



An animal model of cerebral venous insufficiency: implication for neuroinflammation

A. Greco, M. Mancini

IBB, CNR, Naples, Italy

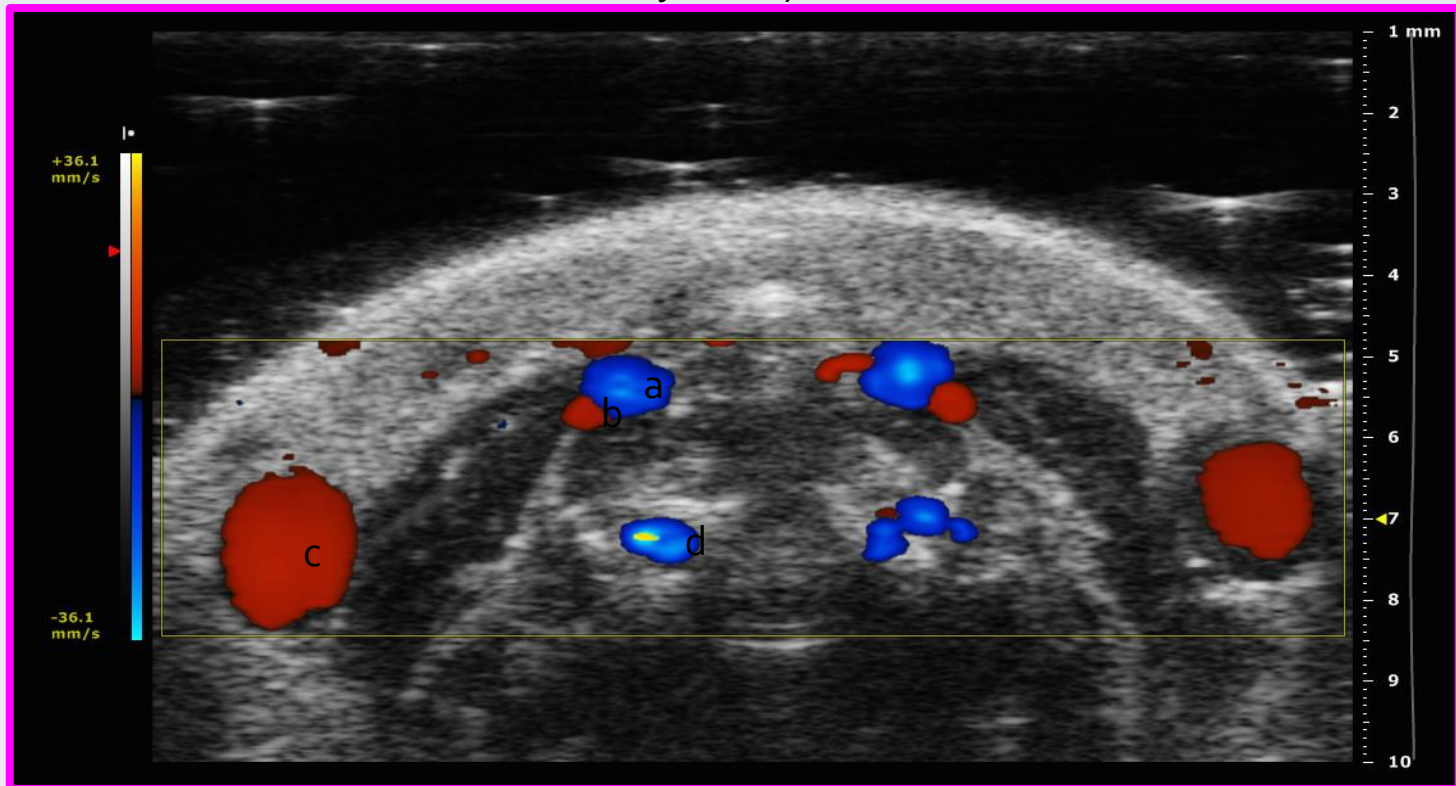
Dipartimento di Scienze Biomediche Avanzate, Università degli studi di Napoli Federico II.



Background

Vascular abnormalities in mice models of neurological disease have been widely investigated.

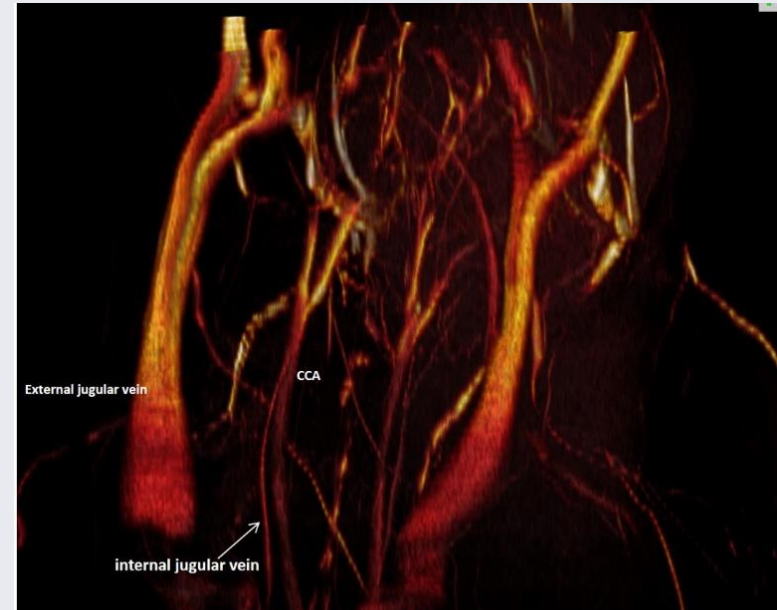
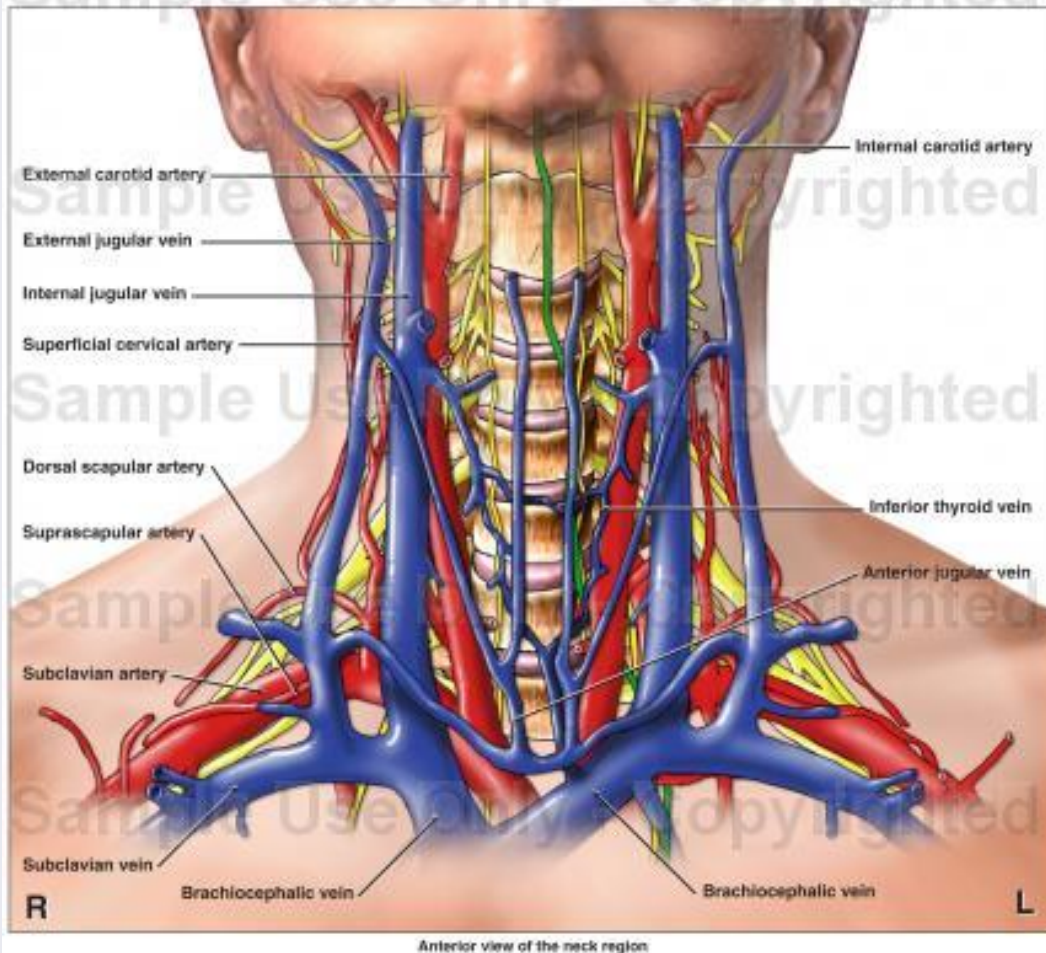
However, most of the studies present in literature are related to the arterial system. The blood returns from the central nervous system back to the heart via several venous systems in the neck and along the spine (extracranial venous drainage system)



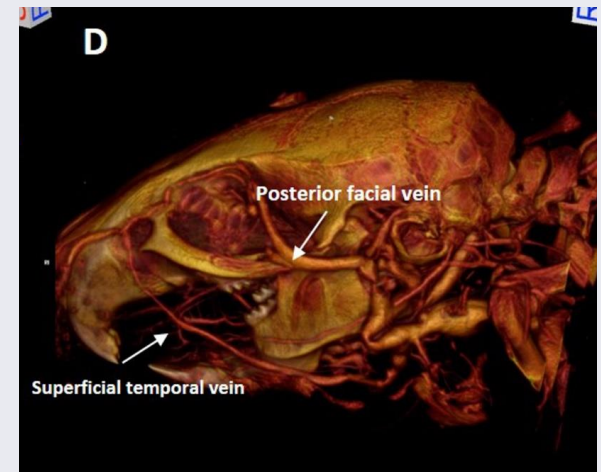
BACKGROUND

The human circulation system differs from that of the mouse in both structure and position.

Anatomy of the Cervical Region (Neck)



MR-Angiography of mice neck

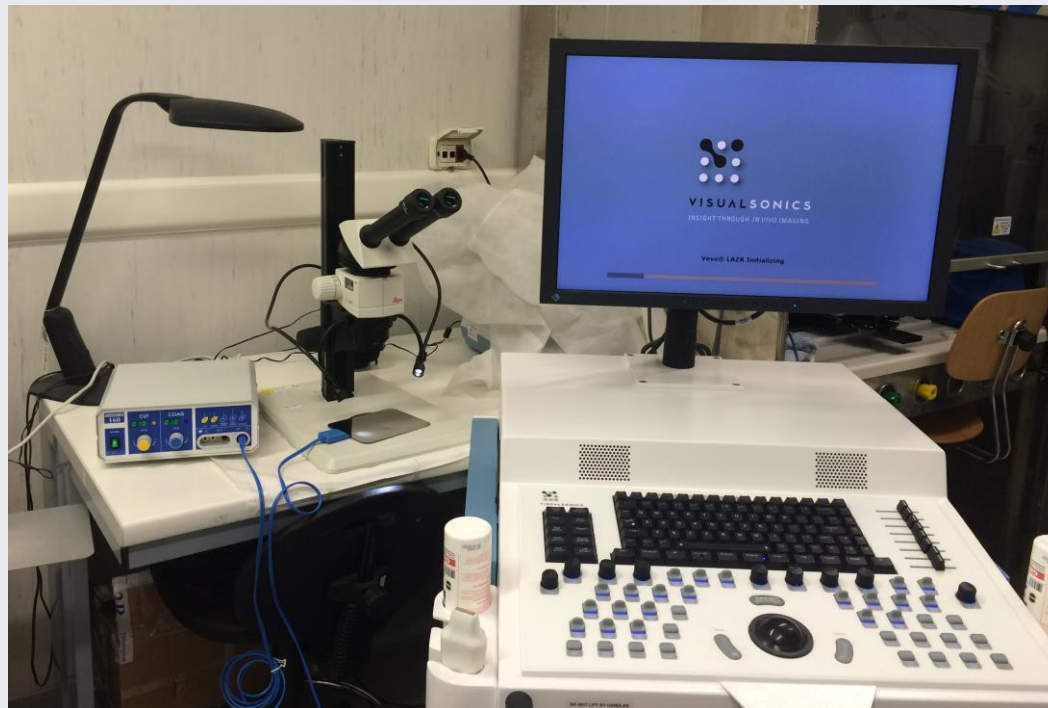


Micro silicon-CT of mouse

AIM

To evaluate an animal model of venous occlusion in order to assess the correlations between venous stasis and the development of neurological diseases.

We used high frequency ultrasound (HFUS) to assess the feasibility of surgical ligation for the induction of venous occlusion in mice.

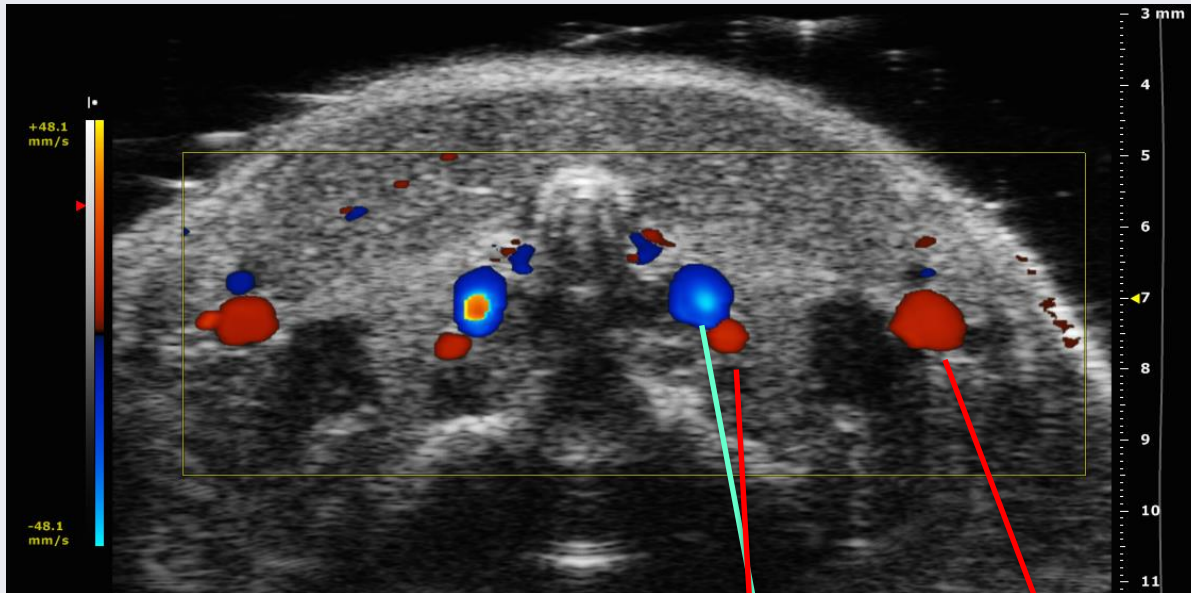


MATERIALS AND METHODS

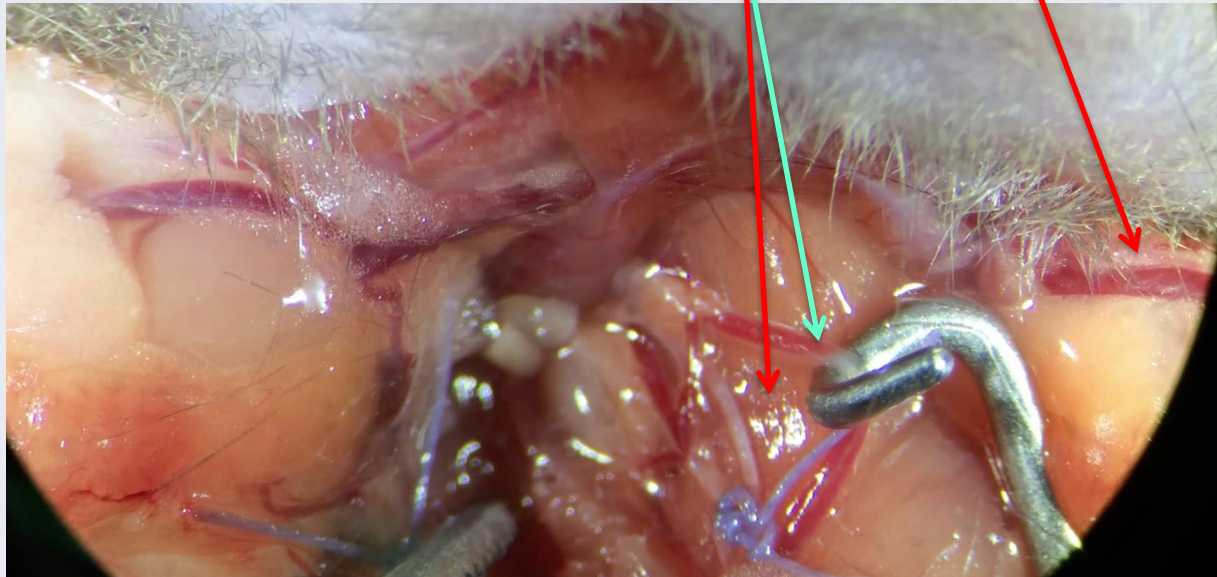
Thirty-eight C57/black, female, 8-12 weeks old:

- 22 mice bilateral ligation of the internal (IJV) and external Jugular veins (EJV)
- 16 mice without jugular veins ligation (“sham-operated group” SG).
- All the procedures were performed under general anesthesia with Isoflurane (2%) in 100% oxygen at 0.8 L/min.
- Blood venous flow was evaluated before and after surgical procedure by Color Doppler HFUS (Vevo 2100, Visualsonics) with a 40 MHz probe.
- .

METHODS



Color doppler
HFUS



Surgical access

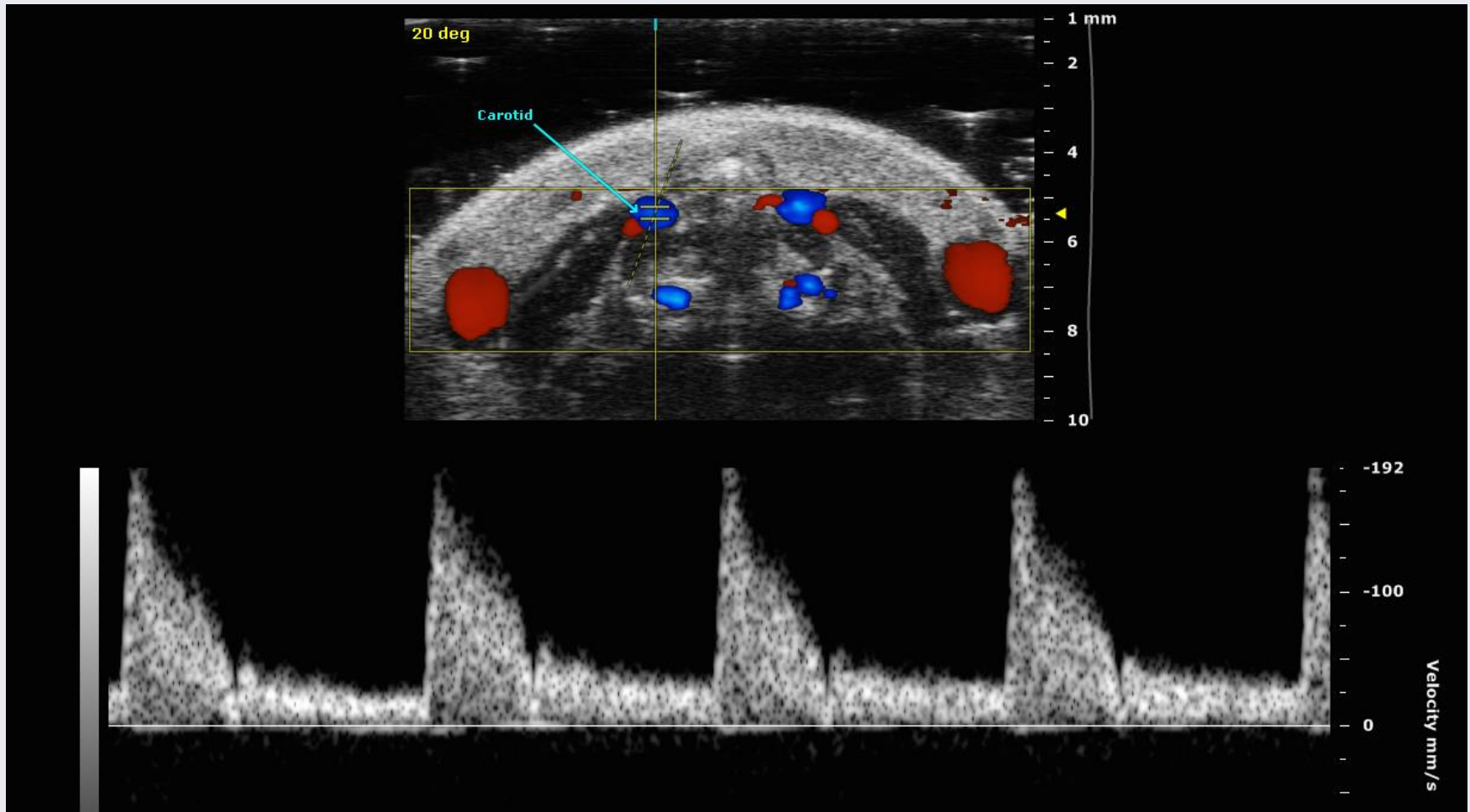
METHODS

Surgery groups

- Eleven mice of LG group were sacrificed 20 days post-surgery to explore sub-acute effects (Sub-Acute ligated group **SALG**) and the remaining 11 were sacrificed after 3 months to explore chronic effects of IJV/EJV obstruction (Chronic ligated group **CLG**).
- At the same way, 8 sham mice were sacrificed after 20 days (Sub-acute sham group **SASG**), and 8 sham mice were sacrificed after 3 months (Chronic sham group **CSG**).

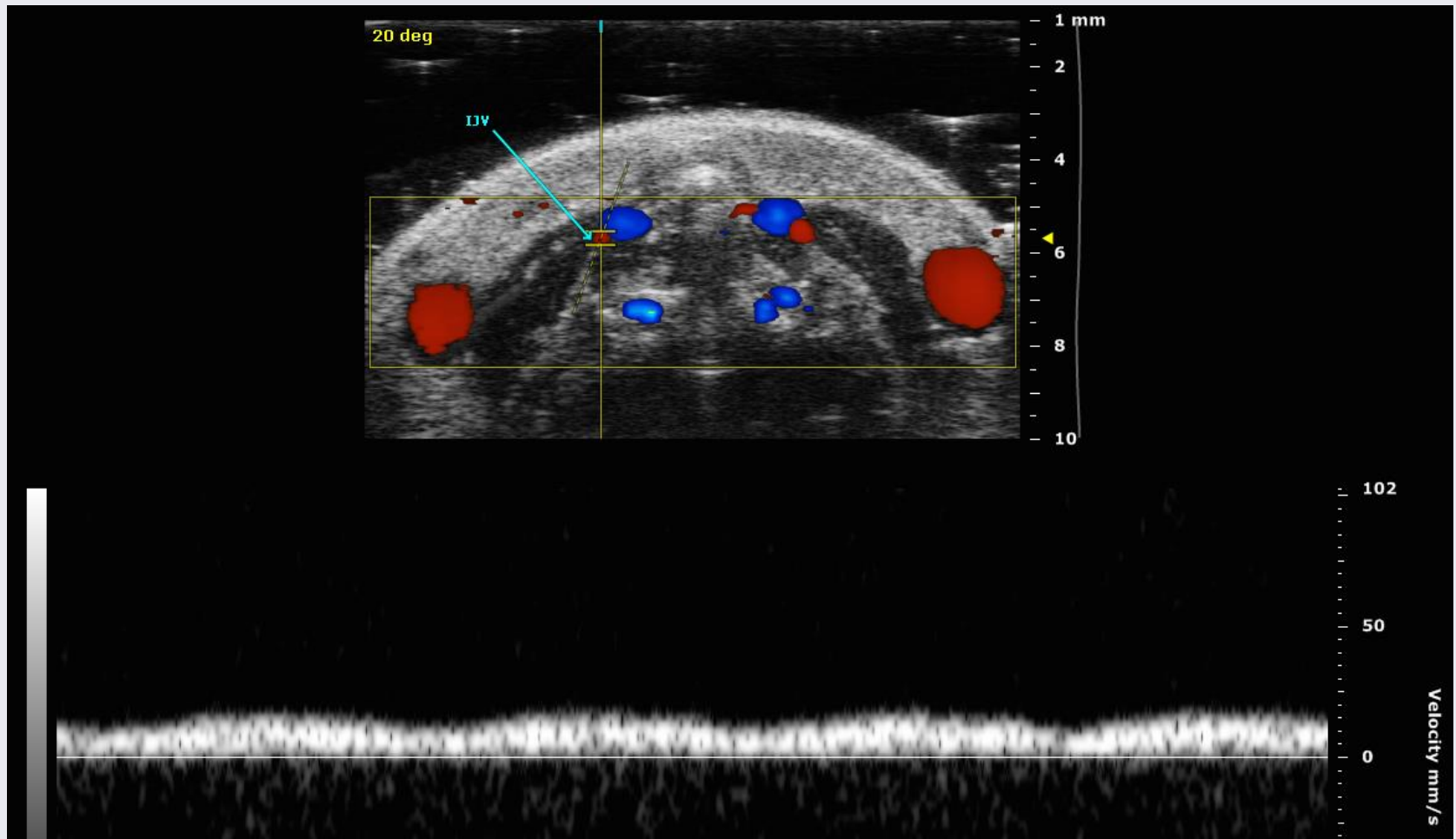
METHODS

Normal Doppler High Frequency Ultrasound of the carotid artery



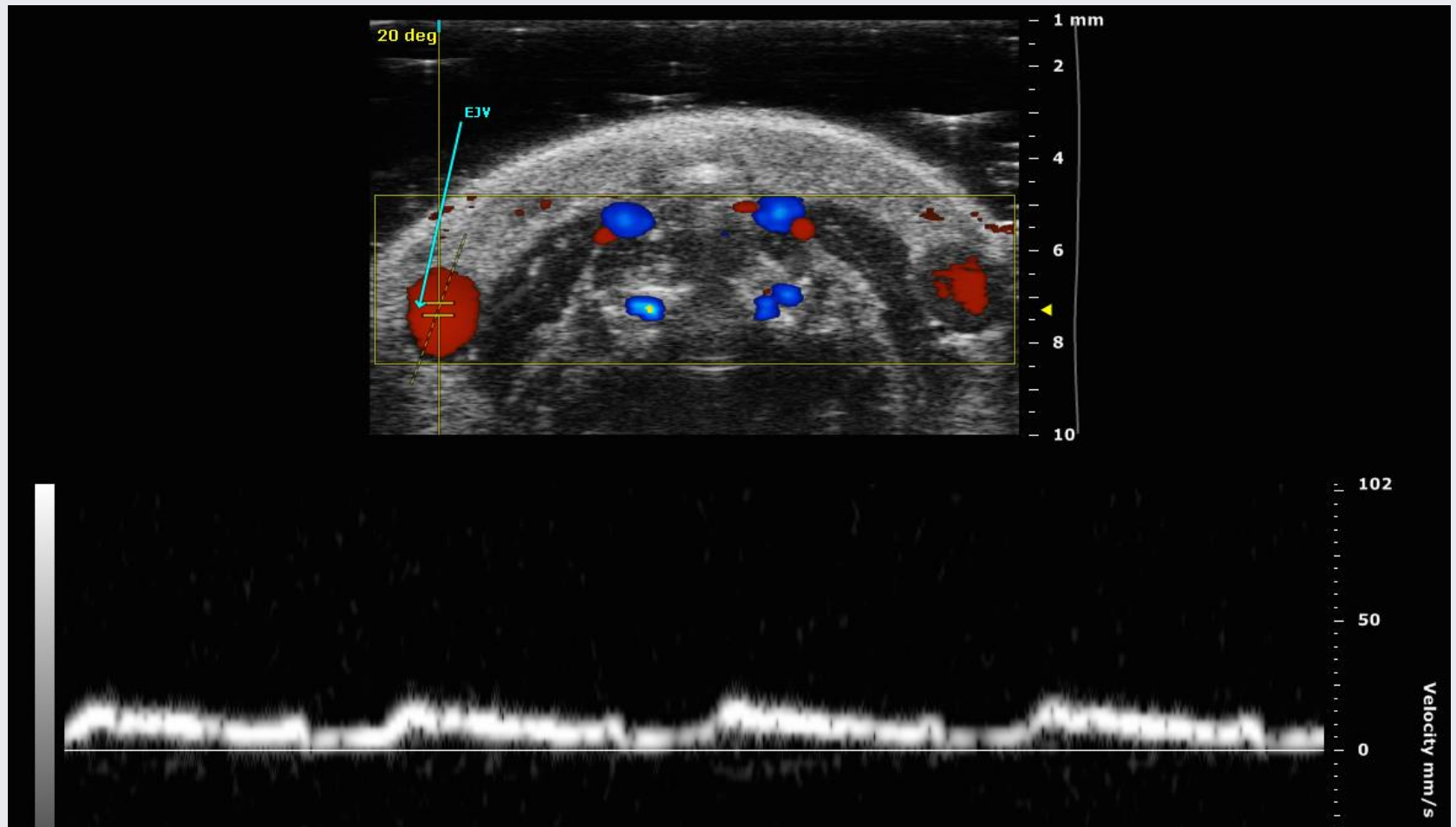
METHODS

Normal Doppler High Frequency Ultrasound of internal jugular vein (IJV)



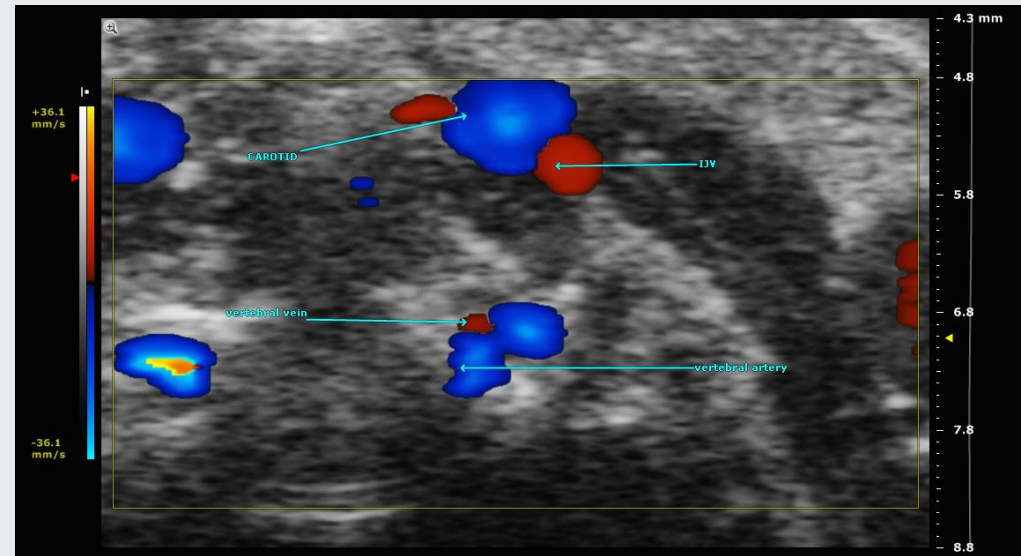
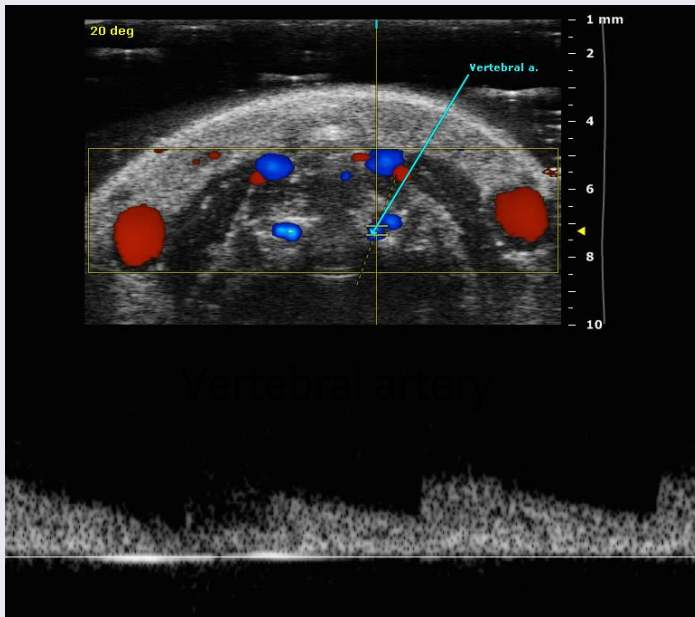
METHODS

Normal Doppler High Frequency Ultrasound of external jugular vein (EJV)

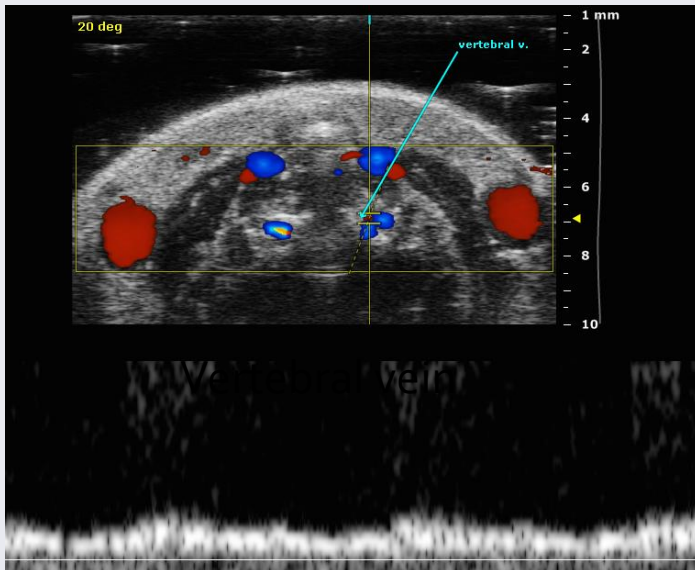


METHODS

Normal Doppler High Frequency Ultrasound of the neck



Zoom of the neck



METHODS

Histology & Immunohistochemistry

Three sections of 5 μm each of the two hemispheres were placed on each slide. The samples were subjected to both histological and immunohistochemical staining using slides in most cases immediately consecutive to define edema, inflammatory reaction, iron and fibrinogen deposit and demyelination.

METHODS

Histology & Immunohistochemistry

Histology

1. Hematoxylin/eosin staining

Immunohistochemistry

2. The Abcam anti myelin ab40390 antibody was used to highlight myelin.

3. the Abcam anti neuro filament ab8135 antibody was used to detect neurofilaments.

4. The Biorad rat anti-mouse F4/80 biotinate MCA497B antibody was used to highlight the presence of macrophages.

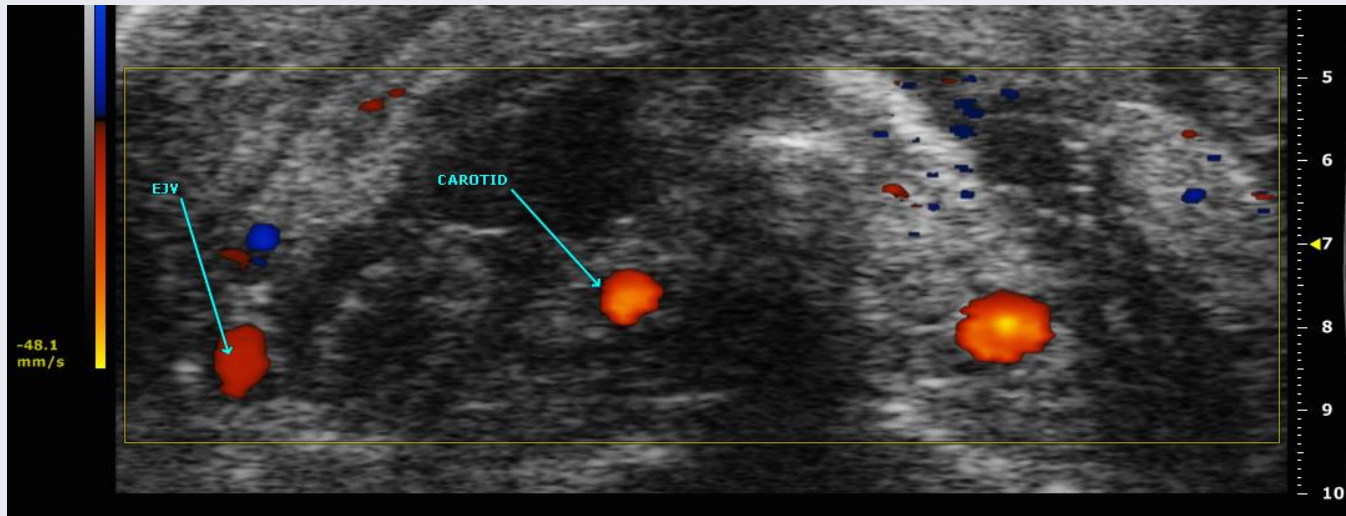
5. Perls staining was used for the detection of iron deposits.

6. Fibrinogen was evidenced in brain tissue samples with an anti α chain fibrinogen antibody.

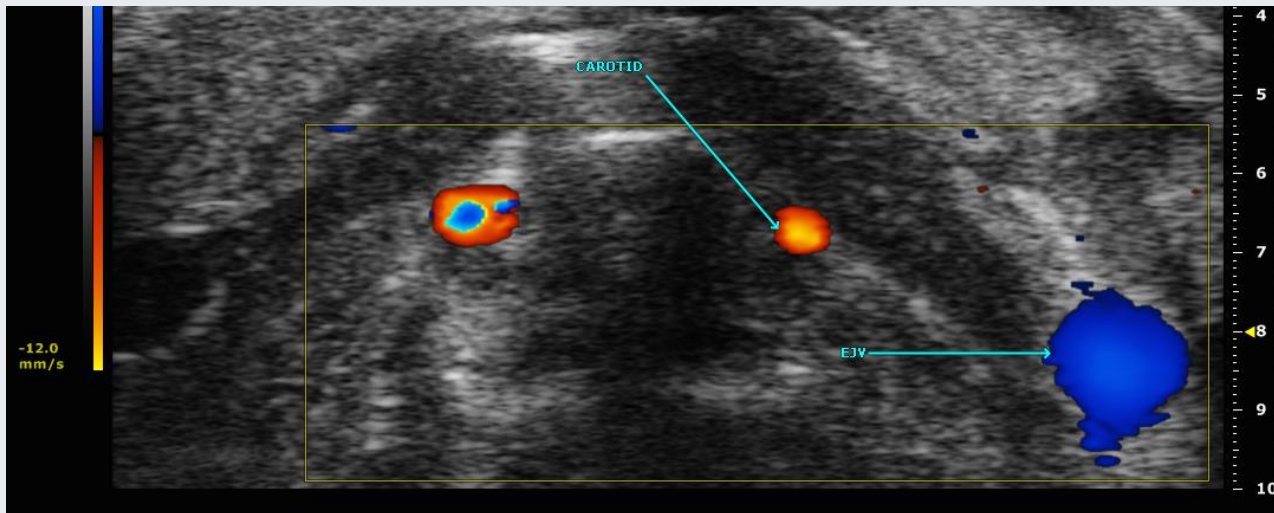


RESULTS

High Frequency Ultrasound Doppler post surgery of IJV



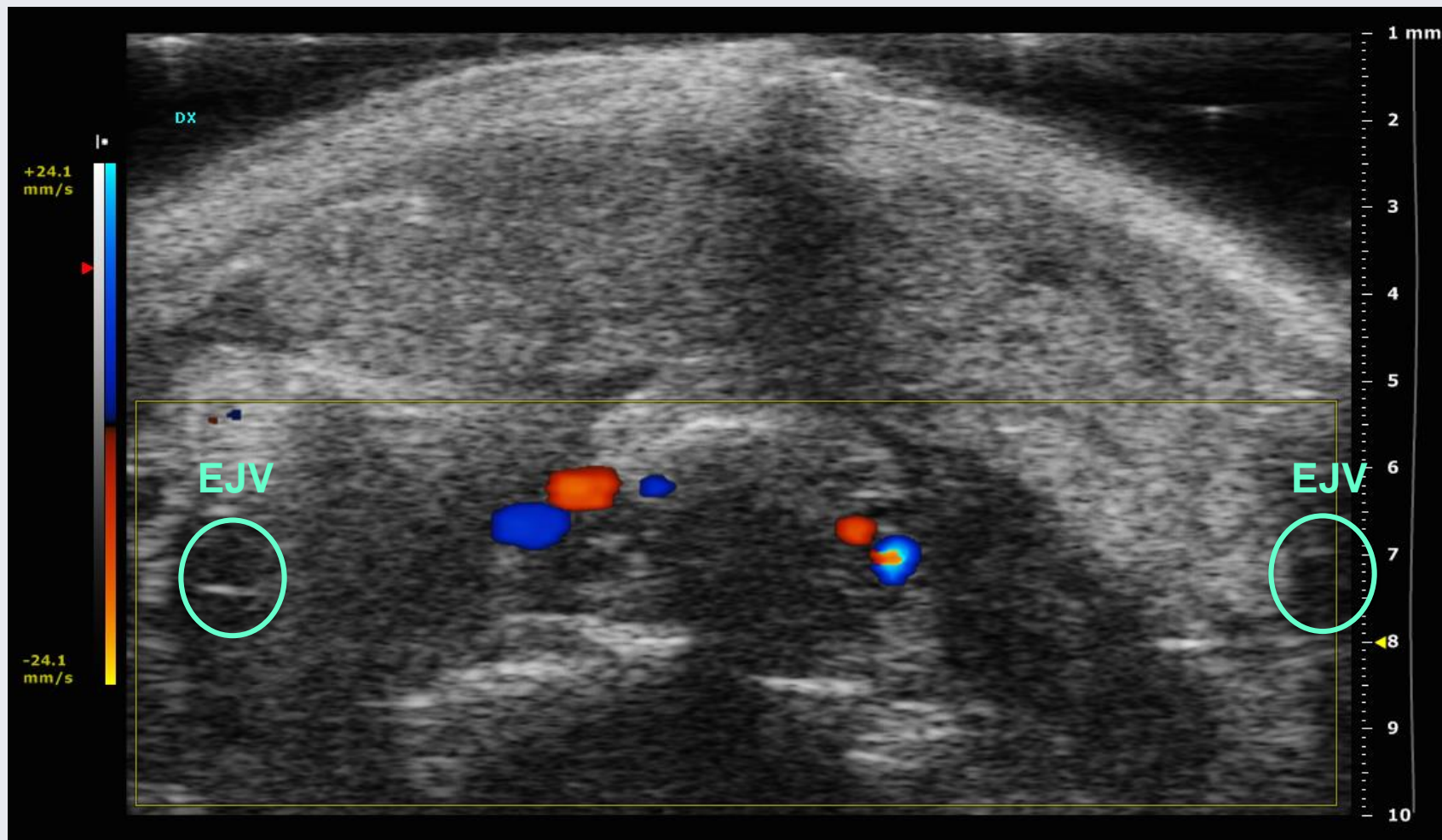
Maximum velocity of blood (48.1 mm/s)



Minimum velocity of blood (12.0 mm/s)

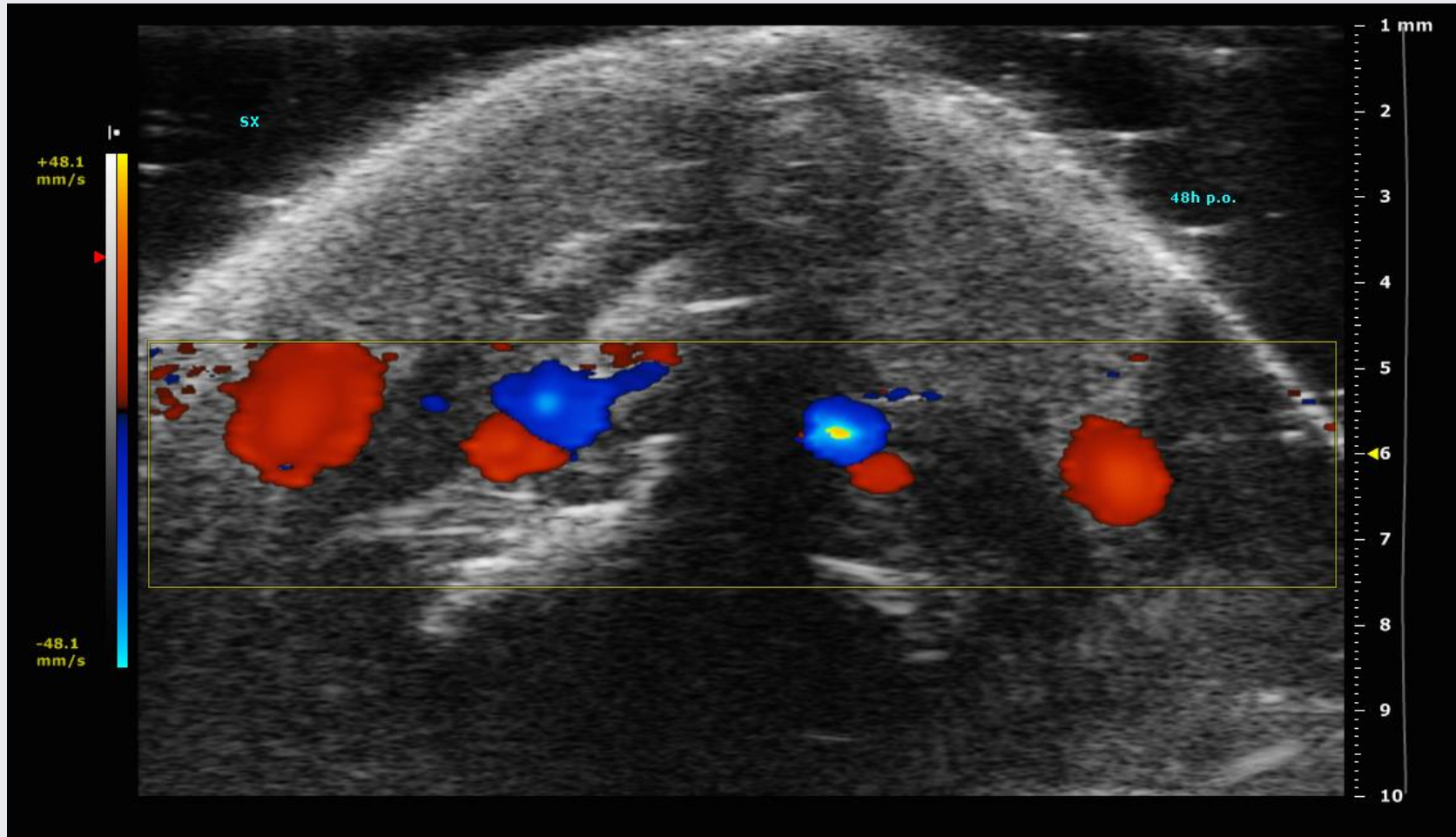
RESULTS

High Frequency Ultrasound Doppler post surgery
of EJVs



RESULTS

High Frequency Ultrasound Doppler post surgery of SHAM group



RESULTS

18/22 survived (four mice died immediately after surgery due to intraoperative complications), and in two mice the bilateral ligation of EJV and IJV was unsuccessful.

Finally, 8 mice of the SALG and 8 mice of the CLG, and all mice of the SG underwent to clinical evaluation and histological analysis.

In 5/22 mice of the LG, collateral veins was demonstrable in the neck

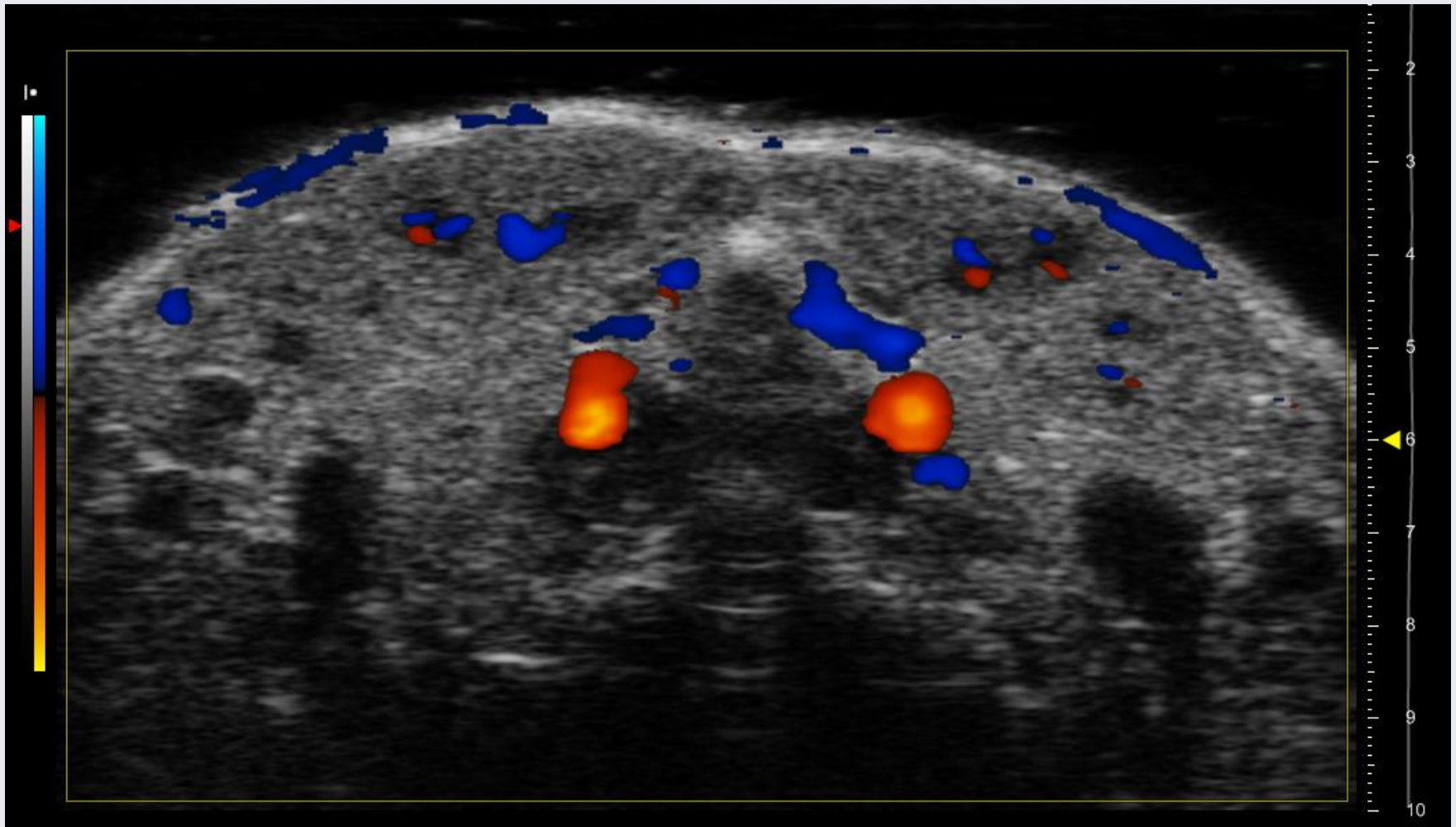
7/22 showed a loss of tail tonicity and of ability of curling the tail, or/and mild hind limb weakness (Grade 1).

These symptoms were present in 4/11 mice of the SALG and in 3/11 mice of the CLG.

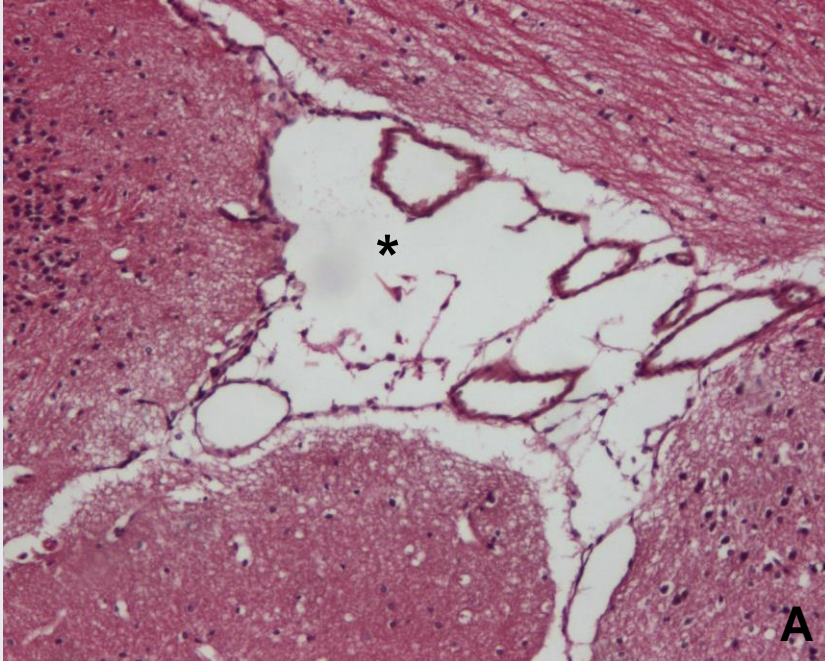
The other mice did not present any neurological sign.

RESULTS

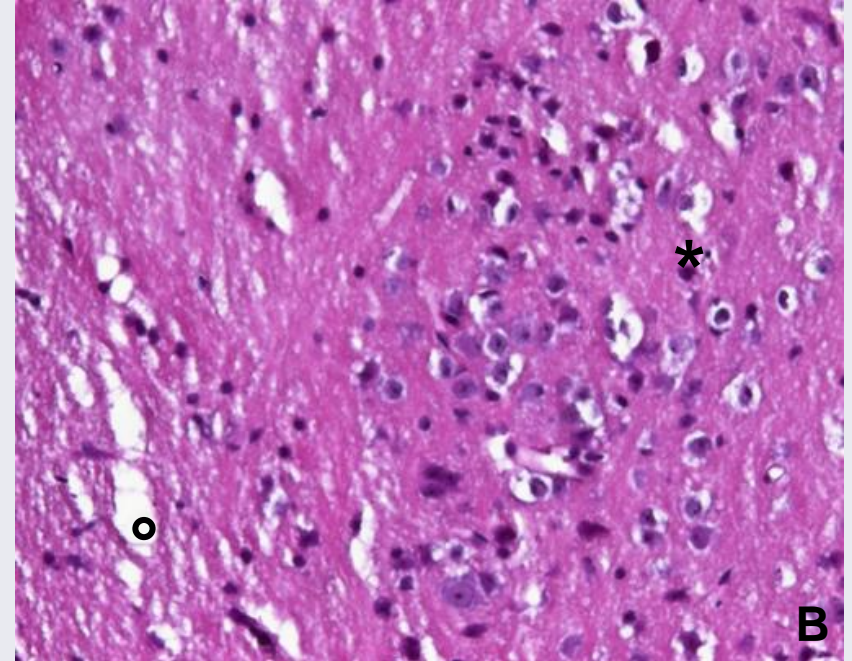
collateral veins



RESULTS

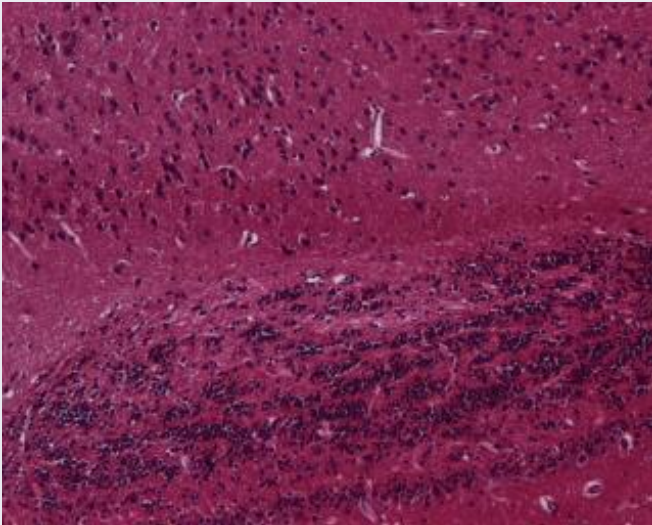


A. Floor of the IV ventricle with choroid plexus residues with clear intra-parenchymal (°) and meningeal (*) edema in an animal of the SALG group. Hematoxylin and Eosin staining (original magnification 10x)

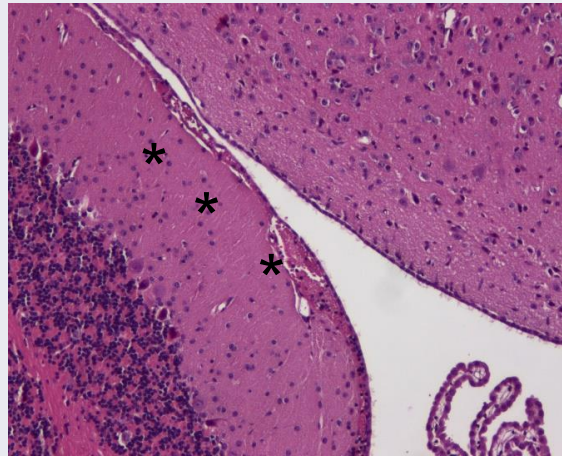


B. Numerous cortical neurons with intra-parenchymal edema at peri-cellular (°) and peri-vascular (*) level in an animal of the SALG group. Hematoxylin and Eosin staining (original magnification 20x)

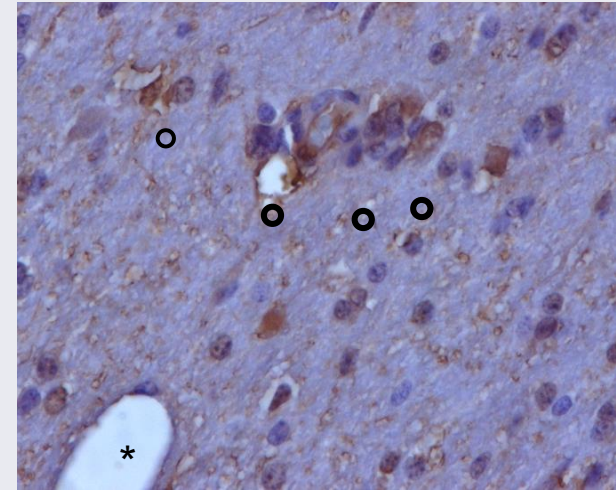
RESULTS



An area of cerebellar cortex of a CSG animal shows a mild edema.



Sub-ependymal microhemorrhages (*) at the level of the IV ventricle of a SASG



A small group of activated microglial cells (°) near a cerebral vessel (*) in the cortex of a mouse of the SASG group, Immunohistochemistry for MCA497B antibody

CONCLUSIONS

Diffuse abnormal accumulation of fluid in the brain parenchyma secondary to obstruction of the jugular veins was found in all animals in the subacute stage and it was still present, although to a lesser extent, in the more chronic stages. Fibrinogen is a major component in the blood that is considered a marker of BBB disruption . The absence of plasma components as fibrinogen, red blood cells and lymphocytes in perivenular space suggest that the BBB is intact.

Multiple micro hemorrhages were also detected. Increase in intracranial venous pressure might have caused brain swelling and meningeal capillary damage leading to micro-hemorrhage.

We also found a ***mild but significantly increase in the number of CD68 positive cells*** isolated or in small groups in located around venous vessels, suggesting the occurrence of mild inflammatory response.

CONCLUSIONS

In line with previous findings we did not find signs of demyelination or major neurological symptoms after surgery.

Overall our and previous results suggest that chronic cerebral venous insufficiency might be obtained in controlled rodent models of JVL able to elicit only a mild inflammatory process. Microglia is the first responders to local parenchymal or vascular damage in the CNS, and their rapid process extension toward injured blood vessels has been proposed as a possible response to the disruption of the BBB.