# Relation between dynamic Gadolinium uptake rate, tumor vasculature and tumor hypoxia in human colorectal liver metastases

H. van Laarhoven[1], J. Lok[2], M. Rijpkema[3], C. Punt[4], T. Ruers[4], J. Kaanders[4], A. van der Kogel[4], A. Heerschap[4]

[1]Medical Oncology, UMC Nijmegen, Nijmegen, Netherlands, [2]Radiation Oncology, UMC Nijmegen, Nijmegen, Netherlands, [3]Radiology, UMC Nijmegen, Nijmegen, Netherlands, [4]UMC Nijmegen, Nijmegen, Netherlands

**Introduction:** Colorectal cancer is one of the most common cancers in the western world and approximately half of the patients will develop distant metastases. Despite advances in surgery and chemotherapy, prognosis of metastasized colorectal carcinoma is still poor. Chemotherapy is effective only in a subgroup of patients. Therefore, early selection of patients who could benefit from chemotherapy is desirable. Since delivery of chemotherapy is dependent on tumor vasculature, dynamic contrast enhanced MRI (DCE-MRI) may provide a useful non-invasive measure for the prediction of treatment outcome and the follow up of therapy. To validate DCE-MRI as a method to characterize tumor vasculature we investigated the relation between dynamic Gadolinium uptake rate measured by DCE-MRI with tumor vasculature and tumor hypoxia measured immunohistochemically in colorectal liver metastases.

**Patients and methods:** DCE-MRI was performed on a 1.5 T Siemens Vision MR system in 19 patients with colorectal liver metastases. All patients were scheduled for resection of the liver metastasis. The patients gave written informed consent and the study was approved by the local ethical committee. 15 ml 0.5M Gadolinium-DTPA (Gd-DTPA, Magnevist®, Schering, Berlin, Germany) was administered intravenously in 6 seconds by a Spectris™ MR injection system (Medrad, Inc.). Using a T1-weighted fast low-angle shot (FLASH) sequence with a time resolution of 2 seconds Gd-DTPA uptake in the tumor and the bolus passage in vessels in the spleen was monitored (TR 50 ms, TE 4.4 ms, flip angle 90°, slice thickness 7 mm, 4 slices, matrix 160x256, FoV 263x350, acquisition time 90 s). Analysis of DCE-MRI data has been described previously (1).In brief, we obtained a vascular normalization function from pixels in the spleen using an automated algorithm based on the concentration of Gd-DTPA (high in blood vessels) and time to bolus passage (short in arteries). Using a physiological pharmacokinetic model (2) the Gd-DTPA uptake rate $k_{ep}$ was calculated per slice. The spatial distribution of $k_{ep}$ was represented in a map. A region of interest (ROI) was applied to the map of Gd-DTPA uptake rate in order to obtain single values of $k_{ep}$ for all tumor pixels. The geometric mean of the Gd-DTPA uptake rate of these pixels was calculated after log transformation and averaged over all slices containing tumor tissue, resulting in an average $k_{ep}$ value (s-1) for the whole tumor.
To determine tumor hypoxia the hypoxic marker pimonidazole was injected in each patient intravenously 12 hours before resection of the liver metastases. Directly after resection a 3 mm transversal slice of the liver metastasis was cut by the pathologist corresponding to the region where DCE-MRI slices were taken. Of this slice 4 sections from the rim and one section from the center were taken (approximately 5x5 mm). Sections were snap-frozen in Isopentane precooled in liquid nitrogen and stored at –80 °C until use. Frozen tumor sections of 5 µm thickness were cut for immunohistochemical staining of vasculature and tumor hypoxia which was analyzed quantitatively using a semiautomatic method based on a computerized digital image analysis system, as described previously (3). In brief, a high-resolution intensified solid-state camera on a fluorescence microscope with a computer-controlled motorized stepping stage was used to scan each tumor cross-section. Whole tumor sections were scanned at 100 magnification. Each scan consisted of 36 - 144 fields of 1.2 $mm^2$, depending on the size of the tumor section. As a final step a contour line was drawn to delineate the tumor area thereby excluding non-tumor tissue from the analysis, using the consecutive H&E stained tumor section to distinguish parenchyma from non-tumor tissue. The vascular density (VD) was then calculated as total number of vascular structures per $mm^2$ of viable tumor area and the hypoxic fraction (HF) as the tissue surface area stained by the hypoxic marker relative to the viable tumor surface area.

**Results & Discussion:** On the T1-weighted MR image recorded just before Gd-DTPA administration the metastases could easily be detected in all patients. In figure 1A a T1-weighted MR image is shown for one patient. On this image a region of interest was drawn to delineate the tumor, which was then applied to the $k_{ep}$-map of Gd-DTPA uptake (figure 1B) to select the single values of $k_{ep}$ for all tumor pixels. Values of $k_{ep}$ for the whole tumor ranged from 0.016 – 0.059 $s^{-1}$ (mean 0.032 ± 0.013 $s^{-1}$). Figure 2 shows a composite image of a tumor section stained for vascular structures and hypoxia. Hypoxic areas were detected at a certain distance from the blood vessels, indicating that hypoxia in these tumors was mostly diffusion limited. Vascular density ranged from 5.86-46.04 $mm^{-2}$ (mean 25.83 ± 10.22 $mm^{-2}$); hypoxic fractions varied from 0.001 – 0.264 (mean 0.128 ± 0.060). A significant positive correlation between vascular density and Gd-DTPA uptake rate $k_{ep}$ was found (Pearson's $r = 0.45$; $p<0.05$) (figure 3). No correlation ($p>0.05$) between hypoxic fraction and Gd-DTPA uptake rate $k_{ep}$ was observed, suggesting that hypoxia indeed is diffusion limited rather than perfusion limited. According to Larsson's model of $k_{ep}$ no distinction can be made between tumor blood flow or the PS product (*p*ermeability of perfused capillaries and the total surfacearea of perfused capillaries). However, the observed positive correlation between vascular density and the Gd-DTPA uptake rate $k_{ep}$ suggests that differences in values of $k_{ep}$ mainly depend on variations in the PS product rather than on variations in tumor blood flow. This is in accordance with pre-clinical studies in a rat glioma model which showed that the Gd-DTPA uptake rate is determined by the perfused vascular surface area (4).

**Conclusion:** A positive correlation was demonstrated between the Gd-DTPA uptake rate $k_{ep}$ and vascular density, indicating that kinetic parameters of DCE-MRI provide a valuable tool for the *in vivo* assessment of tumor vasculature in colorectal liver metastases. No correlation between the Gd-DTPA uptake rate $k_{ep}$ and tumor hypoxia was observed.

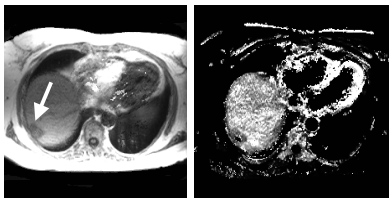

*Fig. 1 T1 weighted image of the upper abdomen (A) and the corresponding map of Gd-DTPA uptake rate $k_{ep}$ (B). The liver metastasis is indicated with an arrow.*

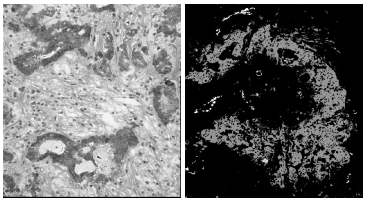

*Fig. 2 H&E staining of a section of a liver metastasis (A) and the corresponding scan of hypoxia (grey) and vascular structures (white) (B).*

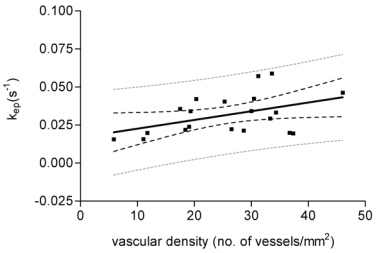


*Fig. 3 Relation between Gd-DTPA uptake rate $k_{ep}$ and vascular density. The regression line (black solid line), 95% regression interval (black dotted lines) and the 95% prediction interval (grey dotted lines) are indicated.*

**References:**
1. van Laarhoven et al., *JMRI* 2003:315.
2. Larsson et al., *MRM* 1990:117.
3. Rijken et al., *Microvasc.Res.* 1995:141.
4. van der Sanden et al., *J.Cereb.Blood Flow Metab* 2000:861.