Imaging of Response to Treatment in Oncology

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Abstract
Imaging plays a crucial role in oncology to assist in the management of patients and selection of drug regimen. Recent advances in imaging techniques allowing to predict and evaluate response to treatments in oncology will be reviewed.

The standard in the evaluation of response to treatment is based on the measurement of lesion size. Functional imaging assesses physiological or molecular processes that may be earlier indicators of early response to treatment.

Dynamic imaging of tumor vascularization assesses the biodistribution of a contrast agent within tumoral tissues. Diffusion-weighted MR imaging can differentiate free water from water restricted by tissues, providing an assessment of tumor cellularity. MR spectroscopy assesses the relative quantity of specific chemical components within normal and tumoral tissues. 18 FDG PET imaging provides an assessment of the metabolic activity of tissues. FDG uptake is proportional to cellular proliferation and number of viable cells within a tumor.

Results from studies assessing the role of these emerging imaging techniques remain preliminary and the medical community must determine their respective role in the routine evaluation of response to treatment in oncological patients.

Key-words: Cancer. Imaging, functional. Therapy, response. RECIST.

Rezsumé
Imagerie de la réponse aux traitements en cancérologie
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L'imagerie en oncologie a un rôle primordial pour guider le thérapeute dans ses choix de traitements. Nous aborderons des nouvelles techniques permettant de prédire et d’évaluer la réponse aux traitements en cancérologie.

La méthode de référence d’évaluation des traitements est basée sur la mesure de la taille des lésions. L'imagerie fonctionnelle ne s’intéresse pas à la taille de la lésion, mais à une caractéristique physiologique ou moléculaire, dont les modifications apparaissent plus précocement en réponse au traitement.

L'imagerie dynamique de la microcirculation suit la biodistribution d’un agent de contraste et analyse la vascularisation tumoraire. L'imagerie par résonance magnétique pondérée en diffusion permet de distinguer l’eau libre de l’eau restreinte dans les tissus, reflétant ainsi la cellularity tumoraire. La spectroscopie par RMN détecte la quantité relative de différents composants chimiques dans les tissus normaux et tumoraux. La tomodigraphie par émission de positons (TEP) au $^{18}$FDG fournit une information sur le métabolisme tissulaire. La capture du FDG est proportionnelle à l’activité proliférative et au nombre de cellules viables dans une tumeur.

Les études testant ces techniques sont cependant préliminaires et la communauté médicale doit aborder une réflexion sur leur rôle dans l’évaluation de la réponse au traitement en oncologie en pratique quotidienne.

Mots-clés : Cancer. Imagerie fonctionnelle. Réponse thérapeutique. RECIST.

modalities. Several ongoing studies involve MRI, CT, US, scintigraphy and PET imaging. Multiple publications already demonstrate the potential value of these imaging modalities in the follow-up of treatment efficacy in oncology patients. However, there is currently no consensus on which of these techniques is or are the most effective, reproducible, accurate and reliable or on the clinical relevance of the measured parameters. Only those imaging techniques that are currently available for clinical patients and could immediately be implemented in the follow-up of oncology patients will be discussed here. Most functional imaging techniques were initially developed for brain evaluation, but newer non-neurological applications, less well known, will be discussed here.

Morphological criteria of reference: changes in tumor measurements

The reference standard for evaluating tumor response to treatment is based on the size of lesions. In 1979, the World Health Organization (WHO) defined criteria based on bidimensional measurements of lesions on CT or plain films (1, 2). The WHO provided recommendations to assist in evaluating anti-cancer treatments in clinical trials allowing comparison of results from different treatment regimens. In spite of subsequent updates, there were persistent difficulties in comparing results from different trials since each trial utilized variations of these criteria. An international committee with members from the European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States and National Cancer Institute of Canada proposed new guidelines in 2000 to evaluate the response to treatment in solid tumors: RECIST (Response Evaluation Criteria in Solid Tumors) (3-5). These criteria, only applicable to solid tumors, are based solely on unidimensional measurements of lesions to simplify and increase uniformity of evaluation criteria in clinical trials. These criteria are now standard in the pharmaceutical industry. It is of interest to note that no radiologist was part of the panel members who defined the RECIST guidelines.

RECIST guidelines

Two examinations are obtained as part of the RECIST protocol. A baseline examination verifying patient eligibility and establishing lesions for follow-up. Follow-up examinations comparing lesion measurements to characterize the objective response to treatment. Acceptable imaging modalities include:

- CT and MRI with slice thickness ≤5 mm,
- Chest X-ray: when lesions are clearly defined and surrounded by aerated lung,
- US: should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions and thyroid nodules.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

Baseline evaluation

All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment. Two types of lesions are defined: measurable lesions and non-measurable lesions. Measurable lesions are lesions that can be accurately measured in at least one dimension with longest diameter ≥20 mm using conventional techniques (chest X-ray) or ≥10 mm with spiral CT scan. Non-measurable lesions include all other lesions, including small lesions (longest diameter <20 mm with conventional techniques (chest X-ray) or <10 mm with spiral CT scan), or lesions with margins that are difficult to define and reliably measure: bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed as malignant and followed by imaging techniques...

To be eligible, all patients must have at least one measurable lesion confirmed as malignant. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Lesions that will be followed up throughout the course of a patient’s treatment are documented on the baseline examination: target lesions and non-target lesions.

Target lesions

All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs should be identified, recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques).

A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

Non-target lesions

All other lesions, i.e., measurable lesions not classified as target lesions and non-measurable lesions, should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Follow-up examinations

The evaluation is based on 4 elements:

- response of target lesions,
- response of non-target lesions,
- appearance of one or more new lesions,
- overall response.

Response of target lesions:

It is defined by the % of change in the sum of the LD of target lesions, taken as reference the baseline sum LD. Four types of response are defined:

- CR (complete response): Disappearance of all target lesions confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met,
- PR (partial response): At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD, confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met,
- PD (progressive disease): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started,
- SD (stable disease): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.
Response of non-target lesions:
It is a subjective assessment. Three types of response are defined:
• CR: Disappearance of all non-target lesions and normalization of tumor marker level, confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met,
• PD: Unequivocal progression of existing non-target lesions,
• SD: Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits.

Appearance of one or more new lesions:
• No: No new lesion,
• Yes: Appearance of one or more new lesions.

Overall response is based on a combination of the above responses, and is summarized in Table I.

Evaluation of a clinical trial
The following parameters may be defined using the RECIST guidelines:
• Confirmation (% of patients with complete and partial response),
• Duration of overall response (measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented),
• Duration of stable disease (measured from the start of the treatment until the criteria for disease progression are met),
• Progression free survival/time to progression (measured from the start of the treatment until the criteria for disease progression are met in patients with stable disease or with complete and partial response).

These parameters may be primary or secondary endpoints in the evaluation of tumor response to treatment in a clinical trial. This should be clearly defined in the protocol by the investigators before the trial begins.

Volumetry
When oncologists redefined the evaluation criteria to characterize tumor response to treatment with the RECIST guidelines, the goal was to simplify the criteria for improved ease of use and reproducibility. Their study, retrospective in nature, and including over 4,000 patients, concluded that complete plus partial response rate comparisons by both the WHO and uni-dimensional RECIST criteria were similar in 97-99% of patients, and that progressive disease rate comparisons by both the WHO and RECIST criteria were similar 92% of patients (4).

This technique is simple and clearly defined. Good interobserver and intraobserver reproducibility has been reported. However, there are limitations. A major drawback stated by radiologists is that nodes are measured along their long axis when studied have demonstrated that short axis measurements are more predictive of malignant nodal involvement (6). In addition, while the use of unidimensional measurements to estimate lesion size and size variations over time is certainly representative for spherical and ellipsoid masses, it may not be for irregular shaped masses. Such lesions could change shape non-uniformly in all three planes, and size changes would not be correctly sampled using a unidimensional measurement (1D).

Because of the wider availability of helical CT units, the question of volumetric versus 1D and 2D tumor measurements is again raised. Two main questions arise: 1) is volumetric measurement more reliable than the 1D measurement of the RECIST guidelines? And 2) is volumetric measurement a predictive factor of response to treatment?

Some studies evaluated discordances in the classification of tumor response to therapy between 1D, 2D and 3D measurements, with very conflicting results (7). Several papers published soon after release of the RECIST guidelines have reported that 1D measurements provided
results comparable to 2D and 3D measurements (3, 8, 9). On the other hand, more recent publications have demonstrated that results were not comparable and that 3D measurements could be a better predictive factor, or an earlier predictive factor, of tumor response. Unidimensional measurements may not be optimal for very irregular shaped lesions (non-spherical and non-ellipsoid) or confluent lesions, when lesion position changes in relation to section plane, and for lesions that non-uniformly shrink in all planes (fig. 1). Theoretical advantages of 3D measurements include improved evaluation of tumor burden, improved evaluation of irregular shaped lesions, and improved accuracy of tumor measurements by addition of a third dimension. Also, the availability of software allowing automated segmentation and volumetric measurements could provide objective measurements and reduce interobserver and intraobserver variability. In a study on chest CT scans of 15 patients undergoing treatment for metastatic colorectal, renal cell, or breast carcinoma to the lungs, the authors measured 2 or 3 lesions per patient with 1D and 2D measurements made using electronic calipers, while nodule volume was measured using a semi-automated segmentation system (7). The concordance was only fair between the three methods, with 1D, 2D, and 3D measurements being concordant 21 of 30 classifications. Level of agreement among the three methods was measured using a kappa statistic (K). When comparing each method, K =0.7, 0.6 and 0.6 at 1 month and 0.2, 0.2 and 0.3 at 4 months respectively for 1D vs 3D, 2D vs 3D and 1D vs 2D (perfect agreement would be K =1). However, only patients with progressive or stable disease were included in this study (no complete or partial response). In addition, response was assessed for individual lesions whereas in current criteria, the sum of measurements is used to compare and it is possible that the rate of discordant results would decrease if patient response was assessed as opposed to specific lesion response. Finally, these studies compare results from 1D, 2D and 3D measurements for classification of tumor response whereas the real question is to determine which of these techniques can most accurately predict outcome and affect patient management. Another group measured the longest diameter and volume of each tumor on MRI in 62 patients with breast carcinoma before and after neoadjuvant chemotherapy treatment (10). The authors demonstrated that MRI tumor volume was more predictive of recurrence-free survival, even though tumor diameter provided good results as well. However, an early change in MRI tumor volume (mean of -35%) was significantly correlated with final MRI volume change in the study. Although a trend was observed, the association with length of recurrence-free survival was not statistically significant. Similarly, early change in MRI tumor diameter (mean of -11%) was not significantly associated with recurrence-free survival. There are pitfalls with volumetric measurement techniques. First, it complicates reading by the radiologist since measurements is 3 planes are required. By adding measurements in 2 other planes, additional interobserver and intraobserver variability is introduced, which could in turn reduce the reproducibility and reliability of the technique. The impact could be limited if measurements were automated or semi-automated and nearly instantaneous. Such techniques are currently available for lung lesions but not for lesions in solid organs where contrast between lesion and tissue is inferior and very variable based on contrast injection. In conclusion, volumetry is a promising technique but technical improvements are needed before it can be routinely used.

Functional criteria

Limitations of morphological measurements

A new therapeutic approach that has been evaluated over the recent years targets tumoral vessels: anti-angiogenesis therapy. The purpose of the treatment is to asphyxiate tumors by depleting its supply of oxygen and other nutrients. By destroying tumor vessels, or by limiting their development, these molecules inhibit tumor growth potential (11). However, most have little effect on tumor size, and current morphological criteria appear ill-adapted to evaluate their efficacy. Such treatments have demonstrated a cytostatic effect (suppression of tumor growth) as opposed to cytotoxic effect (cell death with tumor regression). While few patients usually are responders (10-20%), disease stabilization may be seen in up to 70% of patients (12-15). The final impact of anti-angiogenic drugs on patient survival is not fully established (16) because of insufficient long-term follow-up data. Clinical trials are therefore long since several months, even years, and large patient populations may be required in order for RECIST or WHO criteria to demonstrate the efficacy of these drugs. In addition, morphological criteria may be ill-adapted to assess therapeutic response. Assessment of efficacy of cytostatic inhibitors needs to avoid the pitfall of requiring tumor shrinkage in order to proceed with clinical development. At the clinical stage, an effective treatment could be discontinued based on morphological criteria whereas an ineffective one might be pursued leading to excess risk and costs. Accurate evaluation of treatment response is important especially since anti-angiogenic drugs are costly. An imaging technique able to directly demonstrate the biological or physiological impact of the treatment and predict its efficacy could identify non-responding patients at an early stage resulting causing the ineffective treatment to be discontinued, and adapt dose and delivery schedule to optimize therapeutic response and reduce side-effects for responding patients. Functional imaging evaluates physiological or molecular characteristics, not tumor size. An ideal imaging technique would demonstrate and directly quantify the biological impact of the treatment. Four techniques are reviewed here:

- Dynamic imaging for quantification of tumor microcirculation,
- Diffusion MRI as an indicator of tumor cellularity,
- MR spectroscopy to evaluate molecular components of tumors,
- PET-FDG.

A clinically useful imaging technique should be sensitive to a large spectrum of parameters to be able to demonstrate treatment induced modifications. The technique should also demonstrate measurable differences between normal and tumoral tissues, and between tumoral tissues before and following treatment. It should also have little inter-individual biological variability and high reproducibility because elevated technique-related standard deviation would negatively impact specificity.
Dynamic evaluation of microcirculation

Over the last few years, dynamic imaging techniques allowing evaluation of tumor circulation have been developed for CT, MRI and US. These techniques specifically assess tumor vessels in order to determine the impact of anti-angiogenic drugs, as opposed to indirect and delayed effects on tumor size.

Principle

Dynamic perfusion imaging is based on the imaging evaluation of the biodistribution of a contrast agent acting as a tracer. After intravenous administration, the agent is distributed into tissues based on local microcirculation, then, based on its size, diffuses across the endothelial membrane into the interstitial compartment. Imaging depicts the distribution of the tracer or contrast agent by measuring variations in vessel and tissue enhancement over time. Current imaging systems possess a temporal resolution in the order of a second, which allows sufficient sampling of perfusion phenomenon to create mathematical models.

Current dynamic imaging techniques, and respective advantages and limitations, are described in table II.

Dynamic imaging with injection of contrast material using CT and MRI

Dynamic functional imaging using injected tracers is based on the quantitative analysis of the biodistribution of the intravenously injected tracers in tissues. Tissues are composed of three compartments: vascular compartment corresponding to capillaries, interstitial compartment, and intra-cellular compartment. Contrast agents routinely used in clinical practice have interstitial diffusion and do not penetrate cells or blood cells.

Data acquisition

Perfusion CT and MRI techniques typically require a baseline image acquisition without contrast enhancement followed by a series of images acquired over time after an intravenous bolus of conventional contrast material. Because of current technical constraints, a single lesion can be assessed because the studied volume must be compatible with a temporal resolution of one second. For low molecular weight agents used in clinical practice, the acquisition must proceed over a minimum of 3 minutes to acquire sufficient data to describe transcapillary passage and interstitial diffusion of the contrast agent. As changes in contrast enhancement in this phase are less rapid, images are generally obtained at a lower frequency than that required for first pass studies.

A protocol that aims to use a single administration of contrast material to assess not only perfusion and blood volume but also vascular permeability will usually comprise an initial rapid sequence of images, or temporal resolution of about one second, during the first pass with less frequent images later to reduce data volume and patient irradiation when using CT.

Data analysis

Data are transferred to a workstation where regions of interest (ROI) are defined over a feeding artery and target lesion (fig. 2). These ROI are applied to the entire image dataset to measure signal or attenuation variations over time, which is proportional to the variation of contrast agent over time (directly for CT or indirectly for MRI). A perfusion software then extracts the following perfusion parameters:

- Tissue blood flow,
- Vascular permeability,
- Tissue blood volume,
- Interstitial blood volume,
- Mean transit time.

Tissue blood flow $F$, expressed in ml/min/100 ml of tissue, represents tumor perfusion and corresponds to the flow of blood per unit of tissue volume (fig. 3).

Vascular permeability or endothelial transfer coefficient $K\text{po}$, expressed in ml/min/100 ml of tissue, represents the rate of transfer of contrast agent from the blood to the interstitial space per unit of time and unit of tissue volume (fig. 3).

Tissue blood volume $V_t$ and interstitial blood volume $V_i$ are expressed as % (or ml/ml tissue). The blood volume represents the proportion of vessels within a volume of tissue, but only those vessels containing the injected contrast agent.

The interstitial volume represents the interstitial volume into which the contrast diffuses. Intracellular and red blood cell volumes are “blind” since contrast agents do not diffuse into these spaces (fig. 4).

Mean transit time $MMT$, expressed in seconds, represents the mean time required for a molecule to travel across the capillary bed of a tissue, from afferent arteriole to efferent venule (fig. 5) in the absence of extravasation.

Table II

<table>
<thead>
<tr>
<th>Imaging techniques for evaluation of tumoral microcirculation.</th>
<th>CT</th>
<th>MRI</th>
<th>US</th>
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<tbody>
<tr>
<td><strong>Calculated parameters</strong></td>
<td>– Tissue blood flow</td>
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<td>– Tissue blood volume</td>
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<td>– Interstitial blood volume</td>
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<td></td>
<td>– Vascular permeability</td>
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<td></td>
<td>– Mean transit time</td>
<td>– Mean transit time</td>
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<tr>
<td><strong>Advantages</strong></td>
<td>– Available</td>
<td>– No ionizing radiation</td>
<td>– Available</td>
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<td></td>
<td>– Linear attenuation-concentration correlation</td>
<td>– Contrast injection or spin labeling</td>
<td>– Lower cost</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>– No ionizing radiation</td>
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<tr>
<td><strong>Limitations</strong></td>
<td>Ionizing radiation</td>
<td>Signal-concentration relation more complex and requiring rigorous acquisition parameters</td>
<td>Quantification difficulties (diffusion phenomenon, reflection and absorption)</td>
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<td>– Non standard protocols</td>
<td>– Some organs are not accessible</td>
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<td>– Higher cost</td>
<td>– Operator dependent</td>
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Figure 6 is a schematic representation describing how the different parameters are obtained from time-concentration curves. Results may be obtained pixel-per-pixel to generate parametric maps or as a mean value within a defined ROI. Figure 7 corresponds to a parametric map of tissue blood flow in a patient with retroperitoneal metastases before and after treatment.

Results
In the follow-up of anti-angiogenic therapies, functional imaging has demonstrated its ability to detect biological effects of treatments by depicting early microvascular modifications, before tumor size modifications. In an animal model (17), dynamic MR imaging performed with a high-molecular-weight contrast agent detected and quantitatively measured significant declines in apparent tumor permeability within 24 hours after initiation of therapy (fig. 8). This correlated with histologic analysis on the basis of a corresponding significant reduction in microvessel density, which is a widely used pathologic surrogate of angiogenesis. In addition, our animal results have shown that tumors in responding animals had significantly higher permeability before
**Fig. 5:** *Schematic representation of mean transit time.* Mean transit time MMT, expressed in seconds, represents the mean time required for a molecule to travel across the capillary bed of a tissue, from afferent arteriole to efferent venule. In the figure above, two routes are possible for a molecule symbolized by a circle and a star. At the lower end of the figure, corresponding theoretical transit curves are depicted. The observed curve corresponds to the average of all possible curve with mean transit time being the mean.

**Fig. 6:** *Time-concentration curves.* The volume of an ROI placed over a large vessel is 100% occupied by blood. Its curve (black curve, full line) over time corresponds to the distribution of contrast in the circulation: the first peak corresponds to the first passage, the second peak, smaller, corresponds to recirculation, with progressive dampening to complete elimination of the agent (renal and/or biliary). The volume of an ROI placed over tumor is occupied by vessels (tissue blood volume), interstitial space (interstitial distribution volume) and cells. Intracellular and red blood cell volumes are “blind” or dead spaces since available contrast agents do not diffuse into these spaces. The curve of tumor concentration (grey curve, full line) corresponds to the sum of the curves for tumor capillaries (not drawn) and interstitial space (grey curve, dotted line). An image analysis software generates the tumor curve from ROI placed over the tumor, whereas a compartment analysis software generates curves for the capillary and interstitial compartments. Schematically, perfusion is calculated from the concentration over time slope normalized from the arterial curve. Blood volume corresponds to the relative tissue enhancement peak compared to the arterial peak. The interstitial volume corresponds to the relative interstitial enhancement peak compared to the arterial peak. Vascular permeability corresponds to the slope of the interstitial enhancement curve.

**Fig. 7:** *Parametric map of tissue blood flow in a patient with retroperitoneal nodal metastasis from renal cell carcinoma undergoing anti-angiogenic treatment.*

a Map before treatment: the lesion (arrow) is highly perfused (red pixels) except for a hypo-perfused area of central necrosis (blue pixels).

b Map after 6 weeks of anti-angiogenic treatment: the lesion (arrow) shows only a few peripheral foci of mild perfusion (green pixels).

Mean tissue blood flow before treatment was estimated at 731±272 ml/min/100 ml tissue compared to 190±199 ml/min/100 ml after treatment.
treatment than after treatment compared to non-responding animals (fig. 9). These results suggest that imaging could not only provide a useful means of following-up the effects of treatments on tumors but also could predict tumors that really respond to treatments to select patients that would benefit from continued treatment using these expensive drugs that are not without side effects.

Dynamic imaging with injection of contrast material using US

US also is used routinely in oncology to evaluate response to treatment. It provides real time imaging (18), with high spatial and temporal resolution, allowing measurement of very slow flow (<1 mm/sec) and direct visualization of very small vessels.

The principle of contrast US imaging resides on destruction of microbubbles. A contrast agent is injected (Sonovue®). Bracco Diagnostics Inc., Milan, Italy), and contrast microbubbles in the field of view are destroyed by intense ultrasound. The capillary bed progressively fills with contrast microbubbles. Contrast kinetics allows semi-quantitative evaluation of tissue blood flow and volume (fig. 10).

Microbubble contrast imaging was shown to be useful in the follow-up of anti-angiogenic treatments in humans (19).

Microbubbles used for US do not cross endothelial membranes and cannot be used to assess capillary vascular permeability.

Spin labeling

With this technique, the injection of contrast material is not needed since blood flowing into the slices of interest is magnetically labeled. A modification of the magnetization of arterial protons causes a measurable change in T1 values of tissues.

In arterial spin labeling, protons in arterial blood water are magnetically aligned in the opposite direction from the rest of the protons in blood and tissues by applying a selective saturation pulse. Water readily diffuses across endothelial membranes. After a pre-determined time interval τ, defined as the transit time required to displaced labeled spins from non-labeled spins in the tissues of interest, an image is acquired. The influx of protons with negative signal into the target tissue results in a loss of signal. The difference of signal with a reference image obtained without blood water labeling is proportional to the speed of entry of protons into the tissue, or blood flow (fig. 11).

An advantage of this technique is that it can be performed during a single breath hold whereas perfusion techniques using intravenous contrast agents are acquired over several minutes. Also, because intravenous contrast is not injected, it can be repeated several times, on a single lesion to evaluate reproducibility, or on several lesions in patients with multi-focal disease. On the other hand, only blood flow can be assessed and other parameters of microcirculation cannot be assessed.

This technique was developed for the brain and is almost exclusively used for brain evaluation. However, recent studies have shown that it could be useful for tumor imaging and follow-up of anti-angiogenic treatments (20).

Diffusion MR Imaging

Diffusion weighted MR imaging differentiates water molecules that freely diffuse from water molecules with restricted diffusion. This technique has shown value in the evaluation of tumor response in oncology.

Principle

Two intense and symmetrical gradients are applied to a tissue so that the measured signal intensity becomes inversely proportional to proton displacement or diffusion (21). A first gradient is applied that causes dephasing of water protons relative to their initial position, followed by a subsequent gradient of opposite magnitude. Protons that have remained stationary in between both pulses are rephased and produce signal. Protons that have moved are either only partially or not refocused at all, resulting in loss of signal. The amount of signal loss is related to the motion or diffusion of water protons in tissues. Intracellular water motion is much more restricted due to the presence of multiple structures such as membranes, organelles, and cytoskeleton compared to extracellular water. As a result, the diffusion coefficient is considered to be a representation of the relation between extra- and intracellular water (22). Therefore, the diffusion coefficient of a very cellular tumor would be low. However, after a cytotoxic treatment causing cellular death, water molecules should diffuse more readily with corresponding increase in the diffusion coefficient (23) (fig. 12).

Data acquisition

Diffusion weighted imaging uses an ultra fast spin echo-echo planar imaging (SE-EPI) type pulse sequence allowing the acquisition of images in about 100 msec. The b factor summarizes the influence of the gradients on the diffusion weighted images. The higher the value b, the stronger the diffusion weighting. The factor b is given by $b=\gamma^2 G^2 (Δ-δ/3)$, where $γ$ is the gyromagnetic constant; $G$ is the amplitude of the pulsed gradient; $δ$ is the duration of the individual gradient pulses; $Δ$ is the time interval between the leading edges of the two pulsed gradients. Therefore, the degree of weighting can be increased by increasing gradient amplitude, time duration of individual gradient pulses, or time interval between both pulses.

On current scanners, gradients with intensity values of about 30–35 mT/m can be applied. Several (minimum of two) series of images are acquired using variable b values. The ideal b values to be used in clinical practice have yet to be determined, and will probably vary based on the target organ. Values between $b = 500$ s/mm² and $b = 1,000$ s/mm² seem to provide an acceptable compromise between diffusion weighting and SNR.

Data analysis

Apparent diffusion coefficient values are calculated using the formula: $S = S_0 e^{-(b/b₀)}$, where $S$ is the signal intensity after the diffusion gradients are applied and $S_0$ is the signal intensity before they are applied.

Therefore, $ADC = ln(b₀/b)$

The ADC is a physical constant independent of magnetic field, scanner, sequence or acquisition parameters. It is thus a reproducible value that is non-operator and non-scanner dependent.

The imaging data is transferred to an independent workstation where an ADC map is generated on a pixel-per-pixel basis.

Regions of interests can be placed over target lesions using morphological imaging data. Mean ADC values can then be measured for each lesion, before and after treatment. ADC values are inversely proportional to tumor cellularity (fig. 13).

Results

Animal research has demonstrated the value of this technique for monitoring the effects of a vascular targeting agent on
Changes of tumor permeability during anti-angiogenic treatment. Nude rats with human breast cancers underwent anti-angiogenic treatment using celecoxib (n =17) versus placebo (n =13) during 7 days. Perfusion MRI was performed before treatment, 24 hours after the first treatment and after 7 days of treatment. A significant reduction in permeability was observed at 24 hours (0.83 versus 0.67 ml/min/100 ml) in treated animals versus non-treated animals.

Vascular permeability in responder animals versus non-responder animals receiving anti-angiogenic therapy. Pretreatment permeability values were significantly higher in responder animals compared to non-responder animals. This difference was no longer present after 24 hours of treatment. This functional parameter could be helpful in identifying animals that would be likely to respond to anti-angiogenic therapy before initiation of treatment.

Principle of microbubble contrast imaging. The probe is placed over the lesion of interest. After injection of the contrast agent, a high energy impulse is delivered causing microbubble destruction. Over time, the capillary bed progressively fills with contrast microbubbles. Contrast kinetics allows semi-quantitative evaluation of tissue blood flow and volume: the slope of the curve is proportional to tissue blood flow and the plateau is proportional to tissue blood volume.

Principle of arterial spin labeling. A baseline image is acquired. A selective saturation pulse is applied proximally to magnetically align protons from inflowing blood in the opposite direction from the rest of the protons in blood and tissues. After a pre-determined time interval t, an image is acquired. The influx of protons with negative signal (black circles) into the target tissue with diffusion into the interstitial space results in a loss of signal. A subtraction image is generated. The difference of signal with a reference image obtained without blood water labeling is proportional to the speed of entry of protons into the tissue, or blood flow.
rhabdomyosarcoma in rats (24). After a single injection of combretastatin, the animals (n = 17) underwent DWI at 1 hour, 6 hours, 2 days and 9 days. At 2 days, the ADC values were significantly higher (1.79 vs. 1.26 × 10⁻³ mm²/s, p < 0.0001) indicating tumor necrosis, as confirmed on histologic examination. In contrast, the ADC values of the periphery decreased significantly at 9 days (1.41 × 10⁻³ mm²/s, p < 0.0001). The corresponding histologic specimen revealed regrowth of solid tumor in the periphery in a centrifugal manner.

In human research, Dzik-Jurasz et al. demonstrated that ADC values in rectal adenocarcinomas before treatment (n = 14) were inversely correlated to the percentage of tumor size decrease after chemotherapy-radiation therapy (25). Also, non-responding patients had significantly higher ADC values, indicating less cellular tumors, before treatments compared to responding patients. These results suggest that DWI could help predict patient response to treatment.

Such early results must be confirmed by larger scale studies. However, diffusion weighted MR imaging appears promising in demonstrating therapeutic efficacy in patients prior to completion of a treatment cycle. ADC values can be measured before and during treatment to demonstrate the presence (or absence) of therapy-related changes in tumor tissue architecture.

**MR Spectroscopy**

Magnetic resonance spectroscopy (MRS) is an MR application providing details about the molecular composition of tissues. Initially developed for neurological applications, the technique is now being evaluated for a number of tumors including astrocytoma, and prostate, colon, breast, uterine cervix, pancreas and esophageal carcinoma (26). MRS can be performed on virtually all clinical scanners equipped with the appropriate software packages and coils. Different nuclei can be assessed (1H, 13C, 14N, 19F, 23Na, 31P) but proton (1H) is the most widely used, followed by phosphorus (31P). Proton spectroscopy only requires software and dedicated sequences. Spectroscopy of other nuclei requires dedicated RF coils, and is less frequently performed. This technique detects signal from molecules containing the targeted atom. Based on the surrounding molecular environment, nuclei resonate at slightly different frequencies, allowing evaluation of the molecular component of tissues. Results are displayed on a spectrum showing a series of peaks along an axis labeled in parts per million (ppm) with the ppm scale describing the shift in hertz from a reference peak divided by the frequency of excitation. The different peaks thus correspond to different metabolites, and the height of the peaks is proportional to the metabolite concentration in the sampled tissue (fig. 14a). Absolute quantification is not possible, and peak ratios are routinely used to obtain data that can be used to compare for a single patient or between patients.

MRS provides no spatial information. It cannot pinpoint the source of the signal. As such, morphologic imaging must be used for correlation.

**Data acquisition**

Standard morphologic MR images are first acquired to locate the target organ. Shimming is then performed to optimize magnetic field homogeneity prior to MRS.

MRS can then be performed on an ROI selected from the MR images. MRS localization using 3 orthogonal slices is then confirmed, defining a voxel. The voxel position is displayed on the original MR image. Most frequent MRS acquisition sequences include STEAM (stimulated echo acquisition mode) and PRESS (point resolved spectroscopy) for 1H spectroscopy and ISIS (image selected in vivo spectroscopy) for 31P spectroscopy. Multivoxel spectroscopy using CSI (chemical shift imaging) in 2D (2d-CSI) or 3D (3d-CSI) is now available. Multislice multivoxel acquisitions allow evaluation of a volume but requires phase encoding in each direction. For example, an 8x8x8 pixel matrix requires a minimum of 256 phase encoding steps, for an acquisition time of 8.5 minutes with a TR of 1 second.

Proton (1H) spectroscopy allows quantification of several metabolites, including creatine and phosphocreatine (Cr) and choline (Cho). Signal from lipid molecules (L-CrH₂) can also be detected. Creatine and phosphocreatine are components of the energy metabolism. Choline containing molecules are involved with cell membrane synthesis and metabolism. Therefore, tumors frequently have high levels of choline (27).

In prostate carcinoma, for example, MRS allows relative quantification of citrate, creatine and choline (28). Normal prostate tissue contains high levels of citrate (higher in the peripheral zone than in the central and transitional zones). In carcinomas, the citrate peak is markedly reduced, sometimes undetectable, due to citrate oxidation by tumor metabolism. The choline peak is elevated due to increased cell membrane phospholipid turnover in proliferating tissues. As a result, tumors have an increased choline/citrate ratio (fig. 14a). Because the creatine peak is close to the choline peak, both are difficult to separate. In routine clinical practice, the (choline + creatine)/citrate ratio is used (29).

**Results**

Most research with MRS in oncology was focused on tumor detection, but it seems that MRS is of potential value in the evaluation of response to treatment. In patients with prostate cancer undergoing hormonal therapy (fig. 14b), MRS was able to demonstrate metabolic atrophy correlating with gland atrophy under treatment (30). The loss of citrate and the presence of total metabolic atrophy correlated roughly with decreasing serum prostatic specific antigen levels with increasing therapy. In the absence of citrate, however, residual prostate cancer could still be detected by elevated choline levels. In a study of 16 women with breast carcinoma, MRS was used to predict early response to neo-adjuvant chemotherapy using epirubicin, cyclophosphamide and 5-fluorouracil (31). MRS was carried out before treatment and after the second of six treatment cycles. Early changes of ratio between the water peak (at 4.7 ppm) and the sum of both fat peaks (at 1.3 and 0.9 ppm) after 2 cycles of chemotherapy predicted final volume response in 69% of cases (11/16) while maintaining 100% specificity and positive predictive value. This could allow early and accurate prediction of non-response and would permit an early change to second-line treatment, thus sparing patients unnecessary toxicity, costs and delay of initiation of effective treatment.

**Positron Emission Tomography**

Positron emission tomography with radio-labeled [18F]-2-fluoro-deoxy-D-glucose (PET-FDG) imaging is a nuclear medicine imaging technique assessing tissue metabolism. Whole body PET-FDG imaging has shown its value for diagnosis of several cancers and follow-up during treatment.
**Fig. 12:** Impact of a cytotoxic treatment on diffusion weighted MRI. The diffusion coefficient is a representation of the relation between extra- and intracellular water. The diffusion coefficient of a very cellular tumor would be low because the interstitial volume (free water) is low. After a cytotoxic treatment, the number of cells is reduced and the interstitial volume is increased. The increased proportion of extra-cellular water molecules should diffuse more readily with corresponding increase in the diffusion coefficient.

**Fig. 13:** ADC maps in a rectal cancer before and after radiation and chemotherapy.

a **ADC map from an axial diffusion-weighted image in a patient with rectal carcinoma:** low ADC values (green pixels) suggesting high cellularity. An ROI placed over the tumor shows a mean ADC value of $0.458 \times 10^{-3}$ mm$^2$/sec.

b **ADC map in the same patient after radiation and chemotherapy.** The tumor is smaller and the ADC values have increased due to necrosis as confirmed on surgical biopsy. Post treatment ADC value was $0.812 \times 10^{-3}$ mm$^2$/sec.

**Fig. 14:** MR spectroscopy of the prostate.

a **Proton spectroscopy of normal and tumoral prostate tissue.** Results are displayed on a spectrum showing a series of peaks along an axis labeled in parts per million (ppm) with the ppm scale describing the shift in hertz from a reference peak divided by the frequency of excitation. From left to right, the choline peak is located at 3.24 ppm, and the creatine peak is located at 3.10 ppm. Both peaks are closely located and suboptimally resolved by this technique. The citrate peak is at 2.66 ppm. In routine clinical practice, the (choline + creatine)/citrate ratio is used. In normal tissue, this ratio should be <0.5. In cancers, the citrate peak is reduced, whereas the choline peak is increased. The presence of carcinoma is confirmed when the ratio is >0.8.

b **Proton spectroscopy in a patient after initiation of hormonal therapy.** The spectrum is calculated for each image pixel. All metabolic peaks are reduced, indicating metabolic atrophy and treatment efficacy (Images courtesy of Dr. Taouli, New York University, NY, USA).
Principle

[18F]-2-fluoro-deoxy-D-glucose (a positron emitter) is analogous to glucose and shows uptake in metabolically active cells. Its half-life is about 2 hours. Malignant cells demonstrate marked uptake due to active metabolism and increased transmembrane glucose transporter proteins. Unlike glucose, deoxyglucose is not metabolized and remains trapped within the cells. It was shown that FDG uptake is proportional to proliferation and number of viable cells within a tumor (32).

Data acquisition

After FDG is injected, the patient is positioned in the PET gantry. Disintegration of the radioisotope produces a positron that will soon annihilate with an electron, emitting two collinear 511 keV photons 180° apart. These gamma-ray pairs “in coincidence” are detected by a ring of detectors by using timing circuitry that projects the location of the event producing them along a line between the two detector elements. Spatial resolution remains limited to a few mm.

PET may be combined to CT to combine metabolic data from PET with morphological data from CT.

Data analysis

The acquired images may then be qualitatively assessed for areas of increased tracer uptake. It is also possible to quantify the degree of FDG uptake. Absolute quantification of radioactivity, in KBq/mL, is difficult to measure, and requires calibration of the scanner to convert hits/pixel into MBq/L. As such, a semi-quantitative technique is routinely used in clinical practice based on evaluation of relative tracer uptake on any given point of the image relative to the entire imaged volume, injected dose and patient morphology. Standardized uptake value (SUV) calculation is based on a ratio between tracer uptake and homogeneous distribution of the tracer within the patient using the formula: SUV = QxP/Q_inj where Q corresponds to the uptake at the site of interest (MBq/L), Q_inj corresponds to the injected dose (MBq/L) and P corresponds to the patient’s weight (patient’s volume, assuming a mean density of 1, or density of water). The patient weight may be replaced by the body surface area, probably resulting in a more accurate measurement. Therefore, an SUV value of 1 indicates that the activity is distributed uniformly throughout the volume. A value of 10 in a lesion indicates that the uptake is increased 10-fold. Other values described in the literature are DUR (differential uptake ratio) and DAR (differential absorption ratio), both are synonymous.

It has been shown that these values are correlated and may replace the metabolic uptake rate (MUR) which requires absolute quantification with blood samples and kinetic evaluation (33).

Results

Multiple publications have demonstrated the value of PET in evaluating treatment efficacy. Glucose uptake related to the metabolic activity of tissues is affected sooner than tumor size (fig. 15). However, these studies used quantification methods and post-treatment imaging delays that were variable creating difficulties when comparing studies. In 1999, the European Organization for Research and Treatment of Cancer (EORTC) PET study group issued recommendations on the measurement of [18F]-FDG uptake for tumor response monitoring (32).

This group described the conditions surrounding patient preparation, recommended delays for pre- and post-treatment imaging, and SUV measurement technique. The European Organization for Research and Treatment of Cancer (EORTC) also promulgated guidelines for the standardization of metabolic response categorization based on the RECIST criteria. The proposed metabolic criteria based on changes in SUV are as follow:

- **CMR (complete metabolic response)**: return of FDG uptake in previously documented lesions to a level equivalent to, or less than, residual radioactivity in normal tissues within the organ in question.
- **PMR (partial metabolic response)**: 15-25% reduction in SUV after one therapy cycle or 25% reduction after more than one cycle.
- **PMD (progressive metabolic disease)**: 25% increase in SUV compared to pre-treatment PET or 20% increase in extent of metabolic abnormality suggesting tumor growth or presence of new lesions.
- **SMD (stable metabolic disease)**: not CMR, not PMR and not PMD.

These criteria were established empirically from data from the literature and should be reassessed regularly. The role of PET for evaluation of tumor response may also change with the availability of new fluorine tracers, more sensitive than FDG, such as fluorine-18 fluoromisonidazole (F-MISO), a marker of hypoxia, and FLT (3’-deoxy-3’-18fluorothymidine), a marker of DNA synthesis. These tracers are still at the research stage, and it is difficult which will eventually be used for routine oncologic imaging.

**Strategies and perspectives**

Several techniques are currently available in addition to morphological imaging to assess response to treatment. Table III summarises these techniques and assessed parameter. Results from human studies remain preliminary and the medical community will have to determine how to best assess the value of each of these techniques for routine practice.

**Two possible strategies**

A large number of studies on functional imaging evaluation of tumor response to a wide variety of treatments have been published with each research group proposing a different technique and protocol. However, two strategies seem reasonably foreseeable for routine daily practice. CT is advantageous because it adds functional imaging to a morphological study.

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**Table III Functional Imaging Techniques.**

<table>
<thead>
<tr>
<th>Technique</th>
<th>Tumor parameter</th>
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<tbody>
<tr>
<td>Incremental CT</td>
<td>Volumetry</td>
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<tr>
<td>Helical CT</td>
<td>Vascularization</td>
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<tr>
<td>Perfusion imaging</td>
<td>Cellularity</td>
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<tr>
<td>Diffusion MRI</td>
<td>Molecular profile</td>
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<tr>
<td>MR spectroscopy</td>
<td>Glucose metabolism</td>
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<td>PET FDG</td>
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that is already being routinely performed for work-up and follow-up of oncologic patients. The main drawback of CT is radiation exposure, which may be relative for oncologic patients, except for pediatric patients.

MRI could be used as a “one stop shop” for combined morphological, perfusion, diffusion and spectroscopic imaging. Some tumors could benefit from this approach, namely prostate, rectal, ovarian and breast carcinomas and soft tissue tumors. Drawbacks from MRI mainly are higher cost and reduced availability. Also, acquisition parameters may have a great impact on signal intensity, and it may be more difficult to compare results from different machines or from different protocols. It is likely that manufacturer involvement will be needed in establishing reproducible and comparable protocols.

**Perspectives**

An ideal parameter should:
- be sensitive to a large physiological variability from tumor to tumor while being subject to little inter-patient variability (for example, no impact from variable patient cardiac output or blood pressure on the day of the examination),
- be sensitive to tumor characteristics that will specifically be modified by the treatment (cellularity, capillary permeability),
- allow early quantifiable measurements of treatment-induced modifications, prior to size changes,
- demonstrate dose related modifications,
- show modifications that can predict outcome/survival of the patient/tumor response (for example, steroids for gliomas may provide symptomatic relief but have no impact on patient outcome/survival while modifying blood volume and capillary permeability).

The challenge of functional imaging will be to provide data that will either improve research or patient management. The efficacy of a test is not limited to its diagnostic accuracy or impact on patient management. A new technique must provide a measurable gain in health assessment, important enough to justify its added cost. Imaging provides two categories of data: it measures response to treatment based on type of observed response, dura-
tion of response or duration of progression free response, and the impact for the patient based on global survival, progression free survival, symptomatic improvement and quality of life improvement. Because multiple imaging techniques are available (CT, MRI, US, nuclear medicine, molecular imaging, new biological markers…), the volume of data to analyze will be substantial requiring multi-disciplinary collaboration and implementation of strict analytical methods for decision making (evidence-based medicine).

First, simplified and standardized acquisition and analysis protocols will be needed. Then, validation of these techniques will be required to confirm their value for treatment decision-making process. This subject has not yet been addressed by the scientific community and collaboration between radiologists, nuclear medicine physicians, oncologists and epidemiologists will be needed to determine validation protocols, and needed levels of accuracy for clinical usefulness. Large multicenter standardized studies will be needed to evaluate reproducibility, diagnostic value and clinical applications of these techniques. It is possible that the association of more than one marker is more clinically useful than any single marker alone, for example, the combination of volumetric tumor measurement from morphological imaging and perfusion data from functional imaging.

Ultimately, this rigorous process could help define specific recommendations for each disease with regards to treatment initiation and follow-up.

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References