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Choline as a Biomarker for Cell Proliferation: Do the Results from Proton MR Spectroscopy Show Difference between HER2/neu Positive and Negative Breast Cancers ?

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HER2/neu gene is a member of family of genes encoding trans-membrane receptors for four growth factors, including the epidermal growth factor receptor (EGFR), HER2/neu, HER-3, and HER-4. The intracellular domain of HER2/neu has tyrosine kinase activity which regulates cell growth and proliferation [1-5]. Overexpression of HER2/neu can transform cultured cells into more aggressive phenotype and accelerate tumorigenesis [1,6]. HER2/neu is overexpressed in 20−25% of invasive breast cancers and associated with an aggressive tumor, an early relapse and reduced survival rate [7-9].

It has been found that a tumor cell line overexpressing HER2/neu resulted in an increase in levels of choline-containing compounds (tCho) measured by in-vitro proton MR spectroscopy (MRS), including phosphocholine (PCho), glycerophosphocholine (GPC), and choline [10]. It was postulated that growth factor-mediated activation of the tyrosine kinase cascade can lead to an increase in phosphocholine levels [10]. The proton MRS has been proven very useful in differentiating between benign and malignant breast lesions based on elevated tCho [11-14]. Choline measured by MRS may provide an imaging marker for cell proliferation. Our recently published article [15] analyzing the MR imaging features with respect to HER2/neu overexpression in invasive breast cancer demonstrated a higher choline detection rate in HER2/neu positive compared to negative cancer. The number of patients in that study was however very small and conclusion could not be drawn. Here we reported a larger series study to further investigate the choline expression between HER2/neu +/− cancers.

Sixty-six breast cancer patients (range 32−76 years old, mean 51 years) enrolled from March 2005 to October 2006, who were scanned with the MRI/MRS protocol were included in this study. The inclusion criteria were patients with biopsy confirmed diagnosis of malignant lesions that measured 1.5 cm or larger on MR images. Of the 66 malignant lesions, 41 (77%) were invasive ductal carcinomas, 7 (11%) were invasive lobular carcinomas, and the other 8 (12%) were mixed invasive ductal and lobular carcinomas. HER2/neu status was determined initially using the immunohistochemical staining (IHC), positive in tumors with 3+ staining score, and negative for score of 0 and 1+. For those with IHC 2+ staining, fluorescent in situ hybridization (FISH) was conducted to determine the status.

The examinations were performed on a clinical 1.5T scanner (Eclipse; Philips Medical System, Cleveland, Ohio) with a dedicated four-channel phased-array breast coil. After the MRI study

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was completed, single-voxel MRS was performed using a point-resolved spin-echo sequence (PRESS). The spectroscopic voxel was carefully positioned to maximize the coverage of the contrast-enhanced lesions while minimizing the inclusion of adipose tissue. The voxel size was from 2.4 to 8.0 mL. The absolute tCho concentration was analyzed using as an internal reference method [16]. The tumor size was measured by a radiologist based on the maximum intensity projection (MIP) of the contrast subtraction images.

Of 66 cancers, 45 (68%) were HER2/neu negative, and 21 (32%) were HER2/neu positive. The mean size of 66 malignant tumors was 3.4 cm (range, 1.5 − 8.6 cm). The ^{1}H-MRS result was positive for tCho in 53 (80%) of 66 patients. The measured Cho levels ranged from 0 to 8.5 mmol/kg (mean ± SD, 1.9 ± 1.9 mmol/kg), which were consistent with the previously published value by Bolan et al. [16]. Table 1 summarizes *in vivo* breast ^{1}H-MRS results in HER2/neu positive and negative groups. The choline detection rate was higher in HER2/neu positive group (91%) than in HER2/neu negative group (76%), but not reaching significant level ($p = 0.26$, chi-square test).

HER2 receptor mediates signaling to cancer cells and stimulates proliferation [3-5]. *In vitro* cell line study by overexpressing HER2/neu in MCF7 cells showed higher proliferation rate [17]. Overexpression/amplification of HER2 is associated with tumor aggressiveness and a poor prognosis in breast cancer. Limited literature is available correlating MR imaging features with HER-2 biomarkers expressed in invasive breast cancer. Tse et al. [18] reported 17 out of 19 breast cancer patients showing positive choline detection. The two false negative cases were negative for HER-2/neu oncogene expression, suggesting that a false-negative spectroscopic result may be related to an absence of Her-2 overexpression in carcinoma of the breast. Agrawal et al. [15] also showed more choline detection rate in HER-2/neu positive patients compared to Her-2/neu negative cohort in small number of patients (4/7 vs. 0/8, $P < 0.05$). The case number was too small in the two aforementioned studies. In this much larger series study, the sensitivity of *in vivo* ^{1}H-MRS in HER2/neu negative group, although lower, was not significantly different from that of HER2/neu positive group, and also the absolute tCho levels did not appear to be related to HER2/neu overexpression. Our observation suggests that *in vivo* ^{1}H-MR spectroscopy may play a very limited role for characterizing HER2/neu overexpression in carcinoma of the breast.

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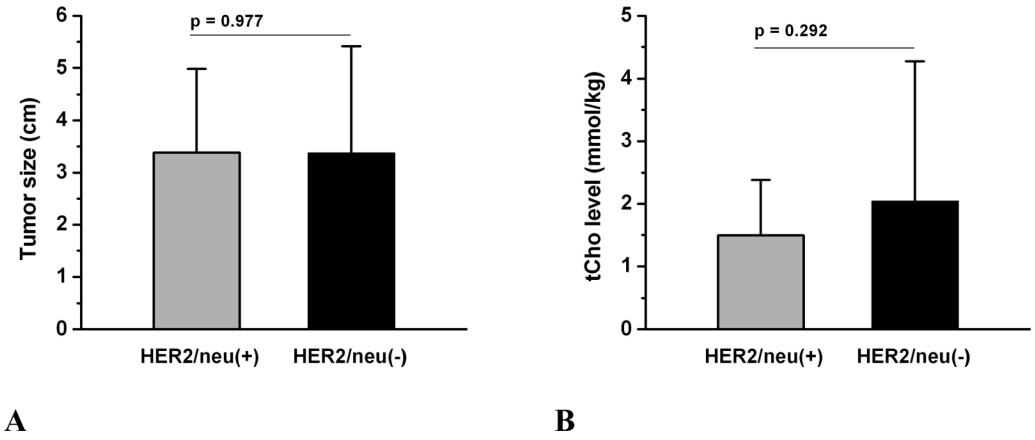

Figure 1.

Comparison of tumor size and total choline-containing compounds (tCho) in HER2/neu-positive and negative groups. There was no significant difference in tumor size (figure 1A) and tCho level (figure 1B) between these two groups.

Table 1

Sensitivity and tCho Concentration in HER/neu Positive and Negative Breast Cancers Using *In Vivo* MR Spectroscopy

	No. of true positives	No. of false negatives	Sensitivity	tCho level (mmol/kg)
HER2/neu				
Positive	19	2	91%	1.50
Negative	34	11	76%	2.03
Overall	53	13	80%	1.87

Note. tCho = total choline-containing compounds.