Online Data Supplement

Metabolic profiling of serum samples by ^{1}H NMR spectroscopy as a potential diagnostic approach for septic shock

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Description of NMR and MS techniques used in metabolomics studies

Nowadays, NMR (Nuclear Magnetic Resonance) spectroscopy and MS (Mass Spectrometry) instruments are the most common platforms used in metabolomics studies. The NMR technique is based on the measurements of the magnetic properties of certain atomic nuclei, e. g. ^{1}H, ^{31}P, ^{13}C, in the metabolites [E1]. In NMR experiments, each chemical compound can be recognized through its unique peak pattern which means that spectral intensities created by individual metabolites can be identified and assigned to that metabolite. In MS experiments chemical compounds are ionized to form positively or negatively charged molecules which are separated and detected according to their mass-to-charge ratio. NMR spectroscopy benefits from being very specific, quantitative and highly reproducible [E1]. Unfortunately, this technique is associated with a low sensitivity compared to the other analytical methods such as GC-MS (Gas Chromatography – Mass Spectrometry) or LC-MS (Liquid Chromatography – Mass Spectrometry). Detailed information about the advantages and disadvantages of NMR and MS techniques are described in Table ET1. In our study, however, using ^{1}H-NMR was sufficiently sensitive to detect significant changes in metabolic profiles between septic shock patients and ICU controls. Moreover, the NMR technique does not require any special and sophisticated protocol in terms of sample preparation. In the most simple case adding H_2O/D_2O and buffer for pH adjustment is sufficient for sample preparation before the NMR experiment [E2]. Therefore, NMR-based metabolomics could be appropriate as a cost effective solution for high-throughput analysis for the diagnosis and prognosis of septic shock in the ICU. Obviously, the assignment of metabolic profiles from NMR, GC-MS and LC-SM data together will further advance the biological understanding of septic shock and hence these methods should also be considered in future studies.

Table ET1. Strengths and weaknesses of NMR and MS methods used in metabolomics studies (adapted from [E3])

Technique	Strengths	Weaknesses
NMR	Non-destructive (sample can be directly measured) No derivatization needed Rapid measurement (few minutes to hours depending on the magnet) High resolution Reproducible	Low sensitivity Congested spectra and overlapped peaks Limited libraries
GC-MS	Sensitive Robust Large available libraries	Requires derivatization and extraction (solvent extraction bias) Less reproducible Thermally unstable compounds Ion suppression effect
LC-MS	Sensitive Usually no derivatization needed Many options of available separation	Solvent extraction bias Limited libraries Expensive when quantitative Ion suppression effect

Additional inclusion and exclusion Alberta Sepsis Network (ASN) criteria for intensive care unit control patients (ICU controls) enrolled in this study.

ICU control inclusion criteria	ICU control exclusion criteria
Elective surgery that does not involve a mucous membrane; Elective spinal surgery; Elective intracranial surgery; Burns > 10% of body surface area within 24 hrs of admission to hospital with no evidence or suspicion of infection; Major trauma (excluding head injuries) with no evidence or suspicion of infection and one of the following: - musculoskeletal injury, - intraabdominal injury, - pulmonary contusion; Pancreatitis, within 24 hrs of admission to hospital.	Surgery involving any mucous membrane; Any suspicion of sepsis (SIRS ≥ 2 criteria); Immunosuppression, including steroid and biologicals within the last 4 weeks; Hematologic malignancy; Pancreatitis > 24 hrs before admission to hospital.

NMR spectral acquisition

NMR spectra were acquired in a blinded fashion using an automated NMR Case sample changer on a 600 MHz Bruker Ultrashield Plus NMR spectrometer (Bruker BioSpin Ltd., Canada). The one-dimensional ^{1}H NMR spectra were obtained using a standard Bruker 1D spectroscopy presutaration pulse sequence (noesypr1d) with optimal water suppression and a mixing time of 100 ms [E4, E5]. Samples were shimmed to ensure a line width at half-height of approximately 0.7 – 0.8 Hz for the 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) peak calibrated to 0.0 ppm. Spectra were acquired with 1024 scans, zero-filled and Fourier transformed to 128k points. For initial inspection, the spectra were manually corrected including phasing, baseline correction and referencing to the DSS peak at 0.0 ppm using the Topspin software program (Bruker BioSpin Ltd., Canada). In addition two-dimensional NMR spectra, including total correlation spectroscopy (TOCSY) and ^{1}H,^{13}C heteronuclear single quantum coherence spectroscopy (^{1}H,^{13}C HSQC), were obtained for randomly chosen samples in order to verify the chemical shift assignments.

References

E1. Dunn WB, Broadhurst DI, Atherton HJ, Goodacre R, Griffin JL: Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. *Chem Soc Rev* 2011, **40**(1):387-426.

E2. Lindon, JC, Nicholson JK, Holmes E, Everett JR: Metabonomics: metabolic processes studied by NMR spectroscopy of biofluids, *Concepts Magn Reson* 2000, **12**: 289-320.

E3. Vernocchi P, Vannini L, Gottardi D, Del Chierico F, Serrazanetti DI, Ndagijimana M, Guerzoni ME: Integration of datasets from different analytical techniques to assess the impact of nutrition on human metabolome. *Front Cell Infect Microbiol* 2012, **2**:156 (1-10).

E4. Weljie AM, Newton J, Mercier P, Carlson E, Slupsky CM: Targeted profiling: quantitative analysis of 1H NMR metabolomics data. *Anal Chem* 2006, **78**(13):4430-4442.

E5. Nicholson JK, Foxall PJ, Spraul M, Farrant RD, Lindon JC: 750 MHz 1H and 1H-13C NMR spectroscopy of human blood plasma. *Anal Chem* 1995, **67**(5):793-811.

Figures and Tables

Figure E1. Examples of ^{1}H NMR spectra of septic shock (orange) and ICU control (black) samples and their spectral overlay.

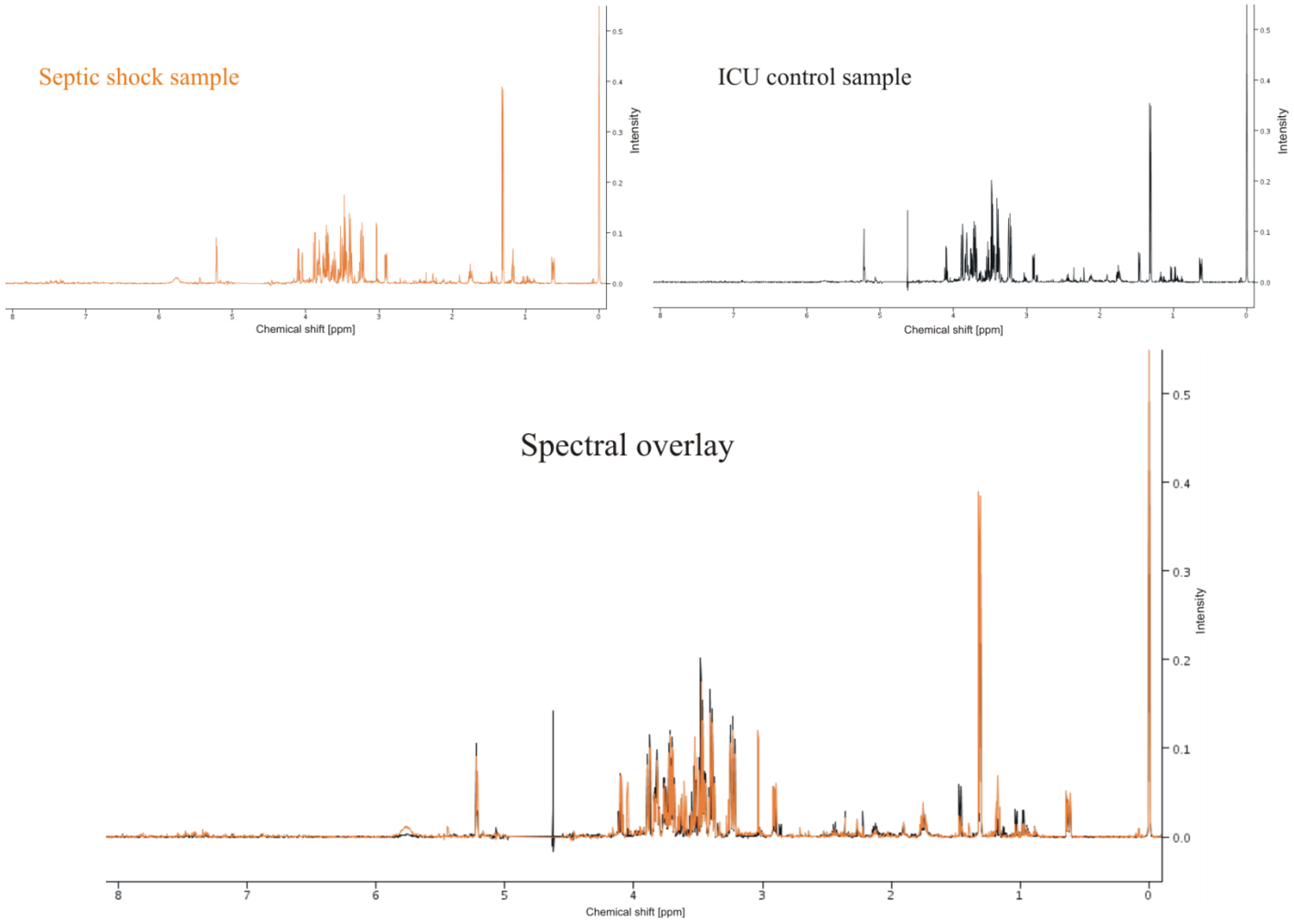
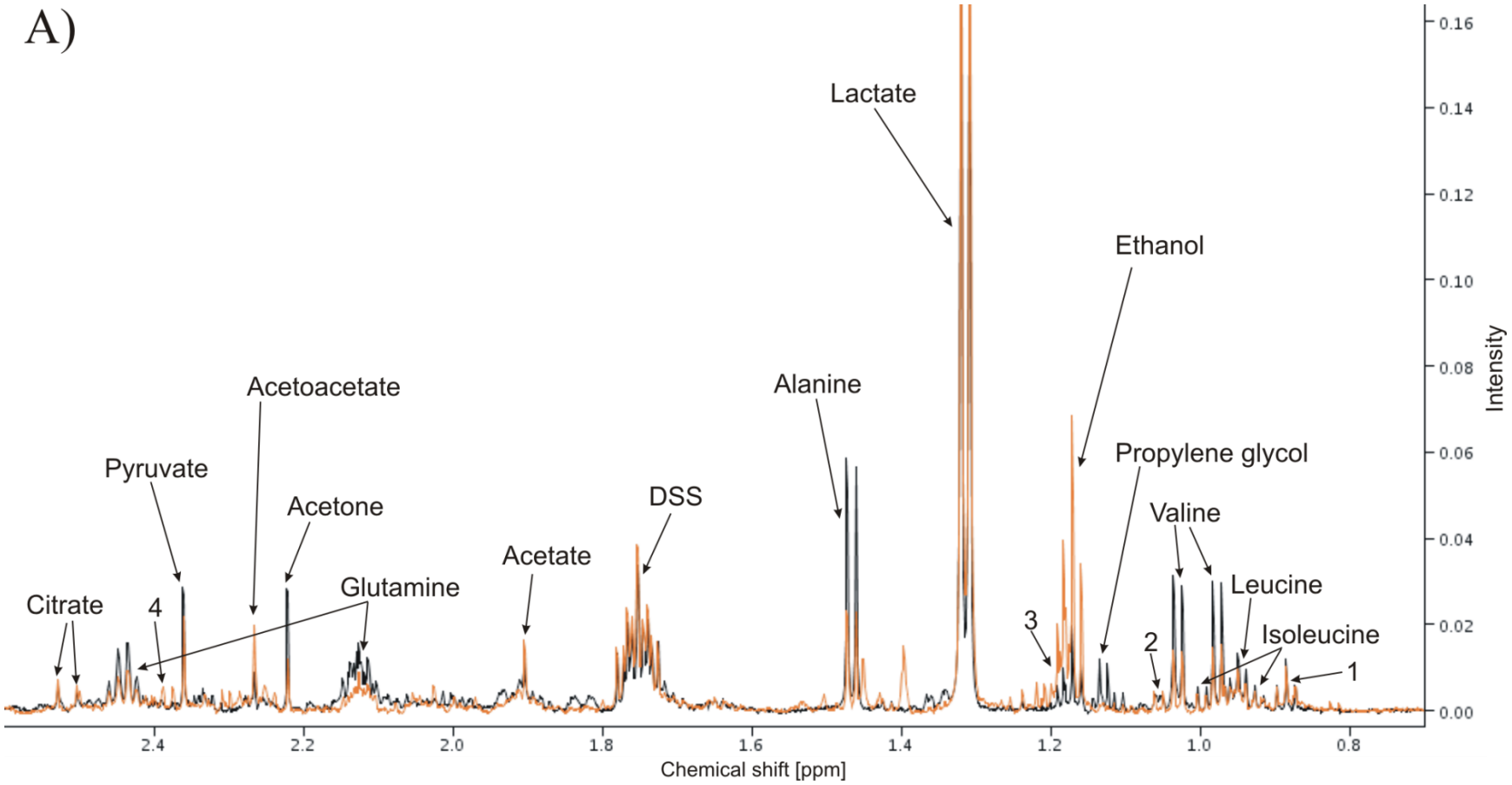
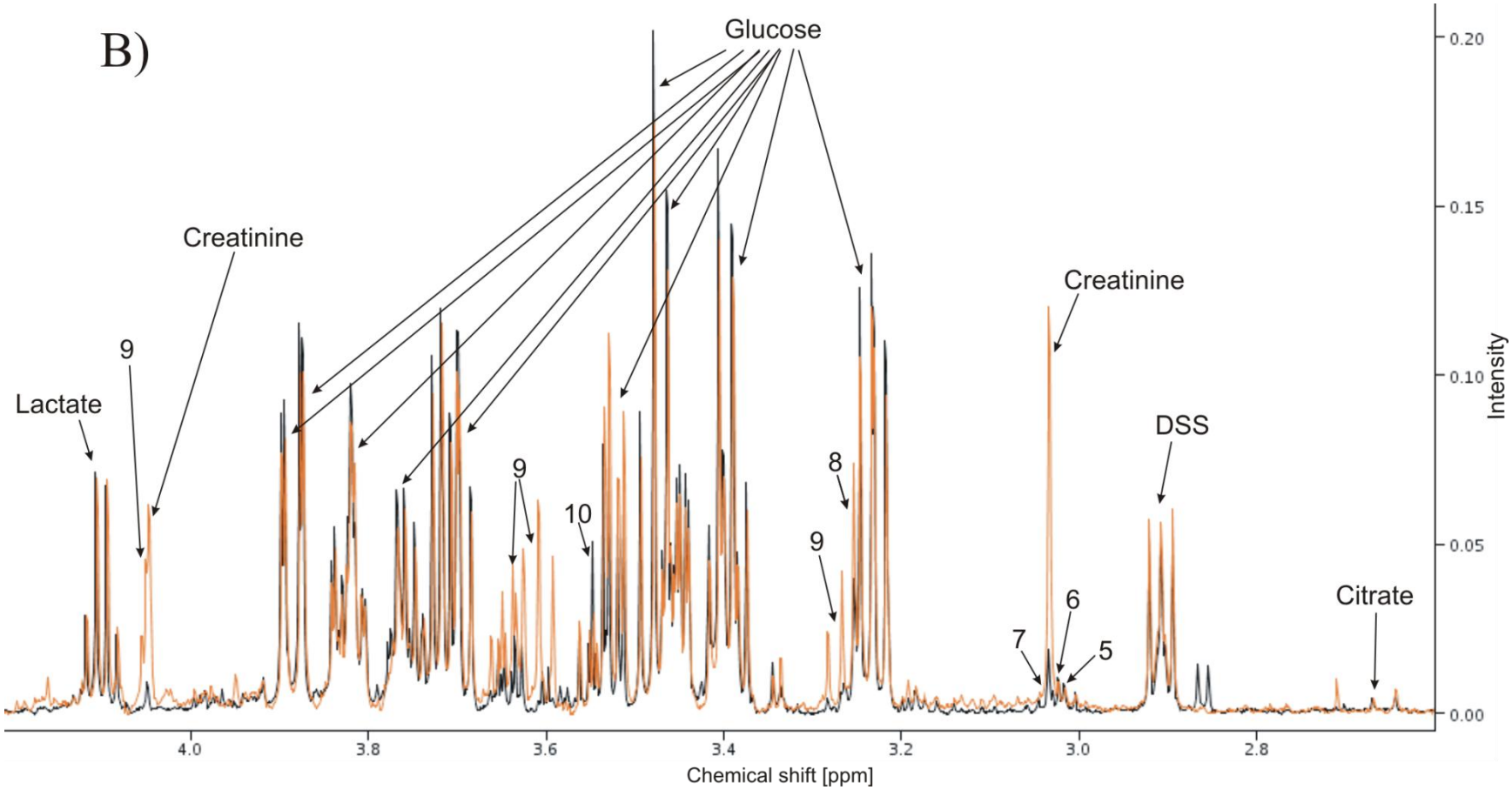


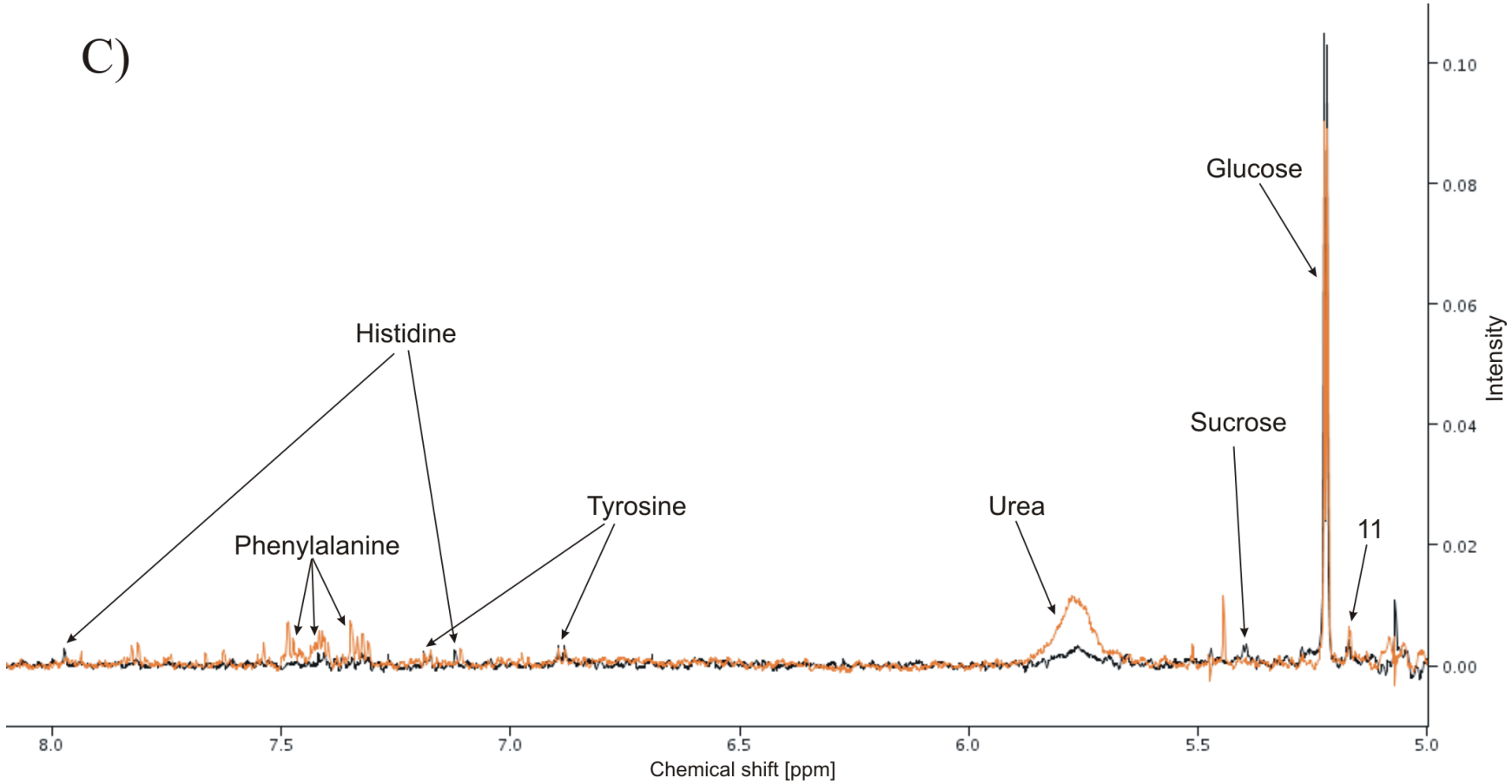
Figure E2. ^{1}H NMR spectral overlays of septic shock (orange) and ICU control (black) samples with the major metabolites assigned in the spectral region of A) 0.7 – 2.6 ppm, B) 2.6 – 4.2 ppm and C) 5.0 – 8.0 ppm.



1. 2-Hydroxybutyrate; 2. 2-Oxobutyrate; 3. 3-Hydroxybutyrate; 4. Succinate.



5. Lysine; 6. Creatine; 7. Creatine phosphate; 8. Trimethyl N-oxide; 9. myo-Inositol; 10. Glycine.


C)
Glucose
Histidine
Sucrose
Tyrosine
Urea
Phenylalanine
11
0.10
0.08
0.06
0.04
0.02
0.00
Intensity
8.0
7.5
7.0
6.5
6.0
5.5
5.0
Chemical shift [ppm]

11. Mannose.

Table ET2. The chemical shifts used to make metabolite assignments in ^{1}H NMR spectra.

Compound Name	Chemical Shifts [± 0.025 ppm]
2-Aminobutyrate	0.97; 1.89; 3.71
2-Hydroxybutyrate	0.89; 1.64; 1.73; 3.99
2-Hydroxyisobutyrate	1.34
2-Hydroxyisovalerate	0.82; 0.95; 2.01; 3.84
2-Oxobutyrate	1.05; 2.75
2-Oxoglutarate	2.43; 3.00
2-Oxoisocaproate	0.92; 2.08; 2.60
3-Hydroxybutyrate	1.19; 2.30; 2.39; 4.14
Acetate	1.91
Acetoacetate	2.27; 3.44
Acetone	2.22
Alanine	1.47; 3.78
Arginine	1.64; 1.72; 1.89; 1.92; 3.24; 3.76; 6.67; 7.23
Asparagine	2.85; 2.94; 4.00; 6.91; 7.62
Betaine	3.25; 3.89
Butyrate	0.88; 1.55; 2.15
Carnitine	2.41; 2.45; 3.21; 3.40; 3.43; 4.56
Choline	3.19; 3.51; 4.06
cis-Aconitate	3.10; 5.69
Citrate	2.53; 2.69
Creatine	3.03; 3.92
Creatine phosphate	3.03; 3.94
Creatinine	3.03; 4.05
Dimethylamine	2.72
DSS (internal standard)	0.00; 0.62; 1.75; 2.91
Ethanol	1.17; 3.65
Ethanolamine	3.14; 3.82
Ethylmalonate	0.87; 1.71; 2.98
Formate	8.44
Fructose	3.55; 3.56; 3.59; 3.67; 3.70; 3.70; 3.79; 3.79; 3.82; 3,89; 3.99; 4.01; 4.10; 4.11
Glucose	3.24; 3.39; 3.40; 3.46; 3.48; 3.53; 3.70; 3.72; 3.76; 3.82; 3.84; 3.89; 4.64; 5.23
Glutamate	2.04; 2.12; 2.33; 2.36; 3.75
Glutamine	2.11; 2.14; 2.43; 2.46; 3.77; 6.87; 7.59
Glycerol	3.55; 3.65; 3.78
Glycine	3.55

Histidine	3.14; 3.24; 3.98; 7.09; 7.88
Isobutyrate	1.05; 2.38
Isoleucine	0.93; 1.00; 1.25; 1.46; 1.97; 3.66
Lactate	1.32; 4.11
Leucine	0.94; 0.95; 1.67; 1.70; 1.73; 3.72
Lysine	1.43; 1.50; 1.72; 1.88; 1.91; 3.02; 3.75
Malonate	3.11
Mannose	3.37; 3.57; 3.65; 3.65; 3.73; 3.76; 3.81; 3.84; 3.87; 3.90; 3.93; 3.94; 4.89; 5.17
Methanol	3.35
Methionine	2.11; 2.13; 2.19; 2.63; 3.85
myo-Inositol	3.27; 3.53; 3.61; 4.06
O-Acetylcarnitine	2.13; 2.50; 2.63; 3.18; 3.60; 3.84; 5.59
O-Phosphocholine	3.21; 3.58; 4.16
Phenylalanine	3.11; 3.27; 3.99; 7.32; 7.37; 7.42
Proline	1.98; 2.02; 2.34; 3.33; 3.41; 4.12
Propylene glycol	1.13; 3.43; 3.54; 3.87
Pyruvate	2.36
Serine	3.84; 3.94; 3.98
Succinate	2.39
Sucrose	3.47; 3.55; 3.66; 3.68; 3.76; 3.80; 3.82; 3.82; 3.83; 3.83; 3.88; 4.04; 4.21; 5.40
Taurine	3.25; 3.42
Threonine	1.32; 3.58; 4.25
Trimethylamine N-oxide	3.25
Tyrosine	3.04; 3.19; 3.93; 6.89; 7.18
Urea	5.77
Valine	0.98; 1.03; 2.26; 3.60

Figure E3. 3D PCA score scatter plot obtained for all 59 samples (septic shock patients – red, ICU controls – green). The groups are well clustered and distinguished along the axes of the three principal components. The sphere describes the 95% confidence interval of the Hotelling's T-squared distribution.