

# MAGNETIC RESONANCE SPECTROSCOPY IN THE MANAGEMENT OF MELANOMA

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## Abstract

The surgical treatment of melanoma has been progressively rationalised during the last two decades. Radical excision of primary tumours and elective (prophylactic) resection of regional lymph nodes have been replaced with more selective procedures that reflect improved understanding of the metastatic potential of individual tumours. Magnetic resonance spectroscopy (MRS) is an evolving technology which has the potential to diagnose many tumours and to characterise their metastatic potential. The Institute for Magnetic Resonance Research and the Sydney Melanoma Unit have developed MRS techniques to diagnose, stage and aid in the clinical management of melanoma. It is anticipated that these techniques will ultimately be used as clinical tools to provide non-surgical diagnosis of metastatic disease in sentinel nodes, either by MRS examination of a simple outpatient fine needle biopsy specimen or by use of an entirely non-invasive *in vivo* MRS assessment. Experience with MRS of primary breast cancers indicates that it may also be possible to predict the metastatic potential of melanoma by spectroscopic analysis of the primary tumour and to distinguish naevi from melanomas thus better selecting patients for surgery.

The last decade has seen the introduction of lymphatic mapping and sentinel node biopsy in an effort to rationalise the management of patients with intermediate and thick melanomas and stage them more accurately. While in the past an elective regional node dissection was recommended for such patients by many melanoma treatment centres around the world, currently only patients with a positive sentinel node are subjected to a complete field clearance at the time of their presentation. Thus many patients are now spared this procedure and its inherent risks and associated morbidity. Although sentinel node biopsy is generally associated with low rates of significant morbidity,<sup>1,2</sup> the development of non-surgical techniques that could determine the disease status of mapped sentinel nodes, and indeed other relatively inaccessible lesions in melanoma patients, would represent a significant advance. Experience with proton magnetic resonance spectroscopy (MRS) in the diagnosis of several primary human cancers has demonstrated that this technology is potentially capable of being developed both to identify metastatic malignancy and to predict those individual primary tumours which have a metastatic phenotype. In the case of MRS examination of a fine needle aspiration biopsy from a primary breast tumour, the method can determine pathology, nodal involvement (without direct biopsy of the nodes), tumour vascularisation, tumour grade and oestrogen and progesterone receptor status.<sup>3,4</sup> A collaborative project has now evolved to explore these and other applications related to the diagnosis and staging of melanoma with MRS.

MRS provides information on the chemical composition of cells and tissues, the changes that occur in the disease process, the host response and the aging process.<sup>5</sup> Proton MRS (on biopsy specimens, ie. *ex vivo*) provides an adjunct to current morphological methods for the diagnosis of human tumour pathology with sensitivities and specificities of at least 95%. Accuracies as high as 99% are obtained when mathematical classifiers are used to analyse the data.<sup>6,7</sup> MRS can also detect changes during tumour development and progression, including altered cellular chemistry ie. not morphologically

manifest.<sup>5</sup> The sensitivity of the MRS method was exhibited in a study of a rat model for lymph node metastasis where malignant cells in lymph nodes were detected with a greater sensitivity than histology.<sup>8</sup> Micrometastases were detected that were not apparent even when the entire node was serially sectioned for examination by histology. The MRS diagnoses were confirmed to be correct by growth of the tumour in nude mice following xenografting of nodal tissue.<sup>8</sup>

*In vivo* MRS yields the chemical information from spectroscopy in one of two forms; single voxel spectroscopy (SVS), where a single area of interest is located by MRI<sup>9</sup> or 2D or 3D magnetic resonance spectroscopic imaging (MRSI) where a 2D or 3D grid of spectra is overlaid on an MR image.<sup>10</sup> A variety of applications, including diagnosis of prostate and breast cancers, has been demonstrated using whole-body magnets and MR spectroscopy at 1.5 Tesla (T) and 3T to monitor altered chemistry. For a review of these applications see Mountford *et al.* 2004.<sup>5</sup>

## Diagnosis of metastatic melanoma in lymph nodes by MRS (8.5T) of fine needle aspiration biopsies (FNAB)

Elective lymph node dissection has now been replaced by sentinel node biopsy (SNB) in most major melanoma treatment centres around the world with blue dye and radioactive tracers used to identify the node/s that receive lymph directly from the area of skin bearing the primary tumour. SNB thereby allows patients with micrometastatic nodal disease to be accurately identified. It is however an invasive surgical procedure with a 5-15% complication rate whilst the histological examination of SLNs requires labour-intensive (expensive) serial sectioning and the use of immunohistochemistry. Patients with intermediate thickness and thick melanomas are known to have metastatic tumour in their related sentinel nodes at rates ranging upwards from 15%.<sup>11</sup>

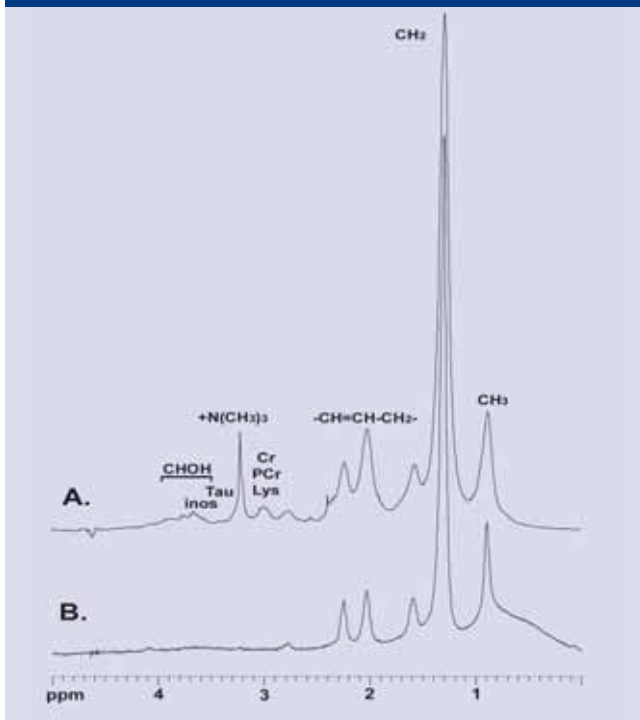
A reliable method of determining regional lymph node status without a need for surgery would reduce patient morbidity and conserve the resources currently applied to surgical SNB and pathological assessment. Our group commenced its experience

with MRS and melanoma by undertaking a study that performed MRS on fine needle aspiration biopsies (FNABs) of sentinel nodes (SNs) procured by conventional surgery. A statistical classifier was then utilised to determine the accuracy with which MRS could identify metastatic tumour in the SNs.

FNABs were obtained from 118 lymph node tissue specimens from melanoma patients undergoing regional node surgery for suspected metastatic melanoma. Each FNAB was assessed using 1D proton MRS analysis at 8.5T.<sup>12,13</sup> Diagnostic correlation was performed between the MRS and histopathological data using a Statistical Classification Strategy (SCS) pattern recognition method designed specifically for biomedical spectroscopy databases.<sup>7,14</sup> The primary data set comprised MR spectra of FNABs from 56 samples containing metastatic melanoma and 62 samples free of metastatic disease. A secondary validation set of duplicate FNABs from a subset of the same tissue samples as the primary data set (24 melanoma-containing, 38 free of metastatic disease) were also classified with the classifier developed from the primary data set to test the variability of FNAB samples from the same tissue, variability in sample handling and storage and variability within the proton MRS measurement procedure.

Typical proton MR spectra of FNABs from histologically benign and malignant specimens are shown in Figure 1. Resonances in the spectra include those consistent with lipid, choline metabolites, creatine, phosphocreatine, lysine, taurine, inositol and carbohydrates.

**Figure 1:**



1D proton MRS (8.5 Tesla) of FNABs of lymph nodes from melanoma patients. A) Node containing metastatic melanoma; and B) Benign node. Reprinted from *Annals of Surgical Oncology* 2005: In press. Stretch JR, Somorjai R, Bourne R, Hsiao MD, Scolyer RA, Dolenko B, Thompson JF, Mountford CE, Lean CL. Melanoma Metastases in Regional Lymph Nodes Accurately Detected by Proton Magnetic Resonance Spectroscopy of Fine Needle Aspirate Biopsies, with permission from Springer.

**Abbreviations:**

**CH<sub>3</sub>** - methyl; **CH<sub>2</sub>** - methylene; **+N(CH<sub>3</sub>)<sub>3</sub>** - N-trimethyl of choline and choline-based metabolites; **-CH=CH-CH<sub>2</sub>-** - acyl chain protons, N- or O-acetyl groups at 2.0 ppm; **Cr** - creatine; **PCr** - phosphocreatine; **Lys** - lysine; **Tau** - taurine; **Inos** - inositol; **CHOH** - carbohydrate residues.

Pattern recognition analysis was applied to the two histopathological categories, ie. free of metastatic disease tissues and tissues containing metastatic melanoma. In the primary data set, the presence of metastatic melanoma was predicted with a sensitivity of 92.9%, a specificity of 90.3% and an accuracy of 91.5%. Six false positives and four false negatives were identified. In the secondary validation set (utilising second FNABs from a subset of the same tissues) the presence of metastatic melanoma was predicted with a sensitivity of 87.5%, a specificity of 90.3% and an accuracy of 89.1%. These results are summarised in Table 1.<sup>13</sup>

**Table 1:**

SCS-based classification of MRS data of FNABs from regional lymph nodes of melanoma patients. Reprinted from *Annals of Surgical Oncology* 2005: In press. Stretch JR, Somorjai R, Bourne R, Hsiao MD, Scolyer RA, Dolenko B, Thompson JF, Mountford CE, Lean CL. Melanoma Metastases in Regional Lymph Nodes Accurately Detected by Proton Magnetic Resonance Spectroscopy of Fine Needle Aspirate Biopsies, with permission from Springer.

	Sensitivity%	Specificity%	Crispness%	*Accuracy%
Primary data set				
Metastatic vs Benign	92.9	90.3	93.2#	91.5
Second data set				
Metastatic vs Benign	87.5	90.3	92.7#	89.1

\* Accuracy for the test was obtained using the crisp data, ie. When specimens classified as fuzzy were excluded. A class assignment was fuzzy if the class probability was less than 75 per cent.

# For the primary data set 8 of 118 samples were classified as fuzzy. For the second data set 4 of 62 samples were classified as fuzzy.

The possibility of non-surgical SN evaluation clearly offers advantages over surgical SN removal. Proton MRS of FNAB specimens accurately predicts the presence of nodal melanoma metastases and thus has the potential to provide rapid, accurate and minimally invasive diagnosis of regional lymph node disease in melanoma patients. Patients could undergo conventional lymphatic mapping and FNAB and MRS performed on the FNA from the marked sentinel nodes. Future development of this technology into a non-surgical technique would offer SN staging without biopsy and histopathologic evaluation including the significant benefits of reduced costs and morbidity.

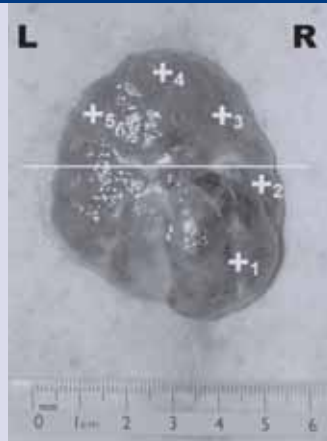
### MRS diagnosis of primary and secondary melanoma on tissue biopsy at 8.5T and *in vivo* at 1.5T

In a study of punch biopsies of benign skin lesions and melanomas, proton MRS at 8.5T distinguished the two pathologies based on the presence of choline based metabolites in the spectra of malignant tissues. Similarly, using tissue biopsies, lymph nodes containing metastatic melanoma have been distinguished from uninvolved nodes based on the presence in the MR spectra of resonances consistent with choline based metabolites. For both primary lesions and lymph nodes this information has also been shown to be obtainable using MRS *in vivo* at 1.5T.

Reporting on a series of case studies, *in vivo* MRS at 1.5T was used to assess a large polypoid cutaneous melanoma (Figure 2) and two enlarged lymph nodes containing metastatic melanoma. Spectra were acquired *in vivo* from voxels wholly within the primary tumour (Figure 3) or metastatic lymph node (Figure 4) and were thus uncontaminated by signals from adjacent tissue. Tissue biopsies (Figure 5) taken after resection of the primary tumours and metastatic lymph nodes were examined by 8.5T MRS and the results compared with the *in vivo* spectra and with spectra from normal skin and a benign skin lesion. There was good agreement observed between the dominant features of 1.5T spectra acquired *in vivo* and 8.5T spectra acquired from resected tissue. However, less intense resonances observed at 8.5T in malignant biopsy tissue were not consistently observed at 1.5T *in vivo*. *In vivo* spectra from primary and metastatic melanoma showed high levels of choline metabolites. An intense lactate resonance was also present in the *in vivo* spectrum of primary melanoma. All 8.5T spectra of biopsies from primary and secondary melanoma showed high levels of choline metabolites and lactate, and additional resonances consistent with elevated levels of taurine, alanine, lysine and glutamate/glutamine relative to normal and benign tissues. Elevated levels of choline, lactate, taurine and amino acids thus appear to be clinically useful markers for identifying primary and metastatic melanoma.

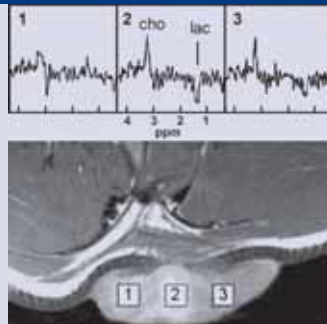
**Figure 2:**

Clinical photograph of primary melanoma (Case 1). The heavily pigmented quadrant can be seen on the inferior right of the tumour. The white line shows the approximate centre of the axial CSI slice shown in Fig. 2. Biopsy sites are marked 1-5. Reprinted from *European Journal of Radiology*, 53/3, Bourne R, Stanwell P, Stretch J, Scolyer R, Thompson J, Mountford C, Lean C. *In Vivo and Ex Vivo Proton MR Spectroscopy of Primary and Secondary Melanoma*.:506-513. Copyright (2005), with permission from Elsevier.



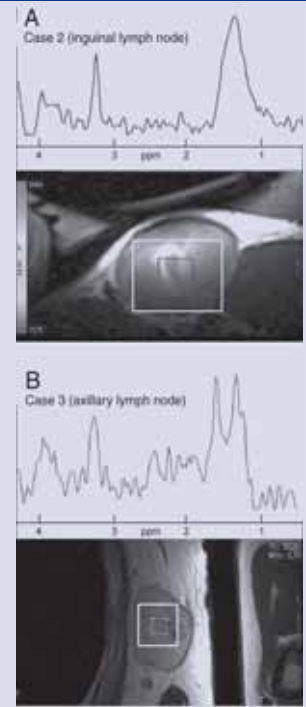
**Figure 3:**

Axial plane CSI of primary melanoma (Case 1). Spectra interpolated from three separate volumes (boxes labelled 1-3) within the tumour are shown. Note the choline resonance (3.2ppm) and inverted lactate resonance (1.3ppm). The approximate centreline of the slice (12mm thickness) is indicated by the line in Fig. 1. Field of view 16x16cm, matrix 16x16, TR=1200ms, nominal voxel size 1.2x1x1cm, global water shim ca. 11Hz, spectral width 1kHz. Reconstruction matrix 32x32. Interpolated voxel size (shown in figure) = 1.2x0.5x0.5cm. Reprinted from *European Journal of Radiology*, 53/3, Bourne R, Stanwell P, Stretch J, Scolyer R, Thompson J, Mountford C, Lean C. *In Vivo and Ex Vivo Proton MR Spectroscopy of Primary and Secondary Melanoma*.:506-513. Copyright (2005), with permission from Elsevier.



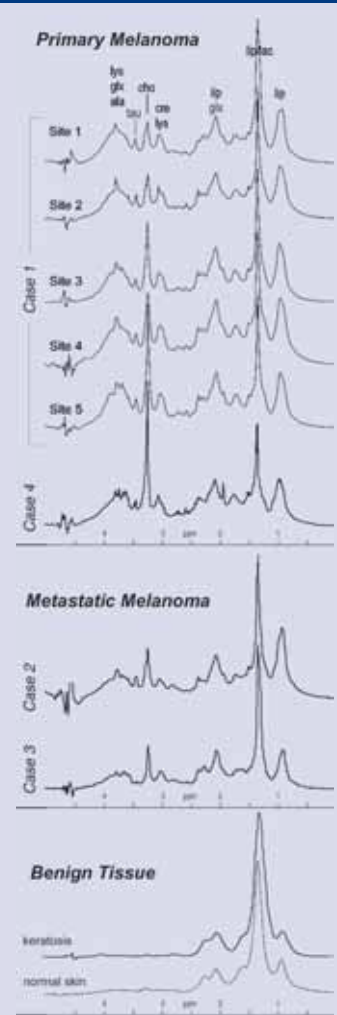
**Figure 4:**

Axial plane CSI of metastatic melanoma in lymph nodes (Cases 2&3). The figure shows interpolated spectra acquired from a 0.3cm<sup>3</sup> volume within A) a 5 x 3.5 x 3cm inguinal node, and B) a 5 x 6 x 3.5cm axillary node. Note the intense choline resonance at 3.2ppm. Acquisition details as for Fig. 2. TE=130ms. Interpolated voxel size (inner rectangle shown in figure) = 1.2x0.5x0.5cm. The outer (white) rectangle represents the CSI volume of interest (VOI). Reprinted from *European Journal of Radiology*, 53/3, Bourne R, Stanwell P, Stretch J, Scolyer R, Thompson J, Mountford C, Lean C. *In Vivo and Ex Vivo Proton MR Spectroscopy of Primary and Secondary Melanoma*.:506-513. Copyright (2005), with permission from Elsevier.



**Figure 5:**

8.5T 1D MR spectra of malignant and benign biopsy tissue. The figure shows: five biopsies from different parts of a large primary melanoma (Case 1) and a single biopsy from a smaller primary (Case 4); two biopsies from two patients with metastatic melanoma (Cases 2&3); and two types of benign tissue (normal skin and keratosis). Note the general similarity of spectra from biopsies of the primary and secondary tumours and the intense choline and creatine resonances relative to the spectra from normal skin and a benign squamous keratosis. The approximate positions of the biopsies from Case 1 are indicated in Fig. 1. 360.1MHz, TR=1s, time domain=8k, 256 transients, pulse angle=60°, spectral width 3600Hz. Reprinted from *European Journal of Radiology*, 53/3, Bourne R, Stanwell P, Stretch J, Scolyer R, Thompson J, Mountford C, Lean C. *In Vivo and Ex Vivo Proton MR Spectroscopy of Primary and Secondary Melanoma*.:506-513. Copyright (2005), with permission from Elsevier.



**Abbreviations:** glx = glutamine/glutamate; tau = taurine; lip = lipid; cho = choline compounds; ala = alanine; lys = lysine, cre = creatine; lac = lactate.

## Conclusions

Proton MRS of FNABs obtained from surgically procured SNs enables accurate diagnosis of metastatic disease in melanoma patients. Proton MRS (on biopsy specimens) has the potential to rapidly and objectively determine the lymph node status of patients with melanoma. Combined with lymphoscintigraphy and ultrasound guided fine needle biopsy of lymph nodes, this MRS technology offers the promise of accurate and minimally invasive assessment of regional lymph node status with high accuracy.

MRI and MRS *in vivo* have the potential to provide preoperative pathological diagnosis of primary and secondary melanoma. In particular, MRI may provide detailed information about primary melanomas including morphological parameters such as tumour depth and satellitosis, while MRS holds potential for determining the malignant potential of a lesion and the status of regional lymph nodes with high accuracy without biopsy.

The maturation of *ex vivo* and *in vivo* MR methodologies to provide reliable non-invasive preoperative diagnosis of melanoma and non-invasive or minimally invasive assessment of lymph node involvement would undoubtedly improve and simplify the clinical management of melanoma patients.

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