Proton Nuclear Magnetic Resonance–Based Metabonomics for Rapid Diagnosis of Meningitis and Ventriculitis

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Background. Reduction of mortality associated with bacterial meningitis and postsurgical cerebral ventriculitis is dependent on early diagnosis and institution of appropriate therapy. Metabonomics rapidly defines metabolic profiles of biological fluids through the use of high-throughput analytical techniques combined with statistical pattern recognition tools.

Methods. Proton nuclear magnetic resonance (1H NMR)–based metabonomics was applied to (1) lumbar cerebrospinal fluid samples collected prospectively from a cohort of patients with bacterial, fungal, or viral meningitis and from control subjects without neurological disease and (2) ventricular cerebrospinal fluid samples from patients with ventriculitis associated with an external ventricular drain and from control subjects. 1H NMR spectra were analyzed by the unsupervised statistical method of principal components analysis.

Results. Metabonomic analysis clearly distinguished patients with bacterial or fungal meningitis (11 patients) from patients with viral meningitis (12) and control subjects (27) and clearly distinguished patients with postsurgical ventriculitis (5) from postsurgical control subjects (10). Metabolites of microbial and host origin that were responsible for class separation were determined. Metabonomic data also correlated with the onset and course of infection in a patient with 2 episodes of bacterial ventriculitis and with response to therapy in another patient with cryptococcal meningitis.

Conclusions. Metabonomic analysis is rapid, requires minimal sample processing, and is not targeted to specific microbial pathogens, making the platform potentially suitable for use in the diagnostic laboratory. This pilot study indicates that metabonomic analysis of cerebrospinal fluid is feasible and a potentially more powerful diagnostic tool than conventional rapid laboratory indicators for distinguishing bacterial from viral meningitis and for monitoring therapy. This should have important implications for early management, reduced empirical use of antibiotics, and treatment duration.

Bacterial meningitis is a medical emergency that must be distinguished rapidly from viral and other causes of acute meningitis. Associated mortality is high, with severe neurological sequelae described in 25% of cases [1, 2]. Prognosis is directly related to the rapidity with which the diagnosis is established and therapy initiated [3, 4]. Identification of the causative organism from cultures of CSF is the most sensitive routine test, but this method is slow. Rapid screening tests (e.g., CSF pleocytosis tests, protein tests, glucose tests, and Gram stain) are nonspecific and/or insensitive. A review of 696 adults with bacterial meningitis found that 12% lacked even 1 abnormality in CSF cell count, protein concentration, or glucose concentration that was predictive of bacterial meningitis [5]. Empirical antimicrobial therapy improves outcomes but adds costs, may be ineffective against emerging β-lactam–resistant pathogens, or is unnecessary (e.g., in cases of viral meningitis), and adverse reactions are potentially problematic. Postsurgical ventriculitis and meningitis are additional diagnostic problems in large urban hospitals, accounting for up to 40% of cases of bacterial meningitis [6]. Ventricular CSF leukocyte counts are typically elevated after ventriculostomy.

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and isolation of bacteria may represent contamination or colonization of an external ventricular drain (EVD), rather than established ventriculitis [7]. Thus, there is much interest in developing rapid, sensitive, and accurate diagnostic methods that distinguish bacterial from viral and other causes of a meningitis syndrome.

Metabonomics is the study of the metabolic composition of biological fluids and tissues [8, 9] and combines high throughput spectroscopic techniques, such as nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry, with statistical tools. A unique metabolic fingerprint reflecting the physiological state of the organism and host environment is generated for each sample. Pattern recognition methods are used to reduce multivariate NMR spectral data into 2 or 3 dimensions, such that clustering of samples on the basis of similarities of biochemical profiles can be observed. This approach was successful in determining the severity of coronary heart disease by analysis of serum samples [10]. Metabonomic studies of CSF samples have been performed in cases of subarachnoid hemorrhage [11], Alzheimer disease [12], Reye syndrome [13], and diabetes mellitus [14]. Early studies of CSF samples included cases of meningitis [14–16]. In the present study, we demonstrate the feasibility of applying metabonomics to rapid diagnosis of community-acquired meningitis and EVD-associated ventriculitis.

PATIENTS AND METHODS

Study population. Surplus CSF samples obtained for diagnostic tests in adults with community-acquired meningitis or EVD-associated ventriculitis and control samples obtained prospectively at Westmead Hospital, Sydney, Australia, between August 2003 and November 2004 were stored at −80°C. Approval was granted by the Human Ethics Committee of the Western Sydney Area Health Service. Written informed consent was obtained from patients or their representatives before CSF analysis. Categorization of patients and control subjects was made independently (by M.O’S.) by review of consecutive CSF sample laboratory results, patient records (for evidence of fever, headache, meningismus, or altered mental status), and imaging studies. Patients who had received therapeutic doses of relevant antibiotic(s) for >18 h (or 1 dose in cases of meningococcal meningitis) were excluded from the study. When necessary, clinicians were contacted for information.

CSF analysis. Measurement of leukocyte counts, protein levels, and glucose levels; performance of Gram stains and special microbial stains; and performance of bacterial, mycobacterial, fungal, and viral cultures were done by standard methods. Cryptococci were identified by a latex agglutination antigen test (Meridian Bioscience) and/or culture. PCR was performed if clinically indicated for enterovirus [17, 18], herpes simplex virus [19], Neisseria meningitidis [20], and Mycobacterium tuberculosis (COBAS Amplicor MTB Test; Roche Diagnostics).

Definitions. CSF leukocyte count >5 × 10⁶ leukocytes/L, protein level >450 mg/L, and glucose level <2.8 mmol/L (or CSF:serum ratio of <0.4) were considered to be abnormal. Bacterial meningitis was defined as neutrophil pleocytosis with a positive culture and/or PCR result. If antibiotics were administered before a CSF sample was collected, a positive culture or PCR result from a blood sample, CSF pleocytosis, and an elevated CSF protein level (with or without a positive Gram stain or PCR result) were required. Ventriculitis was defined as a positive bacterial culture of a CSF sample obtained at least 24 h after insertion of the EVD, neutrophil pleocytosis of ≥10 × 10⁶ neutrophils/L (with or without a positive Gram stain result), otherwise unexplained fever, and at least one of either increased headache, increased neck stiffness, or new impairment of mental status [21]. Viral meningitis was defined as CSF pleocytosis with positive culture or PCR result. Cryptococcal meningitis was defined as abnormal biochemistry and/or pleocytosis with a cryptococcal antigen titer of >1:8 or a culture yielding Cryptococcus neoformans. Tuberculous meningitis was defined as abnormal biochemistry and/or pleocytosis plus a PCR and/or culture result positive for M. tuberculosis. Control subjects were defined as having a leukocyte count of <5 × 10⁶ leukocytes/L, a glucose level of ≥2.5 mmol/L (or a CSF:serum glucose ratio of >0.5), and a normal or near-normal protein concentration. Control subjects for community-acquired meningitis had no clinical or radiological evidence of cerebral pathology.

Sample preparation. CSF samples (600 μL total, containing 400 μL CSF and 150 μL saline or 200 μL CSF and 350 μL saline, plus 50 μL sodium 3-trimethylsilylpropionate in D₂O [1 mg/mL]) were prepared in 5-mm NMR spectroscopy tubes. Sodium 3-trimethylsilylpropionate solution was added as an internal chemical shift standard (1H chemical shift, 0.0 ppm) and to provide a field-frequency lock. The 200-μL CSF samples were analyzed with compensatory acquisition of NMR spectral data for longer times, and therefore, the differential CSF volume did not affect the pattern recognition analysis. To remove systematic variation caused by differences in concentrations between samples, the spectral data were normalized on the basis of the total spectral integral.

NMR spectroscopy. Spectra were acquired at 1H frequency of 400.13 MHz on a DRX-400 widebore spectrometer (Bruker) with a probehead temperature of 25°C. Typically, 96 transients were collected over a spectral width of 4000 Hz with a relaxation delay of 3 s and an acquisition time of 1.5 s. A line-broadening factor of 1.0 Hz was applied before Fourier transformation. For 200-μL CSF samples, the number of transients was increased to 384. Signal assignment was based on 1H–1H correlation spectroscopy (using magic-angle gradients) [22], 1H–13C heteron-
nuclear single quantum coherence, and $^1$H–$^{13}$C heteronuclear multi-bond correlation spectral data for representative samples. Standard Bruker pulse sequences (Xwinnmr software, version 3.5 [Bruker]) were used, with the $^1$H–$^{13}$C Heteronuclear Single Quantum Coherence and $^1$H–$^{13}$C Heteronuclear Multi-Bond Correlation experiments optimized for $J_{CH}$ and $J_{CH}$ of 145 and 6.25 Hz, respectively.

Data reduction and preprocessing. Following phase and baseline correction, each spectrum was reduced to regions of equal width (0.04 ppm) using Amix software, version 3.2 (Bruker Biospin). The region from 4.5 to 0.5 ppm was chosen to represent CSF spectral data, because it contained the most information and enabled complete removal of the broad water resonance from analyses. Integrals were measured for each of the 100 spectral regions, and subdivided spectra were normalized. All spectral data were mean centered to reposition the coordinate system so that the origin was also the mean point of the data set. This improves interpretability of the final model. In addition, Pareto-scaling using a weight of 1/$S_k$ was applied, where $S_k$ is the standard deviation of variable $k$ [23]. This technique facilitates determination of contributions to the multivariate model from both low- and high-intensity spectral peaks.

Principal components analysis (PCA). Pattern recognition was performed using Simca P-10+ software (Umetrics AB). PCA groups data in an unbiased way and is a “unsupervised” approach; inherent clustering behaviour of samples is ascertained with no a priori knowledge of class membership [24].

Of $n$ principal components ($PC$s) identified by the analysis, the first (PC1) is a linear combination of the original input variables and describes the largest variation in the data set. The second (PC2) describes the next-largest variation. When 2 PCs have been defined, they constitute a plane. Projection of the observation vectors in the multidimensional space onto this plane enables the data to be visualized in a 2-dimensional map known as a “scores plot.” This plot reveals inherent clustering of groups of data, based on the closeness or similarity of their input coordinates. A so-called “loadings plot” is used to interpret the scores plot. In the case of NMR spectra, the spectral variables contributing most to the positioning of the observation vector on the scores plot are determined, and those variables that influence any observed separation between members of the spectral data set are identified.

Student’s $t$ test. A 2-tailed, unequal variance $t$ test was computed for the PC scores data. The mean values ($\pm$ SD) and $t$ test probabilities were calculated for patients with bacterial or fungal meningitis, viral meningitis, and ventriculitis, as well as for respective control subjects.

RESULTS

Subjects with bacterial or fungal meningitis (11 subjects), viral meningitis (12), normal lumbar CSF samples (27), bacterial ventriculitis (5), and normal ventricular CSF samples (10) were included in the study. Five patients with bacterial meningitis had received antibiotics up to 15 h before CSF was sampled.

$^1$H NMR spectroscopy. $^1$H NMR spectra for patients were distinctly different from those for control subjects (figure 1). Resonances attributable to glucose, lactate, pyruvate, citrate, glutamine, glutamate, creatine and creatinine, 3-hydroxybutyrate, acetoacetate, acetone, acetate, alanine, valine, isoleucine, and leucine were assigned by comparison of their $^1$H and $^{13}$C chemical shifts (determined from homonuclear and heteronuclear correlation experiments) with literature data. Dominant biochemical changes on visual inspection were an elevated lactate level and depleted glucose level. Concentrations of lower-intensity metabolites that were altered included pyruvate, citrate, acetate, alanine, and the ketone bodies 3-hydroxybutyrate, acetoacetate, and acetone.

PCA of community-acquired meningitis. Pattern recognition provides an objective and simple means of identifying dominant and subtle spectral changes in a large sample cohort. PCA applied to all samples produced a scores plot with excellent separation in PC1 between control and bacterial meningitis classes (figure 2A). Separation between control and viral meningitis classes was highly significant ($P<.0001$). A model comprising 2 principal components explained 91% of the variation in the data, with PC1 and PC2 explaining 72% and 19%, respectively.

There is a suggestion of clustering by pathogen (see *Streptococcus pneumoniae* and *N. meningitidis*), which primarily reflects the extent of derangement of brain or CSF metabolism and the host (metabolic) response but includes a contribution from microbial metabolites, such as acetate and lactate (table 1) [25, 26]. Data from cases with the most marked changes in protein and glucose levels and cell counts lay furthest from data from control subjects. The 3 cases of *S. pneumoniae* meningitis had CSF glucose levels of 0, 0.1, and 0.2 mmol/L and neutrophil counts of $7335 \times 10^6$, $13000 \times 10^6$, and $14880 \times 10^6$ neutrophils/L. Despite high neutrophil counts in many samples (table 1), lipids characteristic of inflammatory neutrophils [25, 27] were not identified in unspun samples, and removal of leukocytes by centrifugation did not affect the NMR spectra. The data point representing 1 patient with enterovirus meningitis mapped with the control group. The patient had a very low CSF viral load, normal glucose level, and low-grade infection (leukocyte count, $7 \times 10^4$ leukocytes/L; glucose level, 2.9 mM).

Loadings plots reveal the most-influential spectral regions (metabolites) responsible for the observed separation. On the basis of a mean-centered loadings plot (data not shown), the metabolites with the highest weighting were lactate and glucose, which were greatly increased and significantly reduced, respectively, in cases of bacterial or fungal meningitis. Additional lower-intensity metabolites were elevated in these patients; those contributing to class differentiation by PCA were
identified from the Pareto-scaled model as 3-hydroxybutyrate, pyruvate, acetate, acetone, isoleucine, leucine, and valine.

**PCA of EVD-associated ventriculitis.** PCA was applied separately to the postneurosurgical group, because ventricular CSF samples were analyzed. The scores plot of PC1 versus PC2 reveals a high degree of separation in PC1 between the 2 classes (figure 2B). A loadings plot of the corresponding Pareto-scaled model revealed that the dominant metabolites responsible for separation in the scores plot were glucose, lactate, 3-hydroxybutyrate, pyruvate, acetone, and amino acids.

**Trajectory case study 1.** A PC trajectory plot of 4 samples collected over a 3-month period from a patient with cryptococcal meningitis is shown in figure 3A. Two pretreatment spectra (samples 1 and 2) were clearly separated from the control multidimensional space (C) represented in the scores plot. Spectra after 6 weeks of therapy with amphotericin B deoxycholate and/or liposomal amphotericin B plus fluconazole therapy (sample 3) and 7 weeks later (while receiving fluconazole), when a relapse was suspected but excluded following further investigation (sample 4), returned to the control space. Clinical parameters reflected the trajectory of disease progression and recovery. The spectrum of sample 2 (figure 1C) illustrates that the major metabolic alterations creating separation in the scores plot were low a glucose level and elevated lactate and alanine levels.

**Trajectory case study 2.** Figure 3B shows a PC trajectory plot for a patient with 2 episodes of ventriculitis due to different pathogens (*Weeksella virosa*, which is a gram-negative bacillus, and *Staphylococcus epidermidis*) over a 6-week period. CSF spectra (samples 1 and 6) at the onset of each infection mapped away from the control space, indicative of metabolic changes caused by infection. Sample 2, obtained a day after initiating meropenem therapy for the *Weeksella* infection, mapped to the control space, as did samples 3 and 4. Sample 5 mapped outside of the control space, suggestive of recurrent infection prior to obtaining the positive *S. epidermidis* sample (sample 6). Sam-
Table 1. Demographic and CSF characteristics of subjects with meningitis or ventriculitis.

<table>
<thead>
<tr>
<th>Subject, by diagnosis</th>
<th>Pathogen</th>
<th>Age, years</th>
<th>Sex</th>
<th>Cell count $\times 10^6$ cells/L, by cell type</th>
<th>Gram stain result</th>
<th>Protein level, mg/L</th>
<th>Glucose level, mg/L</th>
<th>PCR result</th>
<th>Culture result</th>
<th>Prior antimicrobial therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial, fungal, or tubercular meningitis</td>
<td>Cryptococcus gattii</td>
<td>35 M</td>
<td>338 506 0</td>
<td>Yeast</td>
<td>1190</td>
<td>1.4</td>
<td>NA</td>
<td>Positive</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Streptococcus agalactiae</td>
<td>0 M</td>
<td>4575 675</td>
<td>Negative</td>
<td>490</td>
<td>1.9</td>
<td>Positive</td>
<td>Positive</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mycobacterium tuberculosis</td>
<td>30 M</td>
<td>63 83 7</td>
<td>Negative</td>
<td>423</td>
<td>2.4</td>
<td>Negative</td>
<td>Positive</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M. tuberculosis</td>
<td>34 F</td>
<td>19 29 23</td>
<td>Negative</td>
<td>430</td>
<td>2.5</td>
<td>Negative</td>
<td>Positive</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neisseria meningitidis</td>
<td>53 M</td>
<td>1050 50 400</td>
<td>Negative</td>
<td>5090</td>
<td>0.1</td>
<td>Positive</td>
<td>Negative</td>
<td>Yes</td>
<td></td>
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<tr>
<td></td>
<td>N. meningitidis</td>
<td>52 M</td>
<td>1050 50 400</td>
<td>Negative</td>
<td>5090</td>
<td>0.1</td>
<td>Positive</td>
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<td></td>
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<tr>
<td></td>
<td>Streptococcus pneumoniae</td>
<td>62 M</td>
<td>13,000 830 300</td>
<td>Negative</td>
<td>3230</td>
<td>0.1</td>
<td>NA</td>
<td>Negative</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>S. agalactiae</td>
<td>0 M</td>
<td>4500 450 38,250</td>
<td>Negative</td>
<td>2200</td>
<td>1.4</td>
<td>NA</td>
<td>Negative</td>
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<tr>
<td></td>
<td>S. pneumoniae</td>
<td>17 M</td>
<td>735 235 180</td>
<td>Negative</td>
<td>4370</td>
<td>0</td>
<td>NA</td>
<td>Negative</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S. pneumoniae</td>
<td>61 M</td>
<td>14,880 25 290</td>
<td>GPC</td>
<td>6000</td>
<td>0.2</td>
<td>NA</td>
<td>Positive</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Ventriculitis</td>
<td>Staphylococcus aureus</td>
<td>39 M</td>
<td>17,550 0 1800</td>
<td>GPC</td>
<td>ND</td>
<td>NA</td>
<td>Positive</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Staphylococcus epidermidis</td>
<td>49 F</td>
<td>5325 75 950</td>
<td>GPC</td>
<td>ND</td>
<td>NA</td>
<td>Positive</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S. epidermidis</td>
<td>30 M</td>
<td>200 0 1100</td>
<td>Negative</td>
<td>ND</td>
<td>NA</td>
<td>Positive</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S. pneumoniae</td>
<td>60 F</td>
<td>13 0 79</td>
<td>Negative</td>
<td>ND</td>
<td>NA</td>
<td>Positive</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Weeksella virosa</td>
<td>49 F</td>
<td>125 0 2600</td>
<td>Negative</td>
<td>ND</td>
<td>NA</td>
<td>Positive</td>
<td>No</td>
<td></td>
<td></td>
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<tr>
<td>Viral meningitis or meningoencephalitis</td>
<td>Enterovirus</td>
<td>18 M</td>
<td>25 1184 0</td>
<td>GPC</td>
<td>717</td>
<td>2.8</td>
<td>Positive</td>
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<tr>
<td></td>
<td>Enterovirus</td>
<td>32 F</td>
<td>50 118 48</td>
<td>GPC</td>
<td>555</td>
<td>3.1</td>
<td>Positive</td>
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<tr>
<td></td>
<td>Enterovirus</td>
<td>19 M</td>
<td>0 22 2</td>
<td>GPC</td>
<td>366</td>
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<td>ND</td>
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<tr>
<td></td>
<td>Enterovirus</td>
<td>26 M</td>
<td>135 45 14</td>
<td>GPC</td>
<td>608</td>
<td>3.1</td>
<td>Positive</td>
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<tr>
<td></td>
<td>Enterovirus</td>
<td>0 M</td>
<td>28 160 1</td>
<td>GPC</td>
<td>570</td>
<td>2.6</td>
<td>Positive</td>
<td>ND</td>
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<tr>
<td></td>
<td>Enterovirus</td>
<td>33 F</td>
<td>199 110 7825</td>
<td>GPC</td>
<td>1250</td>
<td>2.8</td>
<td>Positive</td>
<td>ND</td>
<td>No</td>
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<tr>
<td></td>
<td>Enterovirus</td>
<td>12 M</td>
<td>562 22 194</td>
<td>GPC</td>
<td>804</td>
<td>4</td>
<td>Positive</td>
<td>ND</td>
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<tr>
<td></td>
<td>Enterovirus</td>
<td>31 M</td>
<td>4 79 4</td>
<td>GPC</td>
<td>768</td>
<td>3.1</td>
<td>Positive</td>
<td>ND</td>
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<td></td>
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<tr>
<td></td>
<td>Enterovirus</td>
<td>31 F</td>
<td>0 7 42</td>
<td>GPC</td>
<td>455</td>
<td>2.9</td>
<td>Positive</td>
<td>ND</td>
<td>No</td>
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<tr>
<td></td>
<td>HSV</td>
<td>79 M</td>
<td>2 59 4</td>
<td>GPC</td>
<td>1675</td>
<td>3.3</td>
<td>Positive</td>
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<tr>
<td></td>
<td>HSV</td>
<td>28 F</td>
<td>0 180 321</td>
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<td>665</td>
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<td></td>
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<td>0 85 0</td>
<td>GPC</td>
<td>587</td>
<td>3.1</td>
<td>Positive</td>
<td>ND</td>
<td>No</td>
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</tr>
</tbody>
</table>

NOTE. GPC, gram-positive cocci; HSV, herpes simplex virus; Mono, mononuclear; NA, not applicable; ND, not done; Poly, polymorphonuclear.

a) PCR of blood and CSF samples had positive results.
b) Specimen clotted; unsuitable for cell count.
c) Insufficient specimen for analysis.
d) Isolated from blood cultures.
e) Gram stain morphology and clinical syndrome suggestive of S. pneumoniae.

f) Not routinely performed from external ventricular drain specimens.
Figure 2. Principal components analysis scores plots of principal component 1 (PC1) versus principal component 2 (PC2), representing proton nuclear magnetic resonance spectral data from control subjects and subjects with bacterial, fungal, and viral meningitis (A) and principal components analysis scores plots of PC1 versus PC2, representing proton nuclear magnetic resonance spectral data from postneurosurgical control samples and samples from subjects with ventriculitis (B). Samples from patients with ventriculitis are labeled SP, WV, SE, SE, and SA. ●, Control samples; ○, Gram-negative bacterial meningitis; ▲, Gram-positive bacterial or fungal meningitis; △, viral meningitis; CG, Cryptococcus gattii; NM, Neisseria meningitidis; SA, Staphylococcus aureus; SB, Streptococcus agalactiae (group B streptococcus); SE, Streptococcus epidermidis; SP, Streptococcus pneumoniae; TB, Mycobacterium tuberculosis; WV, Weeksella virosa.

DISCUSSION

The data presented in this pilot study highlight the potential of metabonomics for rapid diagnosis of meningitis. There were substantial differences in the metabolite profiles of CSF samples obtained from control subjects and patients with cases of bacterial and viral meningitis when analyzed by PCA. Because the prognosis of bacterial meningitis depends on rapid institution of therapy before there is neurological compromise [2, 3], this technology promises to be a major medical advance. Moreover, potentially toxic and/or costly antibacterial drugs can be avoided in patients with cases of viral meningitis.

Routine rapid tests, such as neutrophil counts and CSF and/or serum glucose ratios, are not specific for bacterial or viral meningitis unless the results are very abnormal [27]. Gram
stains are positive in 60%–90% of cases of bacterial meningitis, depending on the pathogen and previous antibiotic therapy [1]. Where sampling of CSF may be delayed, intravenous antibiotics are frequently administered for suspected bacterial meningitis. This may render Gram stain results negative, especially in cases due to *N. meningitidis*, which is very susceptible to penicillin. In the present study, NMR spectra of CSF specimens from 5 patients who received antibiotics 10 min to 15 h before testing (including 3 patients with Gram stain–negative cases and 2 patients with meningococcal meningitis) mapped
with bacterial meningitis, indicating the potential of metabonomics for rapid diagnosis in this important subgroup.

Disproportionately elevated lactate concentrations have been reported for patients with bacterial meningitis. Lactate levels have been proposed as a more sensitive and specific marker than the CSF:blood glucose ratio in cases of postsurgical meningitis [28]. However, it has not been a useful discriminator in several studies (see [29] and references therein). To improve the utility of routine tests, clinical prediction rules for bacterial meningitis were developed and validated that incorporated at least 3 of the following: CSF protein level, neutrophil count, CSF and/or serum glucose concentration, a seasonality surrogate (month), and age [26, 30]. Because concurrent CSF and blood glucose levels are infrequently obtained and seasonality surrogates are restricted to temperate climates, a simpler multivariate regression model was developed for use in children [31]. This yielded sensitivities of 92% (specificity, 98%) and 98% (specificity, 62%), depending on probability cut off points. The potentially higher discriminatory value (100%) of the metabonomic approach in bacterial meningitis results from the generation of characteristic multivariate spectral profiles (fingerprints) comprised of contributions from multiple metabolites of microbial and host origin.

Low glucose concentrations in patients with bacterial meningitis are primarily caused by suppression of membrane carrier–facilitated glucose transport and a switch to anaerobic metabolism (glycolysis) in the brain due to ischemia and cytokine release [26, 29]. The high lactate levels are mainly generated by cerebral glycolysis, with species-dependent, minor contributions from microbial metabolites and little contribution from inflammatory cells [28]. Consistent with a minimal contribution from leukocytes, lipids characteristic of inflammatory neutrophils [27, 33] were not identified, and NMR spectra for CSF supernatants were similar to those for unspun specimens. Impairment of the citric acid cycle caused by reduced production of acetyl coenzyme A results in accumulation of pyruvate and production of amino acids from pyruvate via transamination. Elevated CSF concentrations of pyruvate and amino acids—particularly alanine, isoleucine, and leucine—were evident in bacterial and fungal meningitis. The presence of the ketone bodies 3-hydroxybutyrate, acetoacetate, and acetone suggests a compensatory response to severe glucose depletion and reduced ATP levels.

The metabonomic approach is promising in EVD-associated ventriculitis (including Gram stain–negative cases). Reliance on Gram stain and culture can be misleading, because false-positive results may arise from contamination or colonization of a drain by skin organisms, and CSF cell counts are abnormal after operations [21]. A larger study is needed to test the power of metabonomics to distinguish between contamination and/or colonization, suspected infection, and ventriculostomy infection and/or ventriculitis [21]. Metabonomics also appears to be useful for monitoring therapeutic response. In the 2 cases presented, trajectory plots indicated that samples obtained after therapy mapped toward the control region in concert with a microbiological, biochemical, and clinical response.

Metabonomic analysis is rapid and requires minimal sample preparation. Set-up costs are relatively expensive, but running costs are low. Use of automated sample preparation and transfer systems, together with NMR flow probes and high-field spectrometers, enables up to 300 samples to be analyzed per day. Highly shielded, integrated NMR systems are commercially available, require minimal space (~2 m²), do not require highly trained operators, and are ideal for high throughput analysis of diverse biological samples in a diagnostic laboratory. The platform is suitable for other microbiological applications (e.g., microbial identification [25, 32]). DNA-based techniques provide the major alternative platform to spectroscopic methods. Set-up costs of techniques, such as PCR methods, that use broad-range [34] or species-specific primers are lower, although costs per test are relatively high. PCR methods can be automated but require sample processing and/or preparation and amplification time. Broad-range PCR gives false-positive results in contaminated ventricular CSF samples or colonized EVDs, and species-specific PCR identifies only the limited microbial species targeted by the primers. Microarray techniques under development are likely to be expensive but should overcome the drawback of limited microbial targets and could potentially include epidemiological and resistance markers.

Metabonomics is a potentially valuable diagnostic tool but is complementary to current diagnostic methods, because it cannot yet replace culture for determination of antimicrobial susceptibility. Its ability to provide a specific etiological diagnosis is unknown; however, species of streptococci, staphylococci, enterococci, other bacteria, and fungi are readily distinguished by NMR spectroscopy [25, 32], and early reports using samples from bacterial abscesses suggest that this will be possible [33].

In conclusion, this pilot study has demonstrated the feasibility of metabonomics for rapid diagnosis of meningitis and ventriculitis. Large prospective studies that include additional clinically relevant comparator groups are indicated.

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References


