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**Title:** Immunohistochemical analysis of endobronchial biopsies of patients infected with Puumala hantavirus

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**Body:** Puumala Hantavirus (PUUV) causes nephropathia epidemica (NE) and involves fever, hemorrhagia and acute renal failure but airway symptoms are common. Inhalation of aerosol-containing virus is a common route of infection. Studies have shown the involvement of a cytotoxic cell response, together with activation of endothelial cells however research into this disease in the lung is limited. To investigate the local immune response in endobronchial biopsies of NE patients. We hypothesize that an increased inflammatory response occurs within the lower airways of patients with NE. 17 NE patients and 16 age and smoking-matched healthy controls underwent bronchoscopy. PUUV infection was confirmed using PUUV-specific IgM/IgG in serum. Bronchoscopy was performed 6-14 days after on-set of symptoms and endobronchial biopsies were processed into GMA for IHC and stained for inflammatory cells, endothelial cells and ICAM. Staining was corrected for submucosal area and epithelial length. Activated blood vessels were expressed as the ratio of ICAM+ to EN4+ vessels and corrected for submucosal area.

## Submucosal staining

	NE	Control	p value
Neutrophils	30(14.3)	13.5(11.4)	<0.01
Mast cells	24.6(8.2)	16(9.9)	<0.05
CD8+ cells	12.7(12.8)	3.3(7.2)	<0.05
NK cells	0.66(1)	0(0)	<0.01
ICAM:EN4	0.37(0.22)	0.25(0.14)	<0.05

Results shown as median(IQR)/mm<sup>2</sup>

There was no significant difference in macrophages, eosinophils, T cells, CD4+ cells and B cells within the submucosa or between any cell type within the epithelium. NE involves inflammation of the lung comprised of an infiltration of neutrophils, CD8+ and NK cells and increased ICAM expression in blood vessels. A

cytotoxic response is likely to be important in the pathogenesis of NE.