Dissertation

Volume CT Perfusion (VCTP) Imaging in Evaluation of the Significance in Oncologic Follow-up: Imaging of Metastasizing RCC - Comparison between changes in Perfusion and Changes in Size in the early period of targeted therapy

Submitted by

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Abbreviations

ALARA	As low as reasonably achievable
BF	Blood flow
BV	Blood volume
CA4P	Combretastatin A4 phosphate
CTX	Chemotherapy
CL	Clearance
CR	Complete response
CRC	Colorectal cancer
CT	Computed tomography
CTA	Computed tomography angiography
CTP	Computer Tomography Perfusion
DCE	Dynamic contrast enhanced
DLP	Dose length product, $P_{\mathrm{KL,CT}}$
E	Effective dose
eNOS	Endothelial nitric oxide synthase 3
ESMO	European Society for Medical Oncology
FDG	Fluorodeoxyglucose
FE	Blood flow extraction product
FOV	Field-of-view
GIST	Gastrointestinal stromal tumor
HAFt	Hepatic arterial flow in tumor lesion
HCC	Hepatocellular carcinoma

HIF	Hypoxia-inducible factor
HIF1α	Hypoxia-inducible factor 1-alpha
HNSCC	Head and neck squamous cell carcinoma
HPt	hepatic perfusion in target tumor lesion
I	Tube current
ICRP	International Commission for Radiological Protection
IQR	Interquartile range
IRF	Impulse residue function
LD	longest tumor diameter
L-NNA	Nitric oxide sinthetase inhibitor
MASS	Morphology, Attenuation, Size, and Structure
MEDI522	Monoclonal antibody targeting integrin avB3
mRCC	metastatic renal cell carcinoma
MRI	Magnetic resonance imaging
MTT	Mean transit time
MVD	Micro vessel density
NET	Neuroendocrine tumor
NO	Nitric oxide
NSCLC	Non-small-cell lung carcinoma
p	pitch
PD	Progressive disease
PEI	Peak enhancement intensity
PET	Positron emission tomography
PI	Perfusion index

PR	Partial response
PS	Permeability surface area product
Q	Tube current-exposure time product
RCC	Renal cell carcinoma
RECIST	Response Evaluation Criteria in Solid Tumors
RMSD	Root-mean-square deviation
ROI	Region of interest
RT	Radiotherapy
SACT	Size and Attenuation CT
SD	Stable disease
TDC	Time-density curve
TEF	Tumor extravascular flow
TF	Table feed
TTP	Disease progression time
TVV	Tumor vascular volume
U	Tube voltage
US	Ultrasound
VCTP	Volume Computer Tomography Perfusion
VEGF	Vascular endothelial growth factors
VHL	Von Hippel-Lindau
WHO	World Health Organization

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Abstract

Introduction: Since 2004 when first antiangiogenic agent was approved for the treatment of colorectal cancer, oncologic treatment in many solid tumors has changed. Main targets for oncologic therapy have been switched from heterogeneous proliferating tumor cells to more homogenous tumor vasculature targeted by antiangiogenic agents. Because of different mechanism of action and due to different pattern of treatment response at imaging during follow-up, a current challenge is to find appropriate surrogate biomarkers of the tumor vasculature for appropriate follow up of tumor response to antiangiogenic treatment. Recent work has led to development of non-invasive quantitative dynamic imaging techniques, such as CT perfusion (CTP) that assess early changes in tumor angiogenesis, therewith tumor physiology.

The aim of this thesis was to analyze whether volume CT perfusion (VCTP) imaging of tumors treated with targeted therapy may allow for earlier response assessment than conventional CT imaging with size measurements. An additional aim was to analyze, whether there was any evidence of association between perfusion parameters and clinical outcome.

Material and Methods: VCTP imaging was performed in ten patients with histologically verified metastasizing renal cell carcinoma (RCC) before and one month after initiation of targeted therapy using a 320-slice Volume CT scanner. Blood flow (BF), blood volume (BV) and clearance (CL) were calculated for both time points using compartmental analysis algorithms. In addition, the longest tumor diameter (LD) was measured. Perfusion parameters and LD before and after treatment were compared, and their relative change was correlated against disease progression time (TTP) and associated to tumor response.

Results: For patients who responded to therapy, perfusion parameters have significantly decreased; BF by -37.5%, BV by -28.9% and CL by -50.8% on average. Decrease in tumor size was -9.4%. For non-responders, change in perfusion parameters was inconsistent. Overview of individual cases showed that relative change of BV was the most important perfusion parameter, with highest difference between responders and non-responders (90%). Other parameters increased specificity of perfusion imaging. The most valuable predictor of

TTP for patients who responded to therapy was the relative change of BF, with lowest relative deviation from true value of TTP, confirmed by multiple linear regression analysis.

Conclusion: In the early period after targeted therapy VCTP may be able to discriminate responders from non-responders, sooner than the currently used changes in tumor size. Different percentage change of all three parameters indicated that the therapy has probably affected the physiological processes of angiogenesis in varying degrees and, therefore, all perfusion parameters may have to be considered independently of one another. A decrease of all three perfusion parameters, no matter on percentage change, may be interpreted as positive response to antiangiogenic therapy. Relative change of BV was the most important parameter for detection of non-responders, whereas use of other parameters increased the CTP specificity. In addition, relative change of BF may be a promising parameter for prediction of TTP.

Key words: Volume Computed Tomography Perfusion (VCTP), tumor angiogenesis, response to treatment

Zusammenfassung

Einleitung: Seit 2004, als das erste angiogenesehemmende Mittel zur Behandlung von Darmkrebs zugelassen wurde, hat sich die onkologische Behandlung bei vielen Tumoren geändert. Die Hauptziele der onkologischen Therapie wurden von heterogenen, proliferierenden Tumorzellen auf homogenere Tumorgefäße umgestellt, auf welche die angiogenesehemmenden Wirkstoffe angreifen. Aufgrund des unterschiedlichen Wirkungsmechanismus und des unterschiedlichen Reaktionsmusters in bildgebenden Verfahren während des Follow-up, besteht eine radiologische Herausforderung einen geeigneten Biomarker für das Ansprechen zu finden. Rezente Arbeiten haben zur Entwicklung funktioneller Bildgebungstechniken wie der CT-Perfusion (CTP) geführt, mit denen Veränderungen der Tumorangiogenese und damit der Tumorphysiologie bewertet werden können.

Das Ziel dieser Dissertation ist zu evaluieren, ob die Volumen-CTP (VCTP) das Tumoransprechen auf zielgerichtete Therapie früher als die konventionelle CT-Bildgebung mit Größenmessungen beurteilen kann und ob die Perfusionsänderung als Indikator für das objektive Ansprechen des Patienten verwendet werden kann. Zusätzliches Ziel war zu analysieren, ob ein Zusammenhang zwischen Perfusionsparametern und klinischem Outcome besteht.

Material und Methoden: Die VCTP wurde bei zehn Patientinnen und Patienten mit histologisch verifiziertem metastasierendem Nierenzellkarzinom (RCC) vor und einen Monat nach Beginn der gezielten Therapie mit einem 320-Schicht-Volumen-CT-Scanner durchgeführt. Der Blutfluss (BF), das Blutvolumen (BV) und die Clearance (CL) wurden für beide Zeitpunkte unter Verwendung von Kompartimentanalyse-Algorithmen berechnet. Zusätzlich wurde der längste Tumordurchmesser (LD) gemessen. Die Perfusionsparameter und der LD vor und nach der Behandlung wurden verglichen und ihre relative Änderung wurde mit der Krankheitsprogressionszeit (Time to Progression - TTP) korreliert und mit der Tumorantwort in Verbindung gebracht. Der Vorhersagewert der Parameter wurde mit einer multiplen linearen Regressionsanalyse getestet.

Ergebnisse: Bei Patientinnen und Patienten, die auf die Therapie ansprachen, waren die Perfusionsparameter signifikant zurückgegangen, BF -37,5%, BV -28,9% und CL -50,8%

im Durchschnitt. Die Abnahme der Tumorgröße war -9,4%. Bei Non-Respondern war die Änderung der Perfusionsparameter inkonsistent. Eine Übersicht der Einzelfälle hat gezeigt, dass die relative Veränderung des BV der wichtigste Perfusionsparameter war, wobei der größte Unterschied zwischen Respondern und Non-Respondern besteht (90%). Andere Parameter erhöhten die Spezifität der Perfusionsbildgebung. Der wertvollste Prädiktor für die TTP bei Patientinnen und Patienten, die auf die Therapie ansprachen, war die relative Veränderung des BF mit der geringsten relativen Abweichung vom tatsächlichen Wert der TTP, was durch die multiple lineare Regressionsanalyse bestätigt wurde.

Schlussfolgerung: In der Frühphase nach gezielter Therapie scheint die VCTP in der Lage zu sein, Responder von Non-Respondern zu unterscheiden bevor eine Änderung der Tumorgröße eintritt. Eine unterschiedliche prozentuale Veränderung der drei Perfusionsparameter kann als Hinweis gelten, dass die Therapie wahrscheinlich die physiologischen Prozesse der Angiogenese in unterschiedlichem Ausmaß beeinflusst und daher alle Perfusionsparameter unabhängig voneinander betrachtet werden sollten. Die Abnahme aller drei Perfusionsparameter kann als positives Ansprechen auf die angiogenesehemmende Therapie interpretiert werden. Die relative Veränderung des BV scheint der wichtigste Parameter für die Erkennung von Non-Respondern zu sein, während die Verwendung anderer Parameter die VCTP-Spezifität erhöht. Zusätzlich kann die relative Änderung des BF ein vielversprechender Parameter für die Vorhersage der TTP sein.

Schlüsselwörter: Volume Computerthomographie Perfusion, Tumorangiogenese, Tumor Response auf der Therapy

1 Introduction

1.1 Imaging of solid tumors

Imaging plays an important role in the follow up of oncologic patients, especially in the objective assessment of tumor response to various cancer therapies [1]. Most broadly accepted criteria for evaluation of treatment responses are standardized response evaluation criteria in solid tumors (RECIST), which relay on change in tumor size only [1,2]. Today it is well known that the process of tumor angiogenesis plays a central role in the growth and spread of solid tumors [3]. Hence, tumor vessels have become a main study target for both, therapy and imaging. Angiogenesis is the process of development and formation of new capillary blood vessels through the sprouting from the existing host microvessels. This process of tumor angiogenesis is complex. It is mediated by the alteration in the balance between pro- and anti-angiogenic factors with prevalence of pro-angiogenic factors that links transition from tumor avascular phase “tumor dormancy” to tumor vascular phase enabling progressive tumor growth and metastases [4].

The blood vessels formed by tumor neoangiogenesis differ from vessels in normal tissue by chaotic and immature appearance [5]. Because tumor vessels are of paramount importance for tumor growth and spread, many new oncologic therapies have developed to fight those vessels during the recent years. These antiangiogenic agents are designed to target specific biologic pathways of tumor angiogenesis with high degree of specificity [6,7]. Compared to conventional cytotoxic chemotherapy, which leads to tumor cell death and to actual tumor shrinkage, antiangiogenic agents exert cytostatic nature leading to changes at the level of tumor vessels, altering tumor perfusion and causing tumor stabilization rather than rapid tumor size reduction [7]. Therefore, traditionally used standardized response evaluation criteria in solid tumors (RECIST) that relies on assessment of change in tumor size only, may not be suitable for objective assessment of tumor response at early period of targeted therapy. Instead, functional imaging biomarkers, which assess vascular changes associated with targeted therapy effect, have been developed as means of treatment monitoring, alternatively to morphological criteria (RECIST) [8].

1.1.1 Functional imaging techniques

Among several imaging techniques developed to study microcirculation, the most widely used imaging techniques are dynamic contrast enhanced (DCE)-MRI and CT perfusion. The principle of both methods is acquisition of fast sequential images during intravenous administration of contrast agent in the same volume over time in order to register temporal changes of tissue signal or density. Compared to perfusion imaging with MRI, CTP has the advantage of a linear relationship between contrast media concentration and CT density (expressed in Hounsfield units) in the tissue. This advantage makes mathematical modeling simpler than with MR imaging, in which contrast-signal relation and quantification are more complex [8].

Functional PET and contrast enhanced US have also been considered for evaluation of tumor angiogenesis. Both methods have their own limitations, primarily due to specific properties of contrast agent/radiotracer, but also due to other kind of limitations.

Contrast enhanced US uses gas filled microbubbles as contrast agent, which strictly remain within the vascular system and do not diffuse to the extravascular space [9]. Therefore, it cannot be used to measure capillary permeability, which represents an important biomarker of angiogenesis [10]. Further limitations of US are limited spatial resolution, limited field of view, the short time window available for imaging and its operator dependency[8].

For functional PET, ^{15}O-labeled radiotracers and alternative ^{11}C-labeled radiotracers have been used to quantify tumor angiogenesis. Both radiotracers have short half-life, 2 and 20 minutes respectively, thus requiring on-site production and synthesis in cyclotrons, seldomly available in smaller centers [8]. However, there are studies available, which show that perfusion of tumors and glucose metabolism of tumors (FDG uptake) may not change in parallel in response to therapy. Hence, there is an increasing interest in combining CT perfusion with ^{18}F-FDG PET-CT with the use of iodine contrast agents for the CT component of PET-CT examinations, allowing coregistration of vascular physiology alongside with glucoemetabolism without need for on-site cyclotrons [11].

Therefore, in a view of the fact that CT remains an essential tool in the assessment of cancer patients including diagnosis, staging and therapy monitoring due to relatively low cost, wide availability, easy protocol standardization, short acquisition time and wide spectrum of pathologies that can be imaged, CTP is the preferable technique in quantification of tumor vascularization.

1.2 CT perfusion imaging introduction

Recent work has led to development of non-invasive quantitative dynamic imaging techniques, such as CT perfusion (CTP), which provide both qualitative and quantitative information regarding tumor angiogenesis [12,13]. This method is simultaneously similar and different from conventional CTA. Both require intravenous administration of contrast agent in order to delineate vessels with the difference that CTA acquires a series of images at different anatomical locations across an organ in order to study morphology of the discrete vessels that supply a particular organ or tissue. Conversely, CT perfusion typically requires a series of rapid images over time at the same anatomical location in order to assess functional status of microvessels within those organs or tissues [14].

The measurement of tissue perfusion by using contrast enhanced CT was initially proposed by Axel in 1980 for brain tissue. Back than the technique was constrained by the slow speed of image acquisition and data processing of the commercially available CT systems. Introduction of spiral CT systems in the early 1990s enabled rapid acquisition of images, which was required for reliable perfusion imaging. First implementation of CT perfusion on spiral CT was in 1990 and the first description of the use of this technique in tumor imaging was reported in 1993 by Miles et al. [14]. Although the basic techniques for perfusion imaging have remained the same, recent technological advancements in multidetector CT systems and availability of commercial software have made CTP imaging feasible as technique in an clinical environment [15].

The constraint of limited anatomical coverage of earlier generation CT scanners (e.g. 64-detector row CT with a volume of 3,2 cm in longitudinal axis) has been largely overcome by development of table toggling and periodic spiral techniques of scanning [16]. Furthermore, newer generation CT scanners have a larger number of detector rows (up to 320 detector rows) that allow visualization of larger volumes of up to 16 cm in longitudinal axis, enabling imaging of the whole tumor volume during one gantry rotation without table movement. The key of successful CTP implantation into clinical routine was the development of registration software that corrects motion artefacts in axial and longitudinal direction, which may occur due to respiration or other kind of patient motions, and development of commercial software, which allow pixel-by-pixel calculation of range of physiological parameters of tumor vessels and depiction as parametric maps [14,16].

Within cancer imaging, perfusion CT is readily incorporated into the existing CT protocols, somewhat as the timing sequences during test bolus administration were once performed in conjunction with CT angiography. CTP, in combination with conventional CT that provides morphological information, delivers information about tumor vascularity. Due to the simplicity and speed of acquisition, as well as straightforwardness of perfusion parameters calculation and interpretation, CT perfusion is, in comparison to MRI, a favored imaging technique for assessment of tumor vascularity [17,18].

Basic principles of CTP

Perfusion is the transport of blood to the capillary bed of the tissue volume per unit of time. It is slow in order to permit exchange of blood products between blood and tissue, which is quite different from the concept of velocity within large vessels [10,13].

Fundamental principles of CTP include rapid acquisition of CT images at the same anatomic location (same volume) before and during intravenous administration of contrast agent in order to track its transit and distribution within vascular bed and therewith enabling detailed measurement of temporal changes in tissue attenuation [19]. Tissue attenuation is directly proportional to the contrast agent concentration within the vessels and the tissue. This represents a major advantage for facilitated and straightforward calculation of perfusion parameters within kinetic models. Different kinetic models are available for the calculation of the perfusion parameters. These models consider the physiology of contrast agent dynamics. During passage through the vascular bed, contrast agent shows a two-compartment pharmacokinetic behavior distributing from the intravascular to the extravascular/extracellular compartment. Based on contrast agent distribution, tissue enhancement kinetic can be divided in an early (intravascular) and a late (extravascular) phase, giving a range of measurable quantitative and qualitative vascular parameters, which can be calculated by CTP. In the early phase, tissue enhancement is mainly attributable to the distribution of contrast within the intravascular compartment and is determined by blood flow (BF) and blood volume (BV). In the delayed phase, contrast passes from the intravascular to the extravascular compartment and tissue enhancement results from distribution between these two compartments and is highly influenced by vascular permeability [17,19]. The rate of contrast passage i.e. temporal changes in enhancement depends on several factors: the current morphology of tumor vascular system, and the iodine concentration within the vessels that depends on the type of contrast agent (iodine concentration) and on the mode of contrast agent injection (injection rate, dose) [10]. Temporal attenuation changes are displayed as time-density curves (TDC) which offer quick qualitative insight into contrast agent distribution. The 'raw' images of registered temporal changes in contrast enhancement undergo further computer processing on a pixel-by-pixel basis using kinetic models through dedicated software to generate different quantitative perfusion parameters, which are displayed as colored parametric maps. Depending on the kinetic model different quantitative and qualitative perfusion parameters can be calculated by CTP, which are summarized in Table 1 [13,19,20]. Due to specific characteristics of tumor vessels the main quantitative parameters crucial for differentiation between pathological and normal tissue are regional blood flow (BF), blood volume (BV), and capillary permeability (FE/CL, PS).

Table 1. Perfusion parameters (quantitative and qualitative) calculated by CTP

QUANTITATIVE	
BF (Blood Flow)	"flow rate" of blood through the vasculature per unit volume or tumor mass
PI (Perfusion index)	Percentage amount of BF of a vessel relative to total BF in tissue by organs with dual blood supply
BV (Blood Volume)	Volume of blood within the vasculature in a tumor that is actually 'flowing'
PS (Permeability surface area product)	Diffusion of contrast material across the capillary endothelium and the surface area of the endothelium
FE (Blood flow extraction product)	Unidirectional rate of transfer of contrast material from intravascular to extravascular compartment
MTT (Mean transit time)	Average time taken by blood elements to traverse the vasculature from the arterial end to the venous end in tumor
QUALITATIVE	
Time to peak enhancement	Time from arrival of the contrast agent in major arterial vessels to the peak tissue enhancement
PEI (peak enhancement intensity)	Maximum increase in tissue density

Data from [14,17,21]

Although qualitative parameters are assigned with specific numbers, they are highly influenced by technical factors, including contrast agent characteristics, and patient characteristics, such as cardiac output. Therefore, they cannot be used for intercomparisons. Quantitative parameters, on the other hand, are derived from kinetic modeling of time-density curves. They are expected to depend solely on physiology of imaged region of interest, although the parameters are influenced by the mathematical model which is used for calculation [21].

CTP acquisition

Standard CT perfusion protocol consists of two acquisitions: baseline pre-contrast acquisition (serves as localizer of target lesion) followed by dynamic acquisition of the target lesion or selected volume, respectively. Dynamic acquisition can be divided in early and delayed phase acquisitions according to pharmacokinetic behavior of contrast agents. To enable measurement of temporal changes in tissue attenuation, dynamic acquisition must start before the contrast agent reaches the target lesion in order to determine the baseline tissue density values. Subtraction of the baseline density values from each of the serial dynamic CT studies enables generation of TDC. The selection of the region of interest (ROI) in post processing allows generation of TDC for a particular part and/or whole volume of tumor tissue [10,16,19]. CT perfusion protocols depend on different features, such as the kinetic model used, first pass or delayed imaging, and the organ that will be studied. Therefore, several fundamental steps should be considered when performing CTP [16]. A general brief description of these steps is illustrated in Appendix 2. It includes information on target definition, protocol selection, radiation dose, selection of contrast agent type and mode of its administration, selection of appropriate breathing technique, image and TDC acquisition and processing, as well as analysis and interpretation of CTP. Detailed information is described elsewhere [13,17–19].

Kinetic Models for calculation of CT perfusion

Different kinetic models may be applied for quantification of perfusion parameters. They vary among different CT manufacturers [18,20]. Their basic characteristics are listed in Table 2.

Table 2. Kinetic models used by different CT manufacturers

Vendor	Kinetic model	Perfusion parameters	Principle of model
GE Healthcare	Deconvolution	BF, BV, MTT, PS	Johnson Wilson distributed parameter
Siemens	Compartmental Deconvolution	BF, BV, PEI, TTP, FE BF, BV, MTT, PS	Slope method and delayed modified Patlak Johnson Wilson distributed parameter
Philips Medical System	Compartmental	BF, BV, PEI, TTP	Slope method
Canon Medical Imaging Toshiba	Compartmental Single and double compartment	BF, PI, BV, FE(CL)	Maximum slope (Fick principle) and Patlak

Note—Data from [14,18–20,22]. BV - blood volume, BF- blood flow, FE - blood flow extraction product (CL- clearance), PS –permeability surface area, MTT – mean transit time, PEI peak enhancement image, TTP- time to peak enhancement, PI perfusion index

The most frequently used kinetic models (analysis algorithms) are “compartmental,” that uses maximum slope combined with Patlak, and “deconvolution,” that uses Johnson Wilson distributed parameter for calculation of perfusion parameters. There are some important differences between these two models that are summarized in Table 3. A brief description of each model is presented in Appendix 1. Compartmental and deconvolution modeling methods have been found to be generally comparable, although not directly. The main difference between these two methods is in their validation, theoretic assumptions and their susceptibility to noise and motion. As result, they have different acquisition requirements [23].

Table 3. Kinetic models

Kinetic model	Compa-rtment	Mathematical model	Perfusion Parameter	Features
Deconvolution	Single	Use of arterial and tissue-time concentration curves to calculate impulse residue function for tissue (IRF) instantaneous input function	BF, BV, MTT	Less sensitive to noise.
	Dual	Johnson Wilson distributed parameter -extended deconvolution model Constrained input function	FE (PS)	Enables separation of F and E (PS)
Compartmental	Single	Fick Principle maximal slope or peak height of tissue concentration curve normalized to arterial input function	BF	Sensitive to noise and less sensitive to movement
	Double	Patlak quantifies passage of contrast material from intravascular into extravascular space	FE (CL) and BV Equiv.	F and E cannot be measured separately because they are determined together as the transfer constant (FE) Better for organs having complex microcirculation (kidney, spleen)

Data from [16,19–21] **FE -**Blood flow extraction product – the total unidirectional diffusional flux of the contrast media from the intravascular to the extravascular space across all capillaries (**F** – flow of the blood; **E** – total unidirectional diffusional flux efficiency); **PS** – permeability surface area, **CL** – clearance, **BV** – blood volume **BF** – blood flow. In perfusion imaging a vessel permeability is represented by the blood flow extraction efficacy (FE). FE is measured by both kinetic models, Patlak (represented as CL) and Johnson Wilson distributed model (represented by the PS). In physiological conditions, FE unambiguously depends on flow and permeability (BF and CL/PS). Besides above-mentioned differences between two kinetic models is that deconvolution model is able to provide estimation of F and E independently via Renkin Crone relationship [16]. In contrast, Patlak in compartmental analysis method simulate physiological condition in capillaries and cannot measure flow (F) and total unidirectional diffusional flux efficiency (E) independently, because they are determined together as the blood flow extraction product (FE), represented by CL.

1.3 Clinical Applications of CTP in Oncology

The basis for the use of CTP in oncology is that the peculiar structural characteristics of the tumor microvessels (tortuous, dilated irregular vessel branches, sometimes with blind endings, arteriovenous shunt formations, abnormal fenestrated endothelial cells of the wall, loosely attached or absent pericytes and abnormal, sometimes missing basement membranes) are reflected by tumor vessels' functional abnormalities *in vivo* (increased tumor perfusion, blood volume and capillary permeability), which can be detected by CTP [5,24]. CTP in oncological imaging can be used in many clinical applications, such as tumor detection and diagnosis, lesion characterization (benign or malignant), that in turn enable non-invasive staging, grading, and restaging of the disease. In addition, one of the major applications was found in assessment of tumor prognosis, and in therapy monitoring, therewith enabling noninvasive guidance of the treatment [12–14,16–20].

Functional imaging in tumor characterization is beneficial in differentiation of benign and malignant lesions. In the literature, different tumor types of various organs show increased values of perfusion parameters despite heterogeneity in angiogenesis (between tumor types and within the same tumor). This represents a general hallmark of malignant tumors due to tumor neovascularization [18,25]. This characteristic has been used in differentiation of indeterminate lung nodules [26], distinction between diverticulitis and colon tumor [27], and discrimination between metastatic and benign liver lesions [28–31]. Further application of CTP was recognized in staging and restaging of disease by depicting occult hepatic metastases in colorectal cancer [28,32,33] and discrimination of benign and malignant lymph nodes as it was reported in head and neck cancer [34,35] and gastric tumors [36]. Because of the capability of CTP to illustrate perfusion parameters in form of colored parametric maps, CTP enables easier tumor detection when margins of the tumor are not well delineated i.e. in cases with complex anatomy and/or post treatment changes, or in cases when there is no visible tumor mass (i.e. brain tumors) [18,19]. Additionally, CTP enables better understanding of tumor biology at different growth stages, which can be used as a prognostic biomarker to provide risk stratification and predict tumor response to the therapy. Patients with tumors that had lower blood flow have showed poorer response to chemo- and radiotherapy, and lower overall survival than patients with higher blood flow [21]. In turn, this could be used to guide individualized patient care.

However, major CTP application was found in evaluation of the treatment responses to various cancer therapies, in particular to the new generations of oncologic therapy regimes such as antiangiogenic agents (targeted therapy), not only because of their different mechanism of action compared to chemotherapy (Table 4), but also due to different pattern of treatment response at imaging during follow-up. Nevertheless, CTP showed capability to understand, *in vivo*, the effect of different cancer therapies on tumor vessels, due to their certain mechanism of action [13].

Table 4. Differences between Targeted therapy and Conventional chemotherapy

	Conventional therapy	**Targeted therapy**
Effect of the therapy	Cytotoxic	Cytostatic
Target	Tumor cells	Tumor vasculature
Cellular answer	Apoptosis	Inhibition of cells proliferation: Necrosis
Tumor response	Shrinkage	Early tumor vasculature normalization without significant tumor shrinkage
Change in tumor size with respect to the time	2 months	up to 10 months
Time of response	Late (2 Months)	Early (2–6 weeks)
Follow-up criteria	RECIST	No adequate method or criteria; following suggested: • RECIST? • Size and attenuation criteria (CHOI, mCHOI, mRECIST)? • PERFUSION? • METABOLIC?

Data from [7,8,37]

1.4 Oncologic follow-up of antiangiogenic therapy

1.4.1 CT in Oncologic follow-up – RECIST

Currently, the most widely used morphologic assessment scheme in tumor response are the “response evaluation criteria in solid tumors” (RECIST) [7]. RECIST 1.0 was published in 2000 and offered more precise guidelines for assessment of solid tumors compared to the previously used WHO criteria. RECIST 1.0 has been further revisited in 2009 when RECIST 1.1 was published. Generally, RECIST is solely based on morphologic changes in tumor size and were originally developed to assess tumor response to conventional cytotoxic anticancer therapies. Figure 1 summarizes accurate measurements of target lesions and Table 5 shows a short description of categories of response according to RECIST 1.1.

Measurable lesion
Size ≥10mm in longest diameter

Lymph node
Size ≥ 15mm

Figure 1. Tumor lesions/lymph nodes will be categorized as measurable or non-measurable and target and non-target lesions will be chosen at baseline CT. Total number of target lesions is maximum 5 per patient and maximum 2 per organ. Tumor lesions (metastatic or primary) are always measured in longest diameter in axial plane and must have a minimum size of ≥10mm to be measurable. Lymph node must be ≥15 mm in short axis to be considered pathologically enlarged and measurable when assessed by CT scan. Diameters of selected target lesions are summed to sum of longest diameters, and in further follow up the percentage change of the sum of the longest diameters is evaluated [2].

Table 5. Response assessment classification of target lesions according to RECIST 1.1.

Definition of response	RECIST 1.1
(CR) Complete response	Disappearance of all target lesions and non-target lesions; all lymph nodes must be ≤ 10 mm (short axis)
(PR) Partial response	≥ 30% decrease in sum of diameters, no new lesions and no progress in non-targeted lesions
(SD) Stable disease	Changes between PR and PD
(PD) Progressive disease	>20% and at least 5 mm increase over the smallest sum of diameters; new lesions; unequivocal progression of non-target lesions

Data from [2]

The classification of a response (CR or PR) is based on comparison to the sum of diameters at baseline, while progression is based on a comparison to the smallest of the sum of diameters (nadir) during the trial (including baseline if that is the smallest sum) [38].

A single RECIST-based early assessment of response to targeted therapy may lead to misinterpretations, since the predominant cytostatic effect of antiangiogenic agents does not lead to rapid change in tumor size. Additionally, in some cases tumor can appear larger as consequence of extensive necrosis and simulate progression of the disease according to

RECIST. Regarding limited (slow) tumor shrinkage during antiangiogenic treatment, achieving threshold of -30% may take several months. Therefore, in majority of patients, categorization according to RECIST would be stable disease (SD). Although SD does not imply disease progression, in clinical context these patients stay in the indeterminate group, which includes patients who will show partial response or prolonged stable disease and patient who will show progression in later follow up. This is an important point, because early detection of patients who show outset resistance allows change of therapy to alternative second or third line therapy options [7]. Hence, Thiam et al. proposed optimization of size threshold (from -30 to -10 %) in mRCC, which could earlier reflect patient outcome. While the proposed optimized threshold of -10% showed promising results, the authors also showed some limitations, mainly regarding measurement reliability in small lesions [39,40]. In addition, due to a decrease in tumor attenuation related to therapeutic effects of antiangiogenic agents on tumor vessels, which results in decrease in tumor perfusion, some investigators have proposed criteria, which evaluate both, size and attenuation of the tumor, like CHOI in GIST and mRECIST in HCC treated with antiangiogenic agents [7,41]. Furthermore, there were several suggestions of use such criteria in mRCC including CHOI, mCHOI, SACT and MASS [40,42]. However, comparison between different studies depends on many factors (different CT scanners and CT scanning protocols (kV), contrast agent type, way of contrast agent injection, cardiac output and placement of ROI) which may result in significant variation in attenuation measurements. Although criteria which combined size and attenuation measurements showed correlation with progression free survival, they represent information from static images that cannot offer detailed information about changes in tumor vessel physiology. Besides, increasing number of criteria make their incorporation into daily radiological praxis less practical, because complexity and expenditure of time [7,41].

CTP in Oncologic follow-up

Contrary to conventional CT and morphologic criteria, study of tumor angiogenesis with CTP offers functional analysis of the tumor vessels beside morphologic criteria (tumor size). Changes in tumor vascularization may be affected by various cancer therapies (chemotherapy, radiotherapy, vascular disrupting agents and targeted therapies), in different way, grade and timing due to their different mechanisms of action. CTP studies have shown that various cancer therapies lead to different changes in perfusion parameters in the early period (within first 7–10 days) of the treatment, whereas in long term all cancer therapies should lead to reduction of all perfusion parameters for those patients who responded to therapy [20].

Nevertheless, major need for functional evaluation of treatment response in tumor imaging has been created for antiangiogenic agents, because they are primarily targeting tumor vessels producing limited change in size, despite being clinically effective. CT perfusion can measure vascular changes induced by the treatment non-invasively, *in vivo*, that is in contrast to micro vessel density (MVD), which is a somewhat invasive *ex vivo* method, considered as gold standard for quantification of angiogenesis [17]. Although many studies have confirmed the role of MVD as prognostic indicator in untreated tumors, in evaluation of treatment response MVD has not shown to be an adequate method due to lack of quantitative relationship between alterations in MVD and tumor inhibition during antiangiogenic therapy [43]. However, there were studies that have investigated potential correlation between *in vivo* and *ex vivo* findings and have suggested that peak tumor enhancement (PE) and blood volume (BV) tend to correlate most closely with MVD, because they are not influenced by cardiac output like for example BF. Results from these studies remained relatively modest [16]. Unlike MVD, CTP provides information about functional status of tumor vessels, which is constantly changing. The fundamental processes underlying CT measurement of tumor perfusion are the transport of contrast agent by the blood flow through the tumor vascular bed and exchange of the contrast labeled blood between the intravascular and extravascular spaces. Hence, perfusion parameters provide indirectly information regarding the delivery of oxygen and nutrients to the tumor (regional blood flow), vascular density (regional blood volume), and angiogenesis per maturity of the vessel wall (permeability surface area product, extraction fraction) [20]. However, interpretation of relationship between perfusion parameters and angiogenesis in response to therapy is complex[16,44]. Probable treatment induced vascular changes and their relationship with perfusion parameters are summarized in Table 6.

Table 6. Relationship between perfusion parameters and angiogenesis following treatment induced changes

Changes of tumor angiogenesis and tumor tissue under antiangiogenic therapy	**Changes in Perfusion parameters**
Normalization of tumor vessels, shut down of arteriovenous shunts	↓BF
Reduction of poorly formed basement membranes, pruning of most immature vessels	↓Permeability FE(PS/CL)
Decrease in vessel number (decreased vascular density), decreased vascular tone of tumor vessels	↓BV
Shut down of arteriovenous shunts	↑MTT
Necrosis	↓BF, ↓BV and ↓Permeability

Data from [16,19,20]

Additionally, K.A. Miles has proposed another way for interpretation of perfusion parameters. K.A. Miles suggested that, when combining knowledge of the molecular processes underlying changes in vascular physiology with an understanding of the relationship between vascular physiology and pharmacokinetic contrast agent behavior, CTP may be used as surrogate molecular imaging technique. According to the molecular imaging classification by Blasberg and Gelovani, CTP may represent a surrogate molecular imaging technique, because functional status of tumor vessels depicted by CTP reflect downstream physiological changes that represent end result of molecular processes related to angiogenesis [12]. These processes are described in detail elsewhere [5,11]. Briefly summarized, molecular processes of angiogenesis are activated by hypoxia and/or oncogene mutations (VHL and p53), which upregulate HIF1α, a major transcription factor that increases production of vascular growth factors (the most important are vascular endothelial growth factors –VEGF, and eNOS). In turn, they stimulate angiogenesis leading to increased perfusion [12]. In cases when tumor outgrowths its blood supply, there is additional stimulation of HIF1α that leads to adaptive processes causing downstream-physiological changes in tumor vascularization (further increase in BV and CL/PS) that try to compensate hypoxia, signaling increased tumor aggressiveness and therapy resistance [11]. Therefore, by depicting physiological status of tumor vessels, CTP may provide surrogate information about HIF1α activity (per BF-biomarker of tumor oxygenation), and HIF mediated response to the hypoxia (per BV and PS-biomarkers of hypoxic adaptation), as illustrated in Table 7.

Table 7. CTP as surrogate molecular imaging technique

Molecular processes	End result of molecular processes	Downstream physiological changes in perfusion parameter
Hypoxia and/or oncogene mutation (VHL, p53)		**“Oxygenation”**
↑HIF1α		
activation of	New tumor vessels	↑BF
VEGF		
HIF mediated response to tumor hypoxia		**“Adaptation to hypoxia”**
eNOS	Vasodilatation	↑BV
VEGF (release of prostaglandins)	Increased permeability	↑ PS / EF (CL)

Data from [11,12] **HIF1α** (Hypoxia-inducible factor 1-alpha) - major transcription factor which up-regulates a large number of cellular processes important for the tumor survival and growth. Two processes in particularly: 1) increased HIF-1 activity increases production of vascular growth factors that stimulate new vessel formation and increased perfusion 2) HIF-1 increases expression of Glut-1 glucose transporters and hexokinase which are the major determinates of glucose uptake and metabolism. **eNOS** (endothelial nitric oxide synthetase) converts L-arginine to nitric oxide (NO) which cause vasodilatation and therewith maintenance of vascular tone in tumor vessels. **VEGF** (vascular endothelial growth factor) is a strong angiogenesis inducing factor and by release of prostaglandins VEGF represents powerful permeabilizing factor. In some percent, VEGF together with eNOS, additionally stimulates conversion of L-arginine to nitric oxide (NO) and NO, on the other hand, together with released prostaglandins, supports permeability.

This capability of CTP as surrogate molecular imaging technique was firstly recognized in acute stroke imaging by detecting still viable brain tissue “penumbra” a decrease in BF and compensatory increase in BV, which reflects HIF-mediated vasodilatory protective response (CTP of brain tissue). The vasodilatory role of eNOS which is upregulated by HIF1α activation was also later recognized in tumor angiogenesis and maintenance of vascular tone in tumors [12]. More recently, a concept of BF-BV mismatch associated with penumbra in stroke imaging was demonstrated in a study on head and neck tumors, where tumors with reduced BF and increased BV were indicative for tumor adaptation to hypoxia, which further signify a more aggressive and therapy resistant stage of tumor [12,16,45].

1.4.2 CTP in clinical trials

In clinical trials, CTP is used for *in vivo* understanding of pharmacodynamics of certain therapeutic agents, providing biomarkers for recognition of their biological effect. Hence their application was recognized in different stages of drug development, mostly in early stage for identification of drug biological effect and toxicity (when "go/no go" decision have to be made) and in later stages if the effect is present for selection of optimal biological dose of the drug [15,19,20]. In late phase (III) trials, imaging is used as surrogate for clinical outcome such as TTP and overall survival. Nevertheless, it should be highlighted that use of response biomarkers for different stages of clinical drug trials are different [15].

In the last 15 years, numerous studies have investigated changes in perfusion parameters in response to antiangiogenic therapy across various tumor types, which are summarized in Table 8. Analyzing listed studies, one can see that they have been managed/designed differently. The listed studies have used different CT scanners and different kinetic models. Some studies analyzed one parameter only, few studies two and the remaining studies all perfusion parameters. Finally, they were made in the setting of different therapy concepts: some studies used targeted agents only, some used a targeted agent in combination with radiotherapy (RT) and chemotherapy (CTX) to illustrate their possible interactions. Nevertheless, most of these studies have demonstrated a potential of CTP to identify decrease of perfusion parameters (and therefore decrease in tumor vascularization) in the early period after antiangiogenic therapy as indicator of early treatment response.

Some of these studies have shown correlation with patient survival after treatment (time to progression of disease) indicating that CTP could be used as a response imaging biomarker, providing at the same time morphological information such as tumor size.

Table 8. CTP studies evaluating tumor response to targeted therapy in the last 15 years

Author	Cancer	Therapy	Kinetic model and/or CT manufacturer	Perfusion parameters changed under therapy	Year
Willet et al. [46]	Rectal	Bevacizumab	Unknown	BF, BV/ ↓; PS↔	2004
Xiong et al. [47]	Solid tumors	SU6668	GE	BF, BV / ↓	2004
McNeel et al. [48]	advan. malig.	MEDI 522	GE	MTT↑ [1]	2005
Faria et al. [49]	mRCC	Thalidomide	GE Deconvolution	BF, BV, PS / ↓	2006
Meijernik et al. [50]	Solid tumors	AZD2171+ gefitinib	Siemens- Max.slope	BF ↓	2006
Koukourakis et al. [51]	Rectal	Bevacizumab	Unknown	BF↓	2007
Ng et al. [52]	NSCLC	RT + 4h after CA4P	Siemens- Patlak	PS↑, BV↓	2007
Ng et al. [53]	Solid tumors	L-NNA	Siemens- Patlak	BV ↓	2007
Zhu et al. [54]	aHCC	Bevacizumab	GE-Deconvolution	BF, BV, PS /↓; MTT↑	2008
Schlemmer et al. [55]	mGIST	Imatinib + Sunitinib	Siemens Max.slope & Patlak	PP↓ in responders	2009
Fournier et al. [56]	mRCC	TKI	GE Deconvolution	BF, BV / ↓	2010
Lind et al. [57]	NSCLC	Sorafenib + Erlotinib	Siemens-Max.Slope	BF↓	2010
Ng et al. [58]	Carcinoid Tumor	Bevacizumab + Interferon	GE -Deconvolution	BF, BV / ↓	2011
Fraioli et al. [59]	NSCLC	CTX + Bevacizumab	Siemens Max slope &Patlak	BF, PS / ↓	2011
Frampas et al. [60]	HCC	Sorafenib /Imatinib	GE Deconvolution*	BF, BV / ↔	2011
Yoon et al. [61]	Soft tissue Sarcomas	Bevacizumab Bevacizumab + RT	GE- Deconvolution	BF, BV, PS ↔ BF, BV, PS ↓[2]	2011
Anzidei et al. [62]	CRC liver m.	CTX + Bevacizum.	Siemens - m. Patlak	PP ↔	2011
Jiang et al. [63]	aHCC	Bevacizumab	GE Deconvolution	BF, BV, PS /↓; MTT↑	2012
Sacco et al. [64]	aHCC	Sorafenib	GE	MTT↑	2013
Tacelli et al. [65]	NSCLC	CTX + Bevacizum.	Siemens m. Patlak	TEF↓, TTV↓	2013
Fraioli et al. [66]	NSCLC	CTX + Bevacizum.	Siemens m. Patlak	BF↓, PS↓	2013
Tian et al. [66]	Soft tissue sarcoma	Bevacizumab and Bevacizumab + RT	GE Deconvolution	BF↓	2014
Yao et al. [67]	NET	Bevacizumab + Everolimus	GE Deconvolution	BF↓, BV ↓, PS↓	2015
Kambadakone et al. [68]	Soft tissue sarcoma	Bevacizumab Bevacizumab + RT	GE Deconvolution	BV↓; BF, PS /↔(2W) BV, BF, PS / ↓(10W)	2015
Nyflot et al. [69]	HNSCC	Bevacizumab	Unknown	BF, BV, PS, MTT / ↔	2015
Qiao et al. [70]	NSCLC	gefitinib, erlotinib, afatinib	GE	BF(BP) ↓	2016
Ippolito et al. [71]	aHCC	Sorafenib	Phillips Max. slope	AP t, HP t / ↓	2017
Nakamura et al. [72]	aHCC	Sorafenib	Toshiba Max. slope	AP t ↓	2017
Shen et al. [73]	aHCC	Sorafenib	Toshiba Max. slope	HAF t ↓	2018
Vehabovic-Delic et al. [44]	mRCC	TKI	Toshiba Max.slope & Patlak	BF, PS / ↓; BV ↔	2019

↓decrease of parameter; ↑ increase of parameter; ↔ no change of parameter; [1]MTT increase corresponded with increase of MEDI522 dose; [2]significant change of perfusion parameters (PP); CTX-chemotherapie; CRC – colorectal carcinoma; NET – neuroendocrine tumors; mRCC – metastatic RCC; NSCLC – non small cell lung carcinoma; HNSCC – squamous cell carcinoma of the head and neck; mGIST –metastatic GIST; aHCC – advanced HCC; BF – blood flow; BV – blood volume; PS – permeability surface area; CL – clearance (permeability); MTT – mean transit time; HPI – hepatic perfusion index; PP – perfusion parameters; BP – blood perfusion corresponds to BF; TEF – tumor extravascular flow; TVV – tumor vascular volume; APt – arterial perfusion in tumor lesion (HCC); HPt – hepatic perfusion in target tumor lesion; HAFt – hepatic arterial flow in tumor lesion (HCC).

2 Aims of the study

Regarding the challenges in functional imaging using CTP in follow up of solid tumors during treatment with antiangiogenic agents, the three following aims were defined for this thesis:

2.1 Aim 1) Comparison between changes in Perfusion and Changes in Size in the early period of targeted therapy in mRCC patients

Replacing conventional chemotherapies with new targeted therapies in various tumor types has resulted in increased clinical benefit and low RECIST response rate. The dissociation between clinical benefit and lack of tumor shrinkage have created doubts regarding objectivity of RECIST in oncologic follow-up in the early period of targeted therapy [6]. Besides attempts to improve morphological criteria by optimizing tumor size threshold and additionally evaluating attenuation to tumor size, functional imaging of tumor vessels as primary targets of antiangiogenic agents gained a rising interest in the last 15 years [7]. Since 2004, when the first antiangiogenic agent was approved for therapy of colorectal cancer, the number of antiangiogenic agents as well as the number of indications for antiangiogenic treatments have increased. Many of these drugs have side effects, and the survival benefits of these treatments remain relatively moderate. Possible exception regarding survival benefits has been shown in patients with renal cell carcinoma (RCC) in which the targeted therapy agents have notably improved progression free survival and therewith have made a substantial impact on the therapy options of RCC patients and prognosis of disease [19]. Currently targeted agents have replaced the conventional therapy strategies in the majority of patients with mRCC. However, it is known that for some patients, antiangiogenic therapy may be ineffective. For example, in mRCC there is evidence that approximately 20% of patients show outset resistance. Because of lack of reliable standardized new imaging biomarkers, follow up of antiangiogenic drug efficacy still relies on morphological criteria such as RECIST, which has limitations in this setting [74]. Despite increased progression free survival, the majority of patients are categorized as stable disease according to RECIST.

In this group of patients, RECIST does not allow differentiation between patients who will show real prolonged stable disease from patients who will show partial response or tumor progression in later follow-up [42]. Additionally, according to the ESMO guidelines for mRCC, in the case of RECIST-defined disease progression, there is no clinical evidence that this quantity of progression is a clinically valid end point that could impact treatment modification [75]. Particular effectiveness of targeted therapies makes mRCC to an important setting to explore changes in perfusion parameters in treatment response. Therewith, this study was performed in a homogenous group of patients with mRCC in order to assess the significance of volume computed tomography perfusion (VCTP) imaging in the early follow-up of mRCC treated with antiangiogenic agents.

The aim of this study was to analyze whether with VCTP imaging response to tumor therapy may be evaluated earlier than with CT imaging and size measurements, because it is expected that changes in tumor perfusion precede changes in tumor size under antiangiogenic treatment.

2.2 Aim 2) Correlation between changes in perfusion parameters

The current literature implies that most of the drug treatments, regardless of their type (antiangiogenic or conventional), lead to decrease in BF and BV [16], and that the change of BF and BV is coupled one to another [16,19]. However, when analyzing CTP studies, which have evaluated response to antiangiogenic therapies only, one can note that these studies have shown different results regarding the change of perfusion parameters in response to therapy. These studies have shown that different perfusion parameters can be changed in response to antiangiogenic therapies. Some of them showed decrease in one perfusion parameter only [48,50,51,53,57,66,70,72,73,76] and some of them showed decrease in two [44,47,52,56,59,60,65,71,77] or all three [49,54,55,61,63,68] main perfusion parameters. However, there is still lack of agreement on how perfusion parameters should be interpreted. Because the relationship between perfusion parameters and angiogenesis is complex, and different kinetic models for calculation of perfusion parameters are used, it is questionable whether these parameters should be interpreted in correlation with each other or independently, in comparison to each other [16,44]. Bisdas et al. have proposed that a concept of BF-BV mismatch could be a potential indicator for detection of tumors hypoxic adaptation [45].

The aim of this study was to test the correlation between the different perfusion parameters, to show whether there is any objective correlation between them (before and after therapy) and whether the perfusion parameters should be interpreted in correlation with each other or independently.

2.3 Aim 3) Correlation of changes in perfusion parameters and size with TTP

The recent technical developments of computed tomography, namely increased number of detectors, development of motion correction software, reconstruction techniques to reduce noise and availability of commercial software have improved many of the limitations of early CT perfusion studies and has enabled potential use of CTP in daily clinical routine. Although CT technology has reached maturity, and perfusion parameters have gained acceptance for use as imaging biomarkers in assessment of oncologic response alongside standard response criteria, there is still limited data, which related CT perfusion measurements to clinical outcome [15].

In the last 15 years only few CTP studies, which have investigated response to targeted therapy agents, showed that CT perfusion parameters may correlate with clinical outcome, i.e. in NSCLC [57,70], advanced HCC [72], mRCC [44,49], and in advanced HCC [63].

The aim of this study was to analyze possible relationships between changes in perfusion parameters and change in size with time to tumor progression, which is considered as surrogate end point of overall survival.

3 Materials and Methods

3.1 Patient population

This prospective study has been approved by the Institutional Ethics Committee with informed consent being obtained from all patients. Ten patients, out of which 1 female and 9 males, with average age ± SD of 60 ± 11 years; ranging from 37 to 71 years with histologically verified mRCC were included in the study. Histologically nine patient had clear cell carcinoma and one had undifferentiated carcinoma. One tumor was graded G1, five were graded G2, and two G3. Grading of two tumors was not available in the records. The referral for all patients was obtained from the Division of Oncology, Department of Internal Medicine. Inclusion criteria were: (a) histologically verified mRCC, (b) planned targeted therapy with angiogenesis inhibitors. Exclusion criteria were: (a) contraindications to the intravenous administration of contrast media containing iodine, (b) patients with mRCC but with treatment schedules other than targeted therapy, and (c) patients who participated in clinical and biomedical research with exposure to ionizing radiation in last 10 years. In all patients VCTP was performed within 2 weeks before targeted therapy initiation and one month after initiation of therapy with radiation dose of 10 mSv per exam. Patients who participated in previous clinical trials with radiation exposure in last 10 years were excluded from this study in order to ensure that the overall radiation dose applied in studies does not exceed 30 mSv, which is recommended by federal law.

3.2 CT perfusion protocol

The perfusion protocol was set in accordance to the guidelines for the use of DCE-CT in the assessment of tumor vascular support and was determined according results of a two rounded Delphi process [16]. The selection of the appropriate protocol was made considering the body region being studied, the dynamics of contrast media behavior and the vascularization of the respective tissue.
According to guidelines, all steps in the CTP protocol were considered as follows:

3.2.1 Contrast agent

An important consideration in CTP studies is the appropriate choice of the contrast medium. It is recommended to use a nonionic contrast agent in order to lower the effect of flushing and nausea, which could lead to motion artefacts during data acquisition [14]. Additionally, in order to produce maximal tissue and blood vessel enhancement and, therefore, correct time attenuation curves (Figure 2), the concentration of the CM and the shape of the CM bolus have to be considered. Due to a linear relationship between iodine concentration and tissue enhancement, a higher concentration of CM is preferred (iodine, 370 – 400 mg/mL). Furthermore, it is recognized that CM should be administered with rapid injection rate (at least 4 mL/s), regardless of the analysis method [13,14,16,23].

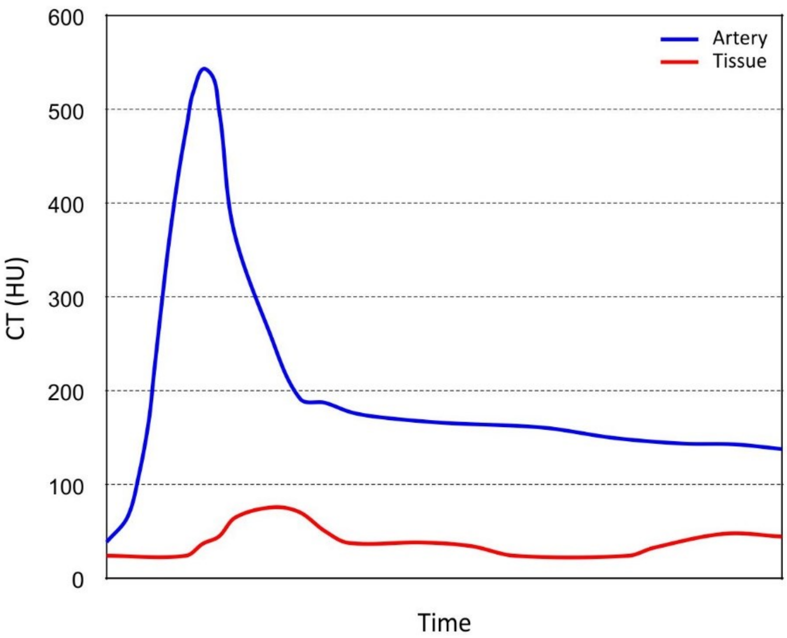


Figure 2. CTP Time-density curve (TDC) –In order to produce high signal-to-noise ratios for time–density curves, appropriate arterial and tissue enhancement is mandatory. The maximizing effect will be achieved using CM with high iodine concentration administered as short sharp bolus, because the increase/slope and the height of the arterial enhancement is directly proportional to the iodine flow rate. Large bolus injection can underestimate perfusion values within tumors [14]. Blue curve: Rapidly increasing TDC of artery represent narrow bolus; Red curve- maximal tissue (tumor) enhancement.

Because the validity of compartmental analysis requires that no contrast medium should leave the tumor region of interest by the time of measurement, we have used a small bolus with high flow rate, which is recommended by the guidelines for the compartmental analysis algorithm [16,23]. According to the recommendations for CT-angiography, dose of contrast agent and injection rate were adjusted to patients body weight in the CT-perfusion protocol (40–60 ml: 50–69 kg body weight, 40 ml; 70–89 kg body weight, 50 ml; and >90 kg body weight, 60 ml) [15].

Contrast agent with 400 mg Iodine /ml (Iomeprol; Iomeron®, Bracco, Milano, Italy), was administered at a flow rate of 6–8 ml/s (50–69 kg body mass, 6 ml/s; 70–89 kg body mass, 7 ml/s; and >90 kg body mass, 8 ml/s), using an automatic pressure injector. Table 9 summarizes how contrast agent dosage and flow rate were adjusted to body weight.

Table 9. Contrast dose and injection rate

Body weight	Contrast concentration	Flow rate
50-69 kg	40 ml	6ml/s
70-89kg	50ml	7ml/s
over 90kg	60ml	8ml/s

3.2.2 Breathing technique

CTP can be acquired using intermittent breath-hold technique or continuous quiet free breathing. Because adequate registration of maximum arterial enhancement is of paramount importance for adequate perfusion calculation and because the compartmental analysis method is very sensitive to movement the quiet free breathing technique was chosen in our study [15,16]. Every patient was instructed to the breathing technique prior the examination.

3.2.3 CTP acquisition parameters and CT perfusion process

All VCTP studies were performed using a 320-detector row CT scanner (Aquilion One, Canon Medical Systems, Otawara, Japan) which allow scanning of volumes up to 16 cm z-axis length. Canon Medical Systems offers several protocols for body perfusion imaging taking the vascularization of the tissue and the body part which will be studied into account. In this study, a body perfusion protocol was chosen. Scanning parameters are summarized in Table 10.

Table 10. Acquisition parameters of the CTP protocol

A. Scanning parameters	**Value**
Tube voltage	100 kV
Tube current	100 mA
Gantry rotation time	0.5 s
Slice thickness	0.5 mm
Detector width	Up to 10 cm (L-FOV)

The process of data acquisition by the body perfusion protocol consisted of two parts: pre-contrast acquisition and dynamic acquisition during contrast application. All in all, the body perfusion protocol contained 21 volume acquisitions, 2 pre-contrast and 19 post-contrast volumes.

Definition of target lesion

A low-dose pre-contrast helical scan was performed to localize the metastatic lesion and to plan the scan protocol for the dynamic part of the CTP study.

The targeted metastatic lesion was primarily chosen by its size and if possible, by its location in an anatomic region with the least motion artefacts.

Dynamic acquisition

After two pre-contrast volumes are acquired, eight volumes are obtained during the early period of the injection protocol with a scan interval of 1 s, followed by seven volume acquisitions with an interval of 2 s and by another four-volume acquisition with a scan interval of 4 s. In total, scanning lasts for 80 s. VCTP acquisitions were obtained without table movement for all patients included in the study. Figure 3 shows the acquisition protocol with a number of volumes and scanning intervals during first pass and delayed phase.

Figure 3. Perfusion protocol with volume scanning distribution in seconds and scan intervals. At beginning two pre-contrast volumes, thereafter eight volumes with 1 s intervals, followed by seven volume acquisitions with a scan interval of 2 s and by further four volume acquisitions with a scan interval of 4 s were obtained.

3.2.4 Radiation dose of the VCTP protocol

The study protocol and scanning parameters followed the ALARA principle, thus dose to patients was As Low as Reasonably Achievable [20,21]. The optimal temporal resolution and image noise were balanced with acceptable radiation burden. Taking the chosen acquisition parameters (Table 10) into account, the effective dose (*E*) received by patients included in the study was calculated.

The corresponding dose length product (DLP or $P_{\mathrm{KL,CT}}$) to patients per volume (10 cm) was 49.1 mGycm. The total DLP of 21 volumes was 644.4 mGycm.

The quick calculation could be done by multiplying P_{KL} value with the appropriate conversion coefficient *k*:

$$E = P_{\mathrm{KL,CT}} \cdot k, \qquad (1)$$

where common value for *k* in body region is 0.015 mSv/(mGycm).

The resulting effective dose per patient was 9.7 mSv.

However, if one wants to take characteristics of specific CT scanner (i.e. filtration, geometric efficiency, etc) and acquisition parameters (tube voltage, collimation, etc.) into account, more precise calculation could only be made with the help of software that utilizes Monte Carlo simulations on computational phantoms.

In this study, we used CT-Expo v2.4(E) (Georg Stamm and Hans Dieter Nagel) to evaluate the effective dose received by patients. The software requires detailed inputs necessary to perform accurate calculations. These are listed in Table 11.

The evaluated effective dose for male patients was 9.1 mSv, while for female patients exposed under the same conditions the effective dose was 11.4 mSv. The difference occurs due to exposure of uterus and ovaries during the examination.

Table 11. Software inputs used for effective dose calculation by CT-Expo v2.4(E) and evaluated effective dose (E) for male and female patients using the tissue weighting factors from International Commission for Radiological Protection (ICRP) Report No. 103.

A. Scanning parameters	**Value**
Age group	Adult
Gender	Male and female
Scanner model	Toshiba Premium/One (BS Large)
Tube voltage, U (kV)	100
Tube current, I (mA)	100
Q_{el} (mAs)	49
Q (mAs)	48.6
Single slice thickness, $N*h_{col}$	100
Table feed per rotation, TF (mm)	0.0
Slice thickness, h_{rec} (mm)	0.5
Pitch, p	1
Number of series	21
Slice position, z^- (cm)	male: 19, female: 17
Slice position, z^+ (cm)	male: 29, female: 27
$P_{KL,CT}$ (mGycm)	644
E (mSv) for male patients	9.1
E (mSv) for female patients	11.4

3.2.5 Pre processing

Imaging registration

Post-processing was performed using body perfusion software (Body Perfusion, Canon Medical Systems) which is provided by the CT manufacturer. Initially, imaging registration was performed to correct for motion between the dynamic volumes. After registration, the superimposed volumes were loaded into the body perfusion software.

3.2.6 Post processing

Perfusion analysis

The single input maximum slope and the Patlak plot model were used for perfusion analysis (quantitative perfusion parameters) of the mRCC for both time points. ROIs were placed manually in the supplying artery and in the metastatic lesion for generation of the arterial input function and lesions time density curve (TDC) (16). Arterial input was defined by placing a ROI within the aorta or the largest available arterial vessel close to the metastatic lesion.

Calculated perfusion parameters were Blood Flow (BF), Blood Volume (BV) and Clearance (CL). Blood flow (BF ml/min/100 ml) was calculated as the maximum slope of the metastatic lesions TDC, normalized for the arterial input function using the single input maximum slope model. Patlak plot model is based on linear regression and was used to calculate the lesion's blood volume (BV; ml/100 ml) and lesion's clearance (CL; ml/min/100 ml), which is the total flux from the intravascular space to the extravascular space. Summary of analysis algorithm used by Canon Medical Systems in this study is represented in Table 12.

Table 12. Analysis Algorithm assessed using Toshiba Medical Systems

Analysis Algorithm	**Mathematic model**	**Perfusion parameter expressed in units**
Single Compartment	SINGLE-INPUT Maximum Slope	BF (ml/min/100ml)
Double Compartment	Patlak Plot Linear regression	CL (ml/min/100ml) BV Eqv (ml/100ml)

*Perfusion parameters are generally expressed for a given mass or volume of tissue so that different tissues can be compared. In cross-sectional imaging a voxel represents one volume element of tissue, therefore the perfusion parameters are expressed per unit of volume [10].

Single compartment with single input uses maximum slope to calculate perfusion parameters and this algorithm depends on high injection rates and it calculates only blood flow (BF). Double compartment uses Patlak plot mathematic model to calculate perfusion parameters and it is appropriate for measurement of vascular permeability (CL) and equivalent blood volume (BV Eqv). All three main perfusion parameters and their definitions have already been explained and demonstrated in Table 1 in the introduction.

At the end of analysis, the perfusion parameters were color coded and superimposed to the grey scale images generating parametric maps for each perfusion parameter. Each pixel displayed on parametric maps represented a parameter value. An example of parametric images in a patient with mRCC is demonstrated in Figure 4.

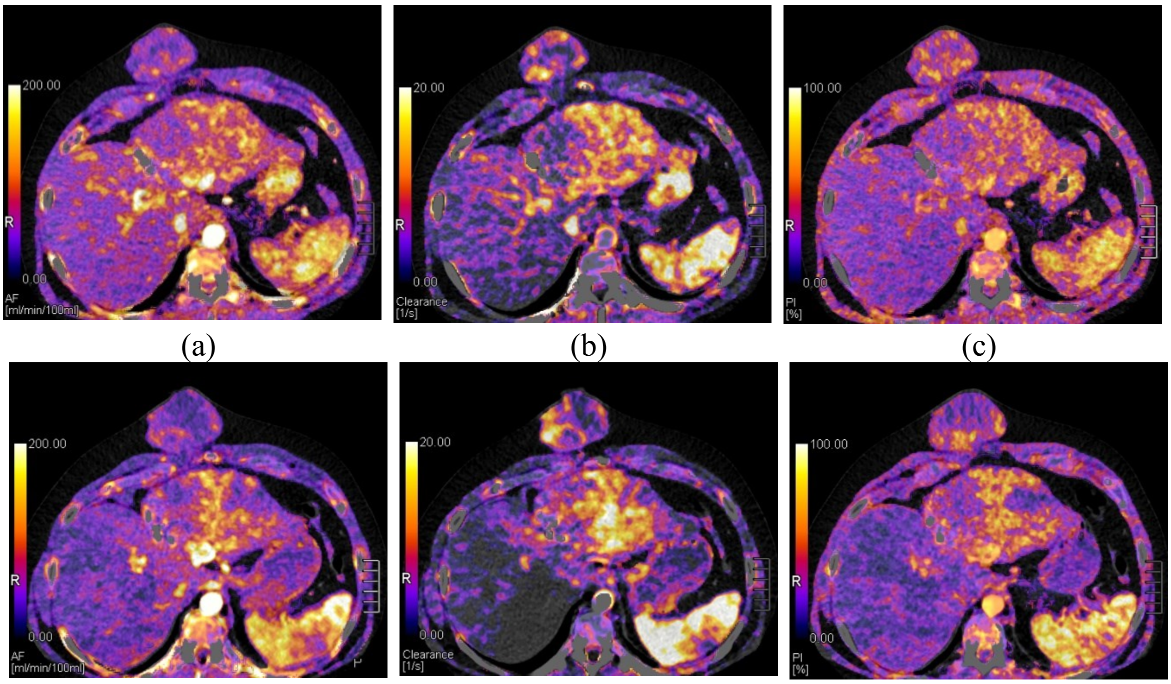


Figure 4 (a-f): 44 year old male patient with undifferentiated Adenocarcinoma in the soft tissue parasternal and in the left liver lobe under Pazopanib therapy. VCTP imaging before (a-c) and 4 weeks after initiation of therapy (d-f) shows minor change in BF from 52,.6 to 43.6 mL/min/100mL (a, d), in BV from 1.0. to 0.8 mL/100mL (b, e) and in CL from 23.4 to 34.4 mL/min/100mL (c, e). The long diameter was 70 mm and 74 mm, respectively (C, E)

3.2.7 CT Morphological analysis and RECIST Definition of the response

On CT gray scale images, the longest diameter of the lesion (LD; mm) was measured before and 4 weeks after targeted therapy in all patients. In cases, in which the metastatic lesion was a lymph node, the shortest diameter was measured. Additionally, all patients continued to be followed up according oncological protocols until disease progression has occurred, which was assessed clinically and by conventional CT examinations. Treatment response was evaluated according to RECIST 1.1 and categorized into four groups: Complete Response (CR), Partial Response (PR), Stable Disease (SD), and Progressive Disease (PD) as summarized in Table 1 in the introduction. Figure 5 shows one patient as an example how the patient was followed and categorized according RECIST between two CTP examinations and on the conventional control CT examination [2].

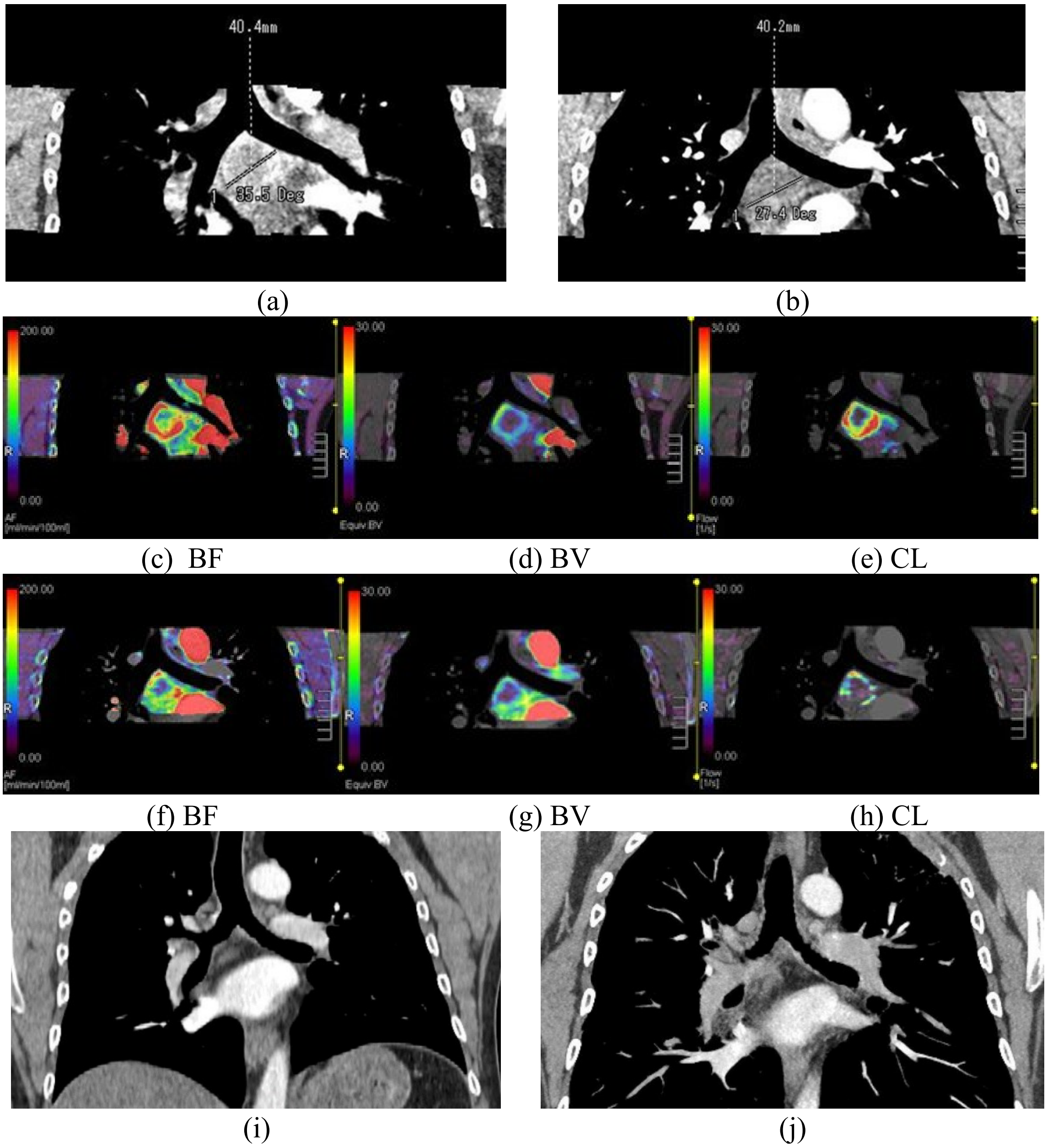


Figure 5 (a-j): (a, b) Subcarinal mediastinal lymph node metastasis of RCC before (a) and 1 month after therapy (b). No significant change in tumor size was observed after therapy, and the patient was categorized as SD according RECIST. (c, d, e) Parametric maps of BF, BV and CL before (c-e) and 1 month after therapy (f-h) show significant decrease of BF (c, f) and CL (d, g) after therapy, whereas no significant decrease of BV (e, h) was found. Follow-up CT 3 months (i) and 10 months (j) after therapy showed disappearance of the metastatic lesion and, therefore, confirmed response.

3.2.8 Time to Progression (TTP)

The interval from the beginning of treatment to disease progression or its spread is commonly defined as time to progression (TTP) (17). It is used as a parameter of treatment efficacy. Disease progression was detected using clinical and imaging evidence, as it was documented in patient medical reports.

3.2.8 Statistical analysis

The variables included in the statistical analysis were blood flow (BF), blood volume (BV), clearance (CL), long diameter (LD), time-to-progress (TTP), all of them expressed with real numbers. We also considered a nominal variable that described disease progression and patient response to therapy. Other, less used parameters were patient age, sex, and time of CT examinations.

A p-value <0.05 was considered statistically significant.

To check normality of distribution we used the Shapiro-Wilk test. All analyzed variables followed normal distribution. Hence, the measure of central tendency used in the study was mean value ($\bar{x}$), while measure of dispersion was standard deviation (σ). Variables were compared one to another using paired sample t-test.

Significance of correlation between variables was tested using Spearman ρ or Pearson correlation test.

Statistical analysis was performed with commercially available software (IBM SPSS Statistics and gnuplot).

4 Results

4.1 Aim 1) Comparison between changes in Perfusion and Changes in Size in the early period of targeted therapy in m RCC patients

4.1.1 Overall change in functional and morphologic parameters

Table 13 summarizes organ sites of metastatic RCC lesions, LD before and after 4 weeks of therapy, relative change of LD (%), and the specific antiangiogenic agent, that patients received. In addition, it shows categorizations of response according to RECIST 1.1 after 4 weeks of treatment. For 10 patients included in the study, LD before therapy initiation ranged from 23 to 85 mm. At 4 weeks after therapy was initiated the change in LD was such that all patients were categorized to have stable disease (SD). The largest increase in lesions LD was 5.7% and largest decrease in LD was -18.2% (mean -3.4%).

Table 13. Anatomic locations of mRCC lesions with antiangiogenic agents that patients received for treatment, long diameter (LD) before and 4 weeks after initiation of therapy, its relative change and categorizations of response according to RECIST 1.1. *Reproduced with modification from Vehabovic-Delic A. et al. J Comput Assist Tomo. 2019 May 1;43(3):493-8) with permission of publisher Wolters Kluwer Health, Inc.*

Patient	Metastasis localization	Therapy	LD before therapy	LD after therapy initiation	Relative change of LD (%)	RECIST 1.1.
1	Hilar lymph node	Pazopanib	23	24	4.3%	SD
2	Lung	Bevacizumab	23	23	0.0%	SD
3	Mediastinum	Sunitinib	41	38	-7.3%	SD
4	Kidney	Pazopanib	85	84	-1.2%	SD
5	Lumbal soft tissue	Pazopanib	25	24	-4.0%	SD
6	Parasternal soft tissue	Sorafenib	70	74	5.7%	SD
7	Parasternal soft tissue	Pazopanib	44	36	-18.2%	SD
8	Retroperitoneal lymph node	Axitinib	67	59	-11.9%	SD
9	Kidney	Pazopanib	58	58	0.0%	SD
10	Kidney	Pazopanib	85	84	-1.2%	SD

Table 14 shows details on the perfusion parameters (BF, BV and CL) and on LD before initiation of treatment and 4 weeks after initiation as well as the percentage changes.

Mean value of pre-therapy BF for 10 patients included in the study was 149 ml/min/100 ml. Four weeks after therapy was initiated BF decreased. Post-therapy mean value was 101 ml/min/100 ml, which corresponds to an average decrease of 28%. This change was found to be significant (Paired samples t-test, $p = 0.13$), and can be seen on Figure 6a.

The second parameter of interest, BV, did not significantly change before and after therapy initiation (Paired samples t-test, $p = 0.878$). The pre- and post- mean values were found to be the same, 5 ml/min/100 ml, ranging from 1 to 9 ml/min/100 ml for all patients included in the study (Figure 6b).

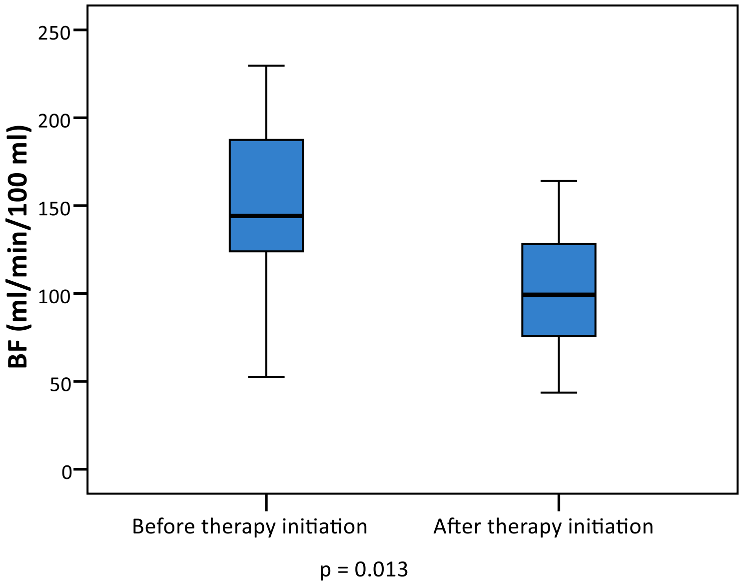
Observable changes were noticed in another CTP parameter, which was clearance (CL). The pre-therapy average of CL of 113 ml/min/100 ml lowered by 44% after therapy initiation. The post-therapy value of 55 ml/min/100 ml was significantly lower (Paired samples t-test, $p = 0.003$). The distribution differences are illustrated on Figure 7a.

In addition to CTP parameters, Table 14 also shows how main numerical characteristics of long diameter (LD) distribution changed before and after therapy initiation. No significant changes according RECIST 1.1 were detected and all patients were categorized as stable disease, which is illustrated in Figure 7b. This was confirmed by the Paired samples t-test (p = 0.086). Overall, we could argue that antiangiogenic therapy of mRCC lesions lead to decrease of BF and CL, while BV remained stable (Figure 8).

Table 14. Mean values ($\bar{x}$) and standard deviation (σ) of CT perfusion parameters before and 4 weeks after initiation of therapy, their relative change Δ and p-values of Shapiro-Wilk normality test. *Reproduced with modification from Vehabovic-Delic A. et al. J Comput Assist Tomo. 2019 May 1;43(3):493-8) with permission of publisher Wolters Kluwer Health, Inc.*

	Pre*					**Post***					**Δ (%)**				
	$\bar{x}$	σ	Min	Max	p	$\bar{x}$	σ	Min	Max	p	$\bar{x}$	σ	Min	Max	p
BF	149	55	53	230	0.95	101	38	44	164	0.92	-28	23	-67	18	0.46
BV	5	3	1	9	0.52	5	3	1	9	0.50	7	65	-88	132	0.05
CL	113	53	23	178	0.23	55	23	24	101	0.56	-44	26	-73	3	0.27
LD	52	24	23	85	0.21	50	25	19	84	0.20	-5.9	8.7	-18	6	0.38

*BF: blood flow (ml/min/100 ml); BV: blood volume (ml/100 ml); CL: Clearance (ml/min/100 ml); LD: long diameter (mm)



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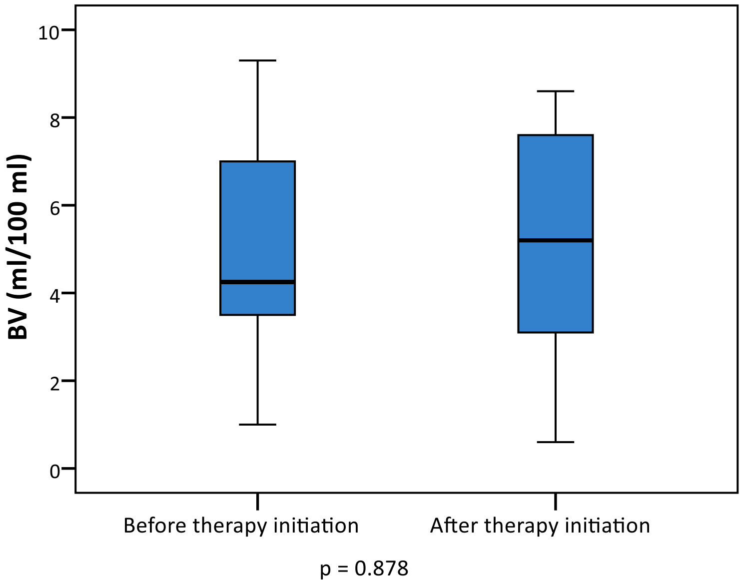
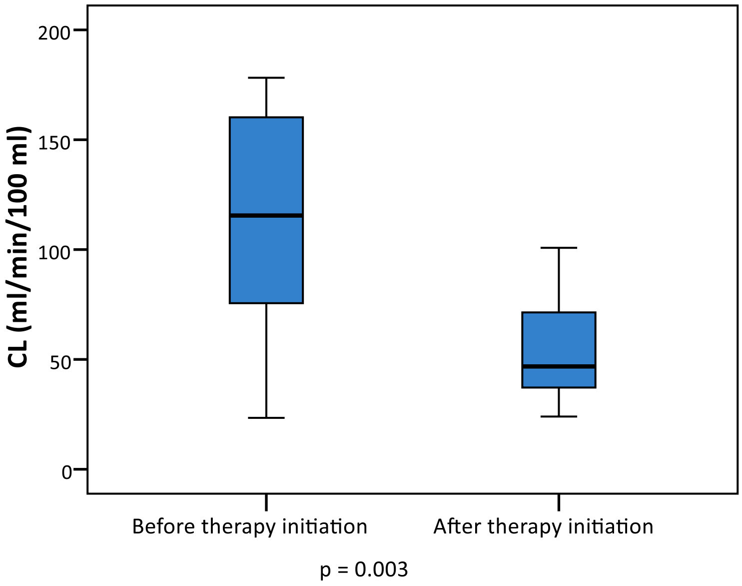


(b)

Figure 6 (a,b). Boxplots of (a) BF and (b) BV before and 4 weeks after initiation of therapy. The decrease of BF was statistically significant ($p = 0.013$), while BV did not significantly change in the whole study group ($p = 0.878$). *Reproduced with modification from Vehabovic-Delic A. et al. J Comput Assist Tomo. 2019 May 1;43(3):493-8) with permission of publisher Wolters Kluwer Health, Inc.*



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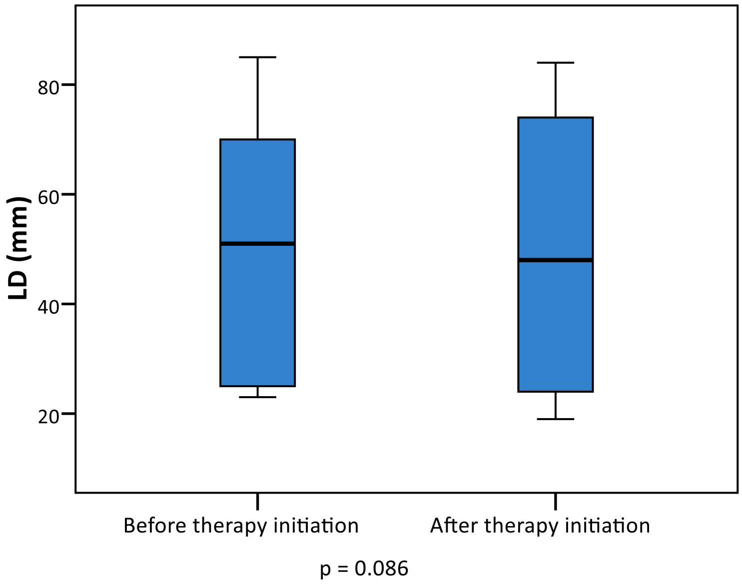


(b)

Figure 7 (a,b). Boxplots of (a) CL and (b) LD before and 4 weeks after initiation of therapy. The decrease of CL was statistically significant (p = 0.003), while LD did not significantly change in the whole study group (p = 0.086). *Reproduced with modification from Vehabovic-Delic A. et al. J Comput Assist Tomo. 2019 May 1;43(3):493-8) with permission of publisher Wolters Kluwer Health, Inc.*

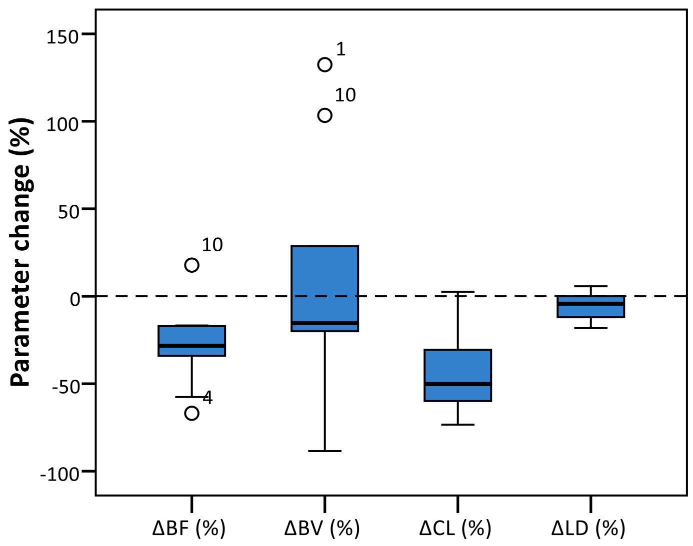


Figure 8. Boxplot of relative change of perfusion parameters blood flow (ΔBF), blood volume (ΔBV) and clearance (ΔCL), and long diameter (ΔLD) before and 4 months after therapy initiation

4.1.2 Comparison with Tumor Response

Follow-up of patients continued after the second CTP examination. All patients initially categorized with SD were later re-evaluated according to the oncological protocols by conventional methods (clinical and/or CT follow-up). Their response to therapy was recorded, as well as time of disease progression (TTP), and according to this follow-up data we were able to investigate the possible predictive value of CTP parameters.

Patients were grouped into two categories, those who responded to therapy (PR or SD), and those who did not (PD). None of the patients showed complete response.

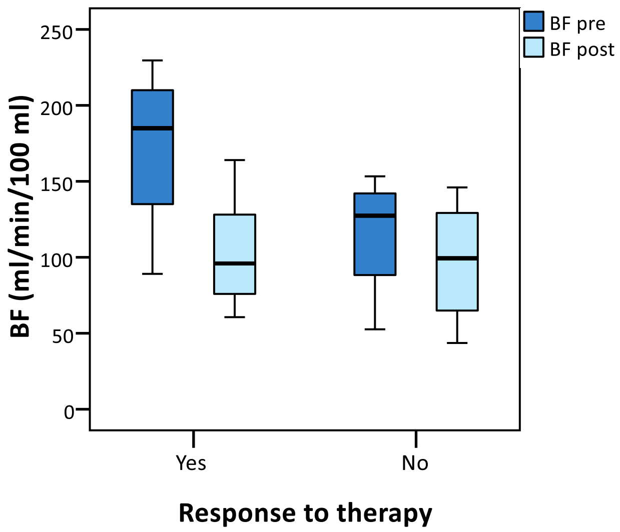
Table 15 shows the best overall response (BOR) category (PR, SD or PD) and time until disease progression occurred. In 6 recorded cases, where PR or SD was observed, which indicates a positive response to treatment, all CTP parameters decreased by more than 10%. In some cases, the change was considerable (>50%). Disease progressed in a later stage, approximately 1.5 years after therapy initiation. The average TTP for responders (PR and SD) was 18.3 months, compared to 3.3 months for non-responders (PD). Noteworthy is the observation in two patients, who have shown good response to therapy and who had longest TTP. First patient (#2), categorized with PR, had a TTP of 34.5 months and patient number 6, categorized with SD, had a TTP of 23.5 months. Both patients showed considerable change in BF and CL (>50%), but BV decreased considerably only in patient #2, whereas in patient #6 it decreased moderately. The findings are somewhat different in four other patients diagnosed with PD on their clinical or CT follow-up. On the first look, CTP parameters seem to be erratic. In cases No. 7 and 8, both BF and CL decreased, BF moderately decreased, and CL has shown considerable change. However, BV considerably increased. In case No. 9, BF and BV moderately decreased, while CL slightly increased. In case No. 10, BF and BV increased and CL slightly decreased.

Table 15. Relative change of blood flow (BF), blood volume (BV), clearance (CL) and long diameter (LD), as well as time to progression (TTP) for each patient included in the study, sorted by category of response after follow-up CT or clinical follow-up

No.	**Response category**	**TTP (months)**	**ΔBF (%)**		**ΔBV (%)**		**ΔCL (%)**		**ΔLD (%)**
1	PR	6.1	↓	-16.7	↓	-11.4	↓	-40.5	-7.3
2	PR	34.5	↓↓	-57.6	↓↓	-88.5	↓↓	-59.9	-12.0
3	PR	10.1	↓	-29.8	↓	-22.5	↓↓	-56.3	-18.2
4	PR[1]	.	↓	-21.9	↓	-12.5	↓	-30.6	0.0
5	SD	13.2	↓	-32.0	↓	-20.0	↓	-44.1	-17.4
6	SD	23.3	↓↓	-66.9	↓	-18.3	↓↓	-73.4	-1.2
Responders average		18.3		-37.5		-28.9		-50.8	-9.4
7	PD[1]	.	↓	-26.7	↑↑	132.4	↓↓	-69.7	4.3
8	PD	6.1	↓	-34.0	↑	28.6	↓↓	-59.4	-11.9
9	PD	2.0	↓	-17.1	↓	-20.0	○	2.6	5.7
10	PD	1.8	↑	17.8	↑↑	103.4	○	-6.0	-1.2
Non-responders average		3.3		-15.0		61.1		-33.1	-0.8

○ = no considerable change (<±10%), ↑ = increase by 10–50%, ↑↑ = considerable increase by more than 50%, ↓ = decrease by 10–50%, ↓↓ = considerable decrease by more than 50%.
[1]Exact time of progression is not known due to lack of data in the records.

Figure 9 and Figure 10 show how different perfusion parameters changed in responders and non-responders. The graphs show that CTP parameters were good indicators of therapy response, as soon as one month after therapy initiation. Significant decrease in BF, BV and CL has been observed ($p < 0.05$). However, according to the t-test, the significant difference was observed in LD, although the relative decrease in responders was less than 10% (Table 15), ranging from 0.0% to 18.2%, which is much lower than 30% as required by RECIST 1.1 for categorization as PR. For the CTP parameters, the relative changes were much higher, ranging from -66.9% to -16.7% for BF, from -88.5% to -11.4% for BV, and from -73.4 to -30.6% for CL in the responders' group (Table 15). Higher relative changes decrease the risk of misinterpretation and could increase sensitivity and specificity. The most obvious difference was the high increase of BV in non-responders (Figure 11). In this patient group, the average ΔBV had a positive value (61.1%). If relative changes of CTP parameters were considered as independent variables, only ΔBV would significantly differ between two groups of patients (t-test, p = 0.027).



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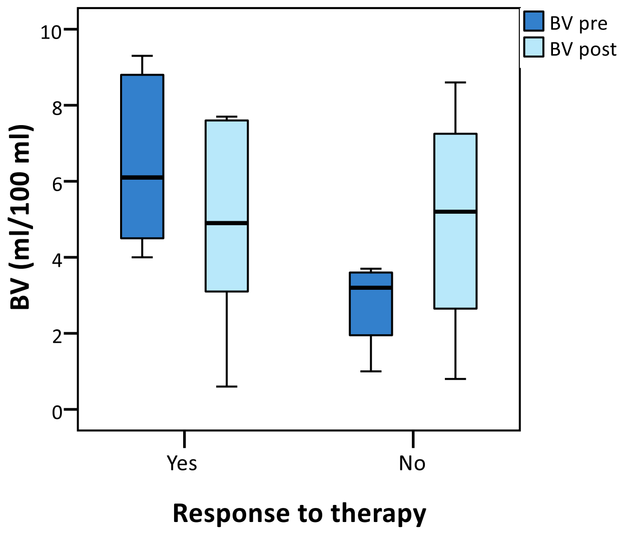
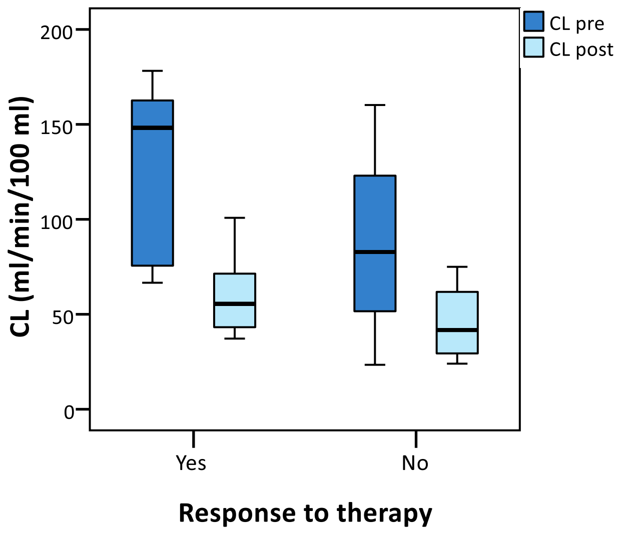


(b)

Figure 9 (a,b). Boxplots of (a) BF and (b) BV before and 4 weeks after initiation of therapy, categorized by response to therapy. The decrease of BF and BV was significant for patients who responded to therapy (p = 0.022 and p = 0.039), but not significant for those who didn't (p = 0.329 and p = 0.148).



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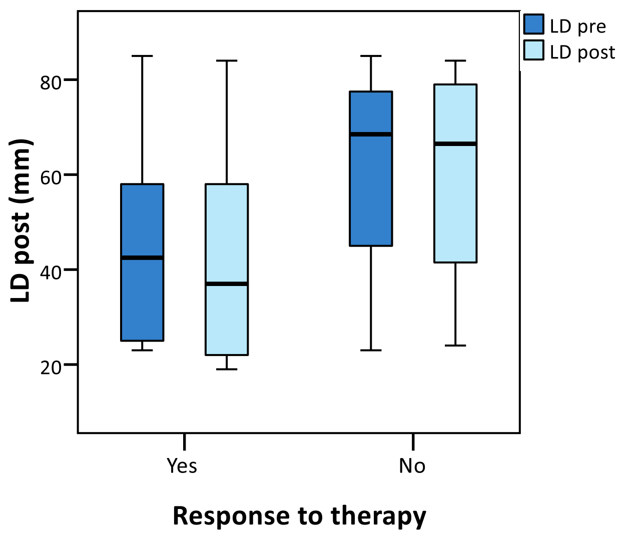


(b)

Figure 10 (a,b). Boxplots of (a) CL and (b) LD before and 4 weeks after initiation of therapy, categorized by response to therapy. The decrease of CL and LD was significant for patients that responded to therapy ($p = 0.008$ and $p = 0.039$), but not significant for those who didn't ($p = 0.207$ and $p = 0.721$).

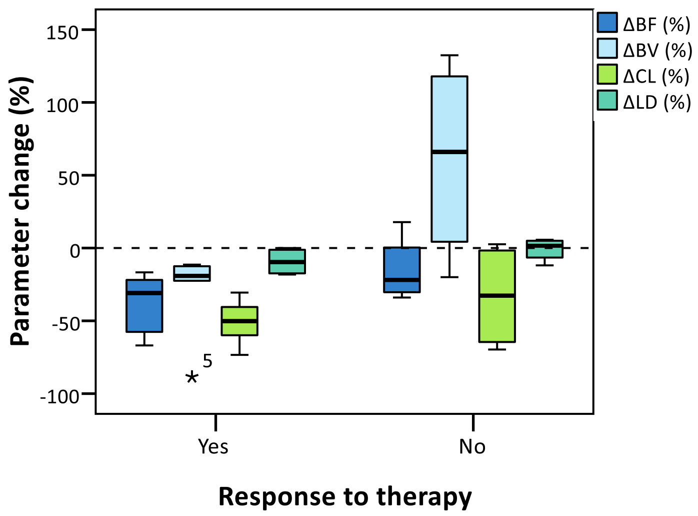


Figure 11. Boxplot of relative change of perfusion parameters blood flow (ΔBF), blood volume (ΔBV) and clearance (ΔCL), and long diameter (ΔLD) before and 4 months after therapy initiation for responders and non-responders. When changes are analyzed as independent variables, t-test was indicating that only ΔBV significantly differed between two groups of patients ($p = 0.027$).

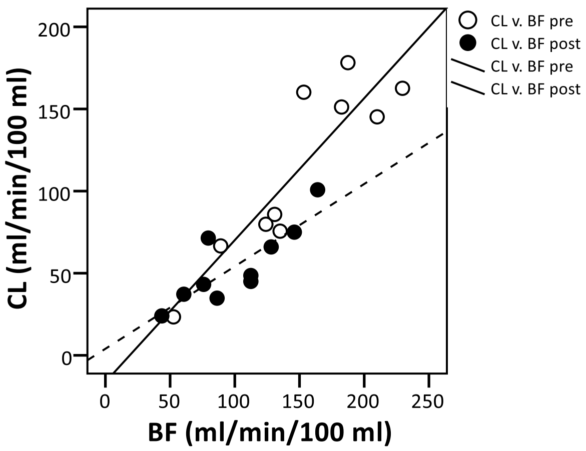
4.2 Aim 2) Correlation between changes in perfusion parameters

A special area of interest was to assess whether perfusion parameters correlate to one another. More precisely, evaluation of correlations was necessary to check, whether one or more prefusion parameters might be redundant, easily explained with another one, therefore not contributing to overall data analysis.

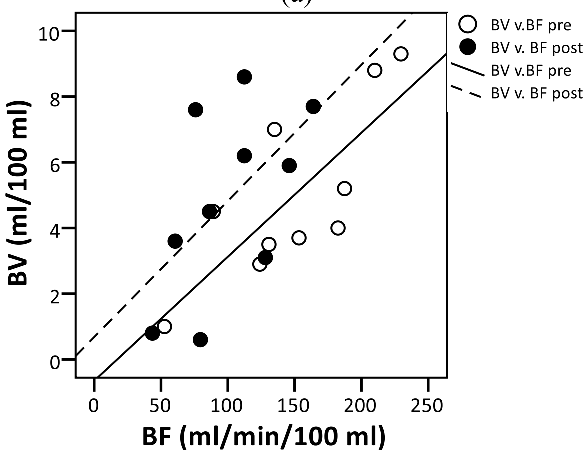
Figure 12a shows correlation between CL and BF, before and after therapy initiation. These two parameters independently decreased over the course of treatment (Δ values in Table 14). However, their absolute values were correlated according to the Pearson correlation test, both before ($r = 0.898$, $p < 0.001$) and after therapy ($r = 0.828$, $p = 0.003$).

Figure 12a shows linear regression curves ($y = a + bx$) for pre- and post-therapy BF (x-axis) and CL (y-axis). The coefficient of determination (R^2) was 0.807 and 0.686 for pre- and post-therapy values, respectively, which shows substantial variances from linear fit model. More interestingly, the slope of the linear curve (b) was different before and after therapy initiation, with values of 0.864 and 0.502, respectively. This illustrates that, although the two parameters were correlated, their values changed independently of one another. Similar conclusion could not be reached for BV and BF correlation graphs (Figure 12b). According to the Pearson correlation test, the significant correlation existed only before therapy initiation ($p = 0.007$), which could not be confirmed after therapy at a 0.05 significance level ($p = 0.091$). R^2 for two regression lines were 0.612 and 0.316 for pre- and post-therapy parameters, respectively. The latter shows that variances between real values and linear model were very high. However, the slopes of the two regression lines are very close ($b = 0.04$), indicating that only one of the parameters influenced the differences in correlation. Understandably, this would be BF, which significantly decreased after therapy initiation.

In a similar fashion, the correlation between CL and BV was tested (Figure 12c). No significant correlation was found, both before and after therapy initiation ($p = 0.112$ and $p = 0.486$). This indicates that changes to these parameters during the therapy course (if any) happened independently of one another.



(a)



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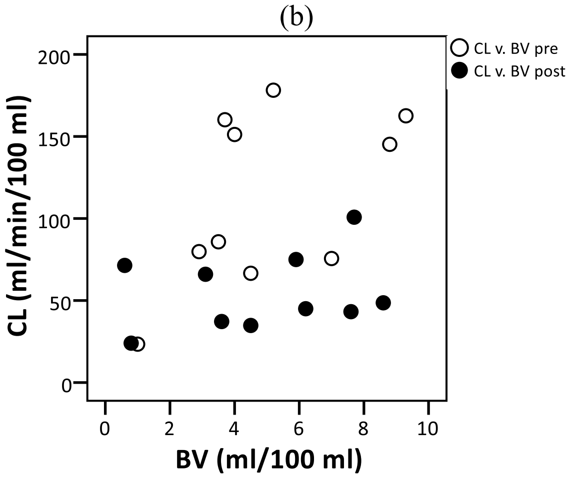


(c)

Figure 12 (a,b,c). Correlation of between (a) CL and BF, (b) BV and BF, and (c) CL and BV before and 4 weeks after therapy initiation, respectively. There was a statistically significant correlation between BF and CL before ($r = 0.898$, $p < 0.001$) as well as 4 weeks after initiation of therapy ($r = 0.828$, $p = 0.003$) and between BF and BV before therapy (r = 0.782, p=0.008). The correlation between BF and BV after the therapy initiation was not significant ($p = 0.091$). The correlation between CL and BV both before and after therapy initiation was not significant ($p = 0.112$ and $p = 0.486$). *Reproduced with modification from Vehabovic-Delic A. et al. J Comput Assist Tomo. 2019 May 1;43(3):493-8) with permission of publisher Wolters Kluwer Health, Inc.*

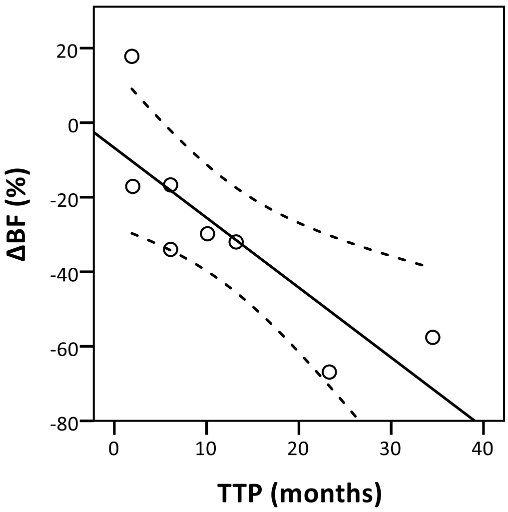
4.3 Aim 3) Correlation of changes in perfusion parameters and size with TTP

In this study we analyzed how changes of CTP parameters, expressed in terms of their relative change (%), correlated with time to disease progression (TTP), which could (apart from actual survival) be considered as a good indicator of treatment success and surrogate of overall survival.

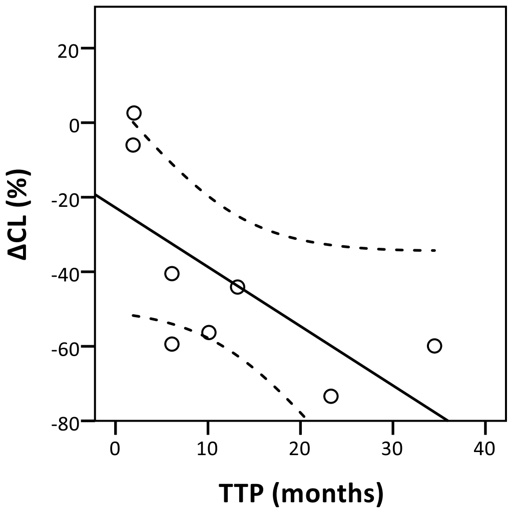
The main goal of this analysis was to assess, whether there was any evidence of association between imaging parameters (perfusion parameters mainly) and clinical outcome. For the acceptance of perfusion parameters as imaging biomarkers in clinical practice, there should be good evidence that CTP parameters predict TTP and patient survival after oncologic therapy. Figure 13 shows the scatter plots of the relative change of BF, CL and BV expressed in percentages, denoted as ΔBF, ΔCL and ΔBV, respectively. Strong negative correlation with TTP was found for ΔBF and ΔCL ($r = -0.838$, $p = 0.009$ for ΔBF, $r = -0.826$, $p = 0.011$ for ΔCL), indicating that a stronger decrease of these parameters was associated with treatment response and meant longer life expectancy of observed patients. However, the Spearman's ρ correlation test showed no significant correlation of ΔBV with TTP ($r = -0.663$, $p = 0.073$).

Figure 13(a,b) shows linear regression lines ($y = a + bx$). Coefficients a and b, as well as root-mean-square deviation (RMSD) of residuals, are presented in Table 16. Coefficient of determination (R^2) of ΔBF and ΔCL line was 0.672 and 0.451. Line slope for ΔBF was $b = -1.81$ %/month. If many uncertainties are put aside, this would mean that decrease of ΔBF by 20% would extend TTP by more than one year. The value of parameter b for the ΔCL regression line was -1.54 %/month. Again, reduction of ΔCL by 20% might indicate extension TTP by one year.

It is worth mentioning that the change of long diameter of lesions (ΔLD) did not correlate with TTP ($p = 0.188$).



(a)



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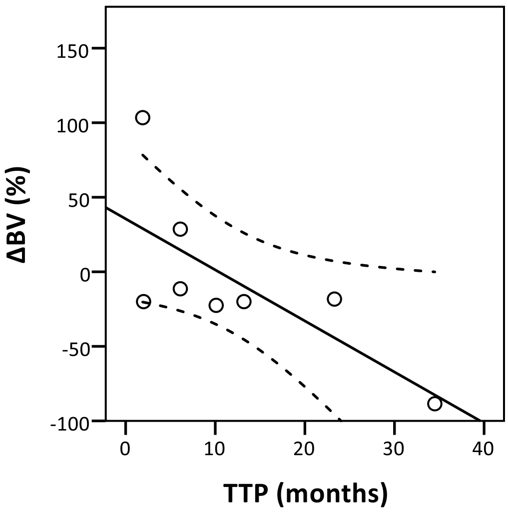

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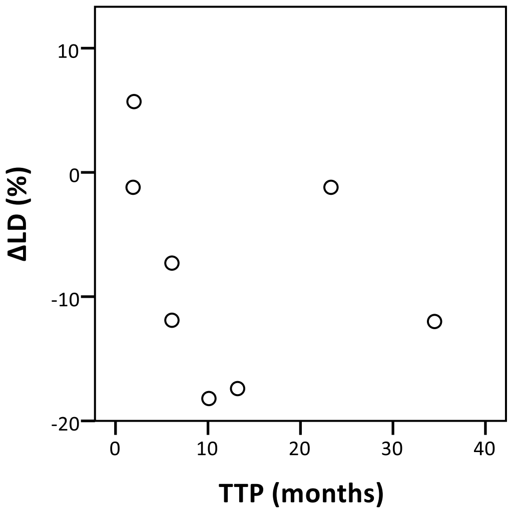


(d)

Figure 13 (a, b, c, d): Correlation between (a) relative change of BF (ΔBF, %) and time-to-progress (TTP, months), (b) relative change of CL (ΔCL, %) and TTP (months), (c) relative change of BV (ΔBV, %) and TTP (months), and (d) relative change of LD (ΔLD, %) and TTP (months). A very strong negative correlation was found between ΔBF and TTP (r = -0.838, p = 0.009) and between ΔCL and TTP (r = -0.826, p = 0.011). Correlation between ΔBV and TTP according to Spearman ρ test was insignificant (r = -0.663, p = 0.073), but significant according to Pearson's correlation test (p = 0.046). Correlation between ΔLD and TTP was not significant (p = 0.188). Dashed lines represent mean 95% confidence intervals.

Table 16. Regression parameters a and b (± asymptotic standard error) and root-mean-square deviation (RMSD) of residuals of fit of relative change of blood flow (ΔBF), blood volume (ΔBV), clearance (ΔCL) and long diameter (ΔLD) versus time-to-progress (TTP) expressed in months.

Parameter	**ΔBF vs. TTP**	**ΔBV vs. TTP***	**ΔCL vs. TTP**	**ΔLD vs. TTP***
a	-8.0±0.2	32.3±0.5	-23.5±0.3	-5.3±0.1
b	-1.81±0.01	-3.24±0.04	-1.54±0.02	-0.216±0.007
RMSD of residuals	13.6	35.9	18.6	7.62

*Not significant

Multiple linear regression analysis

The univariate analysis from the previous chapter suggests two promising candidates for TTP prediction – ΔBF and ΔCL. Because of mutual correlation between these two, we need to explore whether both affect TTP independently, or one of them could be excluded from the prediction process.

Hence, we decided to analyze TTP dependence on CTP parameters using multiple linear regression. The 'stepwise' method, with CTP parameters and ΔLD as independent variables, and TTP as dependent one, suggests that significant correlation exists only with ΔBF, while ΔBV, ΔCL, and ΔLD were excluded because of their lack of significant correlation. In practice, the multivariate analysis has been reduced to univariate analysis. Results are presented in Table 17. The R^2 of this model was 0.672 with standard error of the estimate of 7.1%.

The final result of this model is presented as follows:

$$TTP = 2 - 0.4 \times \Delta BF, \qquad (4.1)$$

where *B* coefficient from Model 1 in Table 17 was used in linear equation (4.1). All coefficients are reduced to 1 significant figure. TTP is expressed in months, and CTP parameters in percentages.

Validity of this model can easily be tested using real patient data. Predicted TTP values and their deviation from the real value is presented in Table 18 for ΔBF, as well as for two other CTP parameters.

Table 17. Results of multiple linear regression on simulated data using ‘stepwise’ model. Independent parameters were blood flow (ΔBF), blood volume (ΔBV), clearance (ΔCL) and long diameter (ΔLD), while dependent variable was time-to-progress (TTP) expressed in months.

Model		**Unstandardized Coefficients**		**Standardized Coefficients**	***t***	***p***	**95% Confidence Interval for *B***	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	1.573	3.916		0.402	0.702	-8.009	11.156
	ΔBF (%)	-0.358	0.102	-0.820	-3.504	0.013	-0.608	-0.108
Excl.	ΔBV (%)	-0.218			-.569	.594		
	ΔCL (%)	-0.003			-.006	.996		
	ΔLD (%)	-0.076			-.287	.786		

Table 18. Results of univariate linear regression analysis for prediction of time to progression (TTP) expressed in terms of absolute values of TTP (months) and relative deviation (%) from true value of TTP.

No.	**Response category**	**TTP (months)**	**Predicted TTP (months) and deviation from the real value (%)**					
			$TTP_{\Delta BF}$	(%)	$TTP_{\Delta BV}^{*}$	(%)	$TTP_{\Delta CL}$	(%)
1	PR	6.1	4,8	-1.3%	13,5	7.4%	11,0	4.9%
2	PR	34.5	27,4	-7.1%	37,3	2.8%	23,6	-10.9%
3	PR	10.1	12,0	1.9%	16,9	6.8%	21,3	11.2%
4	PR[1]	.	7,7	.	13,8	.	4,6	.
5	SD	13.2	13,3	0.1%	16,1	2.9%	13,4	0.2%
6	SD	23.3	32,5	9.2%	15,6	-7.7%	32,4	9.1%
Responders’ mean				0.6%		2.4%		2.9%
Responders’ std. deviation				5.9%		6.1%		8.8%
7	PD[1]	.	10,3	.	-30,9	.	30,0	.
8	PD	6.1	14,4	8.3%	1,1	-5%	23,3	17.2%
9	PD	2.0	5,0	3%	16,1	14.1%	-16,9	-18.9%
10	PD	1.8	-14,3	-16.1%	-21,9	-23.7%	-11,4	-13.2%
Non-responders’ mean				-1.6%		-4.9%		-5.0%
Non-responders’ std. dev.				12.8%		18.9%		19.4%
Total mean				-0.3%		-0.3%		-0.05%
Total std. deviation				8.3%		11.7%		13.0%

*No significant correlation

4.4 Decision flowchart

Based on the results, we could devise a decision-making flowchart (Figure 14) that could be helpful when deciding whether patient is responding to therapy or not.

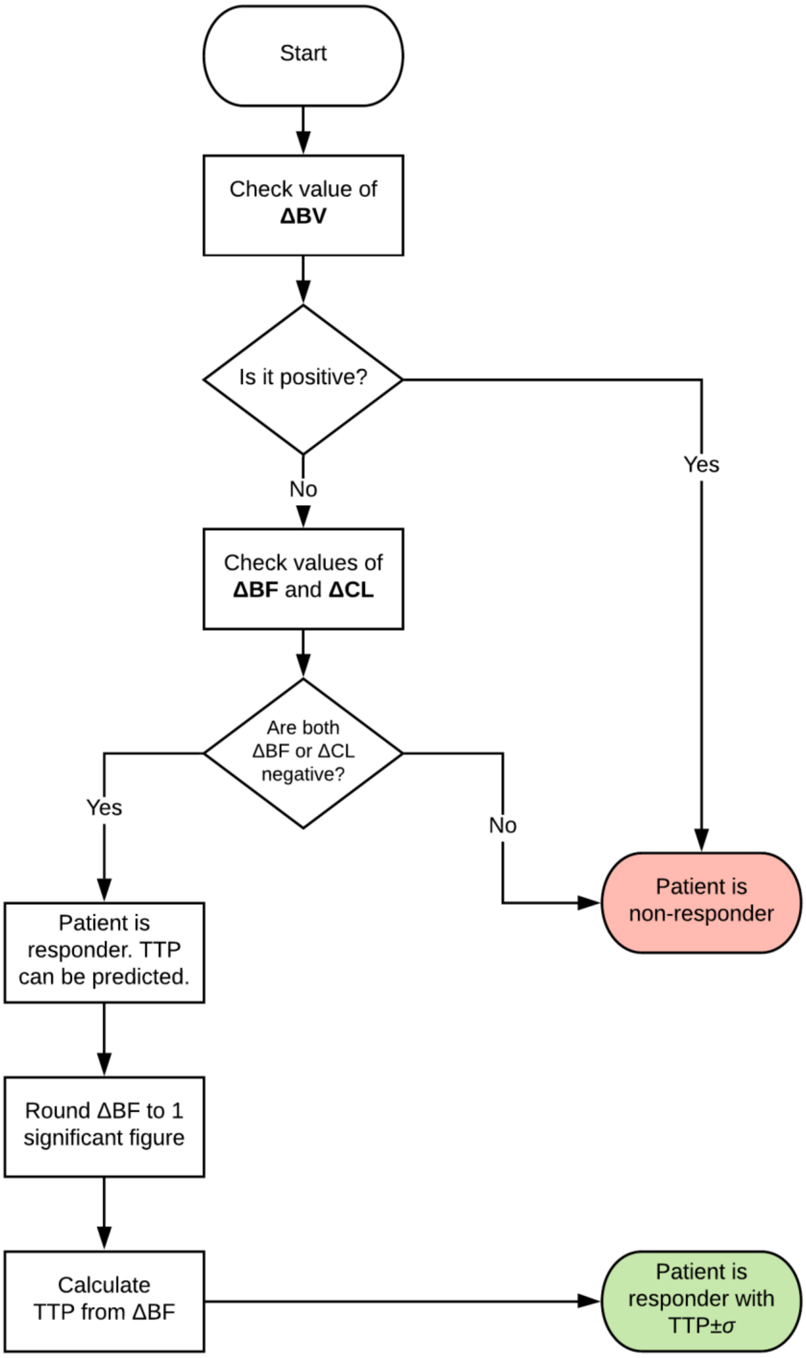


Figure 14. Decision-making flowchart

5 Discussion

It is widely recognized that the traditionally used standardized RECIST are not quite adequate criteria for evaluation of treatment response in the early period of antiangiogenic therapy [15]. Additional limitation of RECIST is lack of reliability in lesion measurements between different radiologists. Even when repeated, measurements made by the same radiologist could account for potential RECIST misclassification of approximately 10% in progressive disease and of approximately 3% in partial response, especially when evaluating poorly demarcated lesions. Possible errors when measuring LD could additionally alter adequate evaluation of treatment response that contribute to further clinical and radiological uncertainty resulting with low impact on patient therapy modification even when PD according RECIST is evident [41].

Therefore, in a light of the increasing use of targeted agents in oncological treatments of a range of solid tumor types, imaging techniques that assess tumor vascular support have gained growing importance in tumor response assessment alongside standard response criteria. Due to its wide availability, simplicity of implementation and interpretation, CT perfusion became a preferable objective functional imaging technique for evaluation of changes in tumor vessels, compared to other functional methods (PET, MRI, US) [8,12].

Since introduction, technology of CTP has evolved, especially in the recent years. New advances allowed implementation of CTP in daily radiological and clinical routine. In the past, different CT manufacturers have provided different analyzing algorithms, and all of them used different acquisition protocols, which was the major obstacle for the standardization of this method [20,78]. However, recent software upgrades by two major manufacturers [20] and consensus guidelines published in 2012 for the use of CTP in assessment of tumor vascular support developed by Delphi process [16] have made steps forward in more standardized approach and facilitated application of CTP in clinical environment.

Despite technological maturity of CTP, there is currently still no consensus on which perfusion parameter is essential to assess changes in tumor vascularity in response to specific treatment. Additionally, there is no agreement on level of significant percentage change in supposed essential perfusion parameters.

This is mostly influenced by the fact that there are differences in perfusion values when calculated with two different kinetic models [78].

Since 2004, numerous CTP studies have investigated the role of CTP in evaluation of antiangiogenic treatment responses. Although these studies have been designed differently, as it is explained in the introduction of this thesis, all of them have contributed to the current understanding of the CTP magnitude in oncologic follow-up. When taking a quick overlook over results of different studies published in the past, without taking different study designs into consideration, one could get the impression that changes in perfusion parameters are inconsistent. Indeed, some of these studies (precisely ten of them) have shown change in one perfusion parameter only [48,50,51,53,57,66,70,72,73,76], while other studies report change in two [44,47,52,56,59,60,65,71,77] or all three [49,54,55,61,63,68] main quantitative perfusion parameters. However, when all details of published studies are taken into consideration, their results are more understandable, and their meaning becomes less inconsistent.

Among ten studies, which have demonstrated a significant decrease in one parameter, seven of them had their study designed to test the change in one perfusion parameter only and, therewith, could not offer any other information. The other three studies were designed to assess treatment response to specific agents, e.g. MEDI522 (monoclonal antibody targeting integrin avB3) [48], L-NNA (nitric oxide sinthetase inhibitor) [53], and specific therapy concepts e.g. combination of radiotherapy and subsequent administration of the vascular disrupting agent combretastatin A4 phosphate (CA4P) [52]. It is worth mentioning that the two latter mentioned studies were designed to test change in only two perfusion parameters, BV and CL (calculated by modified Patlak), due to the expected action mechanism of agents used in treatment. Furthermore, the study by Ng et al. [52], analyzing the effect of RT and CA4P, presented capability of CTP to recognize and clarify specific activity mechanisms of certain agents (in this study RT and vascular disrupting agent), and their positive interaction. However, only one study tested the change in all perfusion parameters and showed change in one parameter. This study primarily evaluated the safety and tolerability of MEDI-522 in patients with therapy for refractory solid tumors, with the secondary aim to test the possible antiangiogenic activity of MEDI-522, demonstrating a tendency of MTT to increase with increasing dose of MEDI-522 [48]. Related to combination therapies, CTP studies by Kambadakone et al. [68] and Yoon et al. [61] have shown that Bevacizumab may augment the efficacy of RT in treatment of soft tissue sarcomas. Both studies demonstrated significant decrease of all main perfusion parameters after combination therapy was applied.

When looking at studies performed on hepatocellular carcinomas (HCC), one can notice that different combination of perfusion parameters was analyzed depending on kinetic models used in the studies. The studies that have used the maximum slope method [71–73] have calculated arterial flow of HCC and surrounding liver parenchyma only, and the studies that have used deconvolution methods [54,60,63,64] have calculated all parameters (BF, BV, PS and MTT) of both HCC and background liver parenchyma. With regard on HCC behavior, it's been identified that this tumor is exclusively supplied by arterial vessels and main parameter for analyzing treatment response is supposed to be blood flow. As expected, all HCC studies, no matter on analyzing algorithm, have shown that blood flow was decreasing after targeted therapy as a sign of good treatment response. Additionally, deconvolution studies [54,63] have demonstrated decrease in BV and PS and increase of MTT as well.

Altogether, we may conclude that lack of assertion on a specific perfusion parameter necessary for evaluation of vascular changes in response to antiangiogenic therapy is not due to differences in kinetic models only, but also due to varying results and differences in study designs from the former abovementioned CTP studies.

Within this thesis, we evaluated the significance of volume CT Perfusion (VCTP) imaging in oncologic follow-up by investigating a homogenous group of patients with highly vascular mRCC treated with TKI. By selecting this group of patients, we aimed to avoid any inconsistencies which might be present in an inhomogeneous group of different solid tumor types.

In the following discussion, observations from this thesis have been presented with regard to other published studies on this topic, and additionally with regard to understanding of relationship between perfusion parameters and tumor angiogenesis.

Comparison between changes in Perfusion and Changes in Size in the early period of targeted therapy in mRCC patients

In this thesis, we explored the potential value of CTP imaging to evaluate response of mRCC to antiangiogenic therapy in the early period of targeted therapy (4 weeks after therapy initiation). Overall, results 4 weeks after targeted therapy initiation have shown, that significant change in perfusion has occurred, whereas the size of the tumor remained stable. The long diameter of the tumors decreased by 4%, which is well below the threshold of -30% that is required to declare partial response when using RECIST 1.1, and it is also much lower than the proposed optimized threshold of -10% [39], as well. Initially, all patients in this study were categorized with stable disease (SD) according to RECIST 1.1, which is in accordance to the literature on early follow-up of RCC after targeted therapy [56,79–81]. In contrast to tumor size, two of three perfusion parameters have shown a significant decrease 4 weeks after therapy, BF decreased by 28%, and CL decreased by 44%, while BV did not show significant change in overall analysis.

However, follow-up of patients continued after the second CTP examination until progression of disease occurred. This follow-up data allowed separation of patients into responders and non-responders which gave the analysis a different approach. We were able to investigate the possible prediction value of CTP parameters.

Responders have shown significant decrease in BF, CL, and additionally in BV (-30.9%, -50.2% and -19.2% respectively). Furthermore, the decrease in LD was also significant by responders, although the relative change of this parameter was lower than 10%. This could imply that tumors showed trends toward size shrinkage by responders, but the change was too low to be characterized according to RECIST 1.1 which requires -30% decrease in size to declare PR. This finding is partly in accordance with the study by Vasudev et. al [74] who have shown that antiangiogenic therapy induces a vascular response in association with tumor shrinkage and clinical benefit. The significant decrease of BF, BV and CL in responders in our study is in accordance with a study by Faria et al., who have used functional CT to evaluate the effect of thalidomide therapy on patients with mRCC. The authors reported a significant decrease of BF, BV, and permeability-surface-area-product (PS), which is comparable to CL in our study, 12 weeks after therapy. Results from this thesis are also in agreement with a study by Fournier et al., who reported a significant decrease of BF and BV after one cycle of targeted therapy. In contrast to that study, CL in our study and PS in the study by Faria et al. have also shown significant decrease. The lack of decrease of PS in the study by Fournier may be explained by the probable insufficient time of scanning for reliable estimation of this parameter. Nevertheless, the findings in this thesis are somewhat different in case of non-responders.

At first glance, CTP parameters seem to be erratic. Regarding surrogate molecular imaging theory proposed by Miles, different perfusion parameters reflect different physiological processes, that represent end result of various molecular processes related to tumor hypoxia [12]. The main effect of antiangiogenic agents is reduction in the number of tumor blood vessels trough the blockage of proangiogenic factors. Among the many proangiogenic growth factors, VEGF is recognized as one with highest importance for the growth of tumor vessels. VEGF represents a direct angiogenesis inducing factor, and through direct stimulation of prostaglandin release represents a powerful permeabilizing factor as well [82]. Inhibition of the VEGF pathway by antiangiogenic agents leads to passive pruning of the leaky and immature tumor vessels and to active remodeling and regression of the remaining vessels, so that abnormal tumor vessels more closely resemble normal vessels. This process is called vessel "normalization". Because of heterogeneity of the tumor vessels, antiangiogenic agents may affect different physiological processes in varying degrees [44,83]. Therewith, physiological explanation of perfusion parameters could be the key for correct interpretation of findings.

In the cases of patients No. 7 and 8, both BF and CL decreased, BF moderately, and CL has shown considerable change, indicating that medication used led to a reduction of poorly formed vascular basement membranes. However, the BV value considerably increased, indicating tumor's adaptation to hypoxia, and growing resistance to the therapy, confirming BF-BV mismatch theory proposed by Bisdas at al.[45] In case No. 9, values of BF and BV decreased moderately, while CL slightly increased, signifying that therapy had no effect on tumor's permeability, which, again indicates its adaptation to hypoxia by release of VEGF and prostaglandins. In case No. 10, BF and BV increased, except for CL, which slightly decreased. Increase of BF and BV signifies continuing angiogenesis and vasodilatation. In another words – unsuccessful treatment. CL, indicator of tumor's vessels maturity i.e. angiogenesis, did not change much; tumor cells received enough oxygen and nutrients necessary for further growth.

Altogether, study results have shown greater percentage oscillation in BV values between responders, -19.2%, and non-responders, 61.1% on average). If CTP parameters are analyzed as independent variables, only ΔBV would significantly differ between responders and non-responders, which could be taken as an indication of how considerable the difference between two averages is. BV decreased in all responders and increased almost in all non-responders with exception of one patient. This implies that positive and negative oscillations in BV values 4 weeks after antiangiogenic therapy may be the reason why BV hasn't been recognized as significant parameter in overall analysis – BV values in responders and non-responders canceled each other.

In summary, when patient cases are analyzed one by one, one could notice how decrease of BV was associated with responders, while increase of BV indicated a patient who has not responded to therapy in three out of four analyzed cases. Therefore, using ΔBV alone would decrease the CTP specificity. This is where two other perfusion parameters show their true value. In the group of responding patients, BF and CL have decreased as well, which lead us into conclusion that responders need to have reduction in all analyzed CTP parameters. For non-responders, ΔBF and ΔCL show variable changes. However, in a single case when ΔBV was negative, ΔCL had a positive value. In the subsequent follow-up, the patient had been categorized as non-responder. ΔBF and ΔCL further reinforce overall test confidence in both groups, responders and non-responders.

Correlation between changes in perfusion parameters

The current literature implies that most drug treatments, regardless of their type (antiangiogenic or conventional), produce decrease in BF and BV [16], and that their change is coupled one to another [19]. In this thesis, we examined results from number of different CTP studies published in the last 15 years, taking only antiangiogenic therapy into consideration. Studies have associated varying changes in perfusion parameters to positive treatment response without agreement on how change in perfusion parameters should be interpreted.

The question of correlation between different CTP parameters might not be essential for tumor progression diagnostics, however, it is necessary to provide an answer whether one or more perfusion parameters might be redundant. If two parameters do not change independently, one of them has no additional value for overall analysis. Therefore, this parameter may be omitted, which makes interpretation of findings more convenient. In literature, there is still lack of agreement on how perfusion parameters should be interpreted.

In this thesis, we found a statistically significant positive correlation between BF and CL before and 4 weeks after initiation of therapy, and positive correlation between BF and BV before therapy. However, significant correlation between BF and BV after therapy initiation was not found. Furthermore, correlation between CL and BV was neither significant before nor after therapy initiation.

Interpretation of relationship between different perfusion parameters and between perfusion parameters and angiogenesis is complex. Different perfusion parameters reflect specific physiological processes of tumor angiogenesis [16,19]. In perfusion imaging, vessel permeability is represented by the blood flow extraction efficacy (FE). FE reflects the total unidirectional diffusional flux of the contrast media from the intravascular to the extravascular space across all capillaries, and it is measured by the Patlak (compartmental model; represented as CL) or the Johnson Wilson distributed model (deconvolution model; represented as PS). CL is comparable with PS, which is the product of the permeability and the total surface area of the capillary endothelium. In physiological conditions, FE explicitly depends on flow and permeability (BF and CL/PS). The Johnson-Wilson model for distribution of blood in tissue has described three regimes for the exchange of the blood solute between the intravascular and interstitial space depending on the relation between BF and CL/PS: (1) when PS $\ll$ BF, the exchange is diffusion limited; (2) when PS $\gg$ BF, the exchange is flow limited; and, (3) when PS is of the same magnitude as BF, the exchange is neither diffusion nor flow limited [15,84].

Regarding this model, nine of ten patients in our study had values of BF greater than CL before and all patients had values of BF greater than CL after therapy initiation, indicating that the exchange of contrast media was predominantly diffusion limited. The overall analysis had shown that CL has decreased 1.7 times more than BF, indicating that in the early course of therapy, normalization of immature vessels was more composed of a reduction of poorly formed vascular basement membranes, which is reflected by the decrease in CL, than on a reduction of arteriovenous shunts, which is manifested by the decrease of BF. This implies that, although two parameters were correlated, their values probably changed independently of one another. The independent change of these parameters is clearly visible on corresponding graphs, where the slope regression line is different. In addition, Patlak as a compartmental analysis method simulate physiological condition in capillaries and in contrast to deconvolution analysis cannot measure flow (F) and total unidirectional diffusional flux efficiency (E) independently, because they are determined together as the blood flow extraction product (FE), which is represented by CL in our study.

According to the literature, BF and BV are often linked one to another [19]. Indeed, the significant positive correlation between BF and BV before therapy has been confirmed in our study too. However, no significant correlation between these parameters was found after therapy initiation, suggesting that therapy has affected these parameters in different degrees. From the correlation graphs it can be seen that, although variances between real values and linear model are very high, the slopes of two regression lines are very close, indicating that only one of the parameters influenced the differences in correlation. This is probably influenced by BF because this parameter has decreased in all responders and in three out of four non-responders. In contrast to this, there is a drastic difference in BV for responders and non-responders, which might be explained as follows. Besides VEGF, nitric oxide synthase (eNOS) is recognized as another important factor in the processes of tumor angiogenesis that are responsible for the maintenance of vascular tone in tumor vessels [12]. A recent study has shown that inhibition of eNOS results in a sustained reduction in tumor blood volume. Having changes in BV of responders and non-responders in mind, this postulation about the effect of eNOS may be confirmed in this thesis [53]. We can see from the study results that therapy has probably affected eNOS in case of responders, who showed decreased BV values, and had no effect on non-responders' eNOS, where we found a considerable increase of this perfusion parameter. Furthermore, we could not find any correlation between CL and BV before and after therapy initiation as well, indicating that these parameters are not associated with one another at both time points. Their changes seemed to happen independently of one another.

Correlation of changes in perfusion parameters and size with TTP

Although CT technology has reached its maturity, and although CTP and perfusion parameters have gained acceptance for use as imaging biomarkers in assessment of oncologic response alongside standard response criteria, data on relationship between CT perfusion measurements and clinical outcomes are still limited [15]. In the last 15 years several CTP studies have investigated response to antiangiogenic agents, and only few of them have shown that CT perfusion parameters may correlate with clinical outcome, i.e. in NSCLC [57,70], advanced HCC [72], in metastatic RCC [49], in advanced HCC [63] ,

In this thesis we analyzed how the change of CTP parameters, expressed in terms of their relative change (%) 4 weeks after therapy initiation, was correlated with disease progression time (TTP), which could (apart from actual survival) be considered as a good indicator of treatment success.

Strong negative correlation with TTP has been found for two CTP parameters, ΔBF and ΔCL, indicating that their decrease means longer life expectancy of observed patients. Looking individually at the relative changes of perfusion parameters, it is noteworthy that patient No. 2 and 6, who have shown considerable decrease (over -50%) in BF and CL after therapy initiation had longest time until disease progressed which was 34.5 and 23.3 months, respectively. If many uncertainties are put aside, the value of parameter *b* in the regression lines of correlation graphs would mean that a decrease of ΔBF and ΔCL by 20% would extend the time until progression of disease by more than one year.

This finding in this thesis is in agreement with a study by Faria et al. [49] who demonstrated same results 12 weeks after treatment. In contrast, Fournier et al. [56] could not find any statistically significant correlation between decrease in perfusion parameters and progression-free or overall survival.

An interesting result was the unexpected lack of significant correlation between ΔBV and TTP, which had seemed to be a very valuable parameter for discriminating responders from non-responders. Another observation was a very wide 95% confidence interval for plotted linear regression lines, when correlations did exist. All predictions stated in the previous paragraph should be taken with a grain of salt.

The univariate analysis suggests two promising candidates for TTP prediction – ΔBF and ΔCL. However, their mutual correlation with TTP had to be explored. For this purpose, the stepwise multivariate correlation analysis has been used. It confirmed the lack of significant correlation between TTP and ΔBV, but also between TTP and ΔCL. The only parameter found to be in a significant correlation with TTP had been ΔBF.

It is worth mentioning that, in our study, the change of long diameter of lesion (ΔLD) did not correlate with TTP. This was expected, because the period of 4 weeks after therapy initiation was too short for a significant change of the diameter of lesions.

Prediction algorithm

In this thesis we analyzed the possibilities of CT perfusion to predict response, beginning from analysis of individual cases to assessing the relative importance of each CTP parameter with multi- and univariate regression analysis. Ultimately, there are two important questions we need to answer: 1) Can CTP detect whether a patient is a responder or a non-responder 4 weeks after therapy initiation, and 2) If so, how long will it take until TTP?

After careful consideration of all available results, we decided to create a decision flowchart with intent to help radiologist interpreting CTP results.

To answer the first question, whether patient is a responder or a non-responder, we considered ΔBV to be the crucial parameter. If its value is positive, the patient is likely to be a non-responder, no matter how other perfusion parameters have changed over the 4 weeks. When ΔBV is negative, values of ΔCL and ΔBF need to be checked. If they are negative too, we may consider therapy to be successful. The importance of ΔBV as a response predictor has been recognized through individual assessment of patients included in the study.

This leaves us with second question – how to predict TTP? The results have shown different possibilities of prediction. Unlike in case of response prediction, ΔBV doesn't seem to be a preferable parameter to be used to estimate TTP. One of the major issues was the lack of correlation between ΔBV and TTP. Other parameters were not perfect either. Hence, we decided to go with the most convenient one, ΔBF – the only parameter found to be significant in the multivariate analysis. Formula based on its relationship with TTP was provided to be used in prediction process.

The proposed algorithm makes use of all CTP parameters, each of them having a specific role in the decision-making process; ΔBV, ΔCL and ΔBF in response prediction, and ΔBF in TTP estimate.

Limitations of the study

The current study has certain limitations. First of all, as already mentioned, the small number of patients may have influenced our results. Second, our results may be limited to the specific type of CT scanner, software, scanning protocol, and contrast agent used in our study. Furthermore, different models used for perfusion parameters calculation in our study and in the literature may contribute to disagreement in results. The software used in our study was based on compartmental analysis, which allowed calculation of BF, BV and CL, but did not offer calculation of further perfusion parameters such as mean transit time (MTT).

6 Conclusions

Several aspects have to be addressed in standardization of use of CTP in oncologic follow-up: Can CTP detect response earlier that the change in tumor size, and if so, which perfusion parameter/parameters could be essential for detection of vascular changes in patient who responded to therapy and how to interpret their change in response to targeted therapy. Additionally, is there evidence of an association between change in perfusion parameters and clinical outcome?

Results from this thesis indicates that CTP is able to detect changes in perfusion of mRCC in the early period of targeted therapy in which tumor size remain stable. Baseline overall analysis did not show that BV is an important perfusion parameter for the response evaluation. However, by classifying response of patients individually (responders and non-responders) we have demonstrated that ΔBV is the parameter with the highest importance for detection of non-responders. Nevertheless, in order to improve CTP specificity all perfusion parameters have to be considered in response evaluation. Only when all three perfusion parameters are decreased, no matter on their individual percentage change, CTP can reliably imply that the patient is responding to the therapy. Different percentage change of all three parameters indicates that the therapy has probably affected physiological processes of angiogenesis in varying degrees and that all three perfusion parameters have to be considered independently. Interpreting CTP parameters individually is probably the most convenient way to make use of all information that can be assessed by CTP, no matter on type of kinetic modeling. This approach may make CTP a real surrogate functional imaging technique of tumor angiogenesis. In addition, BF may be the promising parameter for prediction of TTP. Although this thesis was performed with small sample size, we believe that it has provided some important insights on current understanding of CTP in tumor follow-up, which could contribute to faster incorporation of CTP imaging in addition to routinely performed conventional CT and improve individual patient care.

Appendix 1

Kinetic Models

Compartmental analysis

Single compartment analysis assumes that the intravascular and extravascular spaces are a single compartment and uses Fick principle based on conservation of the mass within the system to calculate perfusion parameters. The single compartment model uses maximum slope of the tissue time density curves (TDC) normalized to the arterial input function to calculate blood flow (BF). Depending on whether the examined organ is supplied by one or two arteries, the "single input" or "double input" maximum slope method can be used. The double compartment model estimates capillary permeability and blood volume (BV) using Patlak analysis, which assumes that intravascular and extravascular spaces are two separate compartments. The Patlak analysis uses linear regression to quantify the passage of the contrast from the intravascular into the extravascular space. Canon, Siemens and Philips are CT providers that use compartmental analysis logarithms in their software packages for perfusion calculation. Compartmental analysis methods are more sensitive to noise and less sensitive to movement because they mainly depend on three images for perfusion measurement: the baseline image and the images immediately before and after the moment of the maximal rate of tissue contrast enhancement [17].

Deconvolution

Deconvolution analysis is based on the use of arterial and tissue time density /concentration curves (TDC) to calculate the impulse residue function (IRF) for the tissue. This method allows calculation of the blood flow (BF), blood volume (BV), and mean transit time (MTT). (7) For the estimation of capillary permeability, a distributed parameter Johnson Wilson model is used, which is essentially an extended deconvolution model [17]. GE Healthcare CT provider use deconvolution analysis within their software package.

Appendix 2

CTP technique implementation

Guidelines for the use of DCE-CT in the assessment of tumor vascular support was determined from results of two rounded Delphi process [16]. Conventional CT perfusion protocol consists of several acquisitions, a baseline pre-contrast acquisition followed by dynamic acquisition during contrast agent administration. When performing a CTP study following steps must be considered:

Definition of target lesion

Baseline low dose helical CT without contrast media application serves as localizer for the selection of the appropriate tumor tissue, which will be studied during dynamic acquisition [13].

Selection of appropriate acquisition protocols

CT perfusion protocols depend on different features, such as the kinetic model used and body region or organ being studied, taking the contrast agent dynamics and the arterial supply in the respective region/organs into account.

Scanning Parameters and radiation dose

It is recommended to use the lowest radiation dose as possible. Therefore, optimal temporal resolution balanced with acceptable radiation burden according to the ALARA principles (dose As Low as Reasonably Achievable) is mandatory for optimal perfusion imaging [20]. Depending on algorithms/kinetic models, there is trade-off between tube voltage (kilovolt peak, kVp), tube current (milli- ampere-second, mAs) and number of images that are acquired during examination in order to reduce the radiation exposure. Employing lower tube voltage (kilovolt kV) has the advantage to decrease radiation exposure and increase attenuation of iodine. However, tube voltage should be adjusted for different body regions and body sizes, because low tube voltage will in thicker body regions or/and larger patients result in excessive image noise even with an increase in tube current [17]. It is recognized that tube voltage potential of 80 to 100 kVp is appropriate for most body applications. The deconvolution method, being less sensitive to noise compared to the compartmental method, allows the use of a lower tube current (50-100mAs). For the compartmental model a higher tube current (100-250mAs) with lower image frequency is preferred for the dynamic study [78].

Contrast agent

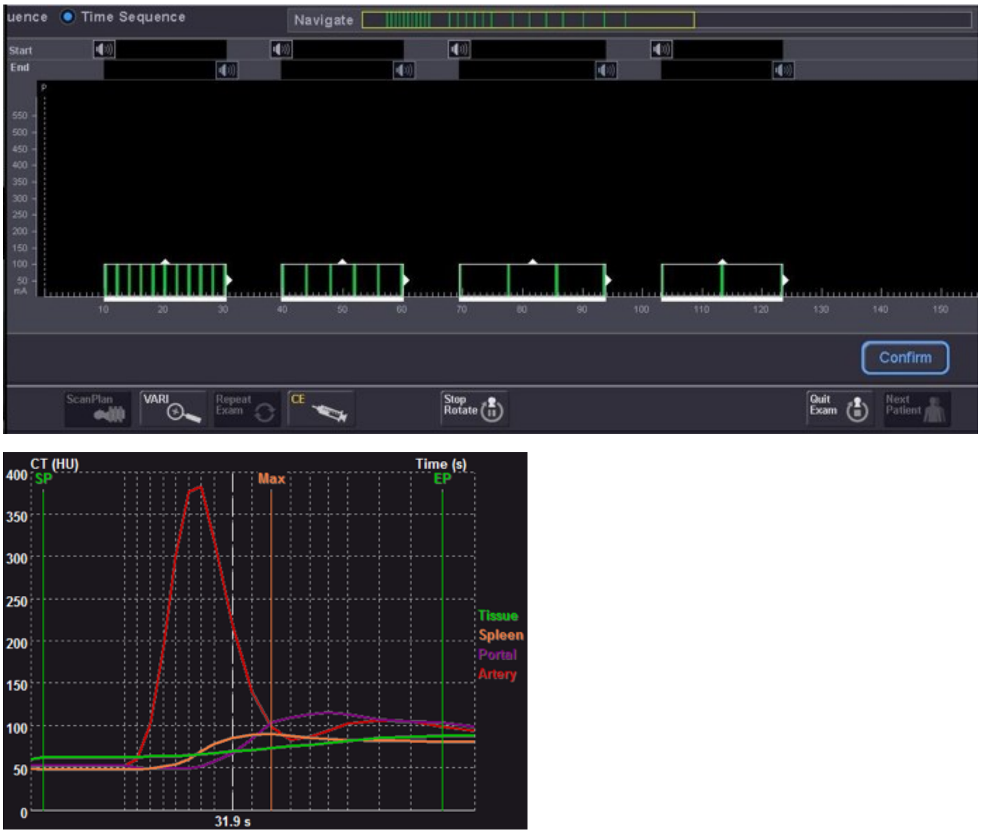
As all CTP techniques are based on intravenous administration of contrast agent one of the important considerations in order to perform adequate perfusion imaging is the choice of contrast agent and mode of injection. Nonionic contrast media are preferred over ionic due to lower effect of flushing and nausea, which could prevent patient movement and motion artefacts during acquisition. In order to achieve fast maximal arterial enhancement, it is recommended to use a short and sharp contrast agent bolus. Therefore, contrast agent should be administered by a rapid injection rate (4-8 ml/s), using contrast media with a high iodine concentration (i.e. 350–400mg iodine/ml), because the slope and the height of the arterial enhancement is directly proportional to the iodine flow rate. Contrast quantity can be reduced to 30-50ml compared to conventional CT in dependence of the body weight. The use of narrow vascular bolus is especially important when using compartmental analysis algorithms, because perfusion measurements are affected by the width of the bolus of contrast medium in the vascular system [15].

Breathing technique

CTP can be acquired using intermittent breath-hold technique or continuous quiet free breathing. Adequate registration of maximum arterial enhancement is of paramount importance for adequate perfusion calculation. Intermittent breath hold technique by patient with restricted cardiac function, may lead to misregistration of maximum arterial enhancement with consequently erroneous perfusion results. Therefore, quiet free breathing technique with using of abdominal straps is preferable, since with this method maximum arterial enhancement will be certainly captured [15,16].

Dynamic acquisition

The Dynamic CT imaging acquisitions may be divided in two phases according /due to the contrast media dynamics (early and delayed). Regarding perfusion parameters being analyzed, dynamic acquisition includes either an early phase or an early phase as well as a delayed phase. Dynamic acquisition is based on preformation of repeated scans over the same target region in high temporal resolution before and during contrast administration, allowing determination of time-density curves (TDC) for the selected area (Figure 1). The early (first pass or vascular) phase is relatively brief and require high temporal sampling with image scanning intervals of 1–2 s. It lasts approximately 30–45 s from contrast arrival. In the early phase, tissue enhancement is attributable mainly to the distribution of contrast within the intravascular compartment and is determined by the blood flow (BF) and blood volume (BV). In the delayed phase, contrast passes from the intravascular to the extravascular compartment and tissue enhancement results from distribution between the two compartments and is highly influenced by vascular permeability. The delayed phase requires lower temporal sampling. Scanning interval is every 5-15 s, and imaging duration depends on analysis model. It can last between 2 and 10 minutes [13,19].



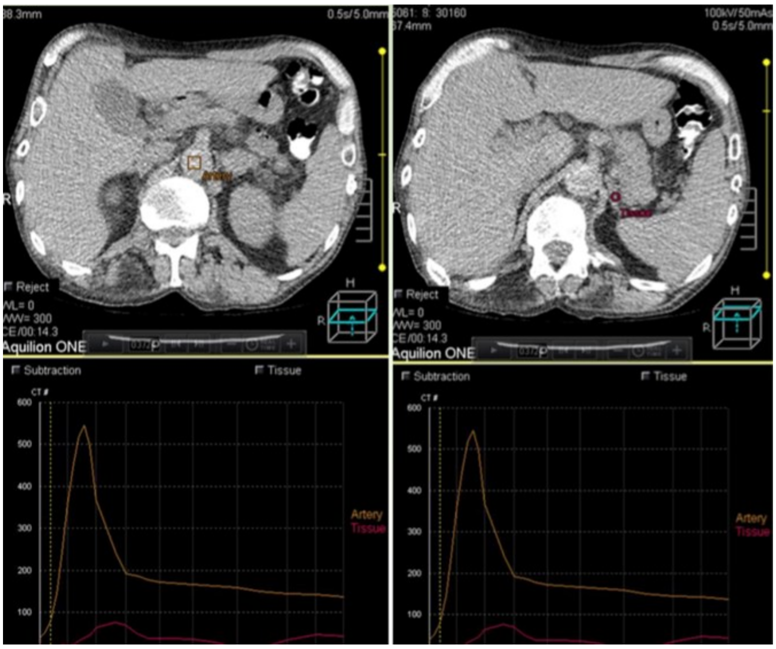
Appendix Figure 1: Figure shows TDCs and scanning interval. At the beginning of the protocol, interval between the scans should be kept short, since the arterial enhancement and input function changes rapidly in this phase. At the delayed phase of the dynamic acquisition, the scanning interval can be increased providing radiation dose reduction. In delayed phase, the arterial contrast has already reached its maximum, and the course of the TDC of the arterial system and the tissue is markedly flattened.

Registration software

The registration software calculates the differences between each image of the different volumes and corrects changes in the contour and position of anatomical structures created by respiratory motions and other motions during scanning.

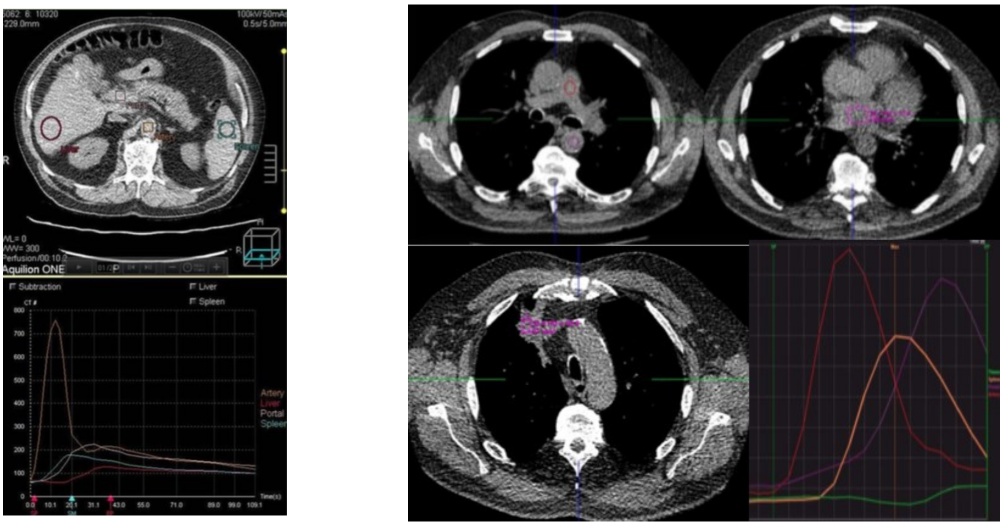
Post-processing

Selection of the arterial input by placing a ROI into the supplying artery is an important step in perfusion analysis. The placement of a ROI into the artery and the tumor tissue that need to be analyzed enable comparison of their TDCs in order to distinguish between quantities of contrast agent in the intravascular compartment and in the extravascular compartment, therewith enabling quantification of perfusion [16]. Figure 2 and 3 shows how the ROI is selected in compartmental analysis algorithms single and dual input maximum slope method respectively.



(A) (B)

Appendix Figure 2. Single Input Maximum Slope Model for adrenal perfusion imaging. After drawing a ROI into the aorta (A) and the left adrenal gland (B) the TDCs were generated. The maximum slope of the adrenal TDC is normalized to the peak of the aortal TDC.



(A) **(B)**

Appendix Figure 3. Dual Input Maximum Slope Model for liver (A) or lung (B) perfusion imaging. (A) Time-Density-Curves (TDCs) of the aorta, the portal vein, the spleen and the liver. The maximum enhancement of the spleen separates arterial blood supply to the liver before the maximum from portal venous blood supply after the maximum. (B) TDCs of the pulmonary artery, the aorta, the left atrium and the lung lesion in the right upper lobe. The maximum enhancement of the left atrium separates pulmonary arterial blood supply to the lung before the maximum from bronchial arterial blood supply after the maximum.

Perfusion analysis and interpretation

CT perfusion provides both qualitative and quantitative information about tumor angiogenesis. Qualitative analysis, also called visual analysis, is the simpler one and offer quick identifications of the area with the highest and lowest perfusion by observing the color maps for each CT perfusion parameter (Figure 4). Furthermore, qualitative analysis of the attenuation time curves allows visual /descriptive evaluation of some parameters such as time to peak enhancement, maximum enhancement and peak slope area under the enhancement curve [21].

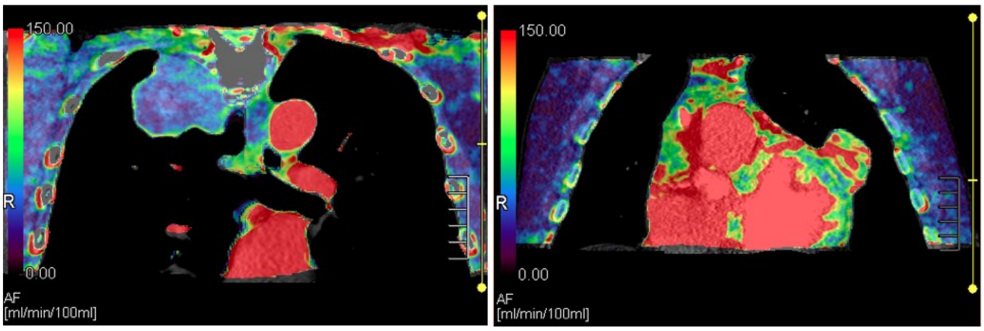


Figure 4. Color/Parametric maps are automatically generated for each perfusion parameter (BF, BV, permeability surface-area PS) and the color can be freely selected and changed by the investigator to maximize the differences between areas with different perfusion. Example show color maps of pulmonary arterial BF in a patient with a histologically verified inflammatory myofibroblastic tumor in the right upper lobe (A) and in a patient with a histologically verified neuroendocrine cancer in the lingua (B). The color scale on the left of each figure displays from the bottom to the top the lowest and highest value. The lesion in B shows a significantly higher pulmonary arterial flow as compared to the lesion in A (green to red color coding in B, blue to green color coding in A).

However, qualitative analysis does not offer any numerical results making comparison of different patients or same patient at different time points impossible. Quantitative parameters are assessed through the fitting of TDCs to the mathematic model so that these parameters can be displayed as parametric maps on a pixel-by-pixel basis. Each pixel represents a numeric value of temporal changes in tumor enhancement. Therefore, by placing the ROI in selected part of the tumor or entire tumor, final numeric values represent average of the numeric values for each voxel within selected ROI [13].

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