# Detection of lipid composition by 7T Proton spectroscopy of ex vivo axillary lymph nodes of 10 breast cancer patients

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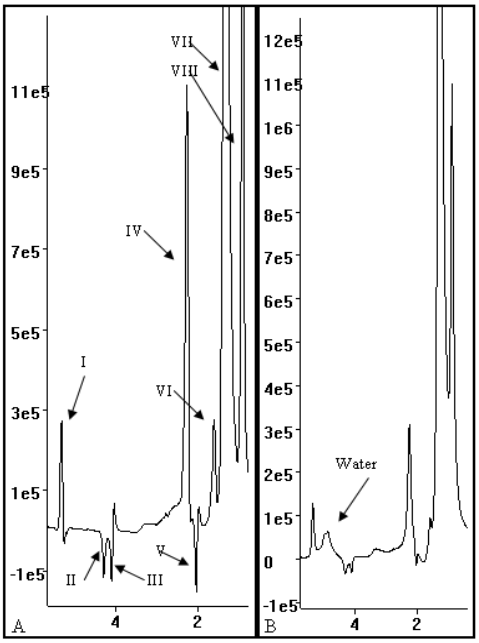


Figure 1. Spectra of the phantom of olive oil and egg yolk (A) mixed with formaldehyde at 24 hours (B). Eight lipid peaks are clearly identifiable on all three spectra. I combination of methine glycerol backbone and methine protons, II methylene glycerol backbone, III methylene glycerol backbone, IV methylene protons α to COO, V methylene protons α to C=C, VI methylene protons β to COO, VII methylene protons and VIII methyl protons. In figure B a water peak is visible at 4.9ppm. The lipid peaks in figure B, although a line broadening effect has occurred, are similar to the peaks in figure A.

**Introduction:** Axillary lymph node status is one of the most important factors determining prognosis in breast cancer patients. Assessment of nodal status currently requires surgical resection. Current MRI techniques cannot discriminate benign from metastatic nodes with high accuracy yet. An alternative may be proton MR spectroscopy ($^1$H-MRS). Malignant tissues have an elevated phospholipid metabolism and malignancy has been associated with altered levels of polyunsaturated fatty acids (PUFA's). Fat composition can thus be seen as a potential marker for differentiating benign from malignant tissue. As a first step towards future non-invasive diagnostic work-up of nodal staging of breast cancer patients, we explored the lipid composition of healthy and metastatic excised sentinel lymph nodes of breast cancer patients at 7 Tesla. The nodes were characterized with 3D multiple voxel $^1$H-MRS on a clinical 7T MR system and verified by pathology analyses.

**Materials & Methods:** Ten consecutive female patients about to undergo surgical nodal staging for the work-up of a histologically proven breast cancer (≥2cm) were included. Following the operation, the nodes were conserved in formaldehyde for 24 hours. During 7T MRI (Philips Health Care, Cleveland, USA), using a T/R head coil with a 16 channel receive coil (Nova Medical Systems), the nodes were submersed in fomblin to provide susceptibility matching. The scan protocol consisted of a high resolution 3D T1w fat suppressed fast field echo (fsFFE) [TR/TE 158/5.59ms, flip angle 35º, FOV 23.6x110x110mm, resolution 0.18mm isotropic]; a $^1$H-MRS from a 3D multi-voxel without water and lipid suppression[TR/TE 1000/118, carrier frequency at the choline (Cho) resonance, resolution 3.0x3.0x5.0mm³, bandwidth 4000Hz, FOV 96x96x25mm³] and a low resolution multislice T1w fsFFE [TR/TE 158/2.4ms, flip angle 35º, FOV 96x96x25mm³, resolution 1.0mm isotropic] which served as background image for MRS. Pathological examination was performed by an experienced pathologist. The nodes were sliced in 4mm sections and numbered. They were paraffin embedded, cut into 3µm thick slices and stained with Haematoxylin & Eosin (H&E). The high resolution 3D T1w scan (voxel volume 5.8 nanoliter) allowed reconstruction along arbitrary planes. This enabled accurate matching of the MRI data to the sectioning plane of the microscopic pathology slides. The content of the MRS voxels was classified into four categories: 1) metastases 2) ≥25% nodal tissue, 3) ≥75% lipid tissue, 4) mixed; <25% nodal tissue and <75% lipid tissue. For all selected voxels, the integral was calculated of water and of 6 distinct groups of lipid resonances (i.e. at 5.4-5.2, 4.3-4.1, 2.8, 2.3-2.0, 1.3-1.6 and 0.9ppm) using 3DiCSI. The ratio of the total of all lipid peaks to the total of all lipid peaks plus the $H_2O$ peak was calculated. Voxels with less than 10% fatty content were excluded from analysis, in order to guarantee sufficient discrimination between noise. Additionally the ratio of each single lipid peak to the total of all lipid peaks was determined. To determine the effect of fixation by formaldehyde on the lipid content of the nodes, a phantom consisting of olive oil and egg yolk was scanned without and with formaldehyde at 24 hours with the same protocol. A statistical analysis was performed by means of a Mann Whitney test. Significance was defined as a p-value <0.05.

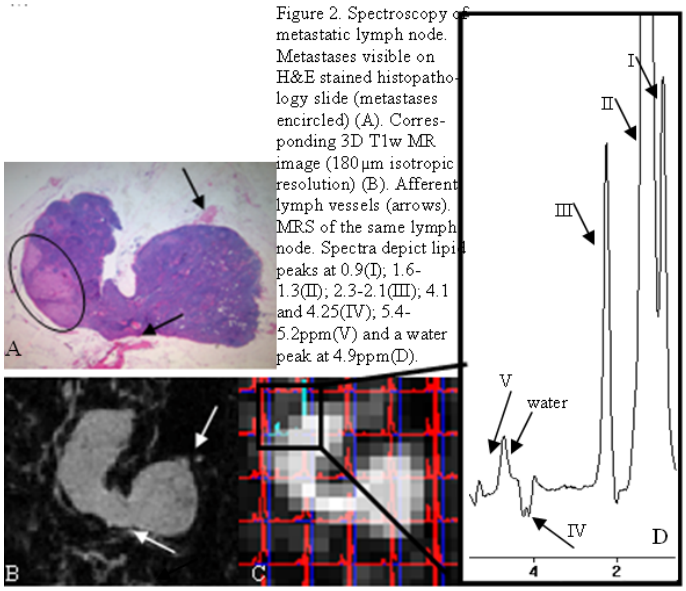


Figure 2. Spectroscopy of metastatic lymph node. Metastases visible on H&E stained histopathology slide (metastases encircled) (A). Corresponding 3D T1w MR image (180 µm isotropic resolution) (B). Afferent lymph vessels (arrows). MRS of the same lymph node. Spectra depict lipid peaks at 0.9(I); 1.6-1.3(II); 2.3-2.1(III); 4.1 and 4.25(IV); 5.4-5.2ppm(V) and a water peak at 4.9ppm(D).

| | Total lipid /Total lipid +H2O | 5.4-5.2ppm /Total lipid | 4.3-4.1ppm /Total lipid | 2.8ppm /Total lipid | 2.3-2.0ppm /Total lipid | 1.6-1.3ppm /Total lipid | 0.9ppm /Total lipid |
|---|---|---|---|---|---|---|---|
| Metastases | 0.97 (±0.03) | 0.00065 (±0.001) | 0.050 (±0.05) | 0.047(±0.01) | 0.039 (±0.05) | 0.35 (±0.3) | 0.55 (±0.4) |
| >25% nodal tissue | 0.79 (±0.2) | 0.10 (±0.2) | 0.029 (±0.4) | 0.0046(±0.01) | 0.16 (±0.2) | 0.46 (±0.3) | 0.25 (±0.3) |
| >75% lipid tissue | 0.91 (±0.1) | 0.14 (±0.3) | 0.03 (±0.04) | 0.0016(±0.003) | 0.07 (±0.1) | 0.35 (±0.3) | 0.41 (±0.4) |
| Mixed | 0.82 (±0.2) | 0.17 (±0.3) | 0.04 (±0.04) | 0.0082(±0.03) | 0.17 (±0.2) | 0.33 (±0.3) | 0.29 (±0.3) |
| P-value meta. vs benign | 0.056 | 0.0018 | 0.43 | 0.40 | 0.029 | 0.94 | 0.052 |

Table 1. ratio of the lipid peaks in respect to the total lipid content per voxel expressed as means ± standard deviation.

**Results:** The phantom measurement showed no effect of the formaldehyde fixation on the lipid resonances other than an effect of line broadening (fig 1). 32 nodes were excised from 10 patients. A total of 6 nodes from 4 patients contained metastases (19%). Of the 32 nodes, 358 voxels were analyzed and all could be matched to pathology (fig 2). Two nodes contained <10% lipid content. Of 356 voxels, 16 (5%) contained metastases. 173 voxels (49%) contained ≥25% nodal tissue, 24 voxels (7%) contained ≥75% lipid tissue and 143 voxels (40%) mixed tissue. Only the methylene and methine protons were significantly reduced in metastatic as compared to benign nodes (table 1).

**Discussion:** This study was designed to assess the potential of in vivo discrimination between malignant and benign lymph nodes using a clinical 7T scanner. It is shown that formaldehyde fixation does not effect the presence of lipid peaks. High resolution MR images were meticulously correlated to pathology slides. Due to the small spectroscopy voxel volume (45 mm³), an accurate detection of lipids in all lymph nodes was possible. 7T $^1$H -MRS was able to non-invasively detect saturated, mono unsaturated and PUFA's independent of the total lipid content. Six groups of lipid peaks were identified in the acquired spectra; methyl protons at 0.9ppm, methylene protons at 1.3 and 1.6ppm, and between 2.0 and 2.3ppm, a small PUFA peak at 2.8ppm, methylene protons from the glycerol backbone at 4.1 and 4.3ppm, and methine protons at 5.2 and 5.4ppm. The results show similar quantitative values for lipid concentrations in benign as in metastatic lymph node tissue; no difference in lipid composition was found when looking at PUFA concentrations or when looking at lipid concentration at the mobile lipid resonances at 4.3-4.1, 2.8, 1.6-1.3 and 0.9ppm. Only the methylene and methine protons were significantly reduced in metastatic nodes, while only a statistical analysis was used which does not correct for clustering of the data. In conclusion we have shown that chemical analysis by $^1$H -MRS at 7T of saturated, mono unsaturated and PUFA's in lymph nodes of breast cancer patients shows only minor differences in composition between benign and metastatic lymph node tissue.