21. medzinárodná vedecká konferencia
Riešenie krízových situácií v špecifickom prostredí
Fakulta bezpečnostného inžinierstva UNIZA, Žilina, 25. - 26. máj 2016

THE MICRO-FLORA OF THE LIVER ORGANS OF BIOLOGICAL SUBJECTS IN THE ALGOR MORTIS PROCESS

Ján Ivanka [*]

ABSTRAKT

Príspevok prezentuje výsledok vedeckého výskumu v oblasti matematizace procese Algor mortis pri stanovení okamžiku úmrtia laboratórneho biologického materiálu. Prehľadnou formou uvádza základné modely matematizácie procesu Algor mortis matematickú regresiu pre výpočet okamihu úmrtia na základe získaných teplotných hodnôt pečeňové hmoty biologického laboratórneho materiálu pre otvorenú a uzavretú vyšetrovanú oblasť peritoneálnej dutiny ľavého pečeňového laloku, lobus sinister. Forenzní špecialisti by nemali byť vyberaví, pokiaľ sa týka druhu dôkazov, ktoré majú k dispozícii. Základom práce forenzných špecialistov by mala byť skutočnosť, že čokoľvek sa im dostane do ruky, musí sa využiť v maximálne možnej miere. Dôkazy získané pomocou najmodernejších technických prístrojov nie sú vždy najkvalitnejšie, než použitie súhrnne iných forenzných metód. Neinvazná metóda urychľuje regresné meranie nestacionárných teplotných polí biologického materiálu.

Kľúčové slová: prevencia**,** trestný čin, exponenciálny model, matematická regresia, aeróbne baktérie, peritoneálna dutina, korekčné faktory

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. 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. Non-invasive measurement method accelerates regression of unsteady temperature fields of biological material.

[*] Ján Ivanka, Ing., Tomas Bata University in Zlín, Department of Security Engineering, Nad Stráněmi 4511, Zlín, 760 05, Czech Republic, +420576035247, ivanka@fai.utb.cz

1   INTRODUCTION

This contribution presents a mathematical model for the identification pathological samples of liver mass which affect the temperature values in the cooling process of biological material. Pathologic clostridium involves approx. 100 species which include common free-living bacteria as well as other significant pathogens. They especially involve: C. botulinum, C. difficile, C. perfringens, C. tetani, Salmonella, Campylobacter, and Haemophilus bacteria. Research was focused on the Aerococcus viridans type of bacteria strains, wherein the bacteria are sometimes encountered as human pathogens - especially in bacterial endocarditis, and further, Micrococcus Luteus Saprothopic bacteria belonging to the genus Micrococcaceae, as well as Staphylococcus xylosus, Pasteurella pneumotropica, and Staphylococcus nepalensis.

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 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 measurement itself and determination of time in the algor mortis process, the 0.00 value was specified as a reference point within the time interval from t_0 to t_{385}, where index 385 represents the 385th minute after the death. The laboratory materials were killed using a humane method. 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 biological material was placed on the XPS type pad which is made of extruded polystyrene tempered to the laboratory ambient temperature of 21.1 °C. 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 Fig.1). The measured temperature values were evaluated using the Launch Guide Ir Analyser programme with visualization of suprema nad infima values of temperature fields. The laboratory measurement of the liver-mass temperature core was chosen due to the fact that the liver of the biological laboratory material is an organ which is located far from the body surface taking up quite a large part of peritoneal cavity.
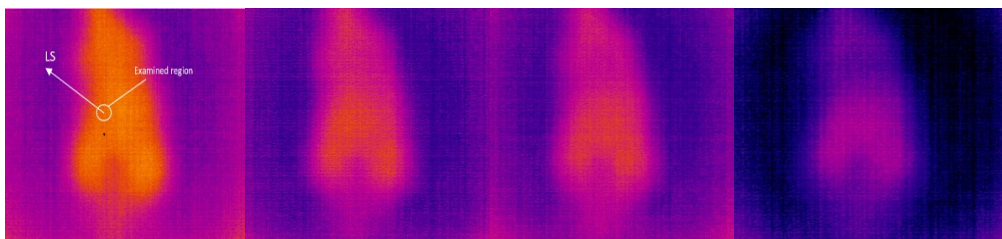
Fig.1 Reference measurement with open wound in the liver region
and the examined region

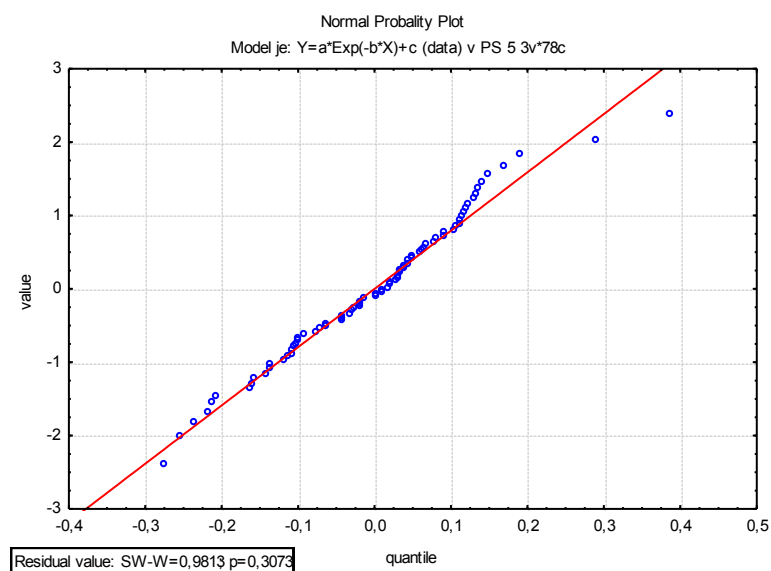# 5 MATHEMATIZATION OF THE DEATH MOMENT DETERMINATION PROCESS

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.

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	18,6542(	0,05445(	342,596	0,00	18,5457·	18,76267
b	0,0099:	0,00007(	130,006:	0,00	0,00978	0,01008
c	19,5582:	0,03669:	533,031	0,00	19,4851·	19,6313:

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

Variable	t-test, single sample> Model: Y=a*Exp(-b*X)+c							
	Mean	SD	N	SE of the mean	constant	t	DF	p
Residual val	0,00000(	0,12244:	78	0,01386·	0,00	0,00000(	77	1,00000(

Normal Probality Plot
Model je: Y=a*Exp(-b*X)+c (data) v PS 5 3v*78c

Residual value: SW-W=0,9813 p=0,3073

Fig. 2 Chart of residues homogeneity test

6 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.

ACKNOWLEDGEMENT

REFERENCE

[1] MURRAY, Robert K., et al. *Harperova biochemie (Harper´s Biochemistry)*. From English (23rd edition) translated by Lenka Fialová et. al. 4th edition in CR. Prague: H & H, 2002. ix, 872 s. ISBN 80-7319-013-3.

[2] VAN DER WAAIJ, D., The ecology of the human intestine and its consequences for overgrowth by pathogens such as Clostridium difficile. Ann Rev Microbiol 1980; 43:69-87

[3] NEČAS, Emanuel, a kol., *Patologická fyziologie orgánových systémů, část II (Pathological Physiology of Organ Systems, Part II)*. 1st edition, Prague: Karolinum Publishing, 2006. 760 p. ISBN 80-246-0674-7

[4] TROJAN, Stanislav, a kol., Lékařská fyziologie (Medical Physiology). 4th edition, Prague: Grada, 2003, 769 p. ISBN 80-247-0512-5

[5] FIXA, B., KOMARKOVÁ, O., Mikroflóra trávicího ústrojí za fyziologických a některých 84 patologických podmínek (Microflora of digestive system under physiological and some 84 pathological conditions). In: Aktuality v gastroenterologii (News in gastroenterology). AZ servis, Prague 1994: 57-82.