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REVIEW

Cerebrospinal fluid metabolomics: detection of neuroinflammation in human central nervous system disease

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Abstract

The high morbidity and mortality of neuroinflammatory diseases drives significant interest in understanding the underlying mechanisms involved in the innate and adaptive immune response of the central nervous system (CNS). Diagnostic biomarkers are important to define treatable neuroinflammation. Metabolomics is a rapidly evolving research area offering novel insights into metabolic pathways, and elucidation of reliable metabolites as biomarkers for diseases. This review focuses on the emerging literature regarding the detection of neuroinflammation using cerebrospinal fluid (CSF) metabolomics in human cohort studies. Studies of classic neuroinflammatory disorders such as encephalitis, CNS infection and multiple sclerosis confirm the utility of CSF metabolomics. Additionally, studies in neurodegeneration and neuropsychiatry support the emerging potential of CSF metabolomics to detect neuroinflammation in common CNS diseases such as Alzheimer's disease and depression. We demonstrate metabolites in the tryptophan–kynurenine pathway, nitric oxide pathway, neopterin and major lipid species show moderately consistent ability to differentiate patients with neuroinflammation from controls. Integration of CSF metabolomics into clinical practice is warranted to improve recognition and treatment of neuroinflammation.

Keywords: cerebrospinal fluid, metabolomics, neopterin, neuroinflammation, nitric oxide pathway, tryptophan–kynurenine

INTRODUCTION

Neuroinflammation is inflammation of the central nervous system (CNS) initiated in response to either infection, autoimmunity, traumatic brain injury, toxic metabolites or degeneration. In the case of acquired inflammation or infection, the inflammatory response is driven by invading immune cells such as infiltrating lymphocytes or monocytes. In addition, inflammation can be

mediated by resident immune cells of the brain such as microglia, which can contribute to neuronal damage or repair.

Encephalitis is inflammation of the brain as a result of viral infection or an autoimmunity. Meningitis is another dangerous inflammatory condition of the meninges surrounding the brain and is caused by invasive viruses and bacteria.[1] The significant mortality and morbidity of encephalitis and meningitis has directed great attention to explore the pathophysiologic mechanisms, and biomarkers for identification.[2,3] In addition, there is increasing evidence that inflammation occurs in common neurodevelopmental diseases such as autism, common neuropsychiatric diseases such as depression, and common neurodegenerative diseases such as Alzheimer's disease. As inflammation is potentially modifiable, novel methods to define brain inflammation are needed.

CEREBROSPINAL FLUID AS A BIOFLUID OF DIAGNOSTIC UTILITY FOR METABOLOMICS

Cerebrospinal fluid (CSF) is the most useful biofluid for analysing brain metabolism and provides a valuable opportunity to detect neuroinflammation in human CNS diseases.[4] The information derived from CSF metabolomics can offer insight into cellular processes, which can further provide deeper understanding of molecular mechanisms of diseases.[5,6] CSF is the closest biological biofluid to the brain and directly reflects the pathophysiological alterations of the CNS.[5] CSF is a colourless filtrated product of blood plasma located in the subarachnoid spaces and ventricles of the brain. The production of CSF occurs mainly in the choroid plexus at a rate of 400–600 mL per day.[7] This is driven by a combination of processes including active transport and diffusion. CSF is mainly composed of water and contains enzymes, metal ions or salts, micronutrients, neurotransmitters, amino acids, glucose, carbohydrates, short-chain fatty acids, alcohols, peptides and low protein content.[8] CSF is circulated within the cranial and spinal arachnoid villus sites and absorbed through the arachnoid villi and into the venous outflow system. The analysis of CSF metabolites, interpretation of metabolite data and subsequent biochemical changes are fundamental to understand neuroinflammatory mechanisms, identify biomarkers, enable prognosis of disease developments and provide treatment strategies.

The workflow for CSF metabolomics analysis involves three major steps: pre-analytical work, analytical work and data processing.[9] The pre-analytical stages require careful handling in the collection, preprocessing and storage steps of CSF to ensure the integrity of the samples before chemical analysis. In the analytical stage, there are multiple steps involved in CSF metabolite extractions and data acquisition using analytical technologies. The data processing stage in metabolomics is composed of (i) feature detection, (ii) retention time correction, (iii) chemical shift (or chromatogram) alignment, (iv) metabolite feature annotation and grouping and (v) metabolite identification. Following data processing, the data quality assessment, including the signal intensity drift correction (within and between batches) and data normalisation, is required prior to statistical analysis. Multivariate statistical methods (such as principal component analysis and partial least squares discriminant analysis) identify relationships between metabolite features and allow sample discrimination or classification. Univariate statistical methods (such as analysis of variance and the Student's *t*-test and the Kruskal–Wallis test) assess the metabolite feature independently.

Standardised CSF sample handling procedures are imperative in the search for reliable biomarkers. It has been reported that delayed storage and blood contamination of CSF result in changes in prostaglandin D-synthase peptides, amino acids and metabolites.[10] CSF samples are recommended to be centrifuged immediately after collection and stored at −80°C. The common extraction methods for CSF metabolites such as organic solvent-based precipitation, ultrafiltration, dilution and solid-phase extraction have been extensively reviewed elsewhere.[11–14] The physicochemical diversity of the CSF metabolome requires the use of multiple instrumental analytical methods and complementary data acquisition modes in order to maximise the metabolome coverage, facilitate metabolite identification and overcome bias from individual techniques. Nuclear magnetic resonance (NMR) [15–17] and mass spectrometry-based methods (such as liquid chromatography and gas chromatography)[18–22] are principal technological platforms employed for metabolomics. The unique strengths in NMR and

mass spectrometry technologies have contributed to the rapid growth of metabolomics and shown to be highly complementary. The importance of combining the analytical techniques for metabolomics has been demonstrated in several studies.[23–25]

The advancement of analytical technologies has led to the demand of different data analysis tools required in the process of extracting relevant information. Data preprocessing software packages, metabolite databases and libraries available for NMR and mass spectrometry (MS) metabolomics research have expanded, with increased dependence on the usage of metabolome repositories and querying platforms.[26] The strategies involved in molecular feature extractions and metabolite annotations have been previously reviewed.[27–30] Advanced statistical tools such as chemometrics have become an essential tool for the extraction of valuable metabolic signature information. Chemometrics has developed into a well-established statistical tool in areas such as multivariate calibration, pattern recognition, multivariate statistical process control and quantitative structure modelling.[31–34]

CEREBROSPINAL FLUID METABOLOMICS: BIOMARKERS OF NEUROINFLAMMATION

The identification of biomarkers is clinically useful for an accurate diagnosis, prognosis and disease management.[35] CSF metabolomics applications that focus on biomarker discovery offer the promise of earlier detection and improved outcomes. In this review, we discuss three main metabolic pathways reported in human studies of CNS inflammation, specifically tryptophan–kynurenine, nitric oxide, neopterin and sphingolipid–ceramide. However, there are a number of other metabolites and pathways associated with inflammatory processes, including biogenic amines, amino acids, neurotransmitters, carbohydrates and lipids. The research in these areas is on a smaller scale, is less consistent and has broader variation of metabolic network coverage across independent studies, which are not discussed in this review.

Tryptophan–Kynurenine pathway

The tryptophan–kynurenine metabolic pathway commences with the conversion of tryptophan into kynurenine (Figure 1), stimulated by indoleamine 2,3-dioxygenase 1 (IDO-1), IDO-2 or a relatively newly discovered IDO-related enzyme.[36] Kynurenine is further metabolised by three main enzymes, kynurenine aminotransferase, kynurenine 3-monooxygenase and kynureninase dividing into three arms generating its metabolites, kynurenic acid (KA), 3-hydroxykynurenine (3-HK) and anthranilic acid (AA), respectively. 3-HK and AA can be converted to 3-hydroxyanthranilic acid (3-HAA) and afterwards interacted with 3-hydroxyanthranilic acid oxygenase to produce quinolinic acid (QA) and picolinic acid (PIC).

The kynurenine pathway is involved in neuroinflammation because of activation of IDO and related enzymes. The activation of IDO, mainly by dendritic cells and macrophages, causes the depletion of tryptophan and an imbalanced formation of neuroprotective and neurotoxic metabolites (Figure 1). The IDO gene expression is regulated and responsive to interferons, which accounts for the increased activity of IDO upon neuroinflammation.

Tryptophan plays a key role in the regulation of protein biosynthesis, immune tolerance, cell growth and proliferation. The depletion of tryptophan causes disruption to systemic homeostasis and psychoneuroimmunological consequences and is observed in a range of CNS diseases with neuroinflammatory mechanisms. Moreover, accelerated breakdown of tryptophan will affect serotonin levels and consequently create vulnerability to neuropsychiatric and neuropsychological diseases.

Human cohort studies (with controls) of the tryptophan–kynurenine pathway as a biomarker of inflammation in CSF are shown in Table 1. As shown, the studies vary in the size of patient and control cohorts (Table 1). The disease states are separated into CNS infections such as encephalitis, meningitis or other infections known to affect the CNS (e.g. hepatitis C, HIV and malaria). Subsequently, studies on MS, a recognised neuroinflammatory disorder of proposed autoimmune aetiology, are reported. Furthermore, Table 1 shows studies of diseases where inflammation is increasingly described, such as in neurodegeneration and mental health, followed by other entities with possible inflammatory associations. As seen in Table 1, there are general trends that inflammation results in decreased tryptophan, elevated kynurenine or

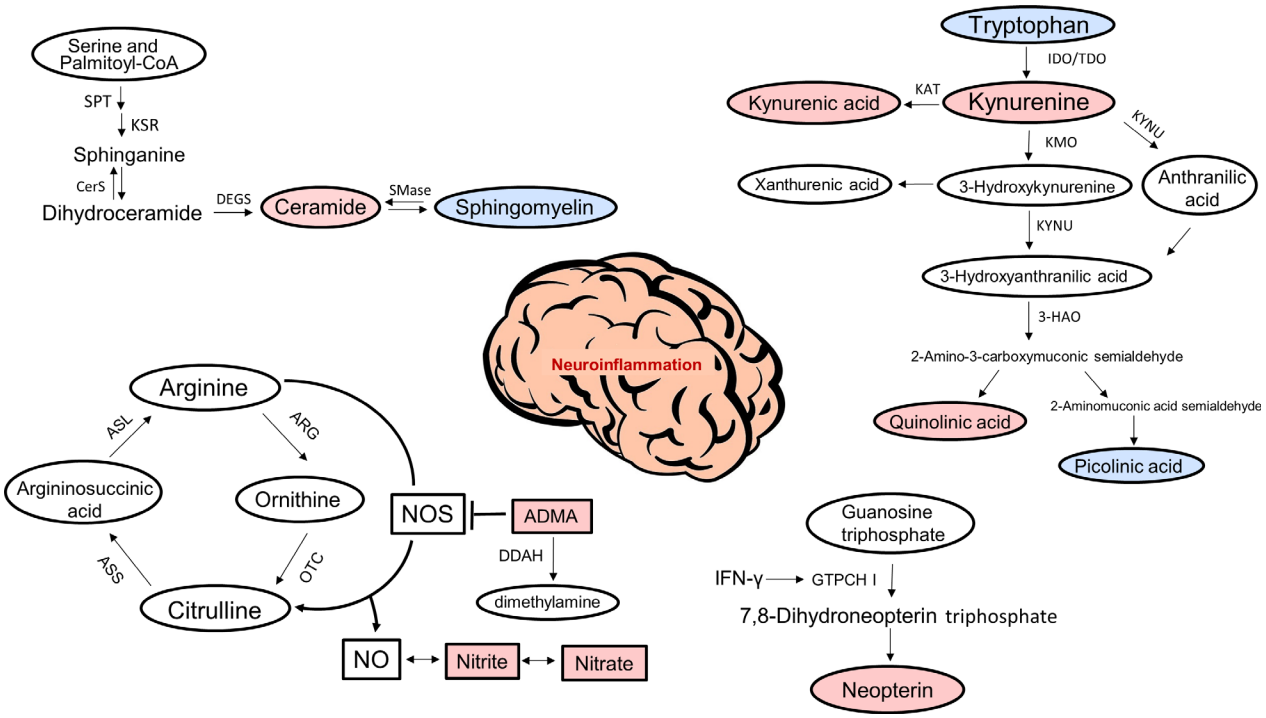

Figure 1. Major pathways involved in neurological diseases with confirmed or suspected neuroinflammation – tryptophan–kynurenine pathway (right above), nitric oxide pathway (left bottom), neopterin (right bottom) and sphingolipid–ceramide pathway (left above). Trends are highlighted in red (representing statistically elevated in patients with neuroinflammation compared with controls) and blue (representing statistically decreased in patients with neuroinflammation compared with controls). Neopterin is the most valuable inflammatory metabolite in the GTP–tetrahydrobiopterin metabolism; therefore, the full pathway is not shown. 3-HAO, 3-hydroxyanthranilic acid oxygenase; ADMA, asymmetric dimethylarginine; CerS, ceramide synthase; DEGS, dihydroceramide desaturase; GTPCH I, guanosine triphosphate cyclohydrolase I; IDO, indoleamine 2,3-dioxygenase; IFN-γ, interferon-gamma; KAT, kynurenine aminotransferase; KMO, kynurenine monooxygenase; KSR, ketosphinganine reductase; KYNU, kynureninase; NO, nitric oxide; NOS, nitric oxide synthase; SMases, sphingomyelinases; SPT, serine palmitoyltransferase; TDO, tryptophan 2,3-dioxygenase.

kynurenic acid, with elevated kynurenine/tryptophan ratio (or decreased tryptophan/kynurenine ratio). Quinolinic acid was almost universally elevated, and picolinic acid was generally reduced when measured. The analysis of CSF metabolites in the tryptophan–kynurenine pathway therefore holds promises as inflammatory biomarkers in the early diagnosis and prognosis of neurological pathologies and provides insights into their pathophysiology. As recently reviewed, it should be highlighted that inflammation induced by activation of IDO and tryptophan 2,3-dioxygenase (TDO) is often inferred as a result of changes in metabolite ratios, rather than actual measurement of the IDO/TDO enzyme protein or activation status.[37,38]

The development of inflammatory-mediated neuropathology is associated with the changes of quinolinic acid levels.[39] Quinolinic acid is an important metabolite inducing immunosuppression and has been hypothesised to induce toxicity in brain cells[40] and interaction with glutamate neurotoxicity.[41] Further studies in common neurological diseases with possible inflammatory mechanisms such as neurodegeneration, neuropsychiatry and neurodevelopmental disorders are therefore warranted.

Nitric oxide pathway

The conversion of arginine to nitric oxide and citrulline is stimulated by nitric oxide synthase (NOS). In the body, there are three isoforms of NOS, whereby inducible NOS (iNOS) is extensively involved in the pathophysiology of inflammation and responsible for the production of nitric oxide.[42,43] iNOS is expressed in microglia cells,

Table 1. Cerebrospinal fluid metabolomics studies reporting tryptophan–kynurenine pathway findings in neurological diseases with confirmed or suspected neuroinflammation

Disease cohort	Description of control group	Analytical platform	Findings					Ref
			TRP	KYN	KA	QA	Other	
Encephalitis, meningitis and infection								
Encephalitis (infectious, autoimmune, unknown, *n* = 10); acute aseptic meningitis (*n* = 25); acute bacterial meningitis (*n* = 6)	NIND (*n* = 42)	LC-MS/MS targeted	↓	↑	↑	↑	↑ PIC; ↑ AA; ↑ 3-HK; ↑ KYN/TRP; ↓ KA/(3HK +QA)	47
Neuroborreliosis (*n* = 34); bacterial meningitis (*n* = 32); multiple sclerosis (*n* = 17); VZV meningoencephalitis (*n* = 15); enterovirus meningitis (*n* = 10); HSV encephalitis (*n* = 9); anti-NMDA-R encephalitis (*n* = 8)	NIND (*n* = 66)	LC-MS/MS targeted	↓	↑			↑ KYN/TRP	56
Enterovirus meningitis (*n* = 10)	NIND (*n* = 19)	LC-MS/MS untargeted		↑				57
Tuberculosis meningitis survivors (*n* = 15); tuberculosis meningitis non-survivors (*n* = 17)	Controls with no infection (*n* = 22)	LC-MS/MS untargeted	↓		↑	↑	↑ AA	58
Cerebral malaria (*n* = 69)	Controls with no infection (*n* = 8)	HPLC-UV targeted		↑	↑			59
Subacute sclerosing panencephalitis (*n* = 32)	Epileptic and other encephalopathy controls (*n* = 43)	GC-MS targeted				↑		60
Hepatitis C treated with IFN-α/ribavirin (*n* = 16)	Hepatitis C – no treatment (*n* = 20)	HPLC-FD, HPLC-UV targeted	↔	↑	↑	↑		61
Bacterial meningitis (*n* = 13) Aseptic meningitis (*n* = 7)	Controls with no infection (*n* = 8)	HPLC-UV targeted	↔	↑	↑		↑ AA; ↑ KYN/TRP	62
Inflammatory neurological disease (*n* = 92)	NIND (*n* = 201)	HPLC targeted	↓	↑	↑	↑	↑QA/KA	63
Tick-borne encephalitis (*n* = 108)	Controls with no infection (*n* = 52)				↑			64
P. falciparum malaria (*n* = 261)	Controls with no infection (*n* = 20)	HPLC-FD targeted			↔	↑	↑ PIC; ↑ QA/KA	65
Herpes simplex virus 1 encephalitis (*n* = 25)	Controls with no infection (*n* = 25)	HPLC targeted			↑			66
HIV-positive patients with virologic control on cART (*n* = 43)	HIV-negative controls (*n* = 23)	UHPLC and GC-MS targeted	↓	↔		↑	↑ KYN/TRP; ↓ PIC	67
HIV-positive patients (*n* = 134)	HIV-negative controls (*n* = 79)	HPLC targeted					↑ KYN/TRP	68
HIV-positive patients with depression and cognitive impairment (*n* = 91)	HIV-negative controls (*n* = 66)	HPLC targeted	↓	↔			↑NEO; ↔ KYN/TRP	69
HIV-1 positive (*n* = 22)	Healthy controls (*n* = 22)	HPLC targeted			↑			70
Multiple sclerosis (MS)								
MS (*n* = 37)	NIND (*n* = 22)	LC-MS/MS targeted	↓	↑	↔	↑		71
	NIND (*n* = 10)	LC-HRMS untargeted		↑	↑			72

(Continued)

Table 1. Continued.

Disease cohort	Description of control group	Analytical platform	Findings					Ref
			TRP	KYN	KA	QA	Other	
Relapsing–remitting MS (n = 30), secondary progressive MS (n = 16)								
Relapsing–remitting MS (n = 20)	NIND (n = 14)	UHPLC-MS targeted			↓	↑	↑ QA/KA; ↓PIC; ↓ PIC/QA; ↓ KA/KYN;	73
Untreated MS (n = 38); RRMS (n = 48)	NIND (n = 20); Other inflammatory neurology (n = 13)	LC-MS/MS targeted	↓	↔	↔	↑	↑ QA/KA; ↑ KYN/TRP; ↔ KA/KYN	74
MS (n = 26); CNS infectious disease (n = 16)	NIND (n = 23)	HPLC-FD targeted			↓		KA lower in MS compared with CNS infection	75
Neurodegeneration								
AD (n = 20)	Controls without AD (n = 18)	UHPLC, HPLC and GC/MS targeted	↓	↔	↑	↓	↑ KYN/TRP; ↑ 3-HK/KYN; ↓ QA/KA; ↓ 3-HAA	76
AD (n = 40)	Controls without AD (n = 34)	LC-MS/MS untargeted	↓		↑	↑		77
Probable mild AD (n = 41); mild AD (n = 24); moderate–severe AD (n = 20); frontotemporal dementia (n = 8); amyotrophic lateral sclerosis (n = 8); progressive supranuclear palsy (n = 8)	Controls non-demented (n = 23)	ELISA kit targeted	↔		↑		↑ KA/TRP	78
Amyotrophic lateral sclerosis (n = 140)	Suspected meningitis (n = 35)	HPLC-UV, GC-MS targeted	↑	↑		↑	↓ PIC	79
Mental health/neuropsychiatry								
Bipolar disorder (n = 163)	Healthy controls (n = 114)	HPLC-UV targeted			↑			80
Depression and suicidality (n = 64)	Healthy controls (n = 35)	HPLC-UV, GC-MS targeted				↑	↑ KYN/TRP; ↓ PIC; ↓ PIC/QUIN	81
Schizophrenia (n = 22)	Controls (n = 26)	LC-MS/MS targeted		↑	↑	↔	↓ QA/KA	82
Schizophrenia on olanzapine treatment (n = 16)	Controls (n = 29)	HPLC-UV targeted	↔	↑	↑			83
Chronic Schizophrenia (n = 23)	Controls (n = 37)	HPLC targeted	↔	↑	↑		↓ TRP/KYN; ↓ TRP/KA	84
Other								
Trisomy 21 (Down syndrome, n = 50)	Controls (n = 50)	UHPLC-HRMS untargeted		↑		↑	↑ formyl-kynurenine; ↑ KYN/TRP	85
Severe traumatic brain injury (n = 28)	Controls (n = 11)	HPLC and GC-MS targeted	↔	↑	↑	↑	↑ QA/KYN; ↑ QA/KA; ↔ AA; ↔ 3-HAA	86

Cohorts separated into subgroups (e.g. encephalitis). ↑represents statistically elevated metabolite in patients compared with controls, ↓ represents statistically decreased metabolite in patients compared with controls, ↔ reports no statistical difference between groups, and blank represents 'not reported or not measured'. Ratios are represented by *x/y* (e.g. KYN/TRP).
3-HAA, 3-hydroxyanthranilic acid; 3-HK, 3-hydroxykynurenine; AA, anthranilic acid; AD, Alzheimer's disease; cART, combination antiretroviral therapy; HIV, human immunodeficiency virus; KA, kynurenic acid; KYN, Kynurenine; MS, multiple sclerosis; NIND, non-inflammatory neurology disease; PIC, picolinic acid; QA, quinolinic acid; TRP, tryptophan.

astrocytes, neurons in CNS, macrophages, endothelial cells at BBB, dendritic cells and neutrophils. The inhibition of iNOS occurs by the endogenous production of asymmetric dimethylarginine. Nitric oxide is further metabolised to reactive nitrogen species, including nitrate and nitrite. Citrulline is recycled to form arginine by argininosuccinate and argininosuccinate lyase, known as the citrulline–nitric oxide cycle. Conversely, arginine can be hydrolysed to produce orthinine via arginase and subsequently converted to citrulline by ornithine transcarbamylase.

Nitric oxide is a critical gaseous molecule involved in neurotransmission, defence mechanisms, and acute and chronic inflammation.[42] The nitric oxide pathway plays a critical role in the regulation of immunoprotective activities defending the body against infectious organisms. However, failure of immune regulation and overactivation of inflammatory pathways can result in disease states. The altered concentrations of CSF metabolites in the nitric oxide pathway have been implicated in a wide range of human diseases associated with inflammation as summarised in Table 2. A variation of analytical platforms, untargeted or targeted approaches and study cohorts have been used (Table 2), and the cohort studies are subgrouped in the same way as Table 1. As shown in Table 2, asymmetric dimethylarginine, orthinine, nitrite and nitrate levels in CSF are generally increased in diseases with confirmed or suspected CNS inflammation. However, it should be noted that the studies differ in methodology and differ in the measured or reported metabolites. Figure 1 depicts the metabolites that are generally elevated or decreased. As is the case for the tryptophan–kynurenine pathway, the activation of iNOS is generally inferred by measuring the pre- and post-metabolites, rather than actually measuring iNOS.

Neopterin

Neopterin is regarded as a valuable early biochemical marker of the cellular immune response during inflammation[44] and is sometimes used in clinical settings.[45] Guanosine triphosphate (GTP) is converted to 7,8-dihydroneopterin triphosphate via the actions of GTP cyclohydrolase I (Figure 1). The activation of T cells induces the enzymatic activity of GTP cyclohydrolase I via pro-inflammatory cytokines such as γ-interferon, leading to the production of neopterin by macrophages and dendritic cells. Neopterin is a direct product generated in the immune activation of γ-interferon able to be detected at low concentrations and practical for clinical assays.[46]

The reported human cohort studies of CSF neopterin as a biomarker of inflammation are outlined in Table 3. The disease states have been classified into CNS infections including HIV, encephalitis, meningitis or other infections affecting the brain (e.g. HTLV-1, HAT). Moreover, studies investigated in MS, neurodegeneration, CNS tumors and autism are reported. CSF neopterin was found to be predominantly elevated in neurological diseases with inflammatory mechanisms. A strong correlation between elevated neopterin and the kynurenine/tryptophan ratio has also been reported.[47,48] Therefore, CSF neopterin serves as a strong inflammatory biomarker for practitioners.

Lipids

Lipids are present in high concentrations in the CNS and play important roles in the cellular structure, cell signalling and energy storage. Sphingomyelin, ceramide, phosphatidylcholine, cholesterol and sulphatides are the most abundant lipid classes in the CNS.[49] Sphingolipids are crucial in the regulation of cellular processes including cell proliferation, apoptosis, autophagy and inflammatory responses. Ceramide is involved in oxidative stress, stimulation of apoptosis and inflammatory processes. Phosphatidylcholines ensure the balance between cell proliferation and death and are key substrates to modulate inflammation and release fatty acids such as linoleic acid and arachidonic acid.

The *de novo* synthesis of the sphingolipid–ceramide pathway commences with the condensation of serine and palmitoyl-CoA by serine palmitoyltransferase and further reduced by ketosphinganine reductase to form sphinganine (Figure 1). Sphinganine is acetylated by ceramide synthase to form dihydroceramide and subsequently converted to ceramide through dihydroceramide desaturase. Alternatively, sphingomyelin is hydrolysed by sphingomyelinases to form ceramide.

The dysregulation of sphingolipids, ceramide, phospholipids and oxylipins has been reported in

Table 2. Cerebrospinal fluid metabolomics studies reporting nitric oxide pathway findings in neurological diseases with confirmed or suspected inflammation

Disease cohort	Description of control group	Analytical platform	Findings				Ref
			ADMA	NO_2^-	NO_3^-	Other	
Encephalitis, meningitis and infection							
Segmental zoster (n = 14); Facial nerve zoster (n = 16); VZV meningitis/encephalitis (n = 15)	Controls with no infection (n = 36)	LC-MS/MS targeted				↓ ARG	87
Tuberculosis meningitis (n = 31)	Controls with no infection (n = 20)	ELISA targeted				↑ NO	88
Streptococcus pneumonia (n = 14); neisseria (n = 22); Haemophilus influenza (n = 9) meningitis	Controls with no infection (n = 7)	Colorimetric assay targeted		↑	↑		89
HIV-infected patients with syphilis infection (n = 33)	HIV-negative controls with no infection (n = 7)	Colorimetric assay targeted		↑			90
Multiple sclerosis (MS)							
Secondary progressive MS (n = 12)	Healthy controls (n = 12)	LC-HRMS targeted	↑				91
MS (n = 14); neuromyelitis optica (n = 9); other neurological disease(n = 26)	Healthy controls (n = 11)	GC–MS/MS targeted	↑			↔hArg/ADMA; ↔ SDMA	92
MS exacerbation (n = 24); MS remission (n = 17); MS progression (n = 20)	Controls with tension headache (n = 19)	CE targeted		↑	↑		93
Relapsing–remitting MS (n = 15)	Healthy controls (n = 15)	absorption spectrophotometry targeted		↑	↑	↑ Peroxynitrite	94
Neurodegeneration							
Amyotrophic lateral sclerosis (n = 52)	Controls (n = 21)	LC-MS/MS targeted	↑				95
Amyotrophic lateral sclerosis (n = 22); Parkinson's disease (n = 22)	Controls without neurodegeneration (n = 28)	NMR untargeted				↑ dimethylamine	96
Amyotrophic lateral sclerosis (n = 22); Parkinson's disease (n = 22)	Controls without neurodegeneration (n = 28)	GC & LC-MS/MS untargeted				↑ ornithine; ↓ ammonia in ALS compared with PD	97
Trauma and acute blood							
Traumatic brain injury (n = 19)	Controls with no infection (n = 5)	LC-MS/MS targeted	↑				98
Acute hydrocephalus because of hypertension (n = 5); SAH (n = 3)	Peripheral neuropathy, ophthalmologic disorders and inactive neurocysticercosis (n = 7)	HPLC-FD, HPLC-UV targeted			↔	↑ citrulline; ↓ARG/citrulline ↔ ARG; ↔citrulline/nitrate	99
SAH with cerebral ischaemia (n = 20)	SAH with no ischaemia (n = 14)	LC-MS/MS targeted	↑			↑ SDMA	100
SAH (n = 40)	Controls with no infection (n = 6)	GC and LC-TOFMS untargeted				↑ ornithine; ↔ citrulline; ↔ ARG	101
Cerebral vasospasm after SAH (n = 24)	Controls with hydrocephalus (n = 6)	ELISA targeted	↑				102
SAH after traumatic brain injury (n = 10); SAH after a non-traumatic injury (n = 5)	Healthy controls (n = 9)	LC–MS/MS targeted	↔			↓ ARG/ADMA; ↑SDMA; ↔ ARG	103

(Continued)

Table 2. Continued.

Disease cohort	Description of control group	Analytical platform	Findings				Ref
			ADMA	NO_2^-	NO_3^-	Other	
Other							
Glioblastoma IDH-WT (n = 7); IDH-mutant (n = 4); Metastatic CNS disease with lung cancer (n = 7); Metastatic CNS disease with breast cancer (n = 5)	Controls with no cancer (n = 8)	LC-MS targeted				↑Argininosuccinic acid in metastatic lung cancer to the CNS; ↑ ornithine in metastatic breast cancer to the CNS	104
Overt hepatic encephalopathy (n = 14)	No neurological disease (n = 27)	LC-HRMS untargeted				↑ ammonia	105
Episodic cluster headache (n = 14)	Healthy controls (n = 11)	CE targeted		↑	↑		106
Ischaemic stroke (n = 88)	Controls (n = 24)	HPLC targeted	↑			↑ SDMA	107

↑represents statistically elevated metabolite in patients compared with controls, ↓ represents statistically decreased metabolite in patients compared with controls, ↔ represents no statistical difference between groups, and blank represents 'not reported or not measured'. Ratios are represented by *x/y* (e.g. arginine/citrulline).

ADMA, asymmetric dimethylarginine; ARG, arginine; hArg, homoarginine; HIV, human immunodeficiency virus; IDH, isocitrate dehydrogenase; MS, multiple sclerosis; NO, nitric oxide; NO_2^-, nitrite; NO_3^-, nitrate;SAH, subarachnoid haemorrhage; SDMA, symmetric dimethylarginine.

a broad spectrum of human CNS diseases with neuroinflammatory mechanisms as described in Table 4. Wide variations of patient and control cohorts have been used for CNS infections, MS, Alzheimer's disease, neurodegeneration and autoimmune disease states. As shown in Table 4, ceramide is generally elevated, whereas sphingomyelins are generally decreased, resulting in an increased ceramide/sphingomyelin ratio. Phosphatidylcholines were found to be elevated in CNS infections including encephalitis or meningitis, but conversely generally decreased in neurodegeneration. In addition, an increase in oxylipins (such as prostaglandin E2, 15-(S)-hydroxyeicosatetraenoic acid, 9-hydroxyoctadecadienoic acid, 9-hydroxyoctadecadienoic acid and dihomo-γ-linolenic acid) is evident during inflammation.

Metabolomics has demonstrated to be a powerful tool in the discrimination of metabolite features between different patient groups and responses to therapeutic interventions. From Tables 1–4, the hypothesis-generating and data-mining-driven approach has shown success in the search of biomarkers for diagnosis, prognosis and monitoring of neuroinflammation in human diseases. To date, most of the studies compare single diseases with controls, and there have been very few studies comparing differences in CSF metabolites between different neuroinflammatory diseases. Such studies are required to determine whether CSF metabolomics can help separate different neuroinflammatory conditions and therefore aid in the differential diagnosis. Whilst at present an ideal biomarker is unknown, a combination of metabolites from the tryptophan–kynurenine pathway, nitric oxide pathway, neopterin and major lipid species may exhibit greater potential for discriminating between different causes of inflammation. More importantly, the metabolite changes identified and quantified as primary indicators in patient cohorts will form a crucial part in clinical translational practice.

CHALLENGES AND FUTURE DIRECTIONS IN CSF METABOLOMICS

Despite the discriminative power of the CSF biofluid, there are many challenges involved in the accessibility of samples from a control population and limited sample volumes. This is because of the invasive nature of the matrix and

Table 3. Cerebrospinal fluid (CSF) studies reporting neopterin findings in neurological diseases with confirmed or suspected neuroinflammation

Disease cohort	Description of controls	Analytical platform	Findings		Ref
			NEO	Other	
Encephalitis, meningitis and infection					
HIV patients on cART neurocognitive impaired (n = 70)	HIV patients on cART neurocognitive normal (n = 29)	ELISA targeted	↑		108
HIV-positive patients (n = 67)	HIV-negative controls with no neurological disease (n = 45)	ELISA targeted	↑		109
Acute HIV Fiebig stage I (n = 9); Acute HIV Fiebig stage II (n = 10); Acute HIV Fiebig stage III (n = 32); Chronic HIV (n = 53)	HIV-negative controls (n = 18)	ELISA targeted	↑		110
Untreated HIV-infected (n = 382); Untreated AIDS with CNS infections (n = 73); Treated HIV patients (n = 233)	HIV-seronegative controls (n = 53)	EIA, RIA targeted	↑		111
Encephalitis (n = 10); acute aseptic meningitis (n = 25); acute bacterial meningitis (n = 6)	Controls with similar symptoms without pleocytosis (n = 42)	LC-MS/MS targeted	↑	Strong correlation between KYN and TRP	47
CNS Lyme disease (n = 5); WNV meningoencephalitis (n = 5); Clinically isolated syndrome of MS (n = 4); rabies (n = 10); histoplasma meningitis (n = 3)	No encephalopathy or encephalitis (n = 25)	NMR targeted	↑	Elevated NEO in rabies, Lyme disease and other neuro-infections	112
Acute encephalitis (n = 30); neurodegeneration (n = 17); febrile seizures (n = 6)	NIND (n = 105)	HPLC-FD targeted	↑		45
Nephropathia epidemica caused by acute Puumala hantavirus infection (n = 23)	Controls (n = 19)	ELISA targeted	↑		113
Tumors of CNS (n = 23); peripheral infections (n = 18); meningitis/encephalitis (n = 6); MS/polyneuropathy (n = 9)	NIND (n = 8)	RIA targeted	↑	Elevated NEO order: Meningitis or encephalitis > tumors of CNS > peripheral infections	114
Human African trypanosomiasis stage 1 (n = 20); Human African trypanosomiasis stage 2 (n = 20)	No history of HAT treatment (n = 16)	LC-MS/MS untargeted	↑		115
Human T-lymphotropic virus 1-associated myelopathy/tropical spastic paraparesis (n = 52)	Human T-lymphotropic virus 1-infected asymptomatic carriers (n = 23)	HPLC targeted	↑		116
Multiple sclerosis (MS)					
MS (n = 61); autoimmune encephalitis (n = 24)	Healthy controls (n = 19)	ELISA targeted	↑	NEO elevated significantly in autoimmune encephalitis	117
MS (n = 37)	NIND (n = 22)	LC-MS/MS targeted	↑		71

(Continued)

Table 3. Continued.

Disease cohort	Description of controls	Analytical platform	Findings		Ref
			NEO	Other	
Clinically isolated syndrome (n = 27); Relapsing–remitting MS (n = 44); Primary progressive MS (n = 15)	NIND (n = 39)	ELISA targeted	↑	Elevated NEO order: RRMS > PPMS > CIS	118
Neurodegeneration					
Alzheimer's disease (n = 20)	Controls without AD (n = 18)	HPLC, and GC/MS targeted	↑		76
Parkinson's disease (n = 22)	Healthy controls (n = 11)	HPLC targeted	↑	Strong correlation between neopterin and KYN/TRP	48
Cognitive impairment (n = 10); delirium and cognitive impairment (n = 40); delirium (n = 40)	Controls (n = 56)	HPLC-FD targeted	↑		119
CNS tumors					
Primary central nervous system lymphoma (PCNSL, n = 21)	Other brain tumors (n = 44), CNS inflammatory diseases (n = 34)	ELISA targeted	↑	Higher neopterin in PCNSL patients with multiple lesions	120
Other brain tumor types (n = 54); pseudotumoral inflammatory lesions (n = 13); PCNSL (n = 28)	Non-tumefactive inflammatory CNS disorders (n = 29)	HPLC-FD targeted	↑	NEO elevated significantly in PCNSL patients	121
Other					
Autism (n = 12)	Other neurological disorders (n = 27)	HPLC targeted	↓	↑ biopterin	122

↑ represents statistically elevated metabolite in patients compared with controls.
Acute HIV Fiebig stage I: HIV present in blood samples and positive in RNA.
Acute HIV Fiebig stage II: positive in RNA and HIV-1 p24 antigen test.
Acute HIV Fiebig stage III: positive in RNA, HIV-1 antigen and EIA.
Human African trypanosomiasis stage 1: the presence of parasites in the blood and lymphatics.
Human African trypanosomiasis stage 2: parasites located beyond the blood–brain barrier in the CSF.
cART, combination antiretroviral therapy; HIV, human immunodeficiency virus; NEO, neopterin; NIND, non-inflammatory neurology disease.

Table 4. Cerebrospinal fluid metabolomics studies reporting lipid findings in neurological diseases with confirmed or suspected neuroinflammation

Disease cohort	Description of control group	Analytical platform	Findings				Ref
			SM	Cer	PC	Other	
Encephalitis, meningitis and infection							
Rabies (n = 11)	Controls without corresponding microbiological assessment (n = 25)	NMR targeted				↑ 3-OHB ↑ glycerol	123
Enteroviral meningitis (n = 10)	non-inflamed–non-infected controls (n = 19)	LC-MS/MS and FIA-MS/MS targeted			↑		124
Bacterial meningitis (n = 32); viral meningitis or encephalitis (n = 34); herpes simplex virus encephalitis (n = 9); varicella-zoster virus meningoencephalitis (n = 15); enterovirus meningitis (n = 10)	Non-inflamed controls (n = 66)	LC-MS/MS targeted			↑		125
Bacterial meningitis (n = 32); Borrelia burgdorferi neuroborreliosis (n = 34); herpes simplex encephalitis (n = 9); VZV meningoencephalitis (n = 15); enterovirus meningitis (n = 10); facial zoster (n = 16); segmental zoster (n = 14)	multiple sclerosis (n = 17); Bell's palsy (n = 11); Gilles de la Tourette syndrome (n = 20); normal pressure hydrocephalus (n = 35)	LC-MS/MS targeted			↑		126
Segmental zoster (n = 14); facial nerve zoster (n = 16); zoster meningoencephalitis (n = 15)	Enteroviral meningitis (n = 10); idiopathic facial paresis (n = 11); normal pressure hydrocephalus (n = 15)	LC-MS/MS untargeted	↑		↑	↑ LPC	87
Multiple sclerosis (MS)							
Primary progressive MS (n = 2); secondary progressive MS (n = 25); relapsing–remitting MS (n = 19)	Healthy siblings (n = 46); controls free from current symptomatic disease (n = 50)	GC-MStargeted				↑ PGE2 ↑ 15(S)-HETE	127
Clinically isolated syndrome or relapsing–remitting MS (n = 41)	Controls free from past and current neurological or autoimmune disease (n = 22)	LC-MS/MS targeted				↑ 9-HODE ↑ 13-HODE	128
Clinically isolated syndrome or relapsing–remitting MS (n = 8); primary progressive MS (n = 4); progressive relapsing MS (n = 1)	No MS (n = 10)	LC-MS/MS targeted		↑			129
MS (n = 20)	Other central and peripheral neurological disease (n = 17)	LC-MS/MS targeted	↓				130
Neurodegeneration							
Alzheimer's disease (n = 19)	controls with subjective memory complaints without dementia (n = 19)	LC-MS/MS targeted			↔	↓ LPC ↓ LPC/PC	131
Mild cognitive impairment (n = 40); Alzheimer's disease (n = 29)	cognitively normal (n = 70)	LC-MS/MS targeted	↓	↑		↓ SM/Cer	132
Alzheimer's disease (n = 29)	Controls with no evidence of cognitive impairment (n = 70)	LC-MS/MS targeted	↓	↑	↓	↑ DhCer ↑ Cer/SM	133
Alzheimer's disease (n = 16); idiopathic normal pressure hydrocephalus (n = 10)	Cognitively normal (n = 10)	LC-MALDI-MS/MS targeted	↓	↑		↓ S1P	134
Parkinson's disease (n = 31)	Neurologically healthy controls (n = 95)	FT-ICR-MS untargeted			↓	↑ ARA; ↑ 10-HDA; ↑ DLGA; ↓ PE	135

(Continued)

Table 4. Continued.

Disease cohort	Description of control group	Analytical platform	Findings					Ref
			SM	Cer	PC	Other		
Other								
Post-operative delirium (n = 40)	Non-post-operative delirium (n = 30)	LC-MS/MS untargeted	↑	↑	↓	↓ PE		136
Progressive multifocal leucoencephalopathy (n = 23)	normal pressure hydrocephalus (n = 8)	FIA-MS/MS targeted	↓					137
Guillain–Barré syndrome (n = 86)	Idiopathic oculomotor nerve palsy (n = 8); brainstem or spinal cord ischaemia (n = 5); idiopathic brachial plexopathy (n = 1); Wernicke encephalopathy (n = 1); Vernet's syndrome (n = 1); motor neuron disease (n = 1); diabetic polyneuropathy (n = 1); nutrition deficiency syndrome (n = 1); pineal gland tumor (n = 1)	NMR, GC-TOF/MS, LC-MS/MS untargeted	↑			↑ LPC ↓ acetate		138

Cohorts are separated into subgroups (e.g. encephalitis). ↑represents statistically elevated metabolite in patients compared with controls, ↓ represents statistically decreased metabolite in patients compared with controls, ↔ reports no statistical difference between groups, and blank represents 'not reported or not measured'. Ratios are represented by *x*/*y* (e.g. SM/Cer).
10-HDA, 10-hydroxydecanoic acid; 13-HODE, 13-hydroxyoctadecadienoic acid; 15(S)-HETE, 15-(S)-hydroxyeicosatetraenoic acid; 3-OHB, 3-hydroxybutyrate; 9-HODE, 9-hydroxyoctadecadienoic acid; ARA, arachidonic acid; Cer, ceramide; DhCer, dihydroceramide; DLGA, dihomo-γ-linolenic acid; LPC, lysophosphatidylcholine; PC, phosphatidylcholines; PE, phosphatidylethanolamine; PGE2, prostaglandin E2; S1P, sphingosine-1-phosphate; SM, sphingomyelins.

the ethical issues concerning the collection of CSF from 'healthy' individuals. Furthermore, variation in sample collection, preparation, analytical instrumentation and data processing can influence the set of observed metabolic changes within a study.[50] The optimisation of the experimental design for metabolomics studies is key to ensure standardisation and improve reproducibility of CSF metabolic biomarkers across studies. Data acquisition is a core area of metabolomics experiments, and analytical instrumentations are constantly undergoing advancements for improved detection consistency, sensitivity of metabolite detections at lower levels and simplified data analysis tools. However, challenges lie in the scanning speed and sensitivity of detection, resulting in limited high quality and quantity of metabolomics data for validation of potential metabolite biomarker identities. Preliminary metabolomics studies predominantly used untargeted approaches and produced semi-quantitative data generally using an internal standard for normalisation, but to successfully translate the research data, there is a growing demand for quantitative metabolomics-driven methods. The current lack of quantitative metabolomics data poses challenges in defining reference ranges and determining abnormal values that are important for the translation to a clinical setting.

The ultimate method for developing metabolomics analysis would be to explore the metabolome with minimal platforms; however, to date there is no single platform able to cover the full metabolome.[51] Further challenges in global metabolomics lie in the identification of metabolites and biological variation in human biofluids.[52] A bottleneck in metabolomics studies is accurate metabolite annotation to perform biological interpretations.[53,54] Over the last decade, metabolite databases and libraries available for metabolomics research have significantly expanded. The human metabolome database (http://www.hmdb.ca) and CSF metabolome database (https://www.csfmetabolome.ca) are currently the most comprehensive databases consisting of chemical, clinical, molecular biology and biochemistry data to support the interpretation of metabolomics data.[55] Chemical and spectral data repositories such as METLIN (http://metlin.scripps.edu), ChemSpider (http://www.chemspider.com), NIST mass spectral library (http://chemdata.nist.gov)

Table 5. Summary of types of information found in metabolite databases and libraries used for metabolomics

Database	Information found in the database
Human metabolome database	Chemical data Clinical data Molecular biology data Biochemistry data
Cerebrospinal fluid metabolome database	Chemical data Clinical data Molecular biology data Biochemistry data
METLIN	Spectral data
ChemSpider	Chemical data
NIST	Spectral data
mzCloud	Spectral data

and mzCloud (https://www.mzcloud.org) are popular avenues used as the benchmark for metabolite identification (Table 5). However, owing to the size of the metabolome, the spectral information stored in databases is limited by the availability of pure standards. Moreover, from a bioinformatics point of view, the evaluation for the similarity of spectra matches cannot be fully automated; therefore, visual inspection is mandatory and should not rely on scores only.

Finally, there is a paucity of studies that measure multiple metabolites in unison, in order to see whether there is correlation or key differences in tryptophan–kynurenine, nitric oxide and neopterin metabolites in different disease states. Given the importance of defining potentially damaging and reversible inflammatory mechanisms in common disorders such as neurodegeneration, neuropsychiatry and neurodevelopment, such large studies are vital to provide diagnostic biomarkers *in vivo*.

CONCLUSION

Metabolomics is rapidly moving in an exciting direction, demonstrating great potential in diagnostic and treatment knowledge of diseases affecting the CNS. There is increasing evidence that the changes in metabolites involved in the tryptophan–kynurenine pathway, nitric oxide pathway and neopterin are strongly associated in a wide range of human CNS diseases with neuroinflammation mechanisms. Such metabolic CSF neuroinflammation biomarkers should be integrated into clinical practice.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

JIngya Yan: Conceptualization; Resources; Writing-original draft; Writing-review & editing. **Unnikrishnan Kuzhiumparambil:** Conceptualization; Project administration; Supervision; Writing-review & editing. **Sushil Bandodkar:** Supervision; Writing-review & editing. **Russell C Dale:** Conceptualization; Funding acquisition; Resources; Supervision; Writing-review & editing. **Shanlin Fu:** Conceptualization; Funding acquisition; Project administration; Supervision; Writing-review & editing.

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