BAFS 2025

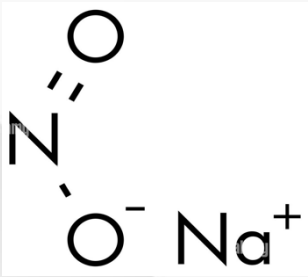
Balkan Academy of Forensic Sciences

LETHAL SODIUM NITRITE INTOXICATION: THE CONTRIBUTION OF IMMUNOISTOCHEMICAL MARKERS

Roberta Bibbò [1], Stefania De Simone [1], Maria Antonella Bosco[1], Alessandro Santurro [2] , Francesco Orsini[1], Giovannni Pollice[1].

1 Department of Clinical and Experimental Medicine, Section of Forensic Medicine, University of Foggia, 71122 Foggia, Italy.
2 Department of Medicine, Surgery and Dentistry, University of Salerno, Baronissi, Italy.

Background and aims: Sodium nitrite intoxication is an increasingly common method of suicide. However, there are no specific signs, routine tests and toxicological tests do not always give reliable results. This study aims to validate scientifically useful methods for the diagnosis of $NaNO_2$ intoxication, testing immunohistochemical techniques of three specific markers of hypoxia: HIF-1α, iNOS2 and VEGF.



Methods: Seven cases of death caused by $NaNO_2$ intoxication were examined; as a control case, a person who died from a stab wound was selected. Immunohistochemical analyses were carried out with specific antibodies to **iNOS**, **HIF-1α** and **VEGF** antigens on autopsy samples of heart, brain and lungs. On all sample collected during autopsy, 4 µm-thick paraffin-embedded sections were cut; they were pretreated by incubation for 120 minutes at 20°C using different substances; then they were incubated with the primary antigen. The sections were coloured in contrast to H&E and observed under an optical microscope.

Results: VEGF and iNOS2 were found to be significantly expressed in samples of cardiac, pulmonary and encephalic tissue in cases of death from nitrate intoxication, while HIF1α showed good expressiveness in lung and brain samples but poor in heart samples. The expression of VEGF, HIF1α and INOS2 was very poor or absent in the control.

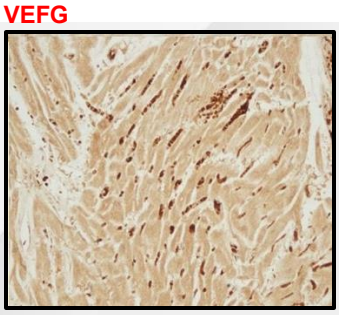
[0]: not expressed.
[+/-]: baseline positivity.
[+]: isolated and disseminated expression.
[++]: expression in groups or diffuse foci.
[+++]: diffuse expression.

Brain Heart Lung

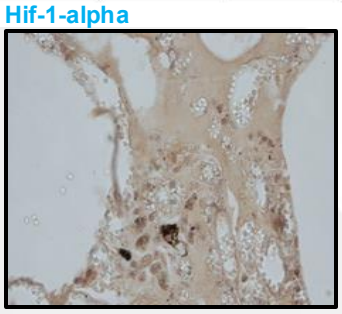
Antigens	CASE 1	CASE 2	CASE 3	CASE 4	CASE 5	CASE 6	CASE 7	Control
Hif-1-alpha	+++	+++	+++	+++	+++	+++	+++	-
INOS 2	+++	+++/-	++	+++/-	+++	++	+++	+/-
VEGF	+++	+++	+++	+++	+++	+++	+++	+/-
Hif-1-alpha	+	+	+/-	+	+	+/-	+	-
INOS 2	+++	+++	+++	+++	+++/-	+++	+++/-	+
VEGF	+++/-	+++	+++	+++	+++	+++	+++	+/-
Hif-1-alpha	+++	+++	+++	+++	+++	+++	+++	-
INOS 2	+++	+++/-	+++	+++	+++/-	+++	+++/-	+
VEGF	+++	+++	+++	+++	+++	+++	+++	+

INOS 2

VEFG



Hif-1-alpha



Discussion - Conclusions: Deaths from suicidal $NaNO_2$ intoxication are a challenge for forensic pathologists. This work highlighted how immunohistochemical techniques play a crucial role in clarifying death mechanisms in sodium nitrite poisoning, showing an increase in oxidative stress affecting the INOS2, VEGF and HIF1α lines.

"BRIDGING SCIENCE, JUSTICE, AND HUMANITY"

BAFS 2025

Balkan Academy of Forensic Sciences

IMMUNOHISTOCHEMICAL MARKERS FOR DIAGNOSING DIFFUSE AXONAL LESIONS

Iuliana Hunea[1], Manuela Ciocoiu[2], Mădălina Maria Diac[1,3], Andreea Velnic[3], Călin Scripcaru[1], Diana Bulgaru Iliescu[1,3]

1 Institute of Forensic Medicine, Iași, Romania

2 Department of Phathophysiology, University of Medicine and Pharmacy "Grigore T. Popa" Iași, Romania

3 Department of Forensic Medicine, University of Medicine and Pharmacy "Grigore T. Popa", Iași, Romania

1.Introductions

Diffuse Axonal Injury (DAI) is a traumatic brain injury where the brain's white matter (nerve fiber tracts) is stretched and torn, leading to widespread damage to the brain's communication networks. Caused by rapid rotational or linear forces that cause the brain to shift and rotate within the skull, DAI often results from severe head trauma in events like motor vehicle accidents or falls. It can lead to prolonged coma, persistent vegetative states, and significant cognitive and physical impairments.

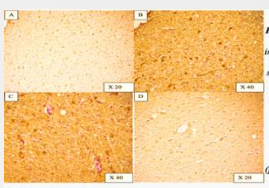
The Adams DAI Classification system grades DAI from I (mild) to III (severe), based on the location and extent of the brain lesions found, such as in the corpus callosum and brainstem.

❑ *AIMES AND OBEJECTIVES:*

- *identify the traumatic moment as accurately as possible*
- *assess the specificity and sensitivity of immunohistochemical markers for DAI*

2.Methods

- semiquantitative analysis of ***anti-β-APP, anti-GFAP, anti-NFL, anti-Spectrin II antibodies, CD 68*** cells for each of the analyzed cases
- on each slide, as many microscopic fields as possible were studied, with a minimum magnification objective, using a grid, the positive immunolabeled cells were counted
- the final mean number of positive cells for each case was determined by counting the cell types listed on each slide, averaging the determinations from each 3/5 case slides
- the density of immunoreactive cells for the above-mentioned antibodies was determined by reporting the final mean number of cells/mm^2 of brain tissue



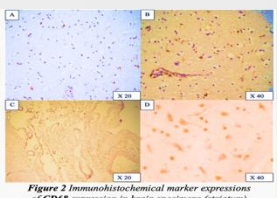


Figure 1 *Expressions of **anti-GFAP** immunohistochemical markers in brain specimens: thalamus at 1 h (A), hypothalamus (B) and striatum at 4 h (c) were intensely positive mainly in TCC neurons compared to the control group (D) (personal collection)*

Figure 2 *Immunohistochemical marker expressions of **CD68** expression in brain specimens (striatum) after 4 h (A) and after 3 weeks with perivascular and perineuronal microglia (B,C), compared to the control group (D) (personal collection)*

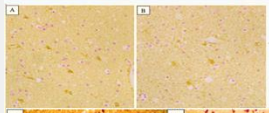


Figure 3 *Anti-spectrin II reaction was positive in patients with TBI(A), unlike those in the control group (B) (personal collection)*

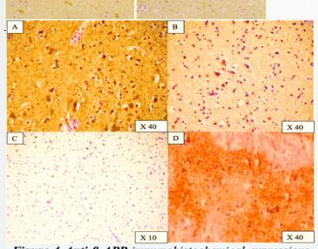


Figure 4 *Anti-β-APP immunohistochemical expressions in brain specimens (thalamus) after 4 h (A), 1 month (B), and 2 months (C) vs control group (D) (personal collection)*

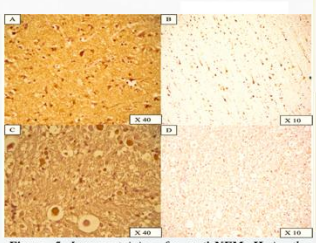


Figure 5 *Immunostaining for **anti-NFM II** in the thalamus at 24 hours (A), hypothalamus at 3 days (B) and striatum at 7 days (C) was mainly positive in DAI neurons, compared to the control group (D) (personal collection)*

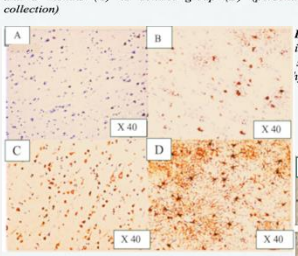


Figure 7 *Expressions of neuronal **anti-spectrin** and glial **anti-GFAP** immunohistochemical markers in brain specimens: thalamus neurons spectrin ++++ (A) and thalamus astroglia GFAP + at (B) one month (personal collection)*

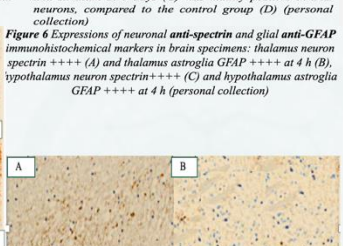


Figure 6 *Expressions of neuronal **anti-spectrin** and glial **anti-GFAP** immunohistochemical markers in brain specimens: thalamus neuron spectrin ++++ (A) and thalamus astroglia GFAP ++++ at 4 h (B), hypothalamus neuron spectrin++++ (C) and hypothalamus astroglia GFAP ++++ at 4 h (personal collection)*

5.References

1. Blumbergs PC, Scott G, Manavis J et al. Stalning af amyloid percursor protein to study axonal damage in mild head Injury. *The Lancet* 1994; 344(8929): 1055-6.
2. Johnson VE, Stewart W, Weber MT et al. SNTF immunostaining reveals previously undetected axonal pathology in traumatic brain injury. *Acta Neuropathol (Berl)* 2016; 131(1): 115-35.
3. Browne KD, Chen XH, Meaney DF, Smith DH. Mild Traumatic Brain Injury and Diffuse Axonal Injury in Swine. *J Neurotrauma* 2011; 28(9): 1747-55.
4. Baines AJ. Evolution of the spectrin-based membrane skeleton. *Transfus Clin Biol* 2010; 17(3): 95-103.
5. Bogoslovsky T, Wilson D, Chen Y et al. Increases of Plasma Levels of Glial Fibrillary Acidic Protein, Tau, and Amyloid β up to 90 Days after Traumatic Brain Injury. *J Neurotrauma* 2017; 34(1): 66-73.

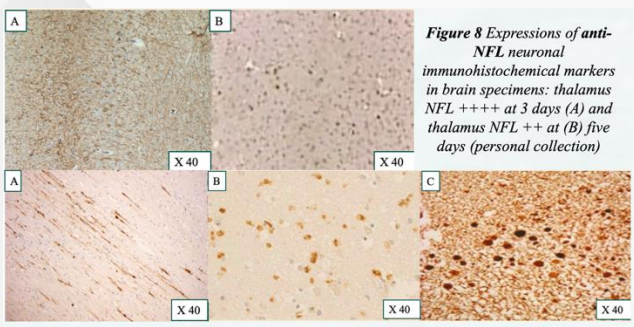


Figure 8 *Expressions of **anti-NFL** neuronal immunohistochemical markers in brain specimens: thalamus NFL ++++ at 3 days (A) and thalamus NFL ++ at (B) five days (personal collection)*

Figure 9*Expressions of **anti-NFL** neuronal immunohistochemical markers in brain specimens: thalamic neuron **NFL** ++++ (A) and **CD 68** +++ and anti-β-APP ++++ at 7 days (personal collection)*

3.Results

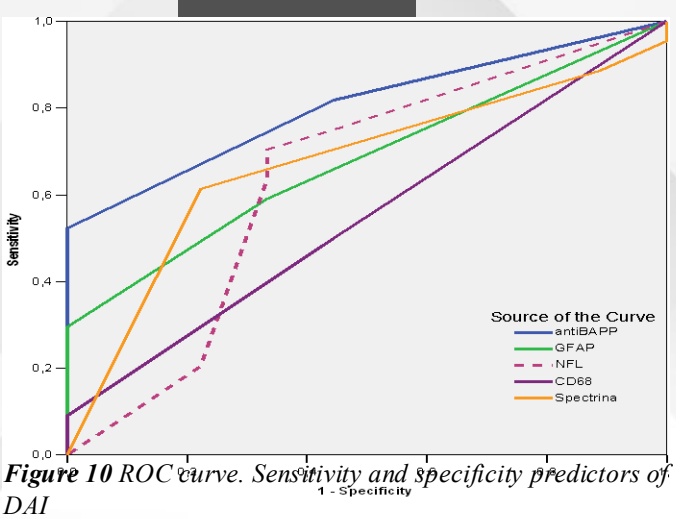


Figure 10 *ROC curve. Sensitivity and specificity predictors of DAI*

- ***antiBAPP***, sensitivity 80% and specificity 60% (AUC=0.803; 95% CI: 0.667-0.909; p=0.001);
- ***GFAP***, with sensitivity 70% and specificity 66% (AUC=0.678; 95% CI: 0.544-0.812; p=0.029);
- ***Spectrin***, sensitivity 60% and specificity 78% (AUC=0.670; 95% CI: 0.528-0.813; p=0.036);
- ***NFL***, sensitivity 68% and specificity 70% (AUC=0.636; 95% CI: 0.469-0.804; p=0.094), without statistical significance.

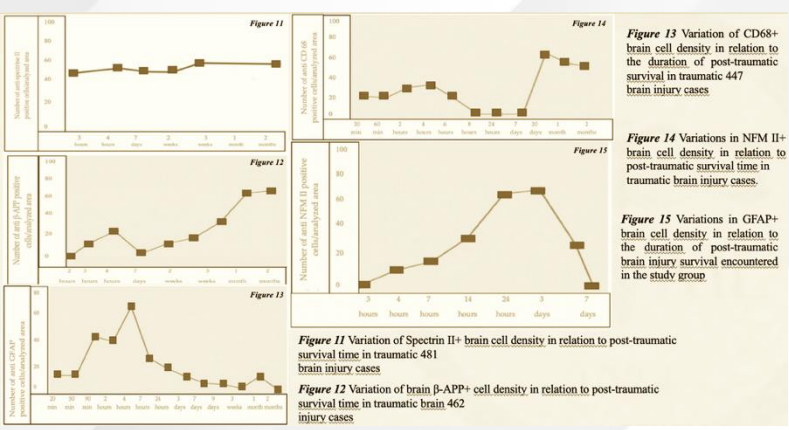


Figure 13 Variation of CD68+ brain cell density in relation to the duration of post-traumatic survival in traumatic 447 brain injury cases

Figure 14 Variations in NFM II+ brain cell density in relation to post-traumatic survival time in traumatic brain injury cases.

Figure 15 Variations in GFAP+ brain cell density in relation to the duration of post-traumatic brain injury survival encountered in the study group

Figure 11 Variation of Spectrin II+ brain cell density in relation to post-traumatic survival time in traumatic 481 brain injury cases

Figure 12 Variation of brain β-APP+ cell density in relation to post-traumatic survival time in traumatic brain 462 injury cases

4.Conclusions

- *The earliest changes were recorded for GFAP and CD68 immunostaining, and the most intense were recorded for spectrin II.*
- *B-APP shows very good predictability for LAD.*
- *GFAP dynamics is chronologically decreasing, from the acute period to the chronic one (>2 weeks).*
- *SNTF immunoreactivity is proportional to that of GFAP in the first hours. In order to assess the post-traumatic moment with greater accuracy, β-APP immunostaining should be completed with that of NF.*
- *Neurofilament and CD68 immunoreactivities are proportional in the first week.*
- *The CD68+ peak is observed in late deaths.*

BAFS 2025

Balkan Academy of Forensic Sciences

Donor-Derived *Strongyloides Stercoralis* Hyperinfection After Kidney Transplantation: Autopsy-Confirmed Two Cases

Mehmet Doğan[1], Yusuf Atan[1,2], Bahri Teker[1,3], İbrahim Üzün[1,4]

[1]Council of Forensic Medicine, Ministry of Justice, Republic of Turkiye, Istanbul, Turkiye
[2]Department of Forensic Medicine, Faculty of Medicine, Bilecik Seyh Edebali University, Bilecik, Turkiye
[3]Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Istanbul Medipol University, Istanbul, Turkiye
[4]Department of Forensic Medicine, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkiye

AIM

This study aims to document fatal cases of **donor-derived Strongyloides stercoralis hyperinfection** confirmed by forensic autopsy, and to emphasize the clinical and medico-legal **significance** of **systematic donor screening.**

BACKGROUND

Strongyloides stercoralis is a soil-transmitted nematode capable of lifelong autoinfection, estimated to **infect 30–100 million** people worldwide[1]. While asymptomatic in most immunocompetent hosts, immunosuppression—particularly corticosteroid therapy or solid organ transplantation—can precipitate hyperinfection and disseminated disease with **mortality rates exceeding 50%**[2].

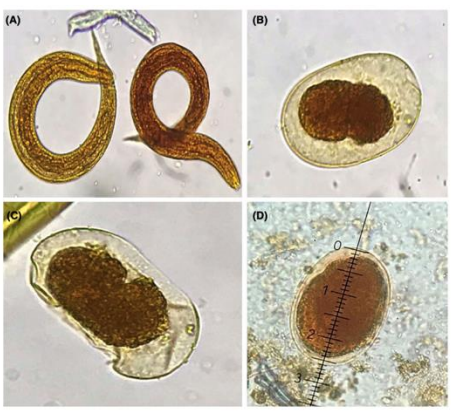


FIGURE 1 *Strongyloides stercoralis* stages found in the microscopic stool analysis. A, *S stercoralis* first-stage rhabditiform larvae (L1) in a concentrated wet mount stained with iodine. Larvae present the characteristic short buccal canal, a rhabditoid esophagus, and a prominent genital primordium (arrow in Figure 2 indicates the genital primordium). B, *S stercoralis* egg in a concentrated wet mount stained with iodine. C, *S stercoralis* egg in a concentrated wet mount stained with iodine. The shell and external membranes present deterioration only a few minutes after slide preparation. D, *S stercoralis* egg in a concentrated wet mount stained with iodine. Size: 65 × 43.8 µm. All images were taken at 400× magnification

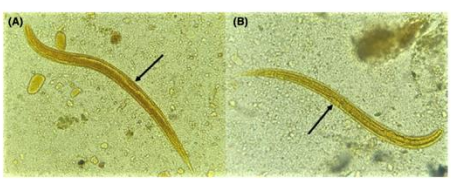
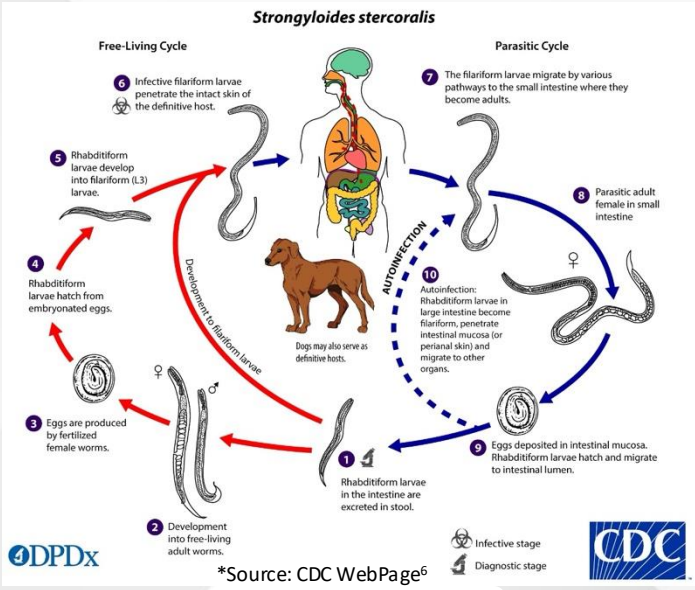


FIGURE 2 A, B, Strongyloides stercoralis larvae in a concentrated wet mount stained with iodine. The arrows points to the prominent genital primordium in both larvae

*Source: Poloni et al.[7]

DISCUSSION

Our series highlights **two fatal donor-derived *Strongyloides stercoralis*** infections with divergent clinical trajectories—one dominated by **gastrointestinal sepsis,** the other by **neurological decline**—consistent with the heterogeneity of disseminated disease reported in transplant recipients[3,4]. **Histopathology confirmed** systemic dissemination in both, but only one case demonstrated **larvae in the transplanted kidney**, a feature exceptionally documented in the literature[5]. Most published clusters have relied on **serology or PCR for diagnosis**[1], whereas our findings provide **direct tissue proof** of graft invasion, thereby clarifying the transmission pathway. This evidence extends prior observations that **renal allografts** can serve as **reservoirs** for donor-derived parasites and underscores the need for routine screening and prophylaxis in transplant settings[4].

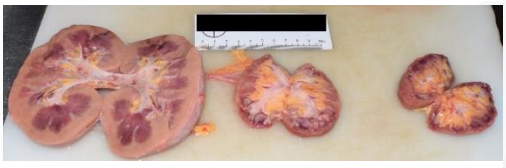


*Source: CDC WebPage[6]

CASE REPORTS

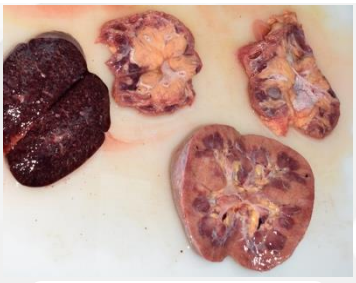
Case 1 (47F): Kidney transplant recipient who developed **persistent fever, diarrhea,** and **neurological decline**. Despite broad antimicrobial therapy, she rapidly deteriorated and died. **Autopsy & Histopathology:** *Strongyloides stercoralis* larvae were identified in the **lungs, brain, and the transplanted kidney,** confirming hyperinfection syndrome with graft involvement.

Case 1



Case 2 (56M): Another kidney recipient from the same donor presented with **gastrointestinal complaints** and **septicemia,** progressing to multi-organ failure and death. **Autopsy & Histopathology:** Disseminated larvae were observed in **the liver, lungs, and brain**, consistent with hyperinfection syndrome.

Case 2



CONCLUSION

Forensic autopsy can uncover hidden transmission routes of **parasitic infections** in **transplantation**, redefining donor safety. Integrating systematic screening into **transplant policy** is both a **preventive necessity** and a **medico-legal obligation.**

REFERENCES

"BRIDGING SCIENCE, JUSTICE, AND HUMANITY"

FATAL FAT PULMONARY EMBOLISM IN CORRELATION WITH SEVERE ANEMIA - AN AUTOPSY CASE REPORT

Antonios Metzikofis [1], Ioanna Abba Deka[2], Leda-Kalliopi Kovatsi [1], Kyriakos Chatzopoulos[2]

[1] Laboratory of Forensic Medicine and Toxicology, Aristotle University of Thessaloniki, Thessaloniki, Greece

[2] Department of Pathology, Aristotle University of Thessaloniki, Thessaloniki, Greece

SUMMARY

- ✓ A case report of a rare case of a fatal fat embolism death.
- ✓ Implementation and investigation of the autopsy and pathology findings and their correlation with the medical records.
- ✓ The first description of Fat Pulmonary Embolism made by Zenker in 1862

METHODS

- ✓ We performed a literature review and discussed the relevant literature.

MEDICAL RECORDS / CHAIN OF INCIDENCES

- ✓ 1st Day: Presents fever and backpain
- ✓ 10th Day : The patient transported to the Hospital with reported backpain. He received painkillers and exit the hospital (under medical admission)
- ✓ 11th Day: The patient was transported to a Clinic with reported acute backpain. During his institution was under clinical/laboratory/imaging screening.
- ✓ The following day, the patient showed respiratory distress
- ✓ He entered the Intensive Care Unit and passed away

SCREENING/LABORATORY RESULTS

- ✓ BONE BIOPSY → Hemophagocytosis and element of reactive marrow
- ✓ BLOOD TESTING →Anemia
- ✓ CT SCANNING → Heterogeneity of the bone marrow of the imaged part of vertebrae, as well as the imaged pelvic bones, without clear focal damage.

FORENSIC INVESTIGATION

- ✓ 42-year-old male
- ✓ limited medical history
- ✓ Death declaration in an Intensive Care Unit of a Private Clinic
- ✓ lack of evidence of criminal activity or suicide

AUTOPSY FINDINGS

- ✓ evidence compatible with previous medical interventions
- ✓ micro petechial hemorrhages
- ✓ cerebral edema
- ✓ pulmonary congestion,
- ✓ secretions in the main bronchi,
- ✓ hepatomegaly & splenomegaly
- ✓ Autopsy findings were supplemented with histopathology samples from brain, lungs & pleura, heart, liver, spleen, adrenal glands and kidneys)

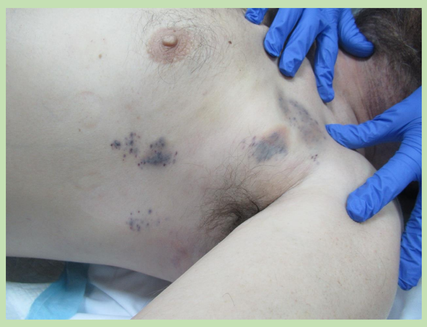


Fig.1. Petechial hemorrhages and ecchymoses

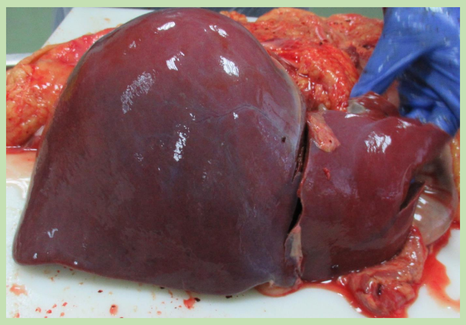


Fig.2. Hepatomegaly with a reported weight of 2470 grams

Fig.3. Splenomegaly with a reported weight of 1410 grams

HISTOPATHOLOGICAL INVESTIGATION

- Histological examination revealed oedematous lung parenchyma, with minor findings of emphysema.
- The alveolar septal vessels were congested, with focal intra-alveolar haemorrhage.
- A significant number of histiocytes were noted, some of which contained hemosiderin deposits, without accumulations of acute inflammatory cells in the alveolar spaces.
- In the lumens of small and medium-sized vessels, an abundance of red and white blood cells as well as fibrin thrombi and lipocytes are observed.

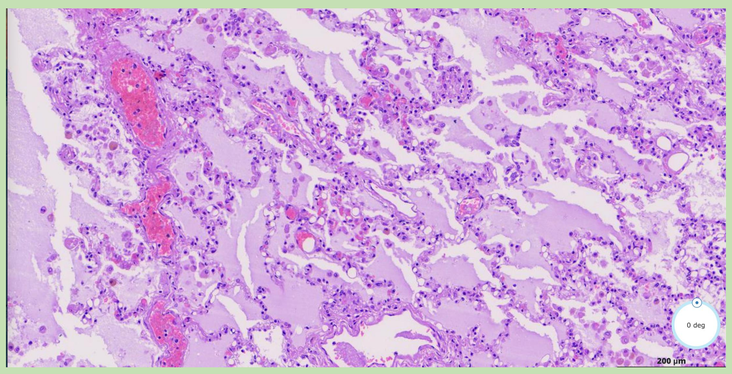


Fig.4: Lung with severe edema, medium and small vessel engorgement and minor emphysematous changes.

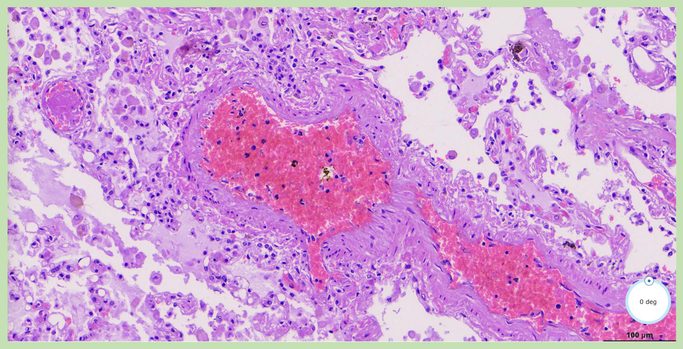


Fig.5: A large vessel (center) filled with an abundance of RBCs and WBC. In the medium sized vessel (top left), a fibrin thrombus is noticed.

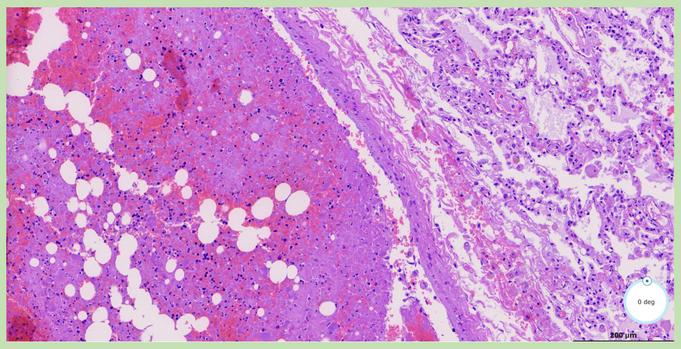


Fig.6: In lumen of the large vessel, besides the abundance of RBCs and WBCs, large vacuolated cells, reminescent of lipocytes are observed.

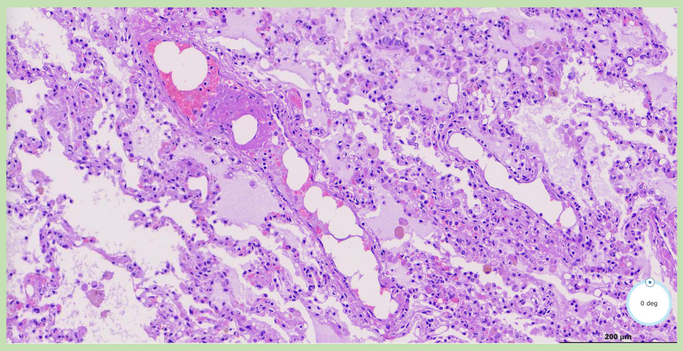


Fig.7: Inside the lumen of the vessel (center), lipocytes, RBCs and a fibrin thrombus are seen.

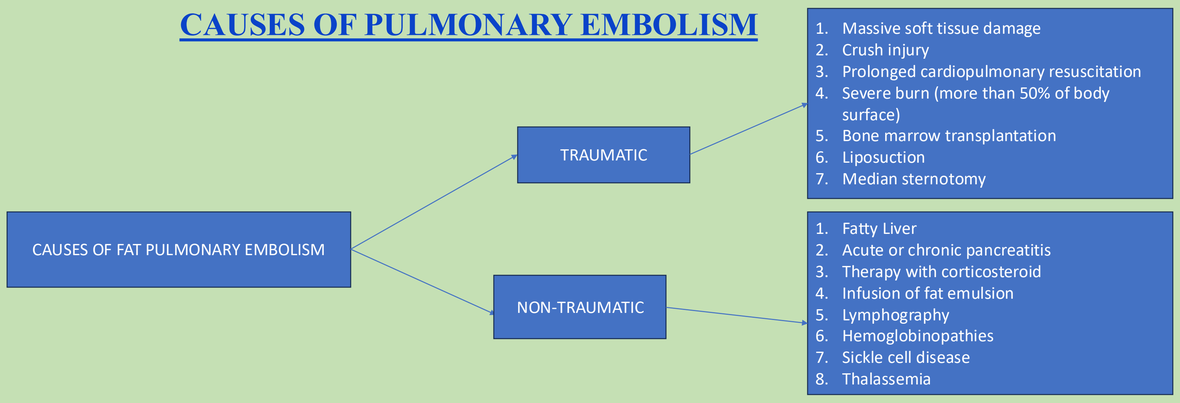
PULMONARY EMBOLISM

- ✓ Death due to fat pulmonary embolism is particularly rare and underdiagnosed.
- ✓ Fat embolism is markedly underdiagnosed, both clinically and at autopsy.
- ✓ The exact incidence rates are difficult to determine precisely, because it is often underdiagnosed or misdiagnosed
- ✓ Marked pulmonary oedema is the pathological marker
- ✓ 2 pathophysiology methods: mechanical and biochemical

BIOCHEMICAL METHOD

- fat globules entering the pulmonary vasculature where they are broken down to free fatty acids
- This fact leading to a microvascular inflammatory response with the development of acute lung injury.]
- (bone marrow is mostly composed of neutral fat and do not display this effect),
- (trauma elevates plasma lipase which precede any rise in free fatty acids.)

CAUSES OF PULMONARY EMBOLISM



CORRELATION OF FINDINGS/ CAUSE OF DEATH

- ✓ Our case is clearly, as we know, is categorized as a non-traumatic type of fat pulmonary embolism.
- ✓ Factors as the heterogeneity of the marrow of vertebrae and the pelvic bones is the primary correlation factor.
- ✓ Hemophagocytosis and anemia is a strong contributive factor in the event.

GRADING

1st method: According to Mason, based in Oil Red-O frozen sections of lung

GRADE	PATHOLOGICAL FINDINGS
Grade 0	no emboli seen.
Grade 1	emboli found after some searching.
Grade 2	emboli found after some searching.
Grade 3	emboli present in large numbers.
Grade 4	emboli present in potentially fatal numbers.

2nd method: According to Scully, the histological severity of embolism graded from 0 to 8,
grade 1 → less than ten small emboli per section
grade 8 →signifying the presence of emboli in a majority of high-powered fields.

HEMOPHAGOCYTOSIS

- ✓ Hemophagocytosis is a term used to describe the phagocytosis of red blood cells, white blood cells and platelets by activated macrophages.
- ✓ Hemophagocytosis can lead to the elevation of free fatty acids, though it's mediated by cytokine-driven lipolysis and lipid metabolism dysregulation, not by the phagocytosis itself.

CONCLUSION

To the best of our knowledge, no confirmed report of fatal pulmonary fat embolism in correlation of hemophagocytosis and anemia has been published in the international literature.

REFERENCES

1. Saukko P., Knight B. (2016). Knight' s Forensic Pathology. New York: CRC Press Taylor & Francis Group
2. Adeyinka A, Pierre L. Fat Embolism. [Updated 2022 Oct 31]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499885/
3. Mason JK. Pulmonary Fat and Bone Marrow Embolism as an Indication of Ante-Mortem Violence. Medicine, Science and the Law. 1968;8(3):200-206. doi:10.1177/002580246800800311
4. Scully R. E. (1956). Fat embolism in Korean battle casualties; its incidence, clinical significance, and pathologic aspects. *The American journal of pathology*, *32*(3), 379–403.
5. Mellor, A., & Soni, N. (2001). Fat embolism. *Anaesthesia*, *56*(2), 145–154. https://doi.org/10.1046/j.1365-2044.2001.01724.x
6. Milroy, C. M., & Parai, J. L. (2019). Fat Embolism, Fat Embolism Syndrome and the Autopsy. *Academic forensic pathology*, *9*(3-4), 136–154. https://doi.org/10.1177/1925362119896351
7. Tang, S., Li, S., Zheng, S., Ding, Y., Zhu, D., Sun, C., Hu, Y., Qiao, J., & Fang, H. (2021). Understanding of cytokines and targeted therapy in macrophage activation syndrome. *Seminars in arthritis and rheumatism*, *51*(1), 198–210. https://doi.org/10.1016/j.semarthrit.2020.12.007

Sudden Cardiopulmonary Arrest Following Colonoscopy in a 41-Year-Old Female with Ulcerative Colitis: A Postmortem Diagnosis of Descending Aortic Dissection

Dr. Gjergji Kaçauni
Forensic Doctor and Histopathology Doctor, Forensic Institute of Tirana, Albania
kacauni@yahoo.com

Dr. Ilir Deçolli
Forensic Doctor, Forensic Institute of Tirana, Albania
ilirdecolli@yahoo.com

Introduction

Colonoscopy is a commonly performed diagnostic procedure with a low incidence of major complications. Fatal outcomes are exceedingly rare and usually result from perforation or anesthetic complications. Aortic dissection is a catastrophic cardiovascular emergency that often presents silently and can be missed if not suspected. Here we report a sudden, fatal case of cardiopulmonary arrest following a routine colonoscopy in a young woman with ulcerative colitis, where the underlying cause was diagnosed only at autopsy.

Case Presentation

- A 41-year-old female with a known history of ulcerative colitis underwent routine colonoscopy under sedation with propofol and atropine. The endoscopic procedure was completed without immediate complications. Shortly afterward, while in the recovery room, the patient suffered sudden cardiopulmonary collapse. Resuscitation was attempted but unsuccessful due to the abruptness of the arrest.
- The patient had no prior history of cardiovascular disease. Family history revealed that her father had undergone surgical repair of an abdominal aortic aneurysm. Unofficial sources indicated possible cocaine use by the patient, which was unconfirmed clinically. There were not found presence of cocaine in the blood based on the toxicologic examination.

Autopsy Findings

- Colon: Dilated with gas, but no perforation or ischemic changes.
- Heart: Normal in size and weight; non-specific epicardial petechiae. Coronary arteries showed minimal atheromatous plaques.
- Lungs: Pulmonary edema with firm consistency, minimal bronchial secretions.
- Brain: venous congestion, mild perivascular and pericellular edema.
- Liver and other organs: Normal on gross examination.
- Descending thoracic aorta: Partial tear of the intima about 1.7 cm and 10 cm below it another tear slightly smaller than the first with the same characteristics. Intramural hemorrhage and periaortic hematoma in this section. No abnormalities noted in the abdominal aorta.

Discussion

- This case represents a sudden unexpected death following a routine colonoscopy. The most likely cause of death was acute dissection of the descending thoracic aorta, which progressed rapidly and silently, leading to fatal cardiopulmonary arrest.
- While aortic dissection is classically seen in older males with hypertension, several risk factors in this case should be noted:

- Family history of aortic aneurysm. Possible cocaine use, which is strongly associated with vascular injury. Chronic inflammatory state due to ulcerative colitis, which may compromise vascular integrity. The use of propofol and atropine during sedation is unlikely to be a direct cause but could have contributed to hemodynamic shifts in a vulnerable aortic wall.
- This case highlights the importance of preprocedural risk assessment, especially in patients with suggestive family history or suspected drug abuse.

Conclusion

- Acute aortic dissection, though rare in young adults, should be considered in cases of sudden postprocedural death, especially when no other cause is apparent.
- A detailed preprocedural evaluation, including family history and substance use, is essential in detecting possible silent risk factors.
- Clinicians should maintain a high index of suspicion for vascular catastrophes, even in routine diagnostic procedures.

References

1. Nienaber CA, Clough RE. Management of acute aortic dissection. Lancet. 2015;385(9970):800-811.
2. Hagan PG, et al. The International Registry of Acute Aortic Dissection (IRAD): new insights into an old disease. JAMA. 2000;283(7):897-903.
3. Parekh D, et al. Sudden death after endoscopy: dissection of the aorta as a rare cause. BMJ Case Rep. 2013.
4. Omura A, et al. Cocaine-induced aortic dissection. J Emerg Med. 2000;18(1):43-46.
5. Vavricka SR, et al. Extraintestinal manifestations of inflammatory bowel disease. Inflamm Bowel Dis. 2015;21(8):1982–1992.

Case report: Fatal subarachnoid Hemorrhage in a 10 years old girl with undiagnosed bilateral middle artery and anterior cerebral artery aneurysm following benzodiazepine use

Dr. Ilir Deçolli
Forensic Doctor, Forensic Institute of Tirana, Albania
ilirdecolli@yahoo.com

Dr. Gjergji Kaçauni
Forensic Doctor and Histopathology Doctor, Forensic Institute of Tirana, Albania
kacauni@yahoo.com

Introduction

Subarachnoid hemorrhage (SAH) in the pediatric population is exceedingly rare, accounting for only 0.5–4.6% of all childhood strokes. Even more rare is the presence of cerebral aneurysms, which in children occur at an incidence of approximately 0.5 to 2 per 100,000 children per year. Pediatric aneurysms differ significantly from adult aneurysms in morphology, location, and etiology. Despite their rarity, aneurysms remain a significant cause of sudden neurological deterioration and death in children when left undiagnosed. Additionally, prescribing sedative medications such as benzodiazepines in pediatric patients with unresolved neurological complaints should be done with caution, given their potential effects on cerebral blood flow and respiratory depression.

Case Presentation

- A previously healthy 10-year-old girl with a history of hyperactivity and high academic performance presented to a pediatrician with non-specific symptoms: insomnia and intermittent headaches. There was no history of trauma, seizures, or systemic illness. She was prescribed a syrup containing a benzodiazepine compound.
- Two days later, while at home, she suddenly collapsed while opening the refrigerator and lost consciousness. Despite resuscitative efforts, she was declared dead on the scene.

Autopsy Findings

- External examination: No evidence of trauma or injury.
- Heart: Normal in size, weight, and morphology; valves and coronaries were unremarkable.
- Lungs, liver, spleen, kidneys, pancreas: Normal in size and consistency; no macroscopic pathology.
- Brain: Extensive subarachnoid hemorrhage, numerous hemorrhagic pigmentations visible on cut section.
- Circle of Willis: Bilateral saccular aneurysms at the level of the middle cerebral arteries (MCA) and anterior cerebral artery. At these levels, inserted intravascular thrombi were also found.
- Histology: Confirmed widespread SAH, cerebral hemorrhage, multiorgan vascular stasis, and mild interstitial myocardial edema. Coronaries free of atherosclerosis.

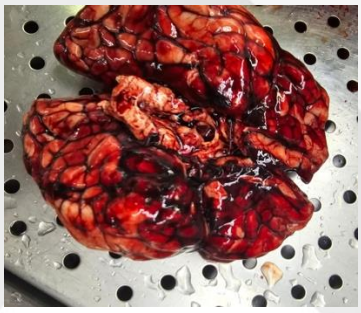


Fig 1. Aneurysm circum willis

Discussion

- Cerebral aneurysms in children are not only rare but often asymptomatic, making diagnosis difficult without neuroimaging. According to pooled data, 60–70% of pediatric aneurysms occur in the anterior circulation, and the MCA is the most commonly involved vessel. Pediatric aneurysms tend to be larger, more complex (fusiform/saccular), and more often multiple, as seen in our case.
- SAH in children accounts for less than 1% of all subarachnoid hemorrhages, and the mortality rate can reach up to 25–50%, particularly when diagnosis is delayed or missed. Sudden collapse and death in children due to aneurysmal rupture have been previously reported.

Conclusion

This report highlights the urgent need for:

- Better awareness of pediatric aneurysm presentations.
- Judicious prescribing of CNS-active medications in children.
- Development of clinical protocols for early neuroimaging in children with persistent neurological complaints.

References

- *Hetts SW, et al. Pediatric Intracranial Aneurysms. Neuroimaging Clin N Am. 2010;20(3):409–426.*
- *Lasjaunias P, et al. Intracranial aneurysms in children aged 15 years or less: review of 59 cases. J Neurosurg. 1997;86(1):43–48.*
- *Lee JY, et al. Pediatric Intracranial Aneurysms: Clinical Features and Surgical Outcome. J Korean Neurosurg Soc. 2008;43(1):46–51.*
- *Mehal JM, et al. Sudden Cardiac Death in the Young: Epidemiology and Causes. Pediatr Cardiol. 2019;40(7):1337–1345.*
- *Smith ER, Scott RM. Spontaneous subarachnoid hemorrhage in children. Childs Nerv Syst. 2004;20(8-9):549–555.*
- *U.S. FDA. Drug Safety Communication: Risk of serious side effects with use of benzodiazepines.*

BAFS 2025

Balkan Academy of Forensic Sciences

Application of Surfactant Protein-A, Hipoxia-inducible Factor 1α and CD-68 in Diagnosis of Plastic Bag Suffocation: an Immunohistochemical Study

Raffaella Campagna [1], Francesco Carpano [1], Elena Giacani[1], Federica Genchi [1], Luigi Cipolloni[1], Christian Pallante [2].

1 Department of Clinical and Experimental Medicine, Section of Forensic Medicine, University of Foggia, 71122 Foggia, Italy.

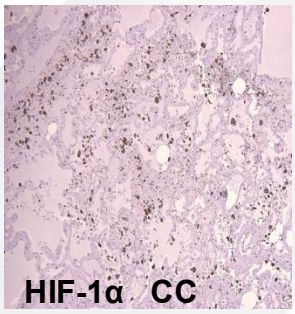
2 Department of Law, University of Foggia, 71122 Foggia, Italy.

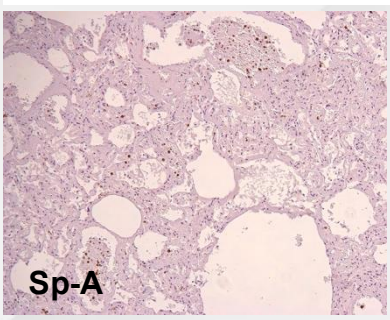
Background and aims: Plastic bag suffocation (PBS) refers to a peculiar type of asphyxia that is uncommon. The mechanism of death can be suicidal, homicidal, or accidental. It represents a challenge for the forensic pathologist because the pathological findings are often nonspecific or absent. An approach to the study of these types of deaths can be represented by immunohistochemistry, in particular by investigating three markers: Surfactant Protein -A (Sp-A), Hypoxia-inducible Factor 1α (HIF-1α) and CD-68. Sp-A is the major surfactant protein produced by alveolar type II cells, usually related to acute respiratory failure. HIF-1α is a transcription factor that plays an essential role in cellular adaptation to hypoxia regulated by the oxygen tissue concentration. CD-68 is expressed by monocytic cells, such as resident macrophages in the lungs; it is used to appreciate macrophage presence and activation and, therefore, signs of respiratory distress. Our study aims to examine the expression of SP-A, HIF-1α, and CD-68 in the lungs as a potential marker of asphyxia in PBSs.

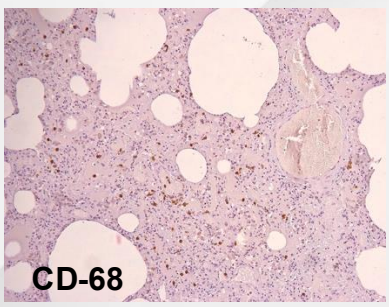
Methods: A total of 8 cases were classified into two groups: 5 plastic bag suffocation (PBS) and 3 controls (CTR) of other types of asphyxial death. The samples were subjected to immunohistochemical investigations. The immunohistochemical investigations used specific antibodies to detect the presence of **Sp-A**, **HIF-1α**, and **CD-68** in the lung tissue.

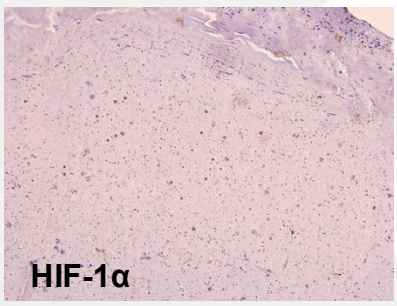
Results: We found a significant positivity for CD-68 and HIF-1α in the cases of PBS compared to the control group, in which they were negative. Moreover, SP-A positivity is higher in the cases of PBS than in control cases.

	CD-68	HIF-1α	Sp-A
CASE 1	-	-	++
CASE 2	+	++	++
CASE 3	+	+	++
CASE 4	+	+	++
CASE 5	+	++	++
Control 1	-	-	+
Control 2	-	-	+
Control 3	-	-	+









Discussion - Conclusions: The expression of Sp-A, HIF-1α, and CD-68 in lung tissue is promising for forensic medicine, as the levels and distribution of these markers could serve as biomarkers for relating an asphixyal death to plastic bag suffocation. Given the small number of cases, further investigation should be performed.

SUDDEN DEATH AND SICKLE CELL CRISIS: A REPORT OF DIFFERENT AUTOPSY CASES

Dr. Reham Eissa[1], Dr..Younis Albalooshi[1], Dr Hazem Metwaly[1], Dr Eslam Feteha[1].
[1]Forensic Medicine Department, Dubai Police, UAE.
Contact: dr.rehameissa@gmail.com

INTRODUCTION

Sudden death represents a perplexing and tragic complication within the realm of sickle cell disease, a genetic disorder primarily characterized by distorted red blood cells that can obstruct blood flow, leading to chronic pain, organ damage, and a host of other health challenges.

While advancements in medical care have increased the life expectancy of individuals with sickle cell disease, sudden death remains a distressing reality that continues to evoke concern within the medical community.

CASE SUMMARY

Herein we present four cases (with African descendent) of sudden and unexpected death with different pre-mortem circumstances. Our first case was an airplane passenger, after almost one hour in the air he felt uncomfortable and died instantly. Two cases were under severe exertion due to running from the police and died shortly after being caught up.Our last case was fighting with some of his friends, died the next day in hospital with renal failure diagnosis.

AUTOPSY FINDINGS

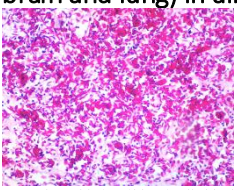
Autopsy was conducted on all cases. Their Lab tests were negative. Lividity was fixed.

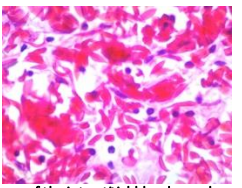
External examination showed minor abrasions and contusions on the bodies of 2nd, 3rd and 4th cases major external injuwith no other ries.

Necropsy revealed the presence of congested internal organs with no gross pathology. So, histo-pathological examination of organs was done.

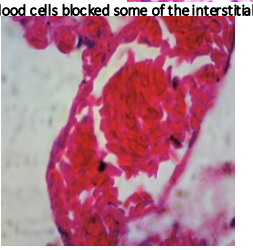
HISTOPATHOLOGICAL EXAMINATION

Histo-pathological examination of H&E stained sections of different organs unleashed intravascular sickling of red blood cells in different organs (e.g. liver sinusoids, spleen, brain and lung) in all cases.





Even the sickled red blood cells blocked some of the interstitial blood vessels.



DISCUSSION and CONCLUSSION

Sickle mutation occurs in sixth position of beta chain and encodes valine instead of glutamic acid. This is responsible for changes in molecular stability and solubility. Distortion of cells into sickle shape is due to haemoglobin polymerisation that occurs in deoxygenated state. Kinetics of sickling suggests that polymerisation occurs in stages. The delay period between deoxygenation and polymerisation is attributable to nucleation process. If the delay time is less than 1 s then the RBCs are stuck in the microcirculation resulting in vaso-occlusion crisis. The delay time is strongly influenced by various factors like temperature, pH, Hb concentration, presence of Hb other than HbS.

Histopathological examination showed widespread sickling of red blood cells. The diagnosis of sickle cell disease was made only after post-mortem examination.

Vaso-occlusive crisis or painful crisis can be precipitated by various factors like infections, low oxygen tension, dehydration, acidosis, extreme physical exercise, physical or psychological stress, pregnancy, alcohol.

TAKE HOME MESSAGE

In cases of sudden death, Autopsy plays a crucial role in identifying the cause of death. The histo-pathologic microscopic examination is essential for determining the cause of death.

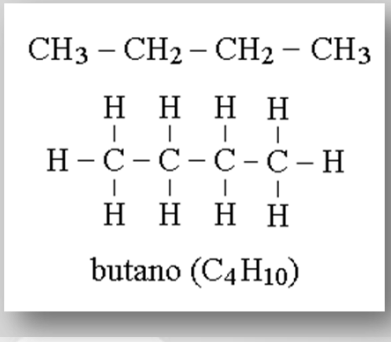
BAFS 2025

FATAL BUTANE INHALATION: THE CONTRIBUTION OF CARDIAC AND BRAIN IMMUNOHISTOCHEMICAL MARKERS.

Maria Antonella Bosco [1], Alessandro Santurro [2], Chiara Fabrello [1], Roberta Bibbò [1], Luigi Cipolloni [1], Angela Procaccino [3].

1 Department of Clinical and Experimental Medicine, Section of Forensic Medicine, University of Foggia, 71122 Foggia, Italy.
2 Department of Medicine, Surgery and Dentistry, University of Salerno, Baronissi, Italy.
3 Department of Law, University of Foggia, 71122 Foggia, Italy.

Background and aims: Butane (C_4-H_{10}) is a highly flammable and explosive aliphatic hydrocarbon. Thanks to its *easy accessibility*, it can become a narcotic substance, causing a *psychoactive effect*. It is usually inhaled directly (sniffing), especially from lighters, camping stoves, and spray cans. Of all inhalants, butane is the one with the *highest mortality rate*, as it exerts its toxicity mainly at the level of the *central nervous system* and the *cardiovascular system*. The rationale for this study is to compare eight cases of fatal butane poisoning with control cases of subjects deceased following head or thoracic trauma to identify antigens that can act, respectively, as cardiac and cerebral markers to support the medico-legal diagnosis of butane poisoning.



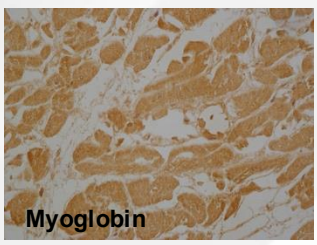
Methods: We identified eight cases of fatal butane poisoning, six men and two women aged between 18 and 52 years. For all the selected instances, hematoxylin and eosin (H&E) staining and immunohistochemical investigations were performed using a panel of antibodies against the antigens **Connexin 43**, **Myoglobin**, **Troponin T** and **Troponin I** on the heart samples (left ventricle, anterior wall) and **MAP2** and **Calbindin** on the brain samples (parietal lobe).

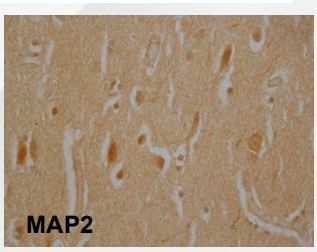
Results: The positivity to the Antigen-Antibody reaction for both brain and cardiac markers was standardized with *semiquantitative interpretation.*

[0]: not expressed.
[+/-]: baseline positivity.
[+]: isolated and disseminated expression.
[++]: expression in groups or diffuse foci.
[+++]: diffuse expression.
[++++]: markedly diffuse expression.

Antigens	Results of semiquantitative analysis of cases							
	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Connexin-43	++	+	+	+	+	+	+	+
Myoglobin	++	++	+	++	+	+	++	++
Troponin I	+	++	++	+++	+++	+++	+	+++
Troponin T	+	+	+	+/-	++	++	++	+/-
MAP2	++++	++++	+++	+	+++	++++	+++	++++
Calbindin	+	++	++	++	+++	++	+++	+++

Antigens	Results of semiquantitative analysis of controls							
	Control 1	Control 2	Control 3	Control 4	Control 5	Control 6	Control 7	Control 8
Connexin-43	+++	++++	++	++++	+++	++++	++	++++
Myoglobin	++++	++++	+++	++++	++++	++++	+++	++++
Troponin I	++	+++	+++	+++	++	+++	+++	+++
Troponin T	++	+++	++	++	++	+++	++	++
MAP2	++	++	+	++	++	++	+	++
Calbindin	+	+	+	++	+	+	+	++





Discussion - Conclusions: The brain markers are more expressed in the cases than in controls. The cardiac markers, in particular connexin 43 and myoglobin, are more highly expressed in the controls than in the cases, which, conversely, are characterized by their depletion. There is still much to be done to be able to compare our case studies with other experiences on the national and international territories, thus expanding the size of the sample, deepening the role of brain and cardiac markers, and proposing a shared methodology both at the autopsy table and in forensic laboratories.

"BRIDGING SCIENCE, JUSTICE, AND HUMANITY"

BAFS 2025

Postmortem diagnosis of accidental hypothermia – macroscopic and microscopic correlations in hypothermia

Andreea-Alexandra Hleșcu[1,2], Mădălina Diac[1,2], Bianca Hanganu[1,2], Iuliana Hunea Zamisnicu[2], Cristian Ștefan Hleșcu[1], Adriana Grigoraș[1,2], Beatrice Gabriela Ioan[1,2], Diana Bulgaru-Iliescu[1,2], Cornelia Amălinei[1,2]

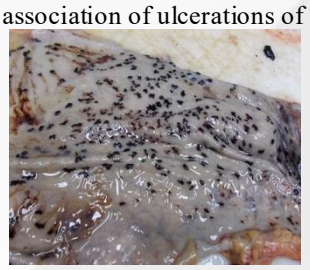
1 Grigore T. Popa University Of Medicine and Pharmacy Iași , Romania
2 Institute of Legal Medicine Iași, Romania

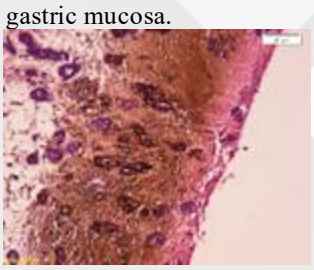
The aim of our study was to detect the histopathological changes in the heart, kidney, brain, lungs, liver and stomach in a group of cases of death caused by hypothermia, in order to obtain new information regarding the mechanism of hypothermic stress and possible associations with medico-legal and toxicological data.

The archived material from the histotheque of ILM Iași was used, all the paraffin blocks of heart, brain, kidney, lung, stomach fragments from 107 necropsy cases. Tissue fragments of brain, heart, lung, stomach and kidney were stained with Hematoxylin-Eosin (HE), periodic acid-Schiff (PAS) and Masson's trichrome stain.

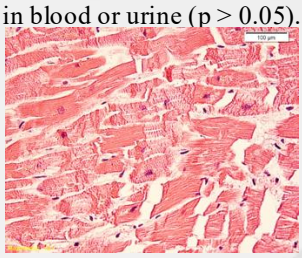
The evaluation of histopathological parameters of hypothermia victims was performed by associating macroscopic and microscopic characteristics with the mechanism of hypoxia and metabolic alterations induced by hypothermic stress and differentiating them from pre-existing ones.

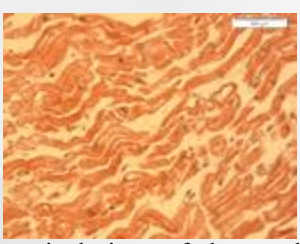
Microscopic study of stomach tissue preparations confirmed the presence of Wischnewsky petechiae identified during necropsy in 47 cases (44.8%), these being expressed in the form of micro-hemorrhages, consisting of blood residues at the level of the lamina propria, without the association of ulcerations of the gastric mucosa.





The heart sections, stained with HE and Masson's trichrome, revealed, besides the lesions observed macroscopically, the presence of vacuolar myocardial degeneration (13.1%) and myocardial contraction bands (95.3%) with a focal wavy appearance, as characteristic signs of myocardial ischemia. Statistical analysis demonstrated the existence of a statistically significant correlation between the presence of myocardial contraction bands and air humidity values (93.5%, $p = 0.001$), but no correlation between cardiac lesions and the level of alcohol in blood or urine ($p > 0.05$).

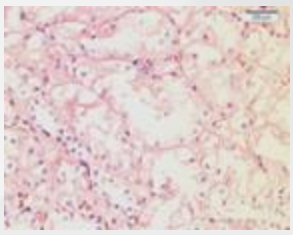


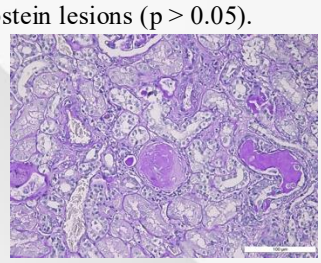


The macro and microscopic lesions of the renal parenchyma in our group were most frequently represented by: passive vascular congestion, Armanni-Ebstein lesions, tubular necrosis, interstitial hemorrhage.

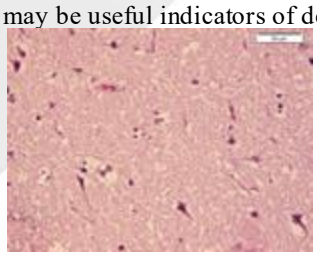
Additionally, a variable thickening of the tubular and corpuscular basement membranes was observed, more evident in PAS staining, associated with amorphous material in the Bowman's capsular space and in the lumens of the renal tubules.

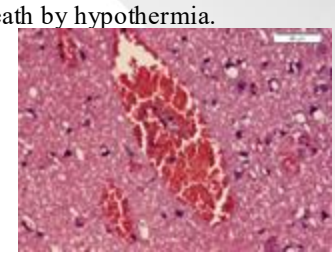
A significant association was identified between chronic alcohol consumption and the microscopic presence of Armanni-Ebstein lesions (52.2%, $p = 0.01$), but no statistically significant association was observed between positive alcoholemia and the presence of renal hemorrhage, tubular necrosis, or Armanni-Ebstein lesions ($p > 0.05$).



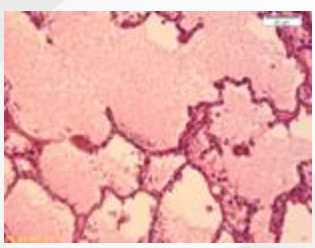


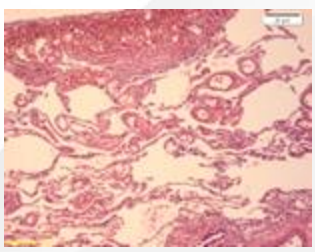
Microscopic examination of brain tissue preparations confirmed the macroscopic autopsy findings, adding additional findings of gliosis, mononuclear cell infiltrate, traumatic brain injury, and focal neuronal ischemia associated with perivascular hemorrhages, showing that they may be useful indicators of death by hypothermia.





At the pulmonary level, macroscopic lesions detected necrotically were confirmed by microscopy, the most relevant being represented by hemorrhagic pulmonary edema, marked diffuse alveolar edema and intra-alveolar and pleural hemorrhages being a result of pulmonary and systemic hemodynamic disorders, associated with microcirculatory lesions, as a consequence of endothelial and alveolar epithelial lesions, as a result of impaired fluid drainage from the alveolar septum.





Microscopic examination is essential and can provide information to confirm findings of suspected hypoxia in the context of hypothermia by highlighting specific organs lesions.

Conclusions

Microscopic examination may encounter difficulties in accurately diagnosing death by hypothermia due to postmortem autolysis and putrefaction, the presence of comorbidities that can alter the occurrence of several types of lesions, like Armanni Ebstein, the length of the survical period, the alcohol consumption, but these can be partially overcome by using immunohistochemical analyses to confirm such a diagnosis with certainty.

References

Ball C.G., Herath J.C.. Earth, Air, Water and Fire: Histopathology of Environmental Death. *Acad Forensic Pathol*. **2018**; 8(3): 641-652

Bjertnas L.J., Nasheim T.O., Reierth E., Suborov E.V., Kirov M.Y., Lebedinskii K.M., Tveita T. Physiological changes in subjects exposed to accidental hypothermia: An update. Front. Med. **2022**; 9: 824395

Wang Q., Ishikawa T., Michiue T., Zhu B.L., Guan D.W., Maeda H. Molecular pathology of pulmonary edema in forensic autopsy cases with special regard to fatal hyperthermia and hypothermia. *Forensic Sci. Int.* **2013**; 228: 137–141

Doberentz E., Markwerth P., Madea B. Fatty degeneration in renal tissue in cases of fatal accidental hypothermia. *Rom J Leg Med.* **2017**; 25: 152-157