

## Online Supplement

### Original Research

#### **KF4 Anti-Chymotrypsin-like Elastase 1 Antibody and Purified Alpha-1 Antitrypsin Have Similar but not Additive Efficacy in Preventing Emphysema in Murine Alpha-1 Antitrypsin Deficiency**

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Zingarelli, MD, PhD<sup>3</sup> Brian M. Varisco, MD<sup>1,2,6,7</sup>

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<sup>5</sup>University of Arkansas at Little Rock, Little Rock, Arkansas, United States

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<sup>7</sup>Arkansas Children's Research Institute, Little Rock, Arkansas, United States

# Supplement: KF4 anti-CELA1 Antibody and Purified $\alpha$ 1-Antitrypsin Have Similar but Not Additive Efficacy in Preventing Emphysema in Murine $\alpha$ 1-Antitrypsin Deficiency

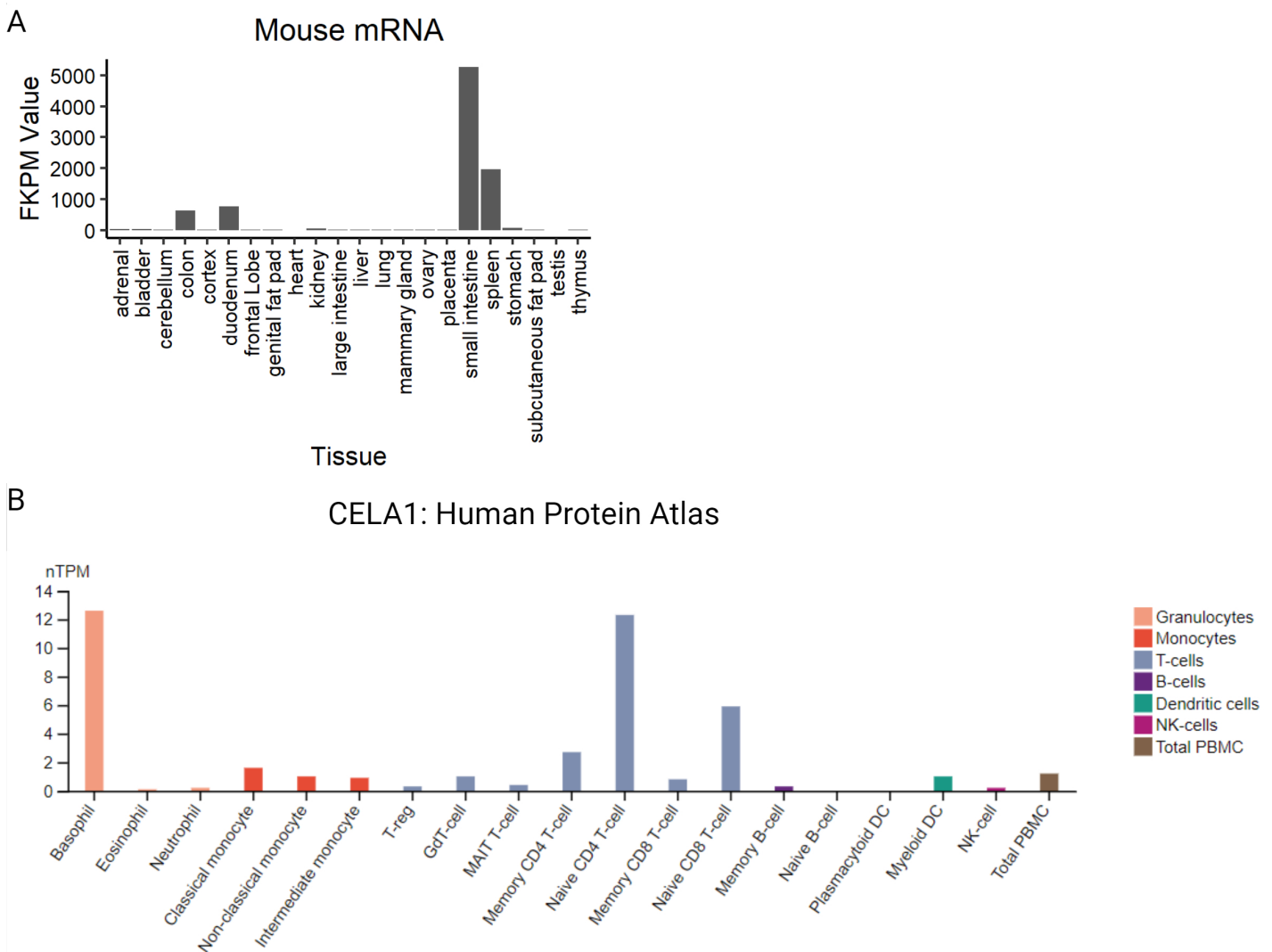
## Supplemental Methods

### Serology

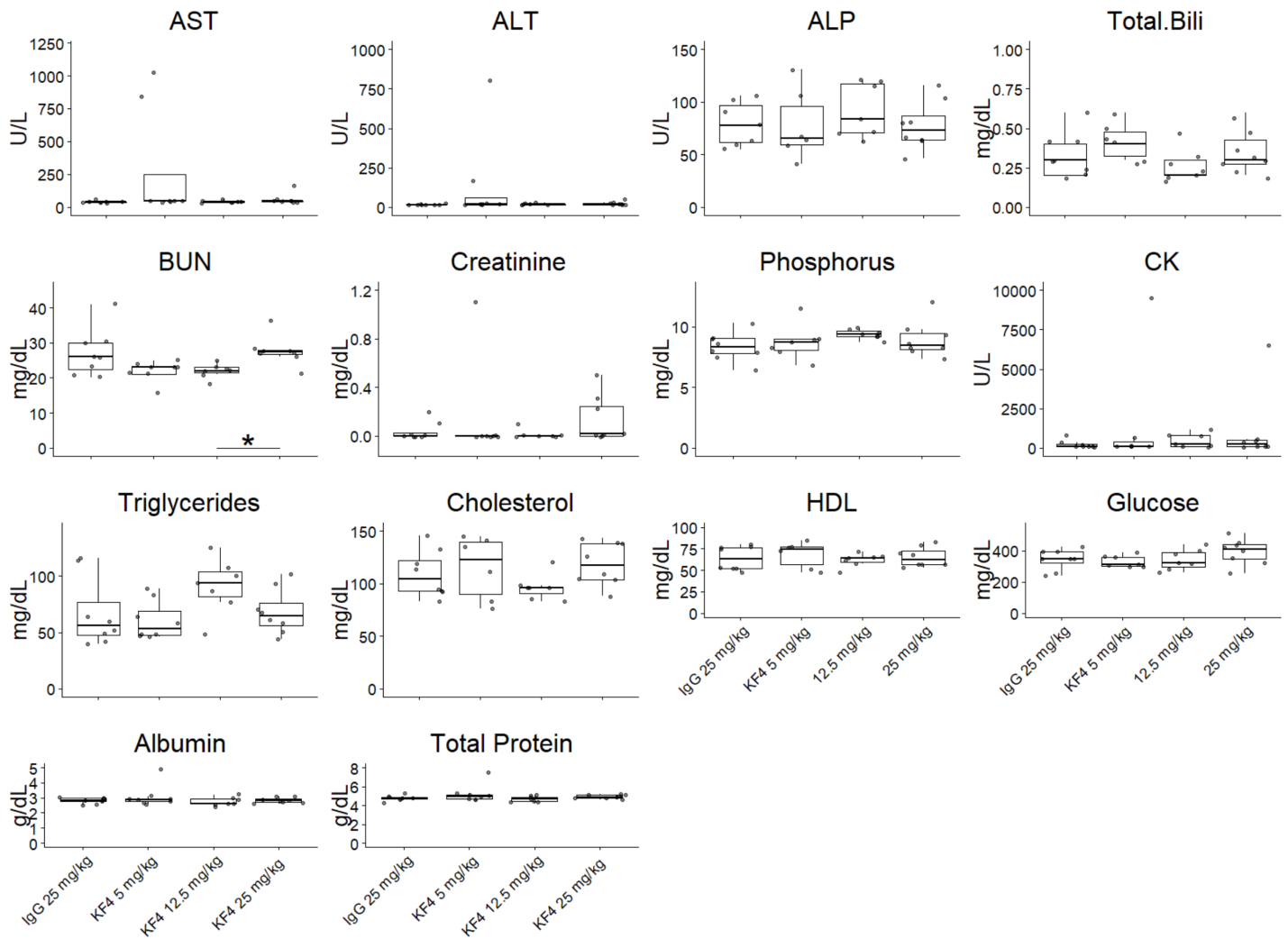
At the time of animal sacrifice, 1 mL of blood was aspirated using a needle in the vena cava prior to exsanguination and major organ harvest. Plasma was stored at  $-80^{\circ}\text{C}$  and sent to IDEXX Bioanalytics for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, blood urea nitrogen (BUN), creatinine, phosphorus, creatine kinase, triglyceride, total cholesterol, high density lipoprotein (HDL), glucose, albumin, and total protein quantification.

## Supplemental Figures

Supplemental Figure 1: CELA1 Expression in Published Datasets. (A) Assessment of mRNA levels of various mouse tissues showed that the highest *Cela1* mRNA levels were in the small intestine, spleen, and kidney. FKPM = Fragments per kilobase per Million mapped reads. (B) To identify what types of cells might be expressing *Cela1* in the spleen, we used a query tool in the Human Protein Atlas which indicated highest expression in basophils and Naïve CD4 T-cells. nTPM = normalized transcripts per million.

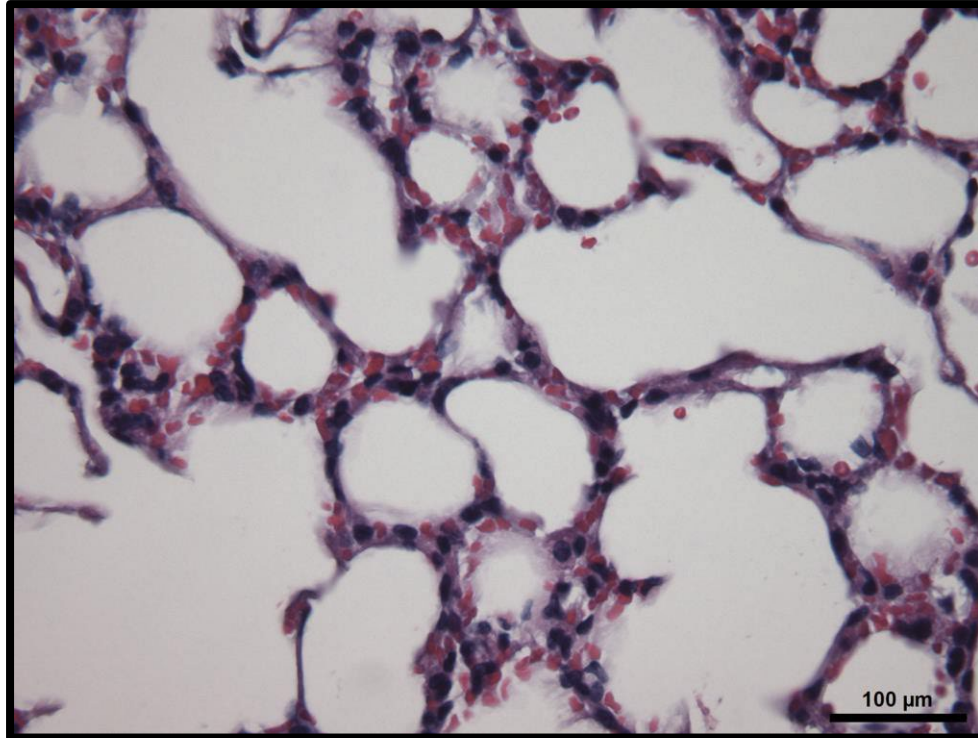


Supplemental Figure 2: Serum Toxicity Analysis. Serology from mice identified no differences in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, creatinine, phosphorus, creatine kinase, triglyceride, total cholesterol, high density lipoprotein (HDL), glucose, albumin, or total protein.

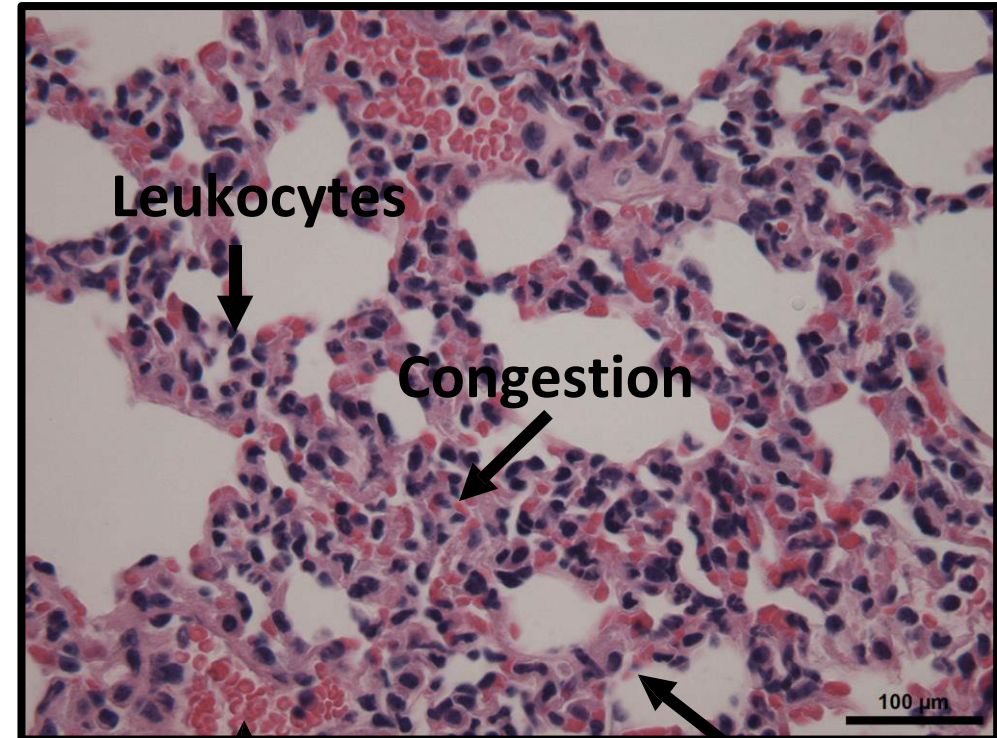


# Histological signs of lung injury

Healthy Lung Tissue

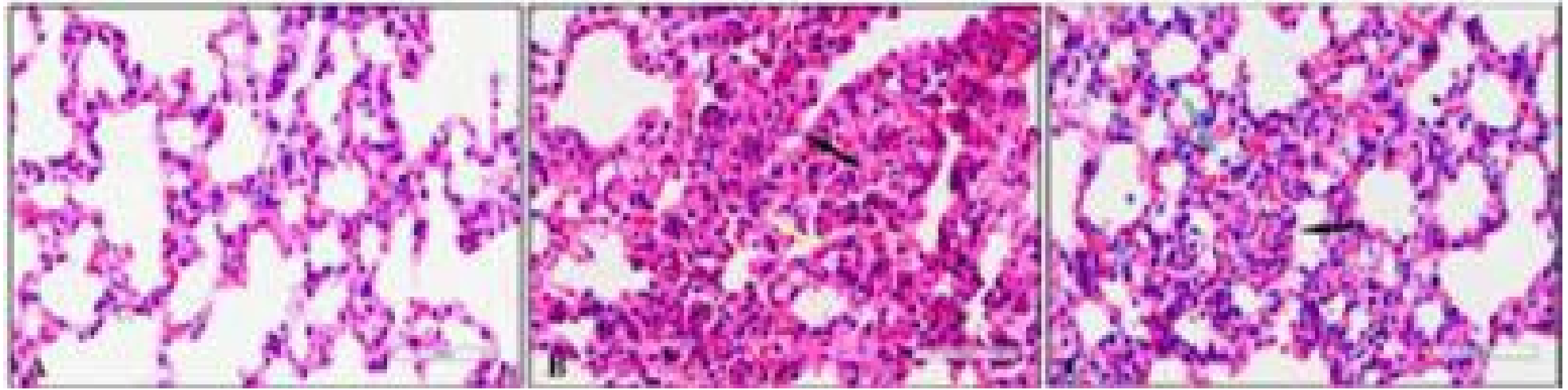


Septic Lung Tissue



Hemorrhage

Thickened Cell Walls



### Scores

0 = no injury

1 = minimal (0-25% of the section)

2 = mild (25-50%)

3 = significant (50-75%)

4 = severe (more than 75%)

Alveolar Congestion or Reduction of Alveolar Space	1	3	2
Hemorrhage	1	3	3
Infiltration of Leukocytes into Airspace or Alveolar Walls	2	3	3
Thickness of Alveolar Wall or Hyaline Membrane Formation	2	4	3
Total	6	13	11



## Scores

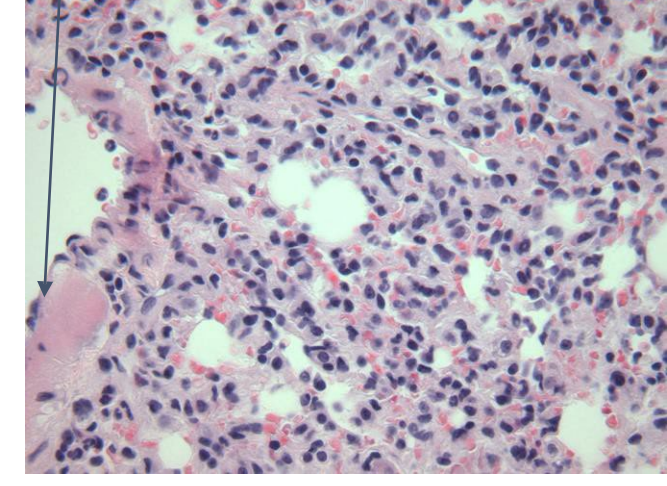
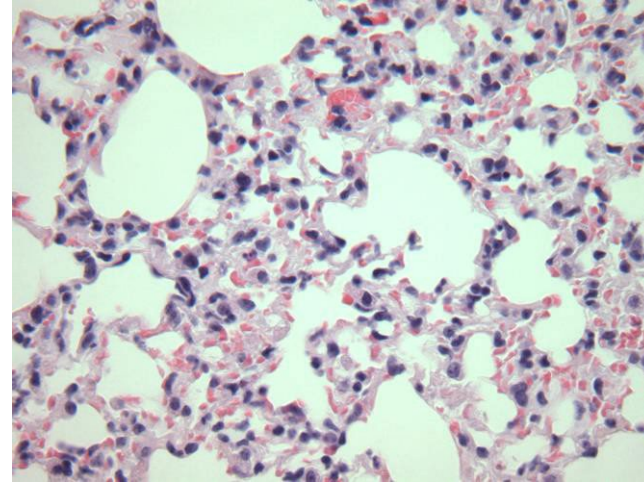
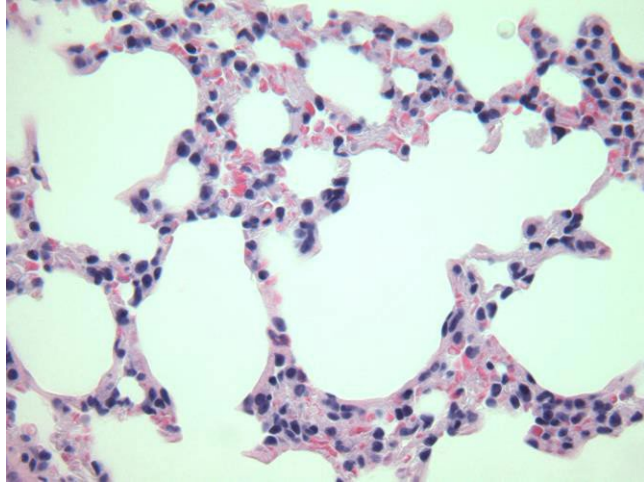
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Hyaline Membranes

	0	1	2	3
Alveolar Congestion or Reduction of Alveolar Space	0	1	2	3
Hemorrhage	0	1	2	3
Infiltration of Leukocytes into Airspace or Alveolar Walls	0	1	2	3
Thickness of Alveolar Wall or Hyaline Membrane Formation	0	1	2	4
Total	0	4	7	11

### Scores

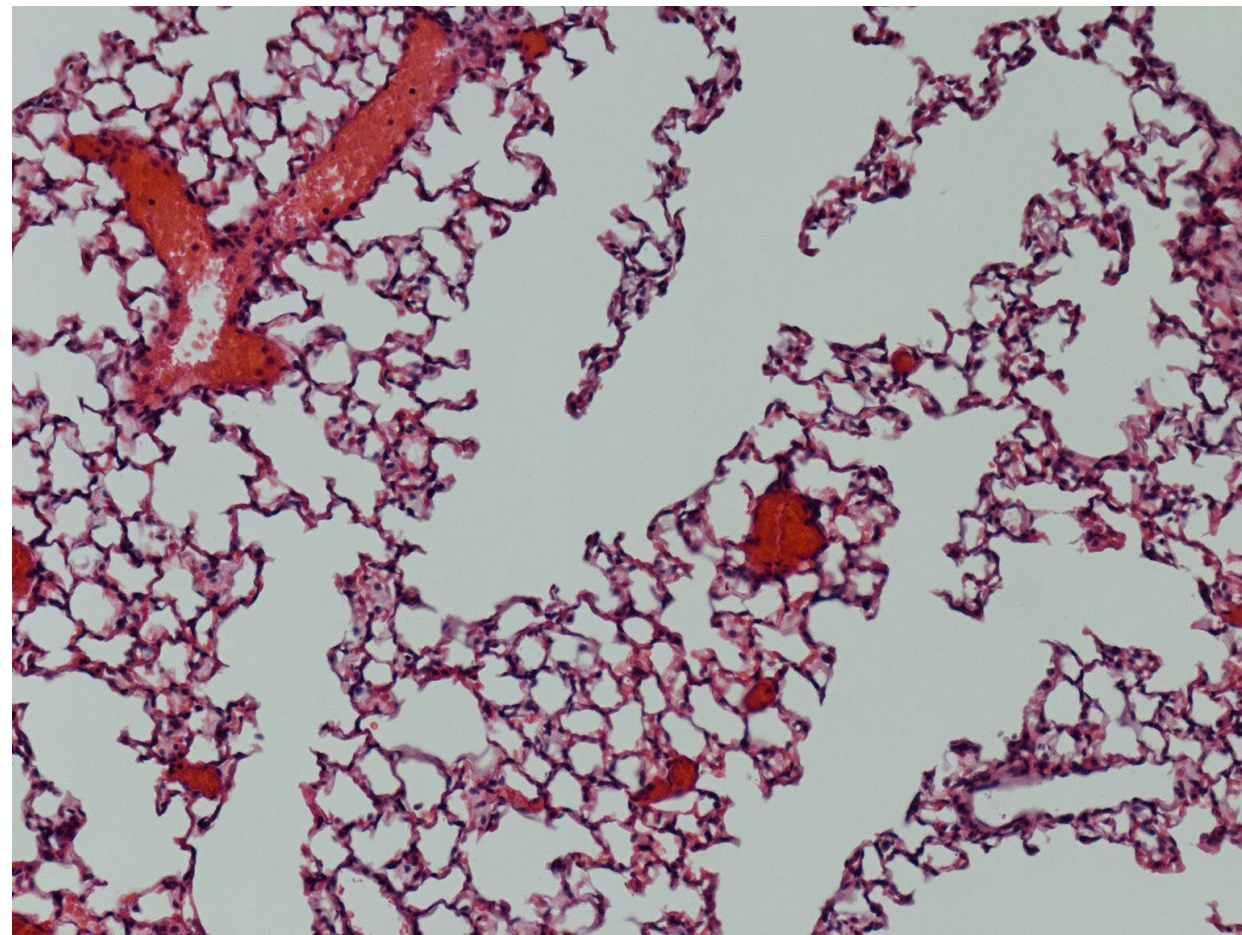
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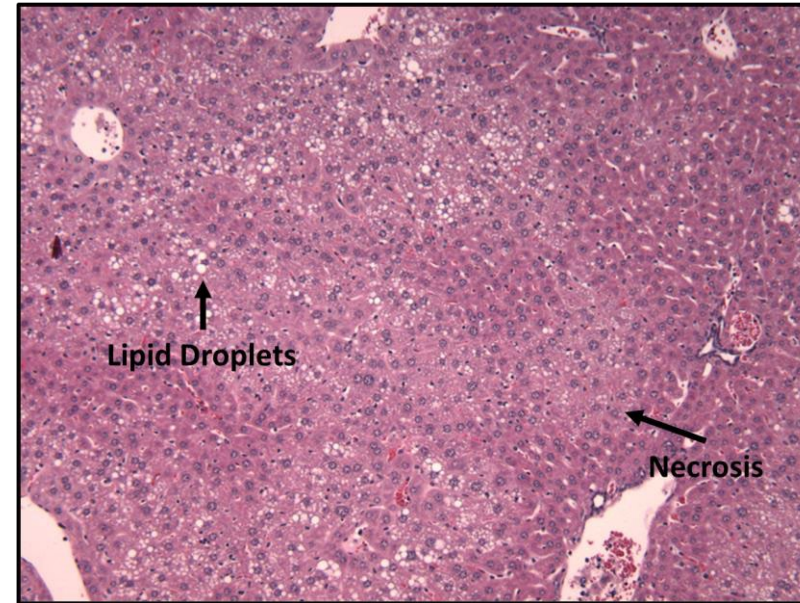
4 = severe (more than 75%)



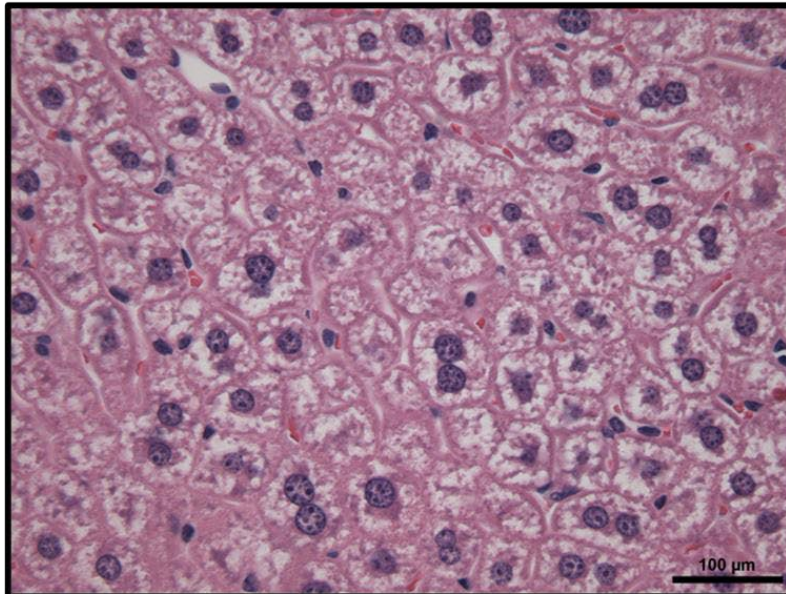


Alveolar Congestion or Reduction of Alveolar Space	0	
Hemorrhage	0	
Infiltration of Leukocytes into Airspace or Alveolar Walls	1	
Thickness of Alveolar Wall or Hyaline Membrane Formation	2 (hyaline membranes)	
Total	3	

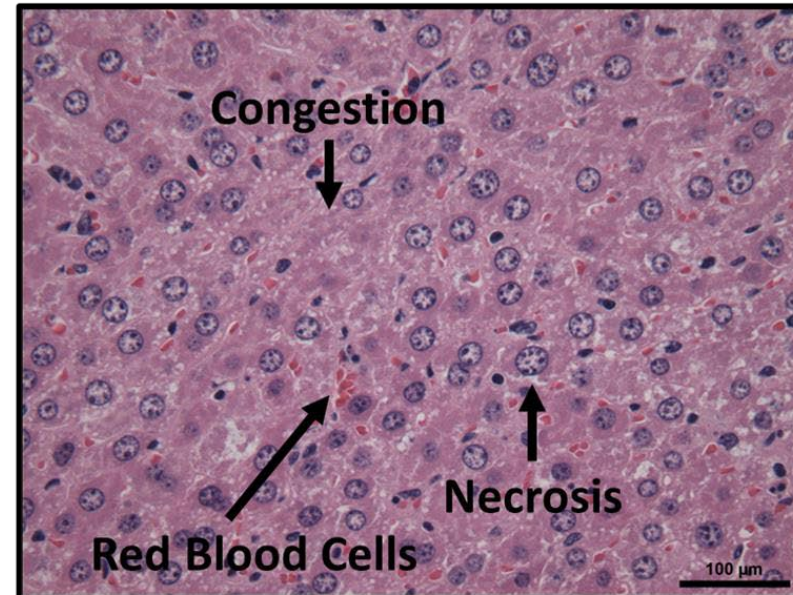
# Histological Signs of Liver Injury



**Healthy Liver Tissue**



**Septic Liver Tissue**





## Scores

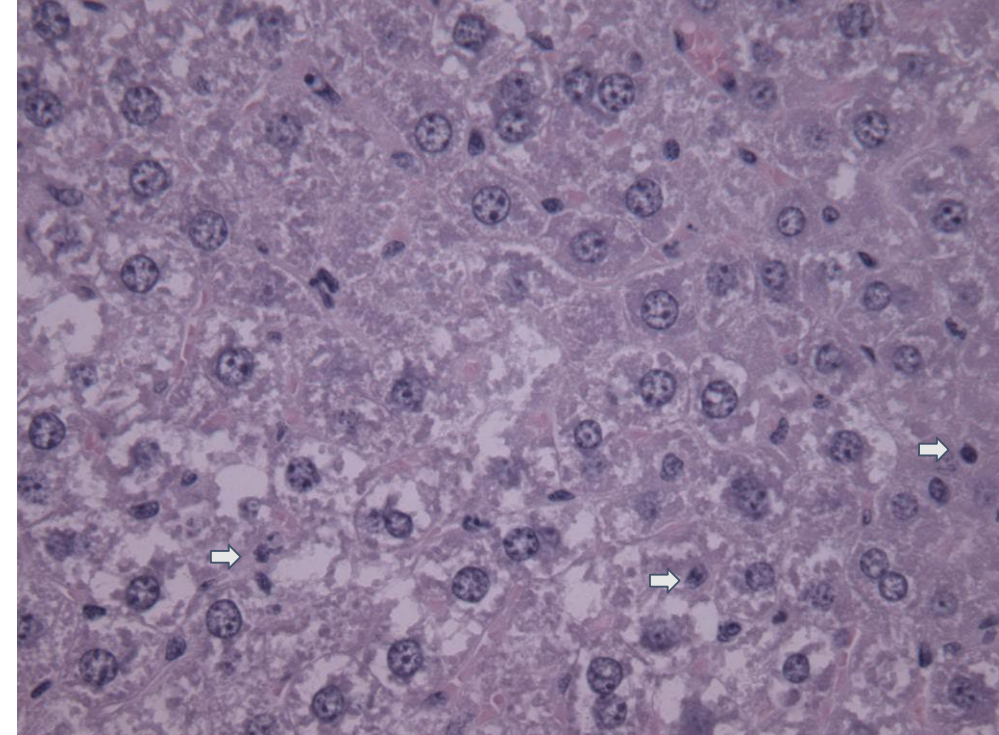
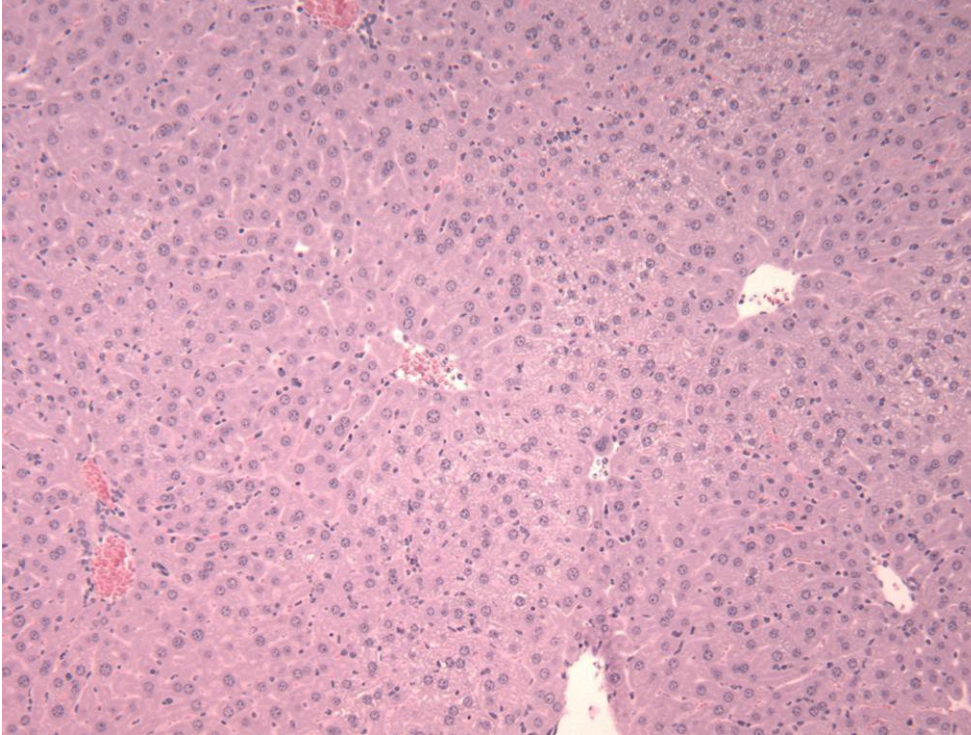
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4 = severe (more than 75%)



10x

40x

Sinusoid congestion and/or edema (10X) 3

Infiltration of red blood and inflammatory cells (10X) 2

Presence of lipid droplets or vacuoles (10 & 40X) 0

Necrosis (10 & 40X) 3

0

3



## Scores

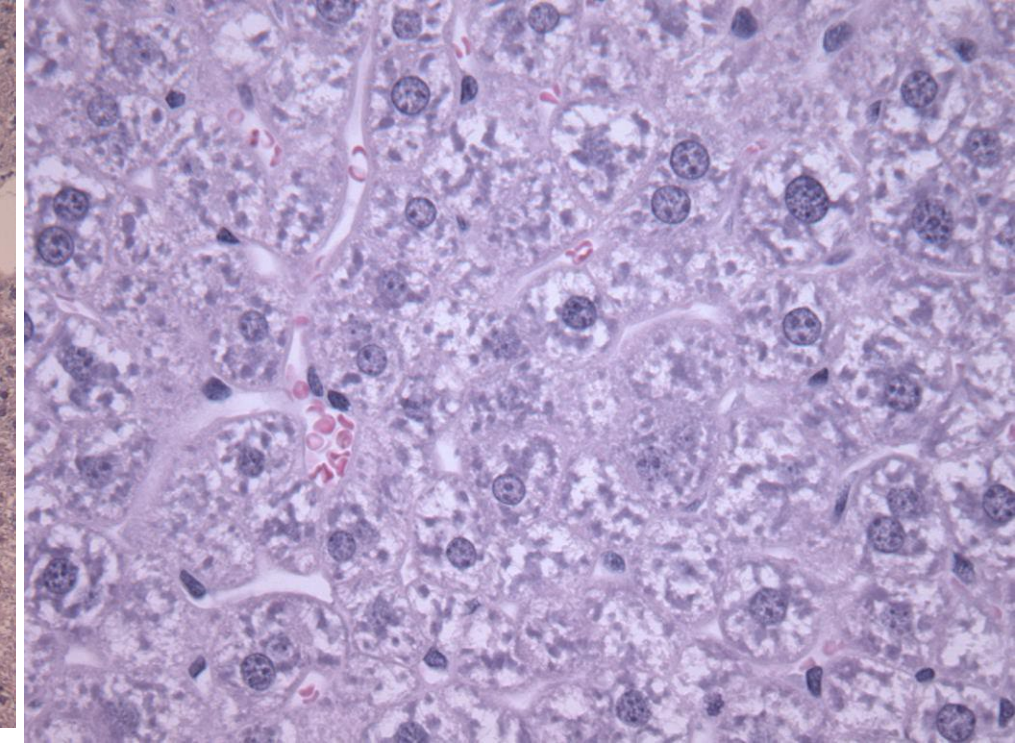
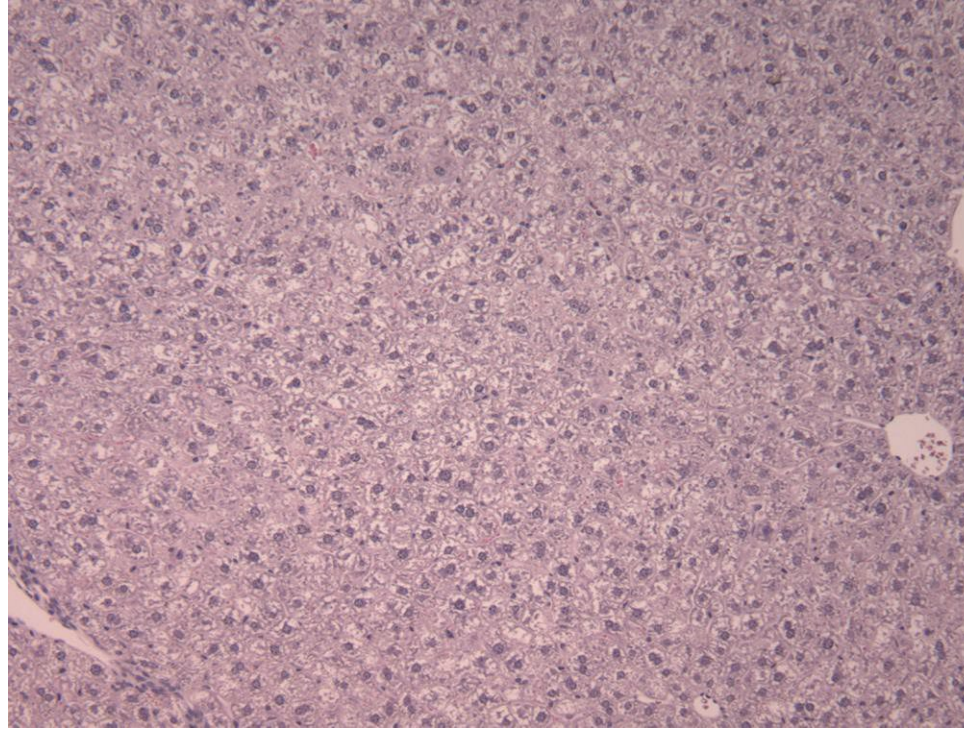
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10x

40x

Sinusoid congestion and/or edema (10X)

0

Infiltration of red blood and inflammatory cells (10X)

0

Presence of lipid droplets or vacuoles (10 & 40X)

0

0

Necrosis (10 & 40X)

1

1



## Scores

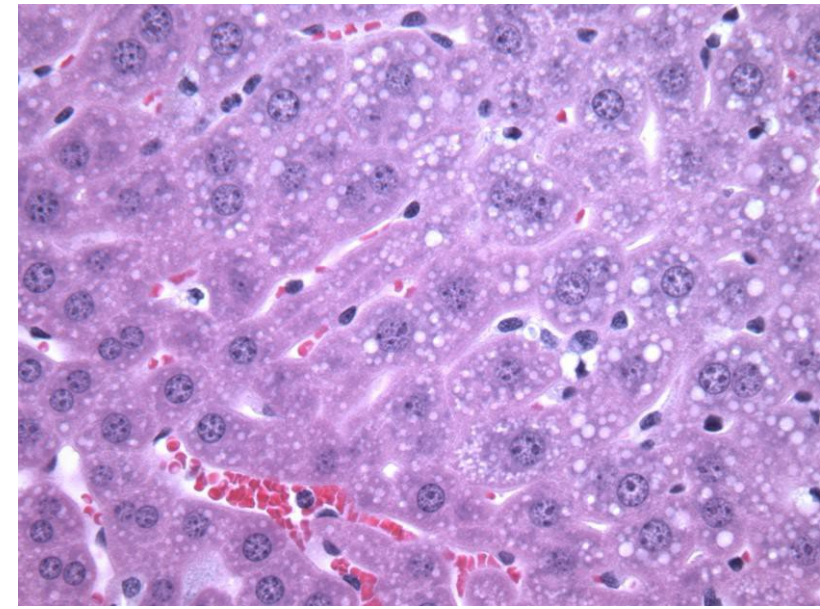
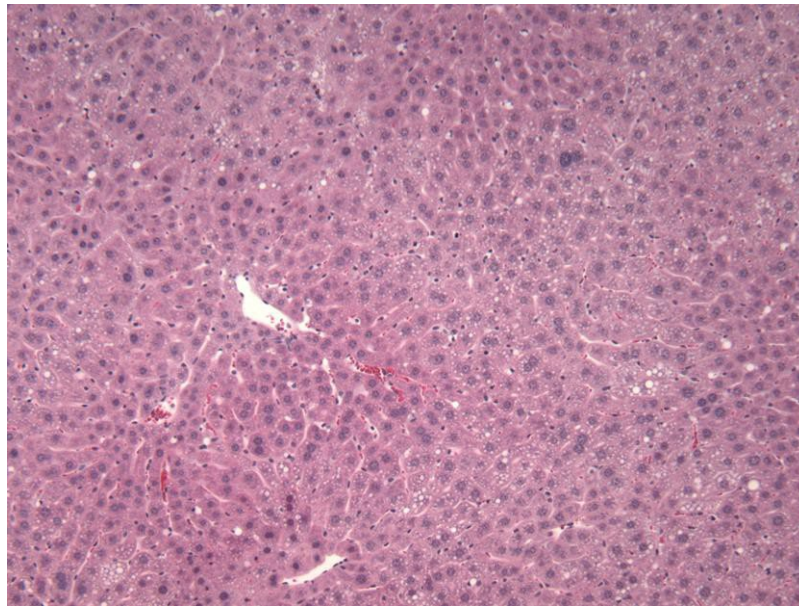
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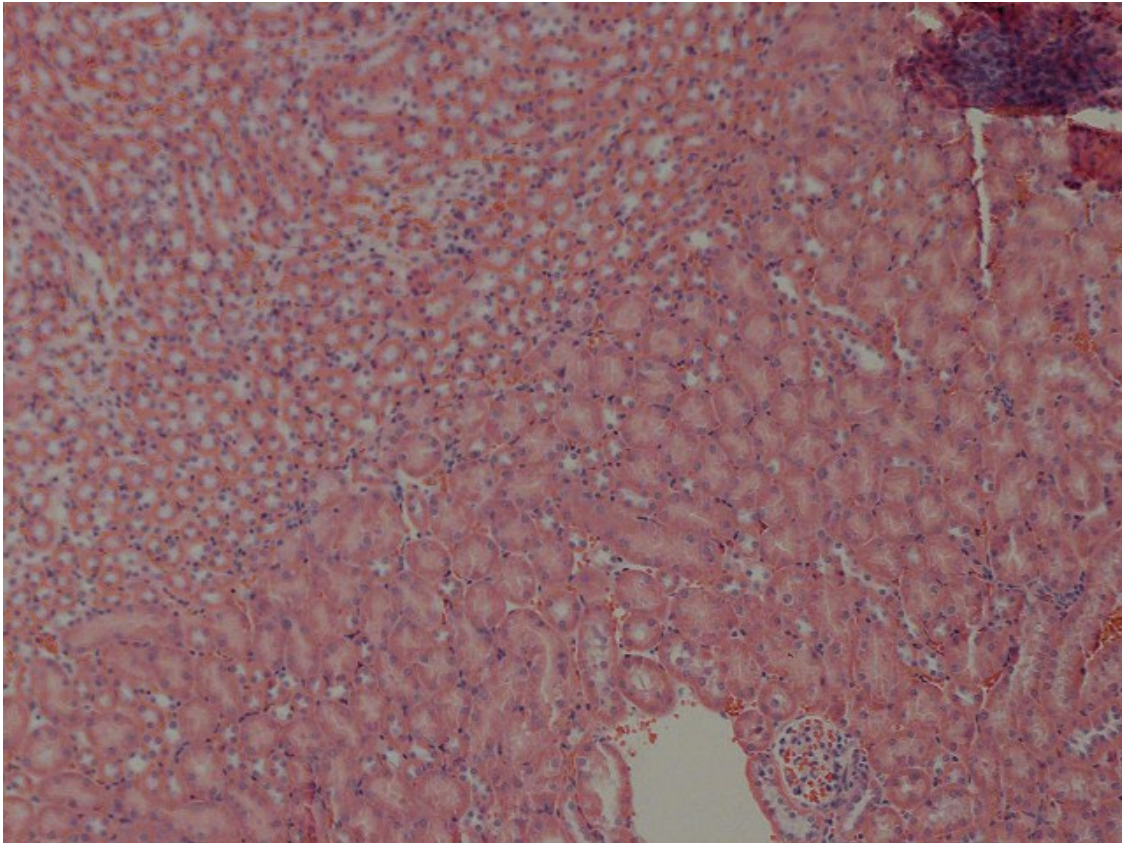
	10x	40x
Sinusoid congestion and/or edema (10X)		3
Infiltration of red blood and inflammatory cells (10X)		3
Presence of lipid droplets or vacuoles (10 & 40X)	3	4
Necrosis (10 & 40X)	3	3
Total		



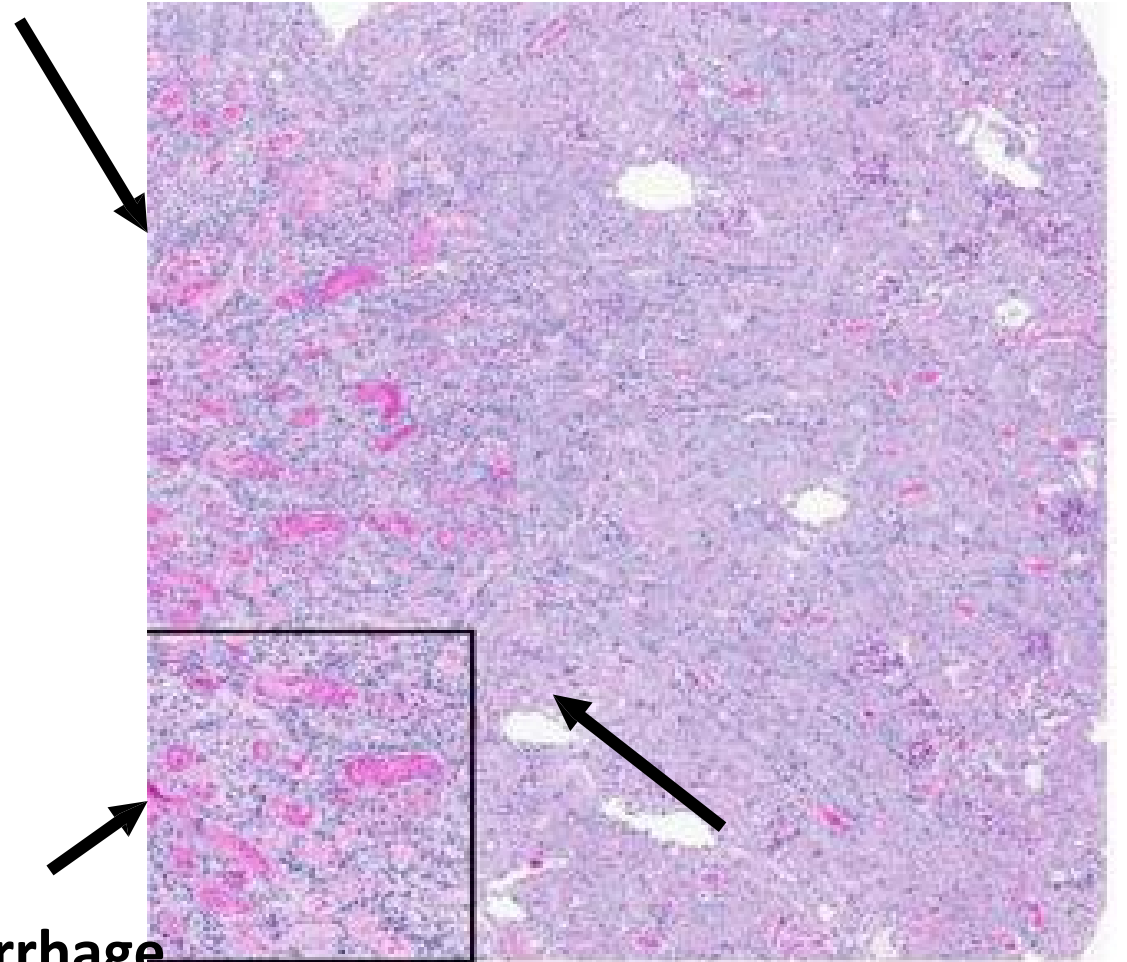
# Histological signs of kidney injury-10X

I/R Kidney Tissue

Healthy Kidney Tissue



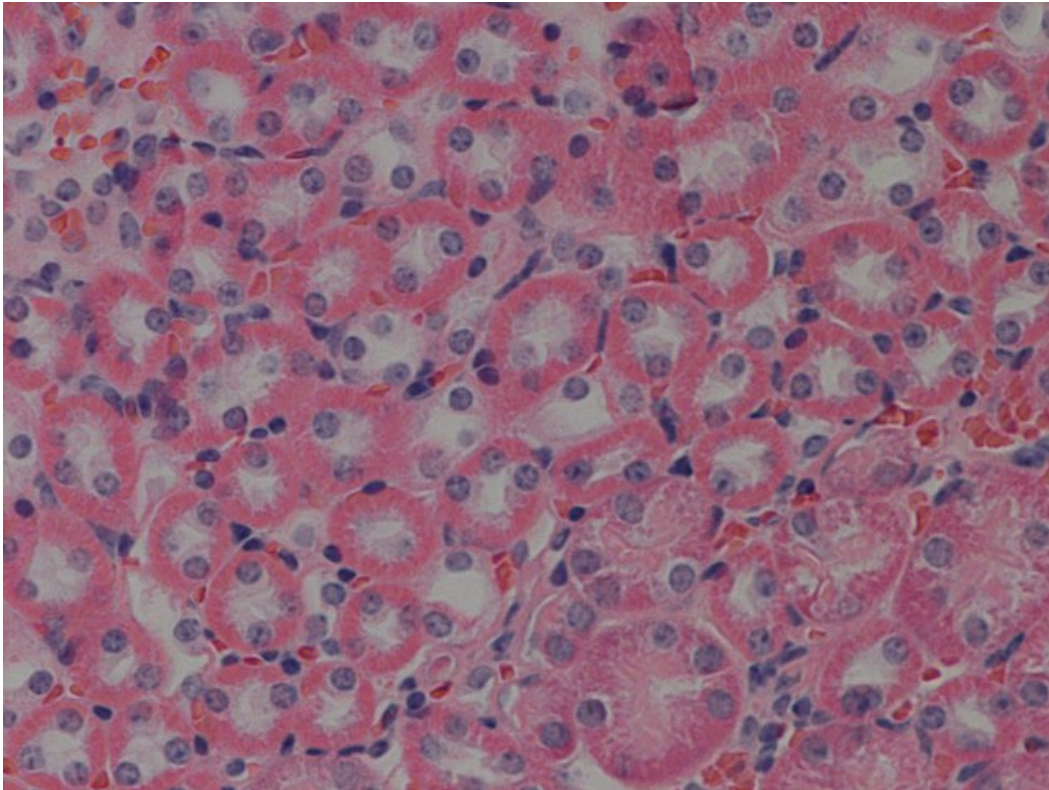
Leukocytes



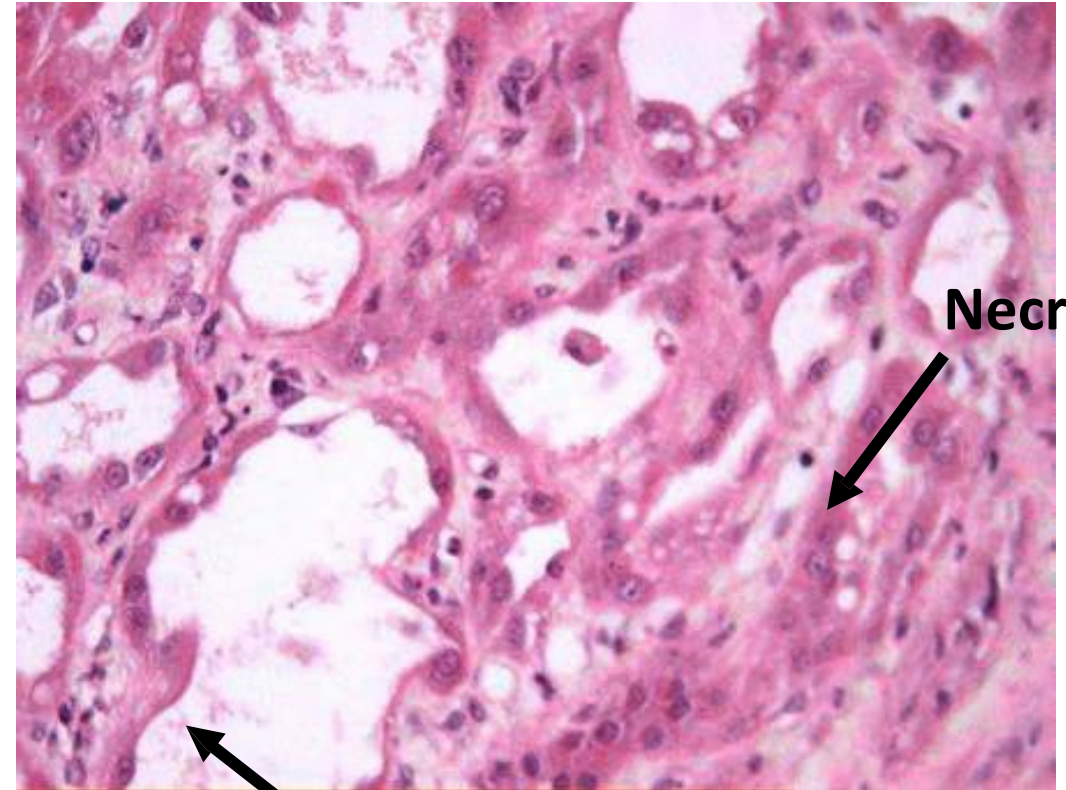
Hemorrhage

# Histological signs of kidney injury-40X

Healthy Kidney Tissue



I/R Kidney Tissue



Necrosis

Loss of Brush Border



## Scores

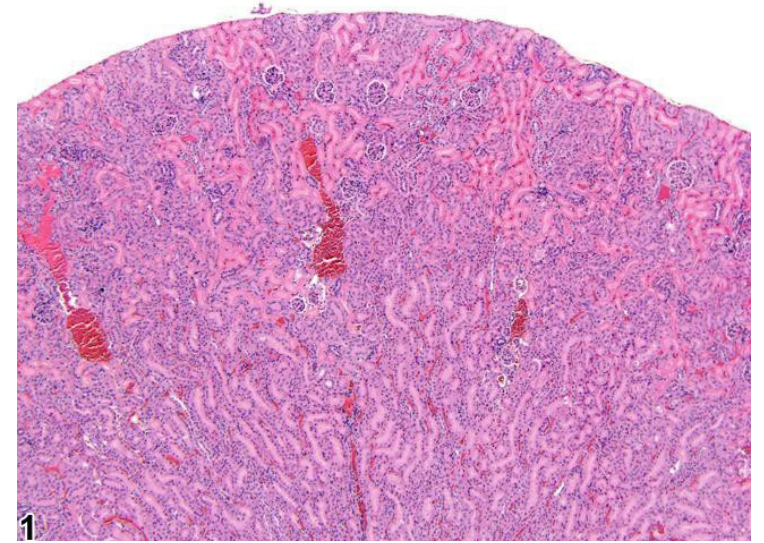
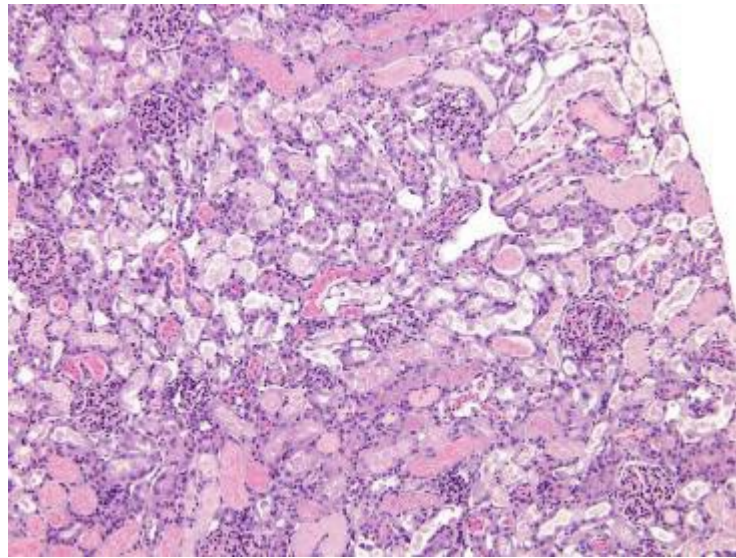
0 = no injury

1 = minimal (0-25% of the section)

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3 = significant (50-75%)

4 = severe (more than 75%)



Loss of Brush Border (40X)

Tubular Necrosis (40X)

Neutrophil Infiltration (10X)

3

4

Hemorrhage/Congestion (10x)

1

3

Total

4

7

## Scores

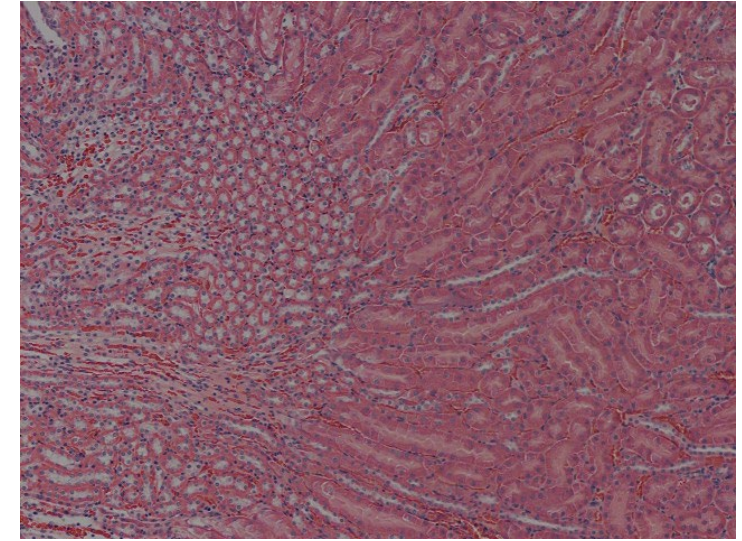
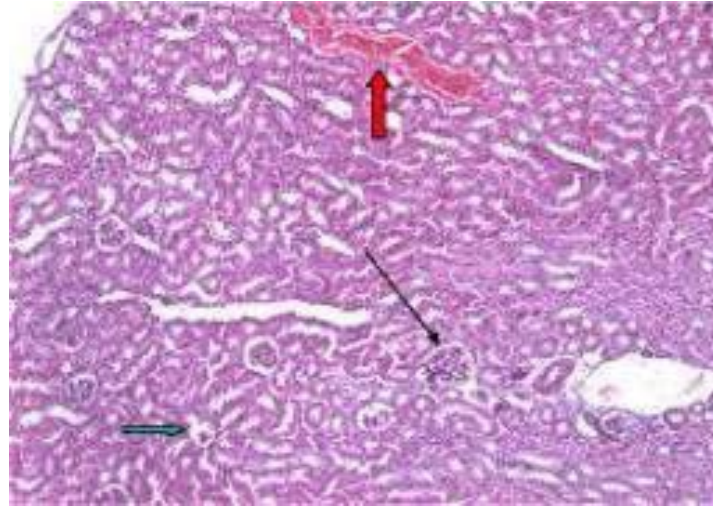
0 = no injury

1 = minimal (0-25% of the section)

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Loss of Brush Border (40X)

Tubular Necrosis (40X)

Neutrophil Infiltration (10X)

0

0

Hemorrhage/Congestion (10x)

1

0

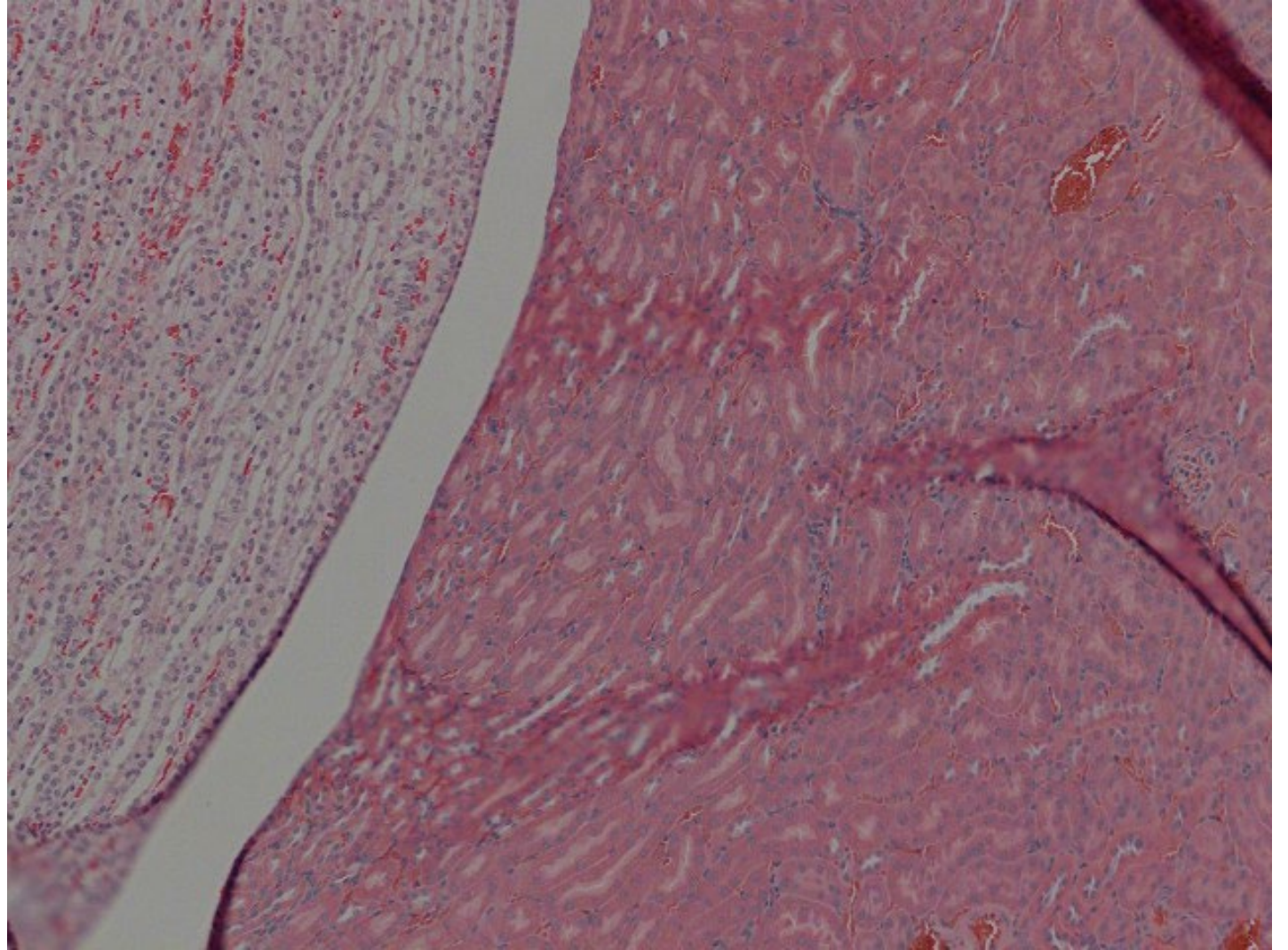
Total

1

0



# Adrenal Gland





## Scores

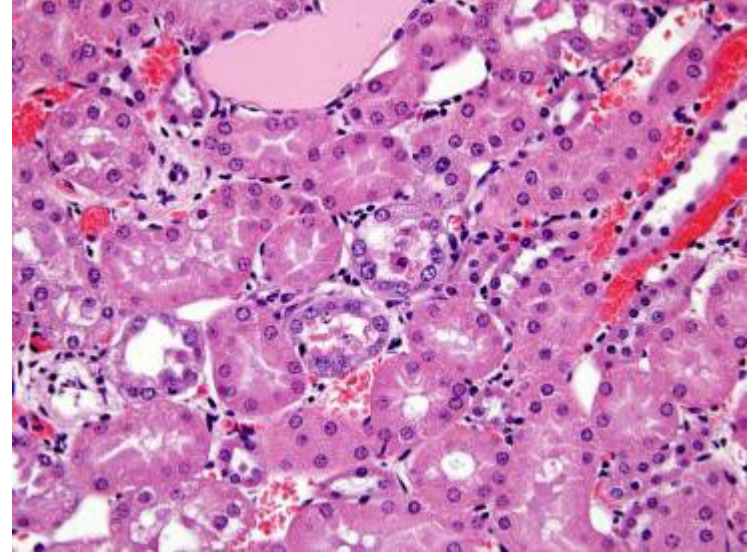
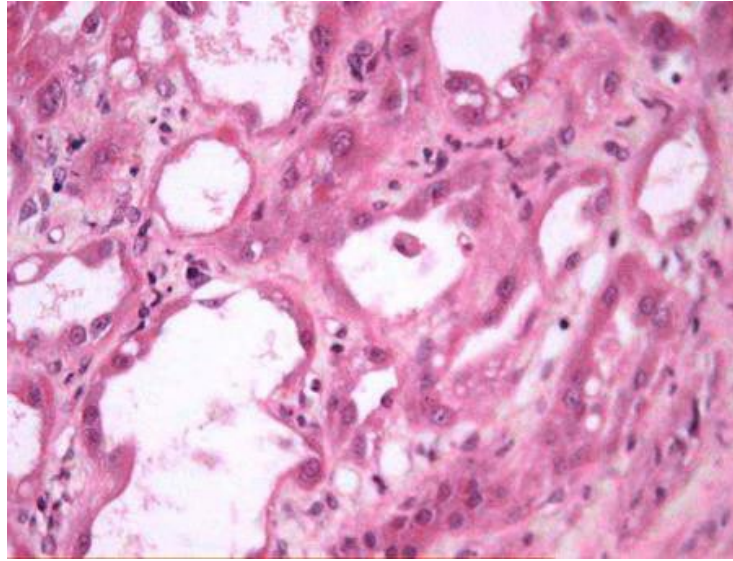
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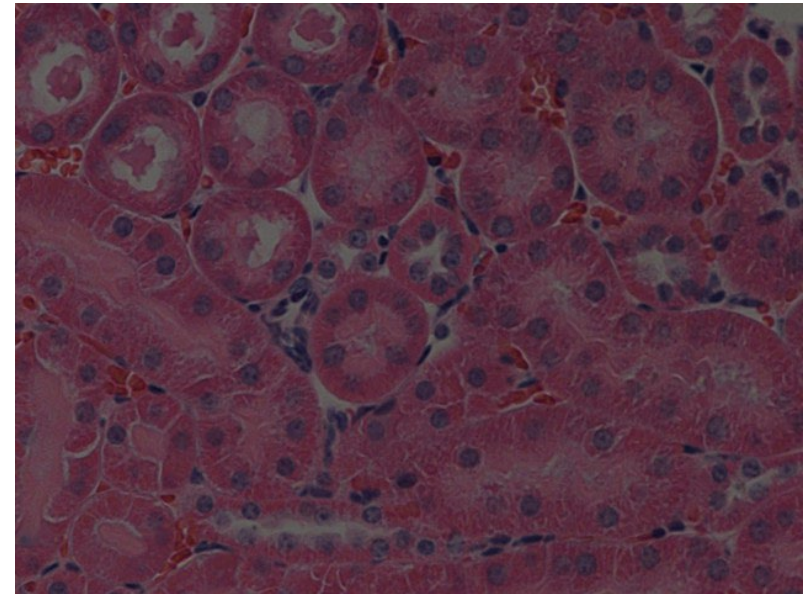
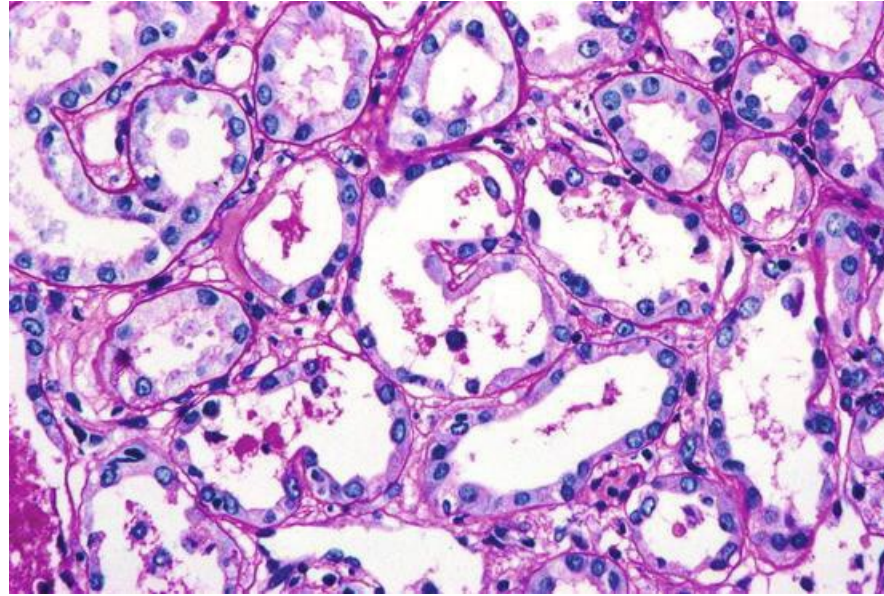
3 = significant (50-75%)

4 = severe (more than 75%)



Loss of Brush Border (40X)	4	2
Tubular Necrosis (40X)	4	3
Neutrophil Infiltration (10X)		
Hemorrhage/Congestion (10x)		
Total	8	5

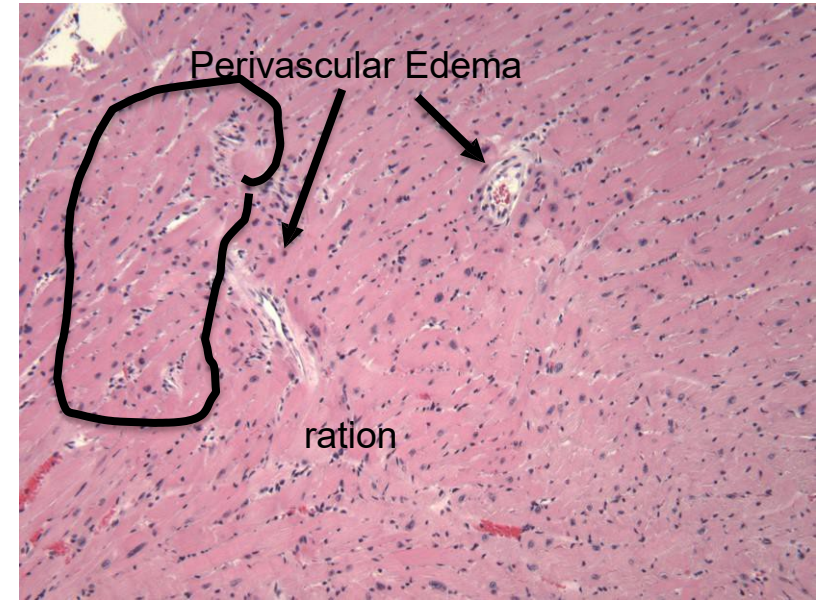
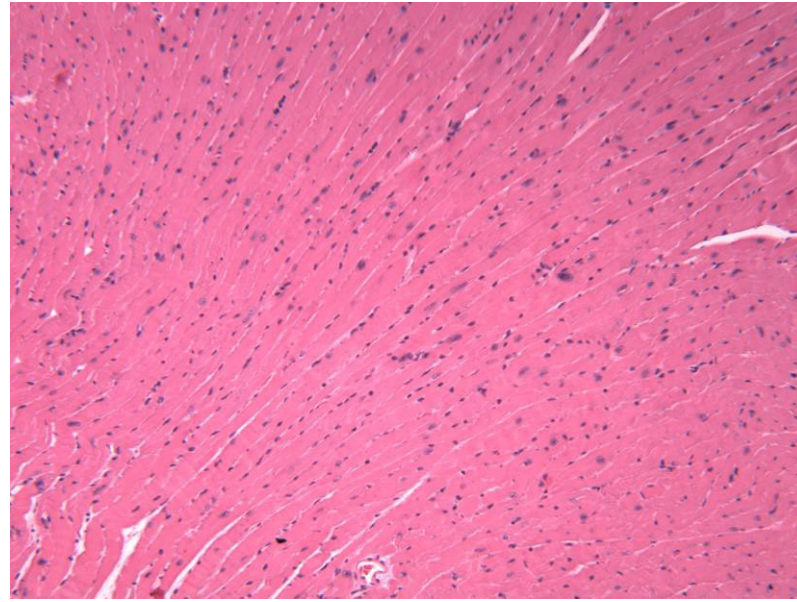
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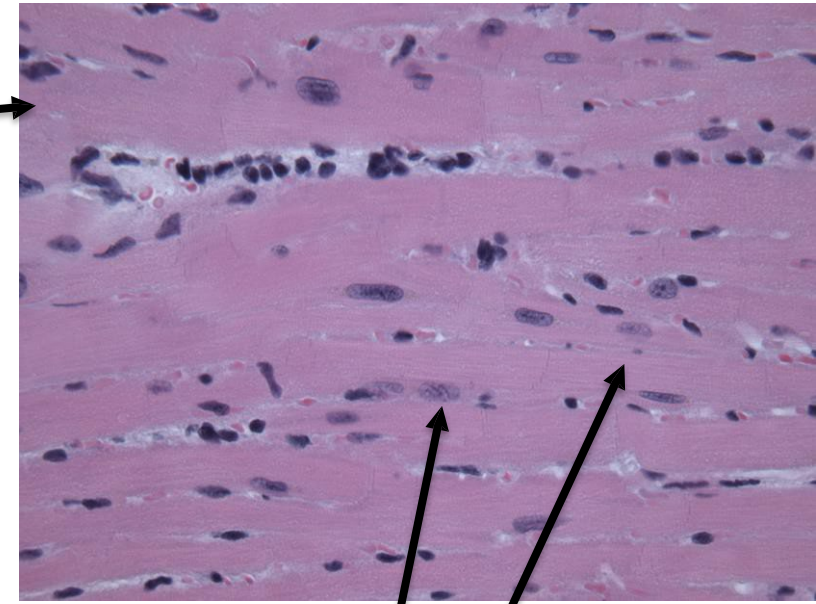
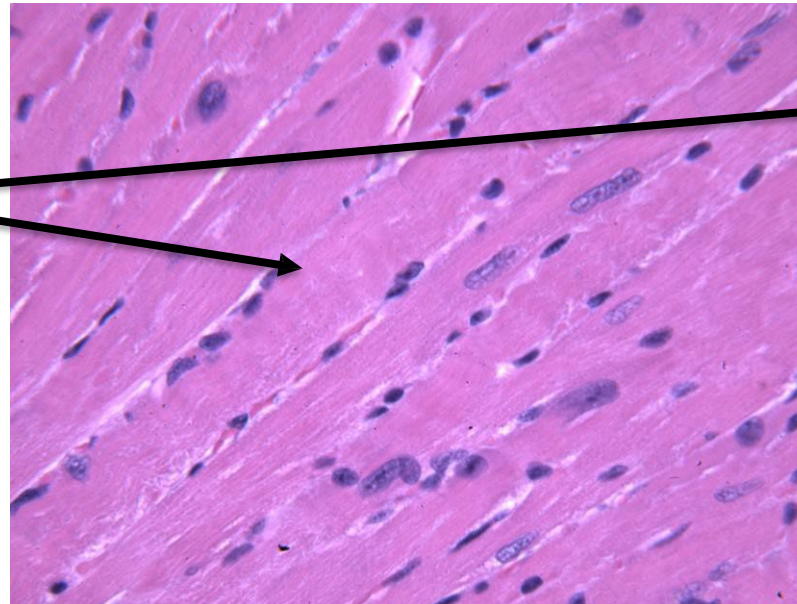
Loss of Brush Border (40X)	4	0
Tubular Necrosis (40X)	2	0
Neutrophil Infiltration (10X)		
Hemorrhage/Congestion (10x)		
<b>Total</b>	<b>8</b>	<b>0</b>



# Histological Signs of Heart Injury



Myofibril Derangement  
(fibrils with bands vs not)



Nuclear Hydrops (swollen, "ghost" nuclei)



## Scores

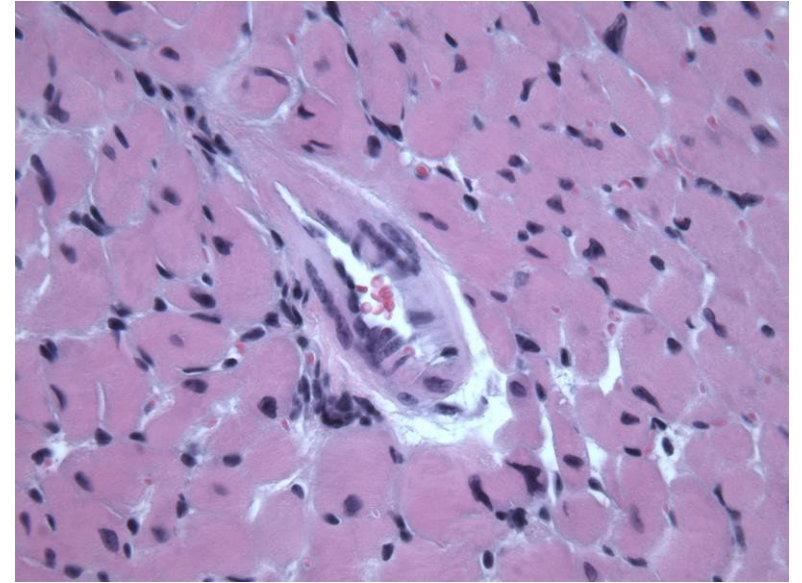
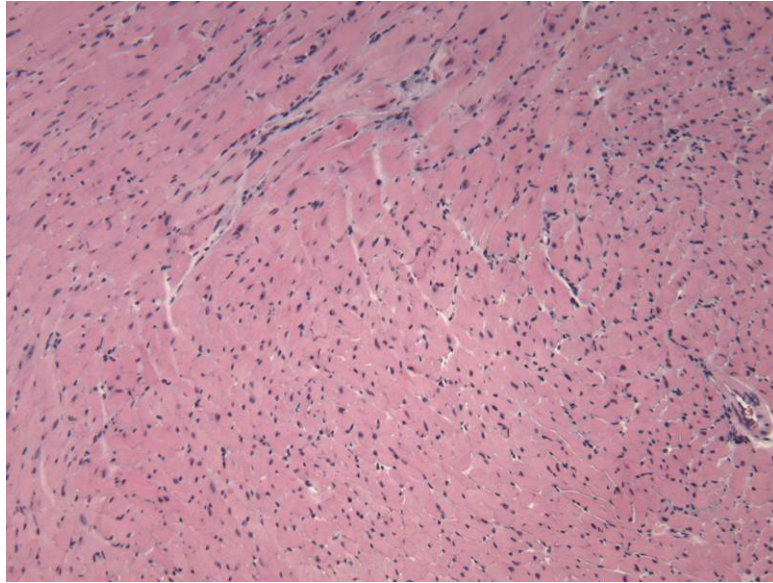
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4 = severe (more than 75%)



	10x	40x
Perivascular Edema (10X)	2	
Myofibril Derangement (40X)		3
Infiltration of Neutrophils (10X)	3	
Nuclear Hydrops (40X)		1

## Scores

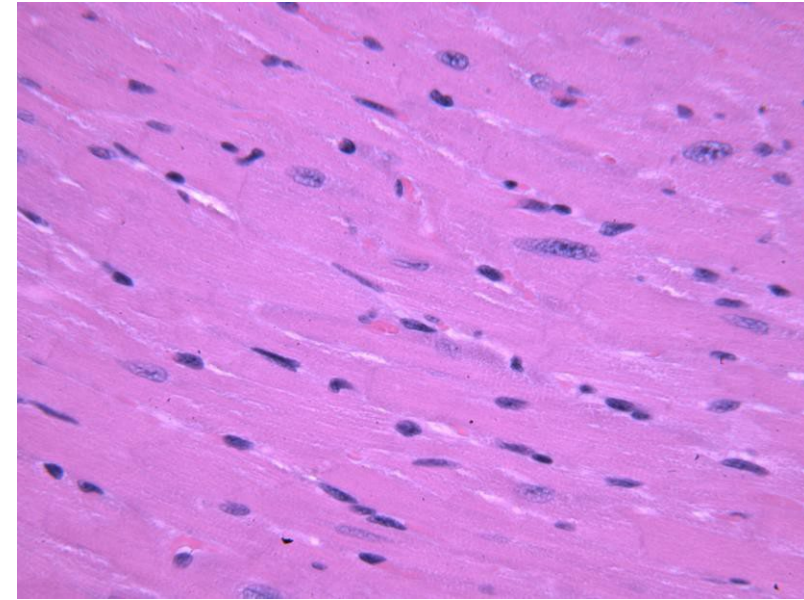
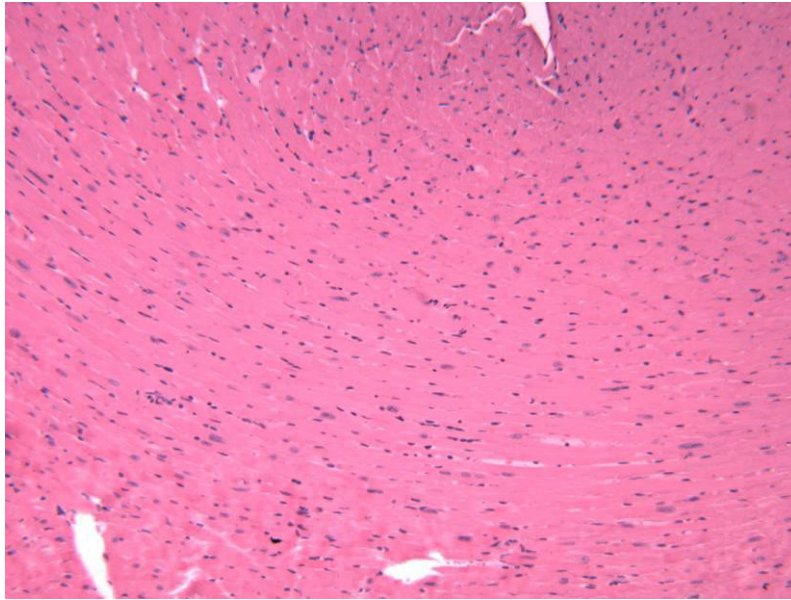
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	10x	40x
Perivascular Edema (10X)	0	
Myofibril Derangement (40X)		1
Infiltration of Neutrophils (10X)	1	
Nuclear Hydrops (40X)		0



## Scores

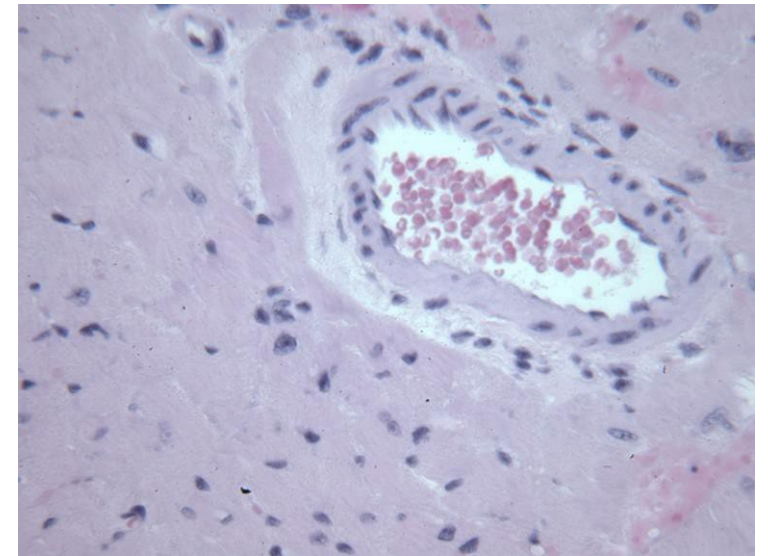
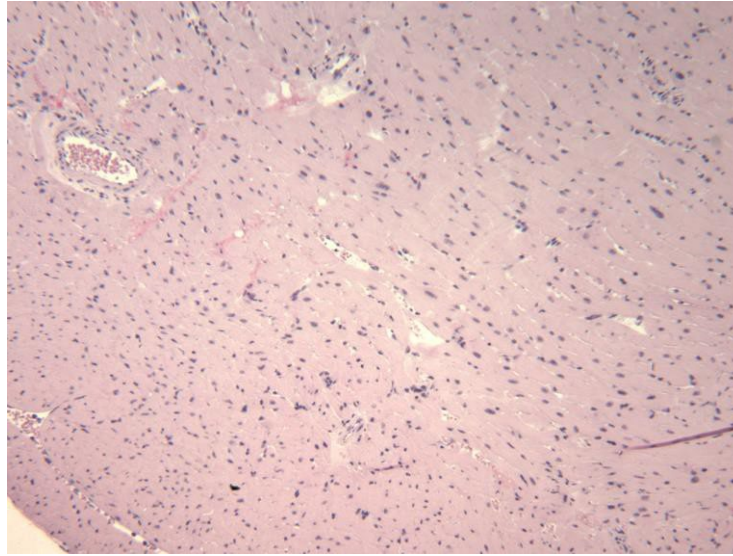
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	10x	40x
Perivascular Edema (10X)	3	
Myofibril Derangement (40X)		3
Infiltration of Neutrophils (10X)	3	
Nuclear Hydrops (40X)		3
Total		

## Scores

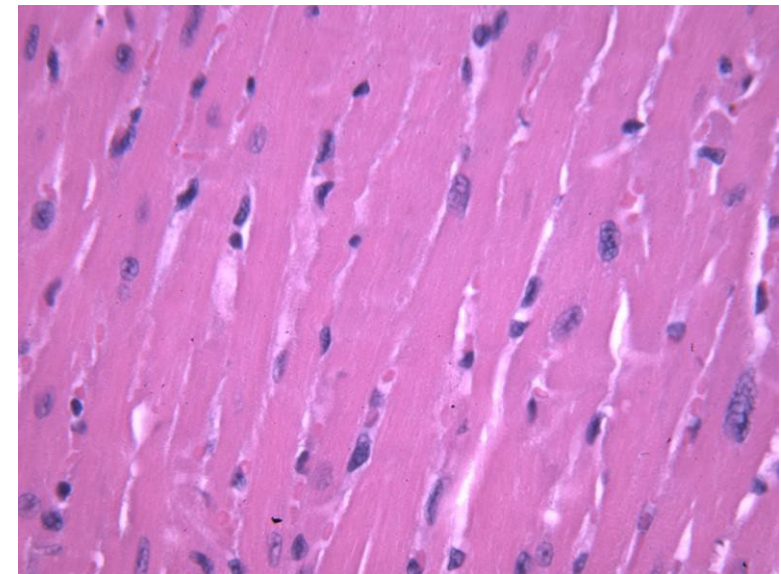
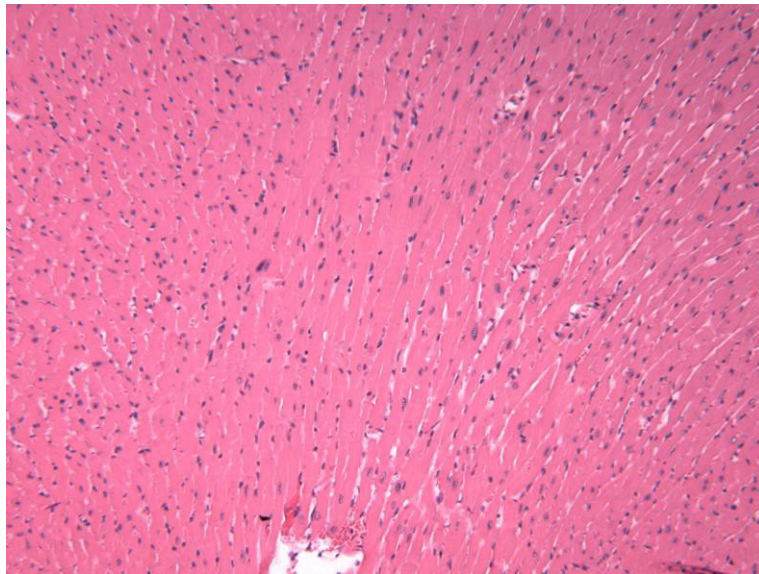
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	10x	40x
Perivascular Edema (10X)	1	
Myofibril Derangement (40X)		1
Infiltration of Neutrophils (10X)	2	
Nuclear Hydrops (40X)		0
Total		