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Mathematization of the death-moment determination process of laboratory biological material

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Abstract: The article presents results of scientific research in the field of mathematization of the algor mortis process in death-moment determination of biological laboratory material. It describes in a clear form the basic mathematization models of the algor mortis process using mathematical regression, for calculation of the death moment based on acquired temperature values of the liver mass of biological laboratory material, for open and closed examined area of the peritoneal cavity of the left liver lobe, lobus sinister. The liver has a large functional reserve; even one third of its tissue is able to provide its basic functions, they also have a large regenerative ability and are located far from the body surface.

Keywords: Exponential model, Mathematical regression, Aerobic bacteria, Peritoneal cavity, Corrective factors

1. Introduction

Forensic specialists should not be too fussy about types of evidence which are available. Their work should be based on the fact that whatever gets into their hands must be made use of as much as possible. The evidence obtained using the state-of-the-art technical instruments are not always more evidentiary and of higher quality than the employment of a complex of other forensic methods. Described below in an abbreviated form is the improvement of quality of the death determination forensic method during the algor mortis process using the state-of-the-art instruments and devices which are nowadays widely used in a number of safety and other technical systems.

2. Algor and rigor mortis

The algor mortis process (in Latin: algor – cold, mortis – death) is defined as a process during which the corpse gradually cools, i.e. its temperature levels with the ambient temperature. It is also typical that the temperature decline is already noticed in agony. According to available information and scientific research, after death the temperature can increase e.g. in feverish diseases, tetanus, electric shock, etc. Generally we can say that the temperature of the body declines to the ambient temperature although the external factors have significant influence, annulling thus the application of corrective factors. The whole process is a process of the body cooling caused by a cease of heat production in the body. Temperature decline in different parts of the body is uneven. First the peripheral parts of the body begin to cool in the process of the temperature decline. When including a large number of corrective factors which influence the cooling process, the mathematical regression is very complicated. The

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temperature of the body core is not equal in all areas when compared to the skin temperature. During the research in the field of algor mortis process, temperature in the liver was measured; in the whole body the liver shows the highest temperature; however there are specialists who prefer measuring the temperature in the rectum.

In translation the "Rigor mortis" means the stiffness of death and it is one of the distinguishable signs of the death which is caused by chemical change in the muscles after the death. In people this process starts in approx. 3 hours, then after 12 hours the body reaches its maximum stiffness which gradually dissipates within 72 hours (i.e. 3 days) after the death. In biochemical terms we can characterize this state as a state when the muscle contraction mechanics become disrupted. Shortly after the death the muscles spend the ATP reserve and the so called "rigor mortis" – the after-death-stiffness comes (with the absence of ATP myosin firmly bonds to actin, i.e. the muscle remains in the continuous contraction condition), which however ceases after a certain time period due to spontaneous degradation (autolysis) of proteins. Bacteria which live in the digestive tract are able to penetrate outside the intestinal wall and to benefit from it. These original mutualists can easily consume sugars and proteins available in the body; this activity produces various gases causing blowing of the dead body, providing thus a potential temperature increase in the measured organ which results in exothermic reactions during which energy – i.e. heat is released.

3. Current state of research and studies

The latest research and new studies made by scientific teams of Rochellys Heijtz and Sven Pettersson indicates that bacteria in the digestive system influence behaviour of the biological laboratory material, i.e. also humans, and the brain activity of the subject is affected as well. Research has also proven that via the nerve connection between the digestive system together with its colonization by bacteria, and the brain, these organs mutually affect their functions. The extent of impact on the liver during the algor mortis process by gram-negative bacterial strains which come from the digestive system has not been described in any study yet. During the liver function failure the homeostasis is disturbed, hormonal disorders occur as well as metabolism and blood coagulation disorders, ascites, kidney failure and brain function disorders which may result in hepatic coma and death. Bacterial translocation has also been described in several experimental models of cirrhosis, where using experimental comparison of intestinal aerobic bacterial flora in the cecal stool of just killed cirrhotic and healthy rats a significantly higher total amount of intestinal aerobic bacteria was established in the cirrhotic rats stool against the healthy rats, and significance of this intergrowing for bacterial translocation has been confirmed. Potential impact of the clostrabial bacteria strains on the liver (hepar) is rather high. Clostridium is a family of gram-positive bacteria belonging to Firmicutes. These are obligate anaerobes which are able to produce endospores. Pathologic clostridium involves approx. 100 species which include common free-living bacteria as well as significant pathogens. They especially involve:

- C. botulinum is a rod-shaped microorganism. It is obligatory anaerobic; oxygen is a poison for it. However it tolerates very small traces of oxygen thanks to the superoxide dismutase (SOD) enzyme which is a very important antioxidation protection for nearly all cells exposed to oxygen. The bacteria can produce endospores under unfavourable conditions, which allow it to survive in inactive state until there are conditions in which it can grow.
- C. difficile is the most serious cause of diarrhoea associated with antibiotics (ADD) and may cause pseudomembraneous collitis, serious intestinal infection often resulting from suppression of normal intestinal flora by antibiotics. The C. difficile bacteria which commonly survive in the body can overgrow. The overgrow is harmful as the bacteria

release toxins which may cause flatulence, constipation and diarrhoea with abdominal pain.

- Clostridium perfringens commonly occurs in infections as a benign component of normal flora.. Its role in the disease is small. The C. perfringens infections are proven in the tissue necrosis, bacteraemia, emphysematous cholecystitis and in gas gangrene, also known as clostridial myonecrosis. Toxin produced in gas gangrene, known as the α-toxin, enters the plasmatic membrane of cells making holes in it, which disturbs normal function of the cell.
- Clostridium tetani causes the disease called tetanus due to its neurotoxin. C. tetani is found as a component of normal flora in the mammal intestines. It can also occur in the human intestines. The C. tetani toxin consists of 3 components: tetanospasmin, which is the neurotoxin itself, tetanolysin has haemolytic properties and enzyme of renin effect. Tetanospasmin is homogenous polypeptide of one antigenic type. When purified, it has a high specific toxicity: 40 pg in 1 ml of broth still represents 2 minimum lethal doses for the mouse. In non-toxic break it cumulates in rods in which it can represent 5-10 % of their weight. During rod autolysis it is released from them already in a toxic form. Using formol it changes to toxoid. Endothermic animals are sensitive to its effect, while the exothermic ones are only sensitive when their body temperature increases. The guinea pig, the mouse and the horse are the most sensitive. The humans are highly sensitive as well.

Neurotoxin production is a unifying element of the C. botulinum species. Seven types of poisons have already been identified and they were assigned a letter (AG). Most of the strains produce one type of neurotoxin, however the strains producing more toxins have been described in scientific articles. C. botulinum produces potentially lethal neurotoxins which are used in diluted form and applied in the nerve area of the facial part to obstruct the muscle motion, slowing thus the ageing effect. Non-pathogenic strains of clostridia can help in the treatment of diseases, e.g. cancer. The research proves that clostridia can destroy the cancer cells in a selective and targeted way. Some strains can enter and replicate within solid tumors. This application of clostridia has been proven in a number of pre-clinical models.

Significant diseases which can affect the process of liver temperature measurement include e.g.: hepatic cirrhosis, a chronic hepatic disease in which the hepatic tissue and the liver blood pool gradually convert. Hepatic cirrhosis is a chronic process of the liver necrosis followed by increased production of connective tissue (fibrotization) and knotty reformation of the liver cells. The original architecture of the liver is completely damaged in this way and the above mentioned blood pool also adapts to the arisen unfavourable situation. The "cirrhosis" name originates in Greek where the "cirrhos" means yellowish. Other causes include metabolic disorders, billiary cirrhosis, cardial cirrhosis, nutrition disorders, damage caused by medicines, and idiopathic cirrhosis. These diseases are not rare in the Czech Republic and they are on the increase all the time. The cirrhotic patients die on average 10 years earlier compared to the total average age, while women die even much earlier. Most often the socially weaker classes of population are affected. Diabetes mellitus represents another pathogenic impacts on the liver; it is a general name of a group of chronic diseases which manifest by saccharides metabolism disorders. Two basic types are distinguished: type I diabetes and type II diabetes which result from absolute or relative lack of insulin. Both the diseases have similar symptoms but different causes. During the initial phases of type I diabetes, the pancreatic cells producing the insulin hormone are destroyed by the patient´s own immune system. It is therefore included in autoimmune diseases. Type II diabetes is caused by reduced sensitivity of the own body´s tissues to insulin.

4. Basic conditions and methodology for laboratory mesurement of the algor mortis process

Laboratory measurement of the biological material was divided according to the algor and rigor mortis into the following phases:

- algor mortis in duration of 6 basic phases, i.e. 0.0 – 6.41 hours,
- rigor mortis – duration of 2.5 phases, i.e. 0.91 – 3.91 hours for the biological laboratory material.

The initial (constant) conditions of the laboratory measurement include:

- the depth of the probe injection into the hepatic tissue is set to 3 mm,
- the ambient laboratory temperature during the measurement performance was within the range from 20.1 to 20.3 °C,
- the air humidity levels in the laboratory room varied within the range of 69 to 72 %,
- infra-video camera Thermo Pro TP8 with configuration, Testo configuration T 735 with certified probe 0602.5792811,
- pad made of extruded polystyrene.

Biological material was used for the laboratory measurement, represented by a biological mass of laboratory rats; specified weight of the individual laboratory biological materials was within the range of 231 to 261 g. The hepatic mass was in the weight range from 8 to 14 g. The measured temperature values were evaluated using the Launch Guide Ir Analyser programme with visualization of suprema nad infima values of temperature fields. 81 samples of biological laboratory material were measured; the reference measurement was carried out on one sample with open wound and 80 measurements with closed puncture wound. (see Figs. 1, 2).

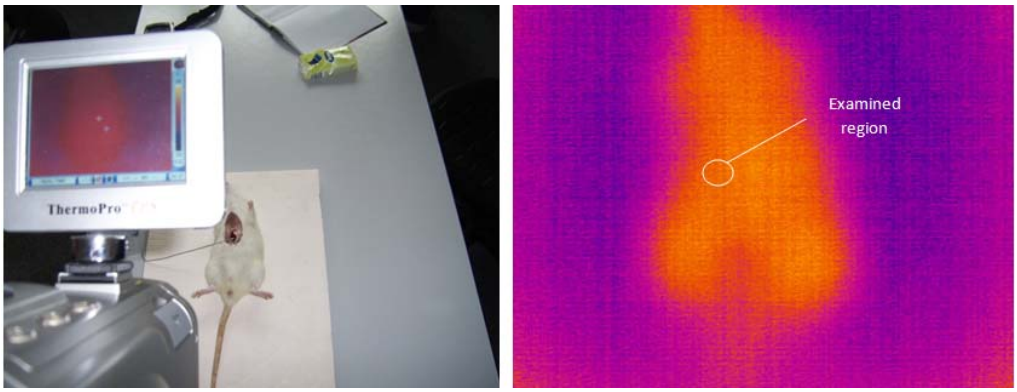

Fig. 1. Reference measurement with open wound in the liver region and the examined region

Used in the laboratory measurement was methodology for short-term measurement of biological material with maximum measurement time up to 385 minutes after the death. The method of laboratory measurement can be described in the following abbreviated form:

- activation and software preparation of the T 735 set with certified probe 0602.5792811. for application;
- activation and software preparation of the infrared video camera Thermo Pro TP8 with the set employing the type 1 filter within the temperature range between -20 to 250 °C;
- humane killing of the biological laboratory material;

- surgical opening of the abdomen, i.e. making a simple surgical cut for insertion of the probe into the peritoneal cavity, i.e. the left liver lobe;
- inserting the active part of the probe into the liver mass;
- sampling and acquiring data within the whole interval of the laboratory measurement of the biological material;
- saving the measurement data and their evaluation.

5. Mathematization of the death moment determination process

Two models were established for mathematical description of the process of death moment determination of the biological laboratory material. The first model (model 1) is designed for the open wound of the biological material – it is an exponential model which is for one variable, so it takes into account neither the liver weight nor the whole biological material weight. The second model (model 2) represents the closed wound with variables which include the liver weight and the biological material weight.

5.1. Function prescription for model 1

Involved in our case is a non-linear model. The loss function is a method for estimating the a, b and c coefficients, where the issue in question was solved using the method of least squares. The model which has a higher index of determination is the more suitable one. If there are more input parameters, the adjusted index of determination is used (it takes into account the number of input parameters), which however is not our case. In our case the model 1 has the value of 99.94%, so 99.94% of the variability could be explained using the model. The R value is a coefficient of correlation (square root of the index of determination) and it confirms how the specified model correlates with the data (see Table 1).

Table 1. Regression parameters estimate

General Growth Model: Y=a*Exp(-b*X)+c
Dependent variable: Y Independent variable : 1
Loss Function: Weighted Least Squares
Coefficient of determination : ,99937853 R =,99968921

	parameter estimates	standard errors	t-value sv = 75	p-value	95% interval	95% interval
a	18,65420	0,054450	342,5961	0,00	18,54574	18,76267
b	0,00993	0,000076	130,0065	0,00	0,00978	0,01008
c	19,55823	0,036692	533,0311	0,00	19,48514	19,63133

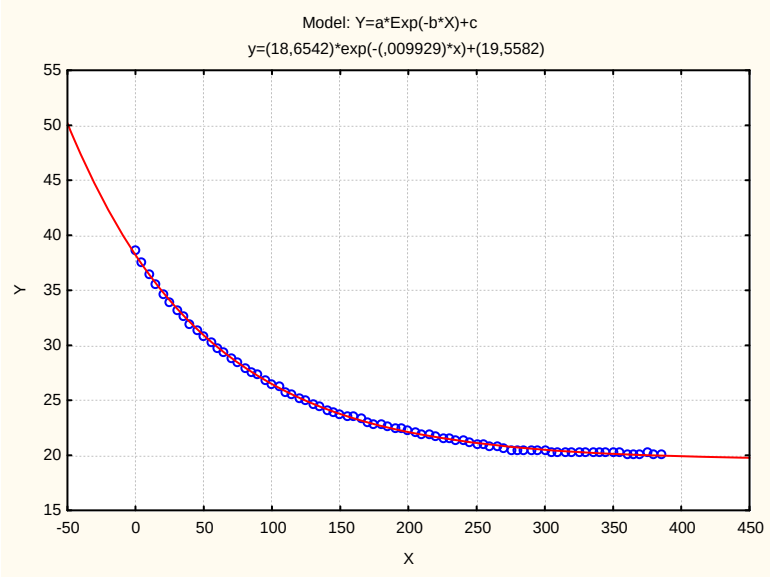

Fig. 2. Chart of the data interpolating curve

On the level of significance α for j = 0,1, ..., p we are testing a hypothesis as follows:

$$H_0: \beta_j = 0 \text{ against } H_1: \beta_j \neq 0 \qquad (1)$$

Test statistics: $T_j = \dfrac{b_j}{S_{b_j}}$ has dispersion t(n-p-1) student´s dispersion, if H_0 is valid.

Critical field: $W = \left(-\infty, -t_{1-\alpha/2}(n-p-1)\right\rangle \cup \left\langle t_{1-\alpha/2}(n-p-1), \infty\right)$.

$T_j \in W \Rightarrow$ H_0 is rejected on the level of significance α. In all the cases the value is lower than 0.05; i.e. on the level of significance of 0.05 we reject the hypothesis on non-significance of regression parameters a,b,c.

Considered as more convenient is the regression function in which the value of test statistics $F = \dfrac{S_R/p}{S_E/(n-p-1)}$ for the test of significance of the model as a whole is higher. Besides, a control of residues has been carried out, which should be (see Fig. 3)

- normally distributed,
- zero mean value,
- constant dispersion.

Table 2. Average residue test (Normality of residues – mean value of residues is 0.00000)

	t-test, single sample> Model: Y=a*Exp(-b*X)+c							
Variable	Mean	SD	N	SE of the mean	constant	t	DF	p
Residual val.	0,000000	0,122445	78	0,013864	0,00	0,000000	77	1,000000

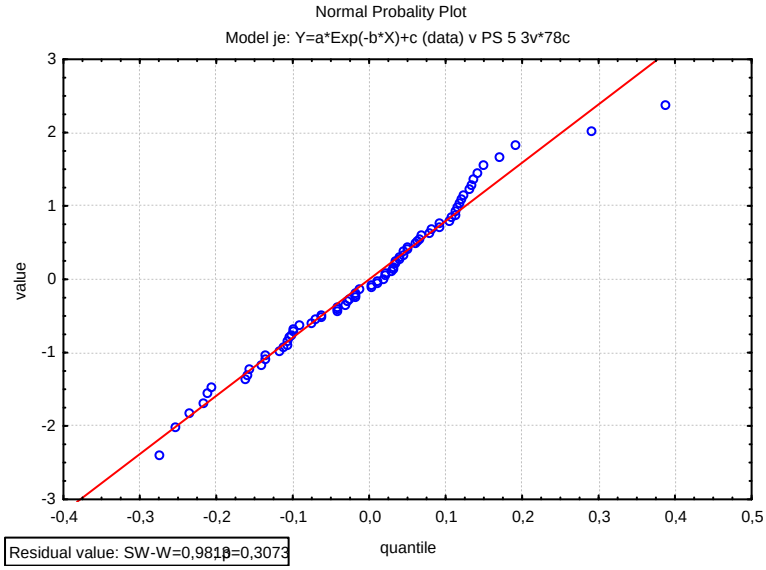

Fig. 3. Chart of residues homogeneity test

5.2. Function prescription for model 2

The second model (model 2) represents a closed wound with the following variables:
Y ……. dependent (temperature)
X ……. independent (time)
Z ……. independent (weight of the biological laboratory material)
M …… independent (liver weight)

Table 3. Resulting values of regression with the dependent variable
(Results of regression with the dependent variable)

N=148	Multivariate regression model Y					
	b*	Standa error	b	Standa error	t(147	p-valu
Abs.va			40,296	1,0951	36,79	0,000
X	-2,697	0,1027	-0,130	0,004	-26,24	0,000
Z	-0,027	0,010	-0,013	0,0051	-2,69	0,007
M	0,034	0,010	0,102	0,030	3,33	0,000
X*Z	0,244	0,1184	0,000	0,000	2,06	0,039
X^2	1,701	0,0162	0,000	0,000	104,5	0,000
X*M	-0,084	0,0337	-0,000	0,0001	-2,52	0,011

The corresponding p-value of this test is 0.00, therefore on the significance level of 0.05 we reject the hypothesis of non-significance of the model as a whole. In this case our model explains 97.5% of data.

Test of residues normality is presented using N – P plot (Normal probability plot). If the data are distributed in a normal way, they are situated along a straight line. (Shapiro-Wilk test). Corresponding p-value indicates that the data have normal distribution. Zero hypothesis in this test is that the data are distributed normally. P-value indicates that on the significance level of 0.05 we cannot reject the zero hypothesis. Residue homoskedasticity is evaluated

based on the chart of residue dependence on the predicted values. In this chart residues should be dispersed uniformly.

6. Verification of the calculated and measured data (model 1 and model 2)

Table 4. Verification of the calculated and measured data in % (model 1)

Measured values	325 min	330 min	335 min	340 min	345 min	350 min	355 min	360 min	365 min	370 min
	20.6	20.4	20.3	20.2	20.2	20.2	20.2	20.1	20.1	20.1
Calculated values	**324 min**	**329 min**	**333 min**	**339 min**	**344 min**	**349 min**	**354 min**	**358 min**	**364 min**	**369 min**
	20.6	20.4	20.3	20.2	20.2	20.2	20.2	20.1	20.1	20.1

Table 5. Verification of the calculated and measured data in % (model 2)

Measured values	325 min	330 min	335 min	340 min	345 min	350 min	355 min	360 min	365 min	370 min
	20.6	20.4	20.3	20.2	20.2	20.2	20.2	20.1	20.1	20.1
Calculated values	**317 min**	**321 min**	**327 min**	**332 min**	**337 min**	**342 min**	**347 min**	**352 min**	**356 min**	**361 min**
	20.6	20.4	20.3	20.2	20.2	20.2	20.2	20.1	20.1	20.1

7. Conclusion

Models 3 and 4 are currently in progress, which solve the issue of correction factors. The article is focused on mathematical expression and determination of the moment of the death in the algor mortis process of biological laboratory material. The exponential model which takes into account neither the liver mass weight nor the biological material weight for the closed examined area is only one-dimensional and it is not suitable for practical application in its full extent. The above mentioned model does not accept the problem of exothermic reactions during which energy – i.e. heat is released. The subject of further research in the above mentioned field will be making mathematical models with the highest precision of the death time calculation with the employment of correction factors and their application on the human body.

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