ORIGINAL REPORT

Evaluation of the reproducibility of a protocol for the pharmacokinetic study of breast tumors by dynamic magnetic resonance imaging

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KEYWORDS
Breast cancer; Magnetic resonance imaging; Pharmacokinetics

Abstract

Objective: To evaluate the reproducibility of a protocol for dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) for the pharmacokinetic study of breast tumors.

Materials and methods: We carried out this prospective study from October 2009 through December 2009. We studied 12 patients with stages II–III invasive breast cancer without prior treatment. Our center’s research ethics committee approved the study. The 12 patients underwent on two consecutive days DCE-MRI with a high temporal resolution protocol (21 acquisitions/minute). The data obtained in an ROI traced around the largest diameter of the tumor (ROI 1) and in another ROI traced around the area of the lesion’s highest $K^{\text{trans}}$ intensity (ROI 2) were analyzed separately. We used parametric and nonparametric statistical tests to study the reproducibility and concordance of the principal pharmacokinetic variables ($K^{\text{trans}}$, $K_{ep}$, $V_\text{e}$ and $\text{AUC}_{\text{ep}}$).

Results: The correlations were very high ($r > .80; P < .01$) for all the variables for ROI 1 and high ($r = .70–.80; P < .01$) for all the variables for ROI 2, with the exception of $V_\text{e}$ both in ROI 1 ($r = .44; P = .07$) and in ROI 2 ($r = .13; P = .235$). There were no statistically significant differences between the two studies in the values obtained for $K^{\text{trans}}$, $K_{ep}$ and $\text{AUC}_{\text{ep}}$ ($P > .05$ for each), but there was a statistically significant difference between the two studies in the values obtained for $V_\text{e}$ in ROI 2 ($P = .008$).

Conclusions: The high temporal resolution protocol for DCE-MRI used at our center is highly reproducible for the principal pharmacokinetic constants of breast.

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Evaluación de la reproducibilidad de un protocolo de resonancia magnética dinámica para el estudio farmacocinético de los tumores de mama

Resumen
Objetivo: Evaluar la reproducibilidad de un protocolo de resonancia magnética dinámica (RM-DC) con contraste para el estudio farmacocinético de los tumores de mama.

Material y métodos: Estudio prospectivo realizado entre octubre y diciembre de 2009, que incluyó 12 pacientes con cáncer de mama infiltrante en estadíos i-iii sin tratamiento previo. Este trabajo fue aprobado por el Comité de Ética de Investigación de nuestro centro. A las 12 pacientes se les realizó 2 RM-DC en 2 días consecutivos con un protocolo de alta resolución temporal (21 adquisiciones/minute). Se analizaron por separado los datos obtenidos en un ROI trazado alrededor del diámetro tumoral mayor (ROI 1) y otro que abarcaba la zona de mayor intensidad de $K^{\text{trans}}$ de la lesión (ROI 2). Se emplearon pruebas estadísticas paramétricas y no paramétricas para estudiar la reproducibilidad y concordancia de las principales variables farmacocinéticas ($K^{\text{trans}}$, $K_{\text{ep}}$, $V_e$ y AUC$_{0\rightarrow\infty}$).

Resultados: Las correlaciones fueron muy altas ($r > 0.80$; $p < 0.01$) en todas las variables del ROI 1, y altas ($r = 0.70-0.80$; $p < 0.01$) en todas las del ROI 2, excepto en $V_e$ tanto en el ROI 1 ($r = 0.44$; $p = 0.07$) como en el ROI 2 ($r = 0.13$; $p = 0.235$). No hubo diferencias estadísticamente significativas entre los 2 estudios para los valores obtenidos de $K^{\text{trans}}$, $K_{\text{ep}}$ y AUC$_{0\rightarrow\infty}$ ($p > 0.05$ para todas ellas), pero sí que las hubo para $V_e$ en el ROI 2 ($p = 0.008$).

Conclusión: El protocolo de alta resolución temporal de RM-DC de nuestro centro es muy reproducible para las principales constantes farmacocinéticas de los tumores de mama.

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Introduction

The dynamic magnetic resonance imaging (DMR) with IV contrast is one image modality more and more widely used in daily practice. Its indication in high risk patients, the preoperative staging of breast cancer, the hidden breast cancer, the detection of relapses and for the evaluation of the neoadjuvant chemotherapy\(^1\)\(^-\)\(^3\) are very well known. Because it is based on neoangiogenesis DMR allows the functional assessment of tumors different than conventional modalities—mammography and ultrasound that are only morphological.

The actual protocols have a high spatial (with cutting thickness close to 1 mm) and temporal resolution (acquisition in approximately 60 s) that allow us to do semiquantitative dynamic studies based on the curves of signal/time intensity. However if temporal resolution is increased the vascular patency can be determined by quantifying pharmacokinetic parameters that describe the passing of contrast from vascular space to the tumor interstitial compartment.\(^4\) To that end it is also necessary to have a specific software capable of estimating these pharmacokinetic parameters.

There are many protocols of pharmacokinetic study with DMR reported in the reference (Table 1).\(^5\)\(^-\)\(^12\) Some of them have even been modified along the study.\(^10\) This technical variability can also make the value of pharmacokinetic parameters change—something necessary to study the reproducibility of different protocols.

The goal of this study is to evaluate the reproducibility and concordance of data obtained through a DMR protocol optimized for the pharmacokinetic study of stages ii-iii breast tumors after the administration of neoadjuvant chemotherapy.

Materials and methods

Patients

Between October and December 2009 we performed one prospective study in our center including 12 treatment naive patients with confirmed histological diagnosis of infiltrating stages ii-iii breast cancer. These 12 patients were part of a phase 2 multicenter clinical trial including 12 hospitals (clinical trial code ML22197/2009-01) sponsored by F. Hoffman–La Roche. This trial enrolled 76 patients and assessed the predictive markers to treatment response with the antiangiogenic drug bevacizumab (Avastin\(^\circledR\), F. Hoffman–La Roche, Basel, Switzerland) and neoadjuvant chemotherapy (docetaxel plus adriamicine) in patients with locally advanced breast cancer. In Table 2 the criteria for the inclusion of patients in the study can be seen. The study protocol was approved by the ethical committee of our hospital and all patients gave their informed written consent.

Technique

To validate the DMR protocol two daily examinations were performed in two consecutive days with identical technical parameters and the patients did not follow any therapies between both examinations. The MRI protocol can be seen in Table 3.

The DMR was performed in one 1.5 T resonance (Magnetom Symphony\(^\circledR\), Siemens Healthcare, Erlangen, Germany). T1-weighted standard f3D echo-spin gradient-enhanced images were modified in an attempt to improve its spatial resolution (150 measurements instead of 6 in a total of 7 min per test with approximately 21 acquisitions per
Table 1  DMR protocols in the reference.

<table>
<thead>
<tr>
<th>Study</th>
<th>Protocol</th>
<th>View</th>
<th>Voxel size</th>
<th>Number of acquisitions</th>
<th>Total time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ah See et al. (2008)⁵</td>
<td>1</td>
<td>Sagittal</td>
<td>0.8 mm</td>
<td>40</td>
<td>8min 5s</td>
</tr>
<tr>
<td>Loo et al. (2011)⁶</td>
<td>1</td>
<td>Coronal</td>
<td>1.2 mm × 1.2 mm × 1.6 mm</td>
<td>5</td>
<td>7min 30s</td>
</tr>
<tr>
<td>2</td>
<td>Coronal</td>
<td>1.1 mm × 1.1 mm × 1.2 mm</td>
<td>5</td>
<td>7min 30s</td>
<td></td>
</tr>
<tr>
<td>Tateishi et al. (2012)⁷</td>
<td>1</td>
<td>Sagittal</td>
<td>1.3 mm × 1.3 mm × 0.8 mm</td>
<td>24</td>
<td>4min</td>
</tr>
<tr>
<td>Rahbar et al. (2012)⁸</td>
<td>1</td>
<td>Axial</td>
<td>0.9 mm × 1.1 mm × 2.2 mm</td>
<td>5</td>
<td>7min 30s</td>
</tr>
<tr>
<td>2</td>
<td>Axial</td>
<td>0.8 mm × 0.9 mm × 1.6 mm</td>
<td>3</td>
<td>7min 30s</td>
<td></td>
</tr>
<tr>
<td>Johansen et al. (2009)⁹</td>
<td>1</td>
<td>Sagittal</td>
<td>1.9 mm × 1 mm × 3–4 mm b</td>
<td>6–7</td>
<td>5min 42s–6min 39s</td>
</tr>
<tr>
<td>Partridge et al. (2010)¹⁰</td>
<td>1</td>
<td>Sagittal</td>
<td>1 mm × 1 mm × 2.2 mm</td>
<td>3</td>
<td>4min 30s</td>
</tr>
<tr>
<td>2</td>
<td>Sagittal</td>
<td>0.8 mm × 0.8 mm × 1.6 mm</td>
<td>3</td>
<td>6min</td>
<td></td>
</tr>
<tr>
<td>El Khouli et al. (2011)¹¹</td>
<td>1</td>
<td>Axial</td>
<td>1.3 mm × 1.3 mm × 5 mm</td>
<td>14</td>
<td>2min 30s</td>
</tr>
<tr>
<td>Baar et al. (2009)¹²</td>
<td>1</td>
<td>Axial</td>
<td>2.3 mm × 2.7 mm × 8 mm</td>
<td>180</td>
<td>24min</td>
</tr>
</tbody>
</table>

DMR: dynamic magnetic resonance imaging.

a This value corresponds to the cutting thickness; the article says nothing about the matrix or the FOV used.

b The protocol was applied to twelve (12) study patients with a cutting thickness of 3 mm and to some other 12 with a cutting thickness of 4 mm.

Table 2  Inclusion criteria.*

- Women between 18 and 70 years old
- Breast cancer histologically tested through skin biopsy
- Stage I or II, naïve to prior therapies
- Eastern Cooperative Oncology Group (ECOG) categories 0 to 1
- Left ventricular ejection fraction (LVEF) ≥ 50% with no signs of cardiac failure


in all patients—the tumor was found through an ultrasound and its location marked on their skin to be able to identify it in the location sequence of the MRI. After the acquisition of three images without IV contrast one bolus of 8 ml gadobutrol (Gadovist®, Bayer-Schering Pharma AG, Berlin, Germany) at 4 ml/s was automatically injected using one automatic injector after which 147 dynamic images could be obtained. Finally subtraction images of lesions were generated.

Images were processed with one specific software (TIS-SUE 4D®, Siemens Healthcare, Erlangen, Germany) capable of evaluating the dynamic uptake of tumor contrast by calculating the pharmacokinetic values in the bicompartmental model of Tofts et al.¹⁰. To assess the concentration of blood contrast or arterial input function (AIF), the software used offers three pre-established categories (quick, intermediate and slow). Given the high temporal resolution of our protocol, the category «quick» was used as the default one in all patients. The following values were analyzed: (1) $K_{trans}$: constant of transendothelial transference. It measures the passing of contrast from the intravascular space to the extracellular space of tumor or in other words it is one measurement for the patency and blood flow of tumor vessels; (2) $K_{ep}$: constant relating the passing of contrast from the tumor extracellular space to the intravascular one. It is the reverse constant to $K_{trans}$, and is related to tumor washout; (3) $V_c$: fraction of tumor volume occupied by the extracellular space; and (4) $AUC_{90}$: area under the curve of signal intensity through time showing variation in tumor blood volume. It was estimated at 90 s which is the standard value of the software used. These parameters are related through the equation $K_{ep} = K_{trans} / V_c$.

In each and every of the two DMR two regions of interest (ROI) were drawn – one of them around the maximum diameter of lesion in subtraction images (ROI 1) and the other one with the smallest possible standardized size in the region of maximum $K_{trans}$ value (ROI 2) that we could identify by analyzing the $K_{trans}$ lesion map of colors without software (Fig. 1). In both cases we measured twice to assess the average values of $K_{trans}$, $K_{ep}$, $V_c$ and $AUC_{90}$. We chose the $K_{trans}$ map because in the reference this value is

Table 3  Dynamic magnetic resonance protocol with IV contrast.

<table>
<thead>
<tr>
<th>Magnetic resonance</th>
<th>1.5 T (Magnetom Symphony®, Siemens Healthcare, Erlangen, Germany)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence</td>
<td>T1-weighted standard f3D echo gradient</td>
</tr>
<tr>
<td>View</td>
<td>Coronal</td>
</tr>
<tr>
<td>Acquisition</td>
<td>Caudo-cranial</td>
</tr>
<tr>
<td>Fat saturation</td>
<td>Without fat-sat pulse</td>
</tr>
<tr>
<td>TR/TE</td>
<td>4.3/1.29</td>
</tr>
<tr>
<td>FoV</td>
<td>320 mm</td>
</tr>
<tr>
<td>Cutting thickness</td>
<td>4 mm</td>
</tr>
<tr>
<td>Matrix</td>
<td>256 × 256</td>
</tr>
<tr>
<td>Acceleration</td>
<td>×2 GRAPPA</td>
</tr>
<tr>
<td>Flip angle</td>
<td>12°</td>
</tr>
<tr>
<td>Number of measure-</td>
<td>150</td>
</tr>
<tr>
<td>Number of cuts per</td>
<td>2</td>
</tr>
<tr>
<td>Total acquisition</td>
<td>7.08 min</td>
</tr>
</tbody>
</table>

In turn the spatial resolution decreased (voxel size of 1.25 mm × 1.25 × 4 mm; 22 cuts) and the fat saturation pulse was eliminated. Given that the entire breast could not be studied through this protocol—especially large breasts
considered to be one of the most relevant ones for the study of changes in neoangiogenesis and the response to neoadjuvant chemotherapy. Only one radiologist (LJP) highly experienced in performing DMR did the readings of all the cases.

Analysis

To study the reproducibility and concordance of the outcomes obtained parametric statistical tests (interclass correlation coefficient and the Student’s t test for paired samples) and non-parametric tests were used (Spearman’s rho and Wilcoxon rank-sum test) with the software SPSS® v.15.0 (Chicago, Illinois, U.S.A.) Prior to this the normality of ROI 1 and ROI 2 variables through the Kolmogorov–Smirnov and Shapiro–Wilk tests was analyzed. $P < 0.05$ was considered significant.

Results

The average maximum diameter of tumors through MRI was 35 mm with a range between 14 and 100 mm. The four variables showed one normal distribution in ROI 1. In ROI 2 only in $K_{ep}$ and $V_e$ the distribution was normal. The outcomes of the statistical analysis can be seen in Table 4. Very high correlations ($r > 0.80$; $p < 0.01$) between the 2 DMR were found in all ROI 1 variables and high correlations ($r = 0.70–0.80$; $p < 0.01$) were found in all ROI 2 variables with the exception of $V_e$ both in ROI 1 ($r = 0.44$; $p = 0.07$) and ROI 2 ($r = 0.13$; $p = 0.23$). The average ROI 1 concordance was higher than that of ROI 2 ($r = 0.77$ vs $r = 0.60$). There were no statistically significant differences between both studies for the $K^{trans}$, $K_{ep}$ and AUC$_{90}$ values obtained ($p > 0.05$ for all) but there were statistically significant differences for $V_e$ values obtained in ROI 2 ($p = 0.008$).

Discussion

In our study we showed that with our high temporal resolution and reduced spatial resolution DMR protocol the concordance of the main pharmacokinetic variables is really good except for variable $V_e$.

The analysis of angiogenesis through DMR in breast tumors is hot at the moment. The reduced tumor neovascularization is one of the most widely studied therapeutic targets during the last years and one of the most promising ones in the neoadjuvant therapy of breast cancer. Hence the importance of the analysis of evolutionary changes occurring in tumor revascularization for which DMR is the most adequate DMR. In our study we did not find statistically significant differences in any variables between the two consecutive tests except for $V_e$ in ROI 2. Concordance of all variables has been high (ROI 2), or very high (ROI 1), except for $V_e$ values. It is possible that the fact of using a predetermined value of non-adjusted AIF for every study might have influenced the poor concordance found in both cases for $V_e$. The software used does not offer the possibility of performing a study prior to the contrast bolus for the analysis of real AIF. According to Leach et al. it is possible to measure the real AIF before the bolus but this increases the time and complexity of the study. Measuring it requires injecting a small amount of contrast media (around 10%) and one optimized image protocol. Another alternative is measuring AIF in one non-tumor region (e.g. muscular tissue) with known pharmacokinetics properties. However to analyze the importance of this study we need to highlight that the most important constants for the analysis of changes in tumor neovascularization are those that have to do with patency and the passing of contrast through the endothelial membrane ($K^{trans}$, $K_{ep}$).

We also performed the segmented analysis of data obtained thanks to one ROI drawn through the tumor...
Table 4  
<table>
<thead>
<tr>
<th>Variable</th>
<th>Student’s t test for paired samples</th>
<th>CCI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median 1</td>
<td>Median 2</td>
</tr>
<tr>
<td>ROI 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K^{trans}$</td>
<td>133.04</td>
<td>134.20</td>
</tr>
<tr>
<td>$K_{ep}$</td>
<td>291.92</td>
<td>271.80</td>
</tr>
<tr>
<td>$V_e$</td>
<td>498.83</td>
<td>513.21</td>
</tr>
<tr>
<td>AUC$_{90}$</td>
<td>14.50</td>
<td>13.15</td>
</tr>
<tr>
<td>ROI 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{ep}$</td>
<td>401.33</td>
<td>374.00</td>
</tr>
<tr>
<td>$V_e$</td>
<td>610.80</td>
<td>733.46</td>
</tr>
<tr>
<td>AUC$_{90}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AUC$_{90}$: area under the curve of signal intensity throughout.
Calculated at 90 s; CCI: intraclass correlation coefficient; $K_{ep}$: constant of contrast passing from the tumor extravascular space toward the intravascular space; $K^{trans}$: constant of transendothelial transport from the intravascular space toward the extracellular tumor space; ROI 1: region of interest drawn around the maximum diameter of lesion in subtraction images; ROI 2: region of interest drawn in the region of maximum value of $K^{trans}$ identified through $K^{trans}$ color map of the lesion provided by the software; $V_e$: fraction of tumor volume occupied by extravascular space.

For the study of pharmacokinetic parameters of smaller tumors the modification of the protocol with a greater spatial resolution and lower temporal resolution could be considered.

In sum the protocol we presented for the assessment of the pharmacokinetic constants of breast tumors is highly reproducible for the values of $K^{trans}$, $K_{ep}$ and AUC$_{90}$, with a higher concordance when assessing the ROI of the tumor maximum diameter. However it does not seem to be adequate for the $V_e$ pharmacokinetic constant even though this result needs to be re-analyzed with a larger number of patients.

**Ethical responsibilities**

**Protection of humans and animals.** Authors confirm that all proceedings and experiments followed relate to the committee of responsible human experimentation ethical rules and regulations in compliance with the World Medical Association and the Declaration of Helsinki.

**Data confidentiality.** Authors confirm that the protocols of their centers have been followed on matters concerning the publishing of data from patients. They also confirm that all patients included in this study have been given enough information and handed over their written informed consent for their participation in this study.

**Right to privacy and informed consent.** Authors confirm that they have obtained the written informed prior consent from patients and/or subjects appearing in this article. This document is in the possession of the corresponding author.
Author’s contributions

1. Manager of the integrity of the study: JE and LP.
2. Original Idea of the Study: JE and LP.
3. Study Design: JE, LP and JGF.
4. Data Mining: JE, LP, JGF and VB.
5. Data Analysis and Interpretation: JE and LP.
6. Statistical Analysis JE, IA and AGL.
7. Reference Search: JE, AGL, AE and LP.
8. Writing: JE, IA, AE, AGL and LP.
9. Manuscript critical review with intellectually relevant contributions: IA, AGL, JGF and VB.
10. Final Version Approval: JE, AGL, IA, AE, JGF, VB and LP.

Conflict of interest

The lab Roche Pharma sponsored the MRI studies presented in this work.

References