Metabolomic profiling in asphyxia: a promising tool

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BACKGROUND

Mechanical asphyxia (MA) is one of the causes of deaths that ultimately brings to cardiac arrest (CA), with high occurrence and dead rates (Zhang et al., 2021)

Up to date, **the diagnosis** of an asphyxiated death is still based on cross comparisons between **histological examinations** and **other circumstances**, but **the findings might be non-specific or**, in some cases, **totally absent**. Moreover, the discrimination between a primitive CA or a CA after asphyxiation depends on factors that lack objective evidence (Locci et al., 2021)

Considering that **biochemical pathway** alterations occur in a **hypoxic environment**, **metabolomics**, the latest "omic" technology, has caught attention to improve our understanding of asphyxia (Fattuoni et al., 2015) by detecting potential and useful **biomarkers for asphyxiated bodies.**

AIM

POTENTIAL BIOMARKERS FOR ASPHYXIATED DEATHS

STUDY DESIGN

1st STEP - Development of LC/MS-MS methods to investigate the metabolic alterations, including the perturbation of amino acids and lipid profiles (*n*=44), occurring in death from asphyxia

2nd STEP - Evaluation of their distribution in lung tissue samples from right and left apex (*n*=14) and right and left base (*n*=14) related to death from asphyxia

3rd STEP - Statistical analysis of lung tissue samples (*n*=17) collected from asphyxiated bodies and different causes of death (control group, *n*=17)

4th STEP - Investigation of the amino acids and lipid profile in other tissues samples, such as myocardium and psoas, from asphyxiated (*n*=17) and control groups (*n*=5)

RESULTS

1st STEP

HOMOGENIZATION
tissue (< 50 mg) in 100 μL water

DEPROTENIZATION
10 μL tissue + ISs + 200 μL IPA for FAs
30 μL tissue + ISs + 90 μL ACN for AAs

DERIVATIZATION
100 μL supernatant + 50 μL of 50 mM of 3-NPH, 50 μL of 50 mM of EDC, 50 μL of pyridine (7%) in 70% methanol at 37°C for 60 min for FAs
60 μL supernatant + 50 μL of pyridine/water 3:2 (v/v) and 5 μL of PITC at 60°C for 60 min for AAs

LC/MS-MS ANALYSIS
add 250 μL 0.5% of HF in IPA and inject 1 μL for FAs
add 400 μL ACN/water 8:2 (v,v) and inject 1 μL for AAs

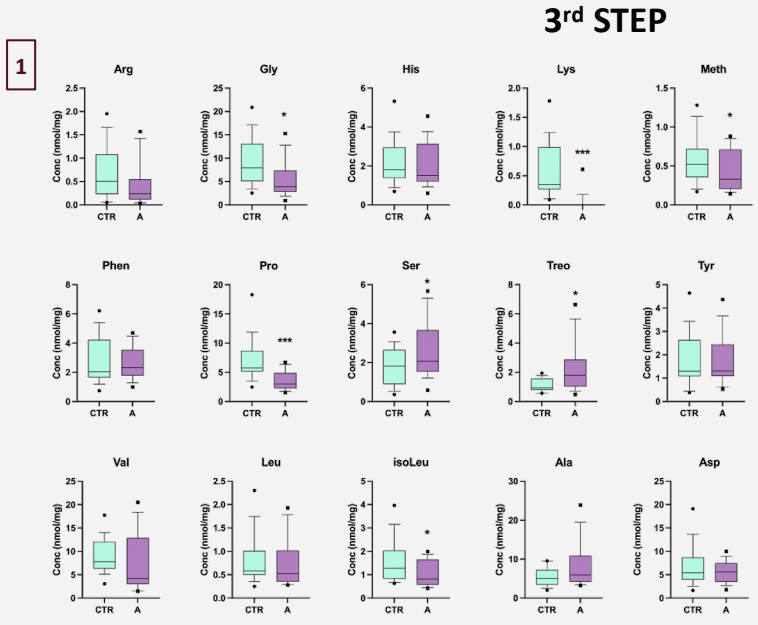
INSTRUMENTAL
QTrap 5500 Triple quadrupole MS (Sciex, Germany) coupled with an Agilent 1200 Infinity pump UHPLC system (Agilent Technologies, USA)

2nd STEP

X X X X

NO STATISTICAL DIFFERENCES BETWEEN DIVERSE LUNG PARTS OF ASPHYXIATED BODIES

3rd STEP



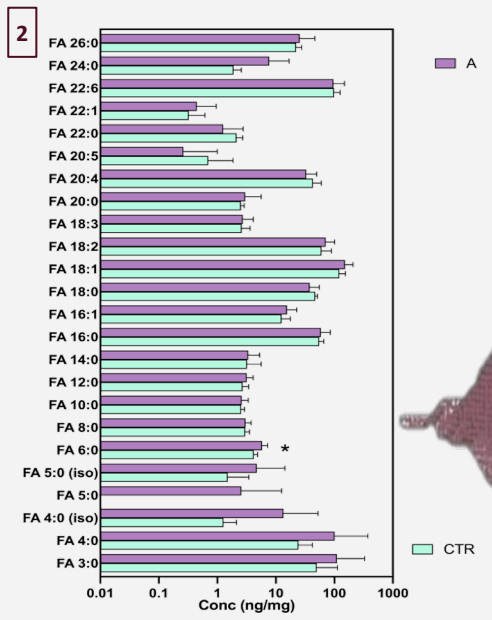
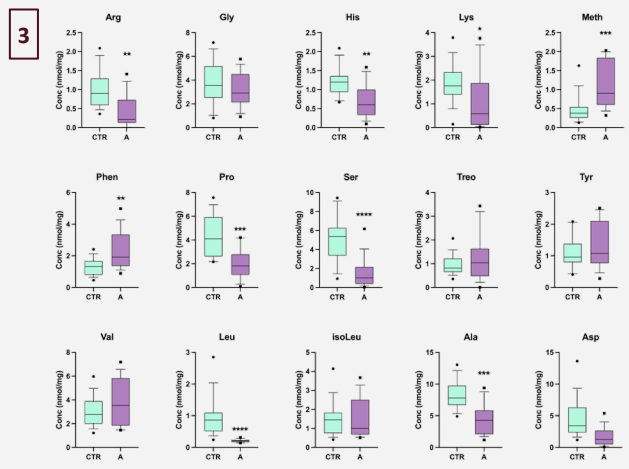
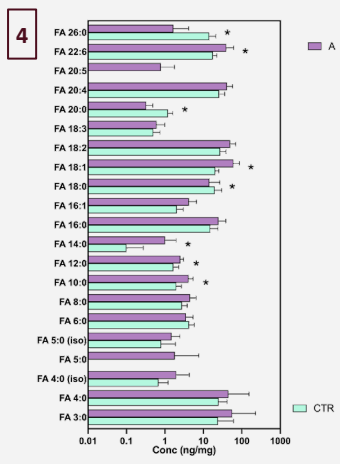
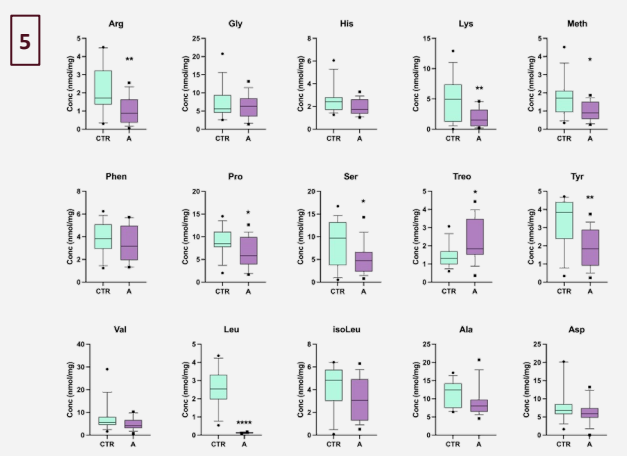


Figure 1. Amino acids (AAs) concentration in control (CTR) vs asphyxia (A) lung homogenates. **Figure 2.** Fatty acids (FAs) concentration in control (CTR) vs asphyxia (A) lung homogenates. The number of carbon atoms and unsaturation are indicated for each species.
Boxes: 25th-75th percentiles; lines: 10th-90th percentiles; crossing lines: median values; separate points: outliers. Statistical tests were performed by unpaired t-test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs CTR

4th STEP







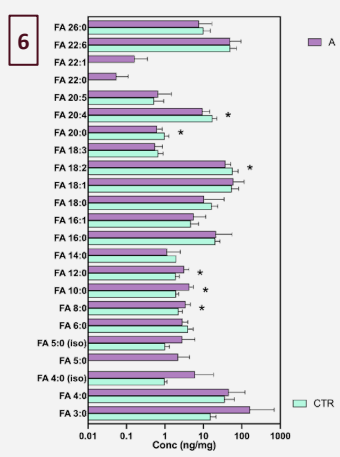


Figure 3-5. Amino acids (AAs) concentration in control (CTR) vs asphyxia (A) myocardium (Fig 3) and psoas (Fig 5) homogenates.
Figure 4-6. Fatty acids (FAs) concentration in control (CTR) vs asphyxia (A) myocardium (Fig 3) and psoas (Fig 5) homogenates. The number of carbon atoms and unsaturation are indicated for each species.
Boxes: 25th-75th percentiles; lines: 10th-90th percentiles; crossing lines: median values; separate points: outliers. Statistical tests were performed by unpaired t-test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs CTR

CONCLUSIONS

1- Two LC/MS-MS methods were developed and validated to identify and quantify AAs and FAs in post-mortem samples
2- No statistical differences between diverse lung parts of asphyxiated bodies
3- Some biomarkers seem to be able to discriminate asphyxia from other causes of death in lung and other tissues
4- Further investigations are in progress in order to expand the number of samples, compare the different matrices and research other biomarkers involved in the hypoxic environment

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E. Locci et al. *Scientific Reports* (2021) doi.org/10.1038/s41598-021-89570-0
C. Fattuoni et al. *Molecules* (2015) doi:10.3390/molecules20047000