In Vivo Proton MR Spectroscopy of the Breast Using the Total Choline Peak Integral as a Marker of Malignancy

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OBJECTIVE. The purpose of our study was to use the total choline-containing compound (tCho) peak integral as a marker of malignancy in breast MR spectroscopy (MRS).

SUBJECTS AND METHODS. Forty-eight single-voxel water- and fat-suppressed 1.5-T MRS measurements were performed in 42 patients, obtaining both absolute tCho peak integral and tCho peak integral normalized for the volume of interest (VOI). Our reference standard was histology for lesions with BI-RADS category 4 and 5 and histology or at least a 2-year follow-up for findings with BI-RADS 2 and 3 and normal glands. Receiver operating characteristic (ROC) analysis, Mann-Whitney U test, and Spearman's rank correlation were used.

RESULTS. Three of 48 measurements (6%) failed. Of the remaining 45 spectra, 18 nonmalignant tissues showed no tCho peak, eight nonmalignant tissues showed a tCho peak integral from 0.99 to 9.03 arbitrary units (AU), and 19 malignant lesions showed a tCho peak integral from 1.26 to 19.80 AU. The diameter of nonmalignant tissues was 16.9 ± 7.4 mm; that of malignant lesions was 15.3 ± 6.9 mm (p = 0.308). At ROC analysis, the optimal threshold was 1.90 AU for absolute tCho peak, with 0.895 (17/19) sensitivity, 0.923 (24/26) specificity, and an AUC (area under the curve) of 0.917 (95% CI, 0.822–1.000); the optimal threshold was 0.85 AU/mL for the normalized tCho peak integral with 0.842 (16/19) sensitivity, 0.885 (23/26) specificity, and an AUC of 0.941 (0.879–1.000) (p = 0.470). A negative correlation (p = 0.011) was found between the VOI and the normalized tCho peak integral of malignant tissues.

CONCLUSION. Breast MRS using tCho peak integral reaches a high level of diagnostic performance.

Proton MR spectroscopy (MRS) allows noninvasive molecular analysis of biologic tissues because of the different chemical shifts of particulate nuclei in a magnetic field. These chemical shifts result from the differential electron density surrounding the nuclei causing different internal magnetic fields in the molecules [1]. Using MRS, a 2D diagram, a signal intensity (or amplitude)-to-frequency spectrum, is obtained [1, 2]. When localized MRS is performed in vivo, compounds contained in the nominal volume of interest (VOI) are shown as resonance signals or bands (the so-called peaks).

Proton MRS has been shown to characterize breast tissues because of the ability to detect the resonances arising from the trimethylammonium head groups of the total pool of water-soluble choline-containing metabolites at 3.2 ppm (more specifically, between 3.14 and 3.34 ppm) that contribute to the total choline-containing (tCho) peak. The tCho spectral profile reflects abnormalities of choline phospholipid metabolism in tumors, as shown in ex vivo studies [3–8] and summarized in several review articles [2, 9–12]. In vivo single-voxel proton MRS has provided a sensitive diagnostic tool for breast cancer in a pilot study [13]. Five studies [14–18] summarized in a meta-analysis showed that, excluding technical failures, a 0.92 sensitivity and a 0.92 specificity can be obtained [19]. Other authors [20–28] later confirmed these findings. Regarding specificity, the tCho peak was not detected in a group of six phylodes tumors studied by Tse et al. [21]. Moreover, an increase in tCho has been associated with overexpression of the HER2/neu gene [4, 21] and with aggressive breast cancer phenotype [29]. Proton MRS was also used for detecting metastasis in axillary lymph nodes.
MR Spectroscopy of the Breast

Subjects and Methods

Study Population

The local ethics committee approved the research, which was carried out in compliance with the World Medical Association Declaration of Helsinki (1964) and its subsequent amendments (1975–2004). All participants signed a written informed consent form. Inclusion criteria were consecutive patients of at least 18 years old who underwent diagnostic bilateral breast MRI showing one or more contrast-enhancing masses measuring at least 10 mm in the largest diameter and at least 7 mm in the remaining two dimensions. Exclusion criteria were previous or current neoadjuvant chemotherapy or radiation therapy, previous needle biopsy or other interventional or surgical procedures in the 3 months preceding the examination in the breast harboring the lesion to be studied, the presence of metallic clips from previous surgery or needle biopsy in the breast harboring the lesion to be studied, and general contraindications to MRI or to the administration of gadolinium-based contrast agents.

Examinations were always scheduled during the second week of the menstrual cycle for premenopausal women [24]. None of the enrolled postmenopausal women was under treatment with hormone replacement therapy. Immediately before the examination and during patient positioning, each patient was carefully instructed to breathe normally and not to move during the entire examination.

MRI Acquisition and Postprocessing

All MR examinations were performed on a 1.5-T unit equipped with 40 mT/m gradients (Sonata Maestro Class, Siemens Medical Solutions) with a dedicated bilateral receiver-only phased-array four-element two-channel coil (one channel per breast) supplied by the manufacturer. Bilateral breast imaging was performed with the following protocol: an axial STIR sequence (TR/TE, 5,400/91; inversion time, 150 milliseconds; 36 slices of 4-mm thickness without an interslice gap; field of view, 350 × 350 mm; matrix size, 256 × 256; acquisition time, 12 minutes 42 seconds). This sequence was repeated with a temporal resolution of 115 seconds; a 3D T1-weighted FLASH dynamic gradient-echo sequence (TR/TE, 11/4.8; flip angle, 25°; 128 coronal partitions of 1-mm thickness without an interslice gap; 1 mm³ isotropic voxel; one unenhanced and four contrast-enhanced acquisitions with a temporal resolution of 115 seconds); and IV injection of 0.1 mmol/kg of gadoterate dimeglumine (Gd-DOTA, Dotarem, Guerbet), followed by a 20-mL flush of saline solution.

Postprocessing consisted of temporal subtraction (enhanced minus unenhanced images) for all the dynamic phases. We generated intensity-to-time and percentage of enhancement-to-time curves, as already described [40]. Lesion diameter was measured on the first (rarely, on later phases) subtracted coronal images and on their axial and sagittal reconstructions; the largest of these three diameters was considered to be a measure of lesion size. Morphologic and dynamic lesion assessment was done according to the Breast Imaging Reporting and Data System (BI-RADS) using the lexicon for MRI [41].

Proton MRS Acquisition and Postprocessing

A single-voxel water- and fat-suppressed point-resolved spectroscopy sequence (PRESS) was acquired at least 15 minutes after the contrast injection. This delay time has already been applied in previous MRS studies of the breast, even after a double dose (0.2 mmol/kg) of a 0.5-M extracellular gadolinium chelate [21]. In addition, the relatively low effect of the extracellular gadolinium chelate in comparison with measurement errors and variability of intracellular tCho levels has been already shown [42].

Before proton MRS was performed, the channel contralateral to the lesion was switched off. Automated parameter optimization consisted of frequency and receiver gain adjustment and gradient tuning. A semiautomatic shimming adjustment was performed to reach a full width at half maximum (FWHM) of the unsuppressed water peak lower than 30 Hz as a quality parameter of the MR signal, as suggested by the manufacturer. If we found values of FWHM higher than 30 Hz, the adjustment procedure was repeated once more. In the case of a value of FWHM again higher than 30 Hz, the quality check was considered unsatisfying and the MRS examination was interrupted, considering the case to be a failure because of technical reasons, patient movement, irregular breathing, or unknown reasons.

The proton MRS sequence was acquired with the following technical parameters: 1,500/136; flip angle, 90°; 512 measurements. The sequence acquisition time was 12 minutes 42 seconds. This relatively long TE (136 milliseconds) was chosen to increase the visibility of the tCho resonance [19] because of the longer T2 of tCho (> 350 milliseconds) in comparison with that of lipids (~100 milliseconds) [5, 43]. Yeung et al. [30] considered “a TE around 135 milliseconds” as “a good compromise for the sensitivity and intense fat signal intensity interference associated with shorter TEs.” Moreover, a strong lipid and water spectral suppression was applied using a frequency-selective inversion pulse surrounded by a spoiler gradient pulse of opposite signs, a method known as “MEGA” [44] or “double BASING” (band selective inversion with gradient dephasing) [45, 46], which also incorporates a motion correction [45].

The nominal VOI was a rectangular prism, positioned by a radiologist with 2 years of experience in breast MRI and MRS. The positioning of the VOI was performed on the basis of coronal
subtracted images, sagittal and axial reconstructions of the subtracted images, or axial STIR images. The minimal size of the VOI along the three dimensions permitted by the sequence was 10 mm, for a minimal VOI of 1 mL. Its axes were always parallel to the coronaI, sagittal, and axial reference planes. The VOI position and size were chosen to encompass each enhancing lesion, limiting as much as possible the inclusion of nonenhancing gland parenchyma or surrounding fat. In such a way, a theoretical filling factor, obtained comparing the lesion volume (calculated as an ellipsoid) with that of the VOI (calculated as a rectangular prism), ranged from 0.38 to 0.70. When a portion of normal gland parenchyma was studied, we excluded from the VOI any visible fat tissue. In this case, the largest VOI diameter was the measure of the size of the normal gland studies, with the filling factor reaching 1.0.

MRS row data were postprocessed on a remote workstation (Leonardo, Siemens AG) using software supplied by the manufacturer (SW Numeris 4, version 2002B, Siemens AG). Post-processing was systematically performed using a locally standardized protocol with the following steps: signal truncated to 1.024 points and then zero-filled to 2,048 points; Fourier transform; Hanning filter; and both automated and manual phase correction. A six-order polynomial baseline correction was performed excluding ranges for lipids (between 0.6 and 2.8 ppm), creatine and tCho (between 2.9 ppm and 3.35 ppm), and water (between 4.4 and 5.2 ppm). A curve fitting using Gaussian function in the range of tCho (between 1.34 and 3.34 ppm) was finally applied to calculate the tCho peak integral. The absolute tCho peak integral was expressed in arbitrary units, and normalized tCho peak integral (absolute tCho peak integral divided by the VOI) was expressed as arbitrary units per milliliter. In case of baseline oscillation with multiple peaks between 3.14 and 3.34 ppm, each of them with an integral lower than 0.09 arbitrary units, the tCho peak was assigned a value of 0.

Reference Standard

All 19 lesions with a BI-RADS category 4 or 5 at MRI were ascertained with surgical biopsy (n = 12) or with 14-gauge core needle biopsy under ultrasound (n = 5) or MRI (n = 2) guidance. The 26 findings assigned BI-RADS 2 (benign) or 3 (probably benign) as well as the volumes of normal gland (BI-RADS 1) were ascertained with surgical biopsy (n = 3), 14-gauge core needle biopsy under ultrasound guidance (n = 7), or at least a 2-year follow-up with mammography (n = 6), mammography, ultrasound and MRI (n = 5), mammography and ultrasound (n = 3), or ultrasound (n = 2).

Statistical Analysis

The distribution of the size of the VOI used for MRS measurement was skewed (not Gaussian). So were the distributions of the absolute and the normalized tCho peak integrals for both malignant lesions and nonmalignant tissues (i.e., benign lesions and normal glands). As a result, all the data were described using not only mean ± SD but also median and 25th and 75th percentiles. For the same reason, a nonparametric test for two independent samples (Mann-Whitney U test) was used for the comparison between the median tissue size (largest diameter) of malignant lesions and that of nonmalignant tissues, between the median of the VOI used for malignant lesions and that used for nonmalignant tissues, between the median of the absolute tCho peak integral of malignant lesions and that of nonmalignant tissues, and between the median of normalized tCho peak integral of malignant lesions and that of nonmalignant tissues.

Receiver operating characteristic (ROC) analysis was performed to find the optimal threshold for both absolute and normalized peak integrals. The optimal threshold was defined using the best compromise between sensitivity and 1 minus specificity, assuming a balanced weighting of the clinical value of the two parameters [47]. Analyse-It for Microsoft Excel (version 1.7.1, available at www.analyse-it.com/download/form.asp [Analyse-It Software, Ltd., PO Box 103, Leeds LS27 7WZ, England, UK]), was used for finding the cutoff and for comparing the area under the curve (AUC) obtained with the absolute tCho peak integral with the AUC obtained with the normalized tCho peak integral.

Considering the skewed data distribution, the correlation between the VOI and the absolute or normalized tCho peak integral was estimated using the nonparametric Spearman’s rank correlation coefficient (Spearman’s ρ). Statistical calculations other than ROC analysis were performed using SPSS software (version 13.0 for Windows, SPSS). Two-tailed statistical tests were always used, and p values less than 0.05 were considered to be significant.

Results

Patients

From February 2004 to January 2005, 42 patients were prospectively evaluated. They were 40 women and two men, ranging in age from 29 to 92 years (median, 61 years; mean, 60 ± 15 [SD] years).

Examination Time

Of the 42 patients, 36 underwent one MRS measurement and six underwent two MRS measurements, for a total of 48 measurements. The total MRI and MRS examination times were no longer than 40 minutes for the 36 patients who underwent only one MRS measurement (centering and scout views, 1 minute; predynamic sequence, 7 minutes; dynamic sequence, 10 minutes; automatic and semiautomatic adjustments and VOI positioning, 5 minutes; MRS sequence, 13 minutes) and no longer than 60 minutes for the six patients who underwent two MRS measurements. The MRS postprocessing and fitting typically required 10 minutes.

MR Spectroscopy

Three of 48 spectra showed values for FWHM larger than 30 Hz at the first and the second evaluation of the spectrum, giving a failure rate equal to 6%. Although the first case was an enhancing homogeneous mass with regular margins of 10 mm in diameter that was no longer visible at the MRI follow-up examinations performed after 1 month and 1 year, the second and third cases were two pathologically proven invasive ductal carcinomas of 10 and 14 mm in diameter. These three cases (numbers 2, 8, and 30 of the series) were excluded from analysis.

Of the remaining 45 spectra from 39 patients were analyzed, with patient age per lesion of 60 ± 15 years (median, 61 years; 25th percentile, 47 years; 75th percentile, 70 years). The VOI distribution was largely skewed, with a mean of 5 ± 12 mL (range, 1–64 mL; median, 2.2 mL; 25th percentile, 1.4 mL; 75th percentile, 3.6 mL). The difference between the median largest lesion diameter of malignant lesions and that of nonmalignant tissues was not significant (p = 0.308). The median VOI used for malignant lesions and that used for nonmalignant tissues were not significantly different (p = 0.407).

Although the absolute tCho peak integral was 1.26 AU or larger for all 19 malignant lesions, the absolute tCho peak integral was considered equal to 0 AU (i.e., not measurable or inferior to 0.09 AU, considered to be indistinguishable from oscillations from the baseline) for 18 of the 26 nonmalignant tissues. Eight nonmalignant tissues showed an absolute tCho peak integral ranging from 0.99 to 9.03 AU (Table 1). The difference between the median absolute tCho peak integral of malignant versus nonmalignant tissues was highly significant (p < 0.001). The difference between the median normalized tCho peak integral of malignant versus nonmalignant tissues was also highly significant (p < 0.001).
MR Spectroscopy of the Breast

Reference Standard
The pathologic standard of reference showed 19 malignant lesions (Table 2). The 26 nonmalignant tissues consisted of 11 pathologically proven benign lesions, eight normal gland tissue, and seven areas of contrast enhancement with negative follow-up (Table 1).

MRS Diagnostic Performance
Figure 1 shows the ROC analysis for both absolute and normalized tCho peak integrals. The AUC was 0.917 (95% CI, 0.822–1.000) for the absolute tCho peak integral and 0.941 (0.879–1.000) for the normalized tCho peak integral. The optimal threshold for the absolute tCho peak integral was 1.90 AU. Thus, considering all lesions with a tCho peak integral equal to or larger than 1.90 AU to be malignant, we obtained a sensitivity of 0.895 (17/19) and a specificity of 0.923 (24/26). The difference

<table>
<thead>
<tr>
<th>Patient–Lesion or Tissue</th>
<th>Age (y)</th>
<th>BI-RADS Category</th>
<th>LLD (mm)</th>
<th>VOI (mL)</th>
<th>Absolute tCho Peak Integral (AU)</th>
<th>Normalized tCho Peak Integral (AU/mL)</th>
<th>Pathology Finding</th>
<th>Negative Follow-Up</th>
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<tr>
<td>1-a</td>
<td>92</td>
<td>1</td>
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<td>73</td>
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<td>—</td>
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</tr>
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<td>13</td>
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<td>—</td>
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<td>82</td>
<td>2</td>
<td>10</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
<td>—</td>
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</tr>
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<td>13</td>
<td>29</td>
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<td>20</td>
<td>8.00</td>
<td>0.00</td>
<td>0.00</td>
<td>—</td>
<td>Normal breast gland</td>
</tr>
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<td>20</td>
<td>8.00</td>
<td>9.03a</td>
<td>1.13a</td>
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<tr>
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<td>18</td>
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<td>6.06a</td>
<td>1.04a</td>
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<td>15</td>
<td>3.38</td>
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<td>—</td>
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<tr>
<td>17</td>
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<td>13</td>
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</tr>
<tr>
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<td>66</td>
<td>2</td>
<td>10</td>
<td>1.00</td>
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<td>0.00</td>
<td>—</td>
<td>Benign contrast enhancement</td>
</tr>
<tr>
<td>19</td>
<td>70</td>
<td>2</td>
<td>20</td>
<td>8.00</td>
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<td>0.00</td>
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<td>Fat necrosis</td>
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<td>17</td>
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<td>Fat necrosis</td>
</tr>
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<td>41</td>
<td>3</td>
<td>17</td>
<td>3.57</td>
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<tr>
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</tr>
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<td>20</td>
<td>8.00</td>
<td>0.00</td>
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<td>Benign contrast enhancement</td>
</tr>
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<td>72</td>
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<td>62</td>
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<td>14</td>
<td>2.18</td>
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</tr>
<tr>
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<td>55</td>
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<td>17</td>
<td>1.44</td>
<td>0.99</td>
<td>0.69</td>
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<td>46</td>
<td>2</td>
<td>13</td>
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<td>0.00</td>
<td>—</td>
<td>Benign contrast enhancement</td>
</tr>
<tr>
<td>31</td>
<td>43</td>
<td>2</td>
<td>12</td>
<td>1.73</td>
<td>0.00</td>
<td>0.00</td>
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<td>Normal breast gland</td>
</tr>
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<td>32</td>
<td>64</td>
<td>2</td>
<td>15</td>
<td>2.34</td>
<td>0.00</td>
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<td>Normal breast gland</td>
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<td>33</td>
<td>45</td>
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<td>38</td>
<td>50.62</td>
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<td>0.00</td>
<td>—</td>
<td>Normal breast gland</td>
</tr>
<tr>
<td>34</td>
<td>61</td>
<td>3</td>
<td>40</td>
<td>64.00</td>
<td>0.00</td>
<td>0.00</td>
<td>—</td>
<td>Benign contrast enhancement</td>
</tr>
<tr>
<td>38 (male)</td>
<td>63</td>
<td>4</td>
<td>11</td>
<td>1.10</td>
<td>1.25</td>
<td>1.14a</td>
<td>Fibrocystic changes in gynecomastia</td>
<td>—</td>
</tr>
<tr>
<td>39</td>
<td>48</td>
<td>3</td>
<td>20</td>
<td>2.00</td>
<td>1.50</td>
<td>0.75</td>
<td>—</td>
<td>Fibrocystic changes</td>
</tr>
</tbody>
</table>

Note—LLD = largest lesion diameter, VOI = volume of interest, tCho = total choline-containing compounds, AU = arbitrary units. Dash (—) indicates not available (pathology findings and negative follow-up) or not applicable (BI-RADS category). All patients were women except for two men, numbers 16 and 38.

*False-positives using cutoff values of 1.90 AU for absolute tCho peak integral and 0.85 AU/mL for normalized tCho peak integral.
between the two AUCs was 0.024, not significant ($p = 0.470$). The distribution of the absolute and normalized tCho peak integrals for nonmalignant and malignant tissues and the discrimination permitted by the optimal thresholds can be seen in Figure 2.

Using the absolute tCho peak integral, we had two false-negatives (one ductal carcinoma in situ and one invasive lobular carcinoma associated with ductal carcinoma in situ) and two false-positives (one atypical ductal hyperplasia and one fibroadenoma). Using the normalized tCho peak integral, we had three false-negatives (all invasive ductal carcinomas) and three false-positives (the same two false-positives obtained using the absolute tCho peak integral and a case of fibrocystic changes in gynecomastia) (Tables 1 and 2). Examples of spectra obtained in a true-positive case and in a false-positive case are shown in Figures 3 and 4, respectively.

**Correlation Between VOI and tCho Peak Integral**

No significant correlation was found between the VOI and the absolute tCho peak integral for either nonmalignant (Spearman's $\rho = -0.096$) or malignant (Spearman's $\rho = 0.052$) lesions. However, although no significant correlation was found between the VOI and the normalized tCho peak integral for nonmalignant tissues (Spearman's $\rho = 0.008$), a significant negative correlation (Spearman's $\rho = -0.571$) was found between the VOI and the normalized tCho peak integral of malignant lesions ($p = 0.011$).

**Discussion**

Our experience shows that single-voxel proton MRS of the breast is clinically feasible. It can be performed after a standard unenhanced and contrast-enhanced breast study in an examination time of approximately 40 minutes, with a relatively low failure rate (6%), similar to the 3% failure rate reported in the meta-analysis by Tse et al. [2] of more than 280 patients.

Our results were obtained in a series of relatively small tissue samples: 19 malignant lesions had a median diameter of 13 mm (with 15 lesions not larger than 15 mm) and a median VOI of 2.2 mL, the same data being 15 mm and 2.2 mL for the 26 nonmalignant tissues. This is an encouraging result considering the diameter of the tumors studied by other authors, ranging from 20 to 47 mm in four older studies [14–16, 18]. Other authors used a diameter larger than 15 mm as an inclusion criteria, reaching a mean size of 33 mm for the malignant and 27 mm for the benign lesions [21] or had a median size of the studied tissues of 21.5 mm [26], 23 mm [28], or 25 mm [30] or a VOI ranging from 1.6 to 9.0 mL [24]. As a reference, the median VOI

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**TABLE 2: Results of In Vivo Single-Voxel Proton MR Spectroscopy at 1.5 T for 19 Malignant Breast Tissues**

<table>
<thead>
<tr>
<th>Patient–Lesion</th>
<th>Age (y)</th>
<th>BI-RADS Category</th>
<th>LLD (mm)</th>
<th>VOI (mL)</th>
<th>Absolute tCho Peak Integral (AU)</th>
<th>Normalized tCho Peak Integral (AU/mL)</th>
<th>Pathology Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-b</td>
<td>92</td>
<td>5</td>
<td>12</td>
<td>1.73</td>
<td>6.64</td>
<td>3.84</td>
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<td>10</td>
<td>1.00</td>
<td>2.50</td>
<td>2.50</td>
<td>Invasive ductal carcinoma</td>
</tr>
<tr>
<td>4</td>
<td>89</td>
<td>4</td>
<td>14</td>
<td>2.55</td>
<td>5.89</td>
<td>2.31</td>
<td>Invasive ductal carcinoma</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td>4</td>
<td>10</td>
<td>1.00</td>
<td>1.42*</td>
<td>1.42</td>
<td>Ductal carcinoma in situ</td>
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<tr>
<td>6-b</td>
<td>73</td>
<td>4</td>
<td>13</td>
<td>2.20</td>
<td>5.92</td>
<td>2.69</td>
<td>Invasive ductal carcinoma</td>
</tr>
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Mean 60.8 — 15.3 2.4 4.5 2.7
SD 14.8 — 6.9 1.2 4.2 4.2
Median 59.0 4.5 13.0 2.2 3.0 1.8
25th Percentile 49.5 — 11.5 1.4 2.4 1.1
75th Percentile 66.5 — 15.1 2.7 5.0 2.4

Note—LLD = largest lesion diameter, VOI = volume of interest, tCho = total choline-containing compounds, AU = arbitrary units. Dash (—) indicates not applicable. All patients were women.

*False-negatives using cutoff values of 1.90 AU for absolute tCho peak integral and 0.85 AU/mL for normalized tCho peak integral.
used by Bolan et al. [23] at 4 T for their in vivo tCho quantification was 1.6 mL, only 27% less than our median VOI at 1.5 T. Thus, our results suggest a potentially relevant clinical implication: Water- and fat-suppressed single-voxel MRS can be diagnostic for lesions of less than 20 mm in diameter—that is, for stage T1 cancers—overcoming one of the limits of previous studies [2].

We found that using an optimized threshold of the absolute tCho peak integral expressed as arbitrary units, 0.895 sensitivity and 0.923 specificity can be reached, with an AUC equal to 0.917. This result is because of the detection of tCho also in seven of 26 nonmalignant tissues, a false-positive rate similar to that already reported at 1.5 T [19, 24, 26] and at 4 T [23, 37]. Our results in terms of sensitivity and specificity are consistent with those of eight previous studies using single-voxel MRS at 1.5 T [14–18, 21, 24, 28]. Pooling the data of these studies, the sensitivity is 149 of 166 (0.898) and the specificity is 97 of 112 (0.866). Thus, we had the same sensitivity and a slightly higher specificity.

Regardless of the limitations intrinsic to the use of the tCho peak integral expressed as arbitrary units, our diagnostic performance could be of value in clinical practice for the possible diagnostic gain in specificity and positive predictive value in comparison with the usual contrast-enhanced breast MRI. A potential clinical application of proton MRS might be to make the MRI-guided breast biopsy unnecessary in cases in which enhancing foci detected only on MRI have an under-the-threshold tCho peak integral, a perspective already suggested [2, 12, 28, 39].

The initial results of five clinical studies using proton MRS of the breast at 1.5 T [14–18] showed its feasibility and a diagnostic advantage using a simple dichotomous approach based on the presence or absence of the tCho peak. Other authors proposed a fixed arbitrary threshold for the signal-to-noise ratio equal to 2 at 1.5 T [21, 24, 28, 30], a method consisting of comparing the signal intensity of the tCho peak with that of the noise measured in regions of the spectrum where no peak was expected, typically inferior to 0 ppm or superior to 6 ppm. However, Jacobs et al. [27], using single-voxel or 2D multivoxel spectroscopic imaging in a small series of nine patients (five malignant and four benign tissues), found a mean signal-to-noise ratio of the tCho peak of 2.0 ± 0.3 in benign tissue and 5.7 ± 1.4 in malignant tissues, with all five malignancies having a signal-to-noise ratio greater than 4.

The possibility of absolute quantification of tCho was explored in vivo at both 1.5 [14, 20] and 4 [23, 37] T. The lowest detectable level of tCho reported at 1.5 T was 0.2 mM
with a TE of 31 milliseconds and 1 cm of resolution [14], whereas a concentration of 2.0 mM was reported by Bakken et al. [20] in a breast tumor. Using an internal reference method developed by Bolan et al. [23], Meisamy et al. [37] obtained an absolute quantification of tCho at 4 T, showing a large difference between the mean concentration of tCho for malignant lesions (2.2 mmol/kg) and that for benign tissues (0.21 mmol/kg). More recently, Baik et al. [38] performed absolute quantification of tCho at 1.5 T using an internal reference; they found a concentration of tCho ranging from 0.76 to 21.20 mmol/kg for 34 spectra in 32 patients with malignant lesions.

The following considerations concerning quantification methods for breast MRS can be proposed. First, absolute quantification of tCho using an internal reference for single-voxel MRS does not reduce the overlap between benign and malignant lesions. In particular, using an internal reference at 4 T with a cutoff of 1.38 mmol/kg, a specificity of 0.94 was obtained with a trade-off in terms of sensitivity, which was reduced to only 0.46 [23]. In a subsequent article, the same group of authors reduced the threshold to 1.03 mmol/kg, obtaining a 0.61 sensitivity and a 0.83 specificity [37]. Other authors found a sensitivity of 0.60 and a specificity of 0.86 using an external reference at 1.5 T with a cutoff of 0.2 mmol/L [14]. A possible reason for this variability is that both quantification approaches need to estimate the T1 and T2 relaxation times of water, lipids, and
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tal carcinoma with a normalized tCho of 19.8 AU/mL (Spearman’s ρ = −0.510; p = 0.031). We have no clear explanation for this. We can only speculate about a lower tCho concentration in some large tumors harboring necrosis or fibrosis and about a higher tCho concentration in some small aggressive tumors. Regarding the latter hypothesis, note that we had one small invasive ductal carcinoma (10 mm in diameter) studied with a 1-mL VOI, which gave the highest level of tCho as both absolute value (19.8 AU) and normalized value (19.8 AU/mL), which was an outlier in both distributions (Fig. 2).

The absence of a significant advantage from normalization could be due to three reasons. First, we had a large number of benign lesions not showing a detectable tCho peak integral without any effect of normalization. Second, the distribution of the size of the VOIs was not sufficiently scattered to permit the normalization to significantly change the results. Third, this result could be due to a too-small sample size. In any event, the normalized tCho peak integral values likely are more realistic than the absolute tCho peak integral values because the VOIs were of different sizes. This view is supported by the higher AUC of the normalized tCho peak integral compared with that of the absolute tCho peak integral. This was not a statistically significant difference. It is only an apparent paradox, suggesting a trend for a better diagnostic performance of the normalized technique.

Using the optimal threshold for absolute tCho, we reported two false-negatives (one ductal carcinoma in situ of 10 mm in diameter and one invasive ductal carcinoma associated with ductal carcinoma in situ of 10 mm in diameter) and two false-positives (a fibroadenoma of 18 mm in diameter and an atypical ductal hyperplasia of 20 mm in diameter). The histologic type of the first false-negative is not surprising because of the possibility of a relatively low level of tCho in ductal carcinoma in situ, which is different from invasive ductal cancer, as already shown from ex vivo MRS of fine-needle biopsy specimens [3] and from in vivo MRS [2, 14, 30]. Yeung et al. [30] reported nine false-negatives, four ductal carcinoma in situ and three invasive ductal carcinoma with an extensive in situ component. Conversely, it is already known that some fibroadenomas may present high levels of tCho at both in vitro [3] and in vivo [15, 18, 28] MRS. False-positive atypical ductal hyperplasia with a high level of absolute tCho (9.03 AU) can be regarded as a borderline high-risk lesion with a high proliferative activity, which is difficult to qualitatively distinguish from ductal carcinoma in situ at pathologic examination [53] and which had already been reported as false-positive at proton MRS [28]. If we consider tCho also as an “indicator of high metabolic activity” [30] or that it is “not exclusive to malignancy” and can be found to be increased also “in proliferating tissues” [9] or in “a benign lesion with high proliferative activity” [21], these two false-positives can be regarded as expected.

Our study has several limitations. In addition to the small sample size already mentioned, we should consider the variable filling factor caused by the impossibility of reducing the VOI below 1 mL, thus almost always including surrounding fat or healthy gland parenchyma. More advanced hardware (e.g., field strength higher than 1.5 T, multi-channel coils) and dedicated postprocessing software could provide MR spectra of better quality than those we obtained. Moreover, the approach of our study, based on the use of arbitrary units, may not allow the application of our cutoff for tCho peak integral to different technical and clinical settings. Finally, the long acquisition time of our MRS sequence (nearly 13 minutes) could have reduced the spectral resolution because of the probability of artifacts from respiratory and other patients motion.

In conclusion, our experience first showed that in vivo 1.5-T single-voxel water- and fatsuppressed proton MRS of the breast can be added as a last phase after unenhanced and contrast-enhanced breast MRI, with an entire examination time not longer than 40 minutes. Moreover, we showed that breast MRS using the tCho peak integral allows high sensitivity and specificity, with an AUC greater than 0.90 for small tissue samples. Studies of large clinical series are warranted to test the added value of proton MRS of breast lesions compared with the established use of breast MRI in clinical practice.

References
3. Mackinnon WB, Barry PA, Malycha PL, et al. Fine-needle biopsy specimens of benign breast le-

choline. The results are affected by possible confounding factors such as the presence of edema caused by cancer, previous invasive procedures or radiation therapy, and variations in cellularity [23].

Second, the independent measurement of an external reference, apart from being relatively time-consuming, is not problem-free. Bolan et al. [48] reported a tCho peak frequency shift due to respiratory artifacts ranging from 10 to 70 Hz (mean, 24 Hz) at 4 T. Considering that the proton MRS signal is an average of multiple signals acquired in possibly different breathing phases, without a frequency correction, both the peak position and height, as well as the peak signal-to-noise-ratio, are affected.

New approaches in metabolites quantification, as reported for proton MRS of the brain using short TEIs with [49] or without [50–52] water suppression, will probably influence future research on proton MRS for breast studies.

A particularly interesting result of our study is the absence of significant change in diagnostic performance in terms of the AUC for using the normalized tCho peak integral. Actually, at the best cutoff, the number of false-negative or false-positive findings changed from four using the absolute tCho peak integral to six using the normalized tCho peak integral (Tables 1 and 2). This was a relatively unexpected finding. In fact, VOIs of different sizes have different proton content and therefore should give different tCho peak integrals: the larger the VOI, the higher the value of the tCho peak integral. As a consequence, the normalization to the VOI should theoretically enhance both sensitivity and specificity, resulting in significantly larger AUC. If we look at the distributions shown in Figure 2, we see that normalization greatly reduced the values of the two false-positives obtained with the absolute tCho peak integral, but not enough to push them below the cutoff. Unfortunately, another benign tissue remained above the cutoff. On the other hand, although the two false-negatives with the absolute tCho peak integral were changed to true-positives by normalization, three cancers of 20–40 mm in diameter were changed to false-negative.

These data agree with our significant negative correlation between the VOI and the normalized tCho peak integral of malignant lesions. Note that this correlation also remained significant, excluding the outlier given by the small (1-mL VOI) invasive ductal carcinoma with a normalized tCho of 19.8 AU/mL (Spearman’s ρ = −0.510; p = 0.031). We have no clear explanation for this. We can only speculate about a lower tCho concentration in some large tumors harboring necrosis or fibrosis and about a higher tCho concentration in some small aggressive tumors. Regarding the latter hypothesis, note that we had one small invasive ductal carcinoma (10 mm in diameter) studied with a 1-mL VOI, which gave the highest level of tCho as both absolute value (19.8 AU) and normalized value (19.8 AU/mL), which was an outlier in both distributions (Fig. 2).

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