ARTICLE

Multiple sclerosis has a distinct lipid signature in plasma and cerebrospinal fluid

Esclerose múltipla tem uma assinatura lipídica distinta no plasma e no líquido cefalorraquiano

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ABSTRACT

The diagnosis of multiple sclerosis (MS) has changed over the last decade, but remains a composite of clinical assessment and magnetic resonance imaging to prove dissemination of lesions in time and space. The intrathecal synthesis of immunoglobulin may be a nonspecific marker and there are no plasma biomarkers that are useful in the diagnosis of MS, presenting additional challenges to their early detection. **Methods:** We performed a preliminary untargeted qualitative lipidomics mass spectrometry analysis, comparing cerebrospinal fluid (CSF) and plasma samples from patients with MS, other inflammatory neurological diseases and idiopathic intracranial hypertension. **Results:** Lipid identification revealed that fatty acids and sphingolipids were the most abundant classes of lipids in the CSF and that glycerolipids and fatty acids were the main class of lipids in the plasma of patients with MS. The area under the curve was 0.995 (0.912−1) and 0.78 (0.583−0.917), respectively. The permutation test indicated that this ion combination was useful for distinguishing MS from other inflammatory diseases (p < 0.001 and 0.055, respectively). **Conclusion:** This study concluded that the CSF and plasma from patients with MS bear a unique lipid signature that can be useful as a diagnostic biomarker.

Keywords: Biomarkers; plasma; cerebrospinal fluid; lipids; multiple sclerosis; myelin sheath

RESUMO

Embora o diagnóstico da EM tenha se modificado na última década, ainda tem como requisito básico a demonstração da disseminação no tempo e no espaço, através do quadro clínico e do exame de ressonância magnética. A síntese intratecal de imunoglobulina pode ser um marcador inespecífico e não há biomarcadores plasmáticos que sejam úteis no diagnóstico da EM, impondo desafios à sua detecção precoce. **Métodos:** Realizamos uma análise lipidômica preliminar por espectrometria de massas, não direcionada, qualitativa, comparando amostras de LCR e plasma de pacientes com EM, outras doenças neurológicas inflamatórias e hipertensão intracraniana idiopática (HII). **Resultados:** A identificação lipídica revelou que os ácidos graxos e esfingolipídios foram as classes mais abundantes de lipídios no LCR e que glicerolipídios e ácidos graxos foram a principal classe de lipídios no plasma de pacientes com EM. A AUC foi de 0,995 (0,912−1) e 0,78 (0,583−0,917), respectivamente. O teste de permutação indicou que essa combinação de íons foi útil para distinguir a EM de outras doenças inflamatórias (p < 0,001 e 0,055, respectivamente). **Conclusão:** Este estudo sugere que o líquido cefalorraquidiano (LCR) e o plasma de pacientes com EM possuem uma assinatura lipídica única, pode ser útil como um biomarcador diagnóstico.

Palavras-chave: Biomarcadores; plasma; líquido cefalorraquidino; lipídeos; esclerose múltipla; bainha de mielina

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Multiple sclerosis (MS) is a complex inflammatory autoimmune demyelinating disease of the central nervous system (CNS), resulting from environmental and genetic interactions. It is the leading cause of permanent non-traumatic disability in young adults[1]. Its diagnosis has changed over the last decade but still remains a composite of clinical evaluation and magnetic resonance imaging (MRI) requirements to prove dissemination in time and space, reflecting the multitude of brain lesions appearing at different moments[2].

To date, markers of intrathecal immunoglobulin synthesis, such as oligoclonal bands or the immunoglobulin G index have been used as diagnostic and/or prognostic tools, but they lack specificity. Also, there has been no plasma biomarker that is a useful tool in MS diagnosis. This somewhat limited scenario imposes some challenges to earlier MS detection[3,4]. An MRI is the only surrogate marker, but it provides indirect and limited information about the disease and its pathophysiological process.

The cerebrospinal fluid (CSF) remains a potential source when looking for a disease biomarker in MS. It is in close contact with the brain and the blood-brain barrier and it is conceivable that the inflammation and the cellular damage intrinsic to the multistep process of MS pathology may be detectable in CSF[5]. Recent studies have shown that the CSF levels of myo-inositol were discriminatory between patients with MS and other neurological diseases and that chitinase-3 levels were predictive of a new relapse and new gadolinium-enhancing lesions[6,7].

In addition, there is a growing body of evidence of the importance of lipids in the pathophysiology of different CNS diseases[8,9]. Indeed, lipids represent a broad group of molecules with critical roles in cell biology and inflammation and are present at a high concentration in brain tissue[10]. Accordingly, lipidomics analyses of the CSF in patients with MS show an increase in molecules associated with oxidative stress and lipid degradation, which may be responsible for the maintenance of the autoimmune process[11]. Moreover, patients with MS have a higher level of ceramide in the CSF, which may be linked to the axonal damage observed in the disease[12]. Ceramide is produced in the CSF through the hydrolysis of sphingomyelin. Curiously, a recently published paper demonstrated that the sphingomyelinase activity is elevated in the CSF of patients with MS[13].

Although the CSF seems the logical fluid to study, one must consider the difficulties in obtaining it. Therefore, a useful biomarker that can be found in plasma is of utmost importance. Several studies have demonstrated that plasma from patients with MS exhibits biochemical profiles distinguishable from other neurological diseases[14,15]. Recently, it has been shown that the presence of sphingomyelin and lyso-phosphatidylethanolamine differentiated patients with MS

from healthy controls[16]. Additionally, the patients displayed metabolites related to oxidative stress[17].

This study aimed to evaluate a lipid profile unique to MS, present in CSF and plasma. Its identification could complement current diagnostic criteria, improve patient care, and guide therapeutic decisions.

METHODS

Study design

We performed a preliminary untargeted qualitative lipidomics analysis comparing CSF and plasma samples from patients with MS, other inflammatory neurological diseases (OIND), and idiopathic intracranial hypertension (IIH). Initially, to investigate whether inflammatory diseases have a specific lipid profile, we clustered the CSF samples from patients with MS and OIND to form an inflammatory group and compared those with the CSF samples from patients with IIH, the noninflammatory control group. In addition, to identify a unique MS signature, we compared CSF and plasma samples from both MS and OIND patients, as separate groups. The institutional ethics committee approved the study (CAEE: 39862114.0.0000.5505/ 951.893) and participants provided written informed consent.

The CSF and plasma samples

Cerebrospinal fluid and plasma samples, frozen and stored at -80°C, collected between 2014 to 2015, were used for the experiments carried out at the Institute of Environmental, Chemistry and Pharmaceutical Sciences, Universidade Federal de São Paulo, between July and December 2016. The CSF was obtained as part of the routine clinical investigation, according to the assistant physician's best judgment, from patients with neurological diseases who were followed at the Neurology Department. When appropriate, a nonfasting blood sample was drawn at the same time as the CSF collection, in order to detect oligoclonal bands by isoelectric focusing, according to standard protocol. After the routine CSF examination and oligoclonal band testing, the remaining CSF and plasma samples were stored for later use, in the experiments described below. There were 42 paired CSF plasma samples from patients with either MS or OIND, and 15 CSF control samples collected from patients with IIH, totaling 57 samples.

All the patients signed a form authorizing the collection of CSF by lumbar puncture, as part of a routine clinical investigation.

Clinical data

We obtained data on the final diagnosis, clinical characteristics and CSF abnormalities by reviewing the patients' medical records. The CSF examination for routine clinical

diagnosis included global and differential cytology analysis, routine biochemical immunological and microbiological analyses.

Lipid extraction

The lipids were extracted based on the Bligh and Dyer protocol with minor modifications[18]. Briefly, 50 μL of the CSF and plasma sample were placed in a microtube with 125 μL chloroform, 250 μL methanol and 50 μL distilled water. This mixture was vortexed for one minute. Then, 100 μL water and 125 μL chloroform were added and centrifuged at $500 \times g$ for five minutes resulting three phases. The upper phase containing the lipids was recovered and transferred to another microtube, which was left open overnight at room temperature for solvent evaporation.

Liquid chromatography-electrospray ionisation mass spectrometry and data processing

The analyses were performed with the ultra fast liquid chromatography system (Shimadzu, Kyoto, Japan), using a C18 column, coupled with a Bruker ESI-micrOTOF-QII mass spectrometer (Billerica, MA, USA). Direct injection was performed using a liquid chromatography system. The mobile phase used was isopropanol/acetonitrile (1:1, v/v) and 10 mmol/L of ammonium acetate with a flow of 200 μL/minute. The spectra were obtained in an Apollo ion source according to the following parameters: 2.0 bar nebulizer gas, dry gas of 8.0 L/minute, the temperature of 180°C and voltage of 4.5kV. The mass/charge ratio (m/z) was detected in scan (100-1200 Da) in positive mode. Sodium formate (Sigma-Aldrich) with a range of m/z 50-1200 was used as the calibration standard.

The acquired spectra were recalibrated in DataAnalysis 4.1 (Billerica, MA, USA) and a list of ions (m/z) and their respective intensities were extracted from each spectrum. The lists containing the ions and their intensities, already separated by group (MS, OIND, and IIH), were processed through the MetaboAnalyst 3.0 online software (www.metaboanalyst.ca) so that the set of ions of each sample was combined generating a single table. For the processing of these data, a mass error of m/z 0.05 was used as tolerance parameter.

Statistical analysis

The table generated after the processing of the individual peaks was used to carry out the multivariate analysis of the data. For this, we used the MetaboAnalyst 3.0 online software. A principal component analysis and a partial least squares discriminant analysis (PLS-DA) were performed on the log-transformed data and standardized by auto-scaling. The principal component analysis was used to observe grouping and the samples discrepancies in general, while the PLS-DA was used to maximize the variations and to guarantee the discriminatory effect of the components based on the values of the variable importance in projection (VIP).

A cross-validation test was applied to validate the method created by PLS-DA and indicated which component of the model was the one that best explained the variations of ions between the groups. Based on the PLS-DA, we selected the ions with the highest VIP score, from the component with the greatest discriminatory effect, as potential biomarkers, according to the adducts and mass tolerance of 0,05 m/z. Those ions that were hyper-represented in the MS group were valued according to their VIP-PLS score. We built a ROC curve for each ion and for their combination. In addition, we performed a permutation test (1000x) and a prediction class probability based on our samples.

The ions selected as potential biomarkers were identified by the online database LipidMaps (www.lipidmaps.org). The identification was performed considering the H^+, Na^+, K^+ and NH_4^+ ions in the positive ionization mode. Compounds with a maximum error of 0.05 m/z were considered.

RESULTS

Clinical characteristics

The clinical data are summarized in Table 1. Briefly, there were 13 patients with multiple sclerosis, eight with OIND, and 15 with idiopathic intracranial hypertension. The mean age of the entire population was 38 years (± 12.9). However, patients with MS were slightly younger (35 ± 10.3 years) than the IIH group. The female-to-male ratio varied from 2.5–4:1. Patients with MS were distributed as follows: two patients (15%) with primary progressive and 11 (85%) with relapsing-remitting MS. The MS group had had at least one relapse before CSF collection and the most common symptom was an acute partial myelitis. Regarding neurological disability, patients with MS were mildly impaired with a median Expanded Disability Status Scale score of 2.5.

The OIND group was heterogeneous and comprised patients with demyelinating, inflammatory, and vascular diseases. The patients with a demyelinating nonMS disease had neuromyelitis optica or idiopathic optic neuritis. The remaining patients with an inflammatory or vascular disease had a final diagnosis of Susac syndrome, rhombencephalitis, Vogt-Koyanagi-Harada, and embolic stroke.

The CSF and plasma samples were collected within 15 days of an acute exacerbation in 7 (53.8%) and 4 (50%) patients of the MS and OIND groups, respectively. The CSF from patients with IIH was normal, whereas patients with an OIND showed a more inflammatory pattern with an average of 18 cells/ml (± 29), predominantly lymphocytes, and higher levels of CSF protein (51.3 ± 30.7). Cerebrospinal fluid oligoclonal bands were present in 11 patients with MS. One patient was not tested for oligoclonal bands.

Table 1. Demographics, clinical and CSF characteristics of the three patient groups.

Variables	MS	OIND	IIH
N	13	8	14
PPMS (n, %)	2 (15%)	(-)	(-)
NMO (n, %)	(-)	2 (25%)	(-)
Gender (%)	F (79%)	F (62,5%)	F (78%)
F/M	4F:1M	2.5F:1M	4F:1M
Age (mean, SD)	35 (±10.3)	38.6 (±15.5)	41 (±13.2)
Disease duration, y (mean, SD)			
up to CSF	5.2 (± 7.7)	3.1 (± 6)	2.3 (±1.7)
Total time	6.9 (± 7.8)	4.9 (± 6.1)	2.3 (±1.7)
Follow up time, y	2.8 (± 4)	1.6 (± 1.4)	N/A
Relapses up to CSF collection (mean, sd)	1.5 (± 1.5)	1.4 (± 0.7)	N/A
First symptom (n, %)			
ADEM like	1	0	N/A
Cerebellar	1	0	N/A
Myelitis	8	1	N/A
Brain stem	1	4	N/A
Optic neuritis	2	3	N/A
CSF			
Collected during relapse (n, %)	5/13 (38.5%)	4/8 (50%)	NA
Cell count (mean, sd)	5.4 (± 6.3)	18.3 (± 29)	1 (± 0.85)
Total protein level, mg/dL, (mean, sd)	30.8 (± 12.)	51.3 (± 30.7)	29 (±12.8)
OCB positive (n, %)	11/12 (91.6%)	1/8 (12.5%)	NA

MS – multiple sclerosis, OIND – other inflammatory neurological diseases, IIH – idiopathic intracranial hypertension, PPMS- primary progressive multiple sclerosis, NMO – neuromyelitis optica, CSF – cerebrospinal fluid, OCB- oligoclonal band, ADEM – acute disseminated encephalopathy.

Lipidomics analysis

We processed the data from the acquired spectra and generated a table containing 733 ions and identified those that were present in at least 50% of the samples (Table 2).

CSF – Inflammation versus noninflammation

To investigate whether inflammatory diseases would have a distinct profile, we compared IIH with MS and OIND. In these groups, the PLS-DA analysis suggested that component 4 of this model was the one that best explained the variations of ions between the samples. Figure 1A shows the group of 15 VIP ions with the greatest discriminatory PLS-DA score. Lipid identification revealed that fatty acids and polyketides were the main class of lipids in the inflammatory signature (Table 2). The area under the curve (AUC) for the inflammatory signature was 0.704 (0.205–0.927) and the p-value for the permutation test, was 0.086 (Figure 1B and 1C).

CSF – MS versus IIH

Additionally, to explore the existence of a disease-specific lipid pattern, we compared MS with IIH. The PLS-DA analysis suggested that component 4 of this model was the one that best explained the variations of ions between the groups. Figure 2A shows the group of 15 VIP ions with the greatest discriminatory PLS-DA score. Lipid identification revealed that mainly fatty acids and glycerophospholipids were the most abundant classes of lipids in the MS signature (Table 2). The AUC for the MS signature was 0.702 (0.458–0.981) and the p-value for the permutation test was 0.08 (Figure 2B and 2C).

CSF – MS versus OIND

After evaluating the different profiles of inflammatory versus noninflammatory diseases, and the presence of an MS pattern, we searched for a specific cluster that would better distinguish MS from other inflammatory neurological diseases.

Table 2. Lipids associated with multiple sclerosis in CSF and Plasma

| Variable	Input Mass	Matched Mass	Delta m/z	Formula	Ion	Name
CSF INF x NINF						
	6.183.572	6.183.976	0.0404	C33H57NO8Na	[M+Na]+	Sterol Lipid
	6.183.572	6.183.168	0.0404	C28H54NO9PK	[M+K]+	Glycerophospolipid
	29.516.225	2.951.329	0.0294	C19H19O3	[M+H-H2O]+	Polyketide
	29.516.225	2.951.904	0.0281	C17H27O4	[M+H-H2O]+	Fatty Acid
	29.516.225	2.952.056	0.0434	C21H27O	[M+H-H2O]+	Sterol Lipid
	64.031.395	6.403.116	0.0023	C35H46NO10	[M+H]+	Polyketide
	66.916.645	6.691.298	0.0367	C28H29O19	[M+H]+	Polyketide
CSF MS X IIH						
	29.516.225	2.952.034	.0411	C16H32O2K	[M+K]+	Fatty esters
	29.516.225	2.951.644	.0022	C16H26O2Na2	[M+2Na-H]+	Fatty esters
	438.178	4.381.887	.0107	C18H34NO10P	[M+H-H2O]+	Glycerophosphoserines
	910.514	910.648	.0134	C11H16O2	[M+2H]2+	Fatty esters
	3.202.338	3.202.152	.0186	C32H63O10P	[M+2H]2+	Glycerophosphoglycerols
	3.202.338	3.202.528	.0190	C41H66O5	[M+2H]2+	Diradylglycerols
	3.202.338	3.202.504	.0166	C37H70O5Na2	[M+2Na]2+	Diradylglycerols
	3.202.338	3.202.580	.0242	C41H70O2Na2	[M+2Na]2+	Sterols
CSF MS X OIND						
	755.363	7.553.613	0.0017	C34H60O16P	[M+H-H2O]+	Glycerophospolipid
	755.363	7.553.613	0.0348	C37H64O13K	[M+K]+	Sterol lipid
	2.941.781	2.942.064	0.0283	C17H28NO3	[M+H-H2O]+	Fatty Acid
	3.881.859	3.882.223	0.0364	C17H36NO5PNa	[M+Na]+	Sphingolipid
	3.881.958	3.882.223	0.0265	C17H36NO5PNa	[M+Na]+	Sphingolipid
	3.881.958	3.882.412	0.0454	C22H36OK1F	[M+K]+	Fatty Acid
PLASMA MS X OIND	8.015.357	8.015.429	0.0072	C47H78O8P	[M+H]+	Glycerophospholipid
	8.015.357	8.015.723	0.0366	C44H81O12	[M+H-H2O]+	Saccharolipids
	8.015.357	8.015.723	0.0366	C44H81O12	[M+H-H2O]+	Fatty Acid
	8.015.357	8.015.487	0.013	C45H78O10Na	[M+Na]+	Glycerolipid
	8.513.786	851.419	0.0404	C42H68O15K	[M+K]+	Prenol Lipid
	55.324.565	5.532.136	0.0321	C24H41O10S2	[M+H]+	Sterol Lipid
	55.324.565	5.532.585	0.0128	C35H37O6	[M+H-H2O]+	Polyketide
	55.324.565	5.532.772	0.0316	C25H46O11P	[M+H-H2O]+	Glycerophospholipid
	55.324.565	5.532.344	0.0112	C25H42N2O7SK	[M+K]+	Fatty Acid
	55.324.565	5.532.562	0.0106	C30H42O7K	[M+K]+	Prenol Lipid
	7.783.713	7.784.056	0.0343	C39H66NO10PK	[M+K]+	Glycerophospholipid

CSF- Cerebrospinal fluid INF- Inflammatory group; N_INF – Noninflammatory group; IIH – Idiopathic intracranial hypertension; MS – multiple sclerosis; OIND – Other inflammatory neurological diseases.

The PLS-DA analysis suggested that component 4 of this model was the one that best explained the variations of ions between the groups. Figure 3A shows the group of 15 VIP ions with the greatest discriminatory PLS-DA score. Lipid identification revealed that fatty acids and sphingolipids were the most abundant classes of lipids in the MS
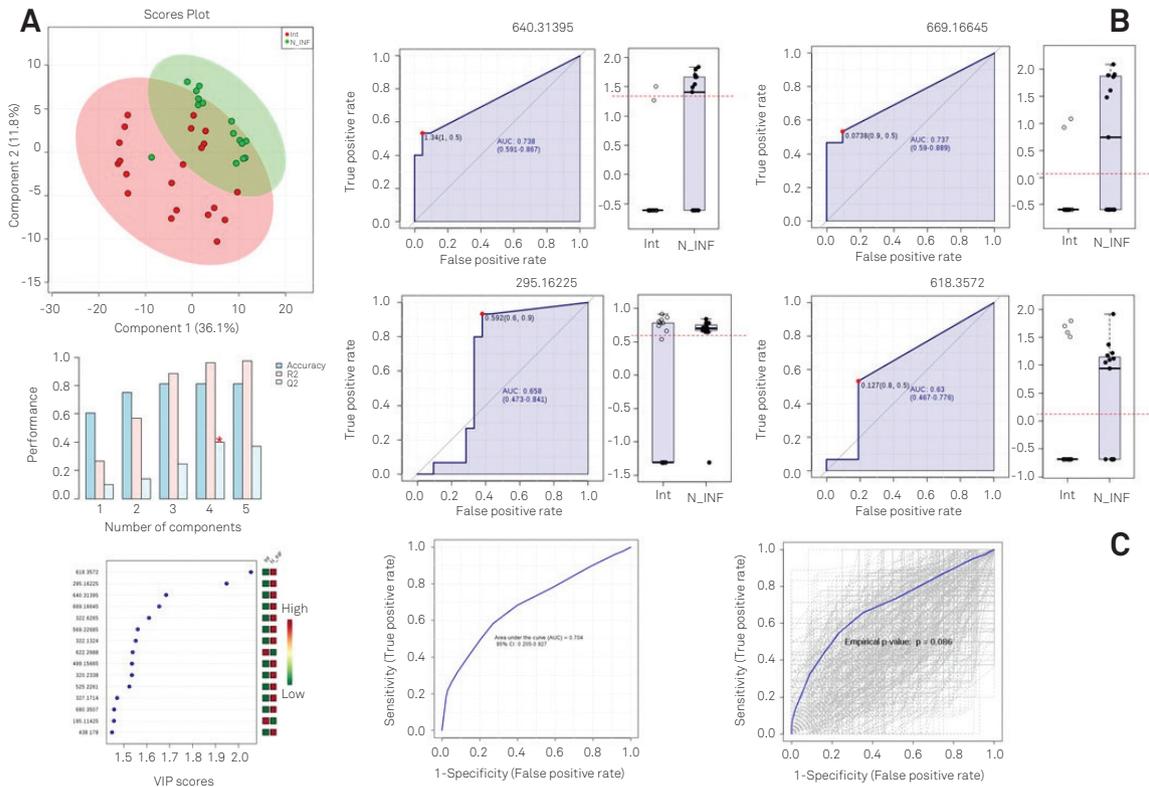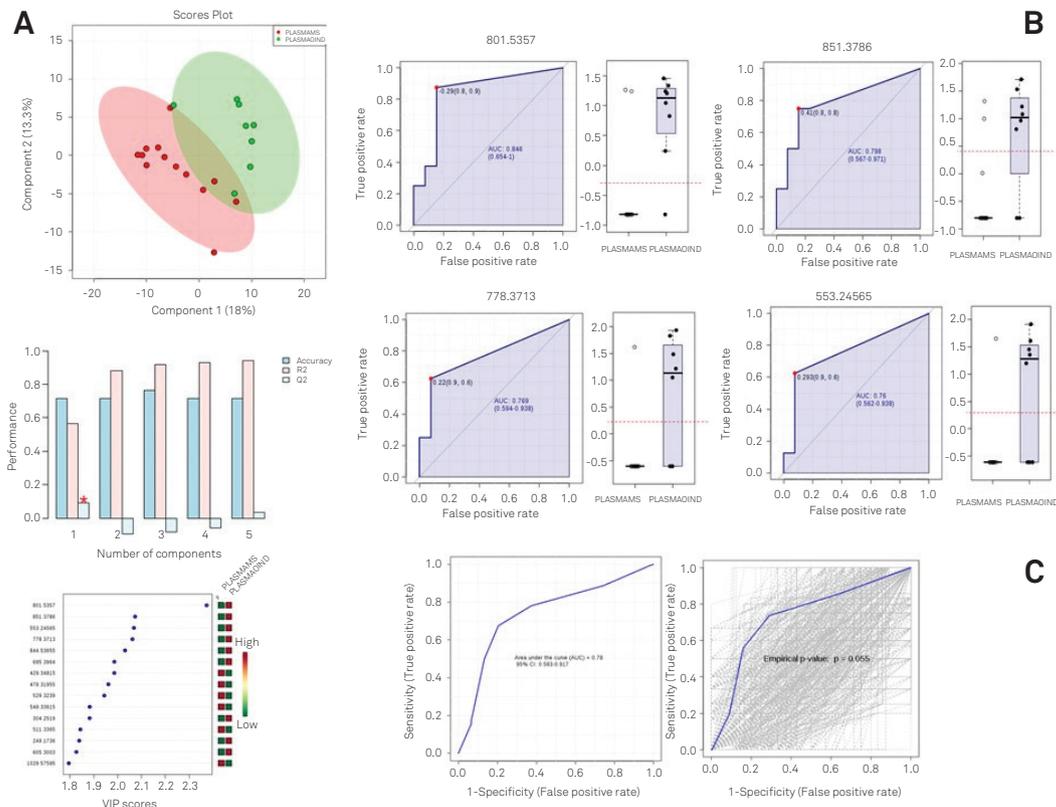
Figure 1. A. PLS-DA analysis comparing CSF of inflammation versus noninflammation (multiple sclerosis and other inflammatory diseases versus idiopathic intracranial hypertension) showing component 4 as the most discriminatory between the groups, and the VIP ions with the higher scores. B. ROC curves for each of the four VIP ions with the higher score. C. ROC curve and the permutation test for the CSF inflammatory signature considering the four ions with the highest scores.
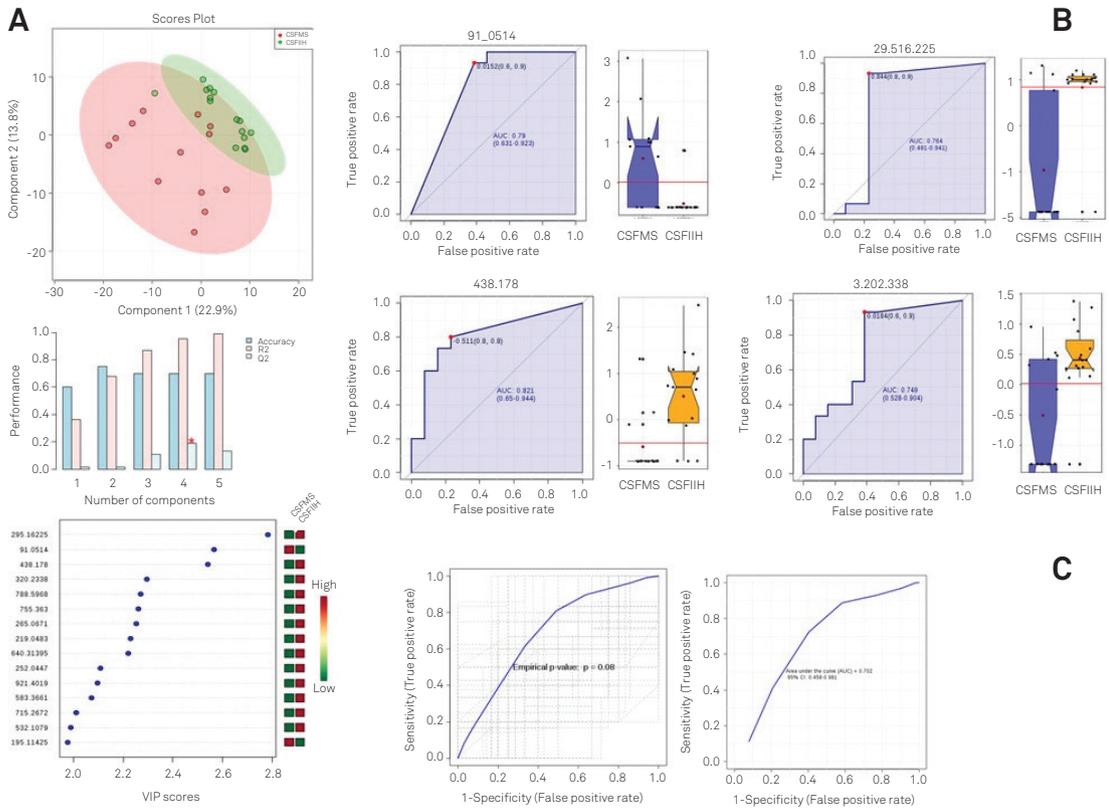
Figure 2. A. PLS-DA analysis comparing CSF of multiple sclerosis versus idiopathic intracranial hypertension showing component 4 as the most discriminatory between the groups and the VIP ions with the higher scores. B. ROC curves for each of the four VIP ions with the higher score. C. ROC curve and the permutation test for the MS-CSF lipid signature considering the four ions with the highest scores.
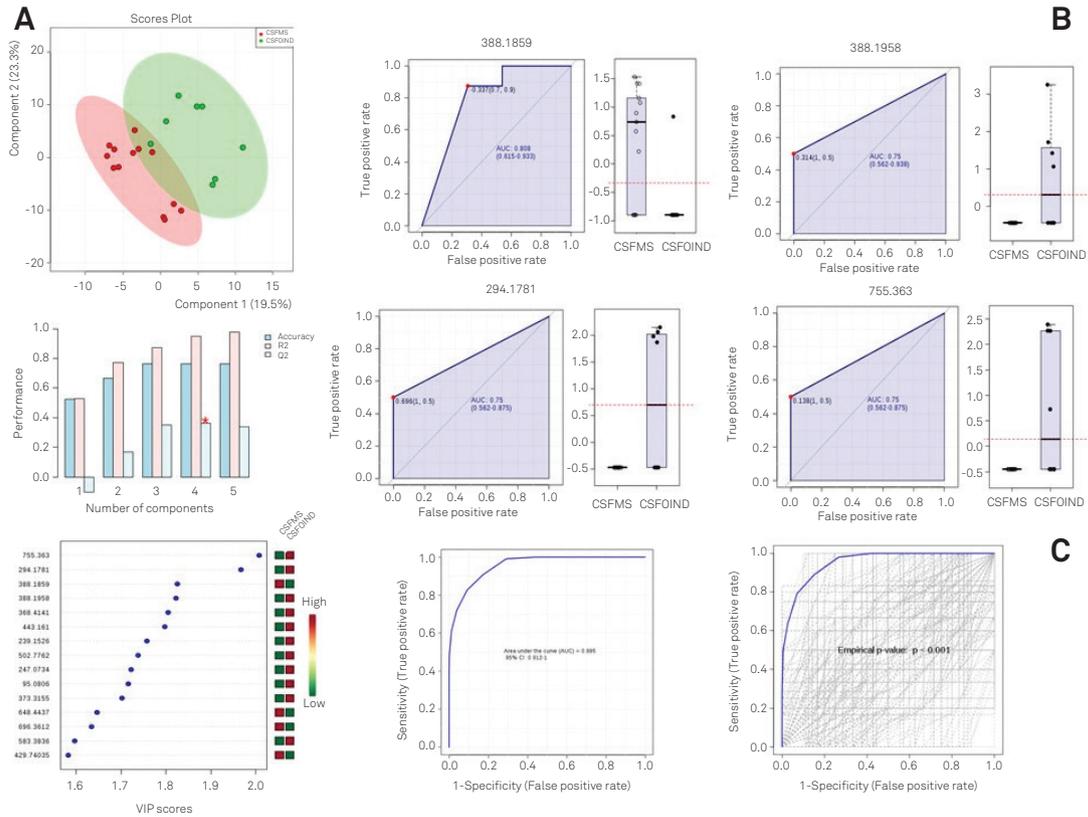
Figure 3. A. PLS-DA analysis comparing CSF of patients with MS versus other inflammatory neurological diseases showing component 4 as the most discriminatory between the groups and the VIP ions with the higher scores. B. ROC curves for each of the four VIP ions with the higher score. C. ROC curve and the permutation test for the MS-CSF lipid signature considering the four ions with the highest scores.

Figure 4. A. PLS-DA analysis comparing plasma of patients with MS versus other inflammatory neurological diseases showing component 1 as the most discriminatory between the groups and the VIP ions with the higher scores. B. ROC curves for each of the four VIP ions with the higher score. C. ROC curve and the permutation test for the MS-plasma lipid signature considering the four ions with the highest scores.

signature (Table 2). The AUC for the MS signature was 0.995 (0.912–1) and the p-value for the permutation test was < 0.001, indicating that this ion combination is useful for the MS diagnosis (Figure 3B and 3C).

Plasma – MS versus OIND

We hypothesized that the inflammatory process observed in the CSF of patients with MS would be reflected in the plasma and that it could be useful in differentiating MS from OIND. Therefore, we performed a lipidomics analysis of 21 plasma samples collected together with the paired CSF samples.

The PLS-DA analysis showed that component 1 of this model was the one that best explained the variations of ions between the groups. Figure 4A shows the group of 15 VIP ions with the greatest discriminatory PLS score. Lipid identification revealed that glycerolipids and fatty acids were the main class of lipids in the plasma MS signature (Table 2). The AUC for the MS signature was 0.78 (0.583-0.917) and the p-value for the permutation test was 0.055 (Figure 4B and 4C)

DISCUSSION

Our results suggest that it is possible to separate patients with MS from OIND based on the lipid profile present in the CSF and plasma. Particularly, in the CSF, the ions had a predictive value of 99% to discriminate patients with MS from patients with OIND. It is interesting to highlight that the lipid profile we obtained from plasma, although discriminatory, showed marginal significance.

Multiple sclerosis and OIND had some degree of inflammation, but it was more pronounced in the latter. Patients in the OIND group had a higher lymphocyte count and a higher level of protein. Curiously, we cannot say that we found an "inflammatory" signature, as the comparison between the inflammation versus the non-inflammation groups, although showing some degree of separation, did not reach statistical significance. Based on the routine CSF analysis, the IIH group did not show any signs of ongoing inflammation, contrasting with the patients with MS and OIND and, despite this, the separation between groups was not clear. The etiology of IIH remains elusive, and one could speculate whether these patients should have been used as our control group.

The identification of the lipids in plasma and CSF showed a mixture of classes from fatty acids to polyketides. Of interest, we emphasize the presence of glycerolipids and sphingolipids in the CSF of patients with MS. Both classes are involved in cell signaling and regulation of biological processes. There is evidence suggesting their role in different diseases such as asthma and breast cancer[10]. In MS, it has been shown that ceramide levels were elevated in MS CSF and this

may be sufficient to cause axonal damage[12]. Interestingly, in an animal model of demyelination, the blockade of acid sphingomyelinase improves remyelination, suggesting the importance of the acid sphingomyelinase/ceramide pathway in MS[19]. Therefore, it is reassuring that on a simple qualitative untargeted analysis, we found the same lipid classes to be discriminative of MS.

The presence of a lipid signature recognizable outside the CNS in patients with MS suggests that the breakdown of the blood-brain barrier is as important as the inflammatory process. The most abundant lipid class we found in plasma from patients with MS were the glycerophospholipids, mirroring what we had found in the CSF. Recent studies have demonstrated that glycerophospholipids are discriminatory between MS and healthy controls. Moreover, these lipids were also linked to a more severe disease[16,20], implying that lipid metabolism may be related to oxidative stress, mitochondrial dysfunction, and degeneration[11]. Thus, the observed lipid profile could mean a degenerative signature rather than an inflammatory one, particularly when one considers that patients with MS, although younger than the control groups, had a longer disease duration and greater neurological disability.

We need to be careful in interpreting these data because the small number of samples may have influenced the results. We did not observe a defined inflammatory pattern, nor did we separate MS from IIH. However, the lack of statistical significance does not mean that there is no biological effect, as we still observed a specific MS signature.

Furthermore, the comparison between the main demyelinating diseases, MS and neuromyelitis optica was not possible, as the OIND group was very heterogeneous, comprising inflammatory diseases of different etiologies and in limited number. This heterogeneity itself could, in part, explain our results.

In addition, the small number of patients did not allow us to establish differences between the clinical forms of MS, and prevented us from performing analysis of lipid profiles and Expanded Disability Status Scale progression.

Our results may have been influenced by the quality of the material. The samples were frozen for up to two years and it is possible that some degree of molecule degradation occurred, as it has been shown that cycles of freezing and thawing can alter the biochemical analysis. Nevertheless, all samples were submitted to the same extraction protocol, handled in the same way, and were equally affected. Likewise, we acknowledge that high resolution mass spectrometry is useful for identifying biomarkers but as we did not acquire MS/MS spectra, the exact identification of lipids was limited.

Moreover, the presence of polyketides, a lipid species that is foreign to mammalian metabolism might be explained by the immunosuppressants or antibiotics some patients were receiving at the time of the sample collection. Clinical information was gathered in a retrospective manner by review of

the patients' files and it should always be considered a potential source of bias.

Our results should be viewed as a first step towards the identification of pathways and molecules that could be useful as diagnostic biomarkers in Brazilian patients. Also, it reinforces the need for quantitative analyses to further investigate the role of complex lipids in the pathogenesis of MS.

We observed a distinctive lipid signature in the CSF and plasma from patients with MS that may be used as a diagnostic biomarker. Understanding MS at the molecular level may be challenging but it is essential for drug development and patient care.

Acknowledgments

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