METABOLIC CHARACTERIZATION OF LOCALLY ADVANCED BREAST CANCER IN RESPONSE TO NAC TREATMENT

Shiva Shojaei Moghaddam[1], Maria D. Cao[1], Guro F. Giskeødegård[1], Hans E. Fjøsne[1], Steinar Lundgren[1], Per E. Lønning[2], and Tone F. Bathen[1]
[1]*NTNU, Trondheim, Sør-Trøndelag, Norway,* [2]*University of Bergen, Begren, Norway*

Purpose: Locally advanced breast cancer (LABC) is a heterogeneous disease, where patients with similar diagnosis can have markedly different prognosis (1). In studies of cancer, metabolic profiling can be applied for cancer characterization, possibly identifying new prognostic and predictive markers, and also for detecting targets for future therapy (2). The purpose of this study was to determine quantitative metabolite changes in LABC patients receiving NAC treatment in order to evaluate weather the metabolic profiling could assist the prediction of prognosis and clinical treatment response.

Methods: 86 patients with LABC enrolled in the period of 1998-2003 were included in this study. The patients received NAC treatment with either epirubicin or/and paclitaxel at 3 weeks intervals for 4-8 cycles. Response to treatment was evaluated using the WHO criteria by the UICC system (3). Treatment response was assessed clinically by comparing caliper measurements prior to NAC treatment and after the last cycle. Patients who died within 5 years after the diagnosis were classified as non-survivors, whereas patients who survived 5 years or more were classified as survivors. Proton high resolution magic angle spinning MR spectra were acquired of biopsies obtained prior to treatment and before surgery. Bruker Avance DRE 600 was used and a single pulse experiment was performed (ZGPR; Bruker). Metabolite integrals were determined by peak fitting (Peakfit v.4.12, Seasolve). Quantification was performed by relating the metabolite area (creatine, choline, phosphocholine, glycerophosphocholine, myo-Inositol, glycine, lactate and glucose) to a synthetic signal using the ERETIC (Electronic REference To access In vivo Concentrations) method. Metabolite concentrations were calculated using the equation:

$$(MET) = \frac{A_{MET}}{A_{ERETIC}} \times \frac{1}{k_{MET}} \times \frac{n_{ERETIC}}{m_{sample}}$$

Where A_{MET}: metabolite area, A_{ERETIC}: ERETIC area, K_{MET}: number of protons of the metabolite signal, n_{ERETIC}: number of moles in the ERETIC signal and m_{sample}: sample mass (gram). The statistical data analysis was performed by SPSS software (SPSS, Chicago, Illions, USA). The Kolmogorov-Smirnov test confirmed non-normal distributed data, and Wilcoxon Signed Ranks tests were used for comparisons. p-values <0.05 was considered significant.

Results: Survivors showed significant decrease in Glycerophosphocholine (GPC) and Glycine (Gly) and increase in myo-Inositol (m-Ino) in response to NAC treatment while non-survivors did not show any significant change in these metabolite levels (Figure 1). Furthermore, significant decrease in Gly and increase in m-Ino levels were observed among the responders whereas the non-responders only showed a significant decrease in Gly level in response to NAC treatment.

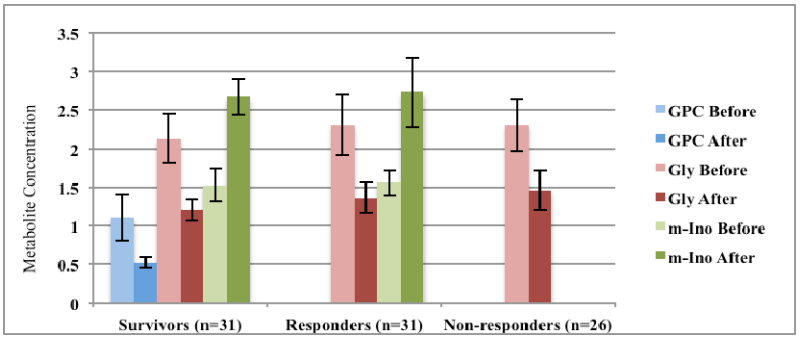

Figure1: The quantification of metabolites from proton HR MAS. This figure only shows metabolites with significant changes after treatment. p-values <0.05 were considered significant. The values are mean metabolite concentration (umol/gr) ± standard error.

Discussion and Conclusion: GPC is involved in cell signaling, lipid metabolism and structural integrity of the cell membrane. The reduced GPC observed in survivors as response to treatment is in accordance with previous finding (4), suggesting its role as a predictor of breast cancer survival. Moreover, the Gly level showed significant decrease among survivors, responders and non-responders. Gly is mainly synthesized from 3-phosphoglycerate, an intermediate of glycolysis (5). Previous findings have identified that increased Gly level is associated with poor survival rates in ER+ breast cancer (6). Thus, decreased Gly in non-responders after treatment was not expected. By definition, non-responders can obtain <50% decrease in tumor volume, which might explain the decreased Gly in this group after NAC treatment. Increased m-Ino levels among survivors and responders were also observed. Previous findings suggest that m-Ino can destruct tumor cells through the chelation and control of cell division (7). However, the molecular mechanisms underlying these observations are not fully understood. Findings from this study indicate that the MR metabolic profile in response to NAC treatment can assist the prediction of prognosis and clinical treatment response in LABC patients.

References: 1. Mathew J, *et. al*, European journal of surgical oncology. 2009 Feb;35(2):113-22. **2.** Kim YS, *et. al*, Future Oncology (London, England). 2008 Feb; 4(1): 93-102. **3.** Hayward JL, *et. al*, Br. J. Cancer. 1977 Mar;35(3):292-8. **4.** Cao MD, *et. al*, BMC Cancer. 2012;12:39. **5.** Geisler S, *et. al*, Cancer Research 2001 Mar 15;61(6):2505-12. **6.** Giskeødegård GF, *et. al*, NMR Biomed. 2012 Nov;25(11):1271-9. **7.** Shamsuddin AM, *et. al*, Anticancer research. 1998 Nov-Dec;18(6A):40916.