A Methodological Investigation of Healthy Tissue, Hepatocellular Carcinoma and other Lesions with Dynamic 68Ga-FAPI-04 PET/CT Imaging

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Short communication

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Abstract

**Background:** The study aimed to establish a $^{68}$Ga-FAPI-04 kinetic model in hepatic lesions, to determine the potential role of kinetic parameters in the differentiation of hepatocellular carcinoma (HCC) from non-HCC lesions.

**Material and Methods:** Time activity curves (TACs) were extracted from seven HCC lesions and five non-HCC lesions obtained from $^{68}$Ga-FAPI-04 dynamic positron emission tomography (PET) scans of eight patients. Three kinetic models were applied to the TACs, using image derived hepatic artery and/or portal vein as input functions. For input functions and the lesions, the according voxel with the maximum standardized uptake value (SUVmax) was taken, for the healthy tissue mean SUV values. The optimum model was chosen after applying the Schwartz information criteria to the TACs, differences in model parameters between HCC, non-HCC lesions, and healthy tissue were evaluated with the ANOVA test.

**Results:** A reversible two-tissue compartment model using both the arterial as well as venous input function was most preferred and showed significant differences in the kinetic parameters $V_{ND}$, $V_T$ and $BP_{ND}$ between HCC, non-HCC lesions and healthy regions ($p < 0.01$).

**Conclusion:** Several Model parameters derived from a two-tissue compartment kinetic model with two image-derived input function from vein and aorta and using SUVmax allow a differentiation between HCC and non-HCC lesions, obtained from dynamically performed PET scans using FAPI.

**Background**

Hepatocellular carcinoma (HCC), the most common primary liver cancer, is a highly heterogeneous cancer(1). The forms of heterogeneity are seen when comparing tumors between patients (interpatient heterogeneity), between different tumor nodules within the same patient (inter tumoral heterogeneity) and between different regions of the same nodule (intratumoral heterogeneity)(2). Although imaging modalities, including ultrasonography, CT, MRI are valuable in hepatic lesion characterization, they still have limitations in distinguishing the functional variables or the differentiation of malignant lesions (1). There were shreds of evidence that limited sensitivity using FDG PET in detecting hepatocellular carcinoma with the false-negative rate approaches 40–50%(3). Thus, the limited diagnostic efficacy of current imaging strategies remains a major challenge in the accurate evaluation of hepatic lesions.

Fibroblast activation protein (FAP) is over-expressed in cancer-associated fibroblasts (CAFs) in several tumor entities, especially in breast, colon and pancreatic carcinomas(4). Recently, its quinoline-based derivatives have been designed into radiopharmaceutical agents(5, 6). FAPI PET demonstrated promising preclinical and clinical results. A few first-in-human studies have found superior tumor-imaging potential of FAPI over FDG PET in different tumor entities(5, 7–9).

Several investigators suggested dynamic PET with kinetic modeling might have potential value in the differential diagnosis other than static quantitative parameters like SUV(10–12). Since the liver has blood...
supply from both the hepatic artery and portal vein, an accurate model is crucial for the following analysis. Few different kinetic models of $^{18}$F-FDG and $^{11}$C-acetate in the liver have been established. In our previous study, we compared several $^{18}$F-FDG dynamic models and introduced a simple two-tissue model using the portal vein solely to differentiate HCC from healthy liver tissues\(^{13}\) accurately.

To our knowledge, the significance of FAPI PET kinetics in the liver has not been reported yet. Since the metabolic process at the cellular level is different between FAPI (binding to FAP and internalization) and FDG (entering glycolysis and accumulation), our previous model\(^{13}\) might not be able to fit FAPI PET kinetics. The objective of this study, therefore, is to establish a kinetic model for $^{68}$Ga-FAPI-04 kinetics in hepatic lesions and to evaluate if the model parameters allow a differentiation between HCC and non-HCC lesions.

**Materials And Methods**

**Patient characteristics**

The study was approved by the Ethics Committee at Peking Union Medical College Hospital. All patients signed informed consent. Eight male patients with 12 available liver lesions (age range, 47-70 y) were recruited in this study. The pathology of these patients was confirmed by surgical resection or by needle biopsy. Among these patients, four patients had been histologically confirmed as hepatocellular carcinoma (HCC), two patients had intrahepatic cholangiocarcinoma, one patient had liver metastasis of gastric cardia adenocarcinoma and one had inflammatory granulomatous.

**PET/CT scan**

PET/CT scans were conducted on a PoleStar m660 PET/CT scanner (SinoUnion Healthcare, Beijing, China) at Peking Union Medical College Hospital (PUMCH)\(^{14}\) for all patients. CT transmission scans (120 kV, 260 effective mA) were performed first for attenuation correction and image fusion. Then, 174-259 MBq $^{68}$Ga-FAPI-04 was administered intravenously and the dynamic PET was performed over the liver region simultaneously. Each PET scan lasted 60 min. Dynamic PET images were reconstructed using a manufacturer (SinoUnion Healthcare, Beijing, China) provided stand-alone advanced research workstation with standard ordered subset expectation maximization (OSEM) algorithm with 2 iterations and 10 subsets. The 120-frame reconstruction protocol consisted of 60 frames of 5s, 10 frames of 30s, and 50 frames of 60s.

**Image analysis**

Delineation of volumes of interest were done on the Hermes Hybrid Viewer tool (Hermes Medical Solutions AB, Stockholm, Sweden). The volumes of interest (VOIs) were drawn manually over all visible lesions and healthy regions within an area far away from any lesion in the liver on the CT image of each
patient. For the image-derived input functions (IDIF), a VOI was drawn within the hepatic artery (here denoted as A) and one in the portal vein (V). Then VOIs were copied to the dynamic PET images. The corresponding concentration time-activity curves (TACs) were extracted. In case of healthy regions, the mean standardized uptake value (SUV) was taken from each region. In case of the lesions and the IDIFs, the voxel with the maximum SUV (SUVmax) was taken, since these values are least affected by partial volume and motion effects (19). Furthermore, the according volume sizes and the uptake of the last five minutes were exported. A representative PET/CT scan is shown in Figure 1.

**Kinetic models**

The kinetic behavior of tracers in the liver is usually described with a model using two input functions(15), since the liver tissue is supplied by both, an arterial and a venous blood input. All IDIFs were fitted with a tri-exponential function starting from the peak maximum and with a linear increase before the maximum. To find the optimum model, three models were applied to all TACs (Figure 2). One reversible two-tissue compartment model “model A” with one input function from the artery using, and second “model V” with one input function from the portal vein. The third “model AV” used both input functions from artery and vein according to the formulas:

\[
\frac{dC_1}{dt} = K_a A(t) + K_v V(t) - (k_2 + k_3) C_1(t) + k_4 C_2(t) \quad \text{Equ. 1}
\]

\[
\frac{dC_2}{dt} = k_3 C_1(t) - k_4 C_2(t) \quad \text{Equ. 2}
\]

with \( C_1(t) \) and \( C_2(t) \) as the hepatic compartment, \( A(t) \) as the artery IDIF and \( V(t) \) as the venous IDIF. Since \( A(t) \) and \( V(t) \) are both contributing to the fraction of blood volume by having a proportion of \( K_a/(K_a+K_v) \) and \( K_v/(K_a+K_v) \) to \( v_B \) (see equ, 3 below), each amount was assessed from their inflow rate constants for the measured compartment \( C_{\text{measured}}(t) \):

\[
C_{\text{measured}} = (1 - v_B)(C_1(t) + C_2(t)) + v_B \left( A(t) \frac{K_a}{(K_a+K_v)} + V(t) \frac{K_v}{(K_a+K_v)} \right) \quad \text{Equ. 3}
\]

with \( v_B \) as the fraction of the measured volume occupied by blood and \( K_a \) and \( K_v \) as the influx rate constant of the aorta and the vein, respectively.

With the rate constants as fit parameters, all model fits were performed according to the least-squares method and optimized with a Levenberg-Marquardt algorithm, implemented to a Java program. Errors of the fit parameters were estimated by calculating the covariance matrix. The residual sum of squares was
calculated for each TAC, as well as the average and standard deviation of all rate constants, furthermore the parameters $V_T = K_1/k_2 (1+k_3/k_4)$ and $V_{ND} = K_1/k_2$ were calculated, with $K_1$ as either $K_a$, $K_v$ or $(K_a+K_v)$ depending on the model. Since to our knowledge no initial parameters are available for FAPI model parameters, every TAC was first fitted with a one-tissue model to obtain values for $K_1$, $k_2$ and $v_B$. For the two-tissue models, these parameters were taken as initial values and a second fit was performed to obtain $k_3$ and $k_4$; note that the latter could become zero thus resulting in a one-tissue model for the according TAC.

**Statistical analysis**

In order to find the optimum model, the Schwartz Criterion (SC) (16) was applied on all models, the percentage of TACs in relation to all TACs showing a model as most preferred was calculated for each model. The ANOVA test was used to find differences between HCC, non-HCC and healthy regions, significant differences between two groups were estimated with the unpaired Student’s t-test. Due to the small sample size, a p-value of less than 0.01 was classified as significant.

**Results**

**Lesion characteristics**

Eight patients with 12 available liver lesions were recruited in this study. The 12 available lesions were separated according to histological results into a group of 7 HCC lesions (group HCC) and a group of 4 other lesions (2 ICC and 2 gastric metastases lesions). One lesion was an inflammation, having a TAC and uptake very similar to healthy tissue, it therefore was treated separately. Lesion characteristics are summarized in Table 1.
Table 1

Standardized uptake value (SUV) and volume size of all investigated lesions, including the preferred model for each lesion. For the healthy region, the average over all regions is presented for SUVmean, size and preferred model.

| Group  | Lesion   | SUVmax | Size [cm³] | Preferred model (Schwartz Criterion) |
|--------|----------|--------|------------|--------------------------------------|
| HCC    | HCC-1    | 2.8    | 6.4        | Model AV                             |
|        | HCC-2    | 4.9    | 1.7        | Model AV                             |
|        | HCC-3    | 6.6    | 2.2        | Model AV                             |
|        | HCC-4    | 14.8   | 20.3       | Model AV                             |
|        | HCC-5    | 6.8    | 0.9        | Model AV                             |
|        | HCC-6    | 8.1    | 1.7        | Model V                              |
|        | HCC-7    | 12.9   | 7.0        | Model A                              |
| non-HCC| ICC-1    | 17.3   | 29.3       | Model V                              |
|        | ICC-2    | 14.8   | 60.7       | Model A                              |
|        | Metas-1  | 10.8   | 16.9       | Model AV                             |
|        | Metas-2  | 9.6    | 4.4        | Model V                              |
|        | Inflammation | 1.3   | 4.8        | Model AV                             |
|        | healthy  | SUVmean| 40.7       | Model AV                             |
|        |          | 0.8    |            |                                      |

**Model selection**

For all TACs, model AV was preferred in 70 % of all cases, in detail in 58 % of all lesions, in 71 % of only HCC lesions and in 88 % of all healthy regions, see Figure 3. All relevant mean parameters derived from model AV are listed in Table 2 for all groups separately. With only one exception, $k_3$ and $k_4$ were zero in case of all healthy regions.
Table 2
Results for the relevant obtained model parameters of model AV for all lesions and healthy regions. The p-value for the ANOVA test for all four groups is also given, results < 0.01 are underlined. Values are presented as mean value plus-minus one standard deviation. Note BP_NP is not presented for healthy regions, since k₃ and k₄ were zero or almost zero.

| Model Parameters | HCC lesions | Non-HCC lesions | Inflammation | Healthy | ANOVA test |
|------------------|-------------|-----------------|--------------|---------|------------|
| $K_a$ [min⁻¹]    | 0.5 ± 0.3   | 0.3 ± 0.3       | 0.0          | 0.5 ± 0.3 | 0.02       |
| $K_v$ [min⁻¹]    | 0.8 ± 0.7   | 0.4 ± 0.5       | 1.6          | 0.6 ± 0.7 | 0.23       |
| $k_2$ [min⁻¹]    | 1.6 ± 0.7   | 0.6 ± 0.2       | 3.6          | 1.5 ± 0.6 | 0.01       |
| $k_3$ [min⁻¹]    | 0.11 ± 0.03 | 0.21 ± 0.09     | 0.01         | 0.01 ± 0.01 | 0.12     |
| $k_4$ [min⁻¹]    | 0.05 ± 0.04 | 0.04 ± 0.02     | 0.03         | 0.01 ± 0.01 | 0.33     |
| $K_i$            | 0.10 ± 0.02 | 0.19 ± 0.09     | 0.08         | 0.01 ± 0.03 | 0.0002 |
| $V_T$            | 3.7 ± 1.4   | 7.6 ± 1.5       | 4.7          | 0.06 ± 0.06 | < 0.0001 |
| $V_{ND}$         | 0.9 ± 0.4   | 1.2 ± 0.3       | 1.0          | 0.8 ± 0.2   | < 0.0001 |
| BP_{ND}          | 3.2 ± 1.3   | 5.6 ± 0.6       | 0.44         |         |            |

Comparison between HCC and non-HCC

No significant differences were found in the SUV uptake according to the ANOVA test between all different groups HCC, non-HCC, inflammation and healthy (p = 0.06), nor between only HCC and non-HCC group (8.1 versus 13.1, p = 0.08), see Figure 4. However, comparing only the SUV uptake from all lesions together (HCC, non-HCC and inflammation) versus the healthy regions, a significant difference was found (9.2 versus 0.8, p = 0.0002). Regarding volume sizes, also no significant differences were found between HCC and non-HCC group (44 cm³ versus 65 cm³, p = 0.06).

Note that for all applied models, the differences in k₃ and k₄ were always significant between lesions and healthy regions, since k₃ and k₄ were equal or close to zero for all models and all healthy regions. For models A and V, there were no significant differences found between HCC and non-HCC lesions in case all rate constants and the macro-parameters $V_T$ (p = 0.08 and p = 0.11, for model A and V, respectively) and $V_{ND}$ (p = 0.73 and p = 0.57). In case of BP_{ND}, a significant difference was found only for model A: 2.9 for HCC and 6.4 for non-HCC, p = 0.0006.

With model AV, significant differences were found between all groups in case of the parameters $V_T$ (p < 0.0001) and $V_{ND}$ (p < 0.0001). Comparing all groups among each other without healthy regions (which
had $k_3$ and $k_4$ of almost or equal zero), a significant difference was also found in $BP_{ND}$ ($p = 0.003$). Comparing only HCC versus non-HCC lesions, the differences were less significant for $V_T$ (3.7 versus 7.6, $p = 0.002$), $V_{ND}$ (0.9 versus 1.2, $p = 0.30$) and $BP_{ND}$ (3.2 versus 5.6, $p = 0.01$).

**Discussion**

No reliable corrections for partial volume or motion effects were available, therefore the values in particular for $K_a$, $K_v$ and $k_2$ may not be taken as real values with a biological meaning. Although these effects are minor in case of the aorta (17, 18), this is certainly not the case for smaller blood vessels like the portal vein. However, to reduce these effects, SUVmax was taken for all IDIFs (19), and also for all lesions due to their partially very small sizes.

Not surprisingly, the preferred model is a model taking venous and arterial input into account, since liver tissue in general is supplied by both (15). Using this model, significant differences could be found between the four investigated groups HCC, non-HCC, inflammation and healthy regions in case of all macro parameters, the binding potential $BP_{ND}$, the total distribution volume $V_T$ and distribution volume of non-displaceable uptake $V_{ND}$. Excluding inflammation and healthy tissue from this analysis, differences were still significant for $BP_{ND}$ and $V_T$, which was not observed for SUV uptake or lesion sizes, suggesting that there are kinetic differences in FAPI kinetic between HCC and non-HCC lesions.

Also not surprising, $k_3$ and $k_4$ were zero for almost all healthy regions, meaning that a reversible one-tissue model described healthy tissue best.

The inflammation lesion was treated separately, since its SUV uptake as well as its curve shape were very similar to healthy tissue having also similar model parameters. A wrong delineation aside from the real lesion due to motion effects can be excluded: a shifting of PET data due to motion effects would most probably affect the whole organ, thus similar curve shapes due to wrong delineations would have been observed also in other, even smaller lesions, which was not the case. Thus, if the delineation was correct, we conclude that FAPI tracer kinetics might be similar to healthy regions in case of inflammation.

A major limitation of this study certainly is the small cohort size. However, this was a first proof-of-concept study showing that IDIFs (with a simple compensation method) are sufficient to derive relevant model parameters from describing FAPI kinetics in liver lesions. Certainly, values like $K_1$ and $k_2$ cannot be taken as biological relevant due to a missing reliable motion and partial volume correction, however, the macro parameters $V_{ND}$, $BP_{ND}$ and mainly $V_T$ show significant differences between all groups. Further studies including a comparison to pathological results, will be conducted.

**Conclusion**

In the present study, we investigated kinetic models for FAPI PET in liver lesions, showing that the consideration of the maximum SUV values for artery and venous image derived input function are
suitable to at least distinguish between healthy regions, HCC lesions and non-HCC lesions in model derived macro parameters.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

BG and HX performed the data collection and analysis, drafted the manuscript. JW helped draft the manuscript. XS participated in the data collection. HZ, MH and XS participated in the design of the study. LH designed the study and revised the manuscript. XL helped revise the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Please contact the corresponding author for data requests.

Ethics approval and consent to participate

All procedures performed in this study were in accordance with the ethical standards of the institution and with the principles of the 1964 Declaration of Helsinki and its later amendments. The study was approved by the Ethics Committee at Peking Union Medical College Hospital. Informed consent was obtained from all participants.

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

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