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THE RELATIONSHIP BETWEEN THE RATE OF DECREASE IN BODY TEMPERATURE AND THE APPEARANCE OF CORPSE STIFFNESS IN WISTAR RATS THAT DIED DUE TO BLOOD LOSS

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ABSTRACT

Every death will be accompanied by signs of death such as rigour Mortis, Algor mortis, livor mortis, and decomposition. In death with massive blood loss, Algor Mortis dan rigour Mortis should appear faster than death without blood loss. It will affect the death estimated time by a forensic doctor. This study is based on the discipline of Physiology and Forensic Sciences that are trying to evaluate Mortis and Algor Mortis in mice as an initial study to look at the relationship between blood loss and signs of definite death. The number of experimental animals used is calculated using the formula experimental Freeder size: (n-1) (t-1) ≥ 15, in which the required sample size is 16 samples divided into two groups of experimental animals. In both groups, we perform anaesthesia using ketamine at a 50-75 mg/kg dose and xylazine at 10 mg/kg administered intraperitoneally (I.P.). The control group will be subject to cervical dislocation, while the other group will have blood drawn as much as ± 30% of the total blood to trigger hypovolemic shock using cardiac puncture techniques. When cervical dislocation is done in both groups, the time required to decrease body temperature and the appearance of rigour Mortis will be observed in both experimental groups. From the research that has been done, it can be concluded that blood loss may be one of the factors that affect the speed of the decline in body temperature of mice to the ambient temperature and the speed of the formation of rigour Mortis. It was proven using Kendall's Tau nonparametric correlation with the results -,770 for body temperature and Pearson correlation with the results -,891 for Algor Mortis. The group that had blood drawn more quickly reached ambient temperature by 19,8 % compared to the group who did not have blood drawn, and groups who had blood drawn reached rigour Mortis maximum score of 32% are faster than the group that did not have blood drawn.

KEYWORDS: Argor Mortis, Rigor Mortis, Blood loss.

INTRODUCTION

Death is the end of life, the absence of life in a biological organism. All living things will eventually die permanently due to natural causes such as disease or unnatural causes such as accidents or murder. Death can be recognized clinically in a person through signs of death, namely the changes in the corpse's body. These changes can occur immediately or within minutes to hours.

Thanatology is the study of aspects related to death; it includes the definition (definition), ways to diagnose, the changes that occur after death and their uses. Thanatology comes from the word "Thanatos, " meaning death, and "logos, " meaning knowledge. In thanatology, several terms are known about death, namely somatic death (clinical death), near death, cerebral death, and brain death (brain stem death). Somatic death (clinical death) occurs due to the cessation of the three life support systems, namely the central nervous system, cardiovascular system and respiratory system, which is irreversible—in comparison, suspended animation /apparent death cessation the three above-defined life systems with simple medical tools. It can still be proven that the three systems are still functioning with advanced medical equipment. Cellular death (molecular death) is the death of organs or body tissues that occurs some time after bodily death. Cerebral death is irreversible damage to both hemispheres of the brain except the brain stem and cerebellum, while the other two systems, namely the respiratory and cardiovascular systems, still function with the help of tools. Brain death (brain stem death) is when there has been irreversible damage to all intracranial neuronal contents, including the brain stem and cerebellum.[1,2]

The importance of studying thanatology is to ascertain the death of a person, determine the time of death, cause

of death, manner of death, and remove or remove organs for donor or transplant purposes. To distinguish post-mortal changes from abnormalities while the victim is still alive. Sometimes doctors can also make mistakes in determining the time of death, especially in homicide cases where the victim is found dead for an undetermined time.

According to the 2014 Criminal Statistics Publication, the number of victims of crime in 2011-2013 was 7,920,860 people consisting of various types of crimes in Indonesia. The number of homicides throughout Indonesia in 2011-2013 was 4309 cases consisting of 1,467 cases in 2011, 1,456 cases in 2012, and 1,386 cases in 2013.[3]

Based on the high homicide rate data above, Indonesia still needs forensic doctors proficient in performing the autopsies needed to determine the time, cause, and manner of death influenced by many factors and, of course, using valid and recognized techniques. One factor that influences the timing of death is the presence or absence of blood loss in the victim.

Physiologically, blood is an essential component and dramatically affects the function of human metabolism, where blood consists of water, electrolytes, nutrients, waste substances, gases, hormones, various proteins, and components of blood cells that play a role in metabolism and inflammation.[4]

In the case of a large amount of blood loss, it will most likely affect the duration of the appearance of definite signs of death such as a decrease in temperature (Argor Mortis), bruising (Livor Mortis / Post Mortem Lividity), stiff corpse (Rigor Mortis), and putrefaction (Decomposition). The presence of blood loss in the victim may obscure and prolong the appearance of definite signs of death. It can be a gap for the murder perpetrators to escape the law because of differences in the time of death interpretation with the perpetrator information. Therefore, it is necessary to know how blood loss in homicide cases affects the appearance of definite signs of death, and the end can increase the accuracy of the estimated time of death. Based on the background described above, the problem can be formulated: a) How is the effect of blood loss on the speed of temperature reduction (Argor Mortis) in rats that die from blood loss? Furthermore, b) How does blood loss affect the duration of the appearance of rigid cadavers (Rigor Mortis) in rats that died from blood loss?. The aims of the study were: a) to determine the difference in the speed with which the signs of a decrease in body temperature (Argor Mortis) appeared in rats that died from blood loss compared to those without blood loss, and b) to find out the difference in the duration of the appearance of rigid cadavers (Rigor Mortis) in rats that died from blood loss compared to those without blood loss.

LITERATURE REVIEW

Thanatology comes from the word "Thanatos," meaning death, and "logos," knowledge. Thanatology studies all aspects of death, including the definition, method of diagnosis, changes that occur after death, and the factors that influence these changes.[1,2]

Death is a process that only living organisms can experience. Medically, death is a process in which the function and metabolism of cells in the body's internal organs stops. Death can be recognized clinically in a person by observing the changes in the corpse's body. In contrast to the state of the body when it is still alive, in the body of the corpse, the processes that occur in the body that are still alive will stop. The respiratory, cardiovascular, and nervous systems will stop causing various manifestations in the corpse's body.[5,6]

Changes that occur after death are divided into quickly and slowly changes. Changes that occur rapidly include cardiac arrest, respiratory arrest, eyes, and skin changes. While the changes that occur further include a decrease in temperature, bruising, stiff corpses, decay, saponification and mummification. In forensic medicine, humans have two dimensions: individuals and collections of various kinds of cells. Individual death is defined simply as the permanent cessation of life, the function of vital organs as a whole, marked by the cessation of oxygen consumption. As a result, one by one, the cells, which are the minor living elements that makeup humans, will experience death (cellular death).[2]

Every cell in the body has a different time to experience cell death due to its cellular metabolism. Cortical neurons need the fastest time, 3-7 minutes after the cells run out of oxygen. In the body, cell death occurs cell by cell, and overall death will occur within a few hours.[7] There are several terms regarding death in thanatology, namely somatic death (clinical death), near death, cellular death, cerebral death, and brain death (brain stem death). Somatic death (clinical death) is characterized by the permanent cessation of the functions of the three life support systems, namely the central nervous system, cardiovascular system and respiratory system.

Suspended animation, apparent death cessation, and the three life systems above are determined by simple medical tools. It can still be proven that the three systems are still functioning with advanced medical equipment. It is due to the decline in vital processes to the most minimal level to maintain life. Near-death is often found in conditions of acute heart attack, hypothermia, drowning, electric shock and excessive anaesthesia.[1,2]

Cellular death (molecular death) is the death of organs or body tissues that occurs some time after bodily death. Each tissue or organ has a different resistance from the cellular death state so that the occurrence of cellular death in each organ or tissue does not coincide.[2] Cerebral death is characterized by irreversible damage to

both hemispheres of the brain. The brain stem, cerebellum, and the other two systems, namely the respiratory and cardiovascular systems, can still function with the device's help.[1] Brain death (brainstem death) is a condition in which irreversible damage has occurred to the entire intracranial neuronal contents, including the brainstem and cerebellum. By knowing brain stem death, it can be said that a person as a whole cannot be declared alive anymore so that that assistive device can be stopped.[1,2] The use of thanatology in the forensic field is the diagnosis of death, determination of the time of death, estimation of the cause of death, and estimation of the manner of death.[2]

Rigour Mortis is stiffness in the body after death caused by the absence of adenosine triphosphate (ATP) in the muscles. At early death, the body experiences muscle relaxation to look weak.[8] However, within 1 to 3 hours after that, muscle stiffness increases, and immobilization of the joints occurs. Muscle flexibility after death can still be maintained because metabolism at the cellular level is still running to break down muscle glycogen reserves that produce energy. This energy is used to convert A.D.P. to ATP. As long as ATP is present, the actin and myosin fibres remain flexible. When the glycogen reserves in the muscles are depleted, energy is no longer formed, actin and myosin will clump together, and the muscles will become stiff.[9]

Muscles need a supply of energy from ATP to contract because the amount available in the muscles can only maintain the function of muscle contraction for a few seconds. Three metabolic pathways maintain the supply of ATP in the muscles, namely the phosphagen system, the glycogen-lactic acid system and the aerobic system. When muscles become anoxia, the oxygen supply is reduced so that ATP is not produced, resulting in the process of aerobic glycolysis that increases lactic acid and pyruvic acid levels. Glycogen levels in muscle decrease, cellular pH becomes six and ATP levels begin to decrease. Normally, ATP functions to inhibit the binding activity between actin and myosin.[10]

At optimal conditions, the phosphagen system can provide energy for use by muscles to contract for 10-15 seconds, and the lactic acid glycogen system provides energy for 30 to 40 seconds and the aerobic system for an indefinite period. Corpse stiffness will occur in all muscles, both striated and smooth muscles, and if it occurs in the limbs' muscles, a rigidity similar to or resembling aboard will be obtained so that energy is needed to fight these forces. Glycogen levels in each muscle are different so that during the breakdown of glycogen into lactic acid and energy at the time of somatic death, there will be differences in ATP levels in each muscle.[11] This situation can explain why the stiffness of the corpse begins to appear in muscle tissue with a small number of muscle fibres. Stiffness is usually seen first in the jaw, then in the elbows and then in the knees. Cadaver stiffness is more significant in men than women because men have greater muscle mass than women.[12]

Rigour Mortis is usually seen 2-4 hours after death in the average person at normal room temperature. Complete rigour Mortis usually occurs after death. The body experiences complete rigour Mortis when the jaw, elbows, and knees can no longer move. It lasts 10-12 hours after death at room temperature 70-750° F.[13] This state will persist 24-36 hours, and after that, the stiffness of the corpse will begin to disappear. Generally, rigour Mortis is seen initially in the facial muscles and spreads to the chest, extremities and then to the rest of the body. The pattern of the disappearance of rigour Mortis also follows the order of appearance. At first, it disappeared on the face and then spread to the chest and extremities. Contraction of the myocardium of the left ventricle causes its walls to become thicker and contain a small amount of blood. Stiffness of the corpse will be accelerated in the presence or at conditions: thin people, before death experienced high fever/inflammation, at high ambient temperatures, and did strenuous physical activity before death.[14] Factors that affect the rigidity of the corpse are muscle condition, age, environmental conditions and manner of death. Stiffness in the body due to rigour Mortis needs to be distinguished from several other processes such as Cadaveric spasm (instant rigour), Heat stiffening, and Cold Stiffening (Freezing).[15]

Decrease in body temperature (Argor Mortis) - A decrease in body temperature occurs due to the cessation of metabolic processes that produce heat, so that heat transfer occurs from an object to a more excellent object (from the body to the environment) through radiation, conduction, evaporation and convection.[16] The graph of the decrease in body temperature is almost in the form of a sigmoid curve or like the letter S. The point is that in the first hours of death, the temperature drop is prolonged because of the glycogenolysis process that produces heat.[17] After that, the decline occurs more quickly and finally slowly Returns. If average, the temperature decrease is between 0.9 to 1 degree Celsius or about 1.5 degrees Fahrenheit every hour, with a note that the temperature decrease starts from 37 degrees Celsius or 98.4 degrees Fahrenheit so that a way can be formulated to estimate how many hours dead bodies with the formula (98.4ºF- rectal temperatureºF): 1.5ºF. Measurements were made rectally using a chemical thermometer (long chemical thermometer).

There are no less than 30 diagnostic criteria that have been compiled to determine a person's death, but there are eight criteria that forensic doctors most widely use, namely.[18] a) The loss of all responses to the surroundings (responses to commands/orders, tactics and so on; b) There is no muscle movement and posture, provided the patient is not under the influence of drugs; c) No pupillary reflex; d) No corneal reflex; e) There is no motor response of the cranial nerves to stimuli; f) No

swallowing reflex or stone when the endotracheal tube is pushed inward; g) There is no vestibule-ocular reflex to stimulation of ice water that is inserted into the ear canal, and h) No spontaneous breathing when the respirator is inflated for a long time even though the pCO2 has exceeded the respiratory excitability threshold (50 torrs).

Research Method

This research is experimental laboratory research that uses experimental animals as experimental objects. The research design schemed to see the definite signs of death in female Wistar white rats (Rattus norvegicus), which were divided into two groups, namely the control group and the treatment group. Both groups were given general anaesthesia using ketamine and xylazine intraperitoneally. The control group will not take blood, while the treatment group will have their blood drawn using a syringe after being sedated. After this stage, both groups were terminated by cervical dislocation. The population of this study were female white rats (Rattus Norvegicus) Wistar strain, while the research sample consisted of 16 female Wistar rats (Rattus norvegicus) obtained from the rat farm of the Faculty of Animal Husbandry, Bogor Agricultural University. Sampling was carried out by calculating the age of female Wistar rats (Rattus norvegicus) from birth to avoid bias due to age and weight variations so that the age of female Wistar rats (Rattus norvegicus) was more than three months. After that, body weight was measured, and gender was confirmed. During standard conditions, all rats were given adequate rat feed and water. This study uses primary data obtained from the duration of the appearance of definite signs of death in the form of a decrease in temperature (Argor Mortis) and a stiff corpse (Rigor Mortis). General anaesthesia (General Anesthesia) can be induced using various drugs and administration methods. Giving just one kind of drug can achieve general anaesthesia criteria such as loss of consciousness, analgesia, reduced reflexes, and skeletal muscle relaxation, but combination administration is more beneficial because the side effects of using just one drug can be reduced. The use of several types of anaesthetics in combination with low doses reduces the effects on all body systems that occur during anaesthesia compared to single agents alone. This research has been declared to have passed the ethical review by ethical clearance from the Health Research Ethics Commission (K.E.P.K.) Faculty of Medicine, Indonesian Christian University, Jakarta, January 17, 2017. Certificate of Passing the Ethical Review is attached at the end of the paper.

RESULT AND DISCUSSION

In this chapter, the author will present the results of research carried out on female Rattus norvegicus Wistar rats conducted in the forensic laboratory of the Faculty of Medicine, the Christian University of Indonesia, in January 2017. In this study, the number of samples used was 16 experimental animals divided into the control group and the treatment group. The control group was given intraperitoneal anaesthesia using ketamine and xylazine and immediately euthanized using the cervical dislocation method. The treatment group was given the same anaesthesia, blood was taken through the heart, and cervical dislocation was performed. After that, the decrease in body temperature and the formation of stiff corpses were observed in experimental animals. The following research results will be presented in pictures and tables containing the observations.

Table 1: Body Weight Distribution of Experimental Animals in Groups A and B.

Group	Body Weight (gram)	Frequency	%	Cumulative %
Group A	139	1	6,25	6,25
	143	1	6,25	12,5
	152	1	6,25	18,75
	155	1	6,25	25
	158	1	6,25	31,25
	159	1	6,25	37,5
	162	2	12,5	50
Group B	122	1	6,25	56,25
	129	1	6,25	62,5
	132	1	6,25	68,75
	133	1	6,25	75
	146	1	6,25	81,25
	150	1	6,25	87,5
	151	1	6,25	93,75
	161	1	6,25	100,0
	Total	8	100,0	

The weighting profile of experimental rats in groups A and B can be seen in table 1. If the two groups are combined and the average weight of the Wistar rats in this study is found, the average weight of the 16 rats used is 147.13 grams with a mean value of 150.50 grams. Minimum weight 122 grams and maximum weight 162 grams. There was a slight difference in body weight in the two experimental animal groups, where the average

body weight of group A was 153.75 while group B was 140.50 grams. The average body weight of group A is 13.25 grams greater than group B. The body weight range in group A is also smaller than group B, which indicates that group A has a more homogeneous body weight than group B. However, this is not the case. have a significant influence on the study results. The author has set the inclusion criteria in this study as the sample used in both groups must have a bodyweight between 120-200 grams. The euthanasia method that the researchers used, namely cervical dislocation, was not allowed if the rat's weight exceeded 200 grams. Weighing is also essential to calculate the dose of anaesthetic used in this study.

Table 2: Laboratory Room Temperature and Humidity.

Hour	Room Temperature Day -1	Room Temperature Day -2	Room Humidity Day-1	Room Humidity Day-2
1	31,1	32,5	60%	61%
2	32,4	32,4	54%	58%
3	31,5	32,2	56%	58%
4	31,4	32,2	61%	57%
5	31,2	32,2	63%	57%
Average	31,5	32,3	58,8%	58,2%

This research was conducted on Saturdays and Sundays at the Forensic Laboratory of the Faculty of Medicine, the Christian University of Indonesia, with room conditions listed in table 2. On the first day, the average room temperature was 31.5oC and 32.2oC on the second day. The humidity in the room used on the first and second days, respectively, was 58.8% and 58.2%. There was no significant difference between the temperature and humidity of the room because the researchers had set the execution time at the same hour for both groups of experimental animals to minimize bias due to the effect of environmental temperature on decreasing body temperature and the formation of stiff corpses. The instrument used is the HTC-1 Thermo Hygrometer with Temperature Range: -50 ~ +70 0 C (-58 ~ +158 Fahrenheit), humidity range: 10% - 99% RH. The accuracy of the instrument used is ±1 0 C (1.8 0 F) for temperature and ± 5% R.H. for room humidity.

Table 3: Execution Date and Time of Rat Death.

No. Sample	Execution Date	Time of Death
1	January 21 2017	13.10 WIB
2	January 21 2017	13.10 WIB
3	January 21 2017	13.10 WIB
4	January 21 2017	13.10 WIB
5	January 21 2017	13.10 WIB
6	January 21 2017	13.10 WIB
7	January 21 2017	13.10 WIB
8	January 21 2017	13.10 WIB
9	January 22 2017	13.15 WIB
10	January 22 2017	13.15 WIB
11	January 22 2017	13.15 WIB
12	January 22 2017	13.15 WIB
13	January 22 2017	13.15 WIB
14	January 22 2017	13.15 WIB
15	January 22 2017	13.15 WIB
16	January 22 2017	13.15 WIB

Note: W.I.B. refers to Western Indonesian Time

This research was conducted on Saturday and Sunday between 21 and 22 January 2017. The research started at 12.00 WIB at the Forensic Laboratory. Researchers need approximately one hour to prepare the necessary tools, including anaesthetics whose dosages have been determined according to existing guidelines. Therefore, the execution was carried out at 13.10 WIB in group A on January 21, 2017, and 13.15 WIB in group B on January 22, 2017. The researchers set approximately the same execution time in both groups to avoid bias in this study so that room temperature and humidity are relatively the same.

Table 4: Details of Blood Loss Factors.

No. Sample	Body Weight	Total Blood Count	Amount of Blood That Should Be Drawn	Amount of Real Blood Drawn	Percentage of Blood Drawn
9	132	8,69	2,607	2,7	31
10	122	8,09	2,427	2,8	34
11	146	9,65	2,895	3,2	33
12	151	9,83	2,949	3	3,5
13	161	10,43	3,129	3,5	33
14	133	8,75	2,625	2,9	33
15	150	9,77	2,931	3	35
16	129	8,51	2,553	2,6	30

In group B, the researchers set a sample for blood collection through the cardiac puncture technique. Before taking blood, the researchers first weighed the weight of the mice in group B to determine the estimated total blood volume obtained using the formula 7% of body weight or can be more accurate using the formula: B.V. (ml) = 0.06 X BB (bodyweight) + 0.77. Table 4 lists the Total Blood Count (JDT) in 8 rats. From the Total Blood Count, the researcher has determined to take at least 30% of the total blood as a differentiating factor in group B. It is necessary to determine the amount of blood the researcher should take. The last two columns in table 4 list the actual amount of blood drawn and are expressed as a percentage of blood drawn. In the statistical results table 4, it can be seen that the average blood taken from group B was 2,963 ml (cc) with a minimum intake of 2.6 and a maximum of 3.5 according to the bodyweight of the rats. If expressed in per cent, the average blood drawn in group B is 32,438%. It was done according to the guidelines on the research methodology for subjecting mice to grade three hypovolemic shocks.

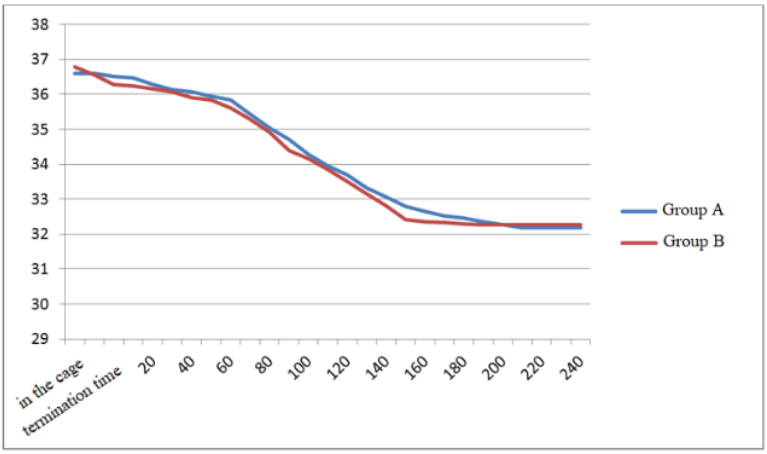

Figure 1: Decrease in Body Temperature Against Time Groups A and B.

At first glance, the decrease in body temperature in groups A and B looks the same. However, if you look more closely, group A starts with a lower temperature of 36.3 0C than group B, 36.787 0C. There was a difference of ±0.4 0C at the start of the study. However, the thing that is striking to note is the rats' temperature at the termination time, where the temperature of group A at the time of termination was 36.512 0C and group B was 36.2750C. It indicated that group B experienced a faster temperature drop than group A during the time course of the rats being anaesthetized until termination.

After the rat was terminated, the rat's body temperature was observed every 10 minutes. At the 10th minute, the body temperature of group A rats was 36,462 0C, and one hour later, the body temperature of the mice became 35.937 0C. There was a decrease of 0.525 0C in the first hour. At the 120th minute, the measured body temperature of the rats was 33.95, meaning a decrease of 1.98 0C in the next hour, and at 180 minutes, the rat's body temperature became 32.512 0C. The decrease in body temperature of mice from 120 to 180 minutes was 1.438 0C. The decline in the last hour was 0.325 0C.

At the 10th minute, the body temperature of group B rats was 36,237 0C, and one hour later, the body temperature of the mice became 35.6 0C. There was a decrease of 0.637 0C in the first hour. At the 120th minute, the measured body temperature of the rats was 33.5, meaning a decrease of 2.1 0C in the next hour, and at

180 minutes, the rat's body temperature became 32.20C. The decrease in body temperature of mice from 120 to 180 minutes was 1.30C. The decline in the last hour was 0.0630C.

Based on the comparison of the temperatures of the two groups, there is one thing in common: the fastest temperature decrease occurs in the first 60 -120 minutes after death. Where in group A the decrease was 1.980C and 2.10C in group B. The decrease in body temperature at the beginning of death and after 180 minutes showed a slow decline. If depicted in a curve, it shows a sigmoid shape (the letter s is inverted). It is undoubtedly following the theory that the decrease in body temperature is slight at first, accelerates in the middle, and finally decreases slowly at the end of the graph of decreasing temperature.

Table 5: Normality Test for Temperature Variables.

	Shapiro Wilk		
	Statistic	**Df**	**Sig.**
No blood drawn	,789	8	,027
Blood drawn	,930	8	,516

Shapiro Wilk is one of the normality tests recommended by many experts if the number of samples is small, namely less than or equal 50 samples. This test is susceptible to detecting any abnormal data distribution. If the value of Sig. > 0.05, then the data is normally distributed, whereas if the value of Sig. < 0.05, the data is not normally distributed. From the study results above, the research data were not normally distributed in the group that was not drawn blood, where the value of p = 0.027 and p = 0.516 in the second group stated that the data were normally distributed.

Table 6: Homogeneity Test of Temperature Variables.

	Sig.
Variance Homogeneity	,095

The homogeneity test is used to determine whether not the variance of several populations is the same. If the value of Sig. <0.05, it is said that the variance of two or more data population groups is not the same, while if the value of Sig.> 0.05, it is said that the variance of two or more data population groups is the same. From the above results, it can be concluded that the data is homogeneous. After that, keep in mind that the data is not normally distributed, so Kendall's Tau test is carried out.

Tabel 7: Mann-Whitney Test.

Mann-Whitney U	
Asymp. Sig. (2-tailed)	,001

The Mann-Whitney test was used to determine whether the mean data in the first and second groups were different. If the Asymp value. Sig. (2-tailed) > 0.05, then the mean of the first group did not have a significant difference. If Asymp. Sig. (2-tailed) <0.05, it can be said that the mean of the two groups is entirely different. The Mann Whitney test is performed if the data is not normally distributed or homogeneous. Therefore it is called a nonparametric test. From the results above, it can be concluded that group A is completely different from group B.

Table 8: Kendall's Tau Correlation Test Blood Loss with Decrease in Temperature.

Kendall's tau_b	**Correlation Coefficient**	**-,770****
	Sig. (2-tailed)	,001

**. Correlation is significant at the 0,01 level (2-tailed)

Because the data source comes from the same subject or in pairs and the data are not normally distributed, Kendall's Tau test is the statistical test chosen. Based on S.P.S.S. processing, it is stated that the significance level used is 0.01, meaning that the confidence level is at 99%. The value of Sig (2-tailed) is 0.001. This figure is smaller than the significance level of 0.01. It means that Ho is rejected, and Ha is accepted, where there is a relationship between blood loss and the rate of decrease in body temperature in rats. The relationship between blood loss and the speed at which body temperature decreases to ambient temperature is expressed by the correlation coefficient -.770, representing a robust negative relationship between the two variables. It means that the more blood is drawn, the shorter it takes for the mice's body to reach ambient temperature.

Theoretically, the blood component in humans consists of 42-45% hematocrit/packed cell volume, and ± 55-58% is blood plasma. Blood plasma is made up of 90% water. Loss of blood also means loss of fluids in the body, whereas water has a large capacity to retain heat. Plasma absorbs and disperses most of the heat generated by metabolism in the tissues. It was proven by measuring rats' body temperature in group B (whose blood was taken), which fell beyond group A at the time of termination, namely 36.512 0C in group B and 36.275 in group A 0C. It was due to ±30% blood sampling causing the rat's body to lose water and interfere with the ability to retain heat so that the group whose blood was drawn would experience a faster temperature drop even before termination. We can also observe this in cases of hypovolemic shock due to bleeding where there will be signs of cold and chills.[19]

Stiffness Score Results Based on Area - The rigour Mortis variable in this study was assessed using a score determined with the Department of Forensic Science, Faculty of Medicine, Christian University of Indonesia. The areas that are assessed for corpse stiffness are divided into four areas. Area 1 (A1) assesses cadaver rigidity at the finger and foot joints. Area 2 (A2) assesses cadaver stiffness in the articulatio cubiti Sinistra et dextra, articulatio humeri Sinistra et dextra (upper

extremity). Area 3 (A3) assesses stiffness in the articulatio talocrural Sinistra et dextra and articulatio genus sinsistra et dextra (lower extremity). The last area, area 4 (A4), assesses joints (articulatio temporomandibularis). The assessment was carried out in each area immediately after termination (0 minutes) and continued every 10 minutes to evaluate the formation of stiffness in the above areas.

The rating scale used is in the form of staging. Stage 1 means the joint is flexible and can move freely without hindrance. Stage 2 means the joint is slightly stiff but still able to move with minimal effort. Stage 3 joints are stiff and require considerable effort to move the joint. Stage 4 joints are entirely stiff and very difficult to move. The assessment is carried out thoroughly in 4 areas based on time. At 0 minutes (shortly after termination), the total score obtained by all rats was 4. It means that in area 1 (A1), area 2 (A2), area 3 (A3), and area 4 (A4), respectively, get a score of 1, stating that there has been no rigidity formed at all for each area that is evaluated. A score of 4 is the minimum score that the researchers set on the corpse stiffness, while a score of 16 is the maximum possible score, which means that each area (A1, A2, A3, A4) gets a score of 4. It means that the rats have experienced maximum stiffness in all four evaluated areas.

It is necessary to calculate the average for both groups to see the stiff formation of corpses in both groups. Data in group A were averaged from 8 samples and then compiled in minutes, as was group B. In the first 30 minutes after termination, there was no significant difference between the two groups, and there was no significant acceleration. However, when more than 30 minutes began to appear, accelerated formation of cadavers in both groups, namely 0.5 in group A and 1.25 in group B and it will continue to increase over time, especially up to 160 minutes in group A and 130 minutes in group B. Group A experienced an acceleration of cadavers not as big as group B where the acceleration of scores from 30-160 minutes was spread more evenly but not as high as the acceleration in group B which peaked at 70-100 minutes which was marked by an acceleration of 1.75 scoring from 70-minutes. 80, 1.25 from 80-90, and 1.75 from 90-100 minutes. If depicted in graphical form, it will look like the image below.

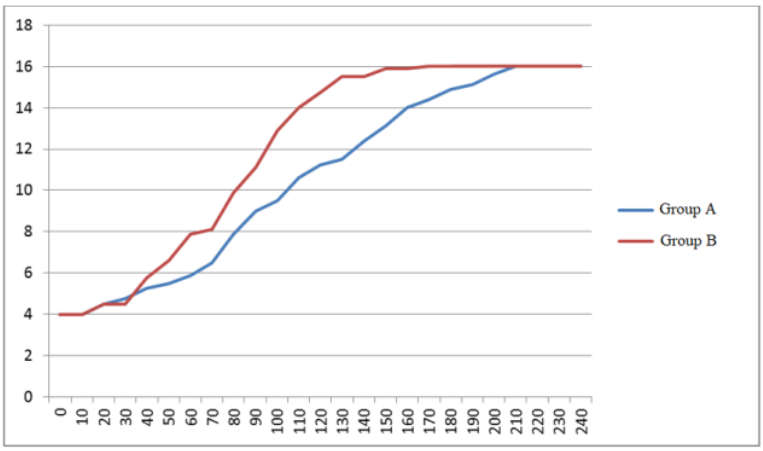

Figure 2: The Speed of Rigid Corpse Forming Against Time in Groups A and B

Table 9: Test of Normal Distribution on Variable Rigid Corpses.

	Shapiro Wilk		
	Statistic	**Df**	**Sig.**
No blood drawn	,866	8	,139
Blood drawn	,876	8	,173

The Shapiro Wilk test in this study was carried out because considering the number of samples as many as 16. The purpose of this test is to detect any abnormality in the data distribution. If the value of Sig. > 0.05, then the data is normally distributed, whereas if the value of Sig. < 0.05, the data is not normally distributed. From the research results above, the research data on rigid corpses were normally distributed in both groups where the p-value is 0.139 (with blood taken) and p = 0.173 (with blood not taken).

Table 10: Test of Homogeneity on Variable Rigid Corpse.

Variance Homogeneity	**Sig.**
	,711

The homogeneity test is used to find out whether the variance of several populations is the same or not. If the value of Sig. <0.05, it is said that the variance of two or more data population groups is not the same, while if the value of Sig.> 0.05, it is said that the variance of two or more data population groups is the same. From the results above, it can be concluded that the data is homogeneous, where the value of Sig. is 0.711. After ensuring that the data is normally distributed and homogeneous, a correlation test is performed using the Independent T-Test.

Table 11: Independent T-Test for Rigid Corpse Variables.

Independent T Test	Sig. (2-tailed)	Mean Difference	Std. Error Difference
	,000	61,250	9,717

The Independent T-Test aims to determine whether there is a difference in the mean (mean) between the two populations by looking at the two samples average on the condition that there is no relationship between the two samples to be tested. In this case, group A was not drawn blood, and group B was taken blood. If the value of Sig. (2-tailed) < 0.05, then there is a significant difference between the mean of the group a and group b, whereas if Sig. (2-tailed) > 0.05, then there is no significant difference from the average of the two data. Based on the data above, it was found that the 2-tailed significance value of 0.000 stated that there was a significant average difference between the two groups.

Table 12: Pearson Correlation Test.

Pearson Correlation	-,891**
Sig. (2-tailed)	,000

The Pearson Correlation test was conducted to find the relationship between the two groups if the data used were parametric (normally distributed data). The number on the Pearson correlation is stated from -1 to 1, where -1 means that it has a perfect negative correlation, and one means that it has a perfect positive correlation. If the two groups have a significant correlation, it will be marked with two stars in the Pearson correlation results. Figures on Sig. (2-tailed) confirmed the significance of the relationship between the two groups if the value was below 0.05.

From the data processing results, the Pearson correlation value is -.891, which means a significant relationship between blood loss and the speed of formation of cadavers. Their relationship is a negative relationship, which means that the more blood is taken, the faster the formation of stiff corpses. The significance value of Sig. (2-tailed) is 0.000. It means the hypothesis is proven because of the p-value <0.05.

ATP levels influence frozen corpses formed theoretically in the tissues and blood vessels. Fresh ATP is needed to break the bonds of myosin and actin after a cycle of vigorous stroke. If there is ATP, it confirms that the cross-bridge is back to normal. If there is no ATP, the myosin will continue to attach to actin to inhibit muscle movement. 4 ATP is needed as a constant energy source for the cycle of contraction and relaxation of skeletal muscles.

Three things might cause cadavers to form more quickly in the blood-losing group than the non-blood-losing group. First, in the case of blood loss, the first pathway (the glycolytic pathway) is the pathway that is disrupted. When blood collection and the plasma fluid is taken, the complete components of blood ranging from electrolytes, hormones, blood cells to blood glucose are also lost from the intravascular circulation. Therefore the supply of glucose to the muscles will also be reduced. If the supply of glucose is reduced, then the ATP produced will also decrease.[20]

Second, blood loss causes a decrease in the total blood volume in the blood vessels. If the blood loss is still within 15-25%, it will not interfere with tissue perfusion, but if the blood loss is >30%, the blood pressure will fall. The vasomotor system cannot compensate for it anymore, releasing catecholamines (epinephrine and norepinephrine) and baroreceptor reflexes (blood pressure receptors) to decrease tissue perfusion. When tissue perfusion decreases, oxygen and glucose will not be delivered to the muscles, so that the muscles will experience a lack of oxygen and glucose as the primary materials for aerobic ATP formation. It causes two things, namely direct tissue damage and a lack of ATP in the local tissue.[21]

Third, aerobic glucose oxidation through the Krebs cycle will produce 32 moles of ATP, while anaerobic glucose metabolism will produce only 2 ATP with lactate products. When tissue perfusion is decreased and uncompensated, anaerobic metabolism happens because oxygen does not reach the tissues properly. As discussed above, that ATP is needed to make muscles not stiff so that the loss of the amount of ATP produced (decrease) due to anaerobic metabolism can cause stiffness of the corpse muscle damage to occur more easily.[22]

CONCLUSION

From the research that has been done, it can be concluded that blood loss can be one of the factors that affect the speed at which the rat's body temperature drops to ambient temperature and the speed at which the body

stiffens. It is evidenced using the nonparametric Kendall's Tau correlation test with a result of -.770 for the variable body temperature and the Pearson correlation test with a result of -.891 for the variable stiffness of the corpse. The Kendall's Tau Correlation Test shows a robust negative relationship between blood loss and decreased body temperature. Pearson's Correlation Test shows a robust negative relationship between blood loss and the rate at which the body stiffens. The group whose blood was taken was 19.8% faster to reach ambient temperature, compared to the group that did not have blood drawn and the group whose blood was drawn reached cadaveric stiffness 32% faster than the group that did not take blood. Budiyanto A, Widiatmaka W, Sudiono S, Winardi T, Abdul Mun'im, Sidhi, et al. Forensic Medicine. Jakarta: Forensic Medicine Department, Faculty of Medicine, University of Indonesia; 1997.

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