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# The Forensic Application Of Proteomics For The Study Of The Time Of Death: An Operative Experimental Model For PMI Estimation

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ABSTRACT

Post Mortem Interval (PMI) estimation is one of the most important questions the forensic pathologist must answer. To date, it is not possible to establish exactly the hour of death, but only to calculate a period (PMI), during which death most likely occurred. In the forensic field, several laboratory methods can be used to perform this calculation more accurately. However, there is still no biomarker that is universally validated and accepted by the forensic community. In the literature, researches about the application of proteomics for forensic purposes are on the increase. Proteomics is a branch of molecular biology that allows the systematic identification of the proteome from a quantitative and qualitative point of view. Below, we propose the operating model of an experimental study currently underway at the Department of Legal Medicine of the University of Catanzaro. The model is based on taking of peripheral blood samples from patients who died at the Intensive Care Unit (AO "Mater Domini" of Catanzaro). The proposed operating model has several advantages including the evaluation, for the first time, of human biological samples from the exact moment of death. The analysis would allow to identify new potential biomarkers expressed in peripheral blood and validate the forensic application of markers already known in the literature. The knowledge of the exact moment of death (time 0) would allow us to evaluate the proteomic profile more accurately on the human model, overstepping the limits of some extrinsic variables evidenced in the literature.

**Keywords:** Forensic sciences, Proteomics, Time of death, Post Mortem Interval, Biomarker, Protein.

**Abbreviations:** PMI (Post Mortem Interval).

## 1. Introduction

Time of death is one of the greatest forensic enigmas. The evaluation of the time of death is performed by the forensic pathologist through the Post Mortem Interval (PMI) estimation, i.e. a time range in which death likely happened. This estimate is based on the calculation of a time interval that is formulated by studying the abiotic and transformative processes occurred on the corpse [1-2]. For the calculation of the PMI, supportive tests such as laboratory analysis on biological fluids, like the measurement of K + concentration in the vitreous humor, can also be used. To date, however, there is no biomarker that has been universally validated by the forensic community for the diagnosis of the time of death. Various scientific studies have been carried out for this purpose. Among them, many methods were analyzed in the literature, focusing on the post-mortem modifications of DNA, microRNA and protein modifications. Specifically, the number of studies proposed on protein analysis for PMI estimation is steadily increasing and have shown promising results.

Proteomics is the discipline that takes care of studying proteins from a systematic point of view. It can provide information about the structure, function, protein-protein interactions and quantitative variations with different

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methods. Among them there are: ELISA assay, Western Blotting, 2D PAGE (Two Dimensional Polyacrylamide gel Electrophoresis) method used in combination with mass spectrometry and LC-MS/MS (Liquid chromatography-tandem mass spectrometry) strategies .

As part of the study of PMI, multiple proteins expressed in various animal and human tissues, such as skeletal muscle, heart and brain, have been analyzed. The scientific evidences published so far in the literature revealed the potential of some proteins as possible biomarkers related to the time elapsed since death [3-5].

The great limitations associated with proteomic investigations so far have to do with the exposure of the corpse to the outside temperature, as it is an extrinsic variable that can alter the kinetics of protein degradation but, above all, the lack of proteomic analysis on humans from the exact time of the death. Indeed, this evaluation has so far been carried out only on tissues and organs of animals and never on humans. This is due to the obvious difficulty of knowing the exact time of death on human (time 0), the inability to obtain in humans an estimation of proteins expressed from the exact time of death and the difficulty of analyzing the corpse for a long-time interval. The exact knowledge of the time of death would allow us to accurately estimate the variation of one or more proteins without the interference of some variables, such as the time elapsed before the corpse's finding and the exposure temperature.

In this paper, we want to propose an operating model, still being applied and analyzed, using human blood as biological sample. This protocol is estimated to be easy to reproduce and it could be used as a reference model for post-mortem proteomic analysis on humans. This protocol, applied on humans, would greatly contribute to the studies of the relationship of one or more proteins with PMI.

## 2. Material and Methods

The proposed operating model was accepted by the Ethical Committee of the A.O. Mater Domini of Catanzaro. The procedure involved taking peripheral venous blood samples from deceased patients at the U.O. of Anesthesia and Resuscitation of the University Hospital of Catanzaro. The samples were taken at the U.O. of Anesthesia, so the exposure temperature of the corpses was the Room Temperature (around 25° C). The blood draws were only performed after the signed informed consent of the patient's family members. Specifically, venous blood drawings from cephalic vein were carried out on patients who have cardiac arrest and then die. From the exact time of death (time 0), blood samples were collected at predetermined time intervals, up to 2 hours after death. This time interval was chosen because corpses can be held by law in the Department of Anesthesia only up to 2 hours after death. Furthermore, the primary aim of the work was to evaluate the so-called "early post mortem interval". The samples were immediately centrifuged (2500 rpm for 5 minutes) and the plasma was extracted and stored in appropriate tubes (2 ml centrifuge tubes) at -80 °. Samples were subjected to proteomic analysis by ELISA immunoenzymatic method ongoing. In our project, the levels of the following proteins will be measured:

- HMGB-1 (High Mobility Group Box 1);
- Cardiac Troponin T..

There are also other candidates:

- Cardiac Troponin I;
- GFAP (Glial fibrillary acidic protein);
- Talin.

24 cases were collected in total. The analyzed cases were broken down by age, sex, comorbidities, and cause of death.

## 3. Results

Although the experimental study is still ongoing, we expect to find consistent results both with the time interval examined and the data already known in the literature. In fact, a review of literature on this topic has already shown that several proteins can undergo quantitative changes in terms of increase or reduction directly proportional to the post mortem interval investigated, but also qualitative ones. According to the available scientific evidences in the literature, the expected results of the study are related to the search for quantitative and/or qualitative alterations from the exact moment of death of some markers, that showed time dependent variations such as:

1. Ubiquitous cellular proteins, like HMGB1 (High Mobility Group Box 1): in serum samples, this protein has already proved to progressively increase with respect to time [6];

2. Specific organ proteins:

   - Muscle proteins due to progressive degradation, such as cTn I (Cardiac Troponin-I) and cTnT (Cardiac Troponin-T) [7-8];

   - Proteins of the brain tissue, such as GFAP (Glial fibrillary acidic protein) or talin, respectively with an increase and a reduction [9-10].

This is the first work that aims to measure the levels of these proteins in human plasma after death. To date, only HMGB-1 has been evaluated on animal serum [6]. These proteins are not typical plasma proteins. This data could have some benefits and some limitations. The benefit is that

the finding of these proteins in the plasma after death is not influenced by pre-existing plasma levels of the same proteins. The limitation is that the proteins could be not detectable in human plasma.

## 4. Discussion

In the literature, it has been shown that several proteins may undergo quantitative variations after death. Some of them exhibit a linear or pseudolinear kinetics of reduction due to the progressive degradation of the protein [8]; others of them show a post-mortem increase [6]. Several proteins expressed in both ubiquitous and specific tissues, whether animal or human, have already been evaluated [3-5].

For example, Kikuchi et al. have shown that the HMGB-1 protein progressively increases after death, using the mouse blood as analytical sample. It is a ubiquitous and abundant protein in the cell nucleus. ELISA assay showed that this protein may also increase up to seven days after death, with temperature-dependent kinetics variations [6]. These results have not yet been validated on a human model.

In the cardiac tissue, it has been shown that both the degraded cTnI intact protein and the degraded cTnT percentage decrease progressively analyzed after death, proportionally to the elapsed time interval. These analyzes were carried out, in the first case on an animal and human model, in the second case on a human model [7-9].

Concerning the brain tissue, interesting results regarding GFAP and talin proteins have been obtained. GFAP is a protein that has shown to increase in human samples of substantia nigra; talin has shown to diminish in the human brains analyzed after death. Both analysis were performed using Western Blotting [10-11].

All the results described and obtained on humans point the limitations related to some variables, such as the temperature, the cause of the death, but above all the time elapsed since death before the analysis was performed.

No research has thus far been conducted to analyze the variation of one or more proteins on the human model from the exact moment of death. For this purpose, we have proposed an experimental operating model under analysis that tries to overcome many of the limitations described.

Indeed, this protocol provided for the taking of peripheral venous blood drains on the corpse knowing the exact time of death (time 0), from patients who had a cardiac arrest and subsequently died. The pros of the analysis of the cardiac arrest group is that we were certain of the exact moment when the cardiac activity stopped. The possible cons is that cardiac arrest could have influence on the levels of some markers that are related to the cardiac damage, such as Troponin T. The analyzed cases were divided by age, sex, comorbidities, and cause of death in order to standardize the data obtained and to better estimate the influence of extrinsic variables on proteic variation in time.

The samples were taken at predetermined standard time intervals and with a constant exposure temperature of the corpse (Room Temperature). In addition, all the samples taken were subjected to the same procedure for analysis, i.e. immediate centrifugation, plasma extraction and subsequent storage at -80 ° C. In addition, the sample that has been selected for analysis (blood) is extremely easy to draw and can also be analyzed with other proteomic methods such as Western Blotting or Mass Spectrometry.

The next step in the protocol involves the performing of ELISA immunoassay for all the samples taken and is still being analyzed. The investigation would reveal the qualitative and quantitative variations of all selected proteins between the different post-mortem intervals. This would allow not only to confirm the data already available literature but also to discover new candidate proteins to become potential biomarkers of PMI.

## 5. Concluding Remarks

Our study has several advantages:

- Identify new potential biomarkers of time elapsed since death, expressed in human blood;
- Verify and evaluate in detail the variation of the proteomic profile of markers already known in the literature;
- Focus on the analysis of the so-called "early post-mortem interval" for forensic purposes;
- Know the kinetics of one or more proteins after death from time 0.

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## References

1] I. Aquila, F. Pepe, C. Di Nunzio, F. Ausania, A. Serra, P. Ricci. J Forensic Sci. 59 (2014) 1665-1667. DOI: 10.1111/1556-4029.12538.

2] I. Aquila, S. Boca, F. Caputo, M.A. Sacco, S. Gratteri, V. Fineschi, P. Ricci. Am J Forensic Med Pathol. (2017) 229-232. DOI: 10.1097/PAF.0000000000000317.

3] S. Pittner, B. Ehrenfellner, A. Zissler, V. Racher, W. Trutschnig, A.C. Bathke, A.M. Sänger, W. Stoiber, P. Steinbacher, F.C. Monticelli. Int J Legal Med. 131 (2017) 479-483.

4] E.E. Foditsch, A.M. Saenger, F.C. Monticelli. Int J Legal Med. 130 (2016) 433-40. DOI: 10.1007/s00414-016-1459-4.

5] S. Pittner, B. Ehrenfellner, F.C. Monticelli, A. Zissler, A.M. Sänger, W. Stoiber, P. Steinbacher. Int J Legal Med. 130 (2016) 1547-1555. DOI: 10.1007/s00414-016-1349-9.

6] K. Kikuchi, K.I. Kawahara, K.K Biswas, T. Ito, S. Tancharoen, N. Shiomi, Y. Koda, F. Matsuda, Y. Morimoto, Y. Oyama, K. Takenouchi, N. Miura, N. Arimura, Y. Nawa, S. Arimura, M.X. Jie, B. Shrestha, M. Iwata, K. Mera, H. Sameshima, Y. Ohno, R. Maenosono, Y. Tajima, H. Uchikado, T. Kuramoto, K. Nakayama, M. Shigemori, Y. Yoshida, T. Hashiguchi, I.

Maruyama. Exp Ther Med. 1 (2010) 109-111. DOI: 10.3892/etm_00000019

7] A.J. Sabucedo, K.G. Furton. Forensic Sci Int. 134 (2003) 11-16. DOI: 10.1016/S0379-0738(03)00080-X.

8] S. Kumar, W. Ali, S. Bhattacharya, A.K. Verma. J Forensic Leg Med. 40 (2016) 16-21. DOI: 10.1016/j.jflm.2016.02.002.

9] S. Kumar, W. Ali, U.S. Singh, A. Kumar, S. Bhattacharya, A.K. Verma, R. Rupani. J Forensic Sci. 61 (2016) S241-2455. DOI: 10.1111/1556-4029.12928

10] A. Crecelius, A. Götz, T. Arzberger, T. Fröhlich, G.J. Arnold, I. Ferrer, H.A. Kretzschmar. Proteomics. 8 (2008) 1276-1291. DOI: 10.1002/pmic.200700728

11] Z.K. Campell, I. Kwon, S.J. Finley, Y. Lee, G.T. Javan. J Forensic Leg Med. 44 (2016) 188-191. DOI: 10.1016/j.jflm.2016.10.020