Magnetic Resonance Imaging 33 (2015) 725–736

Contents lists available at ScienceDirect

Magnetic Resonance Imaging

journal homepage: www.mrijournal.com

Cluster analysis of quantitative parametric maps from DCE-MRI: application in evaluating heterogeneity of tumor response to antiangiogenic treatment

Dario Livio Longo [a,b], Walter Dastrù [b,c], Lorena Consolino [b,c], Miklos Espak [d], Maddalena Arigoni [c], Federica Cavallo [c], Silvio Aime [b,c,*]

[a] Institute of Biostructure and Bioimaging (CNR) c/o Molecular Biotechnologies Center, Via Nizza 52, 10126, Torino, Italy
[b] Molecular Imaging Center, University of Torino, Via Nizza 52, 10126 Torino, Italy
[c] Department of Molecular Biotechnology and Health Sciences, University of Torino, Via Nizza 52, 10126 Torino, Italy
[d] Dept. of Computer Science, University College London, Gower Street, London WC1E 6BT, United Kingdom

ARTICLE INFO

Article history:
Received 12 November 2014
Revised 24 March 2015
Accepted 30 March 2015

Keywords:
DCE-MRI
Gd-complexes
Pharmacokinetic modeling
Clustering
Antiangiogenic
Tumor heterogeneity

ABSTRACT

Purpose: The objective of this study was to compare a clustering approach to conventional analysis methods for assessing changes in pharmacokinetic parameters obtained from dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) during antiangiogenic treatment in a breast cancer model.
Materials and methods: BALB/c mice bearing established transplantable her2+ tumors were treated with a DNA-based antiangiogenic vaccine or with an empty plasmid (untreated group). DCE-MRI was carried out by administering a dose of 0.05 mmol/kg of Gadocoletic acid trisodium salt, a Gd-based blood pool contrast agent (CA) at 1 T. Changes in pharmacokinetic estimates (K^{trans} and v_p) in a nine-day interval were compared between treated and untreated groups on a voxel-by-voxel analysis. The tumor response to therapy was assessed by a clustering approach and compared with conventional summary statistics, with sub-regions analysis and with histogram analysis.
Results: Both the K^{trans} and v_p estimates, following blood-pool CA injection, showed marked and spatial heterogeneous changes with antiangiogenic treatment. Averaged values for the whole tumor region, as well as from the rim/core sub-regions analysis were unable to assess the antiangiogenic response. Histogram analysis resulted in significant changes only in the v_p estimates ($p < 0.05$). The proposed clustering approach depicted marked changes in both the K^{trans} and v_p estimates, with significant spatial heterogeneity in v_p maps in response to treatment ($p < 0.05$), provided that DCE-MRI data are properly clustered in three or four sub-regions.
Conclusions: This study demonstrated the value of cluster analysis applied to pharmacokinetic DCE-MRI parametric maps for assessing tumor response to antiangiogenic therapy.

1. Introduction

Tumor angiogenesis is a key process for solid tumors to survive, grow and metastatize [1]. The development of novel anticancer strategies targeting the angiogenic step calls for imaging methods able to assess the early response to new antiangiogenic treatments, comprising vascular disrupting agents (which destroy existing vessels) or antiangiogenic drugs (which block new vessels formation) [2,3].

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is the methodology of choice for the evaluation of tumor angiogenesis, and it has been proposed as an imaging biomarker of drug efficacy in phase I clinical trials of angiogenesis inhibitors [4]. DCE-MRI allows investigating microvascular structure and function by tracking the pharmacokinetics of an injected Gd-based contrast agent (CA) as it passes through the tumor vasculature. The obtained enhancement patterns reflect vascular perfusion and permeability of the tumor, showing the potential to monitor changes in the tumor microvasculature following antiangiogenic therapy [5–7]. Despite these promising capabilities, clinical adoption of DCE-MRI as an imaging biomarker is still hampered by challenges related to the lack of standardized methods for both image acquisition and quantification.

Two methods are currently employed to analyze DCE-MRI data to yield quantitative (pharmacokinetic modeling) or semiquantitative (shape analysis) results, respectively. In the semiquantitative approach, features directly obtained from the signal intensity time curve (e.g. maximum relative enhancement, initial slope, time to peak, area under the curve) are used to get a simple description of

* Corresponding author at: Dipartimento di Biotecnologie Molecolari e Scienze per la Salute, Centro di Imaging Molecolare, Università di Torino, Via Nizza 52, 10126, Torino, Italy. Tel.: +39 011 6706451; fax: +39 011 6706487.
E-mail address: silvio.aime@unito.it (S. Aime).

http://dx.doi.org/10.1016/j.mri.2015.03.005
0730-725X/

the CA distribution [8]. These parameters depend on a combination of blood flow, permeability, perfusion and blood volume, therefore representing composite information of the underlying physiological processes. A major drawback of this approach is that it is quite susceptible to minor changes in acquisition protocols, sequence parameters and individual examinations, making direct comparison difficult. In the quantitative approach, pharmacokinetic models are applied to contrast agent concentration data to enable estimates of physiological parameters, including plasma volume (v_p), forward vascular transfer constant (K^{trans}) and the reverse vascular transfer constant (k_{ep}). Several pharmacokinetic models have been proposed since the seminal papers by Tofts and Brix [9,10], either requiring a measured/assumed arterial input function (AIF), or neglecting the need for the AIF as in the reference region models [11].

The values of the biomarkers derived from the analysis of the DCE-MRI data strongly depend on the characteristics of the CAs used. This affects the overall ability to assess tumor microvasculature. Clinical studies have been carried out mainly by using small-sized Gd-containing contrast media (i.e. gadoteridol), whereas at pre-clinical level several contrast agents having different size and protein binding capability have been investigated, either at intermediate or high magnetic field (2–4.7 T) [12–15]. Intermediate molecular weight (MW) and macromolecular CAs have been shown to be more sensitive to changes in vascular permeability upon antiangiogenic therapies in comparison to low molecular weight CAs thanks to their reduced extravasation in healthy tissues [12,16,17]. Even though many efforts have been devoted in the last years to optimize the relaxometric properties and the HSA binding affinities of the CA (in order to attain improved contrast enhancement characteristics)[18–21], to date, only one blood pool Gd-based CA has entered into clinical practice. We have recently shown that, exploiting the magnetic field-dependence of the Gd-complexes relaxivity, intermediate MW Gd-based agents show greater performance at 1 T [22]. In addition, high temporal resolution is not a stringent requirement for intermediate MW-enhanced MRI [23,24].

During growth, tumors develop a highly heterogeneous micro-environment, characterized by severe structural abnormalities of the microvasculature network [25]. Furthermore, it has been shown that treatment of tumors with antiangiogenic drugs promote alternative angiogenic growth factor pathways, further contributing to the increased tumor heterogeneity [26]. There is an overall agreement in considering tumor heterogeneity as one of the key factors of the disease. It is directly related to some tumor properties and reflects its ability to respond/escape to therapeutic treatments [4,27]. Conversely, the values of the DCE-MRI estimates are therefore strongly dependent on how the tumor ROIs are drawn and on the applied statistic analysis. So far, there is no consensus on which is the optimal method for tumor heterogeneity assessment. ROIs can be drawn to encompass the entire tumor region, or to split the tumor into regions which are spatially defined (poorly enhanced inner regions or core and strongly enhanced periphery regions or rim) [28]. Consequently, the spatial heterogeneity information is discarded by current quantitative analysis methods employing simple summary statistics (e.g. mean or median values on the whole tumor region) or by pre-defined rigid boundaries between rim and core regions [29]. Histogram analysis is considered to be more sensitive in detecting changes in tumor heterogeneity after treatment, than conventional summary statistics, looking to changes in histogram shape (kurtosis) and asymmetry (skewness), although it does so at the expense of including spatial information [28,30]. Alternative techniques are those based on texture-analysis, providing quantitative estimates of tumor heterogeneity, also considering their spatial distribution [31]. Similarly, novel methods based on clustering approaches, aiming at grouping pixels sharing similar enhancement properties, have been recently proposed. However, to date, clustering methods have only been used for classification of time intensity curve shapes [32] and for discriminating between benign and malign lesions [8,33] or combined with diffusion-based multispectral analysis techniques [34].

The purpose of this study is to investigate the ability of a clustering approach on assessing tumor heterogeneity and thereof changes in the evaluation of the response to a DNA-based antiangiogenic treatment employing a blood-pool contrast agent at 1 T. Within the clustering approach, based on a pixel-by-pixel analysis, the whole tumor has been segmented into several sub-regions according to their enhancement/permeability properties. Moreover, we evaluated if the number of clusters may influence the ability to assess the response to the antiangiogenic treatment. In addition, a quantitative comparison with conventional summary statistics (mean values on the whole tumor or mean values on rim/core tumor sub-regions) and with histogram analysis (skewness and kurtosis) was performed, to test the ability of the clustering approach on the assessment of subtle spatial changes following the therapeutic protocol.

2. Material and methods

2.1. Contrast agent

Gadocoletic acid trisodium salt (B22956/1), a Gd-based blood pool CA with high affinity for human serum albumin (relaxivity of 25 mM^{-1} s^{-1} at 40 MHz in human serum at 298 K [35]) was kindly provided by Bracco Imaging S.p.A. (Colleretto Giacosa, Italy).

2.2. Animal studies and antiangiogenic DNA vaccination

Animal studies were approved by the local ethics committee of our university and carried out in accordance with the EU guidelines under Directive 2010/63. Wild-type BALB/c mice ($n = 12$) were injected subcutaneously in the inguinal region with 1×10^5 TUBO cells (a cloned Her2/neu+ cell line established from a lobular carcinoma of a BALB-neuT mouse [36]). Mice were vaccinated by electroporation with DNA plasmid coding human p80 Amot (pAmot or Angiomotin) and control pcDNA3 (generated as previously described [37]) when tumor mass reached 4 mm mean diameter and, again, 7 days after. Briefly, 50 μg of plasmid in 20 μl of 0.9% NaCl was injected in the quadriceps muscle of anesthetized mice $n = 6$ for both treated (Angiomotin plasmid) and untreated (pcDNA3 plasmid) groups, respectively. Immediately after the injection, two 25 ms trans-cutaneous electric low voltage pulses (150 V amplitude) separated by a 300 ms interval were administered at the injection site via a multiple needle electrode connected to an electroporator (Cliniporator™, IGEA s.r.l., Carpi, Italy).

All animals were maintained under specific pathogen-free conditions inside the animal facility and received standard rodent chow and had free access to tap water.

2.3. MRI protocols

Magnetic resonance images were acquired on anesthetized mice with an Aspect M2 MRI System (Aspect Magnet Technologies Ltd., Netanya, Israel) working at 1 Tesla. The anesthetized animals were warmed with a heat lamp before MRI and then wrapped in warm towels to maintain body temperature and placed supine in a transmit/receive solenoid coil with an inner diameter of 3.5 cm. A phantom filled with diluted gadoteridol (Bracco Imaging SpA, Milan, Italy) was included in the field of view (FOV), close to each animal, as a reference, to allow correction for changes in the instrument performance. After the scout image acquisition, a T_2-weighted (T_{2w}) anatomical image was acquired with a fast spin echo sequence (TR