MR spectroscopy of breast cancer patients for prediction of treatment response – combining in vivo and ex vivo analysis

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Background

The overall prognosis for breast cancer is good, but 45 % of patients with locally advanced breast cancer die within five years (1). Breast cancer care would improve by better stratification of patients to different therapies and more efficient evaluation of treatment effects. Tissue concentrations of total choline (tCho) in breast cancers have the potential to predict treatment response and be a measure of treatment effects (2). We wanted to combine *in vivo* and *ex vivo* MR spectroscopy (MRS) of breast cancer patients assigned for neoadjuvant chemotherapy (NAC) to investigate the correlation between tCho levels detected *in vivo* and *ex vivo*.

Experimental

Patients (n=35) with locally advanced breast cancer (stage T3 or T4) assigned for NAC (FEC: 5-Fluorouracil, Epirubicine and Cyclophosphate) were included in the study. Tumor size was measured clinically the day prior to treatment and after the last chemotherapeutic dose. The Regional Committee for Medical and Health Research Ethics approved the study protocol, and all patients provided written informed consent. *In vivo* ^{1}H MRS was performed before NAC at a 3T Siemens Trio system (Siemens, Germany). The spectra were obtained using the standard PRESS sequence (TE=135, TR=2000 ms and NS=128). Core needle biopsies were taken prior to the first treatment from the same patients. Tissue samples were placed in cryogenic vials and immersed in liquid nitrogen immediately after dissection. *Ex vivo* HR MAS MRS was performed on tissue samples as previously described (3). Peak areas were determined using curve fitting (PeakFit, Seasolve; USA), and concentrations of glycerophosphocholine (GPC), phosphocholine (PCho) and choline were determined using ERETIC (3). Tissue imprints were made on glass slides before sample preparation and HR MAS analysis. The imprints were evaluated by a pathologist after staining with a modified May-Grünwald-Giemsa stain (Color Rapid, Med-Kjemi; Norway) and scored as malignant, suspicious of malignancy, DCIS, non-malignant or of insufficient quality for evaluation. Only patients with biopsies scored as malignant, suspicious of malignancy or DCIS were included in the study, resulting in *in vivo* and *ex vivo* MRS data from 19 patients.

Results

In vivo tCho was detected in 10 of 19 patients (SNR > 2). Tissue metabolites could be quantified using HR MAS in nine of these patients. *In vivo* MRS did not detect tCho in nine patients, where tissue metabolites could be quantified in seven of the HR MAS spectra. Comparing findings obtained *in vivo* and *ex vivo* showed that concentrations of tCho determined by *ex vivo* MRS were significantly higher in tissue from patients with detectable tCho levels *in vivo* (Table 1). Seven of the patients had complete clinical response. These patients had significant higher tissue concentration of GPC (p=0,037) than patients not having complete response (n=12), Figure 1. The higher tCho concentrations in clinical complete responders were not significantly different from those with less than complete response (p=0,066).

Table 1 Tissue concentrations (μmol/g) of choline compounds in biopsies from patients with *in vivo* MRS detectable (SNR>2) and undetectable (SNR<2) levels of total choline.

	GPC	PCho	Choline	tCho
SNR > 2 (N= 10)	1,63 (±2,15)	1,31 (±0,81)	0,60 (±0,37)	3,55 (±2,90)
SNR < 2 (N=9)	0,48 (±0,54)	0,65 (±0,67)	0,24 (±0,18)	1,37 (±1,27)
p (Student's t-test)	0,138	0,068	0,019	0,054

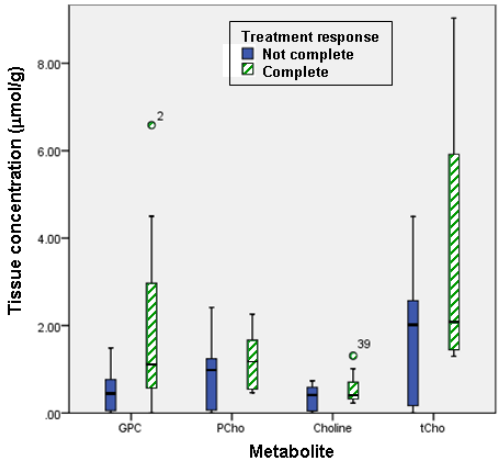

Figure 1 Box-plot presentation of tissue metabolic concentrations in biopsies obtained before NAC for patients with different clinical response.

Discussion and Conclusion

Ex vivo and *in vivo* findings were consistent and demonstrate that *in vivo* MRS and *ex vivo* MRS of breast cancer describe the same features of a tumor. The finding of higher tissue concentrations of GPC in biopsies from patients with complete response to NAC is similar to previous findings in breast cancer biopsies (4). GPC might be a biomarker for treatment response in breast cancer patients receiving NAC.

References

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