



Evaluation du greffon hépatique par spectroscopie RAMAN

Congres Association Française Histotechnologie 2024 – Saint Malot

Dr Edouard WASIELEWSKI

U1242 : Oncogenesis Stress Signaling

Service de chirurgie hépatobiliaire et digestive – CHU Rennes

Dirigé par le Pr Laurent SULPICE



Conflit d'intérêt

- Aucun

Introduction

Matériels et
méthodes

Résultats

Discussion

DEMAND

SUPPLY



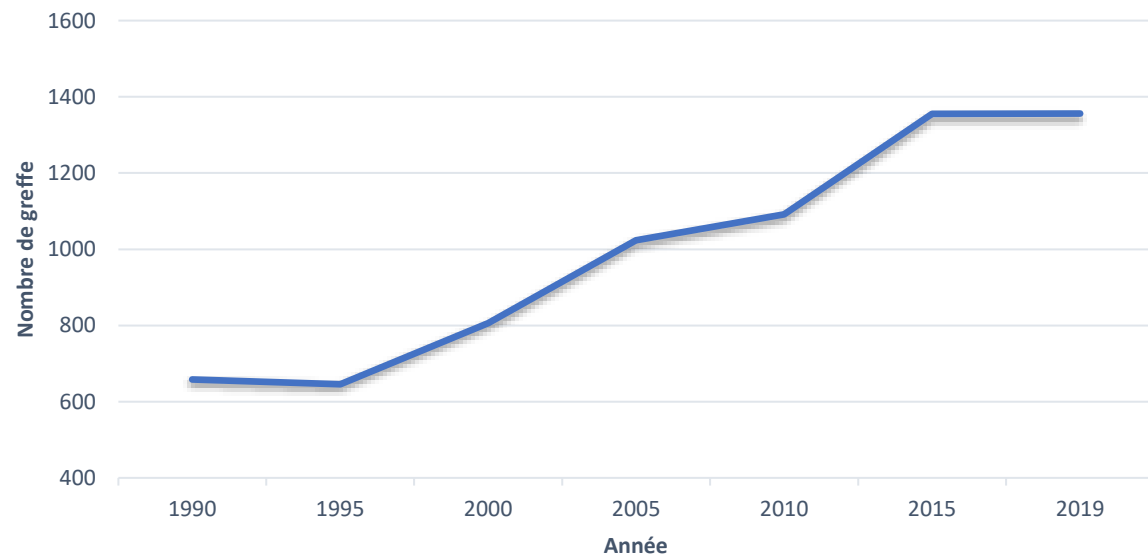
Introduction

Matériels et méthodes

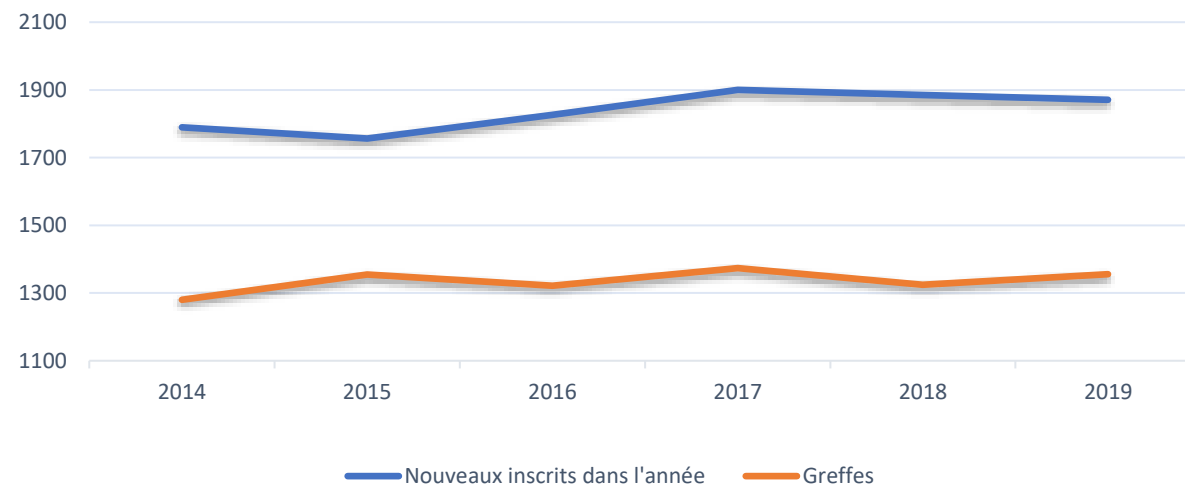
Résultats

Discussion

Evolution de l'activité de greffe



Evolution de la liste d'attente et devenir des candidats en greffe hépatique



ECD (Extended Criteria Donor)

Box 1

Definition of extended criteria donors

Advanced age

Macrovesicular steatosis

DCD

Organ dysfunction at procurement

ICU stay greater than 7 days

Hypernatremia greater than 165

Bilirubin greater than 3

Elevated aspartate aminotransferase/alanine aminotransferase|

Vasopressor use

Cause of death: anoxia, cerebrovascular accident

Disease transmission

HBcAb+

HBsAg+

Hepatitis C Virus

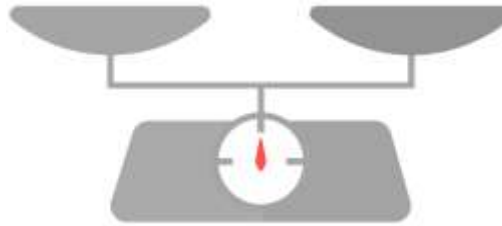
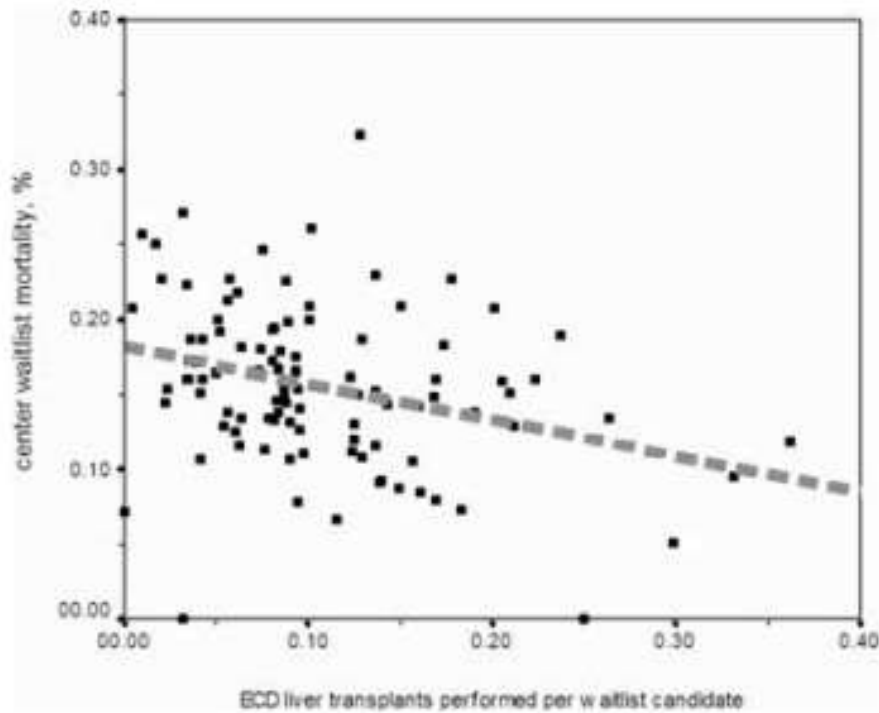
CDC high-risk donors

HIV positive

Extrahepatic malignancy

CIT greater than 12 hours

Pénurie d'organe : EDC (Extended Donor Criteria)



Type of Donor	Primary Risk
DCD	Biliary complication
Advanced age	Delayed graft function
Hepatic steatosis	Delayed graft function
SLT	Vascular or biliary complication
High risk according to CDC	Infectious risk
HCV, HBcAb, or HTLV positive	Infectious risk
Cancer history	Transfer of malignancy

Introduction

Matériels et
méthodes

Résultats

Discussion

- Sélection des greffons = **ETAPE DETERMINANTE**

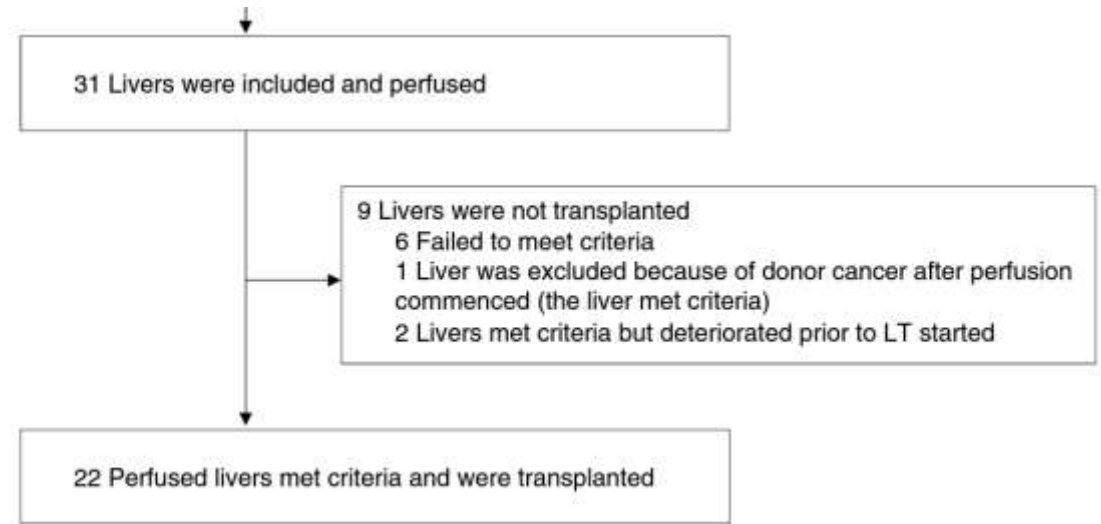
- Score : DRI– D-MELD score – ECD-Score
- Algorithme assisté par ordinateur

}

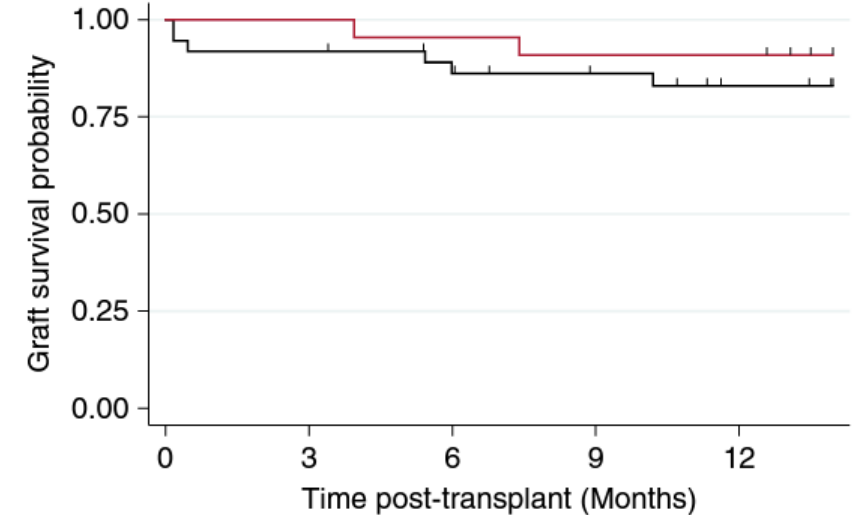
Evaluation subjective par le
chirurgien préleveur

Outils simple et performant pour l'évaluation des greffons per-prélèvement

Sélection :



Kaplan–Meier 1 year graft survival estimates



	0	3	6	9	12
Case-Control = Control	37	34	30	27	23
Case-Control = Case	22	22	21	20	20



Introduction

Matériels et
méthodes

Résultats

Discussion

Peut-on prédire la reprise de fonction ?



Introduction

**Matériels et
méthodes**

Résultats

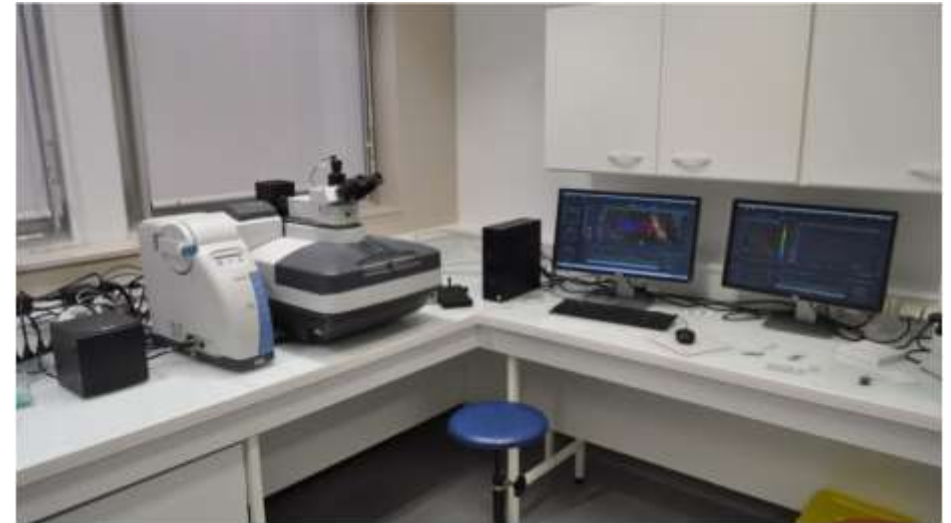
Discussion

Objectif :

Recherche de spectre prédictif de la dysfonction primaire



Biopsie prélevée sur greffon hépatique



Spectromètre Imageur RAMAN DXR2xi –
Plateforme H2P2

Introduction

**Matériels et
méthodes**

Résultats

Discussion

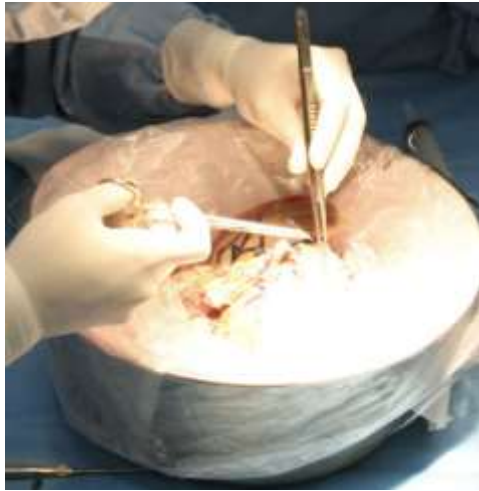
- Inclusion :
 - Novembre 2021 et juillet 2022 – CHU Rennes
 - Greffon de > 18 ans
 - Pour greffe orthotopique
- Exclusion :
 - Split – greffe multi-organe
 - Donneurs vivant
 - Inclusion dans un autre protocole

Introduction

**Matériels et
méthodes**

Résultats

Discussion



Azote liquide

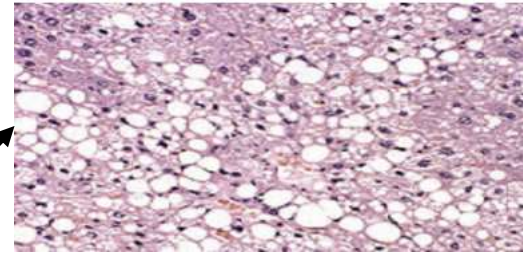


Introduction

**Matériels et
méthodes**

Résultats

Discussion



Analyse anatomopathologique :

- Stéatose
- Fibrose
- Lésion ischémie/reperfusion

Analyse RAMAN :

- Elaborer un modèle prédictif
dysfonction primaire greffon

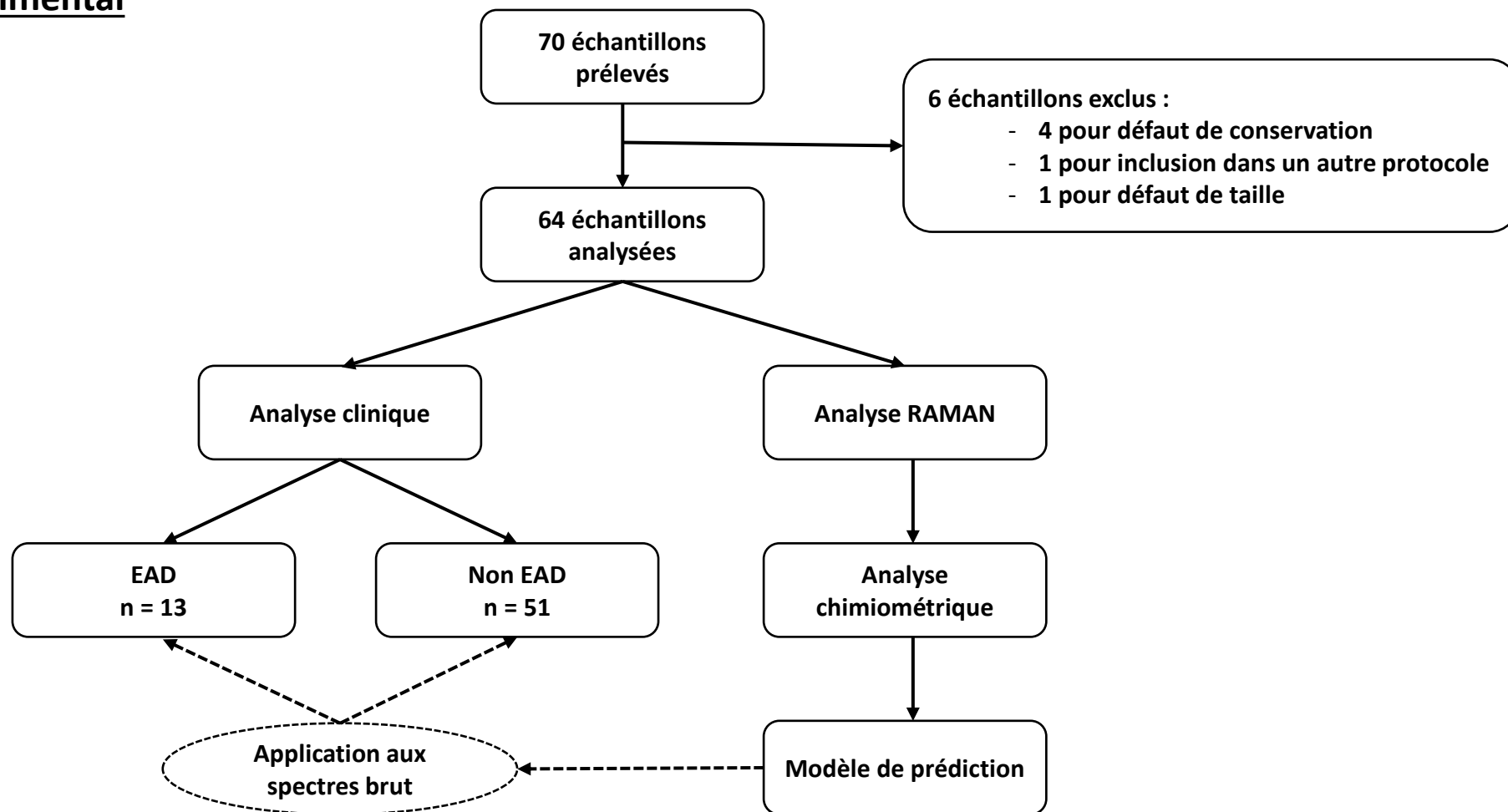
Introduction

Matériels et
méthodes

Résultats

Discussion

Schéma expérimental

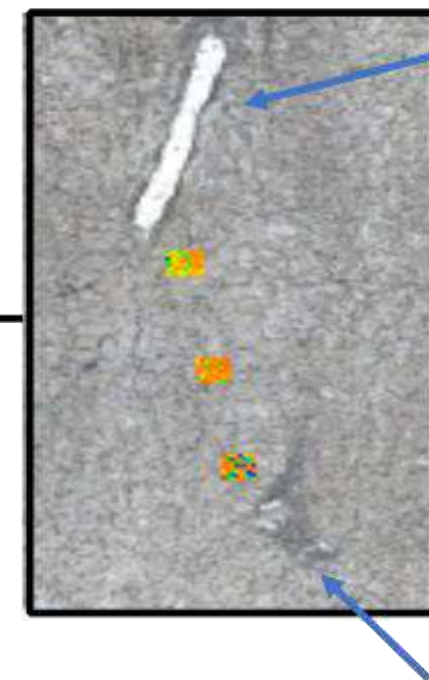
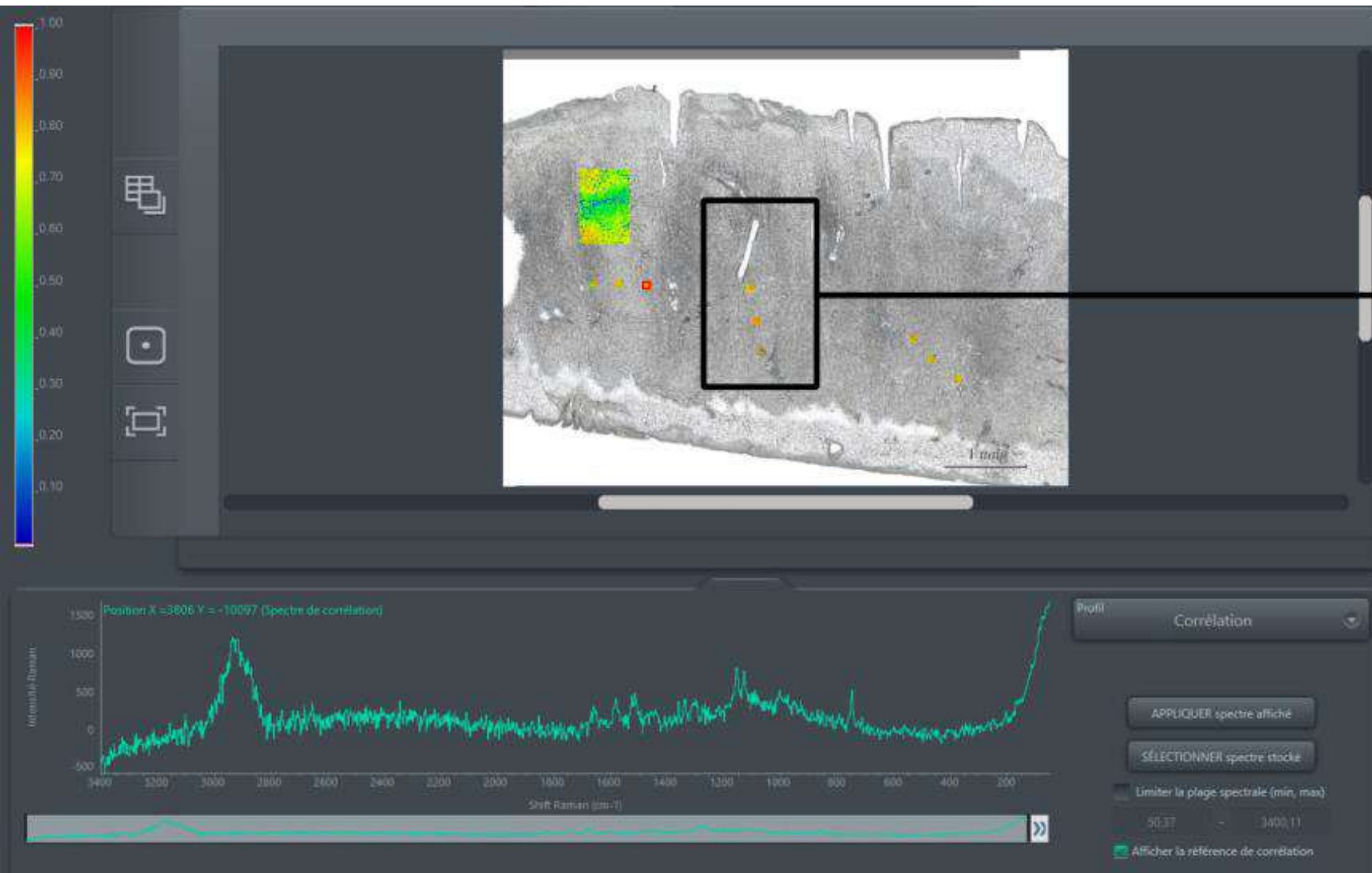


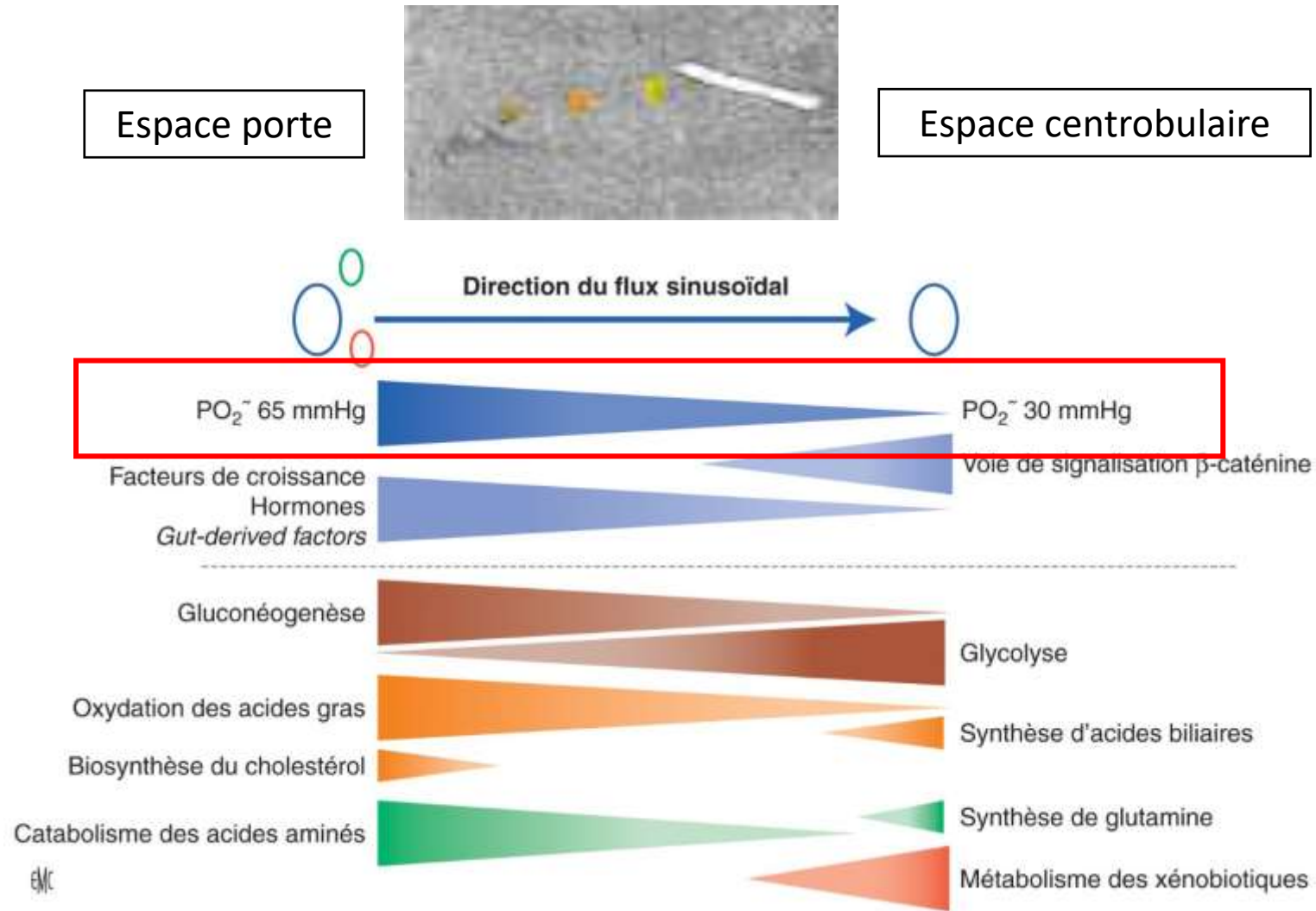
Introduction

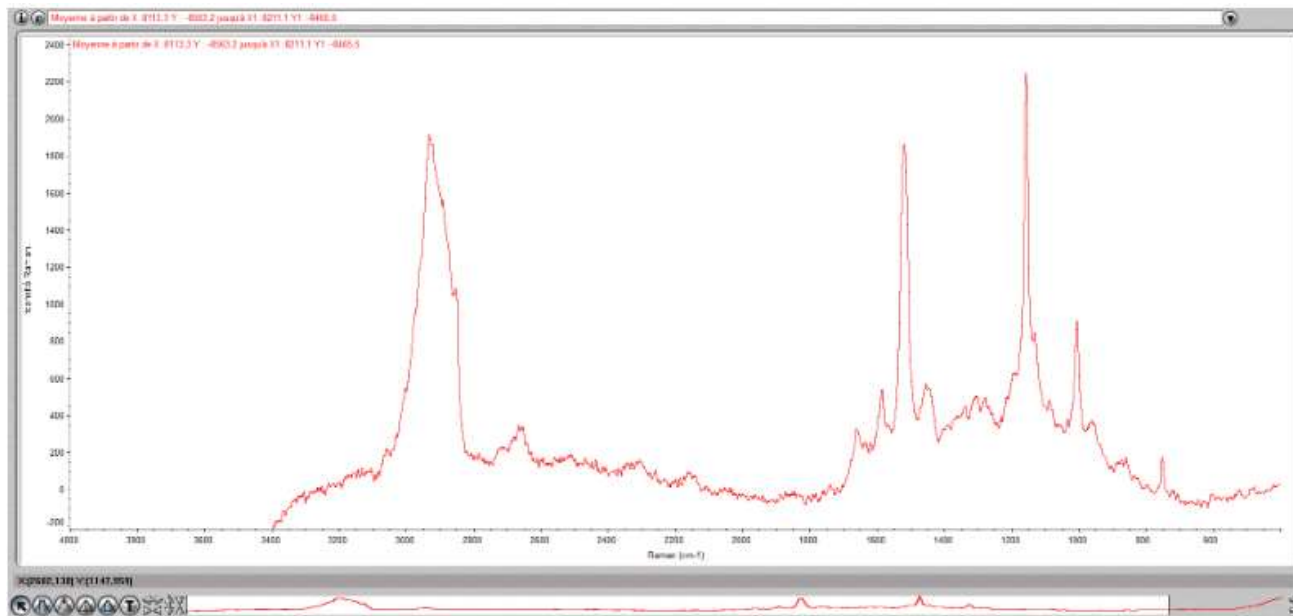
Matériels et
méthodes

Résultats

Discussion





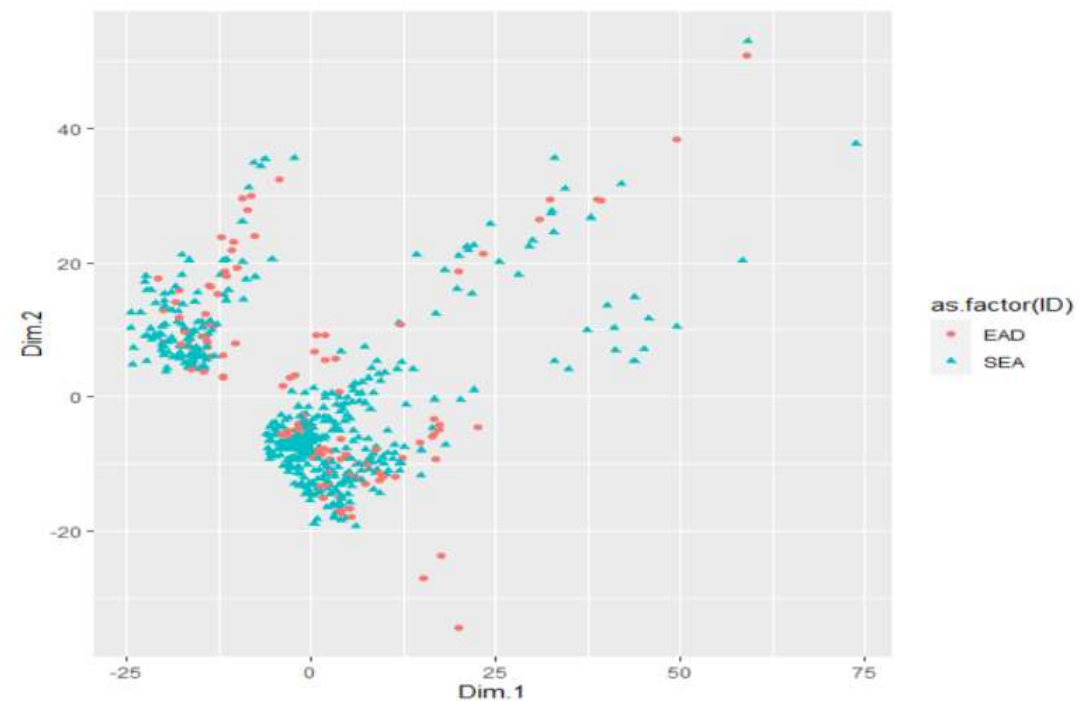
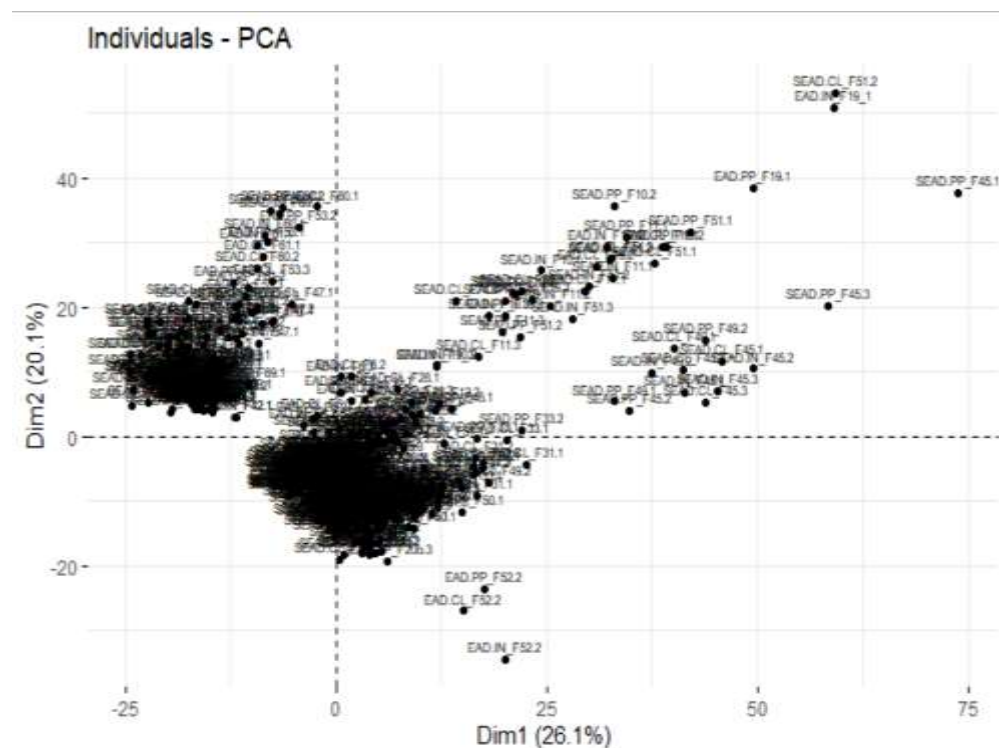
2 types d'informations recueillies :

Exemple d'un spectres

5,83864551E+001	1,99867593E+003
5,22949219E+001	1,97329688E+003
5,42233887E+001	1,94270044E+003
5,61518555E+001	1,91372821E+003
5,80803223E+001	1,88564636E+003
6,00087891E+001	1,85731744E+003
6,19372559E+001	1,84576123E+003
6,38657227E+001	1,80560995E+003
6,57941895E+001	1,76762317E+003
6,77226563E+001	1,74802979E+003
6,96511230E+001	1,71937635E+003
7,15795898E+001	1,68767864E+003
7,35080566E+001	1,66412256E+003
7,54365234E+001	1,64337158E+003
7,73649902E+001	1,60564816E+003
7,92934570E+001	1,55803498E+003
8,12219238E+001	1,52687679E+003
8,31503906E+001	1,49691171E+003
8,50788574E+001	1,46895309E+003
8,70073242E+001	1,43779554E+003
8,89357910E+001	1,37760766E+003
9,08642578E+001	1,34581945E+003
9,27927246E+001	1,31518058E+003
9,47211914E+001	1,27810329E+003
9,66496582E+001	1,23117371E+003
9,85781250E+001	1,18916584E+003
1,00506022E+002	1,15127647E+003
1,02455859E+002	1,11861678E+003
1,04361821E+002	1,08867285E+003
1,06291992E+002	1,06489949E+003
1,08220459E+002	1,04115616E+003
1,10348926E+002	1,01876831E+003
1,12077393E+002	9,94308119E+002
1,14005859E+002	9,3626663E+002
1,15944326E+002	9,1437293E+002
1,17862793E+002	8,82859326E+002

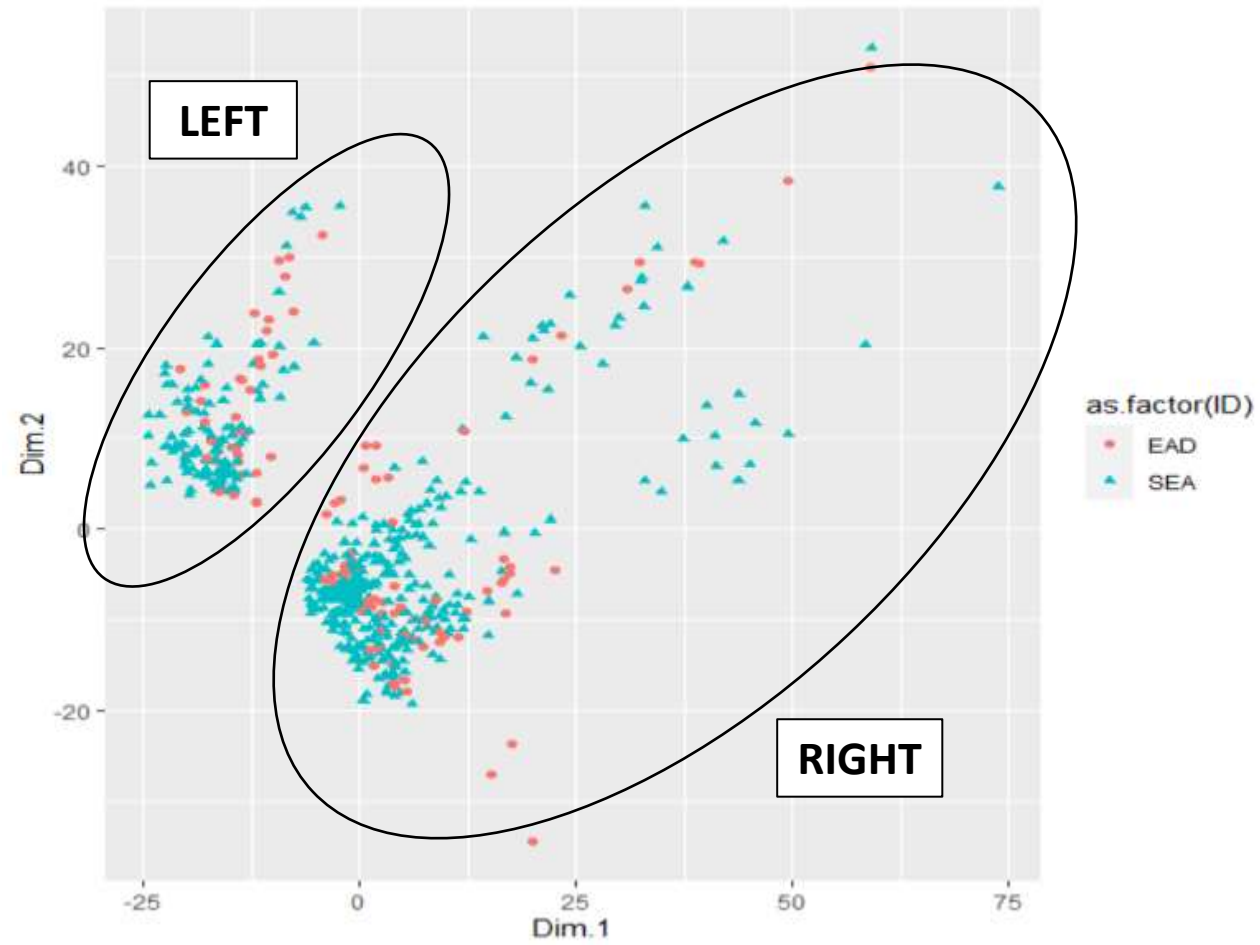
Intensité

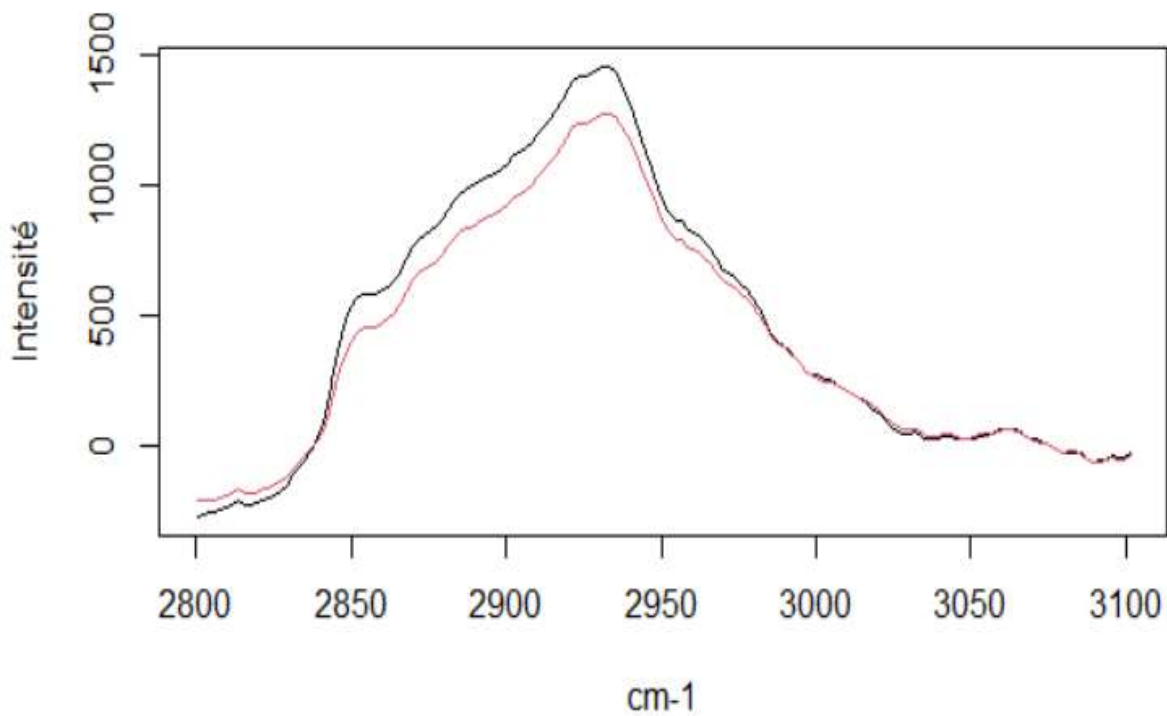
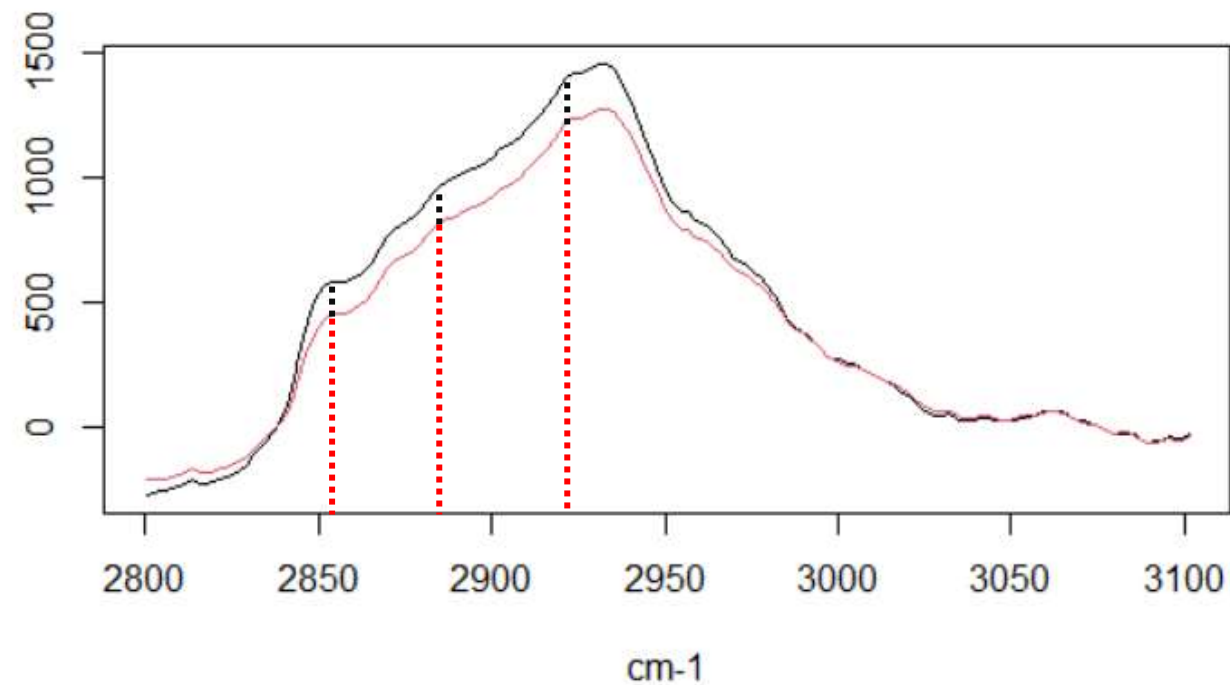
Approche chimiométrique



ACP après détermination des 2 composantes principales

Approche chimiométrique



Approche spectrale**Spectres moyens EAD vs NoEAD****Spectres moyens EAD vs NoEAD**

EAD
No EAD

Introduction

Matériels et méthodes

Résultats

Discussion

Peak Ratio	EAD, N = 106 ¹	NoEAD, N = 454 ¹	p-value ²
Protein/Fatty acid	1.49 (0.12)	1.54 (0.09)	<0.001
Protein/Lipids	2.77 (0.59)	3.24 (0.64)	<0.001

¹ Mean (SD)

² Wilcoxon rank sum test

Zone entre 2800 et 3100 cm⁻¹

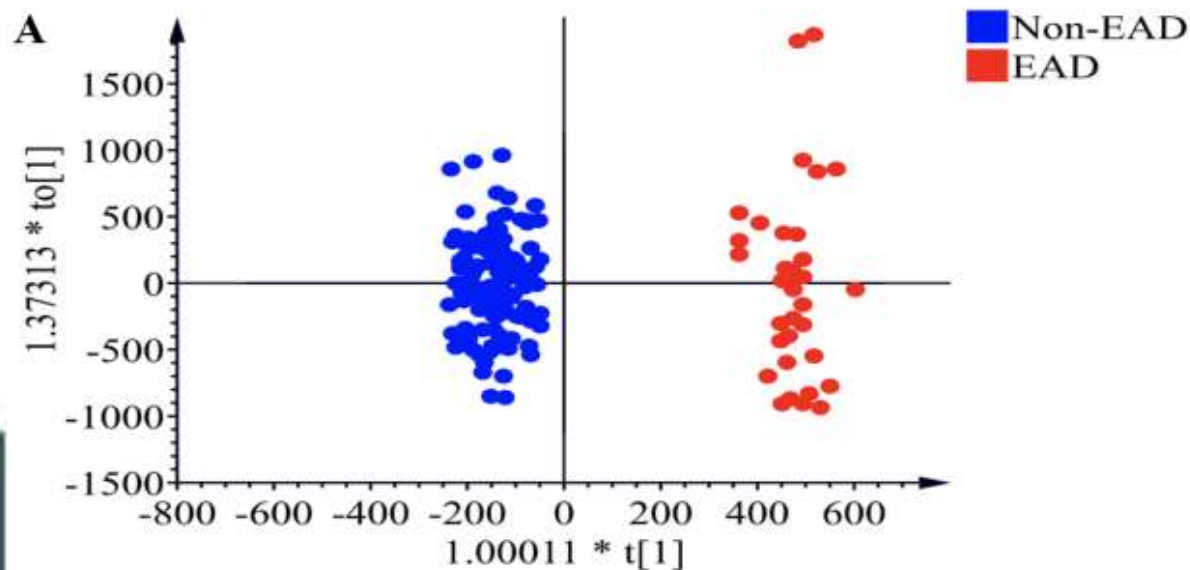
Peak Ratio	EAD, N = 106 ¹	NoEAD, N = 454 ¹	p-value ²
Protein/DNA	3.8 (18.2)	4.4 (10.5)	0.9
Protein/Carotene	0.82 (0.29)	0.91 (0.39)	0.021
Protein/B-Carotene	0.45 (0.28)	0.50 (0.41)	0.8
Protein/AA	1.17 (0.40)	1.28 (0.72)	0.5
Protein/Carotene	0.69 (0.65)	0.62 (0.52)	0.8
Protein/Lipids_1	1.07 (0.17)	1.08 (0.13)	0.6
Protein/Lipids_2	1.07 (0.17)	1.08 (0.13)	0.6
Protein/Vitamin_A	1.48 (0.54)	1.39 (0.40)	0.5
Protein/VitA_1	0.72 (0.26)	0.73 (0.41)	0.3
Protein/VitA_2	1.42 (0.41)	1.49 (0.73)	0.8
Protein/Lipid	1.68 (5.50)	2.23 (1.32)	0.5

¹ Mean (SD)

² Wilcoxon rank sum test

Zone entre 600 et 1800 cm⁻¹

Rôle des lipides dans EAD :



Metabolites	Adduct	EAD (n = 12)	Non-EAD (n = 39)	p-Value	VIP Value
PC ² (16:0-18:1)	M + H	293,234.70 ± 88,445.42	190,835.04 ± 64,727.83	8.80 × 10 ⁻⁸	14.87
Cholesterol linoleate		8247.39 ± 9123.72	23,705.64 ± 10,062.66	1.41 × 10 ⁻¹³	5.94
PC (18:1-18:1)	M + H	109,562.18 ± 32,521.47	86,965.79 ± 23,297.41	0.0004	5.71
PC (16:1-16:0)	M + H	19,371.66 ± 14,900.85	7182.02 ± 5130.27	3.08 × 10 ⁻⁵	5.13
PC (36:4)	M + H	21,621.80 ± 8897.53	11,880.11 ± 5475.14	2.51 × 10 ⁻⁷	4.67
PC (16:0-16:0)	M + H	13,302.07 ± 5827.96	6006.07 ± 3085.78	1.33 × 10 ⁻⁸	4.33
PC (16:0-18:2)	M + H	42,095.65 ± 15,600.17	31,631.49 ± 11,841.82	0.0006	3.76
Cholesterol linoleate	M + NH ₄	1768.35 ± 3256.70	7312.47 ± 3399.90	1.36 × 10 ⁻¹⁴	3.62
LysoPC ³ (16:0)	M + H	1245.59 ± 1282.79	4852.28 ± 2746.80	1.52 × 10 ⁻¹⁹	2.79
PC (18:1-18:0)	M + H	9301.80 ± 3127.75	5999.75 ± 2319.29	6.12 × 10 ⁻⁷	2.57
Cholesterol oleate		1655.40 ± 934.69	3933.22 ± 1373.84	2.79 × 10 ⁻¹⁸	2.38
TG ⁴ (52:3)	M + Na	15,257.06 ± 3186.91	18,383.95 ± 3284.08	1.77 × 10 ⁻⁶	2.20
Met0295		2752.32 ± 2798.94	774.76 ± 766.19	0.0002	2.00
Met0592		3071.09 ± 1361.53	1429.76 ± 1076.40	7.83 × 10 ⁻¹²	1.87
LysoPC (18:2)	M + H	764.32 ± 840.10	2425.17 ± 1466.14	2.32 × 10 ⁻¹³	1.78
L-Acetylcarnitine	M + H	2265.22 ± 2044.40	849.66 ± 824.14	0.0003	1.61
Cholesterol arachidonate		227.70 ± 354.57	1312.62 ± 883.71	7.51 × 10 ⁻²⁰	1.50
Cholesterol		1745.76 ± 640.41	994.24 ± 411.78	5.96 × 10 ⁻⁸	1.32
LysoPC (18:1)	M + H	392.86 ± 576.23	1254.31 ± 834.29	9.18 × 10 ⁻¹⁰	1.23
LysoPC (18:0)	M + H	139.32 ± 229.46	876.64 ± 626.57	1.03 × 10 ⁻¹⁹	1.22
Cholesterol linoleate	M + Na	267.63 ± 856.69	1036.13 ± 854.38	6.75 × 10 ⁻⁶	1.06
PC (32:1)	M + Na	559.23 ± 782.39	44.72 ± 119.20	0.0005	1.02

¹ early allograft dysfunction; ² phosphatidylcholine; ³ lysophosphatidylcholines; ⁴ triacylglycerol.



Rôle des lipides dans EAD :

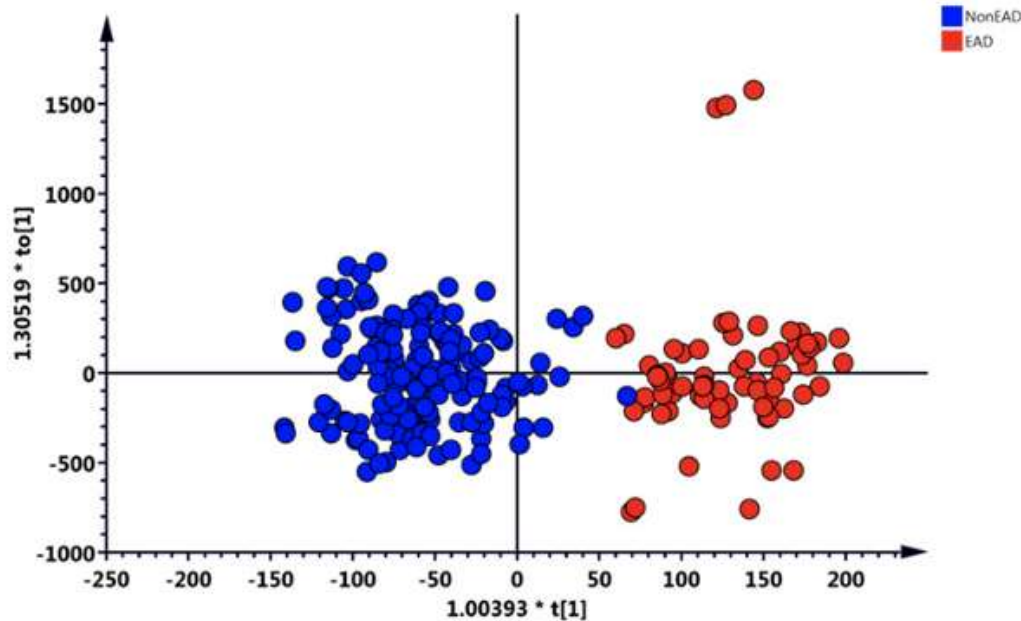
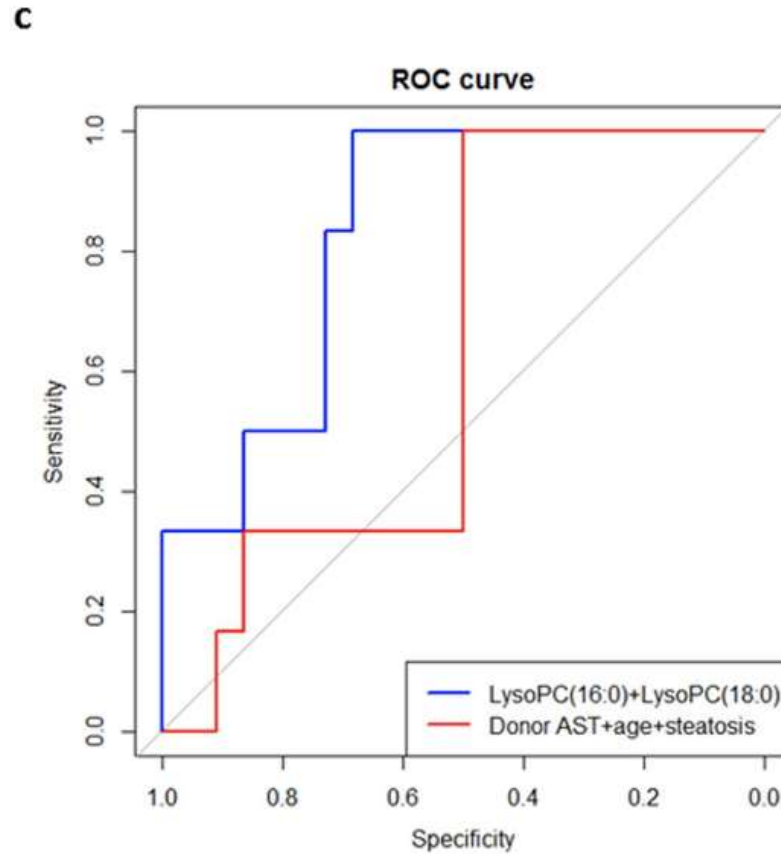
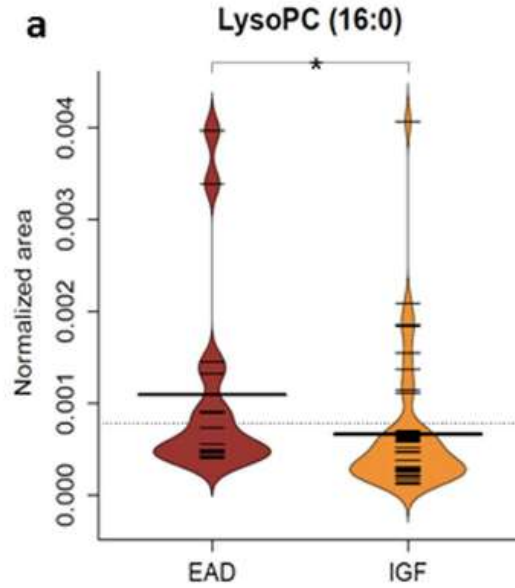


Table 4. Receiver operating characteristic (ROC) curve analysis for individual metabolites in the prediction of EAD

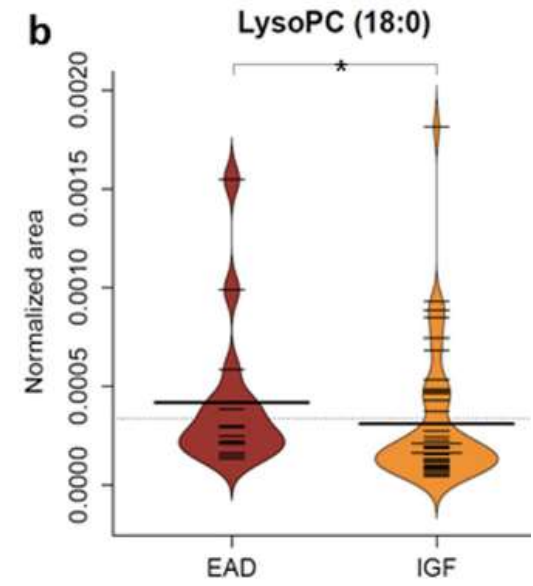
	AUC	Standard error
Betaine	0.686	0.0394
PC (18:0/20:5)	0.630	0.0392
LysoPC (16:0)	0.681	0.0404
Palmitic acid (C16:0)	0.641	0.0401
Combination of betaine, PC (18:0/20:5), palmitic acid and lysoPC (16:0)	0.821	0.0336
Total bilirubin	0.754	0.0438
Combination of metabolites and total bilirubin	0.846	0.0314

Abbreviations: EAD, early allograft dysfunction; PC, phosphatidylcholine; lysoPC, lysophosphatidylcholine; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, internationalized ratio.

Rôle des lipides dans EAD :



	AUC	Accuracy	Sensitivity	Specificity
LysoPC (16:0)+LysoPC (18:0)	0.91	0.82	0.67	0.86
Donor AST+age+steatosis	0.63	0.68	0.33	0.77
LysoPC (16:0)	0.83	0.75	0.33	0.86
LysoPC (18:0)	0.85	0.75	0.33	0.86



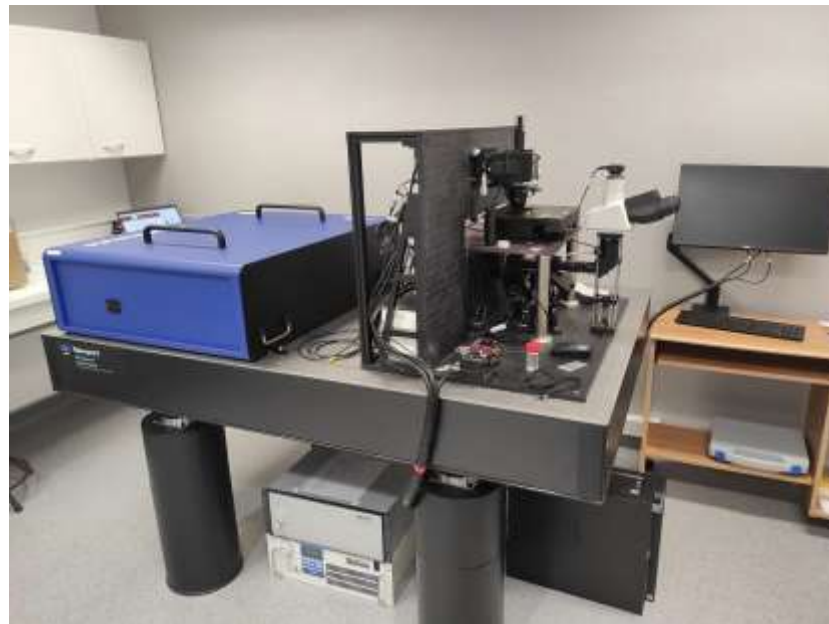
Perspective



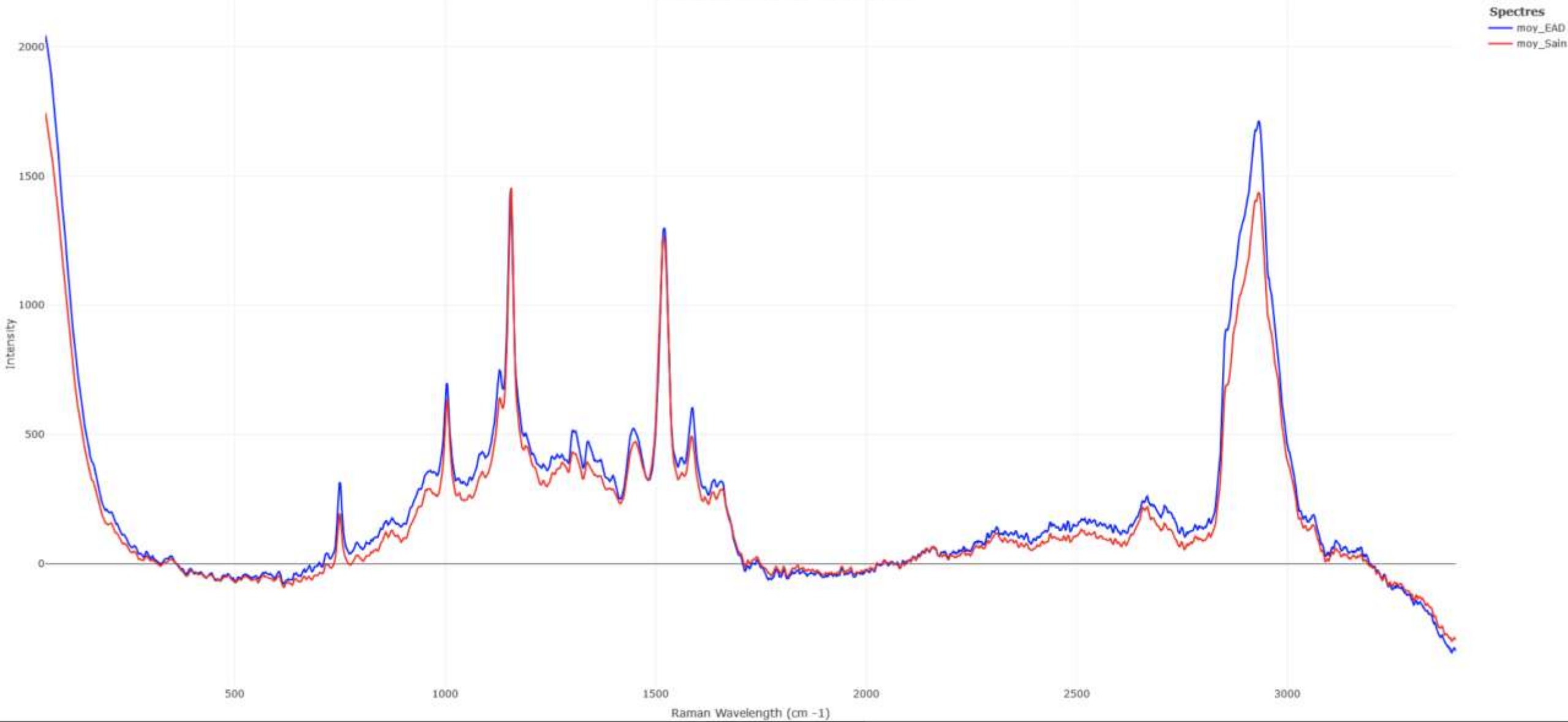
Projet de recherche :



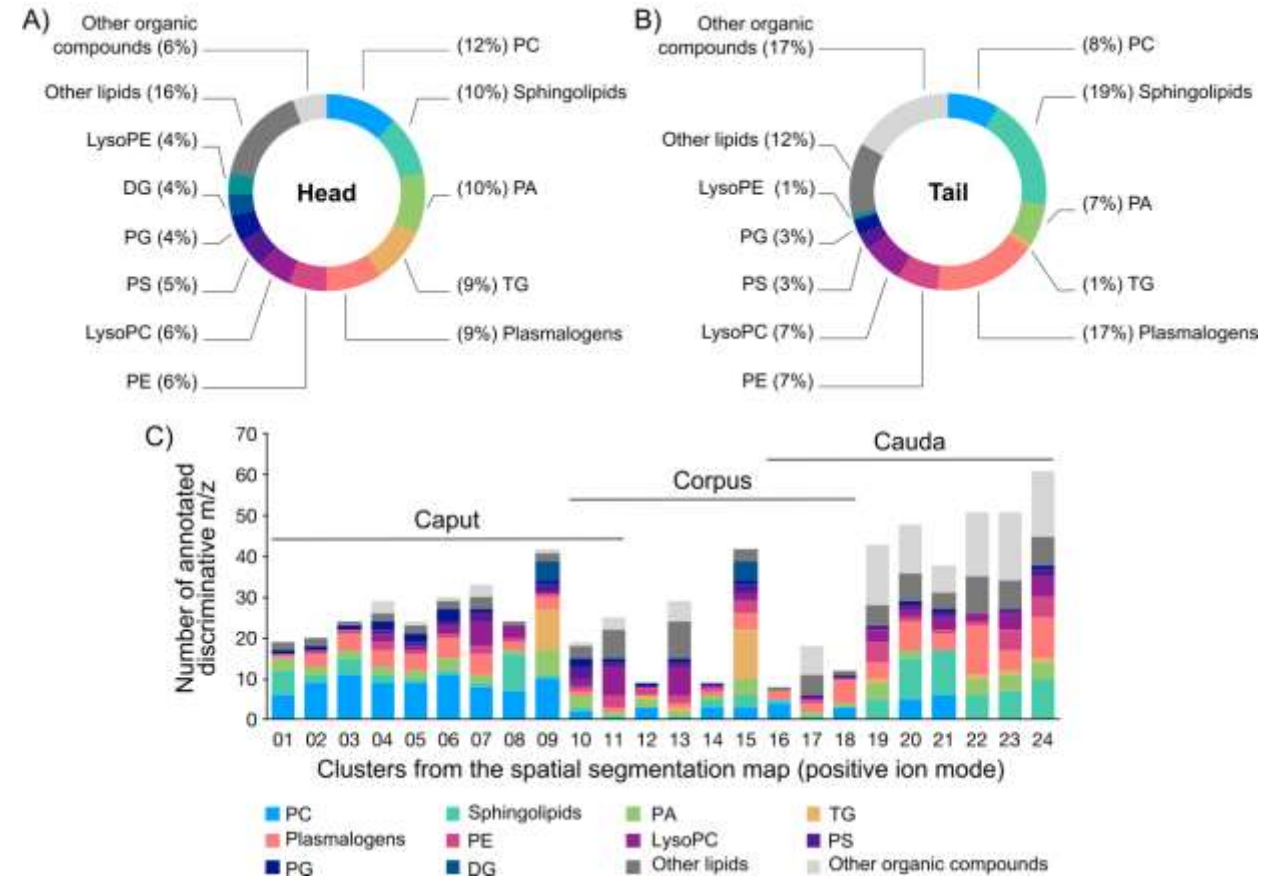
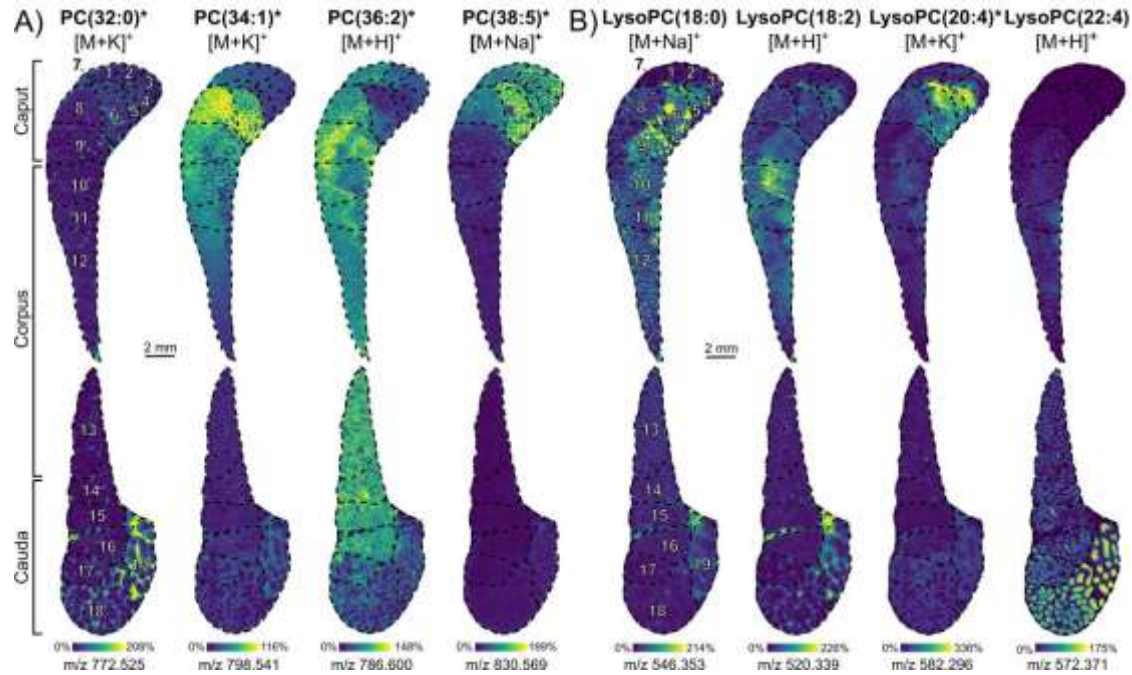
Validation du modèle par SRS



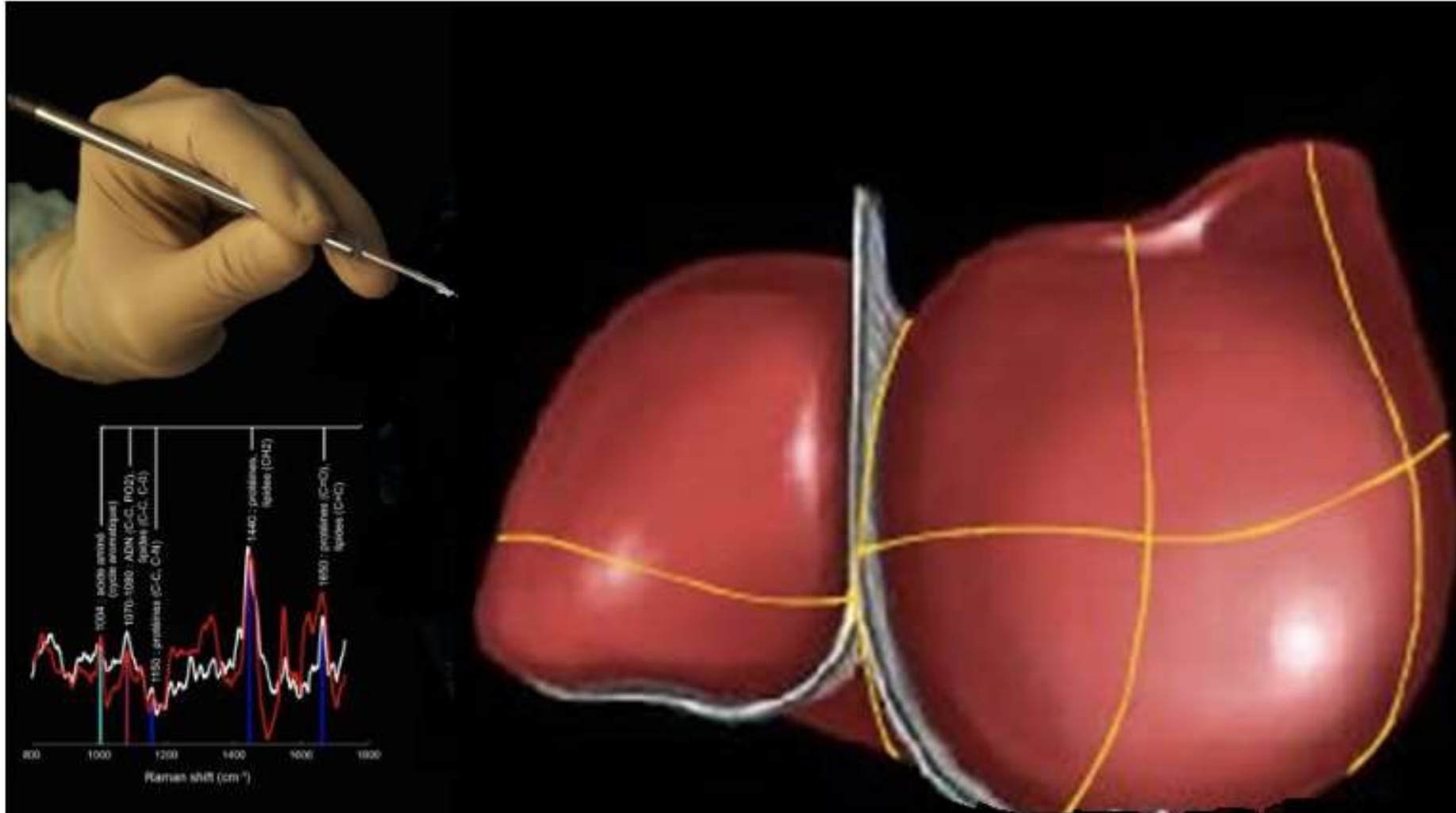
Moyennes de toutes les régions



Lipidomique



Objectif à long terme :





Merci de votre attention

