Intravoxel Incoherent Motion of Colon Cancer Liver Metastases for the Assessment of Response to Antiangiogenic Treatment: Results from a Pilot Study

Ayşegül Öz¹, Sadık Server¹, Bedriye Koyuncu Sökmen¹, Esat Namal², Nagihan İnän¹, Numan Cem Balci³

¹Istanbul Bilim University, Faculty of Medicine, Department of Radiology, ²Istanbul Bilim University, Faculty of Medicine, Department of Medical Oncology, Istanbul, Turkey, ³Department of Radiology, Cleveland Clinic, Lerner School of Medicine, Abu Dhabi, UAE.

Address all correspondence to:
Ayşegül Öz
Istanbul Bilim University,
Faculty of Medicine, Department of Radiology,
Esentepe, Istanbul,
Turkey
E-mail: aoz.ayseguluoz@gmail.com

Short Title: Changes of IVIM Parameters During Antiangiogenic Treatment

Key words: Colorectal carcinoma metastasis · MRI · Bevacizumab · IVIM · DWI
Highlights of the Study

- Intravoxel Incoherent Motion is a diffusion imaging method which is being used clinically for the evaluation of tissue perfusion without the use of contrast media.
- The $f$ (perfusion fraction) value of Intravoxel Incoherent Motion may quantitatively reflect the response of antiangiogenic therapy.
- Intravoxel Incoherent Motion could be added in routine protocols for Magnetic Resonance Imaging to assess response of tumours to antiangiogenic agents.
Abstract

**Objective:** This study was aimed at evaluating the Intravoxel Incoherent Motion (IVIM) parameter alterations of liver metastases of colorectal carcinoma (CRC) during antiangiogenic bevacizumab combination therapy. **Methods:** Twenty-five patients with CRC liver metastases treated with bevacizumab in combination with FOLFOX-or-FOLFIRI protocols were enrolled in the study. MRI was performed in a 1.5T scanner pre-treatment (PT) and at the 3rd, 6th and 9th months of the therapy. Routine abdominal MRI sequences and an IVIM-DWI (diffusion weighted imaging) sequence were obtained. IVIM-DWI sequence was executed with 16 b-values varying from 0-to-1400s/mm². The mean values of apparent diffusion coefficient (ADC), true diffusion (D), pseudo-diffusion (D*), and perfusion fraction (f) of each metastasis were obtained for all b-values and the time-related changes were recorded to analyze the chronologic responses to antiangiogenic therapy. The RECIST1.1 criteria were used for the evaluation of treatment response. **Results:** The diameters of the metastases diminished significantly at 9th month when compared with PT (p = 0.03). D (p = 0.10) and ADC (p = 0.21) values of the metastases increased at the 9th month of therapy. D* was the highest at 3rd month (p = 0.24), and decreased at the 6th (p = 0.97) and 9th months (p = 0.87) of therapy. The f had the highest peak at 3rd month (p = 0.51), and started to decrease after 3rd month. At the 6th month f decreased to lowest values (p = 0.12). **Conclusion:** IVIM parameters, particularly the perfusion fraction, may quantitatively reflect the response of antiangiogenic treatment. The antiangiogenic response manifests after the 3rd month of the therapy before the RECIST-related response.
Introduction

Colorectal carcinoma (CRC) is the second most common cause of cancer death, and its most frequent distant metastatic target organ is the liver. The treatment of metastatic CRC (mCRC) has improved in the last 20 years. Thirion et al. [1] have reported that the addition of leucovorin to 5-fluorouracil (5-FU) increased overall survival to 12 months in mCRC patients. This protocol and its combinations have become the cornerstones for development of new protocols for such patients [1]. Oxaliplatin and irinotecan combinations, known as FOLFOX and FOLFIRI protocols, improved the overall survival to 16 - 18 months. Moreover, targeted antiangiogenic agents have been added to these basic cornerstone protocols, increasing the survival by up to 24 - 30 months [2]. Bevacizumab, widely used for mCRC treatment, is a targeted antiangiogenic agent; it is an antibody to vascular endothelial growth factor (VEGF). Its multiple mechanisms of action result in the inhibition of new vessel growth [3].

The response of mCRC patients to treatment can be evaluated by patient’s physical examination, carcinoembryonic antigen (CEA) levels if initially elevated, and various imaging techniques. Non-invasive imaging techniques play an important role to evaluate tumour responses during antiangiogenic therapies [4].

As antiangiogenic therapy mainly acts on the tumour vasculature, change in tumour size requires a long time. Therefore, biomarkers other than size are needed to assess the early response to therapy. The response to antiangiogenic therapy has been assessed with the use of contrast-enhanced imaging techniques both on CT and MRI [5]. However, contrast-induced nephropathy and nephrogenic systemic fibrosis are major risk factors in patients with diminished renal function. The cancer patients are particularly under risk of contrast-induced renal impairment [6].

Diffusion-weighted magnetic resonance imaging (DWI) has become a preferential
diagnostic tool for the evaluation of liver lesions and parenchymal changes [7]. DWI reflects the water proton mobility in a voxel and provides information about cell microstructure, microcirculation, cell membrane integrity and cell viability [3, 7]. DWI measures the random motion of water molecules in the tumour. When the motion of water molecules is restricted due to high cellularity in the environment, their diffusion is also restricted. After the treatment, the cellularity of the tumour decreases and in some cases necrosis occurs; these changes bring about an increase in the free motion of water molecules and an increase in their diffusion. DWI and apparent diffusion coefficient (ADC) maps are also affected by tissue perfusion of the microvascular capillary [8]. The Intravoxel Incoherent Motion (IVIM) theory of Le Bihan et al. [9] states that increasing the number of b-values leads to a multi-exponential signal drop within each voxel and enables quantitative parameters that measures tissue microcapillary perfusion. This helps to differentiate between true molecular diffusion and microcapillary perfusion [9], and thus is helpful for the diagnosis and the follow-up of the hypervascular lesions. As tumour perfusion changes after antiangiogenic treatments, low and high b-values reflect the changes in microcapillary tissue perfusion and also diffusivity without the need of contrast agent use. The perfusion-sensitive parameters perfusion fraction (f) and pseudodiffusion coefficient (D*), and the diffusion-sensitive parameter, true diffusion coefficient (D) can be measured and mapped separately with biexponential analysis [10]. The perfusion parameters are also obtained by signal intensity (SI) curve biexponential analysis with increasing b-values by the same technique [8].

Bevacizumab therapy is widely used for brain gliomas, kidney, lung, colon, rectum, cervix, ovary and fallopian tube cancers. Recent studies also investigated the changes of IVIM parameters and tumor response in non-small cell lung cancer and gliomas [11, 12]. The value of IVIM for the assessment of the effectiveness of the early treatment has been published in a few reports. In this pilot study, which, to the best of our knowledge is the first, we aimed to
evaluate time-related tumour response to long-term antiangiogenic bevacizumab therapy combined with FOLFOX or FOLFIRI protocols in unresectable mCRC patients with liver metastasis using IVIM-DWI parameters, to also determine the optimal time interval between the onset of therapy and assessment of antiangiogenic affect with the use of IVIM parameters.

**Subjects and Methods**

This study was carried out between March 2016 and August 2018. The single-centre, open-label, non-randomized, prospective study protocol was approved by the Institutional Review Board, Clinical Research Ethics Committee for human investigations. The study was conducted according to the principles of the Declaration of Helsinki, and the International Conference on Harmonization – Good Clinical Practice. All participants provided written informed consent before they were included in the study.

**Patients**

12 patients diagnosed as unresectable CRC with 25 liver metastases were enrolled in the study. Three patients (seven metastases) with poor diagnostic imaging quality due to motion artefacts were excluded from the study. One patient (two metastases) was lost to follow-up. Finally, eight patients (three females and five males) with a total of 16 metastases were included in this study. The mean age was 60 years, range between 41 - 73 years. All patients were treated for a total of nine months by FOLFOX or FOLFIRI protocols combined with bevacizumab therapy.

**MR Imaging**

All patients were underwent MRI before the treatment and then on the 3rd, 6th and 9th months after the start of the therapy. All MR images were obtained in a Siemens, Magnetom Symphony 1.5T scanner (Erlangen, Germany) with a four-channel body coil. Routine pre-contrast upper abdomen MR images were acquired with the use of following imaging
sequences: axial in and out-of-phase (TR:179, TE:2.4-4.8, FA:70) T1 weighted (W) turbo field echo (TFE), axial and coronal T2W single-shot turbo spin echo (SSh-TSE) (TR:1350, TE:92, TSEfactor:281, NEX:1). Subsequently, respiratory-triggered IVIM and conventional DWI sequences were obtained with single-shot echo-planar imaging (SSh-EPI) sequence: IVIM with 16 different b-values of 0 - 100 (average 1), 200 - 300 (average 2), 400 - 800 (average 4), 900-1000 (average 6), 1100 (average 1), 1200 - 1400 (average 6) s/mm². Three orthogonal diffusion-encoding directions were acquired.

T1W Volumetric Interpolated Breath-hold Examination (VIBE) (TR:4.86, TE:2.39, NEX:1, FA:10.0) sequences were obtained after administration of I.V. gadoterate meglumine (Gd-DOTA) 0.2ml/kg (Dotarem, Guerbet LLC, New Jersey, USA). Post-contrast axial dynamic arterial, portal, venous and delayed phase images with fat suppression via the spectral adiabatic inversion recovery (SPAIR) method were performed. The acquisition time for IVIM sequence was 10 ± 3 min, and 40 - 45 min for the total upper abdominal MRI. The images were acquired with a 5 mm/1 mm slice thickness/intersection gap. Imaging matrix was 115×192. The field of view (FOV) varied from 240 to 380 mm (Fig. 1).

Image Analysis

Two radiologists (A.O. and N.I. with 12 and 16 years of experiences respectively in the evaluation of liver MRI) performed blinded assessment for the Response Evaluation Criteria in Solid Tumours (RECIST) analysis. After the longest diameters of the lesions were measured in the axial post-contrast images, evaluation was done according to RECIST guideline (version 1.1) [13]. The tumour response was classified as Partial Response (PR) when at least 30% decrease in the sum of diameters of target lesions was detected. Tumour response was defined as Progressive Disease (PD) when at least 20% increase in the smallest sum of diameters of target lesions with an absolute increase of at least 5 mm or appearance of new lesion was
determined. If the smallest sum of the diameters were not increased enough to classify as PD or decreased enough to classify as PR it was accepted as Stable Disease (SD).

A single radiologist (A. O.) performed the blinded quantitative analysis with the dedicated software (AW Volume Share, GE, USA). ADC map was created automatically from conventional DWI using all 16 b-values. For the evaluation of IVIM values, the SI was measured from all 16 different b-values. Lesions were identified for each sequence. ROIs were positioned in a correlated location between DWI and IVIM scans, covering at least 2/3 of the volume of a single lesion, avoiding from the vascular and biliary structures. The same ROI was propagated to all 16 b-values. The mean size of ROI’s was 9.39 cm² (range: 0.75-54.86 cm²) for lesions and 4.16 cm² (range 3.71-4.81 cm²) for normal parenchyma.

For quantitative measurement of IVIM, the SI were calculated from every 16 b-value between 0 - 1400 s/mm². The following formula was used;

\[ \frac{SI(b)}{SI(0)} = f \cdot \exp(-b \cdot D^*) + (1-f) \cdot \exp(-b \cdot D) \]

D diffusion coefficient, D* pseudodiffusion coefficient associated with blood flow, f the perfusion fraction.

D, D* and f values were postprocessed using a free specific software program from the website [14].

Statistical Evaluation

The lesions were compared to assess the effect of the IVIM parameters at PT, 3rd, 6th and 9th months of the therapy were compared to determine how these parameters change during the antiangiogenic therapy. The goodness of fit of the data to a normal distribution was identified by the Kolmogorov–Smirnov test. The differences in the IVIM parameters and changes in these parameters during the therapy were determined by independent samples t test. All of the statistical evaluations were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) statistical software.
Results

Of the 8 patients with 16 liver metastases included in the study, four patients had SD (2 adenocarcinoma lesion, 2 musinous carcinoma lesion), 3 had PR (11 adenocarcinoma lesion, 2 musinous carcinoma lesion), and 1 had PD (1 musinous carcinoma lesion) according to the RECIST 1.1 criteria [13]. Five patients were male, and the mean age was 60 years (range 41-73 years). Thirteen lesion were adenocarcinoma metastases (11 lesions, 3 lesions were musinous carcinoma metastases.

Results of the quantitative analysis of IVIM parameters and comparison of PT values with post-treatment values after 3, 6 and 9 months are presented in the Table 1 and Figure 2a. The diameters of the metastases were statistically significant diminished at 9th month when compared with PT ($p = 0.03$). Conventional ADC and D values of the metastases increased at the 9th month compared with the PT values ($p = 0.10$, and $p = 0.21$, respectively). $D^*$ value was the highest at 3rd month ($p = 0.24$), and a decrease was found at 6th, and 9th months of therapy ($p = 0.97$, and $p = 0.87$, respectively). The f value had the highest peak at 3rd month ($p = 0.51$), started to decrease after 3rd month. At the 6th month f decreased to lowest values ($p = 0.12$) (Figure 2b). SI measurements were lowest on the 6th month of therapy. IVIM parameter trends for the different disease response groups are summarized in the Table 2.

Discussion

The results of this initial data showed the role of functional IVIM in the assessment of antiangiogenic therapy response without using intravenous contrast agent.

Most studies evaluating the short-term effects of antiangiogenic therapy have shown that successful treatment is reflected by an increase in tumour ADC values [3]. Cui et al. reported that ADC values of responder liver metastases increases after one week of chemotherapy [15]. Significant ADC elevation in responding metastases after long-term
therapies has been shown [16]. Granata et al. evaluated the early response to bevacizumab therapy on the 14th day of therapy, with decreased f value prior to ADC changes [8]. In our study, ADC and D values of the liver metastases increased 9 months after the onset of therapy, reflecting the long-term therapy-induced necrosis within the metastases.

Lower perfusion fraction (f) and lower D* values compared to normal liver parenchyma have been described for malignant lesions. f and D* values have been shown to be significantly correlated with the microvessel density score and microenvironment of the tumour and provide information on tumour perfusion, and that lower f values in tumours correlate with lower histological microvessel density [17]. It has been reported that low D* values are associated with immature and leaky blood vessels, causing high lesional interstitial fluid pressure, resulting in stagnant blood flow [18]. In our study, f and D* values started to decrease after 3 months of therapy. This might suggest that in the first 3 months, normal vascularization caused by antiangiogenic effects of therapy was accomplished, and that after 3 months the destruction of the normal vascularization had started.

Both cellular apoptosis and vascular perfusion changes after chemotherapy affects the lesion size [19]. In our study, the diameters of the metastasis were significantly smaller after 9 months from the start of the therapy when compared to PT. According to the RECIST 1.1 criteria, three patients had PR, four patients had SD and one patient had PD. The patient with PD and two patients with SD had mucinous subtype carcinoma. The relationship between mucinous carcinomas and the evaluation of antiangiogenic therapy warrants further investigation, as our study did not allow this due to small number of included patients.

Also, the number and the values of the b, affects the quality of the IVIM parameters. Recent abdominopelvic IVIM studies have used b-values between 3 to 35 as there is no standardized protocol [20]. The use of increased number of b-values leads to extended
acquisition time and motion artefacts. Respiratory triggered sequences can reduce the respiratory motion artefacts [21]. Although we used respiratory navigation, we excluded three patients due to motion related artefacts. Dyvorne et al. used only four b-values (0, 15, 150, and 800) and reduced the scan time by up to 75% and reduced motion related artefacts [22].

Limitations of our study include small number of patients, imaging sequence related distortions and limitations in the configuration of IVIM mathematical model. The intra- and inter-observer variability was not assessed. In a recent study of Sun H. et al. in rectal cancer patients, there was no significant intra or interobserver difference detected in the measurement of IVIM parameters of the same DWI scan [23].

**Conclusion**

IVIM is a diffusion imaging method which is being used clinically for evaluation of tissue perfusion without the use of contrast media. We suggest that IVIM could be added in routine MRI protocols in unresectable mCRC patients with liver metastasis to assess the tumour response to antiangiogenic agents. The f value of IVIM may reflect the response of antiangiogenic therapy quantitatively at earliest interval. The effects of antiangiogenic therapy became measurable with IVIM after three months of therapy. The evaluation of the performance of IVIM in other types of cancer with different organ metastases merit further investigation on larger populations.
Statement of Ethics

This research complies with the guidelines for human studies and should include evidence and the research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Disclosure Statement: All authors declare that they have no conflicts of interest.

Funding Sources: The study had no sponsors or funding source.

Author Contributions

Conception and design of the work, acquisition, analysis and interpretation of data: Oz A, Server S, Sokmen BK, Namal E, Inan N, NC Balcı. Drafting the manuscript and critical revision for intellectual content; Oz A, Inan N, NC Balcı. Final perusal approval of the manuscript; Oz A, Inan N, NC Balcı.
References


**Table 1.** Quantitative analysis of IVIM parameters and comparison of pre-treatment values with post-treatment values after 3, 6, and 9 months of the 16 liver metastases

<table>
<thead>
<tr>
<th></th>
<th>ADC (10^-3 mm²/s)</th>
<th>D* (10^-3 mm²/s)</th>
<th>D (10^-3 mm²/s)</th>
<th>f</th>
<th>Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>p</td>
<td>Mean</td>
<td>p</td>
<td>Mean</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>Normal parenchyma</td>
<td>0.63±0.13</td>
<td>-</td>
<td>230.63±33.12</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Metastasis</td>
<td>0.99±0.28</td>
<td>-</td>
<td>1.71±0.92</td>
<td>-</td>
</tr>
<tr>
<td>3rd month</td>
<td>Normal parenchyma</td>
<td>0.62±0.11</td>
<td>-</td>
<td>252.78±53.47</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Metastasis</td>
<td>1.01±0.39</td>
<td>0.849</td>
<td>2.10±0.90</td>
<td>0.248</td>
</tr>
<tr>
<td>6th month</td>
<td>Normal parenchyma</td>
<td>0.61±0.15</td>
<td>-</td>
<td>274.71±63.40</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Metastasis</td>
<td>0.97±0.30</td>
<td>0.903</td>
<td>1.69±1.17</td>
<td>0.970</td>
</tr>
<tr>
<td>9th month</td>
<td>Normal parenchyma</td>
<td>0.84±0.22</td>
<td>-</td>
<td>97.97±15.39</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Metastasis</td>
<td>1.14±0.18</td>
<td>0.107</td>
<td>1.76±0.90</td>
<td>0.872</td>
</tr>
</tbody>
</table>

ADC, Apparent Diffusion Coefficient; IVIM, Intravoxel Incoherent Motion; f, perfusion fraction; D*, pseudodiffusion coefficient; D, true diffusion coefficient
Table 2. Quantitative analysis of IVIM parameters for the different disease response groups. Pre-treatment values with post-treatment values after 3, 6, and 9 months of the liver metastases

<table>
<thead>
<tr>
<th>Disease Group</th>
<th>ADC (10-3mm²/s) Mean</th>
<th>D* (10-3mm²/s) Mean</th>
<th>D (10-3mm²/s) Mean</th>
<th>f Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment</td>
<td>PR 0.87 ± 0.40</td>
<td>1.42 ± 2.96</td>
<td>0.86 ± 0.43</td>
<td>0.60 ± 3.06</td>
</tr>
<tr>
<td></td>
<td>SD 1.35 ± 0.32</td>
<td>1.53 ± 0.51</td>
<td>1.42 ± 0.48</td>
<td>1.14 ± 2.23</td>
</tr>
<tr>
<td></td>
<td>PD 0.84</td>
<td>0.59</td>
<td>0.84</td>
<td>0.0063</td>
</tr>
<tr>
<td>3rd month</td>
<td>PR 0.92 ± 0.90</td>
<td>2.50 ± 7.77</td>
<td>0.83 ± 0.99</td>
<td>1.42 ± 2.96</td>
</tr>
<tr>
<td></td>
<td>SD 1.50 ± 0.12</td>
<td>2.02 ± 0.64</td>
<td>1.50 ± 0.12</td>
<td>1.27 ± 1.26</td>
</tr>
<tr>
<td></td>
<td>PD 1</td>
<td>0.97</td>
<td>0.99</td>
<td>0.66</td>
</tr>
<tr>
<td>6th month</td>
<td>PR 0.87 ± 54</td>
<td>1.91 ± 2.47</td>
<td>0.87 ± 54</td>
<td>0.34 ± 2.21</td>
</tr>
<tr>
<td></td>
<td>SD 1.35 ± 0.14</td>
<td>1.71 ± 0.28</td>
<td>1.34 ± 0.14</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>PD -</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9th month</td>
<td>PR 1.13 ± 0.26</td>
<td>1.98 ± 5.01</td>
<td>1.12 ± 0.27</td>
<td>0.75 ± 1.41</td>
</tr>
<tr>
<td></td>
<td>SD 1.19 ± 0.05</td>
<td>0.75 ± 0.11</td>
<td>1.17 ± 0.06</td>
<td>0.02 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>PD -</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

ADC, Apparent Diffusion Coefficient; IVIM, Intravoxel Incoherent Motion; f, perfusion fraction; D*, pseudodiffusion coefficient; D, true diffusion coefficient; PR, Partial Response; SD, Stable Disease; PD, Progressive Disease
Figure Legends

**Figure 1.** Sixty-six-years old female patient with stage IV metastatic colorectal carcinoma.

**Figures a, b, c, and d;** the liver metastases on T2 weighted axial images at PT, 3\textsuperscript{rd}, 6\textsuperscript{th}, and 9\textsuperscript{th} months of therapy, respectively. **Figures e, f, g, and h;** the ADC maps of the lesion. **Figure i, j, k, and l;** the IVIM images of the lesion with 16 different b values (from 0 to 1400 s/mm\textsuperscript{2}) at PT, 3\textsuperscript{rd}, 6\textsuperscript{th}, and 9\textsuperscript{th} months of therapy.

**Figure 2. a.** Time related measurements of ivim parameters b. Time related mean value of SI measurements with increasing b-values

PT, pre-treatment; M3, 3\textsuperscript{rd} month; M6, 6\textsuperscript{th} month; M9, 9th month.
Fig. 1