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Validation of Monitoring Septic Thymus Involution in Mice Using Ultrasound

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***Abstract*— Sepsis is a dangerous condition commonly seen in the intensive care unit (ICU) of hospitals. It causes the thymus, a crucial immune organ, to shrink. This process is known as thymus involution. Although thymus involution is a natural process that occurs as we age, it is accelerated during sepsis. This process is associated with poor outcomes in septic patients, yet it had never been studied using ultrasonography in a septic mouse model. Researchers from the University of Kentucky have validated a non-invasive ultrasound imaging approach to monitor septic thymus involution in a cecum ligation and puncture (CLP) sepsis mouse model. In this study, scientists randomly divided 35 C57BL/6J mice into three groups: baseline, post-CLP at 3 days, and post-CLP at 10 days. In each group, they first obtained estimated thymus area and volumes using 2D and 3D ultrasound imaging. The mice were then euthanized to measure thymus weights ex vivo. The ex vivo weights were correlated with the in-vivo 2D and 3D estimated areas and volumes and proved the reliability of this approach for monitoring thymus changes during sepsis. The study, led by Dr. Xiang-An Li, a professor of physiology at the University of Kentucky, was published in Ultrasound in Medicine & Biology. It has paved the way for further studies investigating the mechanism of thymus involution during sepsis, which is a crucial but poorly understood phenomenon that exacerbates immunosuppression in septic patients.**

***Keywords*—Molecular, Genetic, and Biochemical Nutrition; Pharmacology**

WHEN the coronavirus turned the whole world upside down, many people died from a condition called sepsis caused by COVID-19. This condition is the body's dysregulated immune response toward a viral or bacterial infection (Singer et al, 2016). This dysregulation damages the host's organs, such as the lungs, and ultimately leads to death. Both viral and bacterial infections can lead to sepsis. Although triggered differently, they share similar characteristics, such as an initial overreactive host response followed by immunosuppression due to the depletion of peripheral T cells (Hotchkiss et al., 2001). During sepsis, the immunosuppression is also exacerbated by the shrinking of the thymus, a very crucial organ for T cell maturation (Gruver et al., 2008). This is known as thymus involution. The mechanism of this phenomenon is not fully understood. To investigate the mechanism of why this important immune organ is getting smaller during sepsis, a non-invasive approach to monitor thymus size must be established first. Now scientists at the University of Kentucky have validated a way to monitor this process in a cecum ligation and puncture (CLP) sepsis mouse model. They took advantage of a technology called ultrasound. While this technology has been established for a long time, its value in studying thymus involution in a sepsis mouse model has not been fully exploited.

In 2021, Dr. Xiang-An Li, a professor of physiology, led

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his team in validating an application of ultrasound in monitoring thymus involution during sepsis with a combination of 2-D and 3-D imaging (Ito et al, 2021). "This condition (sepsis) is so deadly in humans. One in every three people who died in hospitals had suffered from it. And because it is so deadly in humans, the CLP mouse model has become a useful tool to understand sepsis and here specifically thymus involution during sepsis," says Dr. Misa Ito, MD, Ph.D., a former Ph.D. student of Dr. Li's lab and the first author of the report published in Ultrasound in Medicine & Biology.

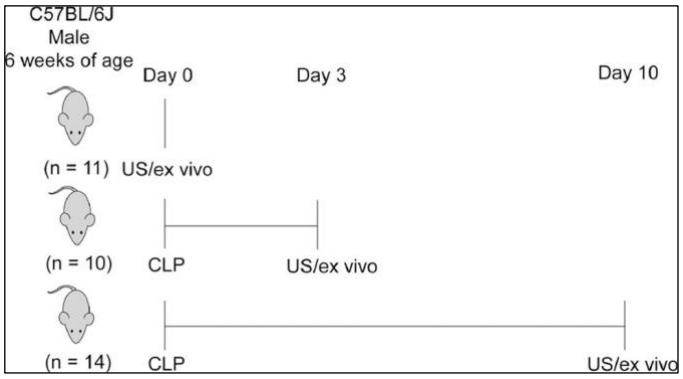

Figure 1. Study Design. 35 male C57BL/6J mice were divided into three groups. 11 mice were used to measure baseline condition without CLP induced sepsis using ultrasonography and then euthanized to weight the thymus. The other two groups had CLP induced sepsis at day 0 and monitored at day 3 and day 10 using the same approach conducted for the baseline group. (Figure reprinted with permission from Elsevier; Ito et al., 2021)

The study divided 35 male C57BL/6J (B6) mice into three groups described in **Figure 1**. The first group had 11 mice and was used to establish baseline thymus area, volumes, and weights. The other two groups were challenged with CLP and monitored survival rates for 3 and 10 days, respectively. The researchers obtained thymus area and volumes via ultrasound imaging (US) on day 3 and day 10 and then euthanized the mice to measure thymus weights (ex vivo).

To validate the reliability of ultrasound estimation, the researchers compared and correlated the estimated thymus area (**Figure 2A**) and volume (**Figure 2B**), with the ex vivo thymus weights at three time points: on day 0 before CLP, and day 3, day 10 after the CLP procedure. Under baseline conditions, the thymus of a 6-week-old B6 male mouse was about the size of two apple seeds with the shape of a butterfly shown in Figure 2C. Later data will show significant reductions in thymus weights upon CLP challenge.

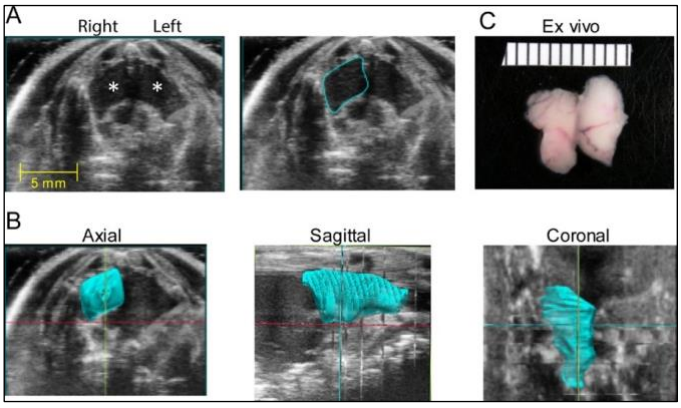

Figure 2. (A) Images of the right and left lobes of the thymus (represented by asterisks) and an outlined right thymus lobe, both in 2D format, are shown for a mouse at baseline (day 0 without CLP). (B) The right thymus is shown in 3D from axial, sagittal, and coronal perspectives. These 3D images were generated automatically using software included in the ultrasound system. Thymus volumes were determined by automatic calculation based on the 2D thymus images that were traced. (C) A thymus image obtained ex vivo displays a similar shape to the 3D thymus images that were reconstructed. (Figure reprinted with permission from Elsevier; Ito et al., 2021)

During sepsis, the thymus weights decreased from day 0 to day 3, followed by a slight increase in thymus weights (**Figure 3A**). This slight increase from day 3 to day 10 may suggest that survivors started to recover, as indicated by the stabilized survival rate from day 6 to day 10 (**Figure 3B**).

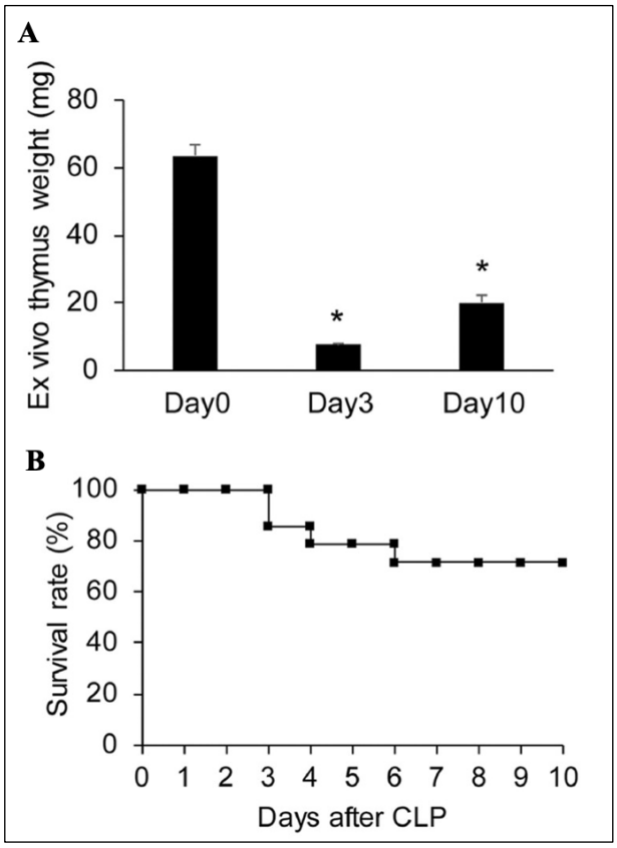

Figure 3. (A) Thymus Weights. 6-week-old (n =11, 10, 14) male at Day 0, 3, and 10. (B) Post-CLP Survival Rate of 6-week-old C57BL/6J male mice (n = 14). (Figure reprinted with permission from Elsevier; Ito et al., 2021)

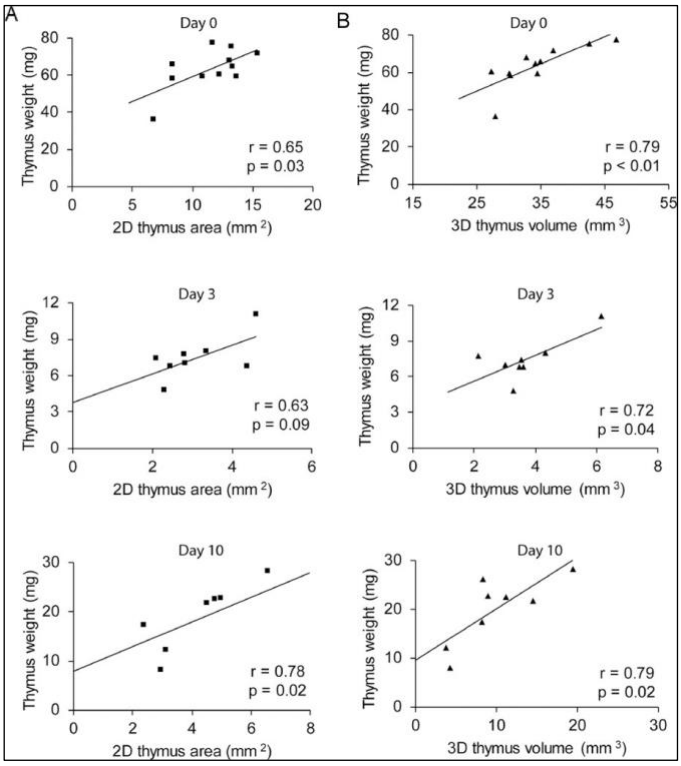

Figure 4. (A) Day 0, 3, 10 thymus 2D area correlations with weights n = 11, 8, 7, respectively. (B) Day 0, 3, 10 thymus 3D volume correlations with weights n = 11, 8, 7, respectively. (Figure reprinted with permission from Elsevier; Ito et al., 2021)

Here, we see significant correlations of 3D imaging estimated volumes with their ex vivo weights at all observed time points in **Figure 4B**. However, for the 2D imaging estimated area, day 3 did not show a significant correlation with their ex vivo weights in **Figure 4A**. This data provided evidence that 3D ultrasound imaging can provide a reliable estimation to keep track of thymus change during sepsis, and 2D ultrasound imaging might be a slightly inferior approach to estimating thymus change.

"Ultrasound in this study is not without limitation," says Dr. Ito, "because these mice are alive, their heartbeats and breathing can introduce challenges to measure via pictures accurately." Nevertheless, this report on using ultrasound to monitor thymus involution in mice with sepsis has laid a good foundation for their subsequent work to dive into a mechanism of why thymus involution happens during sepsis. With this validated approach, scientists will continue understanding more about thymus involution and sepsis. And perhaps, in the near future, the implication of this study and many more that follows will save people from another pandemic around the corner.

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