DYNAMIC SONOGRAPHIC TISSUE PERFUSION MEASUREMENT WITH THE PIXELFLUX METHOD

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INTRODUCTION

This chapter introduces a novel technique (the PixelFlux technique) of color Doppler sonographic perfusion measurement which allows the quantification of tumor tissue perfusion along with a normal ultrasound investigation of the tumor. This technique being non-invasive and non-radiating is patient friendly. It allows an objective calculation of perfusion signals from the tumor; it is inexpensive needs no special ultrasound equipment, and can be made available on the bedside, and frequent reevaluation during therapy is also possible. This chapter is focused on a description of the first results and tries to outline future applications because of the novelty of the PixelFlux technique. Besides this, it is an invitation to researchers and physicians to download the PixelFlux software from the internet (Chameleon-Software, 2004) to try it on their own patients.

Blood flow in a certain minimum quantity and quality is an indispensable precondition of vertebrate life and for the normal functioning of a tissue. It has to meet the metabolic needs of the tissue and is therefore characteristic for a specific tissue. Moreover, perfusion is altered according to functional and structural alterations in tissues. One example for such a state is tumor growth; others might be inflammation, aging, scarring, arterial malperfusion, and venous congestion or transplantation.

The aim of this chapter is to describe the potential usages of perfusion measurement in tumor tissues. Each tumor has an individual history with changing metabolic demands to the hosting organism. Rate of cell division and tumor cell energy turnover lead to a vascular network development that is characterized by a specific branching pattern of vessels and a specific perfusion intensity. Time of metastasis, capacity to

enlarge, and necrosis of central parts may depend on the quality and the amount of tumor perfusion. To understand these processes it is desirable to quantify tumor perfusion.

TUMOR PERFUSION EVALUATION – STATE OF THE ART

Measurement of perfusion related parameters of tumor homoeostasis is a recognized approach to answer questions of tumor biology (Vaupel et al., 2001). The important methods in use are measurement of tumor oxygenation , laser Doppler flowmetry in superficial tumors (Jacob et al., 2005), computed tomography (CT) (Kan et al., 2005), magnetic resonance imaging (MRI) techniques to quantify perfusion and diffusion (Weber et al., 2005), scintigraphy (Wawroschek et al., 2001) , positron emission tomography (PET) (Bruehlmeier et al., 2005) and contrast enhanced sonography (Krix et al., 2005). Some of these techniques are invasive, while, others are restrictive in their application due to technical reasons or are expensive or demanding for the patient.

The organ most frequently investigated is the liver. Here a critical appraisal of perfusion measurement techniques is necessary. Remaining challenges are reduction of radiation with CT-techniques, improvement of spatial and temporal resolution with MRI-techniques, accurate quantification of tissue contrast material at MR imaging, and validation of parameters obtained from fitting enhancement curves to biokinetic models, and applicable to all perfusion methods (Pandharipande et al., 2005). Nevertheless, certain parameters of diverse imaging modalities (MRI and contrast enhanced ultrasound) do correlate with each other (Kiessling et al., 2003), but, physical differences of contrast media (microbubbles vs. MRI contrast agents) may influence depiction of tumor vascularity depending on the branching patterns and vessels' diameter (Galie et al., 2005). So the situation at present is unclear.

Sonography is a method that allows access to many parts of the body without harming or embarrassing the patient, and it is comparatively inexpensive. Simple color Doppler sonography has the capacity to depict blood flow signals in a graduated fashion. Moreover, it is fast enough to monitor rapid changes of perfusion during the heart cycle. There have been many attempts to use traditional sonographic parameters like gray-scale values, and resistance index (RI) (Miyakawa et al., 2005), pulsatiltity index (PI) (Okuyama et al., 2004) and grade of vascularity. To classify vascularity merely subjective scoring systems or computer assisted algorithms have been developed. Pattern of vascularity has also been found useful to discriminate between benign and malignant tumors in lymph nodes and uterine pathologies (Alcazar et al., 2003) but had no pretherapeutic predicitive value in a group of 34 early stage breast cancers (Roubidoux et al., 2005).

The perspective of sonographic and other imaging procedures with respect to prognosis of tumor response to therapy, aggressiveness, and metastatic behavior is not completely clear at present. This might be due to tumor specifics on the one hand, and on the other hand due to limitations of these techniques to reflect the important features of perfusion. Color Doppler parameters are valuable for predicting complete histological response of neoadjuvant chemotherapy in advanced breast cancer. Compared to more expensive MRI and CT or contrast-enhanced sonography, color Doppler sonography may be an effective modality also with regards to health care expenses in less developed countries (Singh et al., 2005). Conventional (i.e., single vessel -) RI and PI may yield inconclusive results in attempts to differentiate benign from malignant tumors (Gallipoli et al., 2005). Clearly, a more refined approach capable of describing momentary changes of perfusion more precisely is needed. Novel approaches referring to both velocity and perfused area in a certain region of interest are necessary to overcome the basic restrictions

and flaws of purely velocity based perfusion velocity estimation of only single vessel measurements. These techniques (RI and PI i.e.) are now more than 30 years old and have definitively fallen back behind the fast development of imaging techniques as offered nowadays. Such techniques as color Doppler perfusion display and 3D blood vessel depiction and even more the ecg-triggered 4D depiction of dynamic flow phenomena in space offer abundant flow information still not used today. Moreover, it would be very useful to observe also the changes of flow velocity and perfused area over a full heart action to achieve a dynamic appreciation of these flow phenomena. It is of foremost importance to refer all perfusion parameters not only to the vessels but also to the entire tumor. This way perfusion drop outs are appreciated according to their real size in relation to the tumor as a whole, and pulsatility parameters like RI and PI can be given for the whole tissue segment under investigation. From a theoretical point of view this is the prerequisite for a more complete understanding of perfusion. Therefore, we extend present conventions for RI and PI, and calculate them as Tissue-RI (TRI) and Tissue-PI (TPI). Moreover, we extend the calculation algorithm for RI and PI from velocity measurements (as this is in use for single vessels for decades) to perfused area and perfusion intensity. Such a method eventually could bring new insight into old questions and would be entitled a dynamic perfusion measurement. It could eventually overcome limitation of a rather static apprehension of perfusion even with contrast techniques. Here the dynamic component of observation may not be precise enough to look at changes of flow during a heart beat. Often the influx of a contrast medium is depicted over a longer period to measure only its saturation.

DYNAMIC TISSUE PERFUSION MEASUREMENT (PIXELFLUX)

If tumor perfusion reflects tumor behavior (growth velocity, metastatic potential, response to chemotherapy, and radiotherapy, involution, necrosis), it is useful to measure tissue blood flow in a reliable, differentiated, and patient-friendly way. To achieve a reliable quantitative perfusion measurement in a tumor it is desirable to yield data on mean flow velocity, mean perfused area, mean flow intensity, pulsatility of velocity / area / perfusion intensity, resistance of flow, spatial distribution of flow across the tissue and quantitative distribution of perfusion intensity throughout the tissue section.

These parameters describe quantitatively the local blood flow and should be recorded in any part of the tumor in an arbitrarily chosen regions of interest. To achieve this goal, we developed the method of dynamic quantitative tissue perfusion measurement (PixelFlux technique). With the PixelFlux software such measurements can be performed in an automated fashion for all sonographically detectable tumors.

Preconditions

Every sonographic imaging depends on external as well as internal preconditions that influence the quality of the image and the image sequence of the video. To achieve comparable results, it is necessary to standardize as many conditions as possible. The most reliable data will be yielded if the ultrasound equipment and its settings are never changed, i.e., one uses the same type of ultrasound machine from the same manufacturer and the ultrasound transducer’s shape and frequency also remain unchanged. Internal presetting of adjustments such as gain, depth compensation, applied frequency, imaging frame rate, spatial and time resolution,

foci, type of color display in distinct hues and others (depending on the equipment's specifications) must not be changed.

In daily routine this is not a problem. Most centers refer patients to specialized ultrasonographers or radiologists who use the same ultrasound equipment over longer periods. This allows long-term follow-ups of single patients and ensures comparability of data from larger populations. Comparability of data from different centers is not provided if differences with respect to ultrasound hardware or presetting of the equipment cannot be ruled out. It is useful, therefore, to carry out such measurements with the most sophisticated equipment available. Before the advent of quantitative tissue perfusion measurement, manufacturers aimed to develop imaging modalities focused on an excellent visual impression for the investigator. PixelFlux advances beyond a simple subjective view of the ultrasound, it extracts imaging data for a quantitative analysis. It is, therefore, crucial to standardize the imaging details of the given equipment. More sophisticated ultrasound machines analyze more precisely and produce more consistent numerical imaging data (our unpublished data). Consequently, to ensure the most reliable measurement, it is necessary to use the most sophisticated equipment.

Workflow

PixelFlux perfusion measurement is a byproduct of the conventional color Doppler sonographic investigation of the tumor. Injection of contrast-enhancing agents (CE) is possible but not necessary (see below).

Procedure:

1. A relevant structure, (e.g. tumor), is scanned with a suitable presetting of all available specifications of the ultrasound equipment and transducer using as

high a frequency as possible to achieve a high resolution and to depict color Doppler signals as sensitive as possible.

2. An imaging plane is selected and transducer is held in this plane.
3. A video clip (preferably DICOM format, avi-format is also possible) with a duration of at least one full heartbeat is recorded.
4. The video clip is transferred to a PC with installed PixelFlux software.
5. The Clip is opened by PixelFlux and the calibration of distances as well as color bar are carried out automatically with DICOM clips.
6. The region of interest (ROI) is cut out. Then the measurement starts. It is completed in 1-5 s depending on the file's size and processor velocity of the PC.
7. A PACS (picture archiving and communication system) – function is included in PixelFlux. It allows review of clips, ROIs, and all measurements as well as export of measurements to a statistical software.

Output

With the PixelFlux technique the following parameters are calculated from a video sequence recording at least one full heart cycle.

1. Mean flow velocity$^{(*)}$ throughout the entire region of interest (ROI)
2. Mean perfused area$^{(**)}$ in relation to the ROI
3. Area of the ROI$^{(***)}$
4. Perfusion intensity throughout the entire ROI: Perfusion intensity [cm/s] = mean perfused area$^{(**)}$ [cm²] * mean flow velocity$^{(*)}$ [cm/s] /area of the ROI$^{(***)}$ [cm²]
5. Tissue Pulsatility Index (TPI) of velocity / of area / of perfusion intensity

TPI = (maximal systolic value – minimal diastolic value) / mean value - "value" may be velocity, area or intensity

6. Tissue Resistance Index (TRI) of velocity / of area / of perfusion intensity

   TRI = (maximal systolic value – minimal diastolic value) / maximal systolic value - "value" may be velocity, area or intensity

7. Spatial distribution of flow across the tissue

   an overlay of false colors upon the ROI shows local distribution of flow intensity

8. Quantitative distribution of perfusion intensity throughout the tissue section

   the whole range of flow intensity (resp. perfused area) over a full video sequence is divided into percentiles. Each interval's fraction of the ROI describes the distribution of perfusion intensity in numerical values.

9. Time lines of above explained perfusion parameters of individual patients can be displayed and statistically evaluated

USE OF CONTRAST ENHANCERS

If contrast enhancers (CE) are used then an additional variable is introduced. Special attention has to be paid to keep factors such as the amount of CE, infusion or injection rate, distribution pattern; and imaging times constant. With high-end ultrasound equipment, perfusion of tumors can often be obtained without the application of CE. This reduces imponderable external influences on the measurements. The application of CE saturation of a tumor in a grey scale image sequences excludes the measurement of the pulsatility parameters inside a tumor, dependent on the heart beat. Contrast enhancer influx is observed over a longer period of time and the gradual rise of tumor contrast gauges perfusion intensity. If a CE is used to enhance color signals in a tumor, care should be taken to avoid

blooming artifacts that are color signals massively splashed across vessel borders and thus cause errors in perfusion quantification.

APPLICATION

Perfusion measurement is desirable in many situations. Some of the numerous pathophysiologic processes accompanied by perfusion changes are outlined below. After opening the video file the first image is automatically calibrated for distances and color hues. Next step is to choose the ROI. Depending on the tissue under investigation, several options are applicable. Region of interest can be drawn free-hand, or geometric shapes are used as a framework for definition of ROI and sub-ROIs. Their selection depends on pattern of vascularization. Often in tumors an irregular branching pattern is found. Freehand ROIs are adequate in most cases. If the tumor has been encircled along its border, then sub-ROIs may be defined. This is helpful in an attempt to correlate perfusion to separate shells of tumor tissue from the center to periphery. Such shells can be defined in a general manner as follows: The sub-ROI is drawn automatically according to the demands of the investigator. To achieve this and to cut out the tumor core it is suitable to use the target mode. One can define a core region with a diameter of 50% of the whole tumor's diameter (any numerical value from 100 – 1% is selectable). This diameter is centered at the balance point of the primary ROI, and required distances are subtracted from this point to the periphery given by the primary ROI. Other possibilities are parallelograms with defined sub-ROIs as slices or parallelographic corners. These ROIs and sub-ROIs allow orientation at anatomical landmarks. For example, in perfusion measurement of the renal cortex, it is useful to look at a complete renal segment fed by a single interlobar artery, otherwise, one would miss a subgroup of cortical vessels and measurement bias would result.

The chosen ROI is valid for all single images of the whole video sequence. Inside the ROI every pixel is evaluated. Number and coloration of pixels change with each image reflecting changing momentary perfusion from systole to diastole. All these changes are numerically evaluated. For each image the perfused area and the mean flow velocity are calculated. Pixelflux is able to detect the beginning and end of a complete heart action. It calculates mean perfused area and mean perfusion velocity from all images of the video from the beginning to the end of the heart cycle. From these data perfusion intensity is calculated.

PixelFlux application in oncology

Tumors differ with respect to their vascularity and perfusion intensity. Both vary with histology and developmental stage of a tumor. In central parts of a tumor perfusion may be different from peripheral regions. Hypoxia is a relevant promoter of therapy resistance in some tumors (Vaupel and Harrison, 2004). This might trigger processes aiming at evasion of tumor cells to bring up satellites. Quantification of intratumoral oxygenation by noninvasive means or direct measurement of tumor perfusion has become pivotal to rate the consequences of new treatments such as hypoxia-mediated gene therapy, application of nonsteroidal antiinflammatory drugs to increase tumor oxygenation (Crokart et al., 2005) or antiangiogenic therapy (Janssen et al., 2005). Others report conflicting results with poor prognosis and response to radiation in head and neck cancers with high perfusion when measured with PET (Lehtio et al., 2004). Distribution of hypoxic tumor parts may greatly differ from individual to individual and with tumor type (Kelleher et al., 1998). It is, therefore, necessary to achieve an individual perspective of every tumor for an efficient therapy especially because tumor vasculature has become a target of tumor therapy. Data about the state of tumor angiogenesis would be most valuable. Such

numerical information could possibly be used to tailor the application of antiangiogenic drugs and to monitor their effects. Tumor vessel network may have some peculiarities such as lacunas, caliber irregularities, arterio-venous shunts, and sudden interruption of vessel branching. These specifics should lead to a deviation from normal flow pattern in tiny tumor vessels. Here could lie an application of tissue perfusion measurement techniques such as PixelFlux. It is reasonable to assume that deviations from a harmonic branching patterns as a consequence of above mentioned peculiarities would produce deviations in flow intensity, flow distribution, pulsatility of flow, and perfused area. In fact, we were able to demonstrate such effects (Scholbach et al., 2005a). In a series of lymph node metastases from oropharyngeal carcinoma we found a significant difference of perfusion intensity between nodes from various N-stages.

Prostate Cancer

Another study investigated the pretherapeutic perfusion of prostate cancer (Rouviere et al., 2004). It could be demonstrated that perfusion intensity prior to high-intensity focused ultrasound (HIFU) therapy had no adverse impact on the formation of posttherapeutic necroses. Perfusion was measured here in the prostate itself and in the surrounding tissue. Perfusion intensity and perfused area did not differ with respect to uniformity of thermic tissue destruction. This way the influence of the vascularization on the effect of thermoablation of tumor tissue could be evaluated. Further studies are needed to describe tumor perfusion with respect to its influence on the abduction of thermal energy during HIFU procedures.

Tumor vascularization influences thermal conduction capacities, nutrient, and oxygen supply alike. Monitoring the extent of vessel distribution and the intensity of perfusion should elucidate individual tumor's metabolic state. One possibility to test this

assumption is to correlate oxygen measurements in tumors with the perfusion measurement. In lymph node metastases from oropharyngeal carcinomas we were able to demonstrate significant correlation of intratumoral oxygenation and sonographically determined perfusion (Scholbach et al., 2005a). In this pilot study we could discriminate sonographically hypoxic metastases from better oxygenated ones with the dynamic perfusion measurement technique.

Head and Neck Cancer

We investigated 24 patients (44–78 years) with cervical lymph node metastases of squamous cell head and neck cancer by color duplex sonography and 17 (46–78 years) with polarography (Scholbach et al., 2005a) . During defined contrast enhancer infusion perfusion intensity , Tissue Pulsatility- (TPI) and Tissue Resistance Index (TRI) were measured. Tumor tissue pO2 was measured by means of polarographic needle electrodes placed intranodally. Sonography demonstrated a significant inverse correlation between hypoxia and perfusion. The extent of hypoxic nodal fraction was weakly but significantly inversely correlated with lymph node perfusion ($r = 0.551$; $p = 0.021$). Nodes with a perfusion intensity of < 0.05 cm/sec showed significantly larger hypoxic areas ($p = 0.006$). Moreover, significant differences of TPI and TRI were demonstrated with different N-stages. In higher N-stages lower TRI and TPI-values were found. TRI-values were as follows: 0,79 in N1, 0,62 in N2 and 0,47 in N3 (differences between stage N1 and N2 $p = 0.028$ and between stage N1 and N3 $p= 0.048$). A similar decline was found with TPI values: 2.07 in N1, 1,16 in N2 and 0,79 in N3 (differences between stage N1 and N2 $p = 0.028$ and between stage N1 and N3 $p= 0.030$). Figure 1 illustrates some output from dynamic perfusion measurement of a metastatic lymph node with PixelFlux . The central part shows the metastatic lymph node encircled by two lines, the outer

one encompassing the outer border of the node and the smaller central circular line enclosing the central 50% of the node. Thereby, two parts of the tumor, the tumor periphery and the center can be looked at separately. All measurements can be done separately in both zones and results can be compared in many ways.

Evaluation of PixelFlux results

The first step is to look at the overall perfusion intensity values as given by the columns on the left
side of Figure 1. It is directly proportional to the product of the perfused area of ROI and the mean blood flow velocity over a complete heart beat. The red column corresponds to the inferior parts of the adjacent diagram depicting the perfusion intensity of the tumor periphery. The green column reflects perfusion intensity of the tumor center. Only by a numerical description of perfusion such a clear distinction of flow becomes possible. It would be impossible to do so by simply visually evaluating moving images displaying tissue perfusion of a tumor.

The second step is to look at the spatial distribution of perfusion (central part of Figure 1). New
possibilities of describing tumor perfusion emerge with the PixelFlux technique. Perfusion signals can
be displayed in false colors signaling local perfusion intensity of the whole video sequence in one
image. Thus, these colors do not only express a momentary state of perfusion but also the mean values over the entire video clip. Hence, an additional information that is not obtainable from a simple

observation of the video itself is acquired. A color spectrum from black to red is chosen with transition over white hues. Distribution of intensity is thereby overlaid as a perfusion relief and can be estimated at a first glance. This may be helpful for surgical decisions to define a border between parts of the tissue having different perfusion patterns.

While interpreting these false colors, it is necessary to keep in mind that color hues are related to the specific video and that they are not absolute. In Figure 1 the tumor center is more evenly perfused with some islands of maximal perfusion (red dots). However, the overall perfusion intensity is less than that in the periphery as is shown by a comparison of the red and green columns. The periphery shows a more focal perfusion in the left lower quadrant where large perfused areas have only weak intensity values (gray and black).

False color coding of perfusion parameters is but one possibility to depict numerical perfusion data.

Another way to do the same is to draw a perfusion intensity distribution diagram. Such diagrams are depicted at the lower margin of the images. The x-axis describes perfusion intensity values from zero to the video's maximum value. The y-axis shows the frequency of occurrence of distinct intensity classes. The whole range of intensity is divided into 30 classes. Each class contains 3,33 % of the whole range and it is assigned a specific value of the y-axis. The curve resulting from this arrangement of perfusion data describes the perfusion intensity distribution of the investigated ROI. If the low-intensity classes prevail, the curve will show a slope from left to right. If the perfusion is more localized in areas or vessels with strong flow, then the maximum of the curve may shift to the right or a second peak, located in the region of higher intensity values, may emerge. Both changes will shift the weighted mean value (also called expected value in statistics) to the right side of the diagram. This weighted

mean value is marked on the x-axis and gives a fast orientation of the distribution curve's shape, when compared to the average value, which is also given. In Figure 1 perfusion intensity distribution in the center is more right-shifted than in the periphery. This tumor's central perfusion is weaker but more evenly distributed. This way different distribution curves are generated characterizing perfusion distribution of the tumor under investigation. A perfusion distribution may be influenced by vascular as well as by interstitial forces. Studies to use such information have not yet been done, but could bring new insight into tumor biology. The synopsis of perfusion intensity values, as given by the columns on the left side, the false color display of perfusion intensity and its graphical description by the distribution curve plus the numerical description of the most important flow data in tables and their course in time-line diagrams, as given in the right side of Figure 1, give a comprehensive numerical and spatial description of a tumor's perfusion. Even more numerical data may be calculated and displayed in larger tables. Actually 34 numerical parameters characterizing perfusion and 15 specifying parameters are offered by the PixelFlux-method.

The third step is to look at cardiac changes of blue and red colored pixels in Figure 1. The time course demonstrates that the perfusion changes along with the heart beat even in the tiniest tumor vessels. The lower part of the image refers to the adjacent color Doppler image of the tumor periphery, and the upper part to the central 50% of the tumor. In both parts, blue pixels, emanating from vessels running away from the ultrasound transducer, exhibit strong pulsations. In the tumor periphery synchronous pulsations of red encoded flow, from vessels going towards the transducer, is visible. In contrast to this, red-encoded flow inside the tumor's center is faster and much less pulsating. In the accompanying tables, above the time

course diagrams, more specified data describing mean values of velocity, area and intensity of red and blue encoded pixels separately, can be found. Moreover, a measure of the extent of the change of these parameters is given as RI and PI values. Resistance index (RI) and PI correspond only loosely to conventional RI and PI. We have coined the term Tissue-RI (TRI) and Tissue-PI (TPI) instead, to mark these differences. With PixelFlux, not only velocity changes in single vessels are recorded, but we advance and record simultaneously velocity as well as area data from a whole ROI. Thereby, we take into account also non-perfused parts. In this way, a more exact average velocity and perfused area recording becomes possible.

We extend the concept of RI and PI to the new data acquisition modality. In the tumor's center of this example the values of TRI of the velocity of red pixels, TRI of the area of red pixels and TRI of the intensity of red pixels are 1 each. This can be easily controlled with the red line of velocity course in the corresponding time line diagram below the data table: red flow velocity values drop down to zero in diastole. This means that perfusion TRI of intensity will also be calculated as zero because it is directly proportional to the product of velocity and area. In these perfusion diagrams in the right part of Figure 1 only time-course of velocity is depicted. Similar curves of heart beat dependent changes of perfused area and perfusion intensity are not given here due to limited space.

Figure 2 shows quite impressively another phenomenon of tumor vascularization. In a single tumor quite different perfusion patterns are found, and are numerically described with the PixelFlux technique. It is a myosarcoma of the thigh extending over nearly 30% of the thigh's length. At the upper pole (upper part of the image), perfusion pulsates only weakly, whereas in central parts of the tumor highly pulsatile signals prevail. This is depicted in the lower part of the image. Perfusion intensity

also differs between these tumor parts but not as strongly as pulsatility. The upper pole (green column) is not as strongly perfused as central tumor areas (red column). This could point to a prospective application of numerical descriptions of tumor perfusion: with PixelFlux the biological peculiarities not only of different tumor types but also of different parts of a tumor could become apparent. This could greatly enhance our understanding of tumor development. With PixelFlux it is possible to observe tumors in clinical settings as often as one desires, because the method being noninvasive and nonionizing is harmless. The oncologist can become quite familiar with the tumor's individual behavior. We can learn how tumor spreading is emerging. Many questions may be investigated. Is low pulsatility of flow a pointer to loose intervascular tissue structures? This could eventually be a sign of fast cell division with development of intercellular matrix falling behind. Is the shift to high pulsatility a signum mali ominis that an imbalance between vascular bed and fed tissue is going to emerge? How do metabolic demands of the tissue modulate perfusion? Is hypoxia the strongest influence or will we discover that metabolites or local factors exert an even stronger influence upon perfusion changes? How would perfusion or tumor nutrition respond to internal conditions of tumor tissue along with nascence, growth, and death of tumor cells? A meticulous numerical description of perfusion could bring new insights for tumor biologists and oncologists and for their patients' therapy. Perfusion intensity and its local distribution could be parameters for such a description. Are there densely packed small vessels or is there a rather wide-meshed lattice of larger vessels? Measurement of pulsation of velocity simultaneously with pulsation of vessels' diameter could describe physical forces and their impact on the tumor tissue. For example, high pulsation of flow velocity with low pulsation of vessels diameter could point to a strong interstitial turgor. Low pulsation of flow velocity with high absolute velocity values could point to a high metabolic

activity of a tumor with a strongly branched vascular tree. Separated examination of different flow direction in conjunction with the above mentioned parameters could allow further insight.

In Figure 3 the drop of perfusion intensity in a liver metastasis after chemotherapy is shown. In the right part of the image a power Doppler sonogram demonstrates the low perfused metastasis with better perfused surrounding tissue. Now that we can quantify perfusion, a numerical comparison of these different tissues is possible and the effect of therapy onto tumors and metastases is quantifiable. This is shown in the left part of the image (Figure 3). Red bars signal pretherapeutic situation with about one third of the lower perfusion intensity in the metastasis. After therapy, the diverging effect onto these different tissues is evident: Perfusion of the metastasis drops to zero, whereas liver tissue on the same day shows only a moderate perfusion drop. This example points to other applications of tumor perfusion measurement. Side effects on other parts of an organ can be measured and the effect of therapy on tumor perfusion is comparable to such side effects. This could be used in the development of new antitumor drugs. Moreover, the effect or dosage of an antitumor drug can eventually be tailored according to the actual effect on both healthy and tumor tissues.

Another application of perfusion measurement is to plan and control surgical measures. In prostate cancer preinterventional perfusion of cancer and normal prostate tissue were measured and compared to extension of HIFU-induced necrosis (Rouviere et al., 2004). In this study no correlation of perfusion intensity with necrosis quality was found. Thus, it seems that thermal energy draining by tumor blood flow is unlikely to affect the success of thermal tumor ablation.

Comprehensive numerical evaluation of a tumor's perfusion becomes feasible. PixelFlux offers the chance to have a quite individual look at each tumor in each

patient. Firstly, the measurement is repeatable at bedside without harm to the patient. A close monitoring can be achieved with no medical or economic restrictions with regard to the imaging procedure. A wealth of numerical information is capable to describe perfusion in detail. Secondly, a tumor can be individually regarded as an organism with differently behaving parts at least as far as perfusion is concerned. Thirdly, behavior of the tumor as well as its environment can be monitored at the same time. Relationships of both tissues and their interactions become observable. All numerical data may be used for individual statistical analysis. One video clip contains ~ 30 to 50 single images with a recording time of ~ 2 to 3 sec.. After transfer to a computer, measurement of a single clip takes ~ 5 sec.. In a short period of time, many clips of a tumor can be evaluated; thus, enhancing the reliability of these measurements. At present the development of this technique is only at its beginning. The method is new, and its potential can only be estimated. Larger studies on various aspects of the PixelFlux technique are desirable. Below we offer some ideas on how to proceed.

Comparison of Results with Other Techniques

There is an urgent need to include vascular pattern of tumors in consideration of their nature as well as a better interpretation of semiquantitative flow data (RI, PI) (Chammas et al., 2005, Gallipoli et al., 2005). Conventional Doppler techniques such as power Doppler (Chammas et al., 2005) or the application of contrast enhancing media (Gallipoli et al., 2005) have led to semiquantitative scoring systems (Marret et al., 2005). Such scores suggest that peritumoral infiltration correlates with subjectively analyzed vessel density (Testa et al., 2003).

Nevertheless, clinical value of conventional Doppler flow parameters or application of contrast media could not be demonstrated (Blanco et al., 2003). Others (Osanai et al., 2003) found a significant correlation of the lowest measured conventional RI in breast cancer with Nottingham Prognostic Index (NPI) (NPI = 0.2 x tumor size (cm) + histological grade (I-III) + lymph node score (1- 3)). Similar results and a significantly lower RI were found in moderately or poorly differentiated tumors and advanced stage tumors. Significantly higher peak systolic velocity (PSV) was found in moderately or poorly differentiated tumors, tumors with larger volume, and advanced stage cervical carcinomas. No correlation was found between RI and PSV and histologic type (Alcazar et al., 2003). This finding corresponds well to our results of the dynamic flow measurement. We found significant lower TRI and TPI (Tissue-RI and PI) in the more advanced cervical metastases of head and neck cancers (Scholbach et al., 2005a). The ambiguity of results in conventional color or power Doppler analysis points to the potential of Doppler interrogation of tumors but at the same time to relevant limitations of the technique. The novel dynamic appreciation of all Doppler signals from any vessel inside a tumor, and the simultaneous automatic calculation of perfusion intensity at each point of the tumor can be a major step forward overwhelming some of the existing limitations. We found a significant inverse correlation of perfusion intensity, as measured with the PixelFlux technique, with intratumor oxygen saturation (Scholbach et al., 2005a). Similar results are reported from direct comparison of oxygenation with CT depiction of tumor flow (Haider et al., 2005).

Inflammation Grading

Some inflammation may accompany tumor and tumor necrosis but it is much more frequently elicited by other stimuli. Whether there exist differences between tumor associated inflammatory hyperperfusion and other causes is unknown at present. It is reasonable to assume that both processes may influence each other. We demonstrated the use of dynamic perfusion measurements to grade inflammatory activity in chronic inflammatory bowel disease (Scholbach et al., 2004a). Inflammatory activity is measured directly via the measurement of hyperperfusion. Then each bowel segment can be classified as quiescent, chronic activated, or acute exacerbated. The same holds true for other inflammatory processes such as arthritis and thyreoiditis (our unpublished data).

Transplantation Medicine

Transplanted organs are balanced between immunosuppression with potentially devastating effects on organ function and rejection with threat of organ loss. Both processes may damage vascular network and can be traced with a meticulous assessment of perfusion (Scholbach et al., 2004b, 2005b). Even separate levels of a vascular tree can be differentiated and evaluated independently. In kidneys a characteristic drop of perfusion intensity accompanied by an increase in overall perfusion pulsatility from central to subcapsular regions has been demonstrated (Scholbach, T. et al., 2004b, 2005b, 2006). Loss of the tiniest vessels preceding loss of an organ's function may be recorded very early and counteraction can be planned.

CONCLUSION AND OUTLOOK

Application of dynamic perfusion measurement is only at its beginning. We and others have demonstrated the feasibility, clinical interest, and direct benefit of

dynamic perfusion measurements in a variety of tissues and diseases (Rouviere et al., 2004; Scholbach et al., 2004 a, b, 2005 a, b, 2006). Researchers and clinicians are invited to develop their own ideas and projects. The focus of this chapter is on oncology, but there are many more fields to discover: inflammation grading, function of transplanted organs, organ insufficiency, adaptation after removal of sister organs, physiologic and pathologic growth and development, microvessel diseases in diabetes, atherosclerosis, vasculitis, aging, microembolization, perfusion response to physiologic stimuli, elasticity examinations of tissues, venous congestion, description of complex venous networks, quantitative analysis of collateral vessels, impact of arterial stenoses on tissue perfusion, and influence of venous outflow congestion on tissues. Downloading of PixelFlux software, necessary for video analysis, is also possible at : www.chameleon-software.de .

In oncology a first step could be to correlate perfusion patterns with the biological properties of a tumor. The potential of metastasis could be related to a shortage of oxygen or nutrient supply inside the tumor. Local growth is dependent on the spread of tumor vessels. Observation of their growth could reveal information on the aggressiveness of the tumor. Only recently, reports have been issued on a local ultrasound guided therapy with ultrasound contrast enhancers, so called microbubbles (Imada et al., 2005, Kessler et al., 2005, Yuh et al., 2005). These spherical containers can be loaded with anti-tumor drugs that will be transported with blood stream. Once they arrive at the tumor, they can be destroyed with a strong ultrasound impulse releasing their load. PixelFlux measurements might predict, which tumors are eligible for such procedures, and where the drug is potentially trapped inside the tiny tumor vessels. Perfusion distribution diagrams could form a comparable basis for selection of specific tumors.

Availability and affordability of a method are at least as important for their broader usage as technical and medical aspects. This is another major advantage of sonographic methods in general. In developed countries the equipment is rather inexpensive (compared to alternative imaging equipment) and can be found in even smaller hospitals or private practices. PixelFlux is a sophisticated method to extract numerical perfusion data from conventional color Doppler images but no new hardware or additional procedure is needed. In most circumstances, no injection of microbubbles is necessary, making the examination itself inexpensive. This offers the possibility of more frequent follow up studies to monitor the effect of antitumor therapies, and to adapt them according to their impact on tumor perfusion. Some contraindications of other imaging modalities cease to exist, allowing inclusion of patients otherwise difficult to investigate. The next step would be a three-dimensional, heart beat-triggered measurement of perfusion in tissue blocks instead of two-dimensional tissue slices. We made this step only recently and can now define the blood flow volume through any horizontal tumor transsectional plane in ml/s (own unpublished results). Until now, only a few but encouraging studies exist with the PixelFlux technique. There are more questions than answers and a united effort of researchers from many centers is necessary to gain the full potential of this promising novel technique.

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LEGENDS

Figure 1: PixelFlux investigation of a lymph node metastasis: **Left part:** perfusion intensity of lymph node periphery (red column) vs. lymph node center (green column) – much stronger peripheral perfusion; **Central part:** color Doppler videos (initial images) with false-color overlay displaying perfusion intensity over time. Maximum perfusion intensity is depicted as red, mean as white and minimum as black coloration. White curve describes distribution of perfusion intensities of the entire ROI. There obvious differences exist between lymph node periphery (image below) and center (image above). Lymph node center has a broader distribution with relatively strong perfusion signals whereas in periphery perfusion intensities are almost entirely restricted to relatively low intensities (always compared to the ROI's maximum value). **Right part:** corresponding time course of lymph node perfusion with numerical values in tables (see text). Red and blue lines corresponding to red and blue color pixels. It is visible at the first glance that differences between both parts of the lymph node exist.

Figure 2: Quantification of perfusion in two different parts of a myosarcoma of the thigh. Upper part: low pulsatility of flow in the tumor's periphery vs. strong pulsating flow in tumor's center (below). Only slightly different intensity distribution curves with little more right shifted curve towards stronger perfusion signals in the upper image (weighted mean value [expected value] at 10,4 resp. 8,7 % (lower image) of the maximum value. The lower part of the figures displays again perfusion intensity, which is stronger in the center of the tumor.

Figure 3: In the left part perfusion intensity measurements are given of both a liver metastasis and its surrounding liver tissue before and after chemotherapy. The right part shows a power Doppler image of the liver metastasis. It can be demonstrated with multiple measurements that metastasis perfusion as well as perfusion of normal liver tissue is dampened by therapy but perfusion of the metastasis has dropped to zero while liver tissue perfusion is maintained at a level of about 75 % of the initial value.

FIGURES

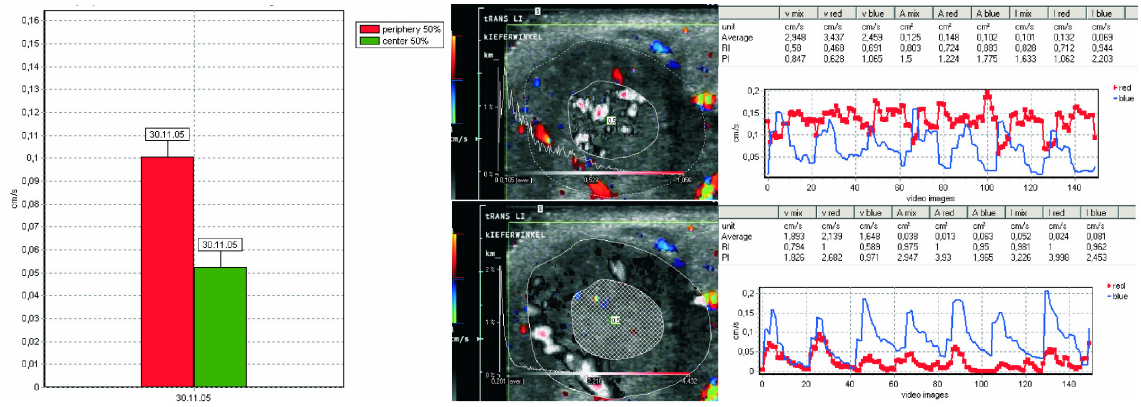
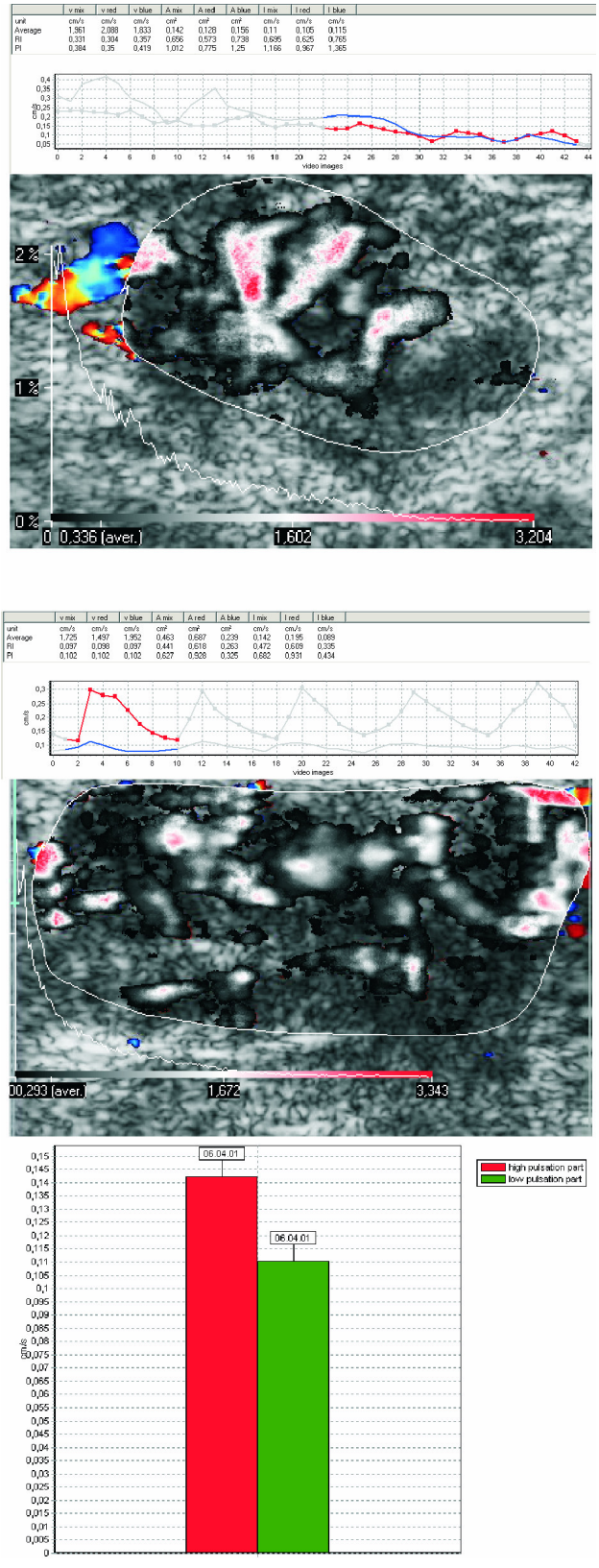


Figure 1


video images
2 %
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0 0,336 (aver.)
1,602
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high pulsation part
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Figure 2

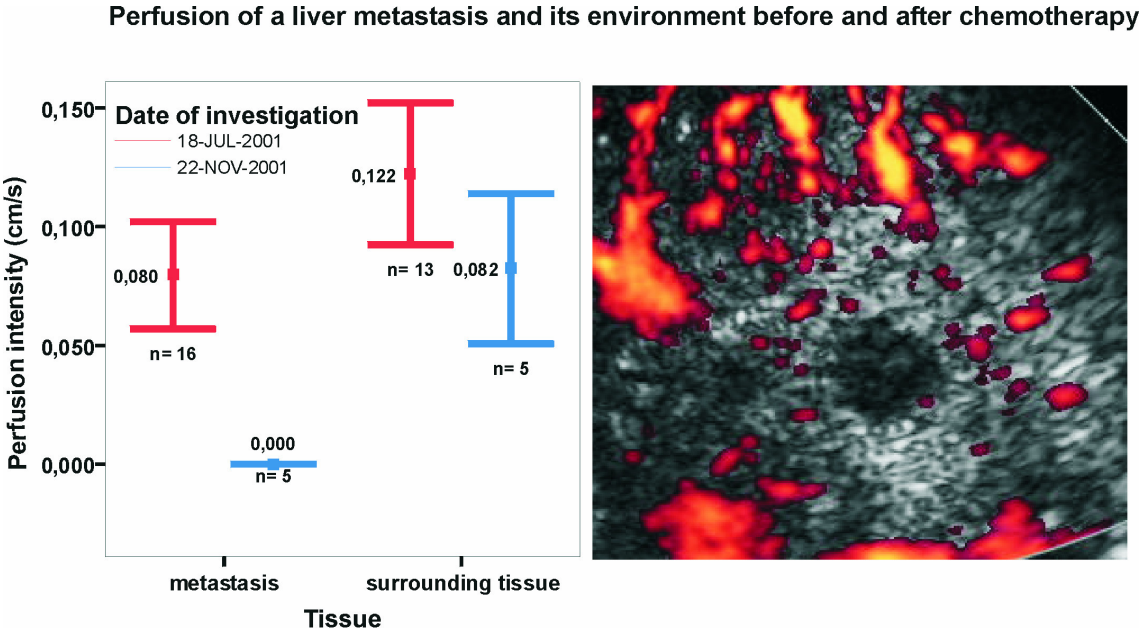
Perfusion of a liver metastasis and its environment before and after chemotherapy
Perfusion intensity (cm/s)
0,150
0,100
0,050
0,000
Date of investigation
18-JUL-2001
22-NOV-2001
0,080
n= 16
0,000
n= 5
0,122
n= 13
0,082
n= 5
metastasis
surrounding tissue
Tissue

Figure 3