

## Morphological Manifestation of Haemolymph Nodes in Mice

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### Abstract

### Original Research Article

The haemolymph node (HLN) has been studied for more than a century and was generally focused on areas of anatomy and histology mostly in human cadavers, larger animals and rats, seldom in mice. In this project, 126 lymph nodes were harvested and studied from different regions of 8 Kunming mice. Out of 8 mice, 3 had the phenomenon of HLN. Within the 3 mice, 9 HLN were found located in: the parathymic, superior vena cava, suprarenal and lumbar regions. Among 9 nodes, 4 of them had either an appearance of HLN or macrophage-erythrocyte-lymphocyte (EML) rosettes in sinuses, 5 had only a few EML rosettes in sinuses without the appearance of HLN. Each MEL rosette was composed of a macrophage in the centre surrounded by numerous erythrocytes and often contained a lymphocyte. Some rosettes were seen without lymphocytes, and some with uncompleted erythrocytes' wreath. It was further observed that deformed erythrocytes were distributed in sinuses. These phenomena might reflect the functional status of the HLN at that time.

**Keywords:** Mouse; haemolymph node; macrophage; erythrocyte; lymphocyte; rosette.**Copyright © 2022 The Author(s):** This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

## INTRODUCTION

It was found that some structures resembling lymph nodes with blood in sinuses rather than the lymph near to the kidney of human subjects in 1884 [1]. Thereafter, these structures were confirmed to exist not only in human subjects but also in animals. They were then named as HLN due to their sinuses being filled with erythrocytes, thereby giving the surface a reddish-brown color [2-4]. Subsequent studies generally focused on areas of anatomy and histology, such as the source, distribution and quantity of erythrocytes in sinuses of HLN mostly in human cadavers, larger animals and rats, seldom in mice [5-13]. Earlier in the century, it was proposed that the appearance of HLN might relate to immune activity of the body, but there were no strong evidence to support this perspective [14, 15]. More recently, a report has shown that HLN could be found in the ilinolumbar region of a tail infected rat [16]. Another article has reported that the HLN model in rats could be established by allografting renal tissue [17]. This will likely open a new era of research on the relationship between HLN and immunity.

In this study, a group of mice were studied and the distribution and histological details of HLN are demonstrated and described.

## MATERIALS AND METHODS

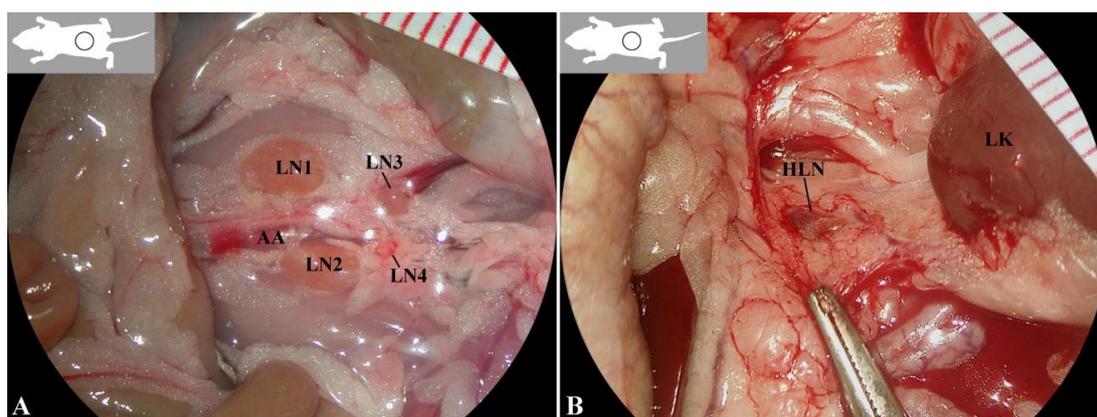
In this study, all animal procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved (#L2021226446) by the Institutional Animal Care and Use Committee of Xuzhou Medical University (Jiangsu, China).

Eight adult male Kunming mice (weighing between 24 to 31g) were obtained from Laboratory Animal Resources, Chinese Academy of Sciences, Shanghai, China. Each mouse was received an intraperitoneal anesthesia with 10% chloral hydrate (0.1 ml/10 g) (CAS# 302-17-0, BBI Life Sciences, Shanghai China) and a total of 126 lymph nodes were harvested from various regions of the mice under a surgical microscope (Leica Microsystems Ltd, Heerbrugg, Switerland). Each lymph node was fixed in 4% polyformaldehyde solution and then was conventionally performed for histological section and hematoxylin-eosin stain (HE). Each slide was scanned by an automatic digital scanner (Pannoramic MIDI; 3Dhistech Ltd., Hungary) and viewed by a Pannoramic Viewer (Pannoramic Viewer 1.1; 3Dhistech Ltd., Hungary) for image analysis.

## RESULTS

A total of 126 lymph nodes were harvested from 8 mice. They had a minimum length of 1 mm and maximum length of 4mm (Fig 1). Some of them looked like a kidney or garden beans, while some presented as a round, oval or irregular forms. In 3 mice, 4 of those nodes had either the appearance of HLN (Fig 1B) or EML rosettes in sinuses; 5 of them had only a few EML rosettes in sinuses (Fig 4, Table 1) without the appearance of HLN. The rest of lymph nodes had no findings of any such changes, whether on their appearance or under histological examination (Fig 1A). The distribution and quantity of EML rosettes in those 9

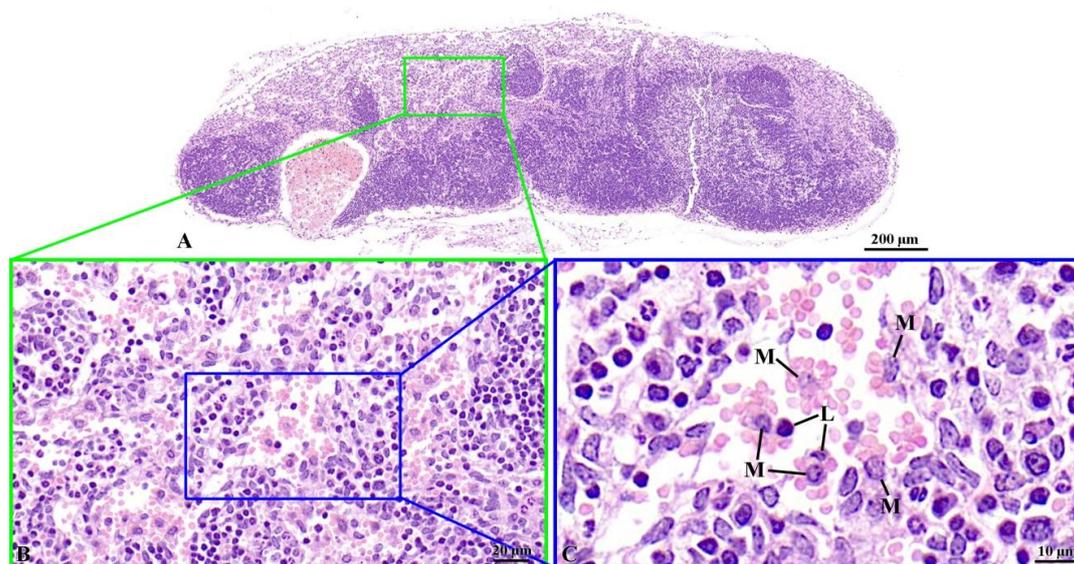
nodes were different. Details have been shown in Table 1 and Figures 2 to 4. EML rosettes were found in the subcapsular, cortical and medullary sinuses of the entire node in 1 case, one side of the node in 2 cases (Fig 2), one third of the node in 1 case (Fig 3) and a small area of the node in 5 cases (Fig 4). Each MEL rosette was composed of a macrophage in the centre surrounded by numerous erythrocytes and often contained a lymphocyte. Some rosettes were seen without lymphocytes, and some with uncompleted erythrocytes' wreath. It was further observed that deformed erythrocytes were distributed in sinuses.



**Figure 1: Lymph nodes and HLN in mice**

A. Ordinary lymph nodes. AA = abdominal aorta; LN1 = left lumbar lymph node; LN2 = right lumbar lymph node; LN3 = left common iliac lymph node; LN4 = right

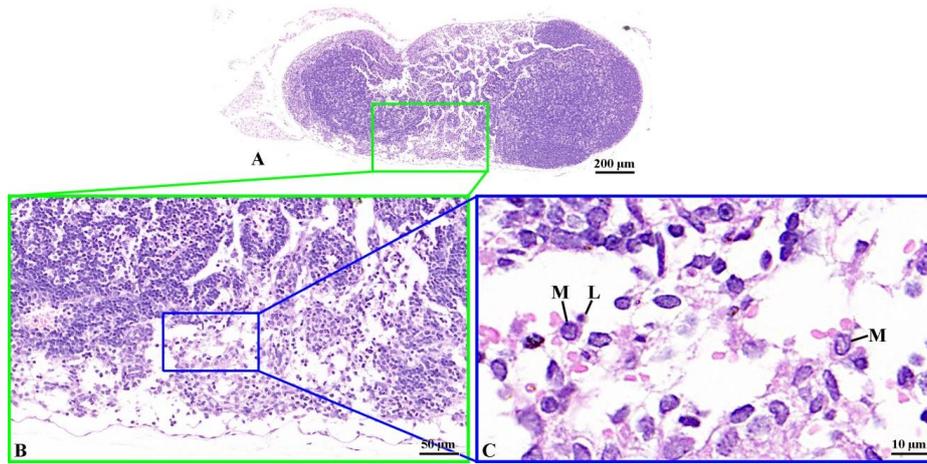
common iliac lymph node. B. HLN = left suprarenal HLN; LK = left kidney.



**Figure 2: Histological results of the left suprarenal HLN of the mouse (I)**

A. EML rosettes and erythrocytes are filled in the upper part of HLN. B. The magnified image in the green boxed area from image A shows numerous EML rosettes and

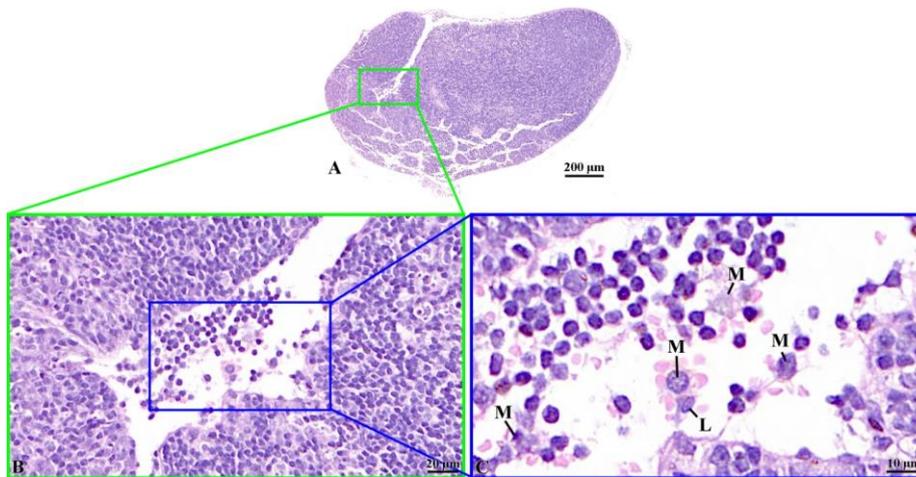
erythrocytes in the sinus. C. The image is magnified in the blue boxed area from image B. M = macrophage; L = lymphocyte.



**Figure 3: Histological results of the right superior vena cava HLN of the mouse (II)**

A. EML rosettes and erythrocytes are filled in the mid-third of HLN. B. The magnified image in the green boxed area from the image A shows numerous EML

rosettes and erythrocytes in the sinus. C. The image is magnified in the blue boxed area from image B. M = macrophage; L = lymphocyte.



**Figure 4: Histological results of the left suprarenal HLN of the mouse (III)**

A. A few EML rosettes and erythrocytes situate in the cortical sinus of HLN. B. The magnified image in the green boxed area from the image A shows a few EML

rosettes and erythrocytes in the sinus. C. The image is magnified in the blue boxed area from image B. M = macrophage; L = lymphocyte.

**Table 1: Distribution and numbers of HLN in mice**

Mouse No	Mouse I	Mouse II	Mouse III
<b>Region of node</b>			
Parathyroid		(1)	
Right side of superior vena cava	(1)	1	
Left suprarenal	1	1	(1)
Right suprarenal	(1)		(1)
Left lumbar		1	

Number without brackets = nodes that have either an appearance of HLN or EML rosettes in the sinuses. Number with brackets = nodes that only have a few EML rosettes in the sinuses without the appearance of HLN.

## DISCUSSION

From earlier literature, it could be seen that HLN was named due to its sinuses (especially the

subcapsular sinus) being filled with erythrocytes, thereby giving the surface a reddish-brown color [1, 2]. Subsequent studies had mainly placed emphasis on the fields of anatomy and histology in human cadavers, larger animals and rats, seldom in mice [3-13]. Research content mostly focused on the source, distribution and quantity of erythrocytes in sinuses of HLN, but neglected the phenomenon and causes of EML rosette

formation. Although it was proposed that the appearance of HLN might relate to immune activity of the body, but there were no strong evidence to support this perspective [14, 15]. Recently, a report showed that the HLN model of rats could be established by allografting renal tissue and so on [17]. Meanwhile, HLN was considered as an immunomorphological organ and MEL rosettes in sinuses were considered as cellular complexes for immune response. In addition, it was found that the nature of HLN was different between individuals; cells in sinuses of HLN were changed quantitatively and /or qualitatively with subsequent days after allografting renal tissue. The phenomenon of cellular changes in sinuses might be the breakthrough for revealing the immune activity of HLN.

From this study, results might be valuable to enrich the knowledge of HLN from previous studies: 1) Out of 8 mice, 3 had the phenomenon of HLN, indicating that HLN did not appeared in every mouse; 2) Within the 3 mice, 9 HLN were found located in the parathymic, suprarenal (right and left), superior vena cava (right) and left lumbar regions, indicating that HLN could appear not only in the suprarenal region, but also in other sites [8-12]; 3) Among 9 nodes, 4 of them had either HLN's appearance (Fig 1B) or EML rosettes in sinuses, 5 had only a few EML rosettes in sinuses (Fig 4, Table 1) without the appearance of HLN. Therefore, we would propose a question: could the latter be called HLN as they do not have HLN's appearance? They could be named lymph nodes with EML rosettes. These phenomena might reflect the functional status of HLN at that time, as described in literature [6, 17].

## CONCLUSION

HLN do not appear in every mouse. HLN can appear not only in the suprarenal region but also in other sites. Some nodes only have a few EML rosettes in the sinuses without the appearance of HLN.

**Declaration of interests:** We declare no competing interests.

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