Biochemistry of death – application of metabolomics in forensic medicine

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"Tenax Vitae" R. Carniello

INTRODUCTION

"(...) in this world nothing can be said to be certain, except death and taxes."
Benjamin Franklin (A letter to Jean-Baptiste Leroy, 1789)

"Death" J. Malczewski

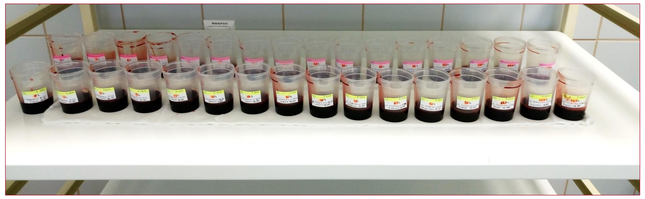
Death affects all people worldwide. Besides the fact, that death accompanies humanity from ages, our knowledge about death it is still very limited. It can be explained by fact that topic of death is not really popular and it is still a bit cumbersome, or it is even taboo. Additionally, for a long time, we have not got adequate tools which could allow us to study these kinds of topics. However, last decades give us brand new technologies and possibilities which give an opportunity to deeper study these areas. Metabolomics can be a great example of it.

Knowledge about the factors which can affect experimental models also has some limitations. Beyond examined factors, such as disease or drug, many other aspects should be carefully taken into consideration - environmental, lifestyle, drugs, microbiota, etc. This problem is particularly noticeable in forensic sciences when single factors, such as temperature or humidity, can have an enormous effect on the obtained results. Mentioned "omics" sciences allow following even the smallest numerous molecular modulations at the same time. Additionally, attention to the influence of environmental exposures resulted in the idea of "exposome". Due to this, the latest research covers a broader view of the experimental results and includes previously omitted factors and their influences.

The aim of our study was the application of metabolomics to evaluate and understand the modulations of small metabolites which take place after death, especially in different time intervals. Additionally, the influence of the external and internal factors on metabolic profiles of blood samples was examined.

MATERIALS AND METHODS

The experiment was performed on blood samples with time, anticoagulant presence, temperature and humidity factors taken into account. Metabolomics profiles and basic clinical parameters were measured in different time points with controlled modification of the aforementioned factors.

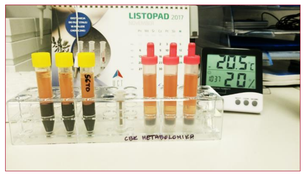


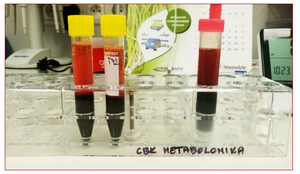
Metabolic fingerprinting was performed by use of liquid chromatography with mass spectrometry detection (QTOF, model 6550 Agilent Technologies, Santa Clara, CA, USA).

Commonly used in forensic sciences, domestic pig (breed: Polish Large White) was chosen as the animal model.

The study protocol and procedures were approved by the Local Ethics Committees.

Fig. 1. Visible differences in hemolysis and hematocrit levels linked to the presence of anticoagulant, observed in both examined groups during material collection.





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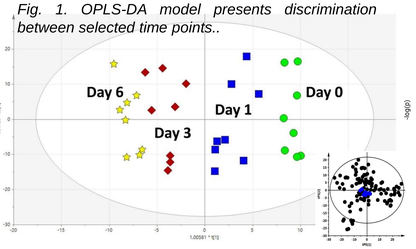
RESULTS

Visible differences in hemolysis and hematocrit levels linked to the presence of anticoagulant were observed in examined groups during material collection and preparation. Performed instrumental and statistical analysis allowed to indicate over thousand metabolites (Table 1) modulated in different time intervals and related to the anticoagulant presence or some external factors like temperature or humidity. Over 200 of them was statistically significant. Detected metabolites were linked with many biochemical pathways, including sphingolipid metabolism, aminoacyl-tRNA biosynthesis, phenylalanine, tyrosine and tryptophan metabolism, porphyrin metabolism, histidine metabolism, sucrose metabolism, glycerophospholipid metabolism, valine, leucine and isoleucine degradation, biosynthesis of unsaturated fatty acids, and others. Some of these processes are probably effects of cells degradation, others can be linked to decay and caused by bacteria metabolism and activity.

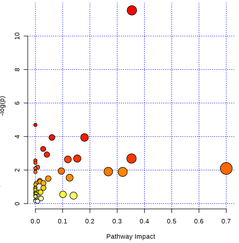
Tab. 1. Selected preliminary results of data reprocessing and univariate statistical analysis (paired Mann-Whitney test)

Time point	Temperature	Humidity	HCT (without EDTA)	HTC (with EDTA)	Features 80% group filter (without EDTA)	FC>1.3 (without EDTA)	Statistically significant (without EDTA)	Features 80% group filter (with EDTA)	FC>1.3 (with EDTA)	Statistically significant (with EDTA)
Day 0 (death)	22.00	39.92	16.5	49.4	581	109	67	398	50	25
Day 1	21.87	40.35	15.9	51.2	565	109	67	390	85	56
Day 2	21.92	39.29	18.1	52.6	599	215	151	403	92	68
Day 3	21.81	38.92	19.8	57.0	599	263	125	408	112	74
Day 4	21.80	38.10	21.7	59.3	589	254	142	417	151	110
Day 5	21.72	36.98	30.7	63.7	581	275	158	409	135	90
Day 6	21.57	38.98	25.2	65.7	598	268	178	418	145	102
Day 7	21.55	40.42	37.2	69.6	597	307	142	405	139	91

Fig. 1. OPLS-DA model presents discrimination between selected time points..



Summary of Pathway Analysis.



Examples of impacted pathways:
- Sphingolipid metabolism
- Pantothenate and CoA biosynthesis
- Citrate cycle
- Butanoate metabolism
- Porphyrin and chlorophyll metabolism

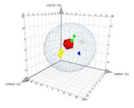


Fig 4. 3D Model for both samples groups scaled by bilivedrin level (1st and 4th day)

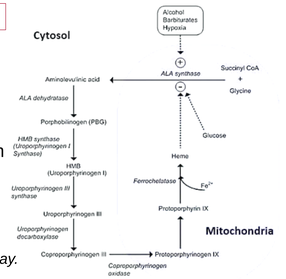


Fig. 3 Porphyrin pathway.

CONCLUSIONS

Preliminary studies demonstrated a relationship between the examined factors, clinical parameters (e.g. hematocrit) and metabolomics profiles. Modified levels of dozens of metabolites like hypoxanthine, bacteria related compounds and lipids (phosphatidylcholines, phosphatidylethanolamines) were detected. Moreover, a link between the presence, addition time of anticoagulant and clinical parameters as well as metabolomics profiles was described.
These findings suggest that death causes wide range scale changes in the cell as well as whole organism metabolism. Some of them will be linked with host biochemistry, others will be the effect of external factors as micro-organisms activity. More studies are needed to explain the processes behind this phenomenon.