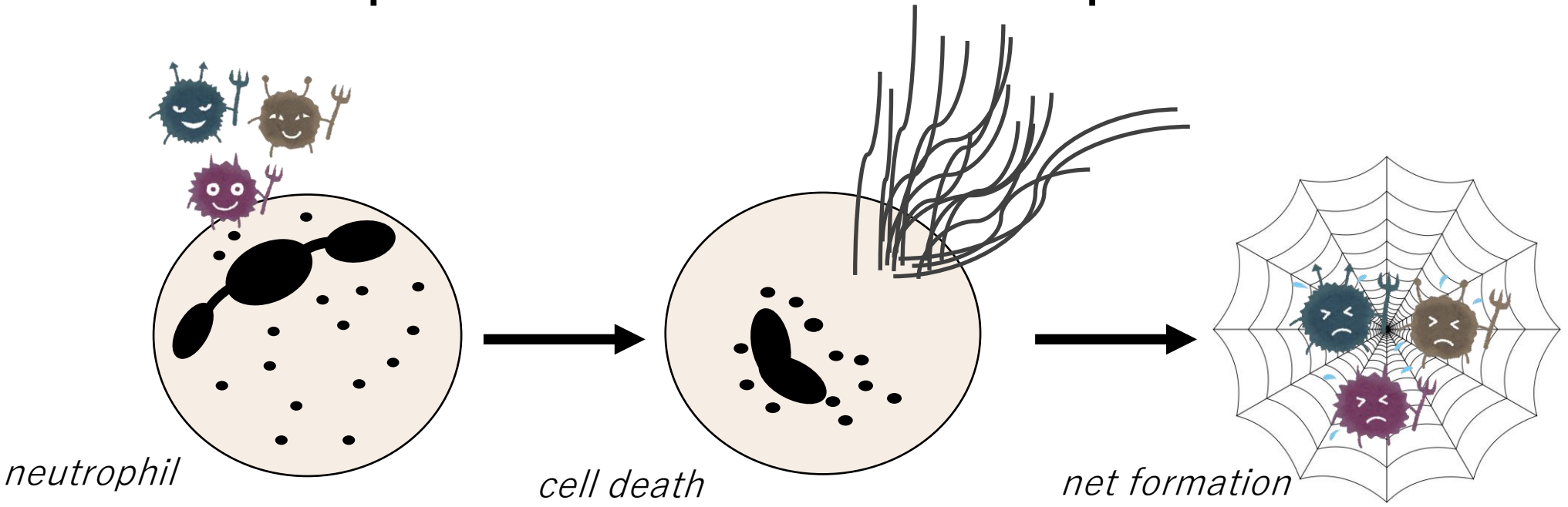
Neutrophil extracellular traps (NETs) and pneumonia

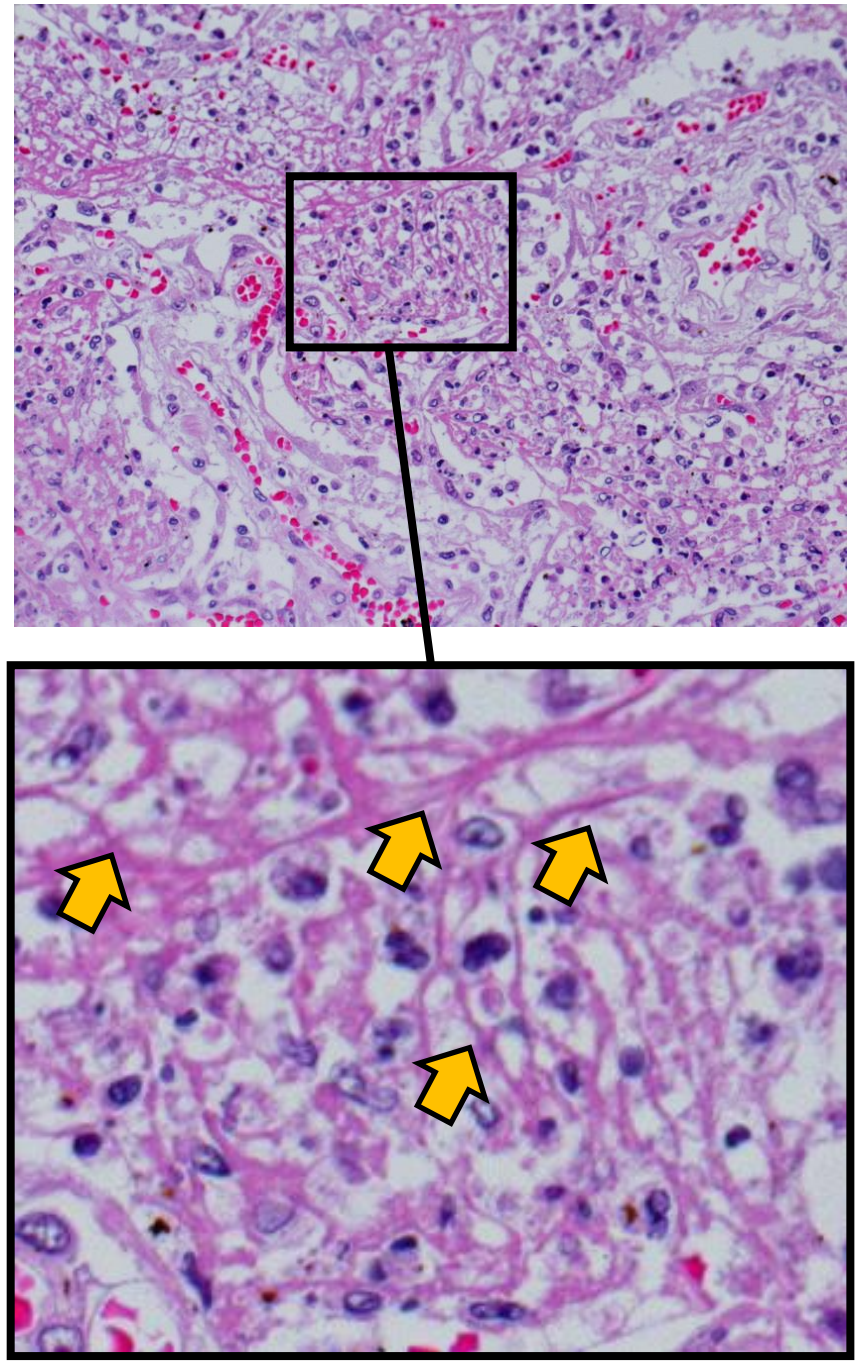
Neutrophils belong to front line phagocytes to kill infected microbial pathogens. One of the important mechanisms of the neutrophil function is the post-mortem formation of neutrophil extracellular traps (NETs), net-like structures entrapping the pathogens. NETs are consistently formed in purulent inflammatory lesions, including pneumonia. Namely, NETs are formed by dead neutrophils upon contact with various pathogens. The main components of NETs are DNA stretches and granular antimicrobial neutrophil proteins, including lactoferrin and myeloperoxidase. The pathogens entrapped in NETs are killed by oxidative and non-oxidative mechanisms. Chromatin and proteases released into the circulatory system may regulate procoagulant and prothrombotic factors and take part in intravascular clot formation. The DNA-complexed granular proteins are also related to autoimmune disorders.

Neutrophil Extracellular Traps : NETs

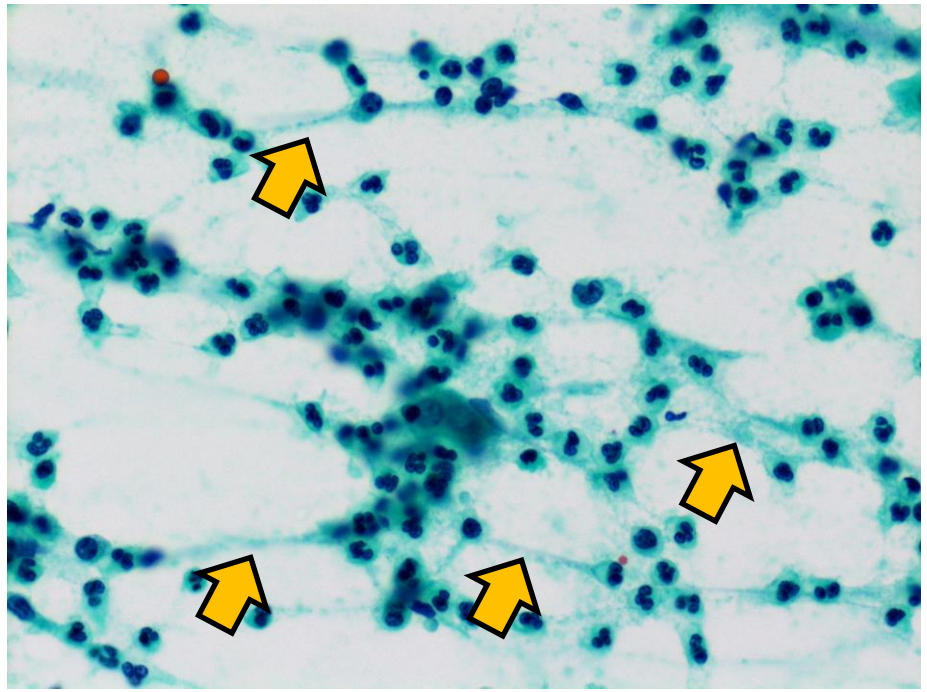


In 2004, Brinkmann and colleagues reported that stimulated neutrophils can produce extracellular fibrils called neutrophil extracellular traps (NETs) that trap to kill microorganisms. NETs are composed of degraded chromatin and granules of neutrophil origin.

In the lesion of purulent inflammation, filamentous structures are seen. NETs or fibrin?



pneumonia (H&E)



cytology for breast abscess (Pap)

Detection of NETs in formalin-fixed, paraffin-embedded sections of fibrinopurulent inflammation

Ref.-1. Shiogama K, et al. Visualization of neutrophil extracellular traps and fibrin meshwork in human fibrinopurulent inflammatory lesions: I. Light microscopic study. *Acta Histochem Cytochem* 2016; 49 (4): 109-116. doi: 10.1267/ahc.l6015

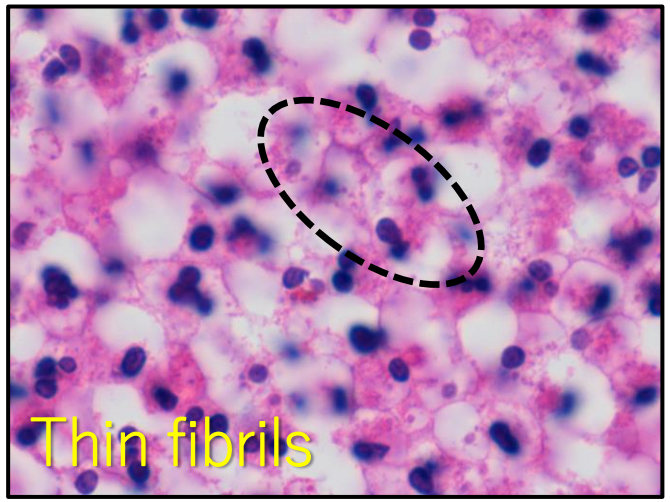
Ref.-2. Onouchi T, et al. Visualization of neutrophil extracellular traps and fibrin meshwork in human fibrinopurulent inflammatory lesions: II. Ultrastructural study. *Acta Histochem Cytochem* 2016; 49 (4): 117-123. doi:10.1267/ahc.l6016

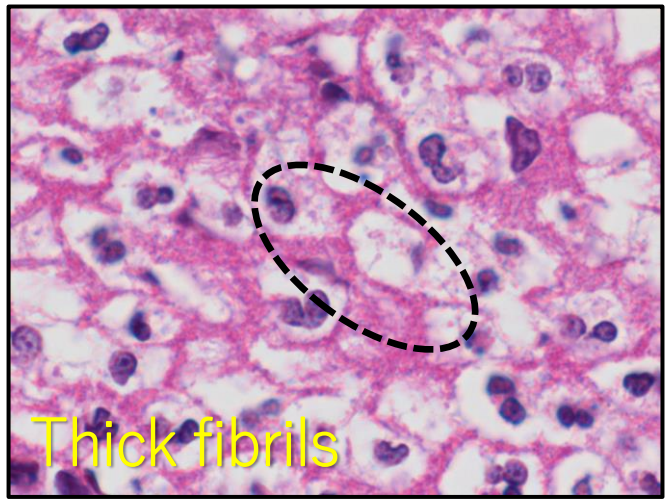
Ref.-3. Onouchi T, et al. Visualizationof neutrophil extracellular traps and fibrin meshwork in human fibrinopurulent inflammatory lesions: III. Correlative light and electron microscopic study. *Acta Histochem Cytochem* 2016; 49 (5): 141-147. doi: 10.1267/ahc.l6028

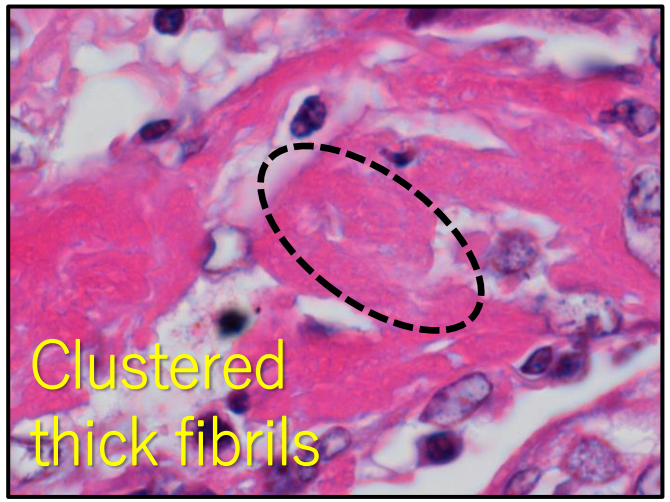
Immunohistochemical markers

Antibodies	Target
Citrullinated Histone H3 (Cit-H3)	NETs fibrils
Lactoferrin (LF)	
Myeloperoxidase (MPO)	
Fibrinogen gamma chain (FGG)	Fibrin fibrils

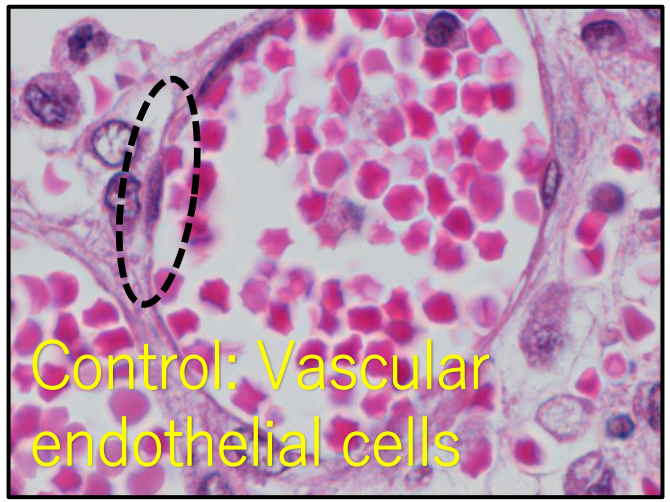
Hypercitrullination of Histone H3 plays an important role in chromatin decondensation.





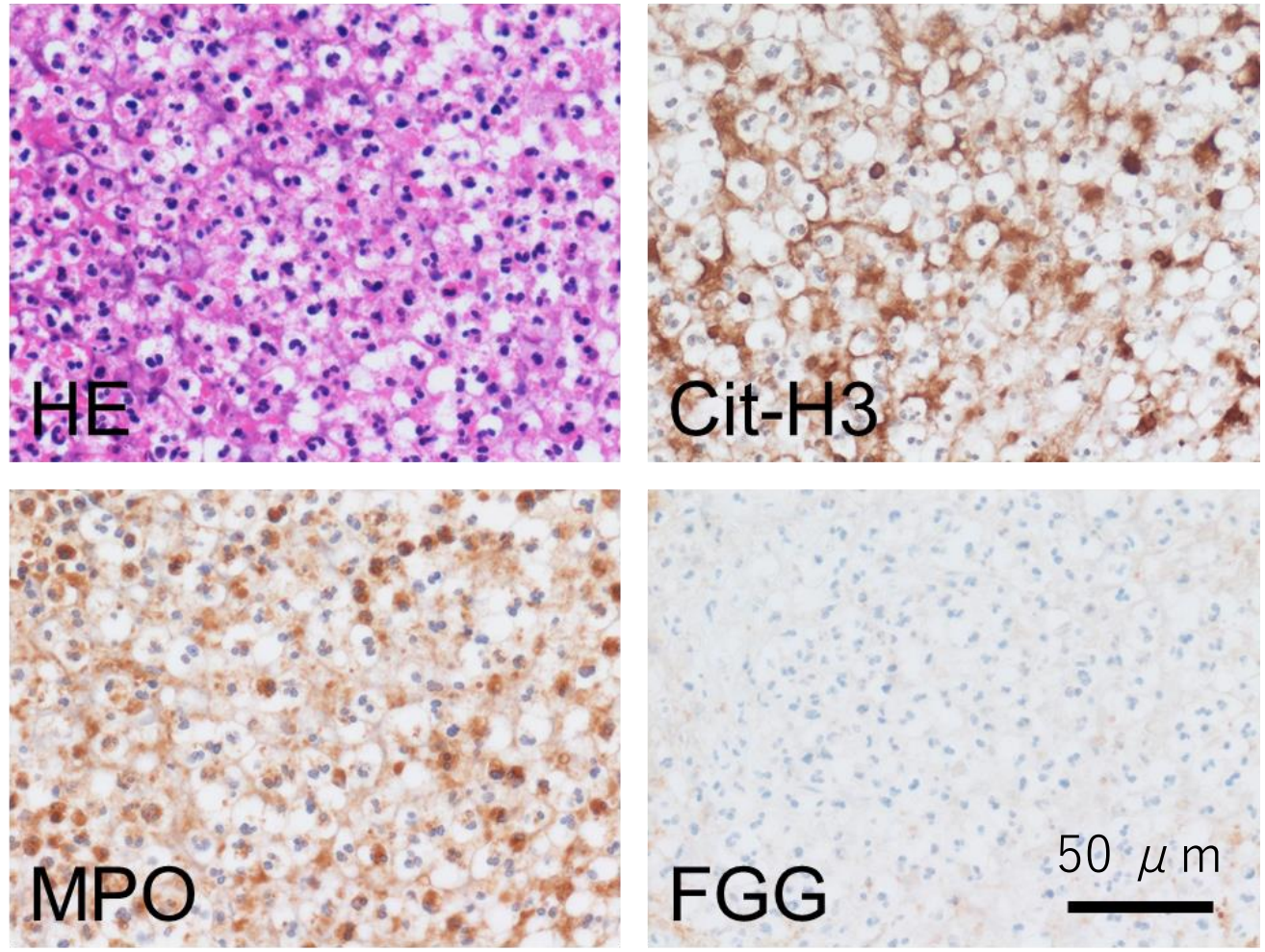


categorization of the fibrils into three types



Thin fibrils

Case 1:
Appendicitis

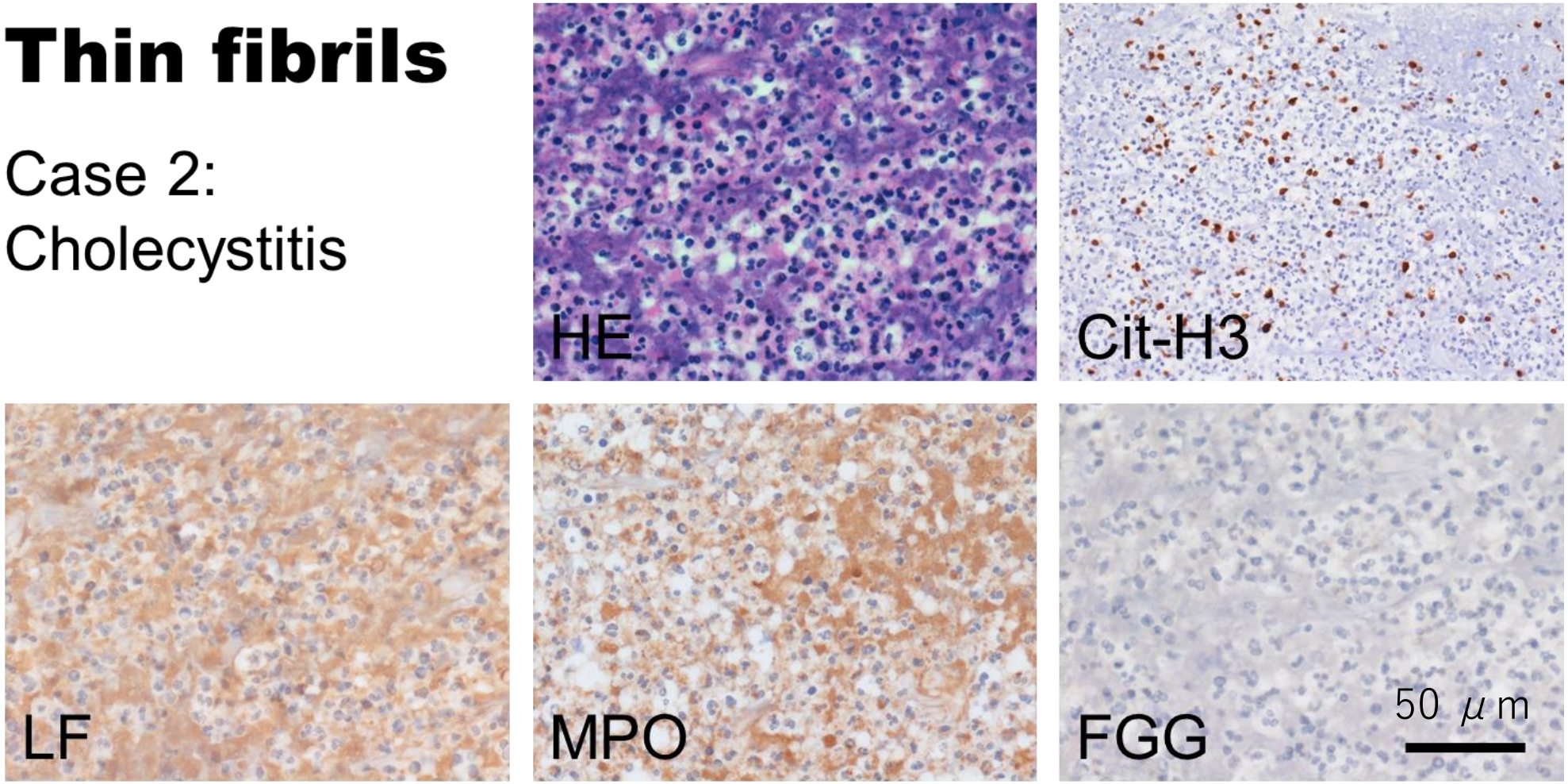


Thin eosinophilic fibrils belonged to NETs.

[Cit-H3 (+) /LF (+) /MPO (+) /FGG (−)]

Thin fibrils

Case 2:
Cholecystitis

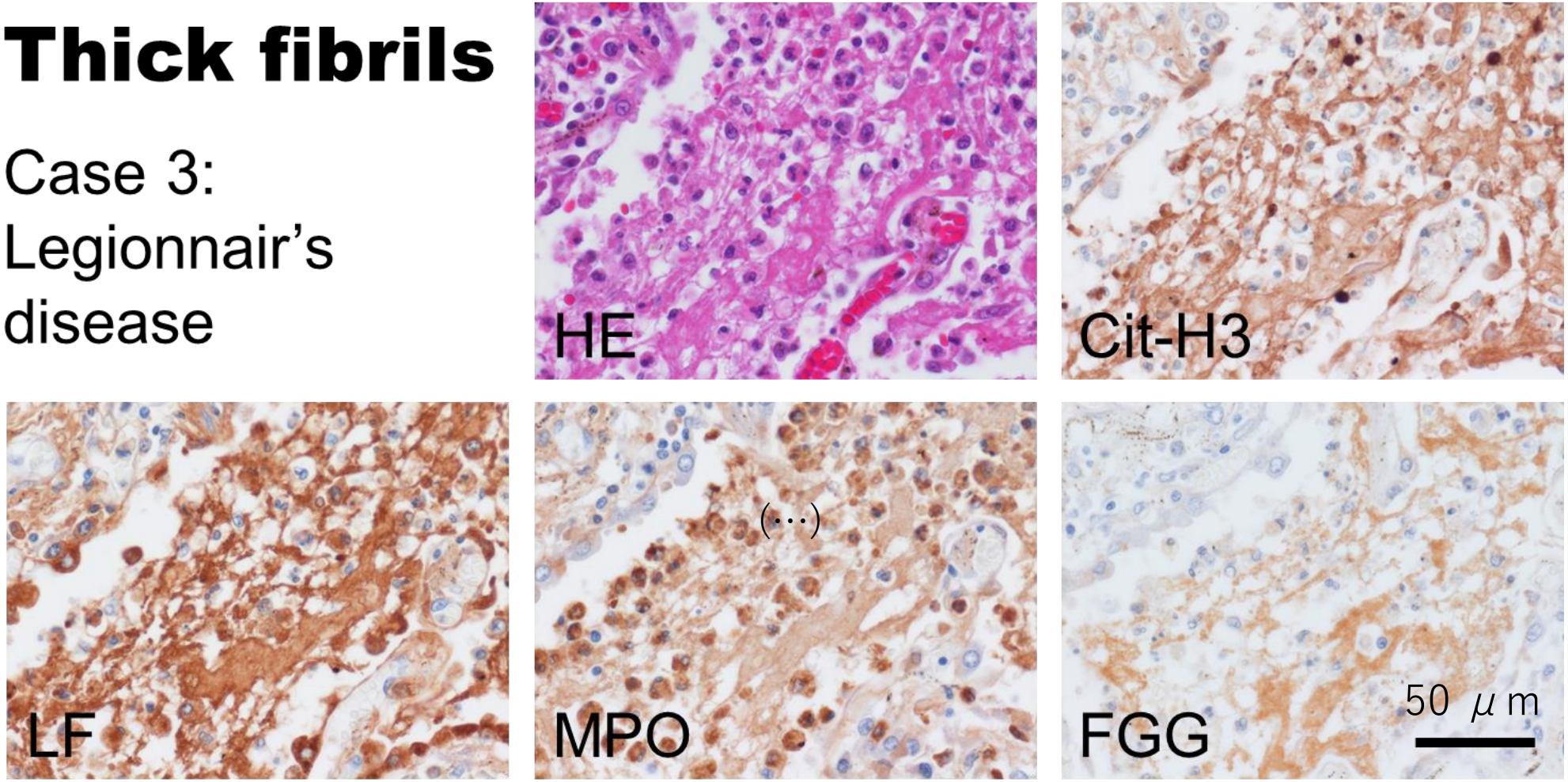


Cit-H3 showed negative immunoreactivity in chromatin-rich basophilic thin NETs fibrils.

[Cit-H3 (−) /LF (+) /MPO (+) /FGG (−)]

Thick fibrils

Case 3:
Legionnair's disease

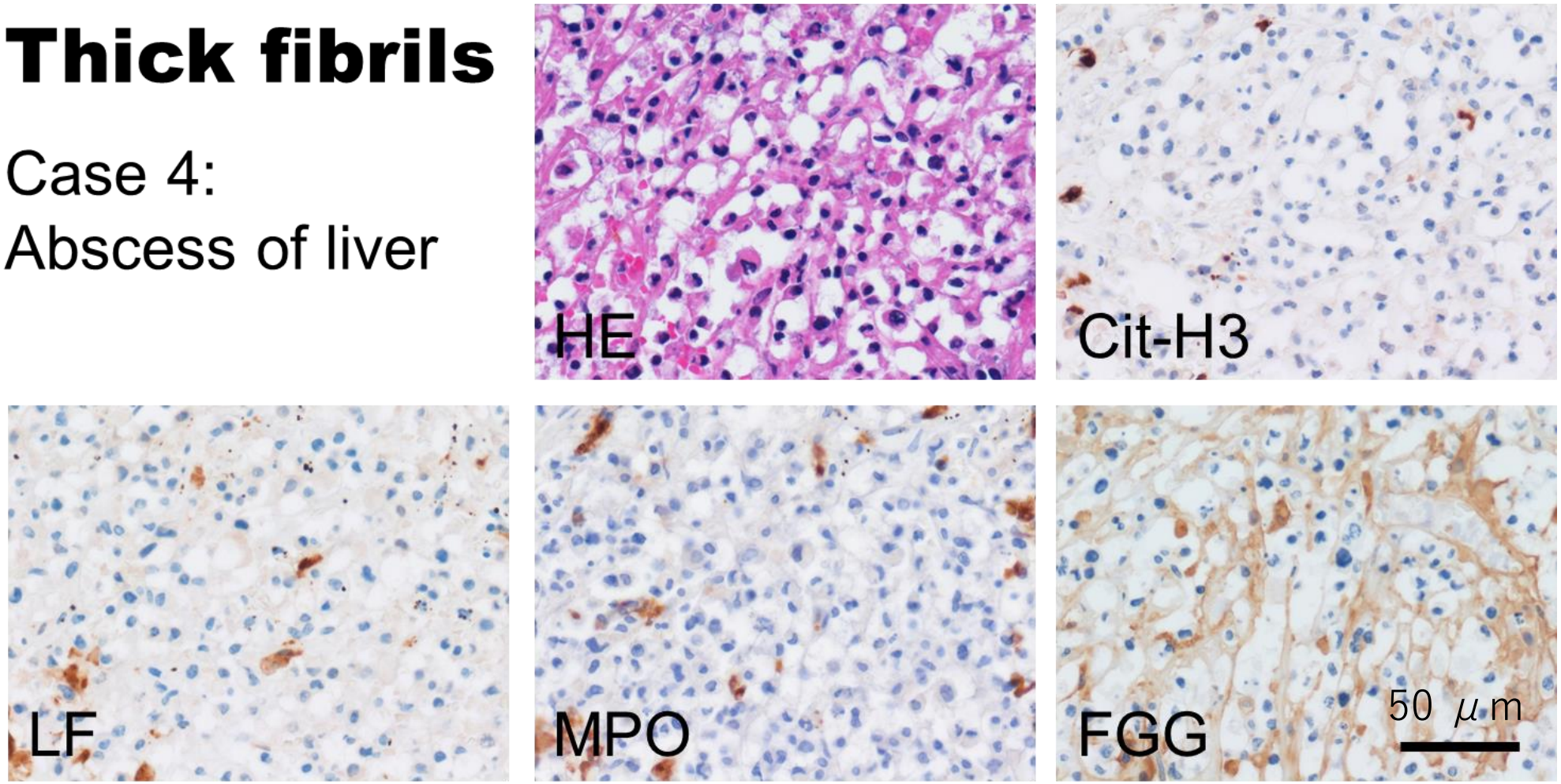


Thick fibrils are composed of both NETs and fibrin.

[Cit-H3 (+) /LF (+) /MPO (+) /FGG (+)]

Thick fibrils

Case 4:
Abscess of liver

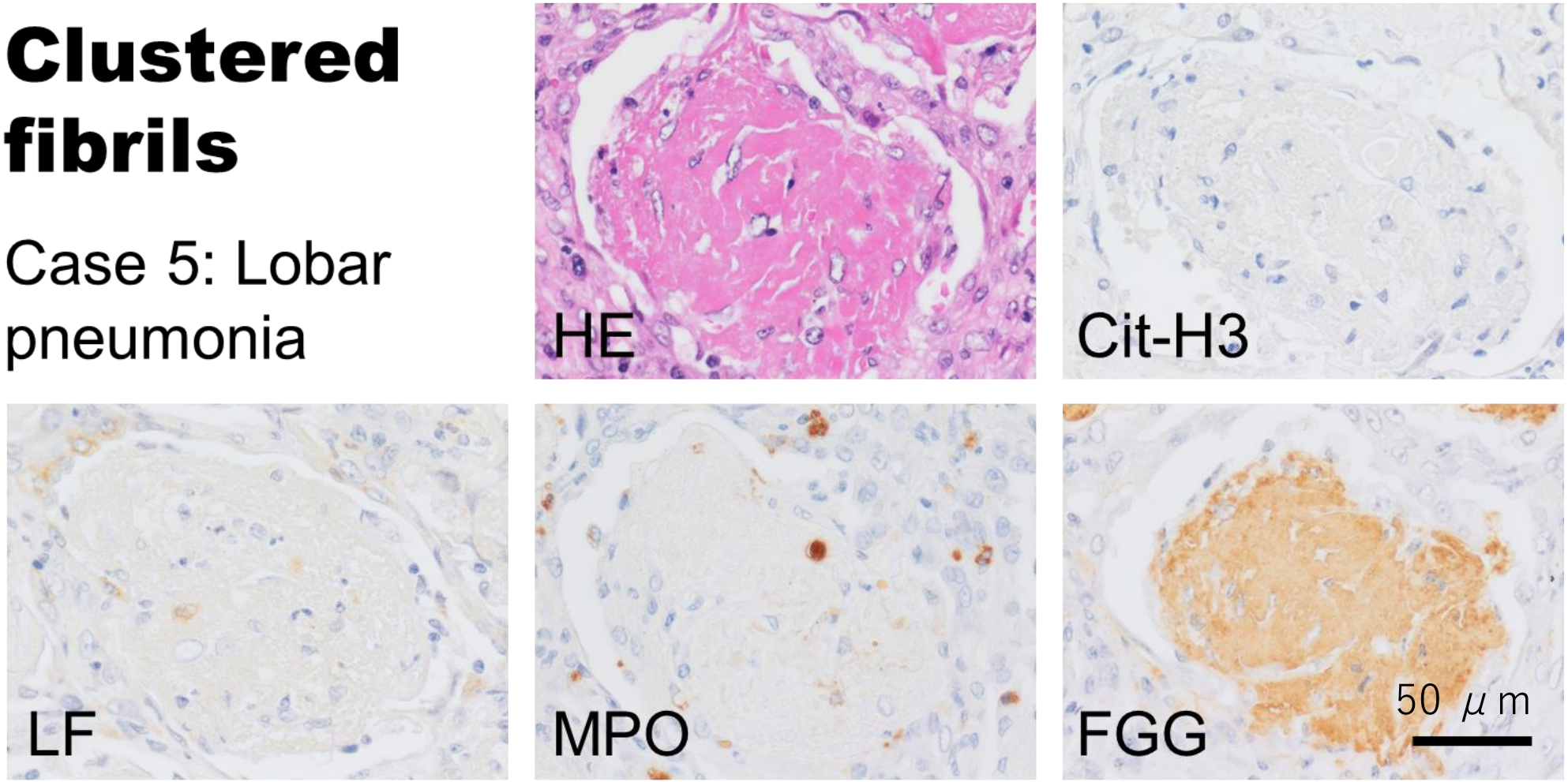


Thick fibrils in this case are solely composed of fibrin.

[Cit-H3 (−) /LF (−) /MPO (−) /FGG (+)]

Clustered fibrils

Case 5: Lobar pneumonia



Clustered thick fibrils are solely composed of fibrin.

[Cit-H3 (−) /LF (−) /MPO (−) /FGG (+)]

Comparison with fibril thickness and NETs markers

	Cit-H3	LF	MPO
Thin fibrils (n=18)	14 (78%)	18 (100%)	17 (94%)
Thick fibrils (n=23)	7 (30%)	18 (78%)	11 (48%)
Clustered fibrils (n=9)	0 (0%)	0 (0%)	0 (0%)

Lactoferrin is the best NETs marker, regardless of fibril size.

Relationship between fibril thickness and LF/FGG immunoreactivities

	NETs & fibrin LF＋/FGG＋	NETs LF＋/FGG－	Fibrin LF－/FGG＋
Thin fibrils (n=18)	7 (39%)	14 (78%)	0 (0%)
Thick fibrils (n=23)	18 (78%)	0 (0%)	17 (74%)
Clustered fibrils (n=9)	0 (0%)	0 (0%)	9 (100%)