60 | 0.18 |
| SUVmax - L | 11.58 ± 4.82 | 9.67 ± 2.58 | 0.77 |
| MTV - L | 77.81 ± 166.44 | 134.69 ± 169.22 | 0.14 |
| TLG - L | 423.25 ± 1101.98 | 672.91 ± 859.16 | 0.06 |
| HI - P | 18.35 ± 9.48 | 14.55 ± 6.04 | 0.12 |

[a]p < .05 is considered significant.

Abbreviations: HI, heterogeneity index; -P, patient; -T, primary tumor; -L, Liver metastasis

| Factor | KRAS/NRAS/BRAF mutation (n = 33) | KRAS/NRAS/BRAF wild-type (n = 27) | *p* value |
|---|---|---|---|
| HI - T | 2.64 ± 0.57 | 2.51 ± 0.64 | 0.25 |
| HI - L | 2.48 ± 0.69 | 2.05 ± 0.31 | 0.01[a] |
| [a]p < .05 is considered significant. | | | |
| Abbreviations: HI, heterogeneity index; -P, patient; -T, primary tumor; -L, Liver metastasis | | | |

# Correlation Between Pet Parameters And Kras/nras/braf Mutation Status

Only HI-L has significant correlation with KRAS/NRAS/BRAF mutation status, and SUVmax-P, MTV-P, TLG-P, SUVmax-T, MTV-T, TLG-T, SUVmax-L, MTV-L, TLG-L, HI-P and HI-T have no correlation with the status (Table 2). HI-L of KRAS/NRAS/BRAF mutation was significantly higher than KRAS/NRAS/BRAF wild-type (2.48 ± 0.69 and 2.05 ± 0.31, respectively, $p = 0.01$).

Then the optimal HI-L threshold was determined for predicting KRAS/NRAS/BRAF mutation. Receiver operating characteristic (ROC) curve analysis showed that when the HI-L cut-off value was 2.12, the prediction accuracy of KRAS / NRAS / BRAF mutation was the highest. The area under the ROC curve was 0.712 ± 0.067. The sensitivity and specificity were 72.7% and 70.4% to predict KRAS/NRAS/BRAF mutation (Fig. 1). These results show that $^{18}$F-FDG PET/CT may be used to predict the KRAS/NRAS/BRAF mutation status in patients with lmCRC. Representative PET/CT images are shown in Fig. 2.

In the multivariate analysis, only HI-L was still significantly associated with KRAS / NRAS / BRAF mutation status (Table 3; odds ratio, 6.78; 95% confidence interval, 1.63–28.08; p = 0.008).

Table 3
Multivariate analysis of KRAS/NRAS/BRAF mutation status.

| Factor | Odds ratio | 95% confidence interval | *p* |
|---|---|---|---|
| HI-L | 6.78 | 1.63–28.08 | 0.008 |
| No. of liver metastatic sites | 0.49 | 0.49–7.08 | 0.358 |

# Discussion

About 20% of colorectal cancer patients initially show metastatic disease, and up to 50% of early tumors eventually metastasize, and systemic chemotherapy is the main treatment for patients with metastatic

CRC(18). Cetuximab and panitumumab, antibodies targeting the EGFR, have been applied to the therapy of metastatic CRC, including liver metastasis. Their therapeutic effect is better than systemic chemotherapy(16). However, the effective rate of treatment is not high. Some researches show that the mutations in KRAS and NRAS, termed extended RAS testing, have resistance to targeted therapy to anti-EGFR in metastatic CRC(19–21). Although $^{18}$F-FDG PET/CT has been widely useful for prognosis of various tumors(22–26), the usual parameters of it might not be used to predict KRAS mutation and exclude it as shown previously(15). Our results has confirmed it in this manuscript. We established a parameter to represent the intra-individual heterogeneity of lesions previously, and it has a good prediction ability in breast cancer model(13). Therefore, we try to predict KRAS/NRAS/BRAF by heterogeneity of lesions. To our knowledge, this is the first study to analyse the association between $^{18}$F-FDG uptake intra-individual heterogeneity among lesions and the KRAS/NRAS/BRAF mutation status in lmCRC. $^{18}$F-FDG uptake intra-individual heterogeneity may apply to informing optimal treatment strategies in patients with lmCRC by to predict effective patients treated with cetuximab and panitumumab.

Our results indicate a significant association between $^{18}$F-FDG uptake intra-individual heterogeneity among lesions and KRAS/NRAS/BRAF mutation in patients with lmCRC. The ROC curve and the area under the lH-L curve manifested that lH-L could potentially be used to infer mutation status in KRAS/NRAS/BRAF. In multivariate analysis, only lH-L was significant predictors of KRAS/NRAS/BRAF mutation in patients with lmCRC.

The underlying biological mechanisms behind these resluts are complex, and they are still under investigation. Studies have confirmd that FDG uptake by tumor cells mainly relys on Glucose transporters (GLUTs) and Hexokinases (HXKs)(27). Upregulation of GLUT 1 and increased levels of HXK II have been implicated in lots of cancers(28–31). Some studies have indicated that GLUT 1 is more important than HXKII for FDG intake of CRC tissues(27). Cell experiments show that glucose uptake increased when GLUT1 expression increased in CRC cell(32). These findings suggest that the positive correlation between lH-L and KRAS/NRAS/BRAF mutation may reflect the role in GLUT1 expression, but they still need to be confirmed in the future.

The main limitations of our study are small sample size and retrospective study, and some of the values HI-L overlap in the two groups. It is impossible that the optimal cutoff values based on small sample size to adapt to all patients. It is difficult for PET/CT to replace the traditional methods for distinguishing KRAS/NRAS/BRAF mutation status in the clinical process. However, our study may promote the development of non-invasive inspection for predicting effective ant-EGFR therapies in patients with lmCRC.

# Conclusion

This study highlights the value of HI-L measured among liver metastatic lesions on pretreatment $^{18}$F-FDG PET/CT scans in predicting KRAS/NRAS/BRAF mutation status in patients with liver metastasis of

colorectal cancer. The results of this study indicate that $^{18}$F-FDG based heterogeneity among liver metastatic disease could help identify patients with liver metastasis of colorectal cancer who could benefit from anti-EGFR therapies.

## Abbreviations

HI: Heterogeneity index; lmCRC:liver metastases of colorectal cancer; FDG:fluorodeoxyglucose; PET/CT:positron emission tomography/computer tomography; EGFR:Epidermal Growth Factor Receptor; GLUT:Glucose transporter; HXK:Hexokinase; ROC:Receiver operating characteristic; SUV:Standardized uptake value.

## Declarations

# Acknowledgments

We are very grateful for the funding supported by National Natural Science Foundation of China (No. 81771861 and 81971648), Shanghai Scientific and Technological Innovation Program (No. 18410711200 and 19142202100), National Key R&D Program of China (No.2019YFC1604605) and Foundation of Shanghai Municipal Commission of Health and Family Planning (No. 20170425).

# Authors' contributions

Shaoli Song and Yingjian Zhang designed this study. The data was supported by Guang Ma, Qiufang Liu, Lingling Pan, Jianping Zhang and Fangsong Zhang. Guang Ma analysed all data, discussed results and wrote the manuscript. All authors read and approved the final manuscript.

# Funding

National Natural Science Foundation of China (No. 81771861 and 81971648), Shanghai Scientific and Technological Innovation Program (No. 18410711200 and 19142202100), National Key R&D Program of China (No.2019YFC1604605) and Foundation of Shanghai Municipal Commission of Health and Family Planning (No. 20170425).

# Availability of data and materials

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

# Ethical approval

All applicable international, national and/or institutional guidelines for the care and use of animals were followed. All procedures performed were in accordance with the ethical standards of the institution or practice at which the studies were conducted. Further information is given in the “Methods” section.

# Consent for publication

Not applicable. All images and data were anonymous.

# Competing interests

The authors declare that they have no competing interests.

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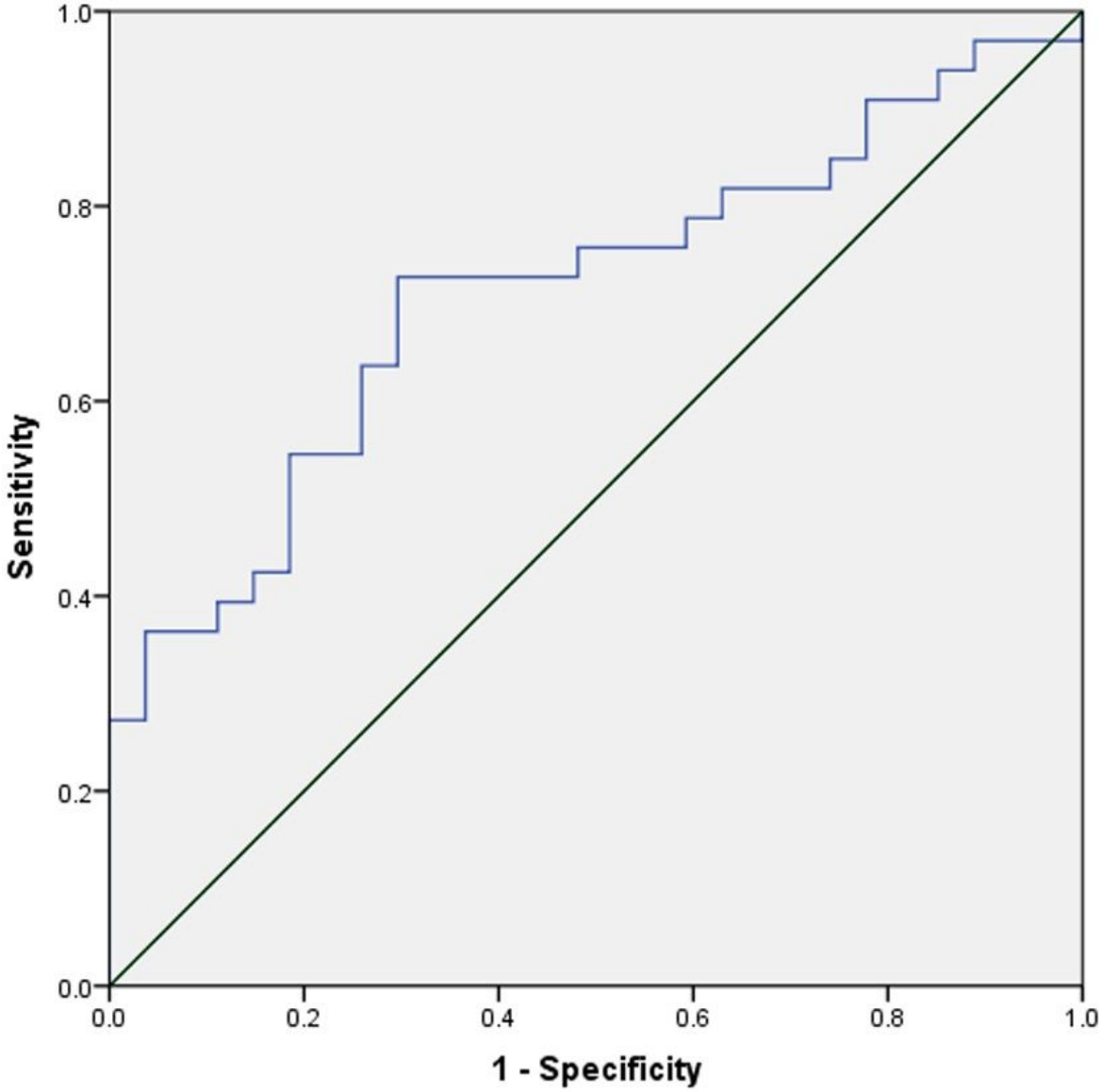
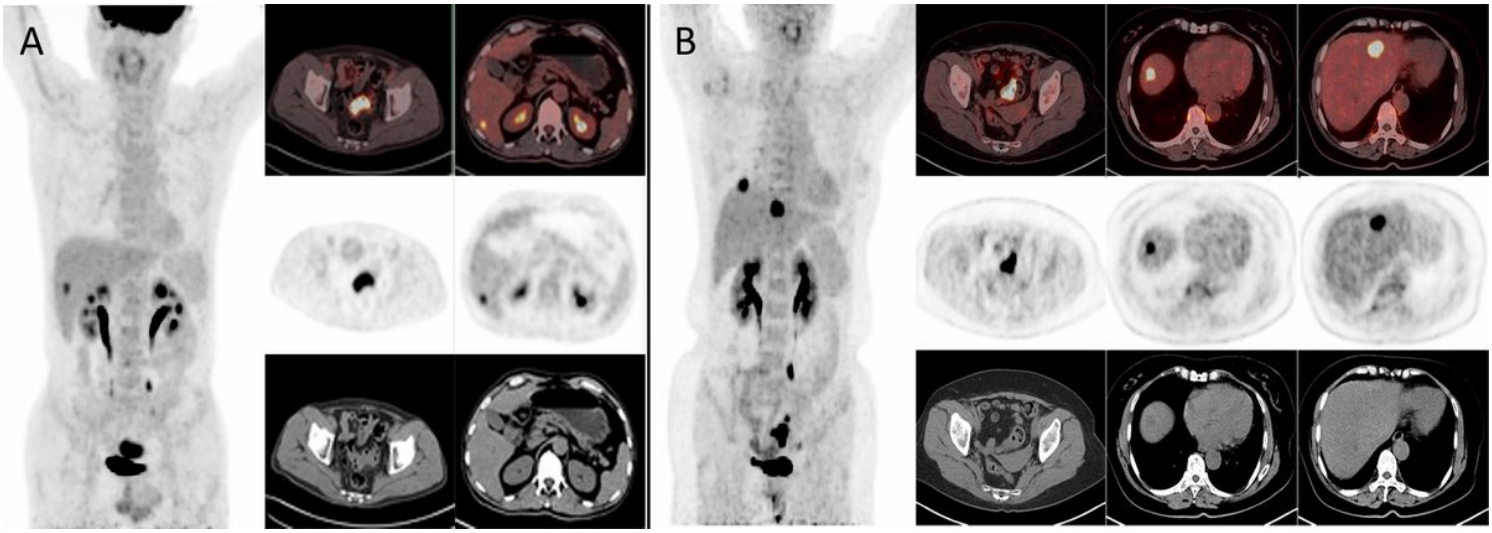
# Figures




## Figure 1

Relationships between 18F-FDG uptake heterogeneity index (HI) and KRAS/NRAS/BRAF mutation status in 60 patients with liver metastasis of colorectal cancer. A: Correlation between HI-L and KRAS/NRAS/BRAF mutation status. HI-L is significantly higher in PD- KRAS/NRAS/BRAF mutation group than KRAS/NRAS/BRAF wild-type group (2.48 ± 0.69 vs. 2.05 ± 0.31, respectively; P = 0.01); B: Receiver operating characteristic curve analysis of the ability of HI-L to predict KRAS/NRAS/BRAF mutation status. Using a HI-L cut-off value of 2.12, the sensitivity and specificity of SUVmax in predicting KRAS/NRAS/BRAF mutation status were 72.7% and 70.4%, respectively. The area under the receiver operating characteristic curve is 0.71 (95% confidence interval 0.58–0.84; p = 0.01).




## Figure 2

Representative 18F-FDG PET / CT imaging of liver metastasis of colorectal cancer showing of KRAS/NRAS/BRAF mutation status. A: KRAS/NRAS/BRAF wild-type; B: KRAS/NRAS/BRAF mutation.