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Assessment of diffusion-weighted Magnetic Resonance imaging to predict the chemotherapy outcome in liver metastases.

Settore Scientifico Disciplinare MED/36

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Index

Abbreviations

CT: Computed Tomography

CA: Contrast agent

MR: Magnetic Resonance

I: Imaging

RECIST: Response Evaluation Criteria in Solid Tumors

DWI: Diffusion-Weighted Imaging

ADC: Apparent Diffusion Coefficient

D: Diffusion Coefficient

ROI: Region of Interest

R: Responder

NR: Non Responder

1.Preface

1.1. MR advances and applications

Two of the most powerful diagnostic tools in use in medicine today are Magnetic Resonance (MR) and Computed Tomography (CT). Each of these, in its own right, is a relatively new technology, rising to prominence within the last 30-40 years and realizing continuous refinement along the way. While both CT and MR essentially provide enhanced images of body structures and/or tissues, they are distinctly different and are most effective in certain situations. During the diagnostic phase of treatment, a physician will assess symptoms and determine whether a need for CT or MR is present. In a time-sensitive situation, particularly trauma, CT may be the initial approach. Then should a CT suggest the need, an MR could follow; however, diagnoses related to soft tissue or organs may indicate the need for an MR early. Both of these imaging modalities have revolutionized the relative ease of diagnosis of many disease processes. They have enhanced a physician's ability to pinpoint and accurately diagnose problems at an earlier stage of development. MR is an imaging modality that uses a magnetic field and radio frequency energy to create a signal which generates images which can be used for diagnostic purposes. It is especially useful for soft tissues including the brain, and for joints and abdominal organs. More recently, biopsies are being performed, particularly in the breast, using MR guidance. CT scans use x-rays and multiple detectors to create images of the same body parts but can be obtained more quickly and are generally more widely available and slightly less expensive. CT remains the most requested imaging tool for evaluation of the chest, abdomen and pelvis, while MR has clear advantages in imaging the brain, musculoskeletal system and spine and has a significant problem-solving role in the abdomen and pelvis when CT is not adequate.

In modern medicine, there is a growing need to diagnose the presence of disease as soon as possible, even when symptoms are not yet present or are minimal, to identify the response to treatment in patients that have been treated, and to detect improvement or worsening of the disease as early as possible. Conventional imaging methods that rely on morphologic or structural data are very precise in the delineation of lesions, but frequently present a limited diagnostic efficacy in the evaluation of response to oncologic treatments. These imaging methods define response to treatment as a reduction of tumor volume, without considering molecular or functional aspects that appear earlier than the structural or anatomic changes. The accurate objective asses-

sment of tumor response has become increasingly important with the rapid and continuous development of new drugs. International guidelines for objective evaluation of tumor response were first established in early 1980 on the initiative of the World Health Organization (WHO) (1). These guidelines were originally based on tumor size determined from the sum of the products of 2D measurements. Since their introduction, the guidelines have been simplified so that 1D tumor measurements may be used (2,3). This new approach has been validated by the Response Evaluation Criteria in Solid Tumors (RECIST) group and integrated into current guidelines for evaluating the tumor response to anticancer therapy (2). However, this morphologic information based on 1D or 2D measurement does not directly reflect biologic changes in tumors and can be misleading in the clinical management of tumors and investigation of new drugs.

In general practice, contrast-enhanced CT is routinely used to monitor tumor response. The degree and pattern of enhancement observed on CT scans are useful for differentiating malignant from benign tumors and identifying post-treatment changes. To a certain extent, the degree of enhancement may reflect the vascular and interstitial volumes of the tumor and may provide information about its biologic behavior (4). Recently, FDG PET has been suggested as a sensitive method for monitoring changes in the glucose metabolism in tumors for the early assessment of metabolic tumor response to anticancer drugs (5). However, FDG PET is costly and available at only limited number of institutions.

1.2 Role of MR-diffusion

MR plays an increasingly important role in clinical use because of its high contrast resolution, lack of ionizing radiation, and the possibility of performing functional imaging sequences. With advances in hardware and coil systems, MR Diffusion-weighted Imaging (MR-DwI) can now be applied to liver imaging with improved image quality. DwI supplies information of water proton mobility. This can be employed to assess the microstructural organization of a tissue like cell density, cell membrane integrity and ultimately cell viability which affect water diffusion properties in the extracellular space. There is growing interest in the application of DwI for the evaluation of the patient with cancer. DwI measurements are quick to perform (typically 1–5 minutes) and do not require the administration of exogenous contrast medium. DwI enables qualitative and quantitative assessment of tissue diffusivity (apparent diffusion coefficient-ADC). Thus, these imaging sequences can be appended to existing imaging protocols without a significant incre-

ase in the examination time. Furthermore, DwI yields both qualitative and quantitative information that can be helpful for tumor assessment.

2. Personal Experience

2.1 Abstract

Purpose: to find a method that allows an early assessment of response to CHT in liver metastases by Magnetic Resonance Diffusion-weighted Imaging (MR-DwI)

Materials and methods: 30 oncologic patients with liver metastases were enrolled from 5 different center and scanned by multi-b MR-DwI before the beginning of CHT (baseline, time 0) and repeated within two weeks (time 1), 20-25 days from the beginning of the first cycle of CHT (time 2), and 20-25 days from the beginning of the second cycle of CHT (time 3). For every metastatic lesion, dimensional variation and ADC values were estimated by fitting procedure. ANOVA and ROC analysis were performed. Sensibility, specificity, positive and negative predictive value and accuracy were obtained for different parameters.

Results*:* 71 metastatic liver lesion were evaluated; 60 classified as responder (R) and 11 as non-responder (NR). Applying a +25% ADC value increase cut-off, on the basis of the ADC fitted variation, of the 60 lesion classified as R: 25-34 and 35 were true R at time 1, 2 and 3 respectively; of the 11 NR lesion, 4-3 and 3 were true NR at time 1, 2 and 3 respectively. Evaluating the diameter changes, 31 lesions showed dimensional stability either at time 1 and 2. 29 metastatic lesions showed reduction in diameter, 8 of whom at time 1 and 20 at time 2. 11 lesions had dimensional increase, 10 at time 1 and 2 at time 2.

Conclusion: an early (20-25 days since the beginning) fine diameter assessment could be a good indicator of the final response to CHT in metastatic liver . A slightly correlation between response to CHT and ADC changes in comparison to the diameter changes of the lesions was found.

2.2 Keywords*:* liver, metastases, MR, diffusion weighted imaging, response, chemotherapy.

2.3 Introduction

Magnetic Resonance Diffusion-weighted Imaging (MR-DwI) allows quantitative assessment of changes in the diffusion properties of water molecules in living tissues by ADC calculation. DwI is increasingly being used in extracranial applications. It is being used more frequently in liver, breast, prostate, musculoskeletal, as well as abdominal and pelvic organ imaging. Parameters derived from MR-DwI are appealing as imaging biomarkers because the acquisition is noninvasive, does not require any exogenous contrast agents, thus enabling its use in patients with renal dysfunction, does not use ionizing radiation yet is quantitative and can be obtained relatively rapidly, and is easily incorporated into routine patient evaluations (6-12)

However, DWI of the liver and other abdominal organs is not without its technical challenges. The liver is a difficult organ to image with MR because it lies in direct contact with the diaphragm, and the left lobe is inferior to the heart. While good breath-holding technique eliminates the motion of the diaphragm, cardiac motion can cause severe artifacts, especially in the left liver lobe. Bowel peristalsis is another source of motion artifact. Another challenge associated with DwI in the abdomen is the occurrence of susceptibility artifacts resulting from fat and gas interfaces in the abdomen. In oncologic imaging, MR-DwI has been linked to lesion aggressiveness and tumor response, although the biophysical basis for this is incompletely understood. For these reasons there is a growing interest in its use, in detection and differential diagnosis of focal liver lesions, in evaluating the degree of fibrosis in patients with chronic hepatitis or cirrhosis (11, 13-16) and it is now potentially useful for an early response evaluation after chemotherapy (CHT) in oncologic patients (17, 18).

Imaging is routinely used for tumor staging and evaluation of response to treatment; tumor size is the main criterion for response evaluation. However, in general, tumor size changes only at the middle or end of a course of treatment and so cannot be used to adjust or change the regimen or measure response during the early period of treatment. Early evaluation and prediction of treatment response may make tumor therapy more efficient and guide individual treatment. Hepatic metastases are the most common malignant neoplasms of the liver and are found in 40% of all patients dying of cancer. Although resection of liver metastases may be beneficial in selected

oncologic patients, most of them are not eligible for surgical treatment because of the presence of multilobar lesions or extrahepatic disease.

Assessing tumor response to CHT is crucial to patient treatment. Currently, this is achieved by monitoring changes in tumor size by using computed tomography (CT) or MR based on RECIST 1.1 and mRECIST (modified-Response-Evaluation-Criteria-In-Solid-Tumor). Unfortunately, this assessment monitors a relatively late event because functional changes occur prior to alterations in size (19) and tumor size assessments are usually undertaken halfway through a course of treatment. Finally, these methods will enable earlier cessation of ineffective treatments, minimizing unnecessary toxicity and expenditure. Response evaluation is commonly performed after 8-9 weeks of treatment. A tool to monitor therapy response early is therefore desirable, as this would prevent unnecessary toxicity and costs.

Early evaluation and prediction of treatment response may make tumor therapy more efficient and guide individual treatment. The RECIST based on lesion size changes at imaging may need to be integrated with quantitative evaluation of functional parameters to accurately monitor disease progression (20, 21). The ADC calculated by MR-DwI, as known, measures the mobility of water in tissues and may thus be sensitive to changes in the tumor microenvironment that occur after treatment. The ADC value is strongly affected by molecular viscosity, the permeability of the membrane separating the intra- and extracellular compartments, active transport and flow, and the directionality of tissue and/or cellular structures that impede water mobility (22-25).

Efficient anticancer treatment results in tumor lyses, loss of cell membrane integrity, increased extracellular space, and, therefore, an increase in water diffusion, whereas viable tumor cells restrict the mobility of water and result in a decrease in water diffusion. In most tumors, including liver metastases, an increase of ADC after the start of treatment occurs in response to treatment, reflecting treatment-induced cell death (26, 27). However, timing is essential to witness this response to treatment: in later stages of treatment, tissue reorganization results in the formation of fibrosis, which could affect ADC values again.

The rationale of our study is that the successful of CHT induces a tumor cell injury, thus weakening the barrier that limits movement of water molecules. The ADC changes may indicate changes in tumor inner structure by detecting the diffusion changes of water.

Given this background, the aim of our multi-center-study was to find a method that allows an early assessment of response to CHT in liver metastases, particularly to satisfy the needs of the

clinical oncologist to be able to recognize soon those patients or lesions that have no clinical benefit at the end of cycle, to program a suitable change of therapy. Moreover our secondary purpose was to investigate the role of DwI as helpful instrument in the final assessment of response to therapy in comparison to perfusion and diameter evaluation.

This method to be clinically used, should be fast, easily feasible, and independent from both the tumor histology and the type of CHT used, to make it easy to program the early control by the clinician and to have a certain temporal variability to obtain high patient compliance. Obviously CHT in use have different durations and different times of action, but our goal is to try to disregard those. Furthermore, with the imaging techniques and analysis advancement, particularly for continuous hardware and software developments in MR (the use of sequences with isotropic voxel increasingly small and the consequent improvement in both the spatial resolution of image and contrast) and new powerful tools for segmentation and automatic analysis of the lesions, it is possible to suppose an evolution of RECIST 1.1 criteria currently in use.

This multi-center study was partially funded by SIRM (Società Italiana di Radiologia Medica) and registered on web site "www.clinicaltrials.gov" with identifier: NCT01411579 and ID: DWIPRECHEMOUT.

2.4 Materials and method

Patients

The local ethics committees approved the study protocol. The aim and nature of this prospective study were explained to the patients, who provided written consent before beginning the examination, according to the principles of the Declaration of Helsinki (revision of Edinburgh, 2000). All examinations were performed after overnight fasting.

Between January 2011 and October 2012, 30 oncologic patients (18 male, 12 female, mean/range age 65/18-80 years) with liver metastases were enrolled from various center in the study (20 in Florence, 3 in Treviso, 3 in Trieste, 3 in Naples, 1 in Brescia): 21 patients with liver metastases from colorectal cancer, 3 from gastric cancer, 6 from mammary adenocarcinoma and 1 from neuroendocrine carcinoma for a total of 71 focal liver metastatic lesions. The inclusion criteria for patients were: age range 18-80 years, non-confluent liver metastases, from every primary carcinoma histotype biopsy/surgical-proven, without intralesional necrosis/calcification involving >30% of their volume; a Karnofsky performance scale score ≥ 60%; ECOG performance status ≤ 2; not being pregnant; and being scheduled to begin a new CHT regimen for their metastatic disease, with no contraindications to CHT. The exclusion criteria were a history of any other malignant disease not included in entry criteria and contraindications to MR imaging.

Imaging technique

All patients were scanned by MR-DwI and contrast agent (ca)-CT before the beginning of CHT (baseline, time 0). The time between the initial MR and ca-CT should not be superior to one week. MR examination will be repeated within two weeks (time 1) and 20-25 days from the beginning of the first cycle of CHT (time 2), and 20-25 days from the beginning of the second cycle of CHT (time 3), At time 3 a ca-CT was performed (table 1).

Table 1: CT and MR-DwI timing control

Time 0	ca-CT and MR-DwI Pre-CHT
Time 1	MR-DwI at 10-15 days from the beginning of the first cycle of CHT
Time 2	MR-DwI at 20-25 days from the beginning of the first cycle of CHT
Time 3	ca-CT and MR-DwI at 20-25 days from the beginning of the second cycle of CHT

Legend: ca-CT: contrast agent computed tomography, MR-DwI: magnetic resonance and diffusion weighted imaging, CHT: chemotherapy.

Ca-CT examination will be performed according to an established protocol by using a 16/64-row equipment according to the centre involved, contrast bolus-track technology, slice-thickness reconstruction of 3 mm, before and after i.v. injection of iodinated contrast agent (3 mL/s), during arterial and portal phase.

All MR examinations were carried out using the following 1.5-T units:

- Gyroscan ACS NT Intera Release 12 (Philips, Eindhoven, The Netherlands) gradient strength, 30 mT/m; slew rate, 120 T/m/s; six-channel phased array multicoil;
- Magnetom Avanto (Siemens, Erlangen, Germany) gradient strength, 45 mT/m; slew rate, 200 T/m/s; 2 phased-array coils with 18 elements.

The different MR equipments employed by the different centers underwent calibration by the use of a dedicated phantom (28) The phased array multicoil will be adequately positioned to cover the upper abdomen of the subject lying in a supine position, the arms extended over the head to avoid artifacts. Patients, fasting from 4 hours, will be instructed to maintain a constant respiration depth, even with the possibility to use exogenous oxygen delivery to avoid deep respiration. All acquisitions will be performed by single-shot sequence to obtain immediately and automatically the ADC-maps.

The protocol, previously established in accordance to the various centers, included the following acquisitions, as performed in the reference center of Florence:

a) T2-weighted half-Fourier single-shot turbo spin-echo (HASTE) free-breath sequence; transverse/coronal plane; TR/TE 810/80 ms; echo-train length 69; slice number 40; slice thickness 5

mm; intersection gap 10%; field of view 300–420 mm; effective matrix size 256 x 165; number signal averages (NSA) 1; total acquisition time 2–3 min;

b) T1-weighted 2D gradient echo in/out phase breath-hold sequence; transverse plane; TR/TE/Flip angle 231-121/14.6-2.3 ms/80°; slice thickness 5 mm; slice number 24; intersection gap 10%; sense factor 1.5; field of view 300–420 mm; effective matrix size 256 x 165; NSA 1; total acquisition time 18 s;

c) Diffusion-weighted echo-planar imaging (EPI) single-shot free-breath multi-b sequences; transverse plane with variable EPI factor; b-value from 0 to 750 s/mm^2 with step of 50, TR/TE/Flip angle 2000/66 ms/90°, slice thickness 6 mm, slice number 12, intersection gap 10%, field of view variable, effective matrix size 128x 64, NSA 4, total acquisition time 3 minutes.

Fat suppression will be obtained by spectral pre-saturation inversion recovery. Isotropic motion probing gradients will be applied for each DwI acquisition and for each b-value will be automatically obtained images and corresponding ADC map. The accuracy of MR-DwI sequences was tested using an MR-DwI phantom study, as recommended (28, 29).

DwImages quantitative analysis

Two radiologists (F.M. and F.P, both with 7 years of experience in interpreting liver CT and MR-DwI) reviewed and analyzed all Dw images and ADC data sets in random. The ADC measurements obtained at baseline and at the different time point during CHT were transferred to an electronic database (Microsoft Excel 2008 for Macintosh; Microsoft Corporation, Redmond, Wash). The reviewer was blinded to the therapeutic response. Three diameter of each marker lesion were measured, and the mean/minimal/maximal ADC±standard deviation (SD) were quantified by circular region-of-interests (ROIs) within the lesion avoiding lesion margins, intratumoral calcification areas, including only the lesions located in the right lobe. The ROI was drawn on the b=50 image for optimal contrast between lesion and background, and then copied over all the other D-weighted images using ImageJ (fig.1) (30).

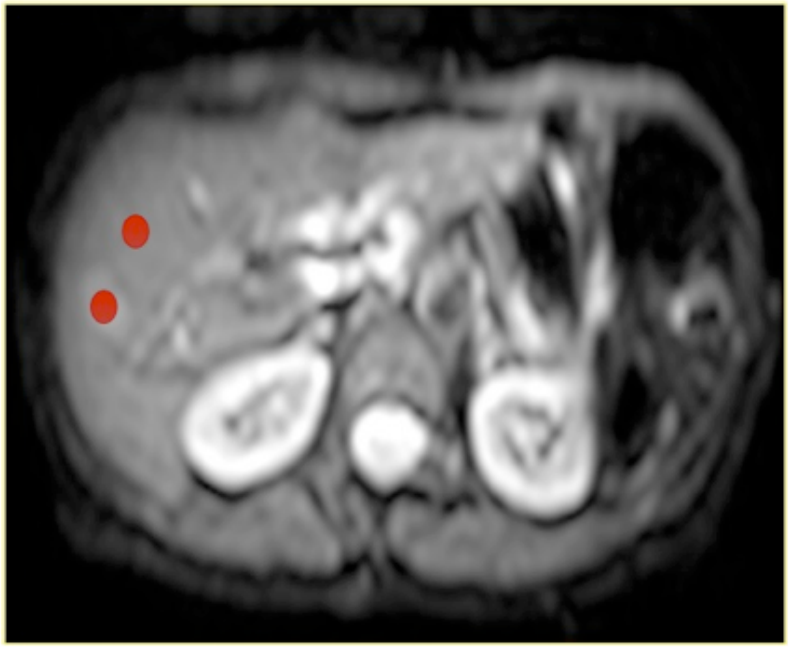

Fig. 1: Sampling method.
A circular ROI was positioned within the metastatic liver lesion and in the surrounding healthy liver parenchyma. The ROI was drawn on the b=50 image for optimal contrast between lesion and background, and then copied over all the other D-weighted images.

Given the high contrast between the liver and the lesion on the low b-value images, no problems occurred drawing the ROIs.

For every focal liver lesion a similar ROI (diameter >2 cm) was drawn in the adjacent healthy liver parenchyma, taking into account to exclude large blood and biliary vessels and artifacts. In patients with multiple focal metastatic liver lesions, at maximum 5 lesions per patient were sampled. All measurements were repeated three times even at the level of the adjacent liver parenchyma (within 3 cm from the lesion margins). Consequently, the absolute values (mm^2/s) of ADC, and the ratio ADC lesion/ADC adjacent liver parenchyma measured at the different times were compared. For every metastatic lesion ADC were estimated by fitting procedure both as absolute value and as ratio between ADC lesion and ADC healthy surrounding parenchyma. Furthermore an ADC with b = 0-750 s/mm^2 evaluation was performed, applying the same ROI-sampling method above described, to reduce the standard error. An evaluation on DwI b=0 s/mm^2 images of the focal metastatic liver lesion's diameter changes was than performed in comparison to ADC value, applying the following scheme:

Diameter	ADC value	R/NR
decrease	indifferent	R
stable	increase	R
stable	decrease or stable or increase < 25%	NR
increase	indifferent	NR

Legend: R responder, NR non-responder

To determine a threshold ADC for use in differentiating non-responder (NR) from responder (R) metastatic lesions, receiver-operating characteristic (ROC) analysis was performed using SPSS version 17 (SPSS Inc.). The authors considered NR all that lesions with a dimensional (sum of the three larger diameters) increase ≥ 5%. The investigators define as R those patients who show an increase of ADC value more than 25% vs. time 0. Changes in tumor size after treatment were calculated by using the formula % Vend = (VB -Vend)/VBx100, where VB was lesion size before treatment (maximum transverse diameter) and Vend was lesion size 20 days after the second administration.

As reference standard, based on the dimensional changes of liver metastases on ca-CT on time 3 vs. time 0 scan, each patient will be classified as R or NR according to RECIST and mRECIST criteria. Afterwards, based on the ADC-values measured during the different MR examinations, the inter/intra-individual ADC-values will be compared to the results of ca-CT to assess the relation between reduction of the liver metastases diameter, evaluated on DwI with b value = 0 s/mm^2 and:

- increase of the ADC-value on time 3 (after the end of CHT), with variation of + 25% vs. time 0
- increase of the ADC-value on time 1 (very early assessment);
- increase of the ADC-value on time 2 (early assessment); and to assess whether the lesions with the highest pretreatment ADC-value present also the highest dimensional reduction and the highest ADC-value at the end of CHT. In all center each evaluation was performed three times by two blinded observers (all trained how to place the ROI by an inter-center conference) to assess the reproducibility of all measurements. The observers who will assess the MR images will be

different from the observers assessing the CT images and will not be aware about the size changes after CHT.

Statistical analysis

For each group, the mean/minimal/maximal ADC±SD were quantified from the maps with multi-b and b 0-750 s/mm^2. Significant differences between means of the different groups were calculated using a parametric test based on analysis of variance (ANOVA), proceeded by the Levene test to verify the homogeneity of variances between groups. The level of significance was set at 5%. Statistical analysis was performed with the SPSS software package (release 17.0.0, SPSS Inc.,Chicago, IL, USA). Then a ROC analysis was performed. Sensitivity and specificity in distinguishing R from NR lesions were calculated on the whole range of ADC, at different time vs. time 0 and on the dimensional percentage changes vs. time 0, with the corresponding Areas Under the Curve (AUCs).

2.5 Results

Seventy-one liver metastatic lesions were evaluated; 60 were classified as R and 11 as NR. In fig. 2-4 are reported the ADC fitted % changes vs. time 0 at different time-point.

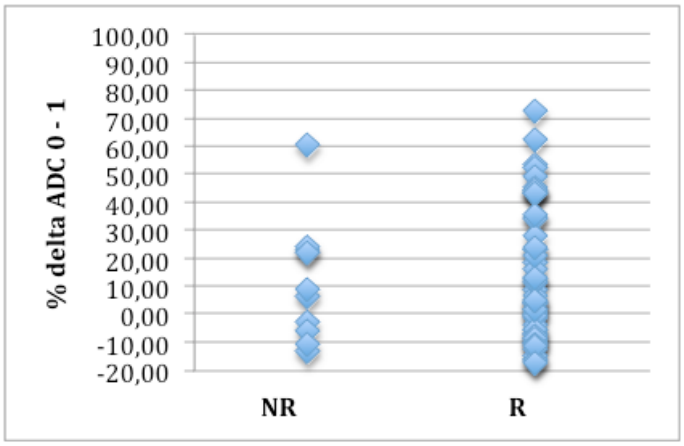

Fig.2: ADC % changes at time 1 for R and NR in comparison to time 0

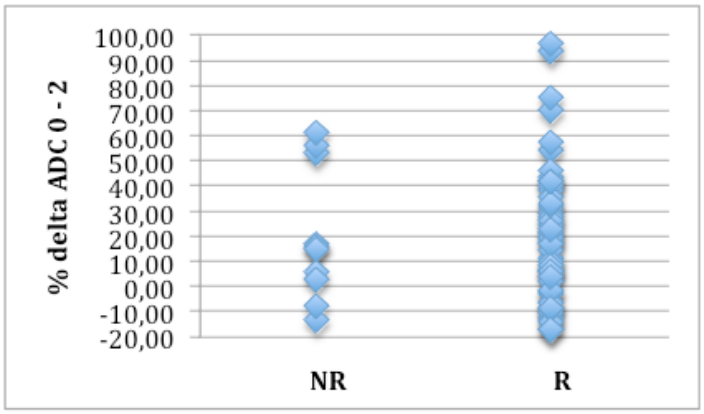

Fig.3: ADC % changes at time 2 for R and NR in comparison to time 0

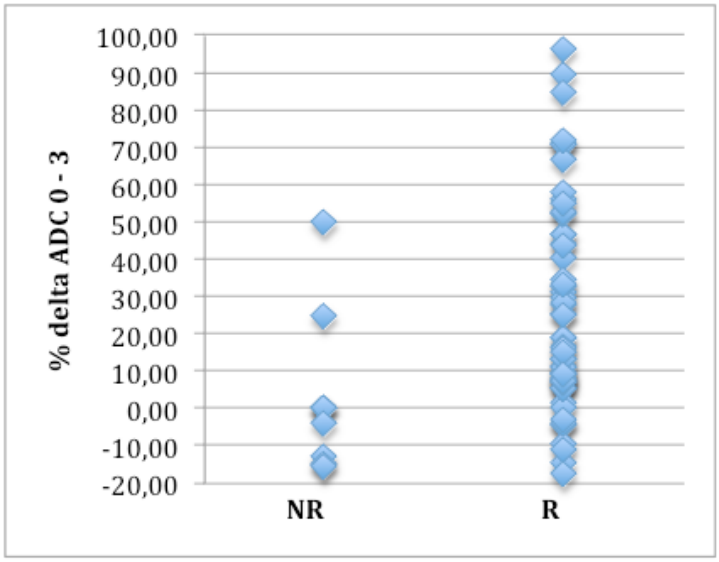

Fig.4: ADC % changes at time 3 for R and NR in comparison to time 0

Applying a +25% ADC increase cut-off, our results showed that, taking into account the only ADC fitted variation, of the 60 lesions classified as R, 25-34 and 35 were true R at time 1, 2 and 3 respectively. Of the 11 NR lesions, 4-3 and 3 were true RN at time 1, 2 and 3 respectively. Evaluating the diameter changes, 31 lesions showed dimensional stability either at time 1 and 2. 29 metastatic lesions showed reduction in diameter, 8 of whom at time 1 and 20 at time 2. 11 lesions had dimensional increase, 10 at time 1 and 2 at time 2. As reported in tables 2-4, sensibility, specificity, positive and negative predictive values (PPV, NPV) and accuracy, obtained at different times respectively for diameter/ADC 0-750/ADC fitted and the combination diameter + ADC fitted value were as follows,

- time 1: 36/91/82 48 for sensibility, 97/23/30/75 for specificity, 67/18/18/92 for PPV, 90/93/90/20 for NPV, 87/34/38/53 for accuracy;
- time 2: 73/91/73/73 for sensibility, 92/27/40/82 for specificity, 62/19/18/96 for PPV, 95/94/89/33 for NPV, 80/37/45/75 for accuracy.
- time 3: 100/91/91/82 for sensibility, 92/35/47/82 for specificity, 69/20/24/96 for PPV, 100/95/97/45 for NPV, 93/44/54/82 for accuracy.

Table 2: Sensibility, specificity positive predictive value (PPV), negative predictive value (NPV) and accuracy at time 1 of the different parameters.

	Diameter	Only ADC 0-750	ADC fitted	Diameter + ADC fitted
Sensibility	36	91	82	48
Specificity	97	23	30	75
PPV	67	18	18	92
NPV	90	93	90	20
Accuracy	87	34	38	53

Table 3: Sensibility, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy at time 2 of the different parameters.

	Diameter	Only ADC 0-750	ADC fitted	Diameter + ADC fitted
Sensibility	73	91	73	73
Specificity	92	27	40	82
PPV	62	19	18	96
NPV	95	94	89	33
Accuracy	89	37	45	75

Table 4: Sensibility, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy at time 3 of the different parameters.

	Diameter	Only ADC 0-750	ADC fitted	Diameter + ADC fitted
Sensibility	100	91	91	82
Specificity	92	35	47	82
PPV	69	20	24	96
NPV	100	95	97	45
Accuracy	93	44	54	82

The ADC fitted mean value for R/NR groups was 1453/1691, 1662/1884, 1765/1591 and 1821/1447 ($\cdot 10^{-6}$ mm^2/s) at time 0, 1, 2 and 3 respectively. Whereas the evaluation of ADC ratio lesion/parenchyma in R and NR groups showed the following data: 0.98-0.98, 1-0.97, 1.05-1, 1.07-0.98 at time 0, 1, 2 and 3 respectively (figures 5 and 6). Our scanner showed good stability, comparable with what has already been reported: repeatability and reproducibility related errors were always < 0.8% (28, 31).

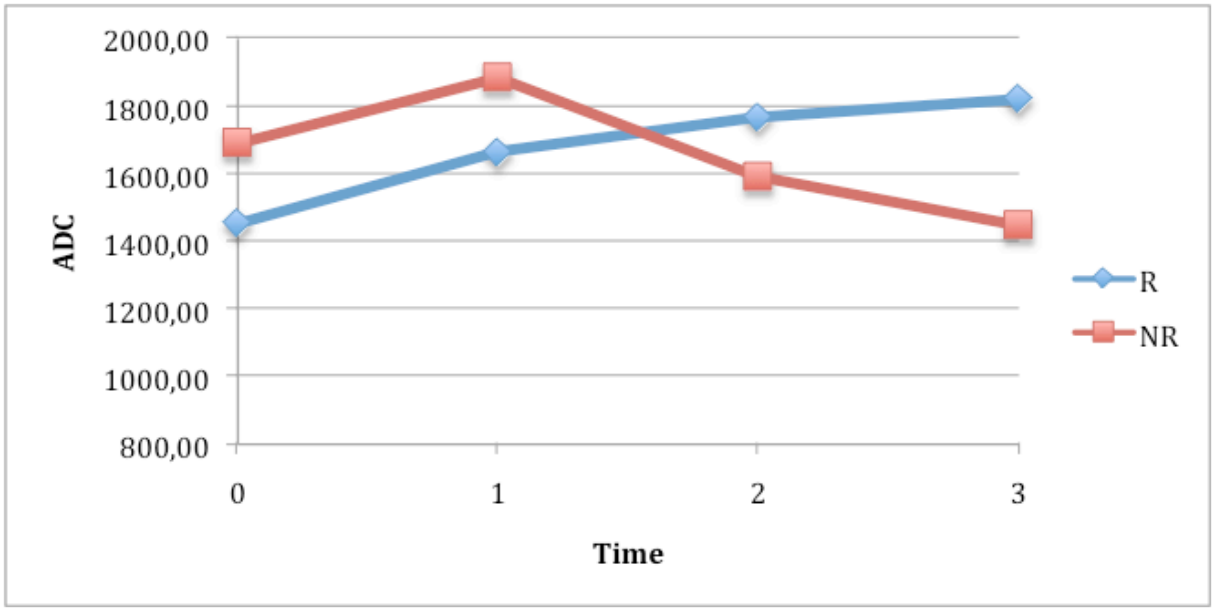

Fig. 5: ADC fitted (mean value) for groups (R-NR) at different time-points.

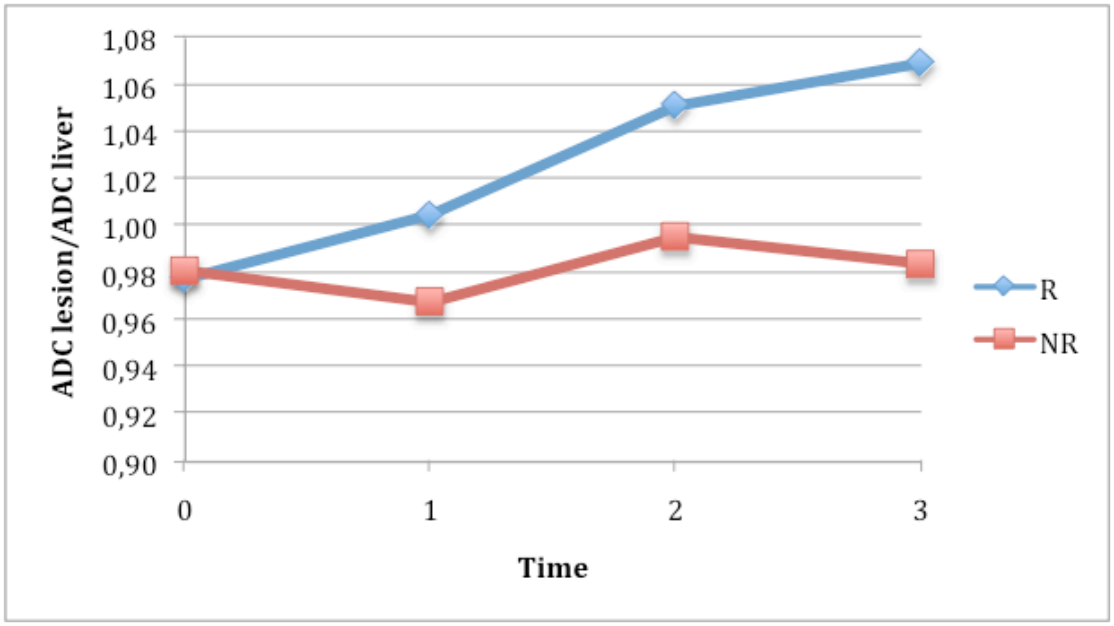

Fig.6: ADC lesion/ADC parenchyma evaluation in different groups (R-NR) at different time-points.

2.6 Discussion

Our results indicate that an early (20-25 days since the beginning) fine diameter assessment could be a good indicator of the final response to CHT in patients with metastatic liver lesion. In fact as reported in results section and in tables 2-4, the diameter show a NPV always ≥ 90 at different time. As known, non-invasive MR-DwI has been used to assess therapy response in animal models and humans (24, 32-35). The results of some animal and clinical studies have demonstrated that, after the initiation of CHT an increase in the ADC value may be observed in cases that respond to treatment (24, 36). The same authors found that the metastatic lesions with low ADC value also had the best response after CHT. In addition, these studies suggested that using MR-DwI performed at day 3 since the beginning of CHT, applying a ROI that includes all the lesion, it would be possible to distinguish the patient R from the NR, reporting that a weak but significant correlation was found between final tumor size reduction and early ADC changes for various systemic CHT against gastric or colorectal hepatic metastases, although in one article there was substantial overlap in the ADCs, calculated at b 0-500 and 150-500, which may limit use of this coefficient in monitoring therapeutic response (14, 18, 19). Other authors reported that the relative change in the minimum ADC values on DwI may be a promising tool for early detection of the response of metastatic liver tumors to CHT, and it may also be useful in judging whether CHT should be continued (14). Therefore, these studies suggested that the DwI appears to be a useful method in early assessment of response to CHT. We have noticed that metastatic lesions show a behavior response to CHT very dissimilar among themselves. As we know the

metastatic lesion shows behavior dissimilar to other lesions, i.e., to lymphomas. Several studies have established that interim FDG-PET scans after 1–3 cycles of CHT provide valuable information regarding early assessment of response and survival in lymphoma, showing a complete response to CHT (37, 38); while metastatic lesion can show an apparent response to CHT during the first cycle of therapy, but resulting NR at the end of the treatment. Furthermore in the same metastatic liver, different lesions can have inhomogeneous response to CHT; we found metastatic nodules classified as R coexisting NR nodules.

As described in introduction section, DwI reveals the microscopic structure of a tumor, such as cell density and necrosis, at the cellular level. The ADC value, which is the quantitative parameter of DwI, can be used to determine the presence of highly cellular or acellular regions within a tumor. For example, a region of high cellularity has a low ADC because the mobility of water protons is impeded. In contrast, cystic or necrotic regions have a high ADC because of the rapid diffusion of water protons. Therefore, ADC may yield indirect clinical information regarding effectiveness after treatment.

We approached the job with good hope, but our results are different from those reported in previous study. As evidenced in our results, the diameter shows better performance in terms of NPV ($\geq$ 90) and accuracy ($\sim$ 90) (tables 2-4) at different time; this parameter can evidence the R lesions. In particular, is not possible to identify an ADC value that delivers statistically significant results in distinguishing R from NR at time 1 or time 2, due to the large overlap of the values. Furthermore, the variability of tumor histotype and the resulting CHT and the various intracellular modifications that occur during the CHT day after day, induces to evaluate different ADC

changes depending on the time exam control. As reported in previous study (28), there is great variability of ADC values measured in the different part of the same liver parenchyma, or in the same patient in different days and/or nutritional conditions. For this reasons it is possible that this ADC variability is also reflected in tumor lesion. We have found that in 3 NR patients there was a different behavior of the various lesions to CHT. We can argue that this variability can be mitigated by normalization of ADC values of the lesion with those of normal parenchyma, although it is possible that this normalization induces an additional error on the single measurement of the ADC lesion. The combination diameter/ADC did not show better results in comparison to the only diameter evaluation (tables 2-4).

The analysis for groups (figures 5 and 6) with the mean ADC ratio values (ADC lesion/ADC parenchyma) at various times seams to give encouraging results, in particular is seen as the lesions R on average show a continuous increase of ADC ratio value on the contrary of the lesions NR.

Our results showed that the R group has a wide increase of ADC value, however it is evident also an overlap between R and NR groups, given that even the lesions NR had an increase of the ADC value. For these reasons, we searched a threshold value of ADC increasing that would allow us to discriminate between two groups. Applying + 25% cut-off ADC value, we obtained better results in terms of sensibility, specificity, PPV and NPV (tables 2-4), distinguishing between those lesions with an increase of ADC in various periods of at least + 25% compared to baseline and those who did not.

These results show that when the ADC value increases, is likely that the outcome of the lesion is good, however, it is possible that the lesion remains stable or regresses even if the ADC does not

increase. In addition, some lesion has progressed despite the increase of the ADC. It should be noted that these results and performances of the ADC analysis, in particular regarding the low NPV, is probably influenced in negative sense by the low number of NR lesions evaluated.

The accuracy of the ADC in the R/NR lesions classification results higher at time 2 (i.e., 20-25 days after initiation of therapy).

Attempting an analysis not only longitudinal but also transversal through the comparison between the mean ADC values of the two groups (R and NR), both in absolute sense and in the ratio ADC lesion/ADC parenchyma, we observed a different behavior of the two groups of lesions, significant at time 2 (figures 5 and 6).

Our results show that the use of DwI in evaluation of the early response to CHT in oncologic patients is still ambiguous and not satisfactory for clinical application. This is probably due to the low signal to noise ratio in DwI sequences, to the presence of artifacts (air/parenchyma interfaces, heart activity, gastrointestinal peristalsis and respiratory movements) which, as we know, invalidate the DwI images quality. We have noticed a diameter reduction at time 1 in R lesion; this may indicate that a dimensional comparison could be performed at 7-14 days from the beginning of CHT. ADC values could be useful for the evaluation of response to CHT in those lesions that show dimensional reduction in association with an ADC increase. However, there is the clinical need to distinguish the NR lesions more than the R, because the NR lesions will be those that need an earliest as possible therapeutic change.

Our study has the following limitations. First, the number of patients is quite limited and inhomogeneous; in particular a small number of R patients were evaluated. Than we choice patients

with liver metastatic lesion from different primary tumor: this is a limitation because of the various CHT protocol applied, with different mode of administration and fixed MR time-points, not adequate to the inhomogeneous group of patients. Although our scanner is not new, it showed good stability, as reported in results section (28, 31).

Exists a bias currently unsolvable: does not exist a unique response to CHT as showed in lymphomas, that is the patient with a good response to CHT will have a better prognosis. In our experience, some patient showed an early response to CHT, becoming NR at the end. Finally technical problems exist involving the ADC calculation in the liver parenchyma and, the well-known DwI limits and the poor reproducibility of the measurements.

In conclusion, MR can provide different tools to perform an early assessment of the response of a liver metastasis to CHT, including the DwI (with the measurements of the ADC values and the ratio ADC lesion/ADC parenchyma) and the dimensional assessment, the last one already in the first month of CHT. None of these instruments, however, can provide an accurate prediction, and major reasons are: low image quality, various artifacts, and measurement errors. However, this tools remain useful instruments for the radiologist, in particular as confirmation when one lesion shows after one month of CHT a diameter change with associated an + 25% ADC value increase, this patient could be probably classified as R. The results of our study show that the only ADC evaluation of focal metastatic liver lesion is not useful in the assessment of response to CHT. We found a little correlation between response to CHT and ADC changes only in comparison to the diameter changes of the lesions. Other studies should be performed to evaluate the role of ADC as useful instrument in identifying NR patient that are those that need a therapeutic change.

3. References

1. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981;47:207-14.
2. Therasse J, Arbuck SG, Eisenhauer EA et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl cancer Inst 2000;92:205-16.
3. Ollivier L, Negrier S, Thiesse P. International criteria for tumour assessment. Cancer I-maging 2000;87:927-34.
4. McDonald DM, Choyke PL. Imaging of angiogenesis: from microscope to clinic. Nat Med 2003;9:713-25
5. Marsden P, Sutcliffe-Goulden J. Principles and technology of PET scanning. Nucl Med Commun 2000;21:221-24.
6. Taouli B, Koh DM. Diffusion-weighted MR imaging of the liver Radiology 2010; 254:47-66.
7. Grobner T. Gadolinium-a specific trigger for the development of nephrogenic fibrosing dermopathy and nephrogenic systemic fibrosis? Nephrol Dial Transplant 2006;21:1104-1108.
8. Sadowski EA, Bennett LK, Chan MR, et al. Nephrogenic systemic fibrosis: risk factors and incidence estimation. Radiology 2007;243:148-157.
9. Marckmann J, Skav L, Rossen K et al. Nephrogenic systemic fibrosis: suspected causative role of gadodiamide used for contrast-enhanced magnetic resonance imaging. Am Soc Nephrol 2006;17:2359-2362.
10. Thomsen HS, Marckmann P, Logager VB. Update on nephrogenic systemic fibrosis. Magn Reson Imaging Clin N Am 2008;16:551-560.
11. Parikh T, Drew SJ, Lee VS, et al. Focal liver lesion detection and characterization with diffusion-weighted MR imaging: comparison with standard breath-hold T2-weighted ima-ging. Radiology 2008;246:812-822.
12. Padhani AR, Liu G, Koh DM, et al. Diffusion-weighted magnetic resonance imaging as a cancer biomarker: consensus and recommendations. Neoplasia 2009;11:102-125.

13. Bonekamp S, Corona-Villalobos CP, Kamel IR. Oncologic applications of diffusion-weighted MRI in the body. J Magn Reson Imaging 2012;35:257-279.
14. Marugami N, Tanaka T, Kitano S, Hirohashi S et al. Early detection of therapeutic response to hepatic arterial infusion chemotherapy of liver metastases from colorectal cancer using diffusion-weighted MR imaging. Cardiovasc Intervent Radiol 2009;32:638-646.
15. Charles-Edwards EM, De Souza NM. Diffusion-weighted magnetic resonance imaging and its application to cancer. Cancer Imaging 2006;6:135-143.
16. De Keyzer F, Vandecaveye V, Thoeny H, Chen F, Ni Y, Marchal G, Hermans R, Nuyts S, Landuyt W, Bosmans H. Dynamic contrast-enhanced and diffusion-weighted MRI for early detection of tumoral changes in single-dose and fractionated radiotherapy: evaluation in a rat rhabdomyosarcoma model. Eur Radiol 2009;19:2663-2671.
17. Chung WS, Kim MJ, Chung YE, Kim YE, Park MS, Choi JY, Kim KW. Comparison of gadoxetic acid-enhanced dynamic imaging and diffusion-weighted imaging for the preoperative evaluation of colorectal liver metastases. Magn Reson Imaging. 2011;34:345-53.
18. Cui Y, Zhang XP, Sun YS, Tang L, Shen L. Apparent diffusion coefficient: potential imaging biomarker for prediction and early detection of response to chemotherapy in hepatic metastases. Radiology 2008;248:894-900.
19. Koh DM, Padhani AR. Diffusion-weighted MRI: a new functional clinical technique for tumour imaging. Br J Radiol 2006;79:633- 635.
20. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45:228-247.
21. Forner A, Ayuso C, Varela M, et al. Evaluation of tumor response after locoregional therapies in hepatocellular carcinoma: are Response Evaluation Criteria in Solid Tumors reliable? Cancer. 2009;115:616-623.
22. Herneth AM, Guccione S, Bednarski M. Apparent diffusion coefficient: a quantitative parameter for in vivo tumor characterization. Eur J Radiol 2003;45:208-213.
23. Kauppinen RA. Monitoring cytotoxic tumour treatment response by diffusion magnetic resonance imaging and proton spectroscopy. NMR Biomed 2002;15:6-17.
24. Chenevert TL, Stegman LD, Taylor JM, et al. Diffusion magnetic resonance imaging: an early surrogate marker of therapeutic efficacy in brain tumors. J Natl Cancer Inst 2000;92:2029-2036.

25. Lyng H, Haraldseth O, Rofstad EK. Measurement of cell density and necrotic fraction in human melanoma xenografts by diffusion weighted magnetic resonance imaging. Magn Reson Med 2000;43:828-836.
26. Koh DM, Scurr E, Collins D, Kanber B, Norman A, Leach MO, Husband JE. Predicting response of colorectal hepatic metastasis: value of pretreatment apparent diffusion coefficients. Am J Roentgenol. 2007;188:1001-8.
27. Mardor Y, Roth Y, Lidar Z et al. Monitoring response to convection-enhanced taxol delivery in brain tumor patients using diffusion-weighted magnetic resonance imaging. Cancer Res 2001;61:4971-4973.
28. Colagrande S, Pasquinelli F, Mazzoni LN, Belli G, Virgili G. MR-diffusion weighted imaging of healthy liver parenchyma: repeatability and reproducibility of apparent diffusion coefficient measurement. J Magn Reson Imaging 2010;31:912-920.
29. Padhani AR, Liu G, Koh DM et al. Diffusion-weighted magnetic resonance imaging as a cancer biomarker: consensus and recommendations. Neoplasia 2009;11:102-125.
30. Abramoff MD, Magalhaes PJ, Ram SJ. Image processing with ImageJ. Biophotonics International 1 2011;1:36-42.
31. Pasquinelli F, Belli G, Mazzoni LN, Grazioli L, Colagrande S. Magnetic resonance diffusionweighted imaging: quantitative evaluation of age-related changes in healthy liver parenchyma. Magn Reson Imaging 2011;29:805-812.
32. Jennings D, Hatton BN, Guo J, et al. Early response of prostate carcinoma xenografts to docetaxel chemotherapy monitored with diffusion MRI. Neoplasia 2002;4:255-262.
33. Galons JP, Altbach MI, Paine-Murrieta GD, Taylor CW, Gillies RJ. Early increases in breast tumor xenograft water mobility in response to paclitaxel therapy detected by noninvasive diffusion magnetic resonance imaging. Neoplasia 1999;1:113-117.
34. Dzik-Jurasz A, Domenig C, George M, et al. Diffusion MRI for prediction of response of rectal cancer to chemoradiation. Lancet 2002;360:307-308.
35. Pickles MD, Gibbs P, Lowry M, Turnbull LW. Diffusion changes precede size reduction in neoadjuvant treatment of breast cancer. Magn Reson Imaging 2006;24:843-847.
36. Thoeny HC, De Keyzer F, Chen F et al. Diffusion weighted MR imaging in monitoring the effect of a vascular targeting agent on rhabdomyosarcoma in rats. Radiology 2005;234:756-764.

37. Lin C, Luciani A, Itti E, Haioun C, Rahmouni A. Whole body MRI and PET/CT in haematological malignancies. Cancer imaging 2007;7:88-93.
38. Haioun C, Itti E, Rahmouni A et al. [18F] Fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) in aggressive lymphoma: an early prognostic tool for predicting patient outcome. Blood 2005 106:1376-81.