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Kidney histopathological findings in fatal pandemic 2009 influenza A (H1N1)

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Dear Editor,

A new pandemic was originated by a novel influenza A (H1N1) virus [1–3]. Severe cases were characterized by acute respiratory distress syndrome (ARDS), shock, and acute kidney injury (AKI) [3]. Lung histopathological changes in fatal cases showed signs of diffuse alveolar damage, necrotizing bronchiolitis, and occasional alveolar hemorrhage [2]. However, histopathological changes in organs other than the lungs are not known. Here we report kidney histopathological findings and describe for the first time the specific kidney cell type targeted by pandemic 2009 influenza A (H1N1) virus infection.

With the approval of our Ethics Committee and with closest relative informed consent, renal biopsies from four patients who died in the intensive care unit (ICU) with diagnosis of confirmed influenza A (H1N1) virus infection were studied by microscopy after hematoxylin and eosin (HE), Masson's or periodic acid-Schiff (PAS) staining. Cell nuclei were revealed by staining with 4',6-diamidino-2-phenylindole (DAPI). Localization of viral antigen and specific kidney cells was carried out by double immunofluorescence (IF) labeling [4] using antibodies (Santa Cruz) specific for either: (1) aquaporin 1, a marker of proximal tubular cells; (2) CD10, a marker of proximal tubular cells; (3) cytokeratin 7, a marker of distal tubular cells; or (4) CD34, a marker of endothelial cells, and a rabbit antiserum specific for influenza nucleoprotein (NP). This antibody was generated by immunization of rabbits with purified recombinant NP and validated by IF, Western blotting, and immunoprecipitation of control and influenza-infected human cells [5]. This antibody is cross-reactive with several influenza A virus subtypes (data not shown). Secondary antibodies were fluorescein isothiocyanate (FITC)-labeled goat anti-mouse immunoglobulin G (IgG) (Santa Cruz) and Alexa 546-conjugated goat anti-rabbit IgG. Sections were studied under confocal microscopy (Leica SP5), and single optical sections are presented.

Only cases 3 and 4 were diagnosed with AKI. Cases 3 and 4 had focal changes consistent with acute tubular necrosis (ATN) in the distal tubules (epithelial cell swelling, individual cell necrosis, and shedding of

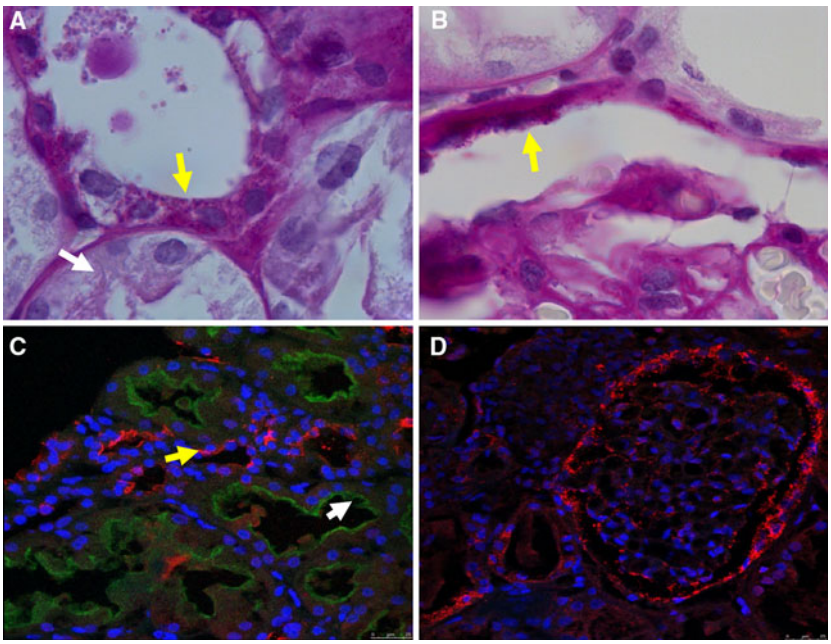

Fig. 1 Representative photomicrographs of kidney pathology (light microscopy), and demonstration of viral nucleoprotein. **a** (HE ×40) (case 3). Distal tubules with necrotic debris and shed necrotic tubular cells in their lumina. Red intracytoplasmic granules are widely seen in epithelial cells (*yellow arrow*). In contrast, a nearby undamaged proximal tubule with normal tubular epithelium and intact brush border is seen (*white arrow*). **b** (HE ×40, PAS stain) (case 3). The edge of a glomerulus shows the Bowman's capsule with numerous red intracytoplasmic granules in parietal cells (*arrow*). **c** Immunofluorescence for proximal tubular cells (CD10) (in *green*, *white arrow*) and viral nucleoprotein (in *pink*, *yellow arrow*) in tubular structures that are not proximal tubular cells. Nuclei are shown in *blue*. **d**. Immunofluorescence for viral nucleoprotein (in *red*) in a glomerular structure. Nuclei are shown in *blue*

necrotic and viable epithelial cells into the tubular lumina). Although post mortem autolysis of renal tubules has features indistinguishable from ATN, we could not identify diffuse damage of proximal renal tubules attributable to autolysis, such as loss of tubular cell brush border or prominent cell vacuolization, in any of the cases reported here. Both biopsies had PAS-positive intracytoplasmic granules in tubular epithelial cells and in the parietal and visceral epithelium of the Bowman's capsule. Endothelial cell lesions were not seen in any of the cases. In cases 3 and 4 there was increased immunoreactivity for viral NP, specifically in the distal tubules and the Bowman's capsule epithelia (Fig. 1), and the level of NP suggested active virus replication in these cells. The predominant cytoplasmic localization of the NP signal suggests these cells are in a late phase of virus infection [5].

In summary, kidney pathological changes in influenza A (H1N1) virus infection are consistent with ATN and persistence of viral infection despite antiviral treatment. Bowman's capsule epithelial cells and distal tubular cells seem to actively replicate the virus.

Conflict of interest None.

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