Differences of Caspase-3 Expression in Liver and Spleen of *Rattus norvegicus* Infected with *Streptococcus pyogenes* and *Acinetobacter baumannii*

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Abstract: *Streptococcus pyogenes* as exotoxin-producing bacteria are the most common cause of pharyngitis infection. *Acinetobacter baumannii* as endotoxin-producing bacteria are found in nosocomial infections. The infections that has already spread to all organs with decreased immune system undergo apoptosis in liver and spleen. Caspase-3 is a death protease most commonly activated by apoptotic mediator. The higher the caspase-3 expression, the higher the severity of the disease, which can cause the organ to experience dysfunction or failure. This study was aimed to observe the expression of caspase-3 in the liver and spleen of *Rattus norvegicus*, infected by *Streptococcus pyogenes* and *Acinetobacter baumannii*. This is a true experimental with a post-test only control-group design. The healthy rats were randomly selected and injected with 1ml (PZ, suspension of bacteri *A. baumannii* and *S. pyogenes*) in the peritoneum in quadrant 3 for each group of experimental animals and observed for 24 hours. After 24 hours, surgery took place for the removal of the liver and spleen. Organ tissues where fixed into a formalin buffer and tissue was prepared for IHC caspase-3.The results showed that the mortality rate of *Rattus norvegicus* infected by *A. baumannii* was higher than those infected by *S. pyogenes*. Caspase-3 expression in the liver of *A. baumannii* group was 48, *S. pyogenes* was 22.5, and the control group was 9.5. The mean value of the caspase-3 index in the spleen of the *A. baumannii* group was 28.5, *S. pyogenes* was 17, and the control group was 4.

1 INTRODUCTION

The infection is caused by bacteria that produces toxins and endotoxins. In general, infectious diseases are caused by bacteria, fungi, viruses, and parasites. *Streptococcus pyogenes* as toxin-producing bacteria is the most common cause of infectious pharyngitis. *S. pyogenes* bacteria is included in Group A streptococci of serology (*Group A Streptococcus*, GAS) (Fidler et al., 2015). *S. pyogenes* bacteria can infect the host body's defenses when dropped or when the organism can penetrate past the host's defense (Ramachandran, 2017).

The bacteria *Acinetobacter baumannii* is an endotoxin- producing bacteria found in nosocomial infections and harmful gram-negative bacteria (Bigot and Suzana, 2017). In recent years the bacteria *A. baumannii* has increased over the nosocomial infections in humans. The bacteria *A. baumannii* is found as a nosocomial infection-causing bacteria in the urinary tract, an infection of a wound, and in vascular surgery, particularly in patients with low immune systems located in the ICU. Research in Indonesia has determined the bacteria *Acinetobacter* as one gram-negative that most often infected are 25.8% (Norhamdani, 2004).

Apoptosis has an important role in bacterial infections of *S. pyogens* and *A. baumannii*. Apoptosis affects the immune cells,which are very important in the course of an infection. Apoptosis can be determined not only on a certain type of bacteria, but in bacterial infections in a variety of species. The regulation of apoptosis is an important aspect of the host cell response against stress, infection, and must be controlled (Ulett and Elisabeth, 2006).

A variety of stimuli can trigger apoptosis from within or outside the cell, for example, infection with microorganisms, cell cycle, or signaling the

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death of cell-surface receptors, development, DNA damage and occurrence of inflammation. Inflammatory Cytokines (TNF) can constantly induce activation of caspase-8, caspase-3, and the fragmentation of DNA through membrane receptors. This is apoptosis pathway activation directly from the extrinsic pathway, called caspase. Metabolic disorders of intracellular reactive oxygen species or overload can cause damage to the mitochondria, which produce cytochrome c release and the activation of caspase-9. Caspase-9 reactivates further trigger activation of caspase-3 and apoptosis. High apoptosis hemeostasis systems cannot keep the organ systems, causing *multiple organ dysfunction syndrome* (MODS). Organ system failure is very harmful to humans and can even cause death (Caspian, 2016). Expressions of Caspase-3 were examined in the liver and spleen of the rat *(Rattus norvegicus)* where the apoptosis occurred.

2 METHODS

The research was done in a purely experimental laboratory using a true experimental research design. Post-tests only Control Group Design (data retrieval is performed after the given treatment) and compared with the control group.

The research was performed in, the Animal Models Department of Biochemistry, Microbiology Clinic Laboratory, Dr. Sutomo Hospital as a place of clinical isolation for the culture of *S. pyogenes* and *A. baumannii*. The Anatomic Pathology Laboratory Faculty of Medicine Airlangga University processed checks and the observation of the expression of caspase-3 in organ livers and lien on the *Rattus Norvegicus*. The research was carried out over three months. (October 2017–December 2017).

The object of the research is the white *R. norvegicus* male wistar strain, aged 3 months with a weight of 200–250 grams. Four *R. norvegicus* were used in this study

Pure liquid bacteria was isolated from *S. pyogenes* at

A. baumannii laboratory clinical microbiology of the Dr. Sutomo Hospital. Expressions of caspase-3 in rats' livers and spleen who had infection with bacteria *S.pyogenes* and *A.baumannii* are detected by IHC method (*Immunohistochemistry*) using the caspase-3 p12 subunits of antibodies.

1. Treatment for animal models:

R. norvegicus were given injections in the peritoniumnya with bacteria *S. pyogenes* and *A. baumanni*. Each anesthetic had 2.52mg and 0.25mg of ketamine dexamethasone, followed by an injection of bacteria *S. pyogenes* and *A. baumannii* of which the doses were 1x109 per CFU/Rat. After 24 hours, if the rat was not dead, it was dissected and the liver and spleen were taken.

2. Tissue Preparation:

The liver and spleen were removed from the treated rats and fixed with 10% formalin. Further cutting of the organs at 1x1x2cm continued using paraffin blocks that are used in the pathology laboratory.

3. Observation of Caspase-3 expression

Observation on the expression of caspase-3 in rats liver and spleen was conducted with primary antibody caspase-3 p12 subunits. Expressions of caspase-3 were observed using the IHC method by using caspase-3 p12 subunit primary antibodies. The cells expressing caspase-3 were counted within five fields of view x1000 magnification and the numbers were compared between two bacteria-treated rats. Cell expression of caspase-3 were calculated on an examination under microscope light with magnification 100x objective with five field of view and magnification of 10x to 40x and photographed for comparison of each liver and spleen.

3 RESULTS

3.1 The results of the expression of Caspase-3 in the liver

The number of cells expressing results obtained demonstrated that the average number of expressions of caspase-3 in the group injected with *A. baumannii* is higher than the group injected with *S. pyogenes* and the control group.

Table 1: The Distribution of liver cells expressing caspase-3 Note SD is Standard Deviation.

Percentage of cell	C(n=4)	*S. pyogenes*	*A.baumannii*
X± SD	9.5 ± 2.38	20.75 ± 7.136	48 ± 21.74
Median	9.5	22.5	48
Min - Max	7–12	11–27	26–70

The number of cells expressing caspase-3 in the livers of rats infected with *A. baumannii* was higher than those infected with *S. pyogenes*

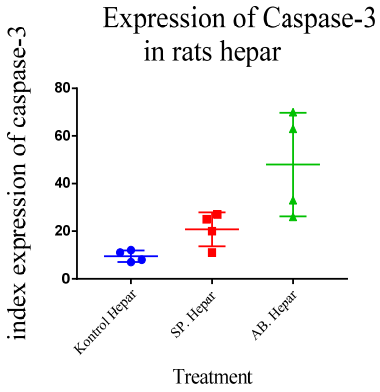

Figure 1: *Box plot* of the number of cells express caspase-3 in the liver infected with *A. baumannii* and *S. pyogenes.*

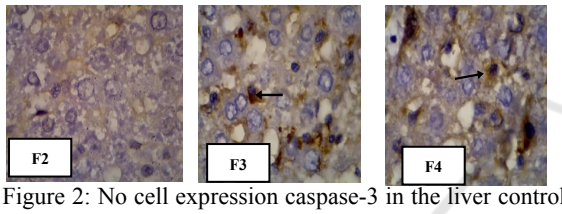

Figure 2: No cell expression caspase-3 in the liver control group, Figure 3: Expression of caspase-3 in rats' liver Hepatocyte Cell Group *A.baumannii* x1000 zoom. Figure 4: Expression of caspase-3 in rats liver Hepatocyte Cell Group *S.pyogenes* x1000 zoom.

3.2 The results of the expression of caspase-3 in the spleen

From the results obtained, the average number of expressions of caspase-3 in the group infected with *A. baumannii* is higher than the group infected with *S. pyogenes* and the control group.

Table 2 : Note SD is Standard Deviation

Percentage of cell	K(n=4)	*S. pyogenes*	*A.baumannii*
X± SD	4.25 ± 0.5	17.75 ± 7.136	27.25 ± 7.58
Median	4	17	28.5
Min - Max	4–5	15–22	17–35

Based on the data in the table above, it can be noted that the expression of caspase-3 in rats in the group infected with *A.baumannii* is the highest, i.e. SD 27.25 ± 7.58, while the expression of caspase-3 in the group of rats' spleens infected with *S. pyogenes* is 17.75 ± 3,403.

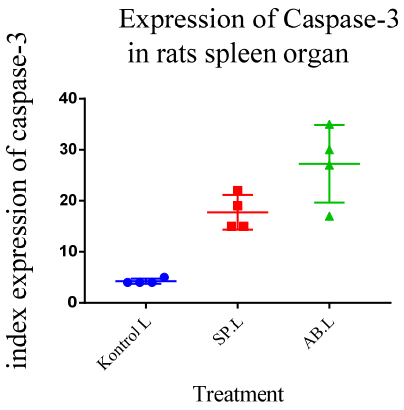

Figure 5: *Box-plot* the number of expression of Caspase-3 in rats' spleens.

The highest median value, i.e. the *A.baumannii* group was 28.5, the second was the *S.pyogenes* group at 17, and the control group was 4. Description of caspase-3 expression was observed under the light microscope with a magnification of x100 and x1000 and the x% fields of view are as follows:

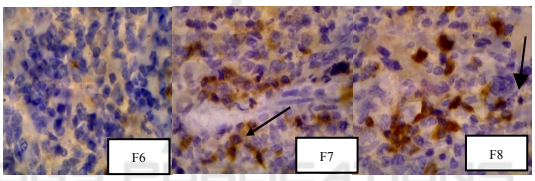

Figure 6: Expression of caspase-3 in the spleen control group, Figure 7: Expression of caspase-3 on rat lymphocyte cells spleen Group *A.baumannii* x1000 zoom, and Figure 8: Expression of caspase-3 in the spleen of rats infected with *S.pyogenes* magnification x1000.

3.3 Expression of Caspase-3 in the liver and spleens of rats infected with *A.baumannii* and *S.pyogenes*

Based on the averages of cells, those that expressed caspase-3 in the livers and spleens of rats infected with *A.baumanii* and *S.pyogenes* showed that the group infected with *A.baumannii* was higher (48) than that of rats infected with *S.pyogenes* (22.5) and the control group (9.5). The average number of cells expressing caspase-3 in the spleens of rats infected with *A.baumanii* was higher (28.5), compared with that of rat infected with *S.pyogenes* (17) and the control group, which was only 4 as shown in Figure 9.

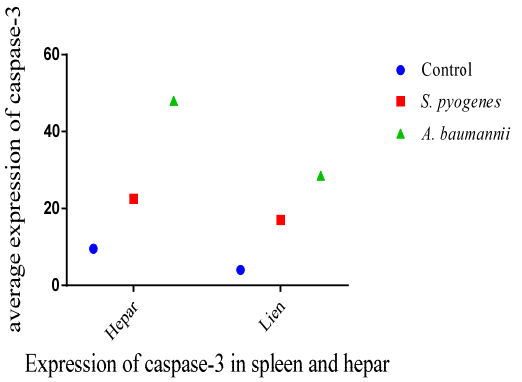

Figure 9: *Box-plot* Average number expression of Caspase-3 in rats' spleens and livers.

4 DISCUSSION

Apoptosis as a type of cell death is highly organized and genetically controlled. It is characterized by a number of different morphological changes, such as cell condensation and marginalization, the shrinking of cells, and plasma membrane blebbing. This is accompanied by biochemical features, such as DNA fragmentation, changes in membrane (e.g. exposure to fosfatidilerin on the outside of the plasma membrane), and specific cell protein degradation, as a result of the activation of a massive amount of intracellular protease and endonuclease (Guicciardi and Scratch, 2005). The cleavage of caspase was mediated by caspase-3 and caspase-7, while the last two caspase activations are generally a function of initiator caspase. Initiator caspase's apoptosis signal pathway determines, after activation of caspase executor, that caspase-3 and caspase-7 can process at least 100 proteins. The cleavage of a caspase-3 substrate can lead to profit or loss of the function of proteins, ultimately causing cellular changes associated with apoptosis (Rogers et al., 2015).

1. The death of rats due to *A.baumannii* and *S. pyogenes*.

A. baumannii produces endotoxin as OmpA capsule (outer membrane protein A) induces apoptosis in human laryngeal epithelial cells. OmpA is purified and localized and the mitochondria and apoptosis are induced through the release of the proapoptotic cytochrome c molecules and the driving factors of apoptosis, suggesting that this is the path where the *A.baumannii* induces damage to the cells of the respiratory tract during infection (Peng et al., 2016). Gram-negative can cause the onset of sepsis and sepsis shock (Girardt et al., 2016).

Streptolisin O is the basic nature of the toxin beta-hemolysis with toxins from Gram-positive bacteria *S.pyogenes*. Streptolisin O is potentially cell poison affecting many cell types including neutrophil, platelets, and organella subsel. This toxin is capable of producing a large cellular immune response that can lead to fatal toxic shock (Regnier et al., 2016). *S. pyogenes* is a species of gram-positive bacteria that contain peptidoglycan cell walls and lipoteichoic acid (LTA) discovered by the immune system as the PAMPs, is in line for the bacteria *S.pyogenes* as a TLR-Peptidoglycan and LTA interaction with TLR-2 produces a signalling pathway via the adapter MyD88 and TRIF activation that can trigger the formation of NF-kB and cytokines expression of MAPKs so it can be (Pyrshev et al., 2017).

2. Expression of caspase-3 in livers of rats infected with *A. baumannii* and *S. pyogenes*

The increased of caspase-3 expression in the livers of rats infected with *A.baumanni* and *S. pyogenes* indicate the death of cells due to apoptosis but no damage to the organ. Hepatocyte death is common in the aftermath of inflammatory disease in the liver. An increase of apoptosis in the liver can be caused by a high inflammatory process, which triggers the apoptotic hepatocyte cell to cause most of the damage to the hepatocytes, mediated by the *reactive oxygen species* (ROS) that initiates inflammatory reactions and the occurrence of apoptosis in hepatocytes (Rinaldi, 2014). In hepatocyte damage, the liver will induce the onset of signals to stimulate the release of monocyte chemoattractant Chemokin protein-1 (MCP-1), which will enhance kupffer cells/macrophages, as well as the release of pro-inflammatory cytokines, such as interleukin (IL)-1 β, IL-1 and Tumor Necrosis Factor (TNF)-α, which can enable the *Nuclear Factor κB* activation (NF-κB activation) and *mitogen-activated protein kinase* (MAPK) (Guicciardi et al., 2013).

3. Caspase-3 expressions in the spleens of rats infected with *A. baumannii* and *S. pyogenes*.

Potential complications from splenic swelling are bacterial infections; this is because the spleen swells, which reduces the number of healthy red blood cells, platelets, and white blood cells in the bloodstream, exposing it to the infection (Bronte and Mikael, 2013).

High bacterial infections activate the immune system to attack the bacteria present in the blood (Tan et al., 2017). The spleen will induce proinflamasi cytokines, such as interleukin (IL)-1 β,

IL6, the tumor necrosis factor (TNF)-α, γ interferon (IFN) and the synthesis of nitric oxide (NO). Patients infected with bacteria undergo overproduction of proinflammatory cytokines, such as, TNF-α, IFN-γ, IL-2, ROS and NO. Excessive TNF-α will increase the production of NO acting as free radicals. In addition, TNF-α can also increase the Intercellular Adhesion Molecule-1 (ICAM-1) and cause obstruction within the brain (Bronte and Mikael, 2017).

5 CONCLUSION

1. Based on the average of cells expressing caspase-3 of rats infected with *S.pyogenes* in hepar was higher (20.75 ± 7.136) than that in the spleen (17.75 ± 3.403).
2. Based on the average of cells expressing caspase-3 of rats infected with *A.baumannii* in hepar was higher (48± 21.74) than that in the spleen (27.25 ±7.58).
3. The highest number of cells expressing caspase-3 was observed in the group of rats infected with *A. baumannii*, compared with that of the group of rats infected with *S. pyogenes*. The liver demonstrated a higher expression of caspase-3 than the spleen.

REFERENCES

Bigot, S., and Suzana P., 2017, The Influence of two-partner secretion system on the virulence of *Acinetobacter baumannii* Virulence, vol.2, pp 1-2.

Bronte, V., and Mikael J.P., 2013., The Spleen in Local and Systemic Regulation of Immunity, Immunity Cell Press, vol.39.

Girardit, T., Thomas R., Fabienne V., and Guillaume M., 2016., Apoptosis-induced lymphopenia in sepsis and other severe injuries, Springer Science New York, vol. 22., pp 295-305.

Guicciardi, M.E., and Gores G.J., 2005., Apoptosis : A Mechanism of acute and chronic liver injury, Rececn Advances In Basic Science, vol. 54., pp 1024-1033.

Norhamdani, 2004, Acinetobacter Baumannii Hemaglutinasi activity of bacteria derived from clinical and Environmental Specimens, Journal of medicine Brawijaya, vol.20, pp 105-109.

Peng, C.H, Xian W.y, Jing Han, Lin Liu, Cheng Zhang, Jing X., Jiu R.N., and Xiang Y.Z., 2016, Preliminary study abaout the mechanism of IL-33 acting on neutrophil apoptosis in rates with *Acinetobacter baumannii* Pneumonia, Intenational Journal Clin Exp Med., vol.9, no.6, pp 8934-8944.

Ramachandran. G, 2017, gram-positive & gram negative bacterial toxins in sepsis virulence vol.5, no.1 pp.213-218

Regnier, E., Philippe A.G., and Guillaume O., 2016, Superoxide anions produced by *Streptococcus pyogenes* group A-stimulated keratinocytes are responsible for cellular necrosis and bacterial growth inhibition, Innate Immunity, Sagepub Journal Permissions, vol. 22, pp 113-123.

Rinaldi, W.A., 2014. The potential granting of Propolis Against Lymphoblasts in Diameter and number of White Male Mice Spleen Pulpa (Mud musculus). Thesis, Faculty Of Veterinary Medicine University Of Airlangga In Surabaya.

Tan, Y., Kai Z., Xiang T., Timothy K., Luxia W., Zhenghui G., Murat A., and Chao Z., 2017, Bacterimes and non-bacteremic pneumonia caused by *Acinetobacter baumannii* in ICUs of South China: A clinical and Microbiology Study, Nature Scientific Reports.

Ulett, G. C., and Elisabeth E.A., 2006, Regulstion of Apoptosis by Gram-Positive Bacteria: Mechanistic Diversity and Consequence or Immunity, Curr Immunol Rev., vol.2, no.2, pp 119-14