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Abbreviations:

GBM = glioblastoma multiforme
 SCS = statistical classification
 strategy

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Identification of *Staphylococcus aureus* Brain Abscesses: Rat and Human Studies with ¹H MR Spectroscopy¹

PURPOSE: To determine the feasibility of a statistical classification strategy (SCS) and the identity of metabolites of bacterial and host origins that potentially contributed to the most discriminatory regions of magnetic resonance (MR) spectra from *Staphylococcus aureus* abscesses of biopsy material from controls, gliomas, and staphylococcal abscesses.

MATERIALS AND METHODS: Human and animal study received ethics committee approval, and informed patient consent was obtained. A rat model of *S aureus* brain abscess was developed. Histologic and microbiologic examination was performed to assess abscess development 3–4, 6–8, and 10–15 days after initiation. Metabolite profiles in pus ($n = 62$) and controls ($n = 37$) were characterized with ex vivo MR spectroscopy and compared with data from rat gliomas ($n = 27$). SCS, optimal region selection, and development of pairwise classifiers allowed MR spectra of abscesses ($n = 42$, day 6–8) to be distinguished from those of glioblastoma multiforme and controls. MR spectroscopy profiles of pus from animal abscesses were compared with in vivo MR spectra from patients with staphylococcal brain abscesses ($n = 7$, aged 6–67 years) and ex vivo pus MR spectra from patients with *S aureus* abscesses.

RESULTS: Histologically confirmed abscesses were present 6–8 days after stereotactic injection of *S aureus* in 42 of 47 rats (89%). MR spectra of abscesses and glioblastoma multiforme in the animal model were similar. Typical metabolites of abscesses due to anaerobe bacteria (acetate, succinate, amino acids) were not detectable in *S aureus* abscesses in rats or humans. MR spectroscopic findings from controls, abscesses, and gliomas were distinguished by means of SCS with an accuracy of 99%. Analysis of the most discriminatory regions with two-dimensional correlation spectra indicated that glutamine and/or glutamate and aspartate potentially contributed to successful classification.

CONCLUSION: *S aureus* is detectable in abscesses with a non-culture-based method in an animal model.

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In developed countries, brain abscesses occur with an estimated incidence of 0.9×10^5 person-years and a mortality of up to 20% (1). Computed tomographic (CT) and magnetic resonance (MR) imaging methods have reduced mortality by improving the detection and localization of brain lesions (2–4). They do not always reliably demonstrate abscesses from malignant tumors, however, especially glioblastoma multiforme (GBM) (5–8). Typically, necrotic or cystic tumors and cerebral abscesses show hypointensity on T1-weighted MR images, hyperintensity on T2-weighted MR images, and perilesional contrast enhancement with gadolinium chelates. The use of diffusion-weighted MR images that show generally lower apparent diffusion coefficients for abscesses than for GBM appeared promising (8,9). However, substantial variability in the apparent diffusion coefficients has

been reported for abscesses (8). A definitive diagnosis is still typically established by means of culture of bacteria from pus obtained with neurosurgical biopsy or inferred by means of culture of infected material from other body sites. Further reduction of mortality from brain abscesses requires more rapid, accurate, and safe diagnostic techniques.

MR spectroscopy characterizes living microbial and mammalian cells by providing information on their chemical constituents rapidly and simultaneously. In vivo MR spectroscopy has been reported to allow the broad group of pyogenic abscesses to be distinguished from malignant gliomas and tuberculoma (5–7,10–13). On the basis of small numbers, analysis by means of resonance assignment of one-dimensional MR spectra resulted in a sensitivity of 90% in one study (13). Acetate, succinate, and amino acids have been identified in cerebral abscesses in humans and were used as bacterial marker metabolites for noninvasive diagnosis by means of MR spectroscopy (5–7,10–13). Bacterial metabolism is diverse, however, and microaerophilic bacteria such as microaerophilic bacteria like *Streptococcus milleri* and *Staphylococcus aureus* do not produce metabolites distinct from those of diseased or normal mammalian tissue in quantities that are detectable in vivo with MR spectroscopy (10).

A recent study demonstrated that subdivision of MR spectra from abscesses according to some causes (tuberculosis, abscesses containing anaerobic bacteria, and other abscesses) was possible (7). However, spectral patterns recorded for GBMs and abscesses caused by microaerophilic bacteria were similar (6,7). For these abscesses, distinction was not possible by means of operator-based analysis.

S aureus is the cause of 10%–31% of cerebral abscesses (2,4,14–16). Early identification of *S aureus* is important, since clinical progression is usually rapid, and empirical antimicrobial therapy may not include first-line antistaphylococcal drugs. On the basis of current data, rapid identification of this subset of abscesses with in vivo MR spectroscopy is not possible.

Multivariate analysis and pattern recognition techniques demonstrate differences in gross spectral characteristics of data from biologic samples without the need to identify individual compounds and are more sensitive than operator-based analysis (17–20). A statistical classification strategy (SCS) was developed

for analysis of complex spectra to diagnose cancer, organ rejection, and speciation of microbial pathogens (17,21–23).

By using a rat animal model, we aimed to test the feasibility of SCS for MR spectra to distinguish between abscesses caused by microaerophilic bacteria and GBM. We also aimed to determine the metabolites of bacterial and host origins that potentially contribute to the most discriminatory regions in MR spectra of biopsy material from normal brain and GBM and staphylococcal abscesses for distinction between these three groups.

MATERIALS AND METHODS

Cell Culture

Five clinical isolates of *S aureus* (97–230-1053, 98–281-2429, SA5, and SA7, Centre for Infectious Diseases and Microbiology, Westmead Hospital, New South Wales, Australia; and ATCC 25923, American Type Culture Collection, Manassas, Va) were cultured in vitro. The identity of isolates, which were all coagulase positive (using rabbit or human plasma) and DNase (ie, deoxyribonuclease) positive, was confirmed with the API ID32 staph test (BioMerieux, Marcy l'Etoile, France).

Isolates were plated onto 5% horse blood agar (Amyl Media, Sydney, New South Wales, Australia) and incubated for 24 hours in 5% CO₂ at 37°C. A single colony was subcultured on horse blood agar or in trypticase soy broth (25 mL, Difco Labs, Detroit, Mich) and incubated for 24 hours at 30°C or 37°C. The effect of different culture media and incubation temperatures on metabolite profiles was studied. These included the following: horse blood agar (30°C and 37°C); brain heart infusion broth (Difco Labs; 30°C and 37°C, standard conditions and strict anaerobic conditions); 1% (wt/vol) isovitalax, 2% (wt/vol) hemin, and 10 mmol/L glucose (Sigma Chemical, St Louis, Mo) in Dulbecco phosphate-buffered saline (Difco Labs; at 37°C); and trypticase soy broth (37°C).

For animal experiments, a single colony was inoculated into trypticase soy broth (25 mL) and incubated for 20–24 hours at 37°C. Bacteria were washed and resuspended at a final concentration of 10⁷ colony-forming units per milliliter either in phosphate-buffered saline or, for MR spectroscopy, in phosphate-buffered saline made up with 99.5% deuterium oxide (Australian Nuclear Science and Technology Organization, Lucas Heights, New South Wales, Australia).

Animal Studies

Rats were anesthetized by means of inhalation of 2% halothane (May & Baker, Degenham, England) in 100% oxygen (medical grade, Commonwealth Industrial Gases, North Ryde, Australia), followed by intraperitoneal injection of ketamine (11.6 mg per kilogram of body weight, Apex Laboratories, Sydney, New South Wales, Australia) and xylazine (1.2 mg/kg, Apex Laboratories). The head was immobilized in a stereotactic frame (David Kopf Instruments, Tjunga, Calif) fitted with a 10-μL Hamilton syringe (Scientific Glass Engineering, Ringwood, New South Wales, Australia) and a flat-ended 30-gauge needle. The lateral and anteroposterior stereotactic coordinates for injection were 3.0 mm lateral and 2.4 mm anteroposterior relative to the “ear-bar zero” (where the ear-bar functioned as the reference point “zero”). Optimal parameters for the development of brain abscesses, including choice of strain of rat (Fisher 344, *n* = 92; Wistar, *n* = 12; and Sprague-Dawley, *n* = 6); strain of *S aureus*; volume, depth, and speed of injection; and inoculum size were established in preliminary experiments. The Fisher 344 rat strain was chosen for subsequent experiments, since abscess formation was similar in Fisher 344 and Wistar rats but less reliable in Sprague-Dawley rats. Animal experimentation was performed according to the Australian National Health and Medical Research Council Guidelines and with ethical approval from the University of Sydney Animal Ethics Committee.

Sixty-seven female Fisher 344 rats (weight, 150–250 g; Animal Research Council, Perth, Western Australia, Australia) were treated according to an optimized protocol and included in the MR spectroscopy study. They were infected with *S aureus* strain 97–230-1053, a clinical isolate from a brain abscess. A small skin incision was made, and a 2-mm burr hole was drilled through the skull. Five microliters containing 10⁷ colony-forming units of *S aureus* was injected slowly at a depth of 3.0 mm below the dura. The needle was withdrawn over 5–8 minutes to minimize reflux of bacteria. Lesion development was monitored 3–4 days (*n* = 10), 6–8 days (*n* = 47), and 10–15 days (*n* = 10) after injection by means of histologic examination. Additional controls included saline-injected rats (*n* = 30) and untreated animals (*n* = 8). Surgical procedures were performed by an author (R.A.), who was assisted by a technician

TABLE 1
Patient Details for Cerebral Abscesses Caused by *S aureus*

Patient No./ Age (y)/Sex	Lesion Size (cm ³)	Lesion Location	Patient Location
1/67/F	24 and 7	Temporal and occipital lobes	Sydney, Australia
2/53/F	10	Frontal lobe	Sydney, Australia
3/10/M	15	Frontal lobe	Lucknow, India
4/18/F	18	Temporal lobe	Lucknow, India
5/23/M	24	Frontal lobe	Lucknow, India
6/6/M	9	Frontal lobe	Lucknow, India
7/13/F	16	Cerebellum	Lucknow, India

Note.—The volume of interest was placed in the center of the lesion on MR images. The size of the volume of interest was adjusted to the size of the lesion to avoid partial volume effects.

under the supervision of a veterinarian (R.M.).

Rats were euthanized by means of CO₂ inhalation. The brain was removed and cut transversely at the site of injection (performed by R.A. and the technician under the supervision of R.M.). One half was used for MR spectroscopy, and the other half was used for confirmation with histopathologic examination. For histopathologic study, brains were fixed in formalin–acetic acid–alcohol, processed, and stained with hematoxylin–eosin or picric acid (Sigma Chemical, St Louis, Mo) and Gram stain procedure. Lesion size, presence, and relative number of microorganisms and type of cellular infiltration were evaluated independently by means of microscopy of stained tissue sections (R.M., T.C.S.). Culture of pus was performed in the Clinical Microbiology laboratory at the Institute for Clinical Pathology and Medical Research, Westmead Hospital, and all cases yielded a heavy growth of *S aureus*.

Human Studies

In vivo MR spectroscopy was performed in seven human subjects with *S aureus* brain abscesses, who met the selection criteria and had been selected over a period of 2 years between January 2001 and December 2002 (Table 1). Selection criteria were the following: (a) A heavy, pure growth of *S aureus* was observed from pus obtained within 2 days of the MR examination; (b) patients had not received antibiotics for longer than 2 days at the time of the MR examination; and (c) abscesses were large enough (at least 2 cm in diameter) to place the volume of interest in the middle of the abscess with minimal “contamination” from surrounding brain tissue.

Ten patients from Westmead Hospital who had cystic GBM were selected randomly for comparison (five women and

five men, aged 57–78 years). Standard T2-weighted MR images were used for placement of the volume of interest. Cerebral MR imaging was performed with 1.5-T MR systems (Magnetom, Siemens, Erlangen, Germany; and Signa LX, GE Medical Systems, Milwaukee, Wis) by using a circularly polarized head coil. Standard T1- and T2-weighted MR images were obtained in three orthogonal planes. MR images were interpreted by radiologists with more than 15 years of experience in brain MR imaging in their respective hospitals (R.K.G. in Lucknow, India; and L.G. in Sydney, New South Wales, Australia). Lesions were hypointense on T1-weighted MR images and hyperintense on T2-weighted MR images and showed perilesional contrast enhancement after administration of gadolinium chelate. Diffusion-weighted MR images were acquired from two patients with *S aureus* abscesses by using diffusion gradient strength ranging from 0 to 1.5×10^{-4} T/cm with increments of 0.25×10^{-4} T/cm.

MR spectroscopy was performed in conjunction with MR imaging. The volume of interest (6–10 cm³) was centered on the lesion. Single-voxel hydrogen 1 (¹H, or proton) MR spectra were obtained by using a stimulated-echo acquisition sequence (repetition time msec/echo time msec, 1500/20, 30 [in Westmead Hospital], 128 signals acquired, 3.13-minute acquisition time) and a long-echo-time point-resolved MR spectroscopy sequence (1500/135, 256 signals acquired, acquisition time of 6 minutes 31 seconds). The water signal was suppressed by using frequency-selective saturation pulses. Free-induction decays were zero-filled to 4096 data points and multiplied with an exponential window function (center, 0 msec; half-width, 150 msec) before Fourier transformation. Zero-order phase correction and polyno-

mial baseline correction were applied to all spectra. Signal assignment was based on ex vivo MR spectroscopy and published data (5,6,11,24).

Patient studies were performed according to the Australian National Health and Medical Research Council Guidelines and with ethical approval from the local Human Ethics Committee (University of Sydney, Australia, and Sanjay Gandhi Post-graduate Institute of Medical Sciences, Lucknow, India). Informed consent was obtained after the nature of the procedure had been fully explained.

MR Spectroscopy of Cell Suspensions and Pus

S aureus suspensions in 500 µL of phosphate-buffered saline made up in deuterium oxide were transferred to a 5-mm MR tube (Wilmad Glass, Buena, NJ). Frozen tissue and/or pus samples were thawed and suspended with 0.15 mL of phosphate-buffered saline made up in deuterium oxide in susceptibility-matched MR tubes (Shigemi, Tokyo, Japan).

Pus was collected from all animals that developed *S aureus* abscesses following the use of the optimized injection protocol. Tissue blocks from the affected cerebral hemispheres, containing 0.05–0.20 mL of pus, were suspended in phosphate-buffered saline and made up in deuterium oxide, snap-frozen in liquid nitrogen, and stored at –70°C for no more than 3 months before MR spectroscopic analysis. Tissue samples from control animals were obtained from 29 animals from the saline injection site 4–6 days after injection (one had died postoperatively) and from the brains of eight untreated animals. Control samples were snap-frozen and stored as for pus samples.

Pus was collected from human subjects (aged 17–73 years) with abscesses in various body sites at Westmead Hospital over the 2-year period. Only pus samples yielding a heavy, pure growth of *S aureus* were studied with MR spectroscopy (12 samples; brain, *n* = 4; surgical wounds, *n* = 3; and pelvis, *n* = 5). Pus was cultured and bacteria identified by the Clinical Microbiology laboratory at the Institute for Clinical Pathology and Medical Research, Westmead Hospital. Pus was collected prior to or within 48 hours of initiation of antibiotic therapy, snap-frozen in liquid nitrogen within 5 minutes, and stored at –70°C for up to 3 months, until required.

Ex vivo ¹H MR spectra from the animal

and human pus samples were obtained (U.H.) at 37°C with an Avance 360-MHz spectrometer (Bruker Biospin, Rheinstetten, Germany) equipped with a 5-mm $\{^1\text{H}, \text{carbon } 13 \text{ } [^{13}\text{C}]\}$ inverse dual-frequency probe. Residual water signal was suppressed by means of selective excitation by using pulse field gradients (25). Chemical shifts were referenced to external sodium 3-(trimethylsilyl) propane sulfonate at 0.00 ppm or internal water (4.65 ppm). One-dimensional spectra were acquired with a spectral width of 10 ppm by using 8192 data points. Free induction decays were averaged over 128 accumulations. A relaxation delay of 1 second was allowed, resulting in a repetition time of 2.14 seconds. An exponential function was applied prior to Fourier transformation, resulting in a line broadening of 1 Hz for ^1H MR spectra of cell cultures and 3 Hz for those of ex vivo tissue and/or pus.

Signal Assignment

Resonances were assigned by means of comparison with published data (5,6,8, 21,23,26) and analysis of $\{^1\text{H}, ^1\text{H}\}$ correlation spectroscopy, $\{^1\text{H}, ^{13}\text{C}\}$ heteronuclear single quantum coherence, and $\{^1\text{H}, ^{13}\text{C}\}$ heteronuclear multiple bond correlation spectra (U.H.).

Because of time constraints for the acquisition of two-dimensional correlation spectra and visual similarity between all one-dimensional MR spectra in the respective sample groups, signal assignment was confirmed for arbitrarily chosen samples, for which sufficient sample material was collected (volume > 10 μL) that was adequate for the acquisition of two-dimensional correlation spectra (cell suspensions, $n = 4$; animal pus, $n = 16$; and human pus, $n = 4$). The human samples included those from the two patients at Westmead Hospital with brain abscesses studied with in vivo MR spectroscopy, one from a surgical wound, and one pelvic abscess. Standard Bruker pulse sequences were used. The $\{^1\text{H}, ^1\text{H}\}$ gradient correlation spectroscopy experiments were reconstructed in the magnitude mode. A total of 2048 data points were collected in the t_2 time domain over a spectral width of 10 ppm. The evolution time (t_1) was incremented to obtain 256 free induction decays, each acquired with 8–16 transients. The repetition time was 1.6 seconds. Sine-bell functions were applied in the t_1 dimension, and Gaussian-Lorentzian functions were applied in the t_2 dimension. Zero-filling was used to ex-

pand the data matrix to 1024 in the t_1 dimension.

The $\{^1\text{H}, ^{13}\text{C}\}$ one-bond shift correlation spectra were obtained in the ^1H detection mode by using a gradient heteronuclear single quantum coherence pulse sequence (27). Heteronuclear single quantum coherence spectra were acquired for some samples to confirm the signal assignment from correlation spectra and to assign signals without proton-proton coupling, unequivocally. The ^1H MR spectral width was 3600 Hz, and the ^{13}C MR spectral width was 15 000 Hz. ^{13}C MR decoupling during acquisition was achieved with the GARP-1 sequence (28). The evolution time (t_1) was incremented to obtain 256 free induction decays, each having 64 transients and consisting of 2048 data points. The repetition time was 1.6 seconds. A sine-bell function was applied in the t_2 dimension, and a Gaussian-Lorentzian function was applied in the t_1 dimension. Zero-filling to 1024 was used in the t_1 dimension prior to Fourier transformation. The $\{^1\text{H}, ^{13}\text{C}\}$ heteronuclear multiple bond correlation spectra were acquired without proton decoupling by using the same parameters as for the heteronuclear single quantum coherence experiments, except for a ^{13}C spectral width of 20 kHz. One-bond and long-range correlation experiments were optimized for $^1\text{J}_{\text{C,H}}$ of 130 Hz and $^n\text{J}_{\text{C,H}}$ of 7 Hz, respectively.

SCS

^1H MR spectra from controls, abscess material (obtained 6–8 days after injection), and glioma were used to develop diagnostic classifiers (R.L.S., B.D.). The MR spectra from the rat gliomas were acquired previously from qualitative results published in reference 26. Pairwise classification was performed as described previously (17,21–23). In brief: Magnitude MR spectra were normalized to the total integral between 0.35 and 4.0 ppm, which contains 1500 data points. The normalized spectra were analyzed by a genetic algorithm-based optimal region selector to identify two to three maximally discriminatory subregions by using first derivatives or rank-orders of first derivatives of the MR spectra (29). By using these regions, pairwise linear discriminant analysis-based classifiers were developed. The robustness of the classifiers was tested by means of bootstrap-based cross-validation by randomly selecting half of the spectra to develop the classifier and the remaining half to test the classifier (30). This process was repeated

1000 times with random replacements. The final classifier was the weighted output of the 1000 different optimized classifier coefficient sets. The classifiers yielded probabilities of class assignments for the individual spectra. Class assignment was called crisp if class probabilities were larger than 0.75 (17). Software developed in-house was used for all steps of the SCS (IBD; NRC Canada, Winnipeg, Manitoba, Canada), as described elsewhere (17,29).

RESULTS

Cell Suspensions

Typical MR spectra from *S aureus* cell suspensions were identical for the five isolates and are shown in Figure 1. The major metabolites are summarized in Table 2.

One-dimensional ^1H MR spectra from horse blood agar plate cultures were dominated by glycine betaine, which accounted for 20%–40% of the total integral. Other resonances arose from lactate (1.31 ppm), glycerophosphocholine (3.23 ppm), choline (3.20 ppm), and amino acid residues (glutamine and/or glutamate, lysine, alanine, leucine, aspartate and/or asparagine, and isoleucine). The acetate resonance was not uniformly present and was always below 2% of the total integral. Metabolite profiles varied with the growth medium. Lactate was the main metabolite in liquid media cultured under anaerobic conditions (Fig 1, Table 2).

Rat Model

Reproducible circumscribed lesions developed in 56 of 67 rats (84%) injected with *S aureus* cell suspensions, according to the optimized protocol. After 3–4 days, ill-defined 1–3-mm-diameter hemorrhagic brain lesions containing Gram-positive cocci were present in eight of 10 animals. After 6–8 days, circumscribed lesions (2–6 mm) containing pus and Gram-positive cocci were present in 42 of 47 animals (89%). After 10–15 days, smaller abscesses (1–4 mm) with well-developed fibrous walls were noted in six of 10 animals.

None of 30 saline-injected animals developed lesions, though small areas of hemorrhage were found in three, and one died of complications of anesthesia.

Rat Biopsies

Representative ^1H MR spectra from control brain tissue, *S aureus* brain abscesses at different stages, and glioma are

Figure 1. Comparison of ex vivo ^1H MR spectra from cell cultures of *S aureus*, animal models, and human pus samples. One-dimensional ^1H MR spectra in phosphate-buffered saline made up with deuterium oxide at 360 MHz were obtained from rat brain biopsy and/or pus samples. *A*, Spectrum from saline-injected controls. *B*, Spectrum from *S aureus* brain abscess after 3 days. *C*, Spectrum from *S aureus* brain abscess after 8 days. *D*, Spectrum from *S aureus* brain abscess after 14 days. *E*, Spectrum from GBM. *F*, *G*, Spectrum from cell suspensions of *S aureus* from horse blood agar cultures (aerobic, *F*) and trypticase soy broth cultures (anaerobic, *G*). *H*, Spectrum from human pus from an abdominal *S aureus* abscess. *I*, Spectrum from human pus from a cerebral *S aureus* abscess. AA = amino acid residues; ac = acetate; bet = glycine betaine; glc = glucose residues; inos = myo-inositol; lac = lactate; lip = triglycerides; lys = lysine; NAA = N-acetylaspartate; $\text{N}(\text{CH}_3)_3$ = choline-containing compounds and glycine betaine; NCH_2 = creatine, phosphocreatine, lysine; tau = taurine.

shown in Figure 1. Metabolites identified with two-dimensional correlation spectra (Fig 2) are summarized in Table 2.

Spectra from controls contained resonances from *N*-acetylaspartate, creatine, phosphocreatine, γ -amino butanoic acid, choline-containing metabolites, *myo*-inositol, and amino acid residues (glutamic acid/glutamine, lysine, alanine, leucine, threonine), and they resemble those from previous studies (24). Lactate resonances of variable but relatively low intensity were found at 1.3 ppm, resulting from anaerobic metabolism occurring in the time between excision and freezing.

Three stages of abscess development, designated early (stage I), intermediate (stage II), and late (stage III), were evident at histopathologic examination and in the MR spectra. Compared with normal brain, stage I was characterized by a marginal increase in the choline-to-creatine resonance ratio (3.2:3.0 ppm) and a decrease of the ratio of *N*-acetylaspartate to creatine (2.0:3.0 ppm). The lactate-to-creatine ratio (1.3:3.0 ppm) was increased in all cases. Lipid resonances were apparent in some spectra. MR spectra from stage II showed a marginal increase in the 3.2:3.0-ppm ratio of 20%–30% (1.2 ± 0.5) and absence of *N*-acetylaspartate resonances. Taurine resonances were increased. Lipid signals were present in all stage II spectra and dominated them in three. In the other stage II spectra, the most intense resonance at 1.3 ppm arose from lactate, which, compared with stage I, was increased in relative intensity. Low-intensity acetate resonances were seen in nine of the 42 spectra. Spectra from stage III were dominated by lipid resonances. *N*-acetylaspartate was absent in all cases. The 3.2:3.0-ppm ratio was similar to stage I and II in four samples and was not detectable in the remaining cases. Taurine and amino acid residues were present in all samples. These included alanine, lysine, glutamine and/or glutamate, asparagine/aspartate, leucine, isoleucine, valine, and threonine. They were of marginally higher relative intensity than in MR spectra from controls and glioma (see also results in reference 26).

Two-dimensional correlation spectra confirmed that the increase of the 3.2:3.0-ppm resonance ratio in spectra from abscesses compared with controls was due to a combination of increased amounts of taurine and phosphocholine. The presence of glycine betaine was confirmed in only four of 16 heteronuclear single quantum coherence and heteronuclear multiple bond correlation spectra (three stage II and one stage III).

TABLE 2
Intensity Distribution of Chemical Shift Regions in One-dimensional MR Spectra

Parameter	No. of Samples and/or Isolates	Spectral Region in One-dimensional MR Spectra (ppm)															
		H α , GB, chol, lac, thr	H α , chol	H α , chol, inos	Tau, chol	Tau, chol, inos	lys, his, cre, asn	asn, asp	gln, glu, lip, NAA	gln, glu, lip, NAA, val	gln, glu, lip, NAA	lip, lys, ac	lip, lys, leu, ile, val	ala, lip	lip, lac, thr	leu, ile, val, lip	leu, ile, val, lip
Cultures																	
<i>S aureus</i> (horse blood agar culture)	5	2	2	1	1	1	2	1	1	1	1	2	2	1	2	1	1
<i>S aureus</i> (anaerobic brain heart infusion broth culture)	5	1	1	1	1	1	2	1	1	2	2	2	2	2	3	2	3
Animal studies																	
Control brain	37	2	1	2	1	1	2	0	1	1	4	1	2	1	2	1	2
<i>S aureus</i> abscess (3–4 days)	8	1	1	2	1	1	2	0	1	1	3	1	1	1	3	1	2
<i>S aureus</i> abscess (6–8 days)	42	1	1	1	2	1	1	1	1	1	2	2	1	1	4	1	2
<i>S aureus</i> abscess (10–15 days)	6	1	1	1	1	1	2	0	1	1	2	1	2	2	4	1	3
Glioma	27	1	2	1	1	1	1	0	1	1	2	2	2	2	4	1	2
Human studies																	
<i>S aureus</i> abscess (ex vivo pus)	12	1	1	1	2	2	1	1	1	1	2	2	1	2	4	1	3
<i>S aureus</i> abscess (in vivo)	7	ND	2	1	1	1	1	0	0	0	2	0	1	1	4	1	3
GBM (in vivo)	10	ND	2	1	0	2	1	0	0	0	2	0	1	1	4	1	3

Note.—Integrals were normalized to the total integral in the chemical shift region of 0.60–4.15 ppm. Most intense metabolites were identified with two-dimensional MR spectroscopy. Intensities are 4, >30%; 3, 15%–30%; 2, 5%–15%; and 1, <5% of the total integral. Assignment of dominating resonances in the respective regions was confirmed with two-dimensional homo- and heteronuclear correlation spectra. Abbreviations for metabolites: ac = acetate, chol = choline-containing metabolites (choline, glycerophosphocholine, phosphocholine), cre = creatine, GB = glycine betaine, H α = α protons of amino acid residues, inos = *myo*-inositol, lac = lactate, lip = triglyceride, NAA = *N*-acetylaspartate, and tau = taurine. All other abbreviations refer to amino acid residues. ND = no data.

Figure 2. Confirmation of the resonance assignment of pus from an *S aureus* abscess in a rat (after 8 days). *A*, One-dimensional ^1H MR spectrum. *B*, Spin-echo spectrum (echo time, 30 msec). *C*, Spin-echo spectrum (echo time, 135 msec). *D*, Two-dimensional ^1H , ^1H correlation spectrum. *E*, ^1H , ^{13}C Heteronuclear single quantum coherence spectrum (optimized to $^1\text{J}_{\text{C,H}} = 130$ Hz). Labeled cross-peaks refer to *bet* = glycine betaine, *gly* = glycerol residue, *lac* = lactate, *lip* = triglycerides, *lys* = lysine, and *tau* = taurine. All other labels refer to amino acid residues, abbreviations of which are expanded in the caption of Figure 1.

SCS

Rank-order first derivatives of MR spectra from rat abscesses after 6–8 days ($n = 42$), saline-injected and noninjected control animals ($n = 37$), and rat gliomas reported in a previous study ($n = 27$) (26) were classified according to SCS, which is based on identification of the most discriminatory regions of the MR spectra by the optimal region selector, followed by pairwise linear discriminant analysis classification (Table 3). Crisp classification

with high accuracy was obtained, indicating separation between the three groups. Although the metabolite profiles from glioma and *S aureus* abscesses appeared similar, an overall crispness of classification of 96% and accuracy of 99% was achieved. Spectral regions identified by genetic algorithm-based optimal region selection and used for the classifications are shown in Table 3. The most discriminatory regions for *S aureus* abscesses versus glioma were at 2.18–2.25

ppm (lipid, glutamine and/or glutamate) and 2.64–2.74 ppm (asparagine/aspartate). For *S aureus* abscesses versus normal brain tissue, they were at 1.33–1.41 ppm (most likely due to increased lipid and lactate in *S aureus* abscesses) and 1.94–2.01 ppm (most likely due to decreased *N*-acetylaspartate and increased glutamine and/or glutamate levels in *S aureus* abscesses).

An independent test set containing MR spectra of control tissue ($n = 5$), glioma

TABLE 3
Classification of MR Spectra from the Animal Models by Using SCS

Parameter	No. of Animals	Crisp (%) [*]	Fuzzy [†]	Sensitivity (%)	Specificity (%)	No. of Misclassifications	Overall Accuracy (%)	Chemical Shift Regions Identified by the Optimal Region Selector
<i>S aureus</i> abscess	42	100	0	100	100	0	100	1.33–1.41
Versus control	37		0			0		1.94–2.01
<i>S aureus</i> abscess	42	96	0	98	100	1	99	2.18–2.25
Versus glioma	27		3			0		2.64–2.74
Glioma	27	100	0	100	100	0	100	1.73–1.83
Versus control	37		0			0		3.68–3.72

^{*} See Materials and Methods.

[†] Refers to samples that were not assigned to one of the classes with a probability of more than 75%.

($n = 6$), and abscess ($n = 6$) was compared against the classifiers; this resulted in correct classification except for one glioma, which was classified as indeterminate.

Human MR spectroscopic Studies

Figure 3 shows MR images and spectra from patients with *S aureus* brain abscess. MR spectra from patients with *S aureus* abscesses were compared with representative spectra from cystic GBM (Fig 3). Control MR spectra from the contralateral hemisphere of patients with staphylococcal abscesses were normal for the location of the lesion and age of the patients (data not shown) (31). MR spectra from all seven staphylococcal abscesses lacked *N*-acetylaspartate and were dominated by resonances from lipids and lactate, similar to spectra from patients with GBM (Fig 3) (31,32). The presence of lactate was confirmed with phase inversion in MR spectra acquired with an echo time of 135 msec. No resonances from choline- or creatine-containing metabolites were present in five of the seven *S aureus* abscess spectra, and in two, the choline-to-creatine ratio was increased marginally (1.1 and 1.3, respectively). No resonances arising from acetate, succinate, or amino acid residues were detected, in contrast to spectra from mixed anaerobic bacterial abscesses (5–7,11).

Diffusion-weighted MR images from two of the seven patients resulted in apparent diffusion coefficients of 0.65 and $1.31 \times 10^{-3} \text{ mm}^2 \cdot \text{sec}^{-1}$.

Human Pus Samples

MR spectra from human pus samples are shown in Figure 1, and results are summarized in Table 2. The metabolite profile was independent of collection site (brain, $n = 4$; surgical wounds, $n = 3$; and pelvis, $n = 5$). All MR spectra were dominated by lipid and lactate signals.

Resonances from amino acid residues (alanine, asparagine and/or aspartate, isoleucine, glutamine and/or glutamate, leucine, lysine, valine, threonine) were of low and variable relative concentration (Table 2). Small amounts of acetate were detected in six of the 12 samples. Taurine was identified in all samples and confirmed by means of $\{^1\text{H}, ^1\text{H}\}$ correlation MR spectroscopy (3.28–3.50 ppm). Glycine betaine was confirmed in five samples by using heteronuclear single quantum coherence and heteronuclear multiple bond correlation spectra. These MR spectra resembled those of the pus from stages II to III of the rat brain abscesses (Fig 1). MR spectra of the four samples collected from *S aureus* brain abscesses were analyzed by the SCS classifiers developed from the MR spectra of the animal models and were classified correctly as *S aureus* abscess.

DISCUSSION

A reliable rat model of brain abscess due to *S aureus* was developed by using stereotactic intracerebral injection of an isolate of *S aureus* obtained from a human brain abscess. Agar beads or other “adjuvants” used by others in experimental brain abscesses (33) were not used in our model. Although this resulted in a marginally lower success rate, the histologic appearance and size of the abscesses were similar to those reported previously (33). Importantly, interference with MR experimental results due to the adjuvant materials was avoided. Evolution from an early histopathologic stage of cerebritis to late mature abscess formation with central purulent material and a defined capsule over a period of 15 days was accompanied by changes in metabolite profiles in concurrent MR spectra. As with pus from human staphylococcal abscesses, MR spectra from mature abscesses in rats

were dominated by lipid and lactate. The neuronal marker *N*-acetylaspartate was absent, and signals from creatine- and choline-containing compounds were much reduced, indicating that this experimental model was representative of human staphylococcal abscesses.

The respective contribution of metabolites of bacterial and host origin to the MR spectra were determined by comparing metabolite profiles of *S aureus* cultured under reducing conditions (representative of the abscess environment) with those of pus samples from experimental and human brain abscesses. MR spectra from *S aureus* cell suspensions were dominated by glycine betaine, a constitutively expressed osmotic protectant (34) that was increased under conditions of increased osmotic stress. Large amounts of lactate were released into the growth medium under culture conditions similar to the abscess environment. *S aureus* is a facultative anaerobe that produces lactate as its predominant metabolic end product (35). Acetate, which is often referred to as a “marker” metabolite for in vivo diagnosis of bacterial abscesses at MR spectroscopy (5–7,10–13), was either absent or present in only very small amounts.

The metabolite profile of staphylococcal pus from humans and rats was distinguished from that of cultured *S aureus* by the presence of substantial amounts of taurine and lipids and relatively little glycine betaine. Lactate resonances were comparable to those in spectra of *S aureus* cultured anaerobically. Lipid resonances are characteristically present in MR spectra from patients with brain abscesses (5–7,10). They most likely arise from the mass of activated and damaged neutrophils that comprise virtually all of the host cells in pyogenic brain abscesses, since they dominate MR spectra from activated (36) and apoptotic (37) neutrophils and necrotic tissue (38). Taurine

Figure 3. Representative in vivo MR images and spectra from patients with cerebral abscesses caused by *S aureus* and GBM. MR images and spectra were acquired with clinical 1.5-T MR imagers from (a) patient 4, (b) patient 5, (c) patient 1, and (d) a patient with a cystic GBM. Transverse T2-weighted MR images (2200/80) are shown in the top row. MR spectra were acquired from the volume of interest centered within the abscess and outlined on the images. Middle row: stimulated-echo acquisition sequence (3000/20 or 30). Bottom row: point-resolved MR spectroscopy sequence (3000/135). Volume of interest, 8–12 cm³.

was also prominent in spectra of activated neutrophils; its role is not understood (36). Glycine betaine was abundant in cultured cells, but in pus samples, at most, trace amounts were seen.

Abscesses caused by anaerobic bacteria have been distinguished from those caused by microaerophilic bacteria (like *S aureus*) based on metabolites detectable with MR spectroscopy (acetate, succinate) (7,10). However, the relative amounts of lipids and lactate, though derived from different cell types, overlapped in MR spectra from staphylococcal abscesses and GBMs. Diagnosis based on such subjectively selected metabolites or metabolite ratios was not possible. Indexes often used for inter-

preting clinical MR spectra, such as choline-to-creatine ratios, were similarly unsuitable because of substantial overlap between the two categories. In contrast, pattern analysis by means of a genetic algorithm-based optimal region selector, which selects the most discriminatory regions between the classes of interest (29), resulted in the development of successful classifiers. Resonances in the regions selected by using the optimal region selector were assigned by using two-dimensional MR techniques. These indicated that glutamine and/or glutamate and aspartate were the compounds most likely responsible for distinguishing between GBMs and *S aureus* abscesses, whereas

lactate was most likely responsible for successful distinction of GBM and *S aureus* abscesses from normal brain tissue. SCS has the advantage of objectivity, sensitivity, and comparison against a set of classifiers developed from a large (and hence more representative) data set for each diagnostic class (17).

Human MR images acquired from *S aureus* brain abscesses and GBMs were not distinguishable (hypointensity on T1-weighted MR images, hyperintensity on T2-weighted MR images, and ring enhancement after administration of gadolinium chelate). Diffusion-weighted MR images resulted in a relatively wide range of apparent diffusion coefficients for the *S aureus* abscesses, as reported previously (8).

This is most likely due to variability in the viscosity of the abscess fluid, depending on the age of the abscess. Although apparent diffusion coefficients are in most cases lower in GBMs than in abscesses (8,9), this finding is not specific and does not allow different etiologies to be distinguished.

Although the classification of MR spectra from pus of staphylococcal abscesses and GBM in an animal model was successful, the results are not directly translatable to in vivo diagnosis in humans. More in vivo MR spectroscopy data from *S aureus* abscesses are required for the development of reliable SCS classifiers. Systematic collection of spectra from clinical cases is slow because of the relatively small number of cases.

MR spectra obtained in vivo from *S aureus* brain abscesses were dominated by resonances from lipids and lactate and resembled spectra of GBM. Typical features of MR spectra from anaerobic bacterial abscesses, such as succinate or acetate and increased amino acid resonances (5–7,10), were absent. Thus, interpretation of in vivo MR spectra by means of resonance assignment could allow us to distinguish between abscesses due to *S aureus* and those where anaerobic bacteria are present, but not between *S aureus* abscesses and GBM. The success of the SCS on MR spectra from pus to distinguish between GBM and *S aureus* abscess in rats and the similarity of human ex vivo and in vivo MR spectroscopy data from abscesses are promising for diagnostic applications. It now remains to be seen if adequate numbers of in vivo MR spectra from patients with *S aureus* abscesses can be collected to develop robust classifiers.

If so, cerebral *S aureus* abscesses might no longer be mistaken for malignancies, as is currently the case by using conventional imaging modalities. An early and correct diagnosis will reduce the high morbidity and mortality rates that occur when diagnosis is delayed. Introduction of in vivo MR spectroscopy as a noninvasive method of diagnosis of infective lesions in the brain will reduce the risk and expense of unnecessary surgery or biopsy and expedite patient treatment decisions.

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References

1. Calfee DP, Wispelway B. Brain abscess. *Semin Neurol* 2000; 20:353–360.
2. Mathisen GE, Johnson JB. Brain abscess. *Clin Infect Dis* 1997; 25:763–781.
3. Haimes AB, Zimmerman R, Morgello A, et al. MR imaging of brain abscesses. *AJR Am J Roentgenol* 1989; 152:1073–1085.
4. Sharma BS, Khosla VK, Kak VK, et al. Multiple pyogenic brain abscesses. *Acta Neurochir (Wien)* 1995; 133:36–43.
5. Remy C, Grand S, Lai ES, et al. 1H MRS of human brain abscesses in vivo and in vitro. *Magn Reson Med* 1995; 34:508–514.
6. Dev R, Gupta RK, Poptani H, Roy R, Sharma S, Husain M. Role of in vivo proton magnetic resonance spectroscopy in the diagnosis and management of brain abscesses. *Neurosurgery* 1998; 42:37–42.
7. Garg M, Gupta RK, Husain M, et al. Brain abscesses: etiologic characterization with in vivo proton MR spectroscopy. *Radiology* 2004; 230:519–527.
8. Leuthardt EC, Wippold FJ, Oswood MC, Keith MR. Diffusion-weighted MR imaging in the preoperative assessment of brain abscesses. *Surg Neurol* 2002; 58:395–402.
9. Nadal Desbarats L, Herlidou S, de Marco G, et al. Differential MRI diagnosis between brain abscesses and necrotic or cystic brain tumors using the apparent diffusion coefficient and normalized diffusion-weighted images. *Magn Reson Imaging* 2003; 21:645–650.
10. Himmelreich U, Dzendrowskyj TE, Bourne R, Mountford CE, Sorrell TC. MR spectroscopy and infectious diseases. *MAGMA* 2000; 11(suppl 1):197.
11. Grand S, Passaro G, Ziegler A, et al. Necrotic tumor versus brain abscess: importance of amino acids detected at 1H MR spectroscopy—initial results. *Radiology* 1999; 213:785–793.
12. Gupta RK, Vatsal DK, Husain N, et al. Differentiation of tuberculous from pyogenic brain abscesses with in vivo proton MR spectroscopy and magnetization transfer MR imaging. *AJNR Am J Neuroradiol* 2001; 22:1503–1509.
13. Shukla-Dave A, Gupta RK, Roy R, et al. Prospective evaluation of in vivo proton MR spectroscopy in differentiation of similar appearing intracranial cystic lesions. *Magn Reson Imaging* 2001; 19:103–110.
14. De Louvois J, Gortvai P, Hurley R. Bacteriology of abscesses of the central nervous system: a multicenter prospective study. *BMJ* 1977; 2:981–984.
15. Mampalam TJ, Roseblum ML. Trends in the management of bacterial brain abscess: a review of 102 cases over 17 years. *Neurosurgery* 1988; 23:451–458.
16. Lakshmi V, Rao RR, Dinakar I. Bacteriology of brain abscess—observations on 50 cases. *J Med Microbiol* 1993; 38:187–190.
17. Somorjai R, Dolenko B, Nikulin A, Nickerson P, Rush D, Shaw A. Distinguishing normal from rejecting renal allografts: application of a three-stage classification strategy to MR and IR spectra of urine. *Vibr Spectrosc* 2002; 28: 97–102.
18. Hagberg G. From magnetic resonance spectroscopy to classification of tumors: a review of pattern recognition methods. *NMR Biomed* 1998; 11:148–156.
19. Holmes E, Nicholls AW, Lindon JC, et al. Development of a model for classification of toxin-induced lesions using ¹H NMR spectroscopy of urine combined with pattern recognition. *NMR Biomed* 1998; 11:235–244.
20. Poptani H, Kaartinen J, Gupta RK, Niemitz M, Hiltunen Y, Kauppinen RA. Diagnostic assessment of brain tumors and non-neoplastic brain disorders in vivo using proton nuclear magnetic resonance spectroscopy and artificial neural networks. *J Cancer Res Clin Oncol* 1999; 125:343–349.
21. Himmelreich U, Somorjai RL, Dolenko B, et al. Rapid identification of chemical characterization of *Candida* species using nuclear magnetic resonance spectroscopy and a statistical classification strategy. *Appl Environ Microbiol* 2003; 69:4566–4574.
22. Mountford CE, Somorjai RL, Malycha PL, et al. Diagnosis and prognosis of breast cancer by magnetic resonance spectroscopy of fine needle aspirates analysed using a statistical classification strategy. *Br J Surg* 2001; 88:1234–1240.
23. Soper R, Himmelreich U, Painter D, et al. Pathology of hepatocellular carcinoma and its precursors using proton magnetic resonance spectroscopy and a statistical classification strategy. *Pathology* 2002; 34:417–422.
24. Remy C, Arus C, Ziegler A, et al. In vivo, ex vivo, and in vitro one- and two-dimensional nuclear magnetic resonance spectroscopy of an intracerebral glioma in rat brain: assignment of resonances. *J Neurochem* 1994; 62: 166–179.
25. Hwang TL, Shaka AJ. Water suppression that works: excitation sculpting using arbitrary waveforms and pulse field gradients. *J Magn Reson* 1995; 112:275–279.
26. Himmelreich U, Dzendrowskyj T, Allen C, et al. Cryptococcomas distinguished from gliomas with magnetic resonance spectroscopy: an experimental rat and cell culture study. *Radiology* 2001; 220:122–128.
27. Palmer AG 3rd, Cavanagh J, Wright PE, Rance M. Sensitivity improvement in proton-detected two-dimensional heteronuclear correlation NMR spectroscopy. *J Magn Reson* 1991; 93:151–170.
28. Shaka AJ, Barker PB, Freeman R. Computer-optimized decoupling scheme for wideband applications and low-level operation. *J Magn Reson* 1985; 64:547–552.
29. Nikulin A, Dolenko B, Bezabeh T, Somorjai R. Near-optimal region selection for feature space reduction: novel preprocessing methods for classifying MR spectra. *NMR Biomed* 1998; 11:209–217.
30. Efron B, Tibshirani R. An introduction to the bootstrap: monographs of statistics and applied probability. Vol 57. New York, NY: Chapman & Hill, 1993.
31. Danielsen ER, Ross BD. Magnetic resonance spectroscopy diagnosis of neurological diseases. New York, NY: Dekker, 1998.
32. Negendank W. Studies of human tumors by MRS: a review. *NMR Biomed* 1992; 5:303–324.
33. Flaris NA, Hickey WF. Development and characterization of an experimental model of brain abscess in the rat. *Am J Pathol* 1992; 141:1299–1307.
34. Kunin CM, Rudy J. Effect of NaCl-induced osmotic stress on intracellular concentrations of glycine betaine and potassium in *Escherichia coli*, *Enterococcus faecalis* and staphylococci. *J Lab Clin Med* 1991; 118:217–224.
35. Schlegel HG. Allgemeine mikrobiologie. Stuttgart, Germany: Thieme, 1992.
36. May G, Szelma K, Sorrell TC, Mountford CE. Comparison of human polymorphonuclear leukocytes from peripheral blood and purulent exudates by high resolution 1H MRS. *Magn Reson Med* 1991; 19:191–198.
37. Wright LC, Groot-Obbink K, Delikatny EJ, Santangelo RT, Sorrell TC. The origin of ¹H NMR-visible triacylglycerol in human neutrophils: high fatty acid environments result in preferential sequestration of palmitic acid into plasma membrane triacylglycerol. *Eur J Biochem* 2000; 267:68–78.
38. Zoula S, Herigault G, Ziegler A, Farion G, Decors M, Remy C. Correlation between the occurrence of H-1-MRS lipid signal, necrosis and lipid droplets during C6 rat glioma development. *NMR Biomed* 2003; 16:199–212.