Original article

Determination of the time for maximal response to neoadjuvant hormone therapy for prostate cancer using magnetic resonance with spectroscopy (MRSI) and dynamic contrast enhancement (DCEMR)

Alessandro Sciarra, M.D.\textsuperscript{a,}\textsuperscript{*}, Valeria Panebianco, M.D.\textsuperscript{b}, Stefano Salciccia, M.D.\textsuperscript{a}, Danilo Lisi, M.D.\textsuperscript{b}, Andrea Alfarone, M.D.\textsuperscript{a}, Alessandro Gentilucci, M.D.\textsuperscript{a}, Ulderico Parente, M.D.\textsuperscript{a}, Susanna Cattarino, M.D.\textsuperscript{a}, Roberto Passariello, M.D.\textsuperscript{b}, Vincenzo Gentile, M.D.\textsuperscript{a}

\textsuperscript{a} Department of Urology, University Sapienza of Rome, Rome, Italy
\textsuperscript{b} Department Radiology, University Sapienza of Rome, Rome, Italy

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Abstract

**Purpose:** To determine the time-dependent metabolic and angiogenic changes that occur in prostate cancer (CaP) during neoadjuvant hormone therapy (HT), using a combination of MRSI and DCEMR analysis.

**Materials and methods:** This is a prospective study on a population of non-metastatic CaP submitted to neoadjuvant HT prior to radiation therapy. All cases homogeneously received a 6-month period of neoadjuvant HT using leuprorelin acetate 7.5 mg every 28 days. In all cases, a MRSI/DCEMR study was performed at baseline (pretreatment) and at regular intervals (4, 12, 24 weeks) during HT. Serum PSA was measured at baseline and at the same intervals (4, 12, 24 weeks). All MRI examinations were performed on a commercially available 3 T scanner.

**Results:** There was a significant \((P < 0.01)\) time-dependent loss of all prostate metabolites during HT. In regions of CaP no significant variation in the absolute value of metabolites was reported at 1-month interval and a higher variation was observed at 24-week compared with 12-week interval. A complete metabolic atrophy was a common feature (30\%) at a 24-week interval of HT, but not at short (4-week 0\%), and lower at an intermediate interval (12-week 10\%). At DCEMR, onset time and time to peak parameters significantly \((P < 0.05)\) increased at 12- and 24-week intervals.

**Conclusions:** To individualize neoadjuvant HT courses prior to definitive treatment, the combination of MRSI and DCEMR may represent a valid noninvasive method, and the addition to PSA data could be used to better assess the time-dependent efficacy of HT in our patients. © 2011 Elsevier Inc. All rights reserved.

**Keywords:** Prostate cancer; Hormone therapy; Magnetic resonance; Spectroscopy

1. Introduction

In recent years, hormone-deprivation therapy (HT) has been increasingly used as neoadjuvant therapy for patients with non-metastatic prostate carcinomas (CaP) \cite{1–4}. However, the optimal use of HT in the treatment of non-metastatic CaP remains controversial since neither the best duration of therapy nor the best type of agent is defined. In particular, the neoadjuvant application of HT to radiation therapy (RT) has been associated with better results in terms of recurrence and progression-free survival \cite{1}. To individualize neoadjuvant HT prior to definitive therapy, a noninvasive method of assessing the effectiveness of therapy and measuring the presence and local extent of residual CaP is required. Serum prostate specific antigen (PSA) as a hormone-dependent biochemical marker of prostate metabolism can be used to monitor the response during neoadjuvant HT \cite{5,6}.

Recently, many studies \cite{7–10} underlined the high diagnostic accuracy of combined proton 1H-magnetic resonance spectroscopic imaging (1H-MRSI) and dynamic contrast
enhanced imaging (DCEMR) in the management of CaP. The advantage of MRSI is that it provides metabolic information from substances (citrate, choline, creatine), which are characteristics for prostate tissue [8]. DCMRE is based on the assessment of tumor neoangiogenesis, which is an integral feature of tumors [7]. The aim of this study was to determine the time-dependent metabolic and angiogenic changes that occur in CaP during neoadjuvant HT, using a combination of MRSI and DCEMR analysis.

2. Materials and methods

This is a prospective study on a population of non-metastatic CaP submitted to neoadjuvant HT prior to three-dimensional (3D) conformal radiation therapy. A research protocol that involves the use of MR imaging with MRSI and DCEMR for the management of CaP was implemented at our center. From January 2007 to May 2010, 430 cases were included and submitted to MRSI and DCEMR analysis. Local ethic committee approval was obtained for studies involving combined MRSI and DCEMR examinations of the prostate.

In the present study, from June 2009 we analyzed the use of MRSI/DCEMR in 20 consecutive patients with non-metastatic CaP submitted to neoadjuvant LHRH-analogue therapy prior to 3D-RT. An informed consensus was obtained from all patients in our Urologic and Radiologic Center for inclusion. Inclusion criteria for the study were: histologically confirmed adenocarcinoma of the prostate at prostatic biopsy; clinical stage T2/T3 - N0- M0. Exclusion criteria for the study were previous prostatic surgery, radiation, hormones or chemotherapies, contraindication for MRI studies. Clinical characteristics of the population are described in Table 1. The study design is shown in Fig. 1. All cases homogeneously received a 6-month period of neoadjuvant HT using LH-RH analogue monotherapy with subcutaneous depot injection of leuprolide acetate 7.5 mg (Eligard; Astellas, Milan, Italy) every 28 days. In all cases, a MRSI/DCEMR study was performed at baseline (pretreatment), (at least 6 weeks after biopsy and within 2 weeks prior to HT), and at regular intervals (4, 12, 24 weeks) during HT. Serum PSA was measured at baseline and therefore at the same intervals (4, 12, 24 weeks) within 24 hours before MRSI/DCEMR procedures.

2.1. MRI and MRSI/DCEMR

All examinations were performed on a commercially available 3 T scanner (Magnetom Avanto; Siemens Medical Solutions, Erlangen, Germany), equipped with surface phased array (Body Matrix; Siemens Medical Solutions) and endorectal coil, filled with 70–90 ml of air on the basis of patient tolerance (e-Coil; Medrad, Pittsburgh, PA, combined with Endo-Interface; Siemens Medical Solutions). Morphologic imaging of the prostatic gland was performed by acquiring turbo spin echo (TSE) T2-weighted sequences in the axial, sagittal, and coronal planes, with the use of optimized parameters for a better spatial resolution. The technique used for MR imaging, H-MRSI, and DCEMR of the prostate has been previously described [9].

At H-MRSI, a point-resolved spectroscopic sequence was obtained with the use of 3D chemical shift imaging (CSI) sequence with spectral/spatial pulses optimized for quantitative detection of choline and citrate. DCEMR images were acquired by using a Gradient-Echo (GRE) T1 weighted sequence during intravenous (i.v.) contrast agent (c.a.) administration immediately following completion of an intravenous bolus injection of 1.0 mmol/ml of gadobutrol (gadovist; Bayer Shering Pharma AG, Berlin, Germany). Contrast was administered with a power injection (Spectris; Medrad) at 3.0 ml/s and was followed by a 15 ml saline flush. The 3D volume was acquired with the same positioning angle and center as the transverse T2-weighted sequence, covering the entire prostate gland.

2.2. Data analysis

All MR studies were interpreted by 2 independent radiologists (V.P. and R.P.) with extensive experience in this field. Both readers knew that the patient had a biopsy-proven CaP, but they were unaware of the patient treatment status and of all other clinical findings.
As in previous studies [11,12], for comparison of MR with pathologic data, the peripheral zone of the prostate was divided into sextants according to the following criteria: the base was defined as the upper third, which extended from the vesical margin of the prostate to the axial level with the largest transverse diameter; the mid-region was defined as the central third from the axial level to the level of the ejaculatory duct orifices at the veru montanum; the apex was defined as the remaining inferior portion of the prostate. The left and right sides of the prostate were separated by the median sagittal plane through the veru montanum. The location of MRSI voxels and DCEMR areas used for the analysis was correlated with the sextants defined by MRI. For each available voxel, absolute values (ppm) of choline (Cho), creatine (Cr), and citrate (Cit) were calculated. Ratio value from Cho plus Cr to Cit was obtained in all patient groups.

Voxels were classified as suspicious if the (Cho + Cr)/Cit ratio was >0.8 and then localized in the peripheral zone according to the described site scheme. Voxels with elevated ratio overlapping with high intensity T1-weighted areas were not considered suspicious but were referred to as artifacts from post-biotic hemorrhage [13].

The dynamic MR post-processing procedure was performed in 10 minutes per patient and the same radiologists reviewed the subtracted DCEMR images on the basis of maximum and minimum enhancing regions. Functional dynamic imaging parameters were estimated via the SI-T curves modeled with the 3 main enhancement records (ERs): onset time of signal enhancement (OT), time to peak (TTP), and peak enhancement (PE) [14].

MRSI and DCEMR data were superimposed on the corresponding T2-weighted images for correlation with anatomical imaging and localization. Only the peripheral zone of the gland was evaluated for possible cancer. As in previous studies [5,11] for the analysis of MRSI and DCEMR parameters, cancer and healthy tissue in the peripheral zone of the prostate were anatomically discriminated based on a combination of biopsy and T2-weighted MRI findings. A distinct low T2-weighted signal intensity lesion was identified as cancer if there was a corresponding positive biopsy finding in the same sextant. Spectral voxels were considered to be in a region of cancer if at least 75% of the voxels overlapped the low T2-weighted signal intensity lesion and the sextant area resulted positive at biopsy. Spectral voxels not including a low T2-weighted signal intensity lesion and in a sextant with a negative biopsy finding were considered healthy. In particular, in the present experiment: 6.1% of areas positive at prostate biopsy resulted negative at MRSI/DCEMR; 92.0% of areas suspicious at MRSI/DCEMR corresponded to an area of CaP at biopsy.

2.3. Aim of our MR analysis

Our analysis was focused on the variations in MRSI/DCEMR parameters from baseline to increasing HT duration, either in healthy or in CaP sites. As in previous studies [5,15], spectral voxels in which the choline and citrate peak area-to-noise ratios were below 5 were considered to be absent of metabolites and were termed “metabolic atrophy.”

2.4. Statistical analysis

Statistical data analysis was performed with the statistical Med Calc Software Demo for Windows, ver. 9.3 (Mariakerke, Belgium). Comparison within and among groups were performed using the $\chi^2$ test with the Yates correction for continuity and Bonferroni correction for multiple tests involving the same data (significance level $P < 0.05$). A 2-tailed Student’s $t$-test applying the Bonferroni correction for multiple tests involving the same data was also used.

3. Results

All cases in the study concluded the 6-month period with neoadjuvant HT, and all MRI studies were performed. MRSI/DCEMR variations during HT failed to demonstrate statistically significant associations with patient age, pretreatment PSA serum levels, and biopsy Gleason score ($P > 0.05$).

3.1. MRSI

There was a significant ($P < 0.01$) time-dependent loss of all prostate metabolites during HT, resulting in the complete loss (metabolic atrophy) following longer periods of therapy.

Comparing healthy to CaP tissue, at baseline, healthy tissue demonstrated high levels of citrate, intermediate levels of choline and creatine, and normal ratio. Conversely, regions of CaP demonstrated a high reduction in citrate and increase in choline and ratio. The amount and time-dependent variation in metabolites during HT significantly differed from healthy to CaP tissues in the short (4 weeks) and intermediate (12 weeks) interval but not in the long term interval (24 weeks) (Figs. 2 and 3).

In particular, the time cause of loss of prostate metabolites was delayed in CaP tissue compared with healthy tissue (Figs. 2 and 3). In regions of CaP, a no significant variation in the absolute value of metabolites was reported at 1-month interval, and a higher variation was observed at 24-week compared with 12-week interval. Citrate levels decreased faster than prostate choline and creatine levels, resulting in an increase in the ratio.

Individual patients showed a variable metabolic response to HT. Table 2 shows that a complete metabolic atrophy was a common feature (30%) at 24-week interval of HT, but not at short (4-week interval 0%) and lower at an intermediate interval (12-week interval 10%). On the contrary, the percentage of patients with undetectable citrate but detectable choline-creatine increased faster (4-week interval 5%; 12-week 35%; 24-week 65%) (Table 2).
At all time points during the study, the reduction in citrate levels and the presence of metabolic atrophy significantly correlate ($r = 0.4530, P < 0.01$) with serum PSA level variations. In particular, patients with detectable citrate had significantly ($P < 0.05$) higher (at 24-week interval, mean: 3.5 ± 0.5 ng/ml) PSA levels than those without detectable citrate (at 24-week interval, mean 0.85 ± 0.9 ng/ml). All patients with metabolic atrophy showed PSA levels below 0.4 ng/ml (at 24-week interval, mean: 0.15 ± 0.1 ng/ml). However, not all cases with PSA levels <0.4 ng/ml demonstrated complete metabolic atrophy (6/12 cases = 50%).

### 3.2. DCEMR

Only OT and TTP but not PE showed a significant ($P < 0.05$) time-dependent variation during HT. Comparing healthy to CaP tissue, at baseline healthy tissue showed higher values of OT and TTP and lower values of PE compared with CaP regions ($P < 0.05$). In regions of CaP, at 4-week interval only TTP values significantly ($P = 0.020$) increased, whereas at 12- and 24-week intervals, both OT and TTP values significantly ($P < 0.01$) increased compared with baseline.

### 4. Discussion

The present study suggests that the combination of MRSI and DCEMR represents a valid noninvasive method of assessing the effect of a neoadjuvant course of HT on prostate tissue (Fig. 4). We showed that during 6 months of neoadjuvant HT, there is a significant time-dependent loss of all prostate metabolites (MRSI) and a significant modification in some dynamic angiogenetic parameters (DCEMR) that start at 1-month interval but become significant only at 3-month and progressively continue till a 6-month interval. More information is obtained from MRSI than from DCEMR analysis, and at 6-month interval 30% of cases reached a complete metabolic atrophy. MRSI/DCEMR modifications significantly correlated with serum PSA variations during therapy. In particular, all cases with metabolic atrophy showed PSA levels below 0.4 ng/ml but not all (50%) patients with PSA level <0.4 ng/ml demonstrated a complete metabolic atrophy.

Compared with previous studies [5,15,16], the advantages of our study are: (1) a prospective and not retrospective analysis [15,16]; (2) the evaluation of MRI modifications at different HT intervals in the same patient and not in different patients for each HT interval [5,15]; (3) for the first time in the literature, we used the combination of MRSI to DCEMR analysis to verify not only metabolic but also dynamic angiogenic modifications in prostate tissue during HT.

There are limitations to this study. We performed MRI analysis at standard time intervals of HT (1-, 3-, 6-month intervals) because it was not ethically approved to perform a higher number of MRI procedures in the same patient during a 6-month interval. Our population was limited to 20 cases and do not consent to stratify results on the basis of clinical parameters such as Gleason score of the tumor. However, it has been obtained from a larger population of cases examined in MRI studies. Following the European Guidelines (EAU) [17], we used neoadjuvant HT only in...
patients selected for RT and not for radical prostatectomy as definitive treatment. In this way, we had no possibility to compare MRI results with histology obtained after surgery.

As in previous studies [5], our patients showed an individual variable metabolic response to HT. This aspect sustains the possibility to individualize neoadjuvant HT intervals prior to definitive therapy, through the data obtained at MRSI/DCEMR study, as a noninvasive method of assessing the time-dependent modifications induced by HT in prostate tissue. An important question is whether a complete metabolic atrophy (MRSI) is always necessary during neoadjuvant HT to start definitive RT treatment. Cases with a complete metabolic atrophy showed also a complete PSA response and, therefore, this is the point of best response to HT and then more suitable to switch in the definitive treatment. However, it is possible that also lower metabolic responses may represent a reasonable point to switch in RT treatment. Only a complete clinical evaluation of cases after the entire neoadjuvant HT+RT definitive treatment could answer this question.

The usefulness of MRSI in describing HT effects on CaP is anticipated by the fact that prostatic citrate metabolism is under hormonal control [18] and that there is a time-dependent increase in prostatic tissue atrophy during HT on histology studies [19]. Ackerstaff et al. [20,21], in androgen-dependent prostate cancer cell lines, showed that HT produces a significant reduction in citrate levels followed by a significant increase in choline levels in the first 13 months and than by a significant reduction in choline levels in >13 months of treatment. According to these data, during HT our patients showed a faster reduction in citrate than in choline.

Also Mueller-Lisse [5,15], in 65 CaP cases who underwent either short, intermediate, or long term HT, showed a significant time-dependent loss of prostatic metabolites (earlier in citrate, later in choline), resulting in the complete metabolic atrophy in 25% of cases in >16 weeks of therapy.

For the first time in the literature, in the present study we combined MRSI with a DCEMR analysis to obtain results in this type of patient. DCEMR is based on the assessment of tumor angiogenesis. A number of features of tumor vascularity are characteristics of malignancy, such as chaotic structure, arterovenous shunting, and high permeability [7]. Furthermore, because the amount of interstitial spaces is greater in a cancerous tissue than in a normal tissue, there is a larger difference in contrast material diffusion and enhancement [7]. The histologic atrophy induced by HT in CaP tissue reduces tumor vascularity and vascular permeability. This basis is the rationale to complete MRSI with DCEMR analysis in patients submitted to HT. In our study, we showed at DCEM a significant increase in OT and TTP values according to longer intervals (3- and 6-month interval) of HT, as possible indicators of a reduced angiogenic activity in the tumor.

5. Conclusions

Serum PSA is currently the main method of choice among clinicians for the assessment of the efficacy of HT in
CaP. To individualize neoadjuvant HT courses prior to definitive treatment, the combination of MRSI and DCEMR may represent a valid noninvasive method, and the addition to PSA data could be used to better assess the time-dependent efficacy of HT in our patients.

Future prospectives from this study are represented by: (1) a comparative evaluation of different types of HT on MRSI/DCEMR variables; (2) a comparative evaluation of RT alone versus HT+RT on MRSI/DCEMR variables.

References