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Illustrated histopathological features of fatal dengue cases in Colombia

Fatal dengue histopathology

Histopatología ilustrada de casos fatales de dengue en Colombia

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Jaime E. Castellanos: Results analysis and manuscript revision.

María Leonor Caldas: Project design, analysis of results and manuscript revision.

Dengue is the most important arboviral disease in humans in tropical and subtropical countries and considered endemic by the World Health Organization. It has been estimated that 390 million cases occur annually, 96 of them clinically diagnosed as symptomatic dengue fever (1). Although most infections are asymptomatic, the more severe forms of dengue (formerly called hemorrhagic or shock syndrome) could result in organ failure or death (2), with around a half million cases reported each year, an estimated average of 10,000 fatal cases per year (between 1990 and 2013) and a peak in 2010 (11,302 fatal cases) (2). Dengue incidence has seen a 30-fold increase in the last 50 years (3), making it a major public health concern currently.

In Colombia, dengue virus infection has an endemic and epidemic behavior with a steady increment in the last 20 years. The Colombian national surveillance system reported the highest historic peak of dengue cases in 2010 (157,000 cases) and 9.777 severe dengue cases, with a worrying 217 fatalities (2.28% lethality rate) (4). During 2011, there was a decrease in dengue cases, but the lethality rate increased to 3.75% (5). The burden of the 2010 dengue epidemic was 14-fold higher than those of the years 2011 or 2012 (57.017 vs. 3.989 disability-adjusted life years were lost, respectively). Additionally, the estimation of the 2010 epidemic costs rose to US\$ 65.5 million, almost 4-fold higher expenses compared to a regular endemic or epidemic year. Approximately 30% of these costs corresponded to those involving the loss of income due to fatalities.

Dengue is caused by a virus that belongs to the *Flaviviridae* family of the genus *Flavivirus* and presents four antigenically different serotypes (DENV-1 to 4). Each serotype is capable of producing an asymptomatic infection or an infection with

clinical signs and symptoms ranging from a mild febrile disease to a severe infection characterized by an imbalance of endothelial function leading to massive plasma leakage, severe hemorrhage and multi-organ failure (6). The infection could be fatal, involving different organs such as the liver, brain, spleen, lungs, and kidney (7).

Different cell types can be infected by dengue virus and pathological manifestations can be variable (8,9). Most severe cases show damage in the vascular endothelium which results in plasma extravasation and hemorrhage, liver function impairment with high transaminase levels and histologic alterations (10-12).

Histopathological analyses of fatal cases indicate that the liver and spleen are the organs most affected during dengue virus infection. In the liver, it is common to find small necrotic foci, microvesicular steatosis, hyperplasia and apoptosis of Kupffer cells, lymphocyte infiltration in the portal tract and Councilman bodies (necrotic foci, acidophilic bodies and pyknotic nuclei) (13-16), although occasionally intranuclear glycogen can be found (17).

Regarding the spleen, histopathological analysis shows interstitial edema, white pulp vascular and cellular congestion with reactive hyperplasia (13,18). Atypical alterations have also been reported in the kidneys, lungs, heart, and brain in which hemorrhage, edema and leukocyte infiltrate are observed, with no specific morphological findings for each tissue (8,19-22).

On the other hand, immunohistochemistry for DENV antigens has revealed different distribution patterns, from the location of antigens in a single organ per case to the presence of antigens in multiple organs (8,9,18,22,23).

This work aims to describe, illustrate and compare different histological alterations found in 95 fatal confirmed dengue cases using 87, 42, 32, 37, 22, and 16 samples of liver, spleen, kidney, lung, heart, and brain, respectively. The study was carried out based on the review of histopathological slides with hematoxylin and eosin staining from 95 cases obtained of the pathology archive of the “Instituto Nacional de Salud” of Colombia (INS). Confirmation of death due to dengue virus infection was performed by molecular techniques and by an analysis of the clinical history of the cases.

The main alterations found in the liver were necrosis (78.2%) and hyperplasia of Kupffer cells (82.7%), while in the spleen, reactive plasmacytosis (69%) and vascular congestion (92.9%) were the most observed findings. Edema was the most frequent alteration found in the lungs (83.8%) and brain (68.8%).

Interestingly, most heart (77.3%) and kidney (65.3%) tissues had a normal histopathological aspect, without any other specific finding (see figures 1-12).

Figure 13, illustrates in more detail the frequencies of the alterations found in each tissue.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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The organs and its histopathology alterations

The liver

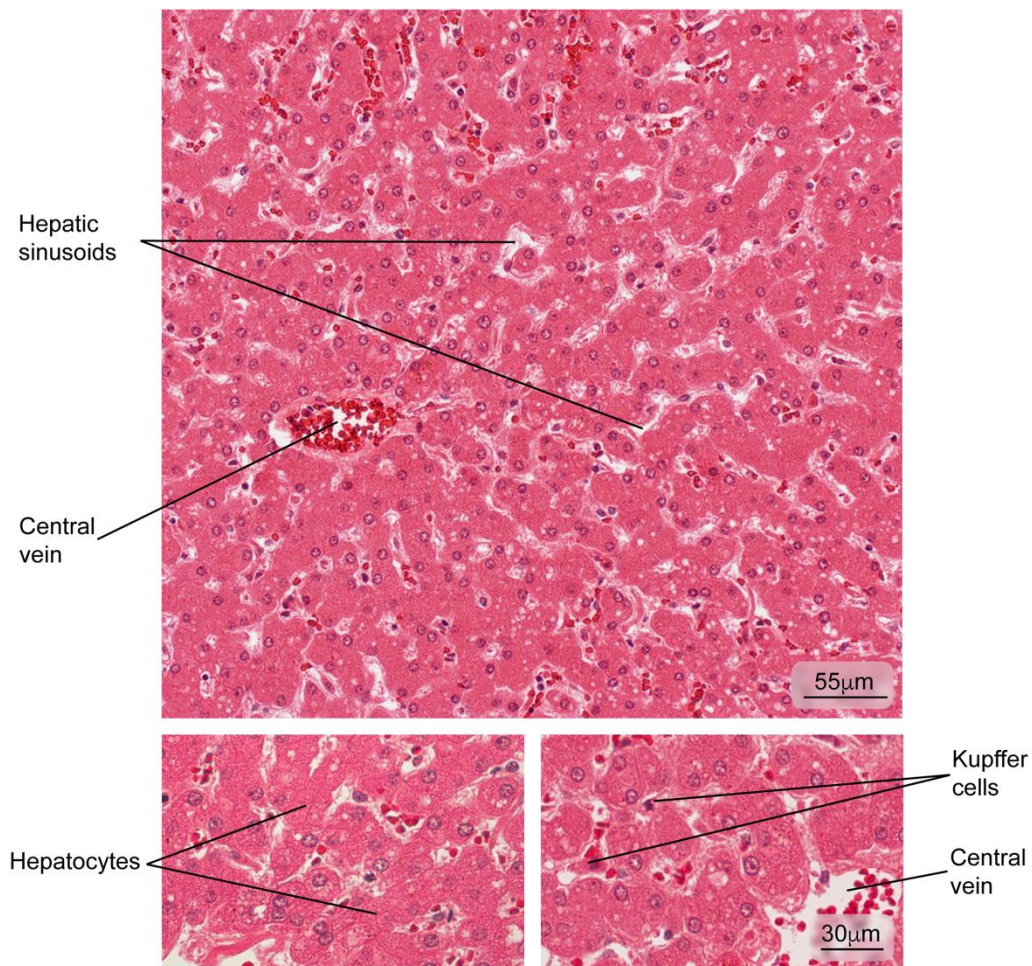


Figure 1. Normal liver tissue. Part of a hepatic lobule is observed; note the radial distribution of the hepatocyte plaques from the central vein. Some phagocytic cells (Kupffer cells) and hepatocytes in greater magnification are observed. H&E staining.

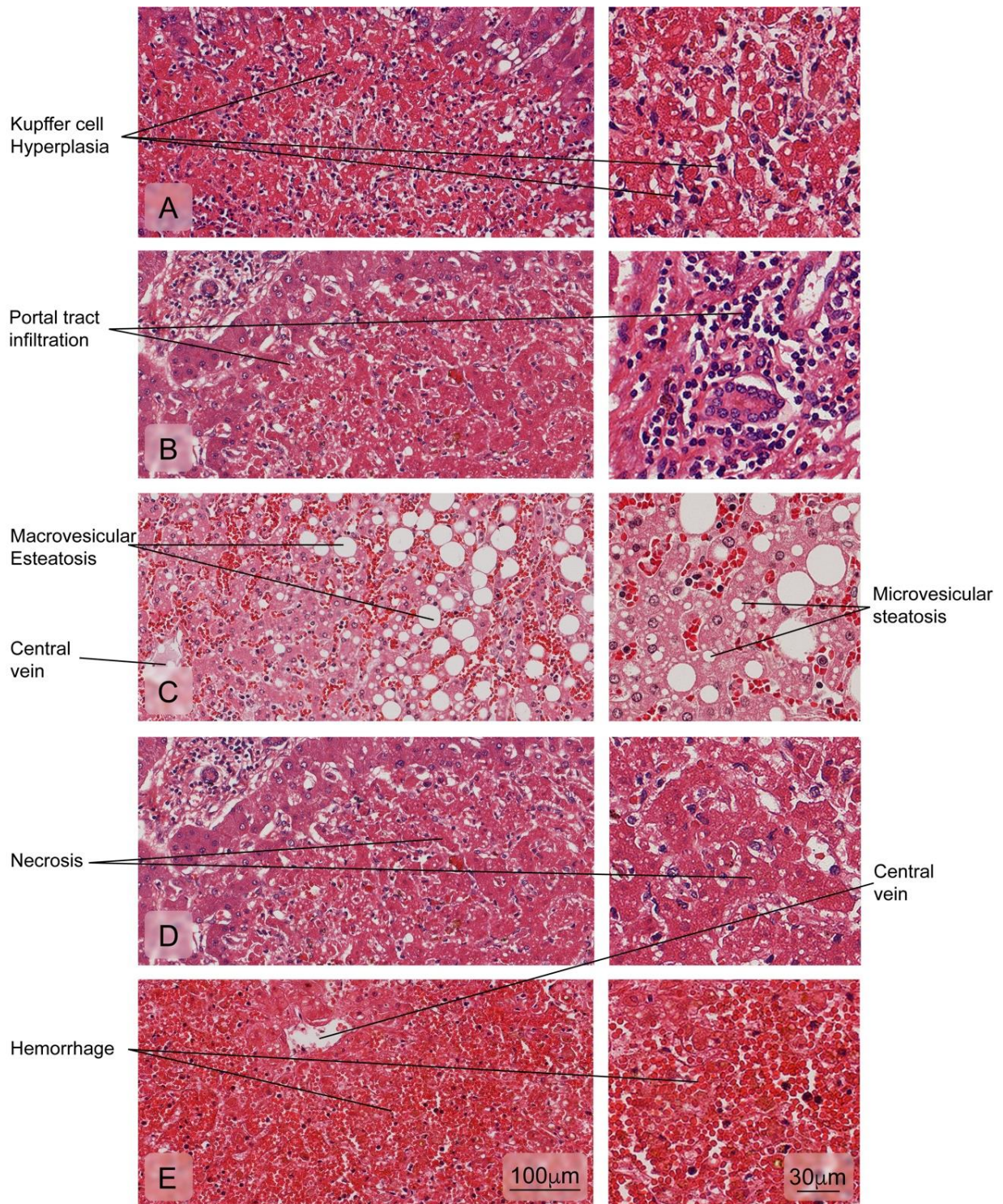


Figure 2. Alterations of the liver tissue showing: A) Kupffer cell hyperplasia, B) portal tract leukocyte infiltration, C) hepatic fatty degeneration (macro and microvesicular steatosis), D) image shows an area of necrosis with loss of radial

arrangement of hepatocyte plaques. A pale eosinophilic stain, pyknotic or absent nuclei are observed; and E) hemorrhages. H&E staining.

The spleen

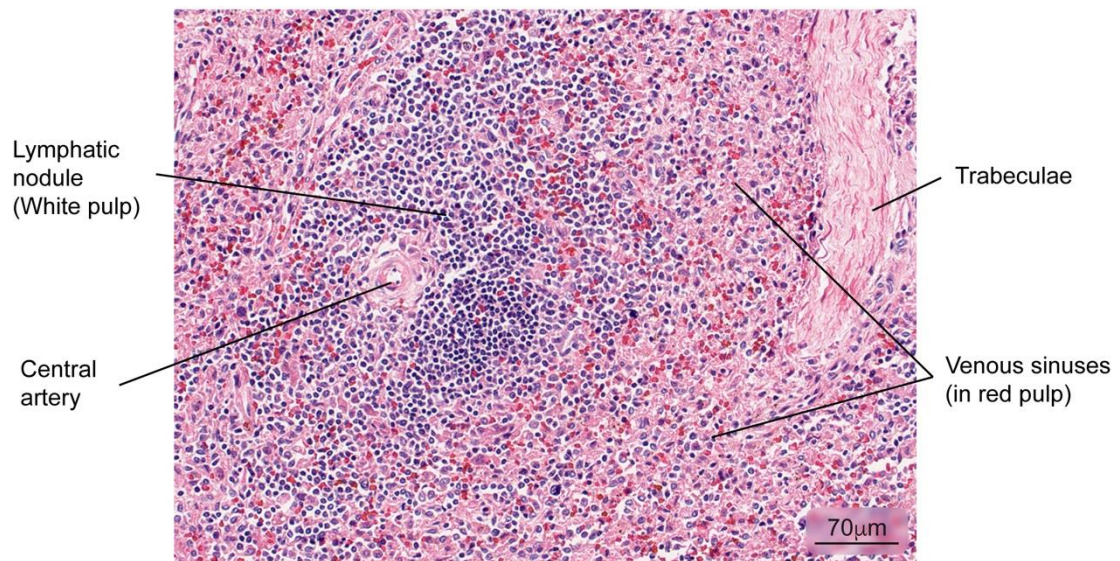


Figure 3. Normal splenic tissue. A small lymph node with a peripherally located central artery is observed. Connective tissue trabeculae are evident. H&E staining.

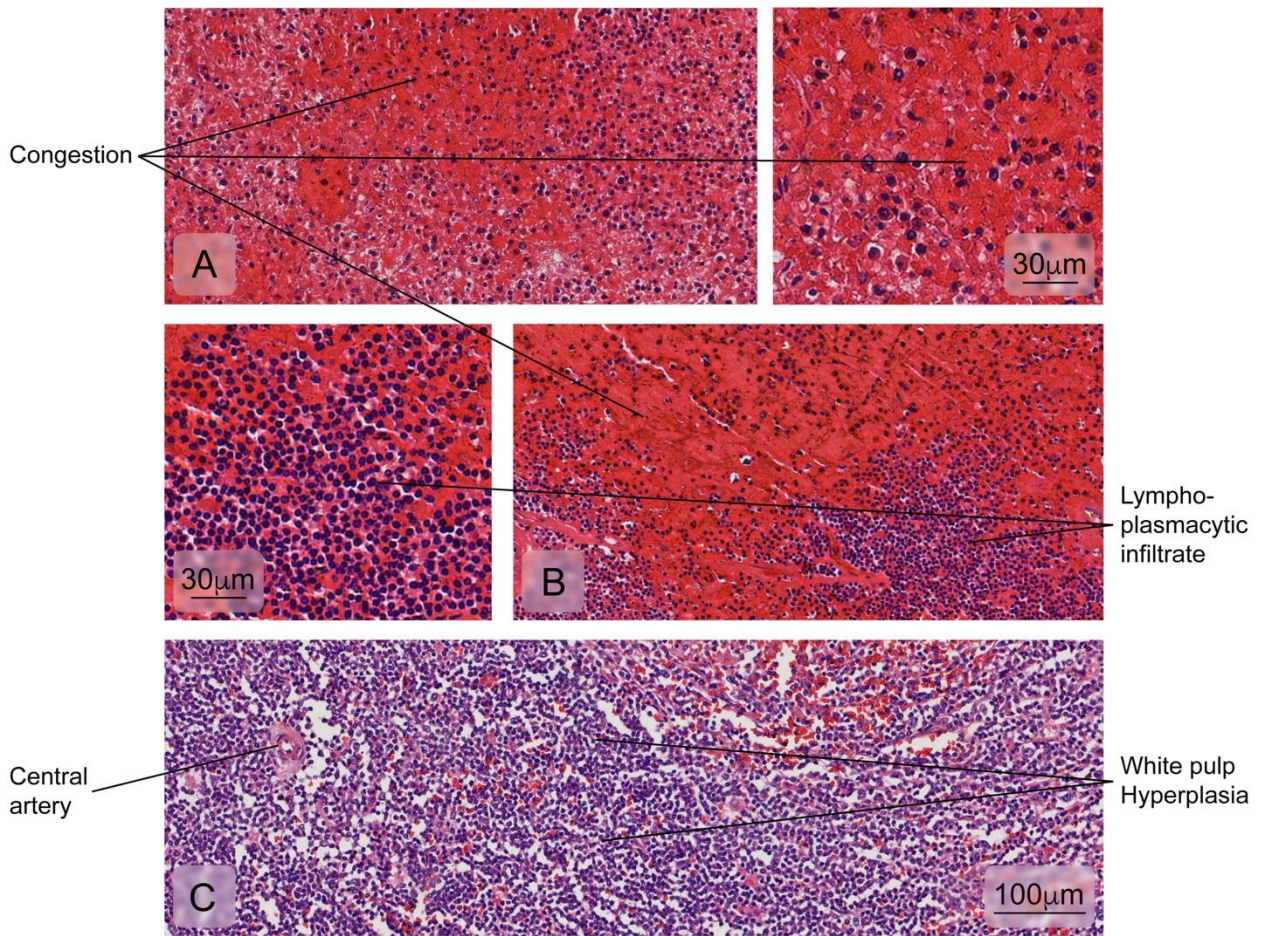


Figure 4. Different kind of splenic tissue alterations are illustrated in: A) venous sinuses congestion in the red pulp, B) lymphoplasmacytic cell infiltration in the red pulp and C) white pulp hyperplasia. H&E staining.

The Kidney

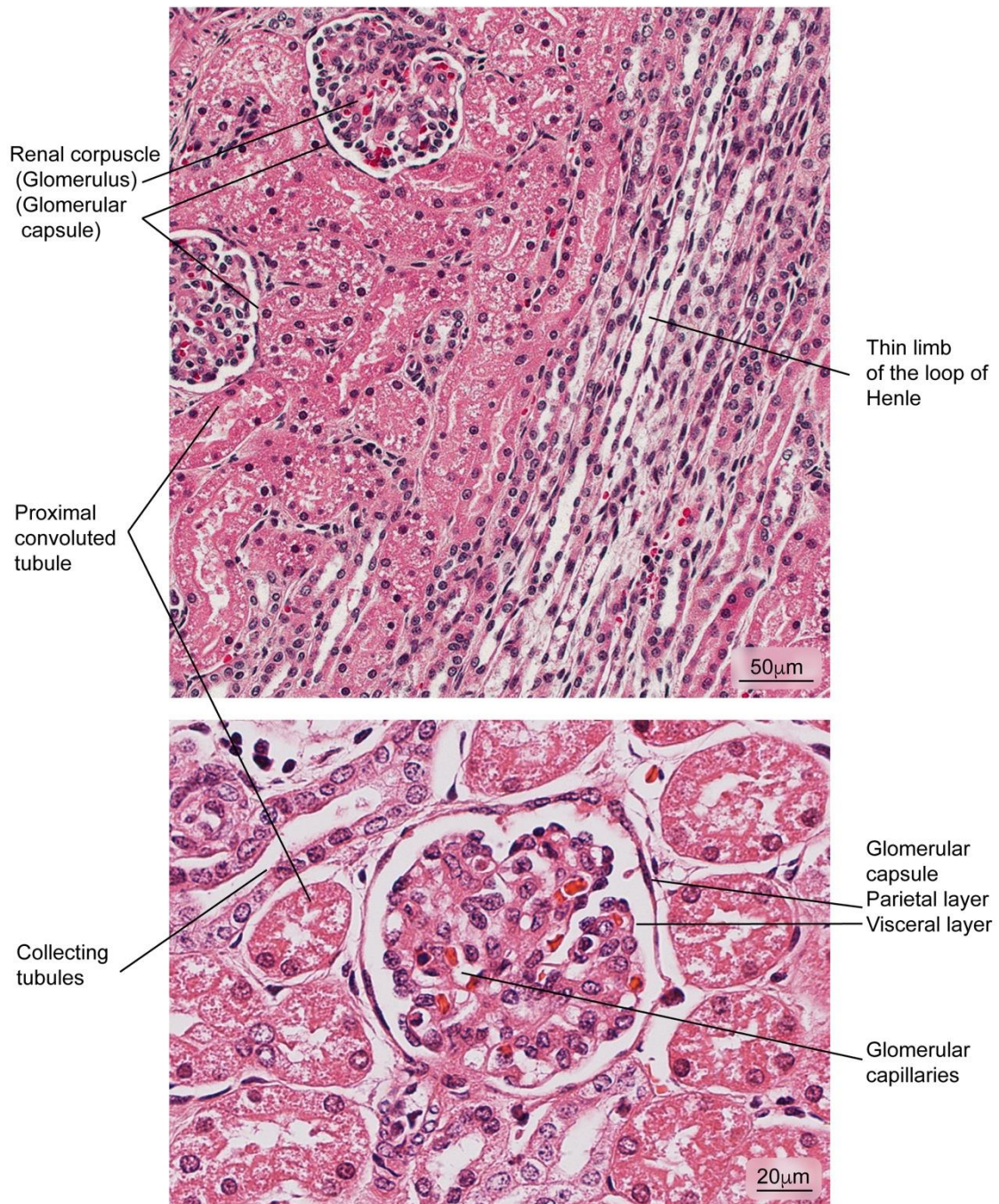


Figure 5. Aspect of normal kidney tissue. Renal corpuscles are observed in medullary areas and renal cortex; the glomerular capsule is shown in detail. H&E staining.

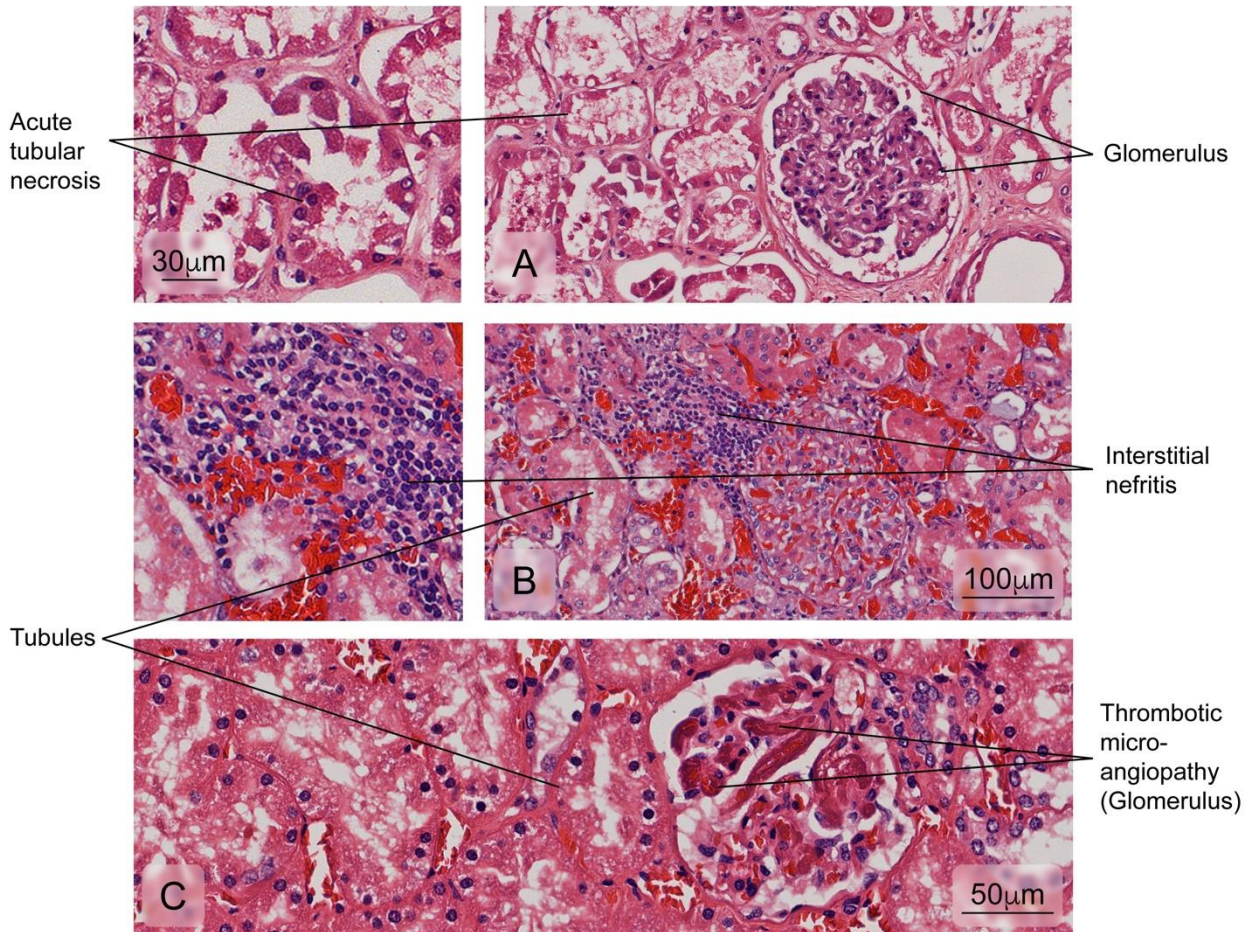


Figure 6. Alterations observed in renal tissue of cases are shown. A) Necrosis of the tubular epithelium, B) leukocyte infiltration of the interstitial space surrounding the renal tubules (interstitial nephritis), and C) vascular lesions accompanied by intraluminal platelet thrombosis obstructing the vascular lumen (thrombotic microangiopathy). H&E staining.

The heart

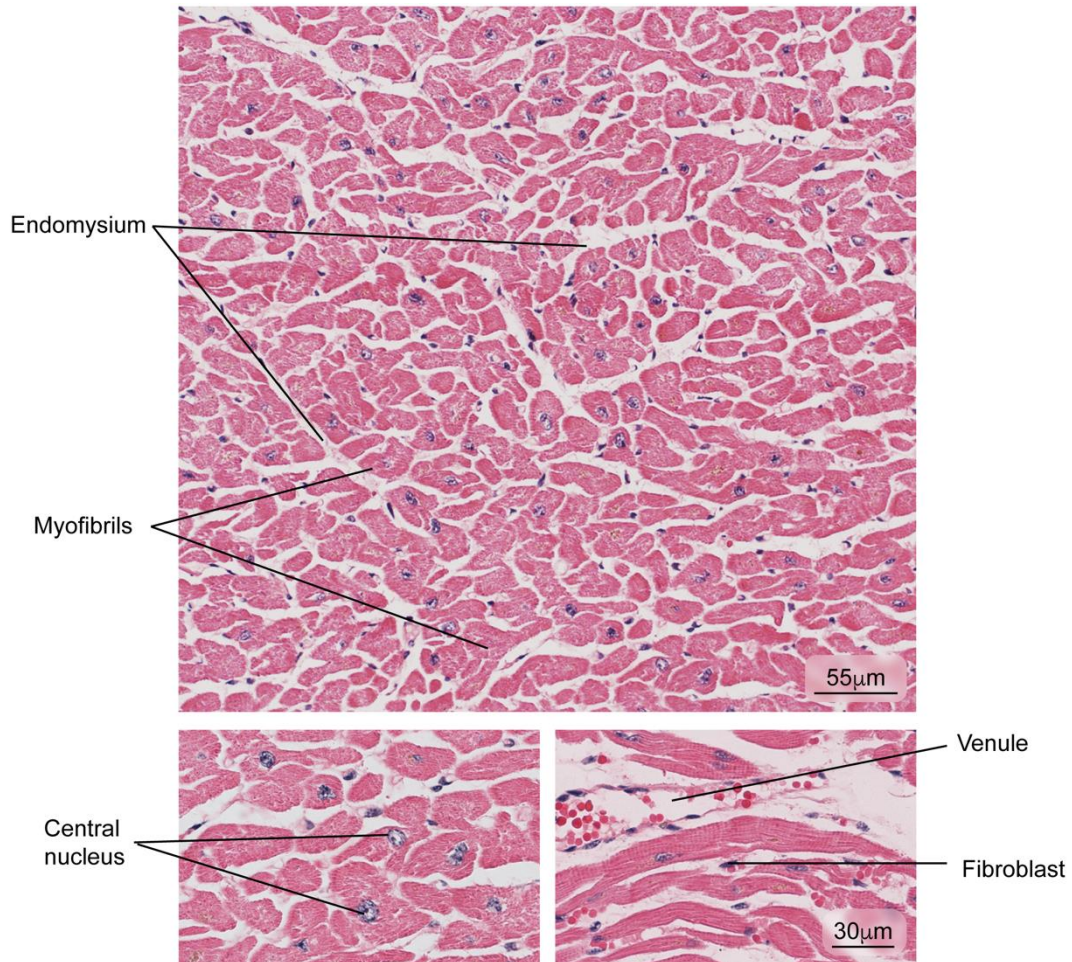


Figure 7. Aspect observed in those patients with normal cardiac muscle tissue. The myocardium composed of cardiac muscle fibers, venules and fibroblasts in the endomysium is observed. H&E staining.

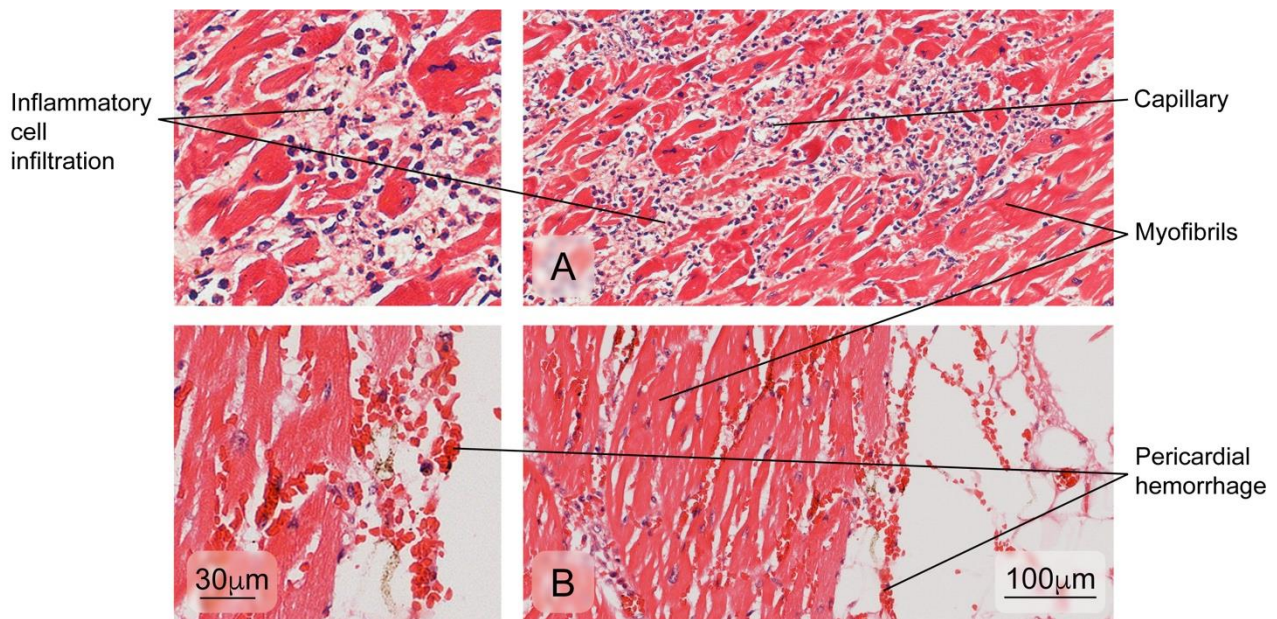


Figure 8. Aspect of cardiac muscle tissue in dead patients with anomalies. It can be observed A) inflammatory cell infiltration of the myocardium and B) pericardial hemorrhage. H&E staining.

The Lung

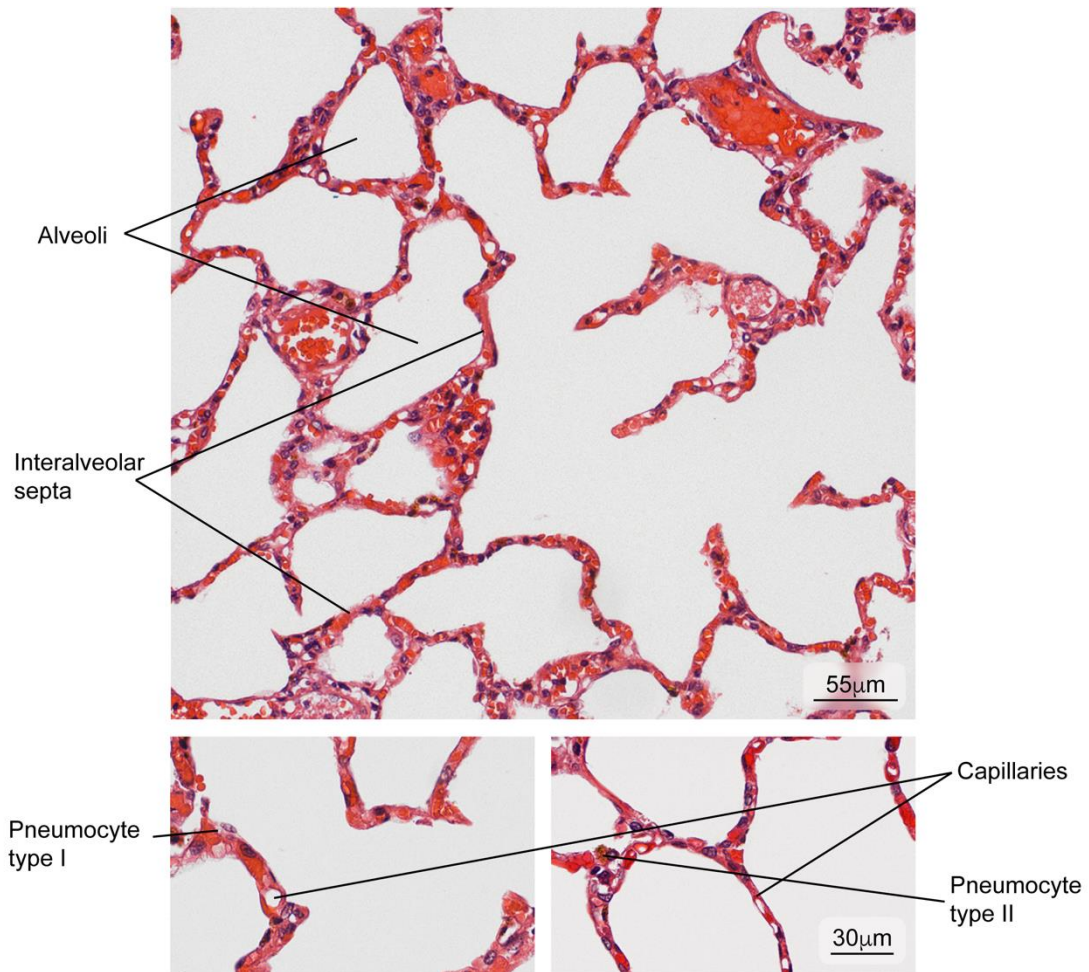


Figure 9. Normal lung tissue. An intrapulmonary structure is shown where gaseous exchange occurs (alveoli), and the main cell types in the interalveolar septum (type I and II pneumocytes) are also observed. H&E staining.

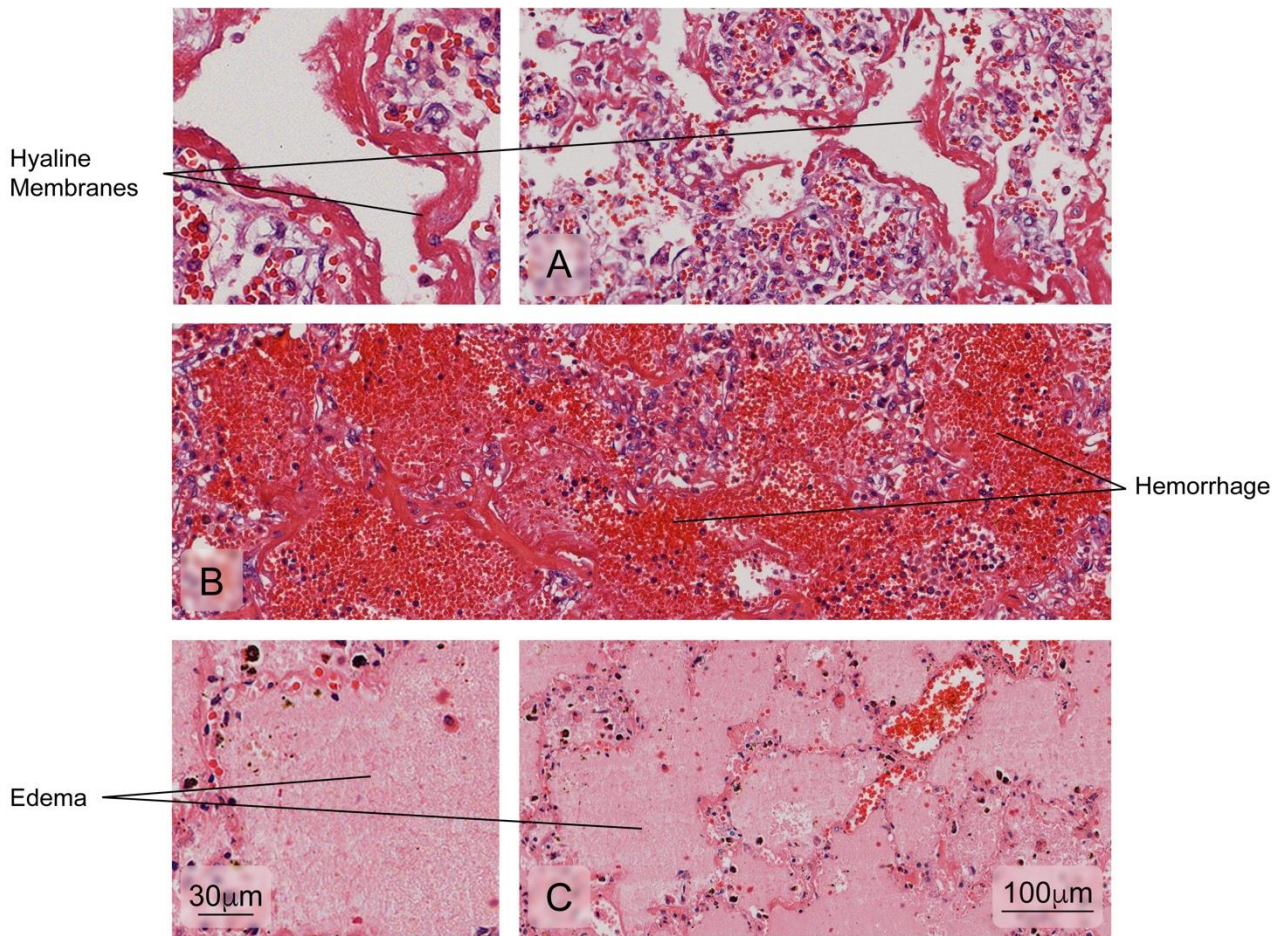


Figure 10. Alterations found in lung tissue of fatal dengue cases. Abnormal findings were observed: A) formation of hyaline membranes characteristic of diffuse alveolar damage (DAD), B) severe hemorrhages in the alveoli and C) presence of serous fluid in alveolar areas (edema). H&E staining.

The brain

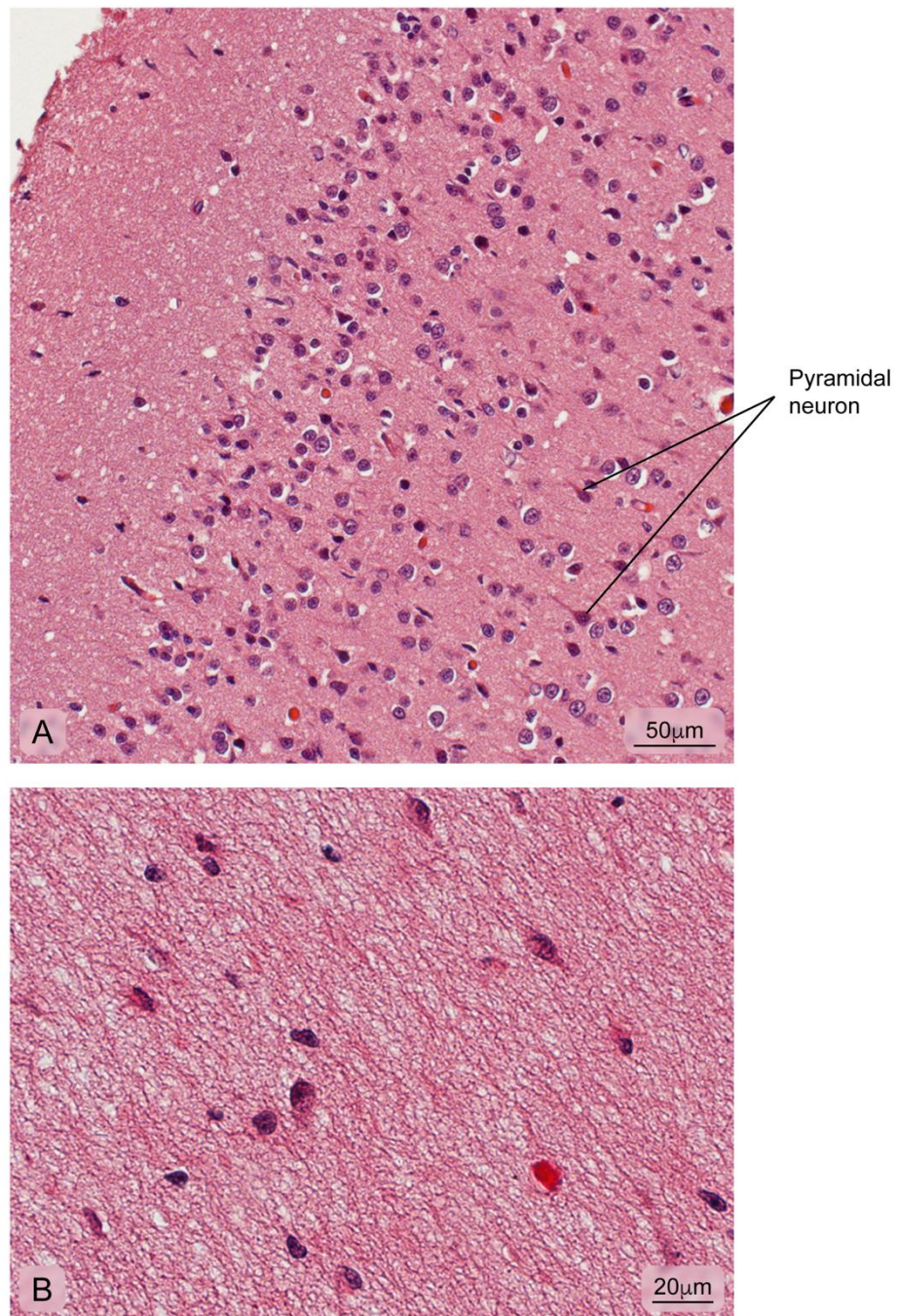


Figure 11. These images show normal cerebral cortex. In panel A) cerebral cortex outermost layer showing different kind of neuronal bodies, and B) a more internal part of the cortex in which the integrity of the neuropil is observed. H&E staining.

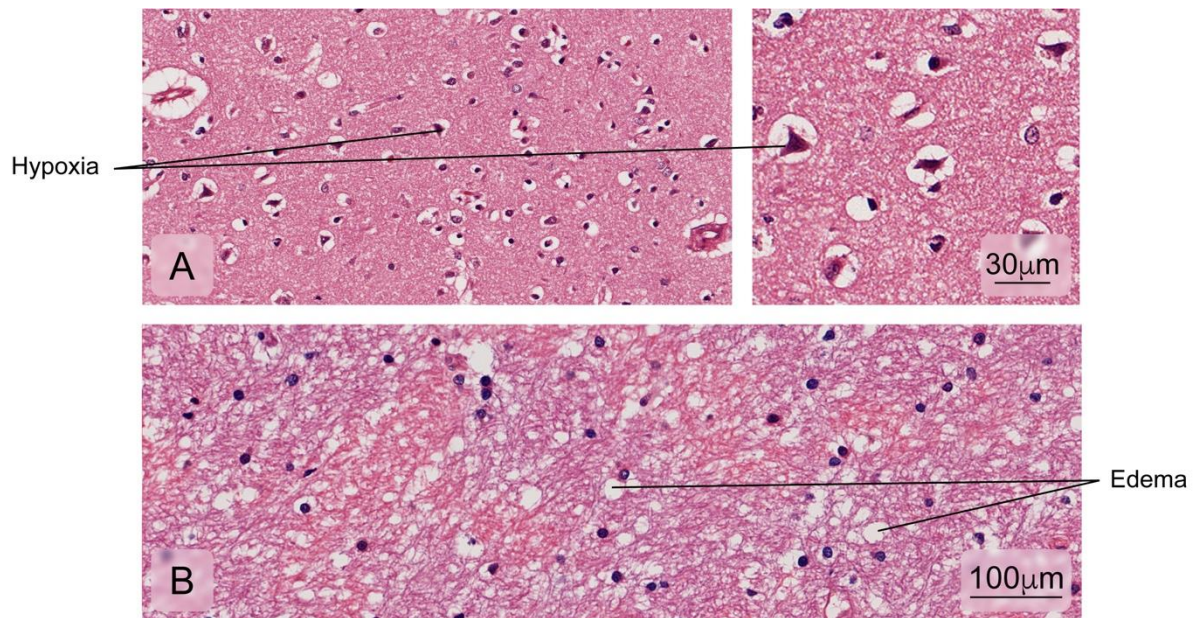


Figure 12. Alterations found in brain tissue-cerebral cortex. Some of the fatal cases presented A) decreased neuronal size due to retraction of the cytoplasm, with nuclei pyknosis and hyperchromasia associated with hypoxic cortical changes and B) edema. H&E staining.

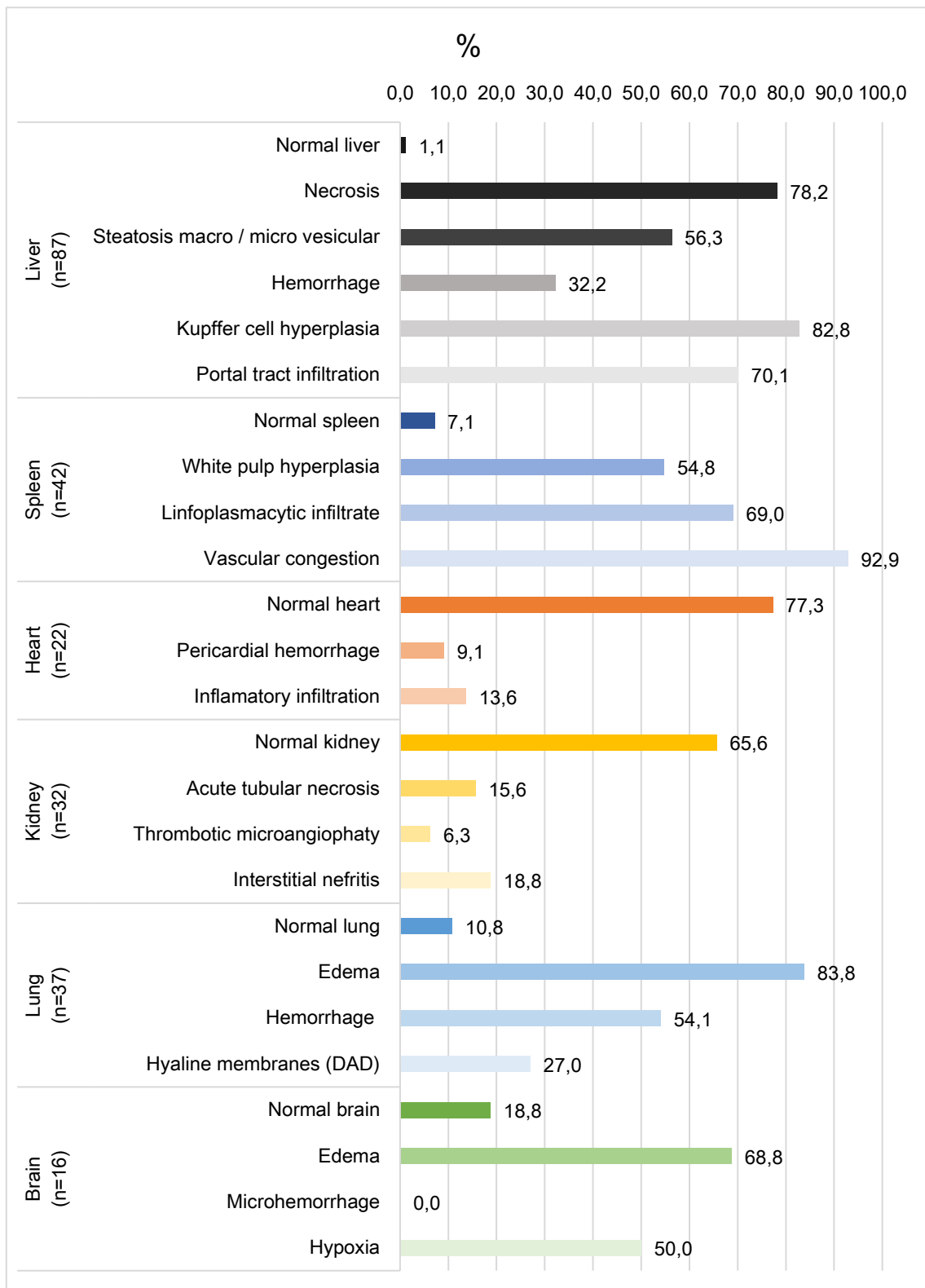


Figure 13. Percentage frequencies of different abnormalities evaluated in tissues from fatal cases