

ESTABLISHMENT OF FORENSIC ARCHEOLOGY IN THE REPUBLIC OF AZERBAIJAN AND ITS APPLICATION IN THE EXUMATION OF HUMAN REMAINS BELONGING TO PERSONS GONE MISSING DUE TO THE CONFLICT



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Key words: Forensic Archaeology, Forensic Anthropology, Institute of Archaeology and Anthropology of the ANAS, Karabakh war, Azerbaijan, ICRC, exhumation, archaeological excavations.

At the end of 80s of the XX century due to socio-economic and political problems in the USSR the central government became weaker and as a result the conflicts on national grounds broke out. One of such conflicts arose between the Republics of Azerbaijan and Armenia. Thus, population of Armenian descent living in Karabakh region of Azerbaijan raised the demands for the region to be annexed to the Republic of Armenia. Subsequently, war broke out and tens of thousands of people lost their lives in the conflict, over a 600.000 Azerbaijani people had to flee their homes and became IDPs, while more than 3900 people went missing, whose fate is still unknown today. Negotiations that lasted over 30 years and implementation of UN Security Council Resolutions 822, 854, 874 and 884 yielded no results.

On 27 September 2020, a 44-day, II Karabakh war broke out and as a result, thousands more people died, and 6 military servicemen went missing. The war ended with the Armenian side signing an act of capitulation.



Figure 1. In situ human remains found in the premises of Shusha Prison

On 13 July 1993, the State Commission on Prisoners of War, Hostages and Missing Persons was established by the Decree of the President of the Republic of Azerbaijan to coordinate the search and identification process of missing persons. The State Commission comprises the State Security Service, Prosecutor's Office of the Republic of Azerbaijan, Institute of Archaeology and Anthropology of the Azerbaijan National Academy of Sciences (ANAS), Ministry of Defence, among other pertinent organizations.

A forensic archaeology and anthropology expedition was set in the Institute of Archaeology and Anthropology of the ANAS in connection with the search and recovery of people who went missing due to conflict.

In the past, on separate occasions archaeologists and anthropologists of ANAS were invited by the authorities to participate in the exhumation of victims of 1918 genocide and Soviet reprisals. However, these activities were mostly sporadic and carried out using traditional field archaeological techniques.



Figure 2. Exposing human remains buried in Edilli

The Azerbaijan delegation of the ICRC with its great expertise played a crucial role in the development of this area. With the support of the ICRC, the archaeologists and anthropologists of the Institute attended trainings in Türkiye, Germany, South Cyprus, Serbia and learned about forensic archaeology and anthropology (**Figure 3; 4**). In addition, within the Pilot Project of the ICRC the Working Group of the State Commission carried out search in the liberated areas of Karabakh and remains belonging to more than 500 individuals were exhumed and handed over for examination.



Figure 3. Training on scene analysis with commingled remains

On 14 September 2022 the expedition team started the investigation work in an area 50 m away from the outermost house of Edilli village, 250 m to south-east from the village cemetery (N.39033.8170 E.047004.1220). The first excavation site was 1200 sq. m. comprising 48 quadrants each 5x5 m. In the next stage the excavation site was expanded to include 6 more areas of suspicion with 5x5 quadrants. During the excavation AKM bullets, a buckle, an unexploded F1 grenade, fragments of a military uniform, buttons, missile pieces, personal effects, a fragment of a human pelvic bone were found on the surface level.

On 17 September, a skeleton was found at 0.20-0.25m depth in the 10th quadrant. The excavation work started on 15 September 2023 and continued until 28 November with breaks. Considering the relief, 875 sq. m., 35x25 was covered. During the excavation skeletal remains belonging to over 30 individuals were found (**Figure 2; 5**). The remains were exhumed and collected with the participation of the Military Prosecutor and forensic experts of the Association of Forensic Medical Examination and Pathological Anatomy.



Figure 4. Practical forensic anthropology training in Serbia



Figure 5. Exhumation operations of a mass grave in Edilli

Excavations were carried out both by traditional methods, i.e. manually and using machinery such as an excavator. The teeth of the bucket of the excavator were removed in advance to avoid any damage to skeletal remains. Parallel trenches at 0.5 m distance were excavated with the excavator. The operator removed earth at 15 cm depth under the archaeologists' supervision. If something suspicious was noticed in the soil the operator was stopped and the archaeologists checked that area, if a bone, piece of fabric or any object was found, the machine was removed from the area and the excavation was continued manually.



Figure 6. Mass grave with remains belonging to 30 individuals in Shusha Prison

During the investigation of Yukhari Seyidahmadli village, the expedition members found skeletal fragments belonging to several individuals on the surface. Initially, the area was cleared of mines and like in Edilli excavation work was started with the help of an excavator using trench method. During the excavation, human remains belonging to more than 10 individuals were found. The expedition continued its work in Ashaghi Seyidahmadli village, where presumably a mass burial site was. As a former frontline, the area was heavily mined, and the investigation works started upon clearance by the ANAMA staff, however, nothing was found.

In June 2023, excavation works started in Shusha prison, where a mass grave with human remains belonging to 30 individuals who went missing in relation to the conflict was located (**Figure 1; 6**). The investigation and exhumation works continued in 2024 as well, with human remains belonging to more than 20 individuals found from Shusha, Khojaly, Askeran, and Malibayli areas and were handed over to the pertinent authorities.



Figure 7. Meeting with YTB in Ankara, securing full-ride scholarships for Azerbaijani students

In May 2023, the International Conference on Forensic Archaeology and Anthropology was jointly held in Baku by the Institute of Archaeology and Anthropology of the ANAS, State Commission on Prisoners of War, Hostages and Missing Persons and the ICRC. Forensic archaeologists and anthropologists from around the globe participated at this event and shared their experiences.

Notably, over these years the ICRC supported the development of the forensic archaeology and anthropology in Azerbaijan through material support and capacity building activities. Namely, with the support of the ICRC, an agreement was reached between ANAS and Ankara University of Türkiye (**Figure 7**), allowing 3 Azerbaijani nationals to receive fully-funded admissions to the forensic anthropology degree programs at Ankara University. In 2024, the Forensic Archaeology Department was established at the Institute of Archaeology and Anthropology of the ANAS. The purpose for the establishment of the department is to trace people disappeared in Karabakh wars, locate, and exhumate their remains applying methods of modern forensic archaeology.



CHALLENGES FACED IN THE FORENSIC MEDICAL EXAMINATION PROCESS OF SKELETAL REMAINS OF CONFLICT-RELATED MISSING PERSONS IN AZERBAIJAN



Məhkəmə Tibbi Ekspertiza və Patoloji Anatomiya Birliyi

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Keywords: Azerbaijan, Forensic Medical Examination, SOP, commingled remains, missing persons

Azerbaijan is engaged in a significant and complex process to account for approximately 4,000 missing persons from the Karabakh conflict of the 1990s. The “Unification of Forensic-Medical Expertise and Pathological Anatomy” Ministry of Health of Azerbaijan Republic (UFMEPA), operating under the aegis of the Azerbaijan's State Commission for Prisoners of War, Hostages, and Missing Persons, plays a crucial role in the forensic medical examination of human remains. This effort is part of a broader multi-institutional initiative aimed at identifying and recovering the missing individuals.

The process started in 2021, when approximately 100 skeletal remains were recovered from the Karabakh region and subsequently sent to the UFMEPA laboratory for forensic examination.

The skeletal remains presented for examination comprised various types of cases, including human remains handed over by opposite side, those discovered during repair and construction activities, and those exhumed as part of planned operations. A significant proportion of these remains were commingled, presenting a unique set of challenges for forensic experts.

The forensic examination of these skeletal remains faced several challenges. One of the primary issues was the lack of experience in identifying and processing commingled human skeletal remains, particularly on a large scale. The forensic experts' prior experience in dealing with such complex cases was insufficient, and the existing procedures were not adequately developed to handle the scale of this task.

The initial approach to documenting the findings was based on textual reviews. There were no standardized forms, coding systems, or Standard Operating Procedures (SOP) in place. This lack of standardization hindered the efficient processing and documentation of the remains. Furthermore, the conditions for the reconstruction and re-Unification of bones, following the determination of the Minimum Number of Individuals (MNI), were unfavorable due to inadequate laboratory facilities and resources.

The laboratory faced significant constraints, including insufficient space for storage, a lack of specialized equipment, and the general inadequacy of facilities.

These infrastructural limitations compounded the difficulties faced by forensic experts, making it challenging to effectively manage and process the remains.

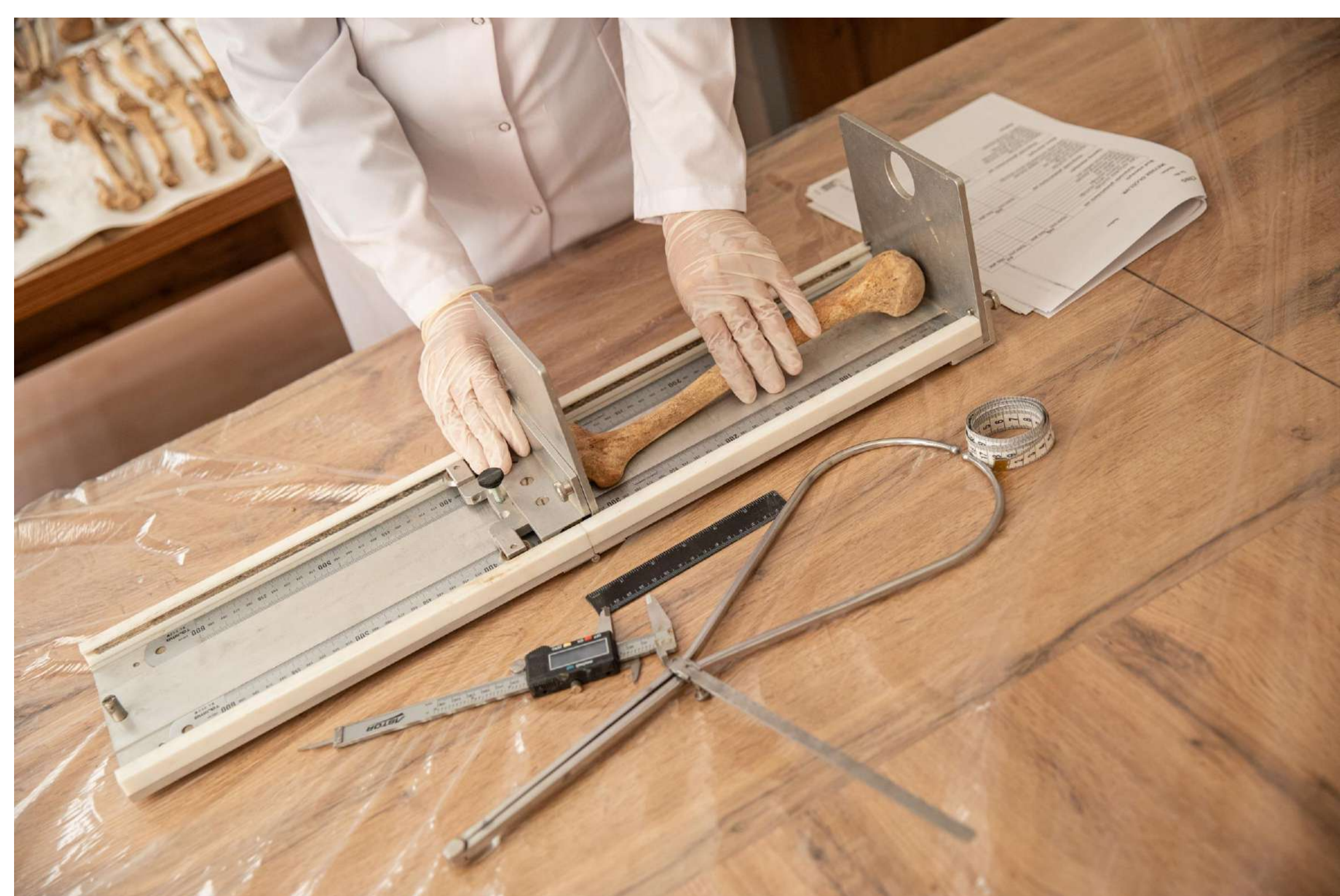


Figure 1. Measuring a humerus with an osteometric board

Government Response and Improvements

Recognizing these challenges, the government allocated a separate building specifically for the forensic examination of conflict-related skeletal remains. This new facility was equipped with enhanced infrastructure, including larger examination rooms, a dedicated storage room for skeletal remains, and updated equipment. A special table for DNA sampling was also provided to improve the efficiency and accuracy of the examination process.

Capacity Building and International Collaboration

The International Committee of the Red Cross (ICRC) played a pivotal role in addressing these challenges. Through capacity-building initiatives and material support, the ICRC facilitated the adoption of internationally accepted forensic best practices and new laboratory methods. This support included the development of new forms and coding systems, as well as the validation of several documents and SOPs. The collaboration with the ICRC also involved international exposure for forensic experts. Experts from Azerbaijan traveled to the Republic of Cyprus and Serbia to gain insights into best practices in human remains exhumation and laboratory examination.

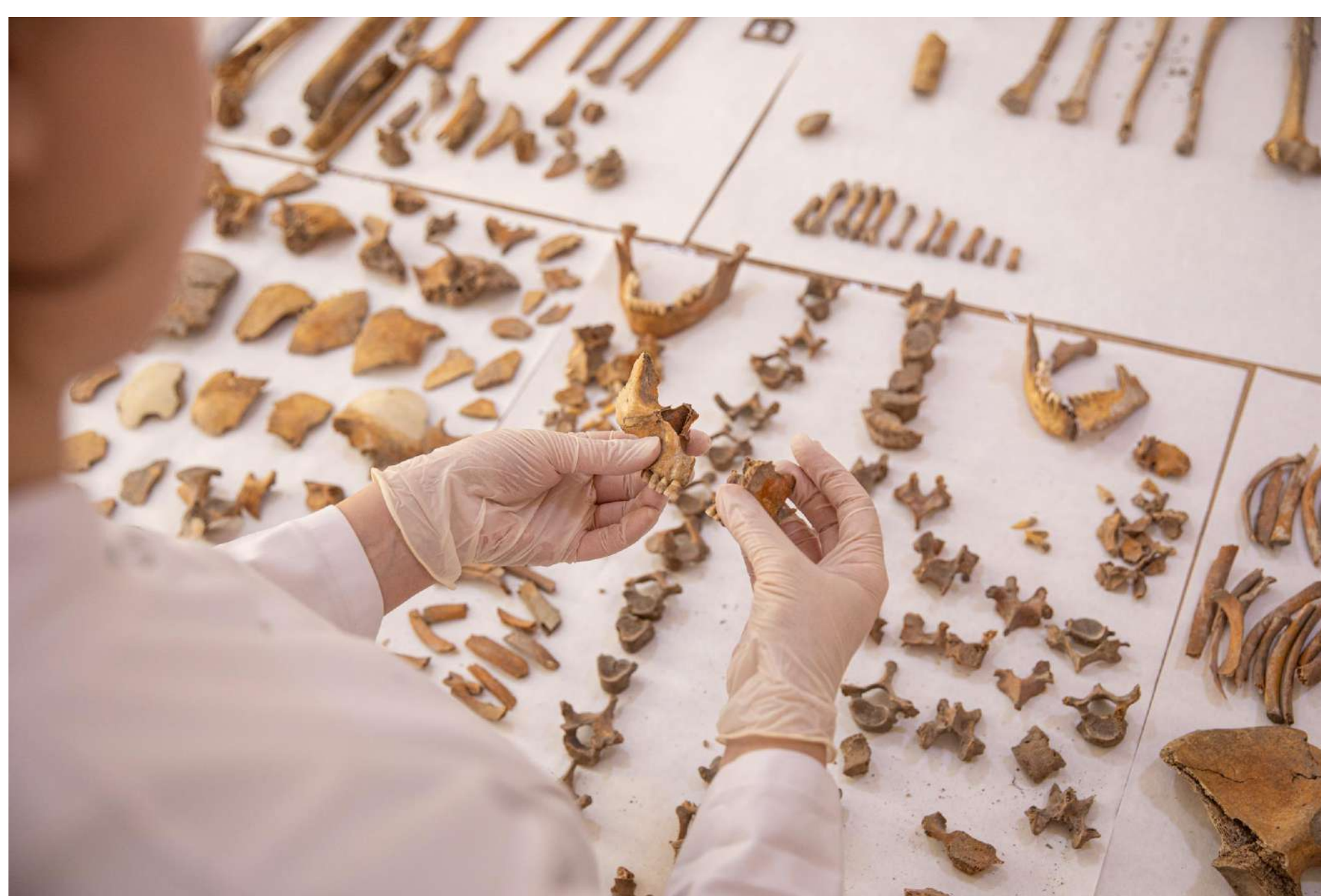
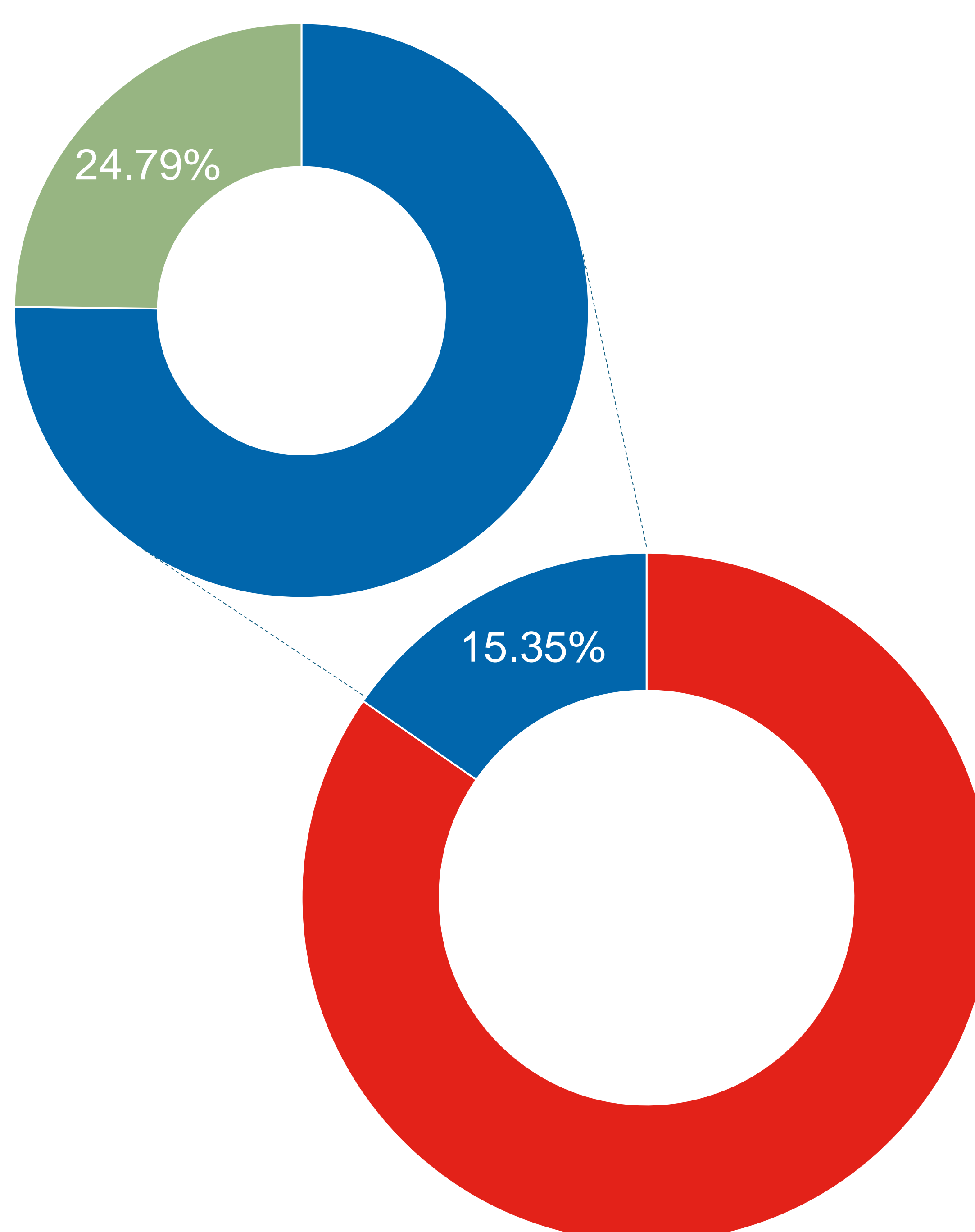


Figure 2. Anthropological examination of commingled human remains



Total number: **3967**
Total examined: **609***
Total identified: **151**

**May include non-conflict related cases, to be determined after DNA analysis.*

Figure 3. Caseload scope pertaining to missing persons in Azerbaijan



Figure 4. Cutting bone samples inside a fume hood

We express our gratitude to the ICRC delegation in Azerbaijan and all members of its Forensic team for their support!



Additionally, leading anthropologists, were invited to Azerbaijan to share their extensive experience in handling commingled human remains.

The adoption of advanced forensic expertise standards has greatly improved the efficiency and effectiveness of the examination process.

Innovations and Outcomes

A major advancement was the creation of a new SOP regarding Bone/teeth sampling for DNA Analysis. Enhanced techniques for the reUnification of commingled remains were developed, which alleviated the bottleneck workload and improved the overall identification process.

The establishment of a dedicated forensic examination facility, coupled with the support from the ICRC and the integration of global forensic standards, has markedly improved the process. The development of new SOPs, forms, and techniques has facilitated a more effective examination and identification process, providing a critical contribution to resolving the cases of missing persons from the Karabakh conflict.

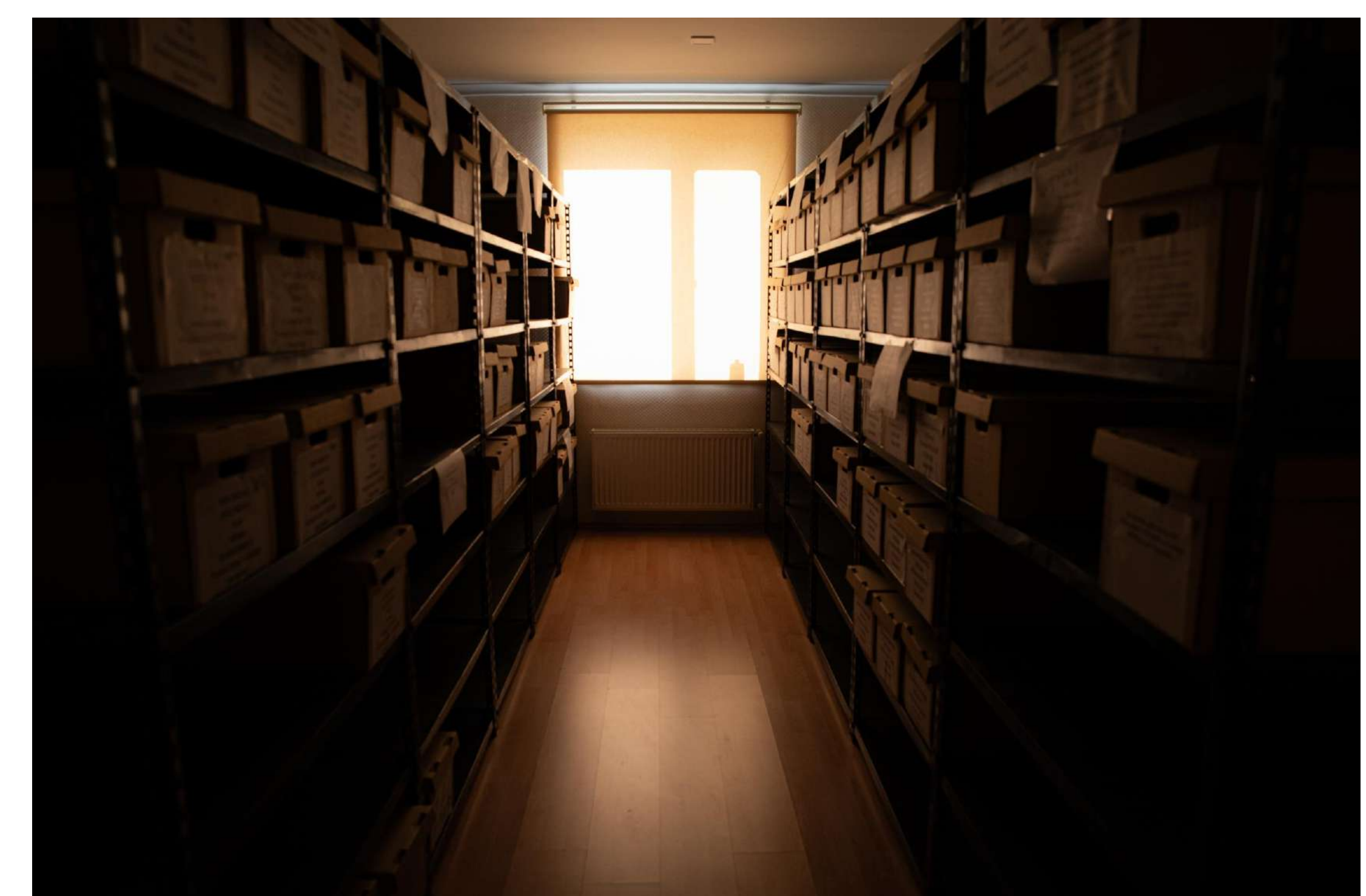


Figure 5. Storage room for skeletal human remains at the UFMEPA

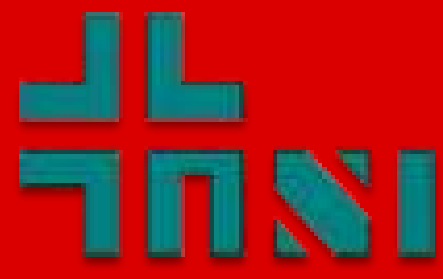
These efforts underscore the importance of continued investment in forensic infrastructure and expertise, as well as the value of international cooperation in addressing complex forensic challenges. The advancements achieved in Azerbaijan serve as a model for other regions facing similar issues and highlight the critical role of forensic medicine in conflict resolution and human rights efforts.



Figure 6. Filling anthropological forms



Faculty of Medicine
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A quantitative approach for detecting drugs from multiple classes in human blood by scheduled MRM LC-MS/MS



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BACKGROUND

Forensic laboratories are increasingly using LC-MS/MS techniques to screen biological specimens for various drug classes. However, most methods are restricted to certain drug classes and use variable and time-consuming sample preparation procedures.

AIM

The development and validation of an LC-MS/MS method to quantitatively determine drugs from different drug classes, including new psychoactive substances (NPS), in whole blood, utilizing a simple and rapid liquid-liquid extraction procedure suitable for routine analyses and medico-legal death investigations.

Drugs included (100 total)

Amphetamines

Amphetamine
Methamphetamine
MDA
MDMA

NPS

2-FDCK
ADB-Butinaca
Benzylone
Brorphine
Eutylone
Flubromazepam
Mephedrone

CNS Stimulants

BE
Caffeine
Cocaine
Cocaethylene
EME
Ephedrine
Nicotine

Antiepileptics

Topiramate
Carbamazepine

Antidepressants

Agomelatine
Amitriptyline
Amoxapine
Bupropion
Citalopram
Clomipramine
Desipramine
Desmethyl-citalopram
Duloxetine
Fluoxetine
Fluvoxamine
Imipramine
Maprotiline
Melatonin
Mirtazapine
n-Desmethyltrimipramine
Nor-triptyline
o-Desmethylvenlafaxine
Paroxetine
Protriptyline
Sertraline
Trimipramine
Venlafaxine

Antipsychotics

Aripiprazole
Amisulpride
Chlorpromazine
Clozapine
Haloperidol
Nor-clozapine
Olanzapine
Paliperidone
Quetiapine
Risperidone
Sulpiride

Opiates & Opioids

6-MAM
Buprenorphine
Codeine
Dextropropoxyphene
Fentanyl
Methadone
Morphine
Norbuprenorphine
Nalorphine
Tramadol

Anaesthetics

Ketamine
Nor-ketamine
Lidocaine
Other Drugs
Atropine
Cimetidine
Cyclobenzaprine
LSD
Mescaline
Mitragnine
Paracetamol
Procainamide
Propranolol
Sildenafil
Tadalafil
Verapamil
Zolpidem
Zopiclone

Benzodiazepines

Alprazolam
a-Hydroxyalprazolam
Bromazepam
Clobazam
Clonazepam
Chlordiazepoxide
Diazepam
Flunitrazepam
Lorazepam
Midazolam
n-desmethylflunitrazepam
Nitrazepam
Nor-diazepam
Oxazepam
Prazepam
Temazepam
Triazolam
***Internal Stds**
Clozapine-D4
MDMA-D5
Morphine-D3

MATERIALS & METHODS

Instrumentation

Dionex UltiMate 3000 UHPLC system

- **Accupore C18 column**, 2.6 μ m, 50 x 3 mm, 30°C
- **Eluent A**: 10mM Ammonium Acetate pH=3.5/ 0.1% Formic Acid in H₂O
- **Eluent B**: Acetonitrile/ 0.1% Formic Acid
- **Injection volume**: 1 μ L
- **Flow rate**: 0.5 mL/min
- **Run time**: 6.5 min

Sciex 5500+ QTrap™ MS/MS

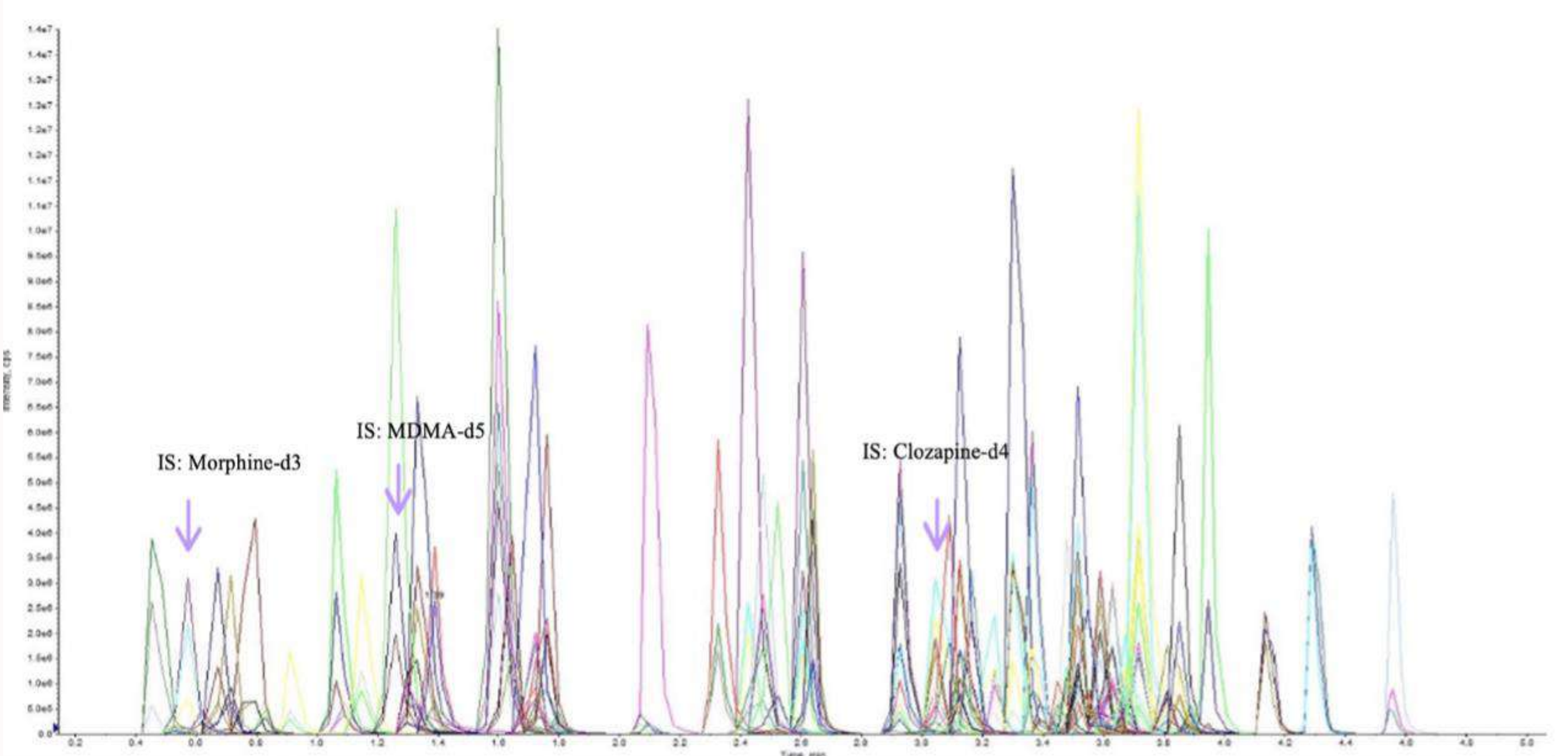
- **ESI** +5500 V, 550°C
- **G1/G2**: 90 psi
- **Curtain**: 55 psi
- **MRM window**: 20 sec
- **Cycle time**: 0.2 sec
- **3 MRMs per analyte**

Extraction procedure

- 200 μ L of whole blood fortified with target analytes + 20 μ L IS solution
- 200 μ L carbonate buffer (1 M; pH 9.5)
- Stirring + vortex, 10 min
- 1 mL 0.1 M HCl/ methyl-t-butyl ether
- Stirring + centrifugation 10,000 rpm, 10 min
- Supernatant transfer, evaporate N₂ 40°C
- Reconstitute 50 μ L A:B (88:12%)

RESULTS

- **LOD**: 0.01 - 5 ng/mL
- **LOQ**: 0.05 - 20 ng/mL
- **Matrix Effects**: 75% for all analytes
- **Inter-day precision**: 3-15%
- **Intra-day precision**: 7-18%
- **Post-Preparative Stability**: > 95%
- **Selectivity**: No interferences and no **Carry Over**
- **Linearity**: 0.05 - 500 ng/mL (R² > 0.99)



Total Ion Chromatogram of 100 drugs (250 ng/mL)

CONCLUSION

A **rapid, sensitive, specific, effective** and **reliable** method was developed, based on the application of LLE engaged to LC-MS/MS, for routine identification and quantitation of multiple drug classes from whole blood, was developed, and validated (Standard Practices for Method Validation in Forensic Toxicology, 2019) for use in routine forensic casework.



Publication: D. Florou, M. Di Rago, A. Orfanidis, D. Gerostamoulos, V. A. Boumba. (2024). A broad-spectrum LC-MS/MS method for screening and quantification of 100 analytes in clinical and autopsy blood samples, *Journal of Chromatography B*; 1247, 2024, 124323





CASE REPORT

FATAL INTOXICATION WITH TRAMADOL IN COMBINATION WITH ALPRAZOLAM



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• Background and Aim

We report two cases of fatal intoxication with tramadol in combination with alprazolam. Three foreign girls were working as escorts in the city and had spent their night entertaining in clubs. The next day, one of them found her roommates dead in their apartment; one was found in the bedroom and the other one near the bathroom. During the death scene autopsy, medicinal drugs (Tramadol, Xanax), vitamins, condoms, lubricants and alcoholic beverages were found in their apartment.

Method

A full autopsy was performed revealing signs of general asphyxia in both girls. Femoral blood, urine, vitreous humour (VH) and stomach with content were sent to the Toxicological Laboratory, while specimens from heart, lungs, pancreas and kidneys were sent to the Histological Laboratory. During the toxicological investigation, head-space-GC/FID analysis for alcohol, screening analysis for drugs of abuse by EMIT-immunoassay method as well as GC/MS analysis for determination of medicinal drugs, drugs of abuse and other substances was performed. Histo- pathological examination were performed by H-E method.

• Histological findings

Fig. 1 Histological changes in heart, case with tramadol/alprazolam intoxication

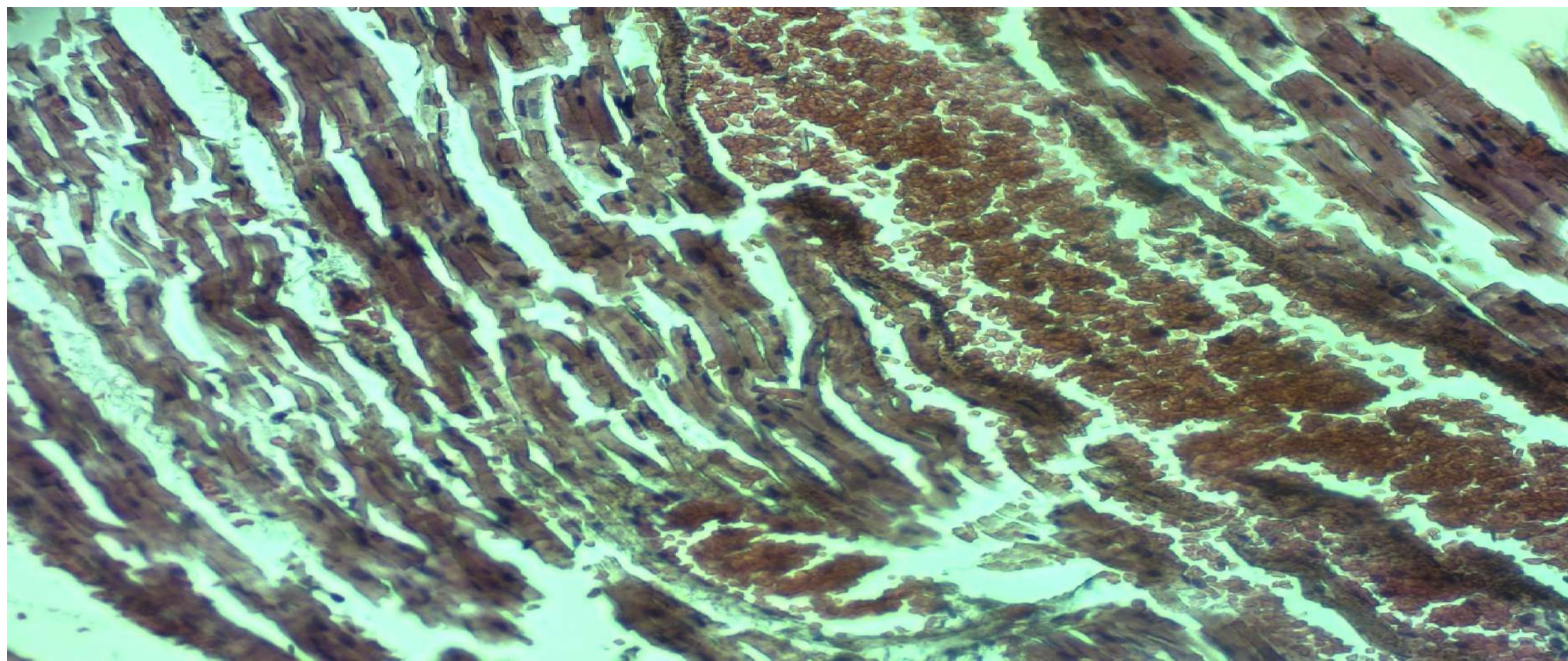


Fig. 2 Histological changes in lungs, case with tramadol/alprazolam intoxication

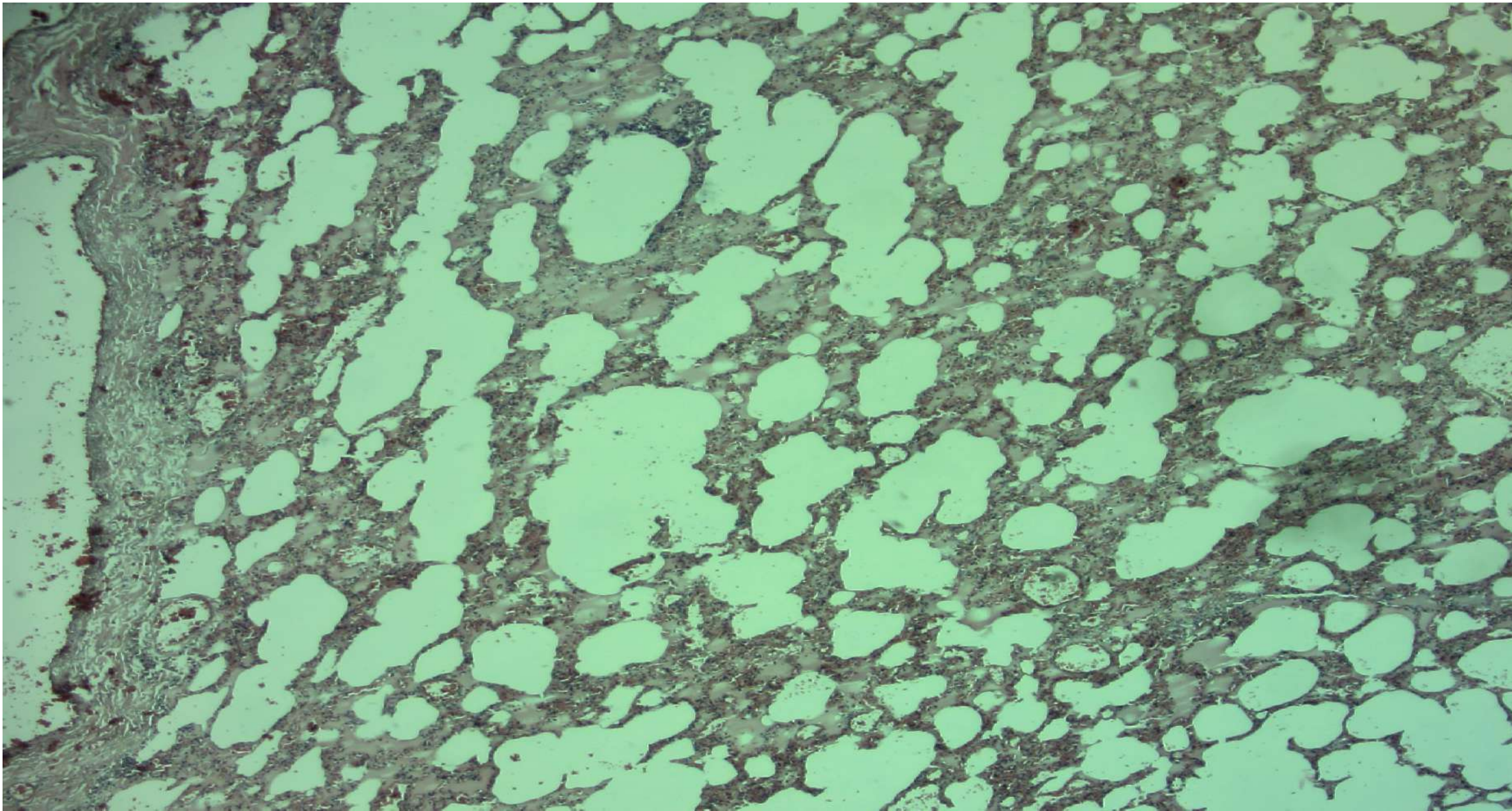
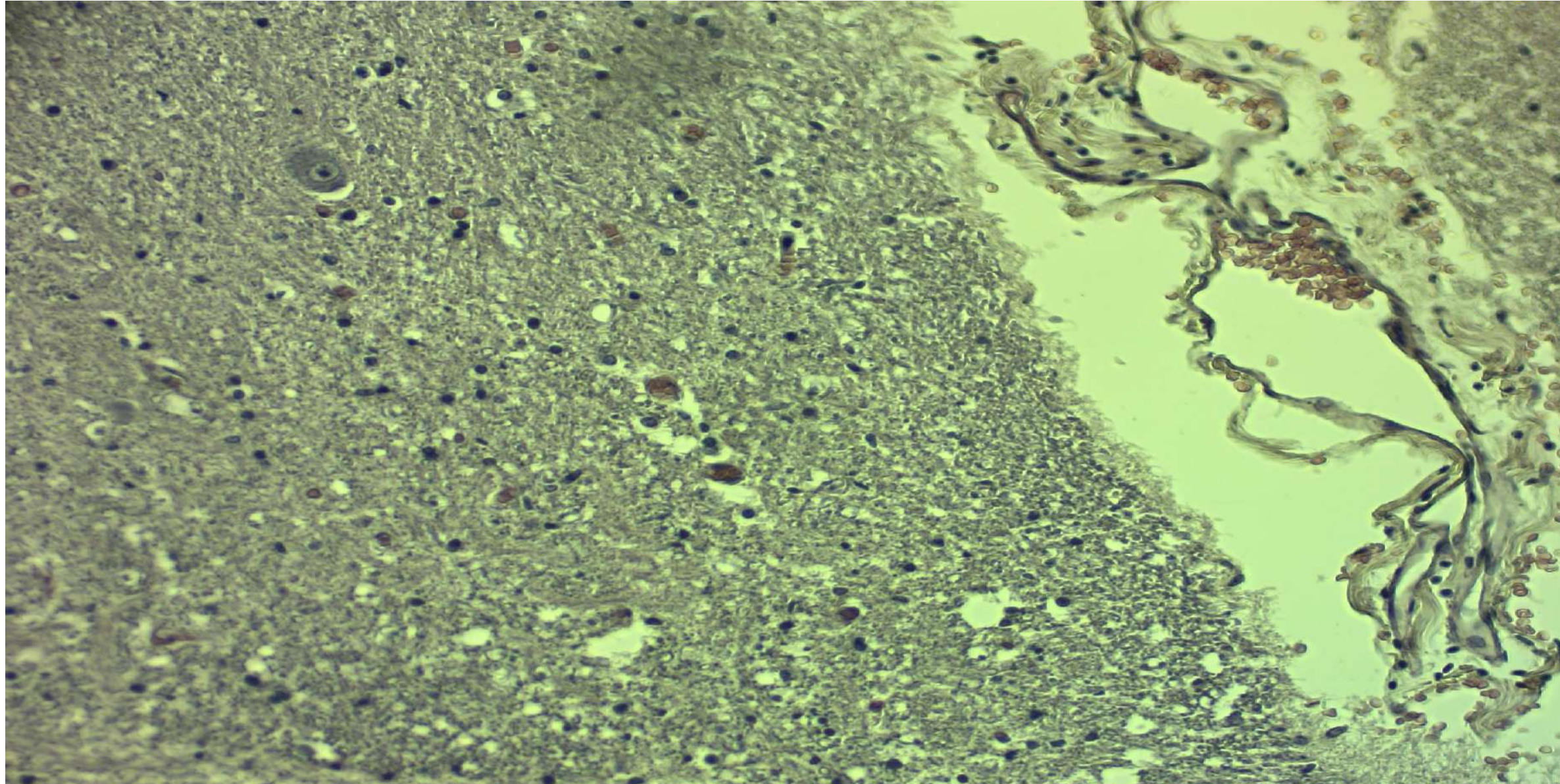


Fig. 3 Histological changes in brain, case with tramadol/alprazolam intoxication



• Toxicological findings

In both cases, no alcohol was found in blood and VH samples. Immunoassay blood and urine analysis was negative for opiates, cocaine, cannabis, and amphetamines and positive for benzodiazepines. Also presence of paracetamol, temazepam, ibuprofen were detected in the urine samples.

During GC/MS analysis, tramadol, O-desmethyltramadol and alprazolam were detected in all biological samples, and they were quantified in blood and VH samples

Sample	Tramadol (ug/ml)	O-desmethyl tramadol (ug/ml)	Alprazolam (ug/ml)
Blood 1	5.1	0.92	0.167
VH 1	2.5	0.58	0.038
Blood2	7.5	0.84	0.276
VH2	3.5	0.48	0.025

• Conclusions

In scientific literature, blood levels of tramadol higher than 1µg/mL are considered toxic, and may indicate contribution to the cause of death, while blood concentrations above 2µg/mL are considered lethal. For alprazolam blood concentrations above 0.1µg/mL are considered toxic. The presence of tramadol and alprazolam in blood samples of the two cases at concentrations above these limits, indicate that the cause of death for both cases was drug intoxication with tramadol and alprazolam causing respiratory failure.

Fig. 4 Photos of death scene autopsy

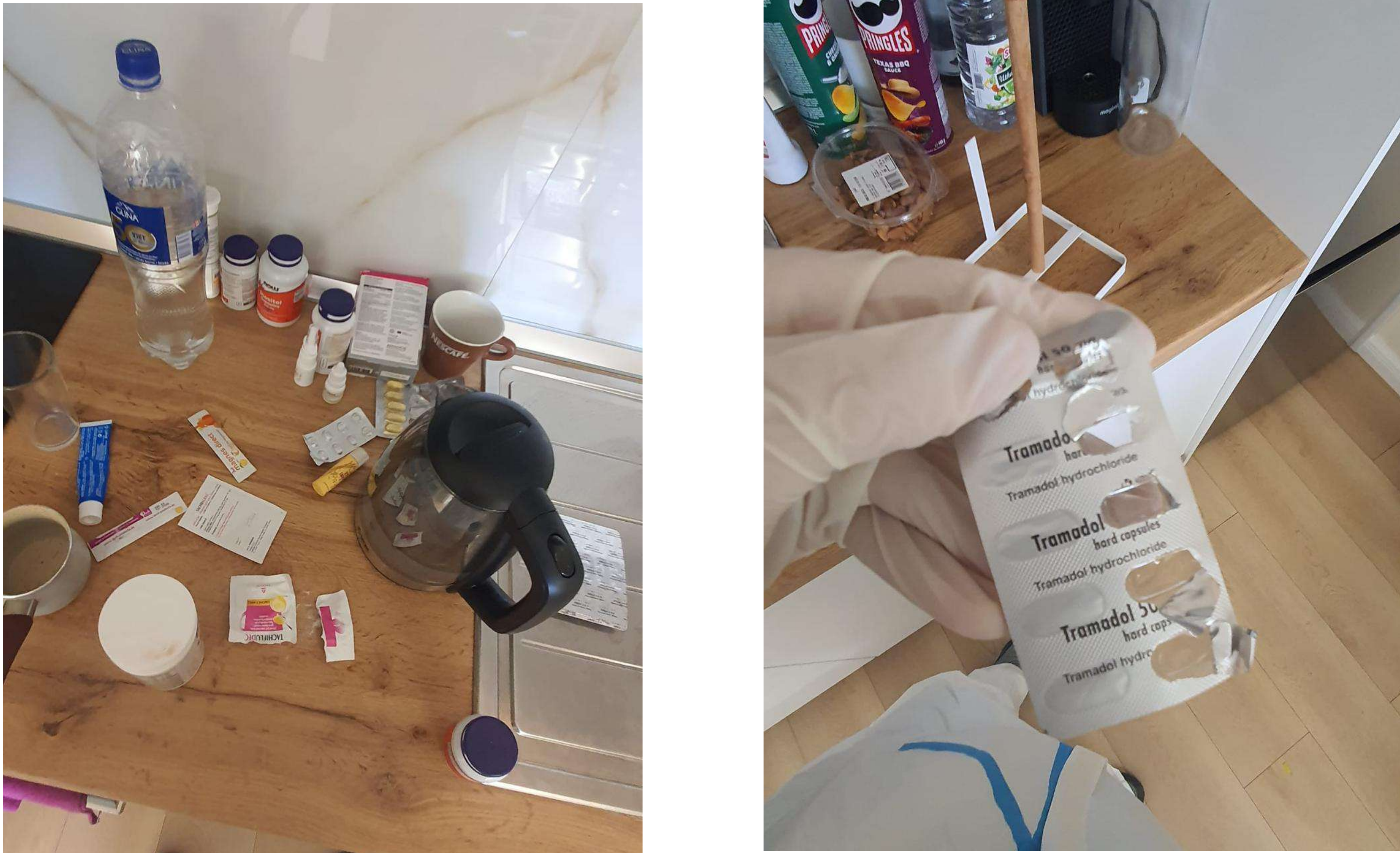
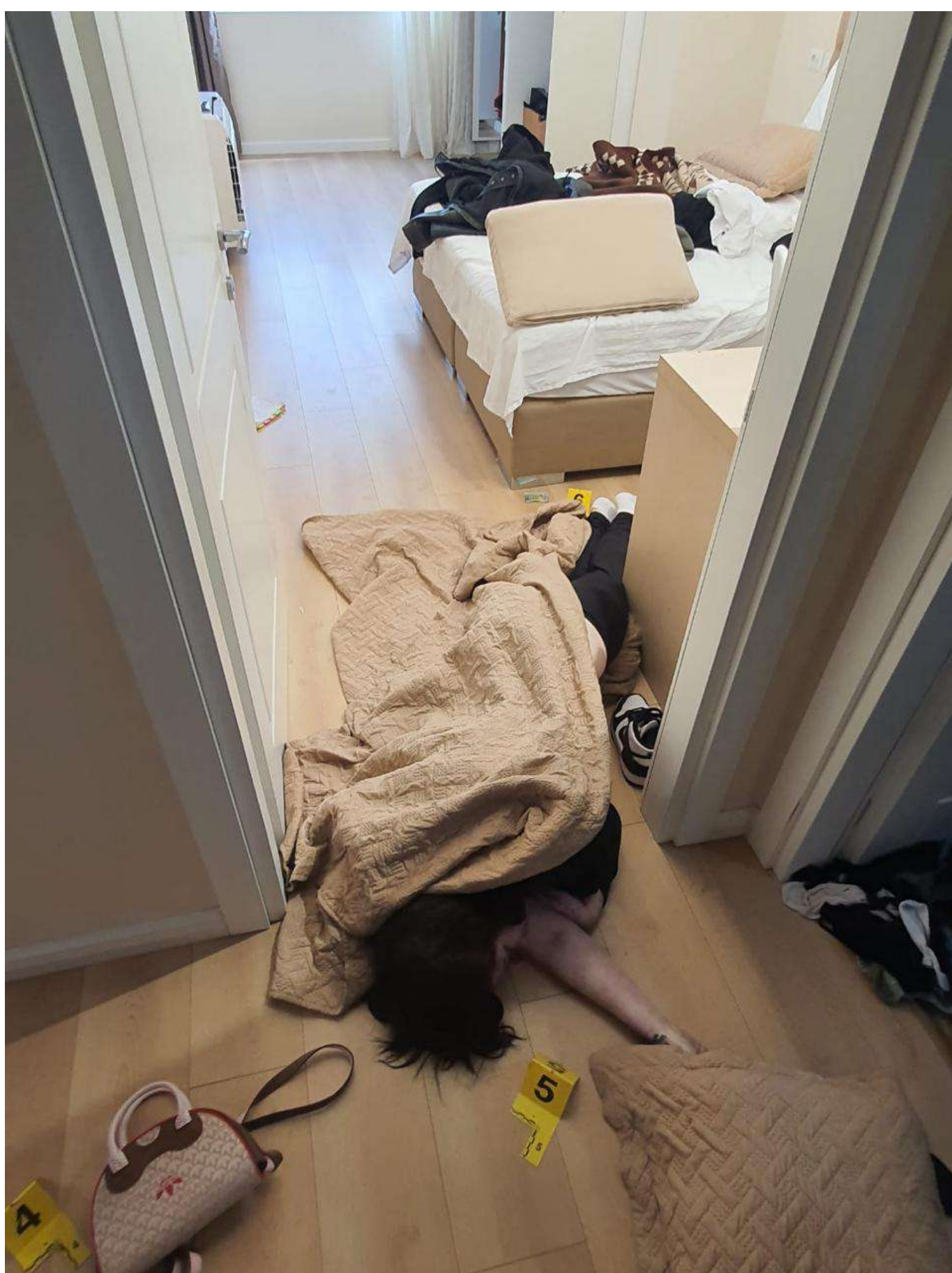


Fig. 5 Photos of the bodies found in the apartment





CASE REPORT:

ASPHYCTIC DEATH IN INDOOR POOL

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• Background and Aim

After alcohol and some kind of drugs, carbon monoxide is the most widespread cause of death in forensic pathology. We are presenting a case of fatal intoxication of carbon monoxide. In the indoor pool area of a hotel a family of four person 2 men and 2 women were found dead. The hotel used gas heating system to heat the indoor pool. The bodies were found in different positions of the pool away from each other. The autopsy revealed externally cherry post mortem staining in all corpses.

Method

A full autopsy was performed revealing signs of general asphyxia and pink colour in the hypostasis areas, red pinkish blood in all corpses. Blood from femoral vein and stomach with content were sent to toxicological laboratory and specimens from brain, heart, lungs, pancreas, kidneys were sent to the histological laboratory. In the toxicological laboratory qualitative and quantitative analysis of alcohol, narcotic drugs, organophosphates, cyanide, carbon monoxide were performed by (UV spectrophotometer, GCHS and GCMS). Histopathological examination were performed by H -E method.

• Histological findings

Fig. 1 Histological changes in heart case with carbon monoxide

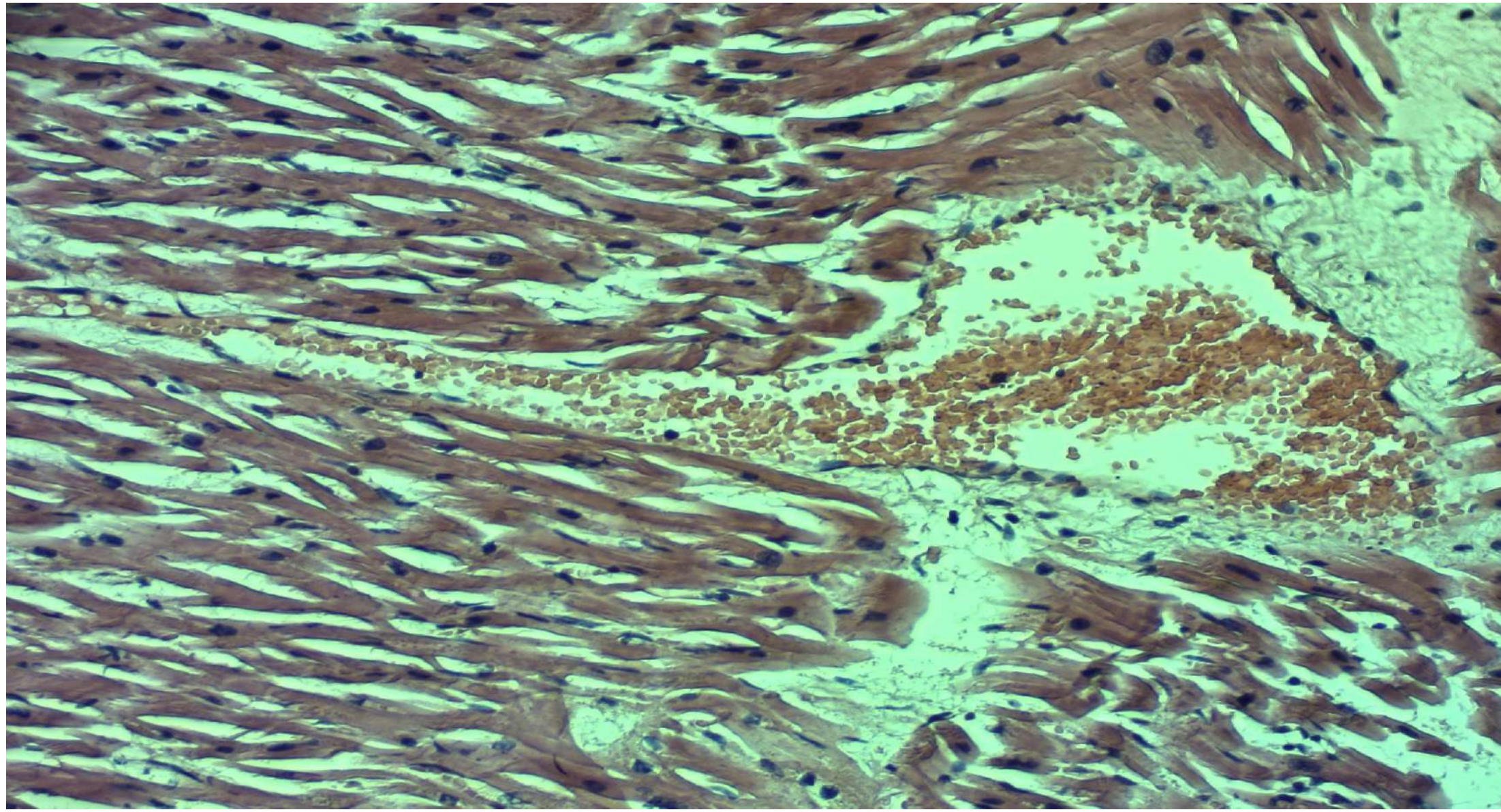


Fig. 2 Histological changes in lungs case with carbon monoxide

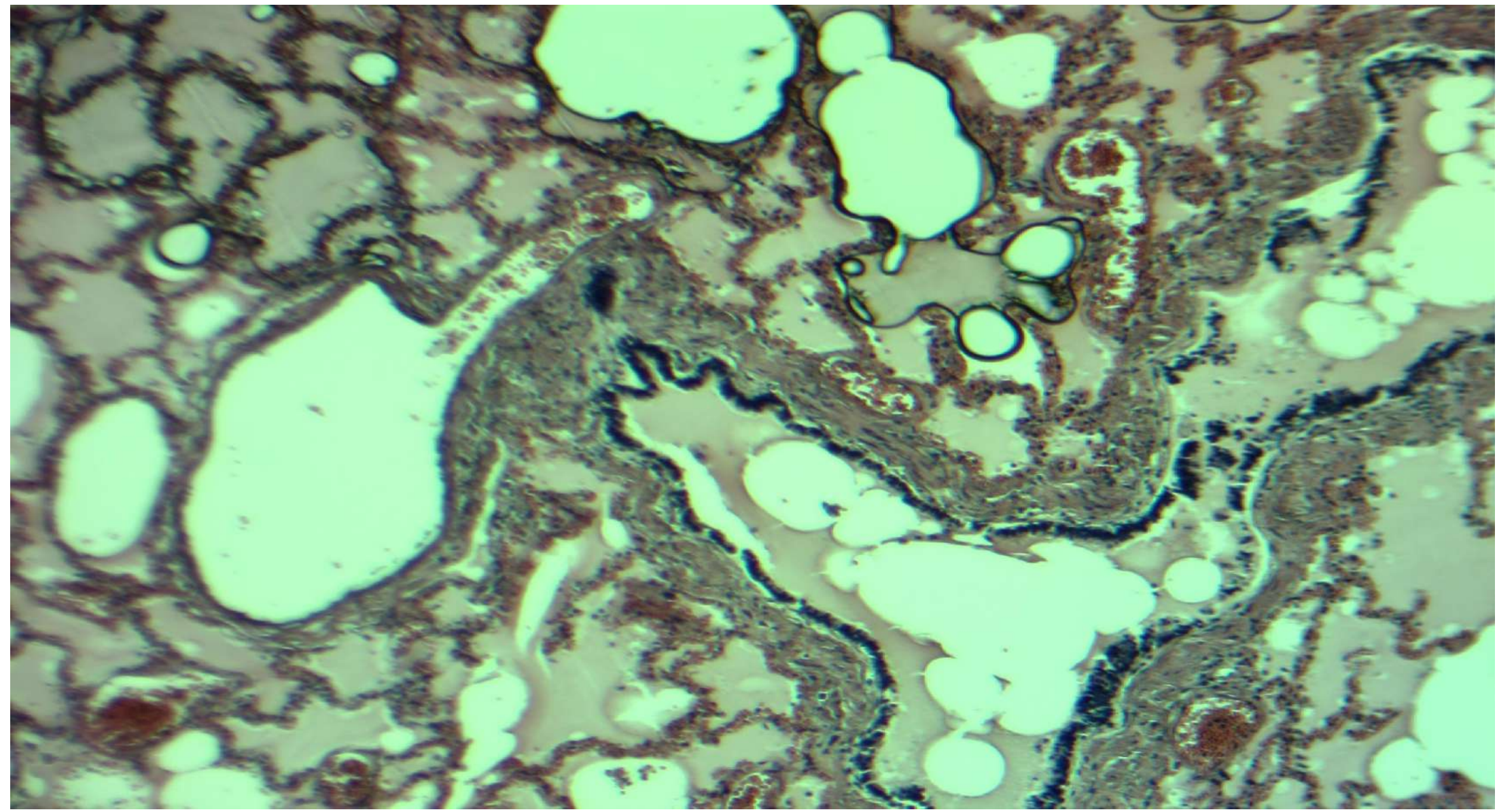
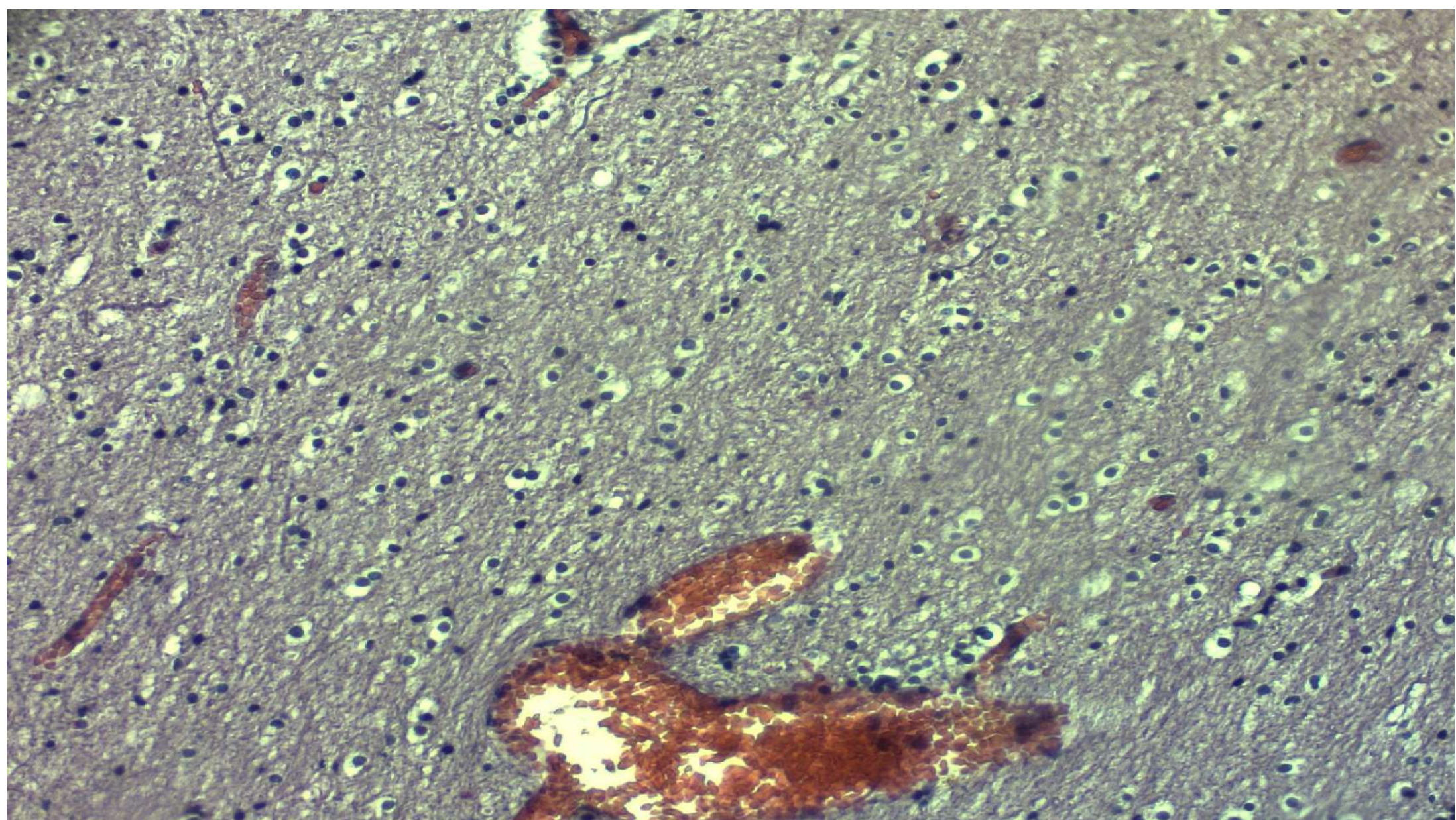


Fig. 3 Histological changes in brain case with carbon monoxide



• Toxicological findings

During the toxicological investigation, head-space-GC/FID analysis for alcohol, screening analysis for drugs of abuse by EMIT-immunoassay method as well as GC/MS analysis for determination of medicinal drugs, drugs of abuse and other substances was performed. Blood alcohol concentration, narcotic drugs, cyanide and organophosphates were negative for all cases.

Analysis of blood performed by spectrophotometer revealed presence of carbon monoxide

Cases	Concentration of HbCo
Corpse 1 Woman	77%
Corpse 2 Woman	79%
Corpse 1 man	76%
Corpse 2 man	76%

• Conclusion

The cause of death of four corpses was intoxication from carbon monoxide causing respiratory failure and cellular anoxemia these because carbon monoxide has an affinity for hemoglobin that is 200 - 300 times greater than oxygen.

Fig. 4 Red pinkish colored stain of corpses In the autopsy room



Fig. 5 Photos of the bodies found in the indoor pool



APPROACH TO POST MORTEM DETERMINING OF GESTATIONAL AGE OF ABORTED FETUS. A CASE REPORT.

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Background: The examination of an aborted fetus is relatively uncommon in forensic practice. We present a case of a 14-year-old female with mental retardation who was admitted to the Obstetrics and Gynecology department with a history of sexual assault resulting in pregnancy. The precise date of the assault was indeterminate; however, clinical data indicated that the pregnancy was in the 5th lunar month. A surgical abortion was performed for medical reasons. The primary objective of the forensic examination was to ascertain the gestational age of the fetus, which was critical for establishing the date of the criminal act and confirming the victim's minor status at the time of the assault. A comprehensive literature review revealed a scarcity of relevant publications, with few addressing systematic protocols for determining gestational age in the context of fetal autopsy.

Aims: This report aims to underscore the necessity of a multi-method approach in deriving conclusions of high evidentiary value and to summarize an algorithm for determining gestational age for medico-legal purposes.

Methods: The medical records were reviewed. Fetal radiographs were obtained prior to the autopsy. A thorough external examination and morphometry were performed, followed by an internal examination of the organs. Biological specimens were collected for histological and DNA analysis. Ossification was assessed based on the radiographic findings, and both descriptive and metric features of the fetus were analyzed (Fig. 1 and 2). The histological evaluation specifically focused on determining the number of glomerular generations formed, which is pertinent for estimating gestational age (Fig.3).

Results: The analysis of the collected data indicated the gestational age of the fetus to be between 20 and 21 weeks.

Discussion - Conclusions: The determination of gestational age post-mortem necessitates a multifaceted approach, incorporating imaging techniques for ossification analysis, detailed external and internal examinations with morphometric assessments, and histological evaluations. Such a comprehensive methodology is essential for providing accurate and reliable conclusions in medico-legal contexts.

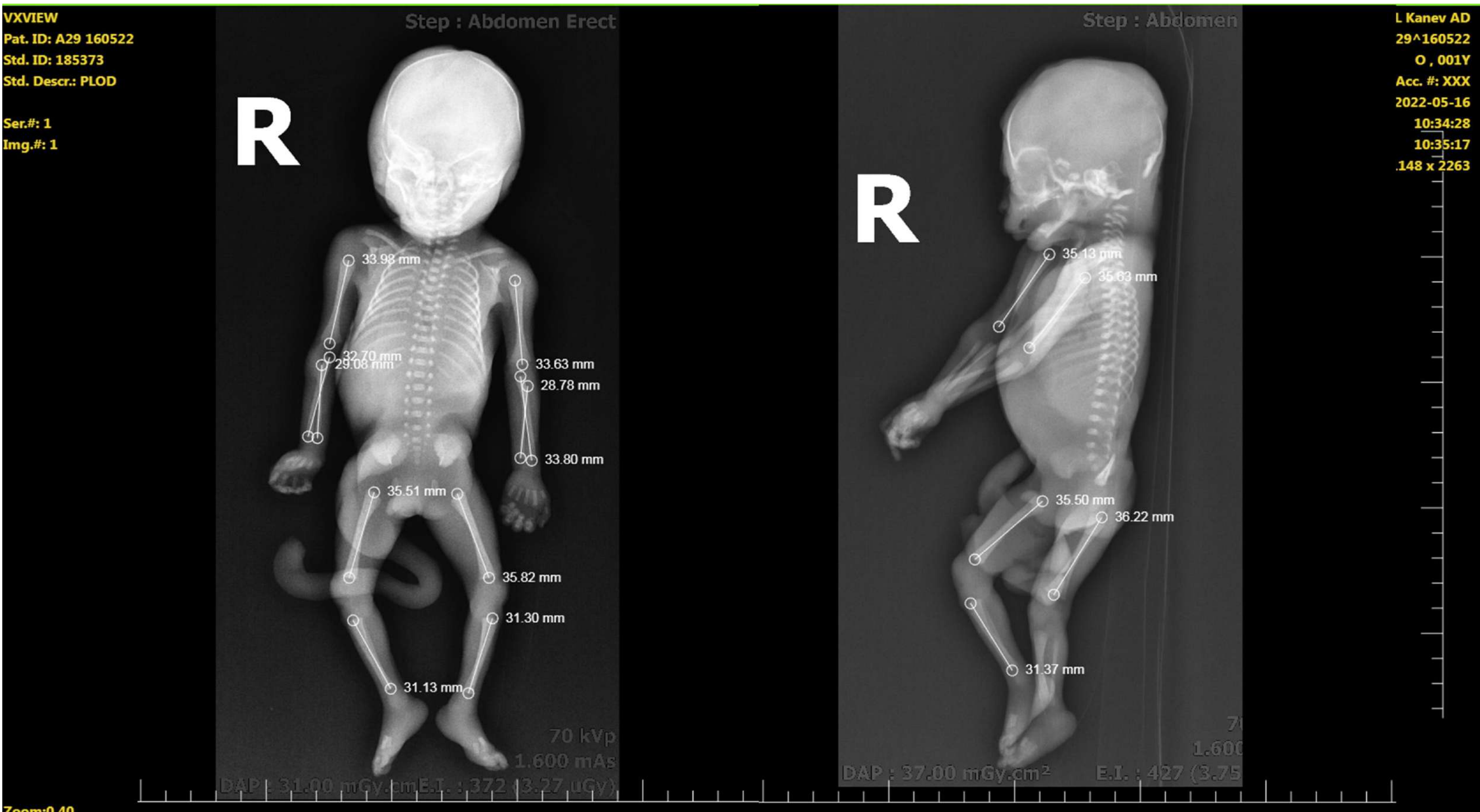


Fig. 1: Radiographs of the fetus: Ossification of the facial bones, frontal and occipital bones, as well as the base of the cranium; long bones, ribs and vertebral bodies, which are closer to the arches; teeth are visible; calcaneus is hardly visible with very low density; there is no ossification of the sternum and the pubic bones. Based on the assessment of the ossification, the gestational age is 21 g.w., and based on the metrical assessment of the skeleton, - 20 – 23 g.w. All analysis was in accordance with *Fetal Radiology – A Diagnostic Atlas, 2nd Edt. 2010, Springer*

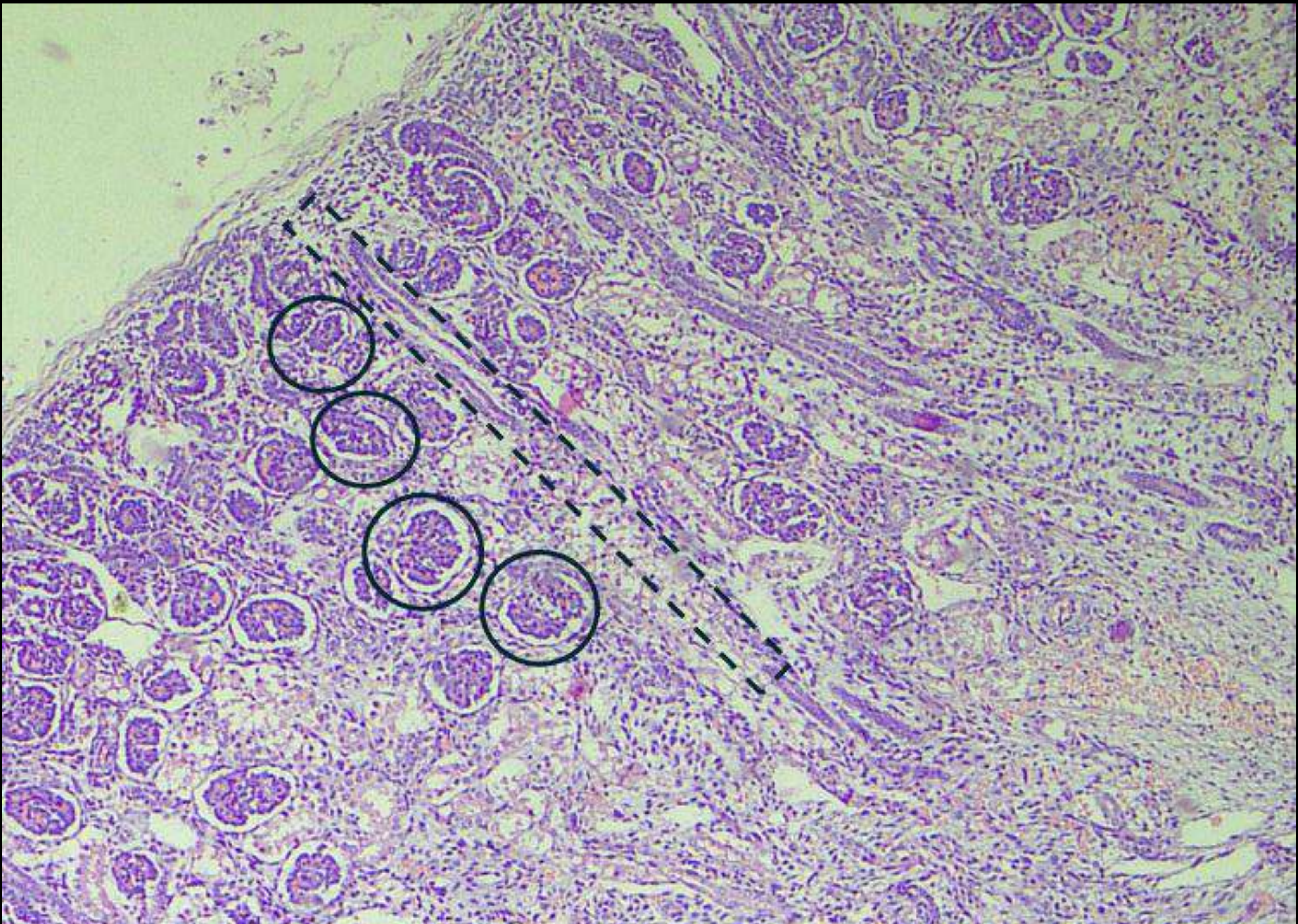


Fig. 3: Histological assessment of the kidneys. Counting glomerular generations: This method counts the number of glomeruli formed along a medullary ray from the corticomedullary junction to the outer renal cortex, including the glomeruli that form after ureteric branching is complete. In our case, the mean count of glomerular generations in 5 medullary rays was 4.4. This corresponds to circa 23 g.w. HE, 4X. Data from: *T.Yee Khong, Roger D. G. Malcomson (eds.) - Keeling's Fetal and Neonatal Pathology, Macerated Stillbirth - Springer International Publishing (2015)*



Fig. 2: Some of the indicative descriptive features: vernix caseosa and lanugo; short, thin and soft nails; the palpebral apertures were still closed with normally developing bulbs in the orbits. The results correspond to 20 – 22 g.w. Metrical features: Weight – 364 g; Height – 25,3 cm; Crown-Rump length – 17,5 cm; Foot length – 3,6 cm; Head circumference – 17,7 cm; Chest circumference – 15,3 cm; Inner canthal distance – 1,5 cm. The internal examination showed normal development of all organs; the lateral and olfactory sulci were well developed and the parieto-occipital and central sulci were short and shallow. The results correspond to 20-21 g.w.

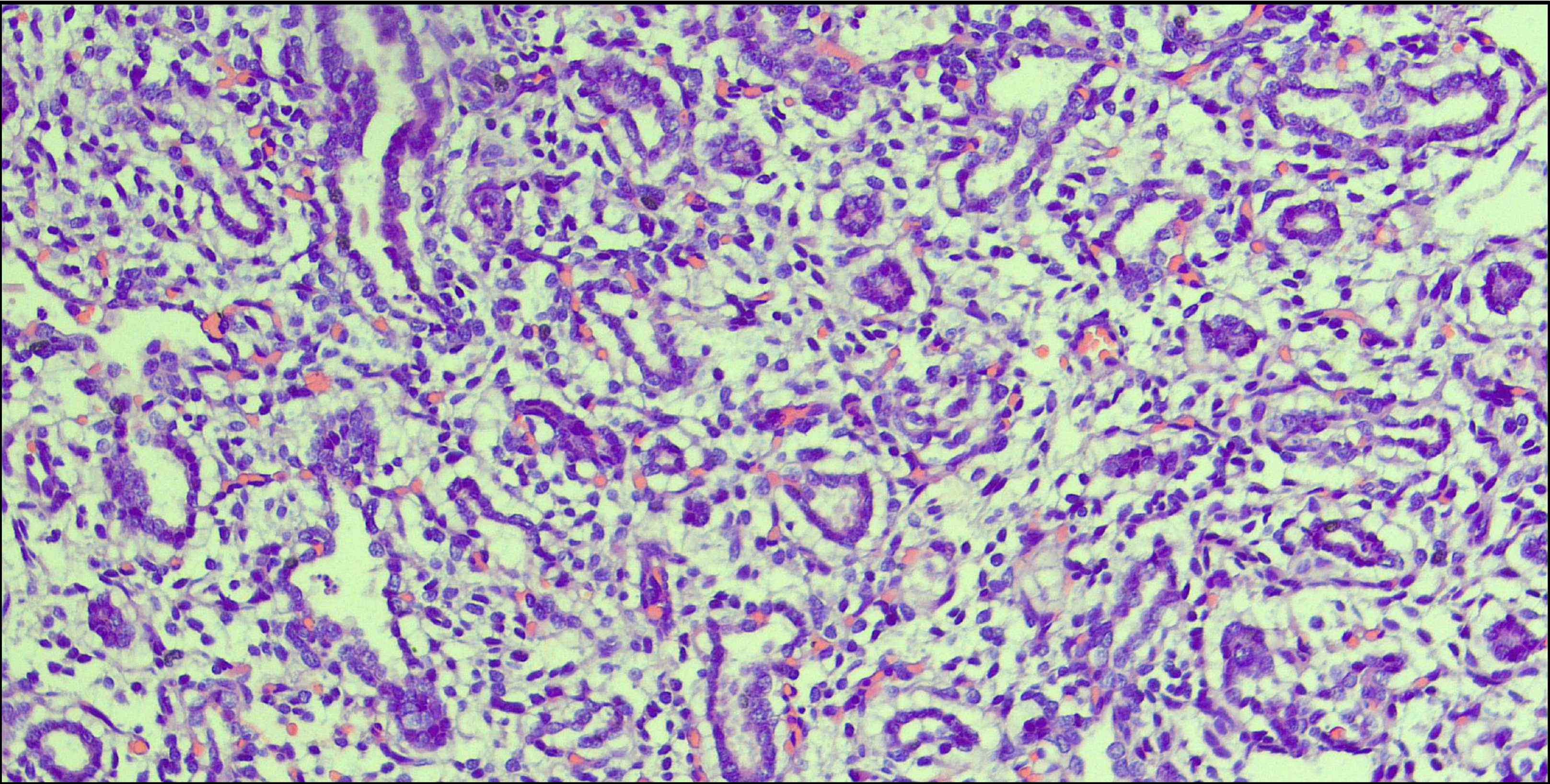


Fig. 4: Histological assessment of the lungs. Shown here is lung tissue in late canalicular phase of the embryonal development. Canaliculi spaces lined by cuboidal glycogen-rich epithelium can be appreciated. Capillaries can be seen pushing into the walls of the canaliculi thus forming the blood-gas barrier. HE, 20X. Data from: *T.Yee Khong, Roger D. G. Malcomson (eds.) - Keeling's Fetal and Neonatal Pathology, Macerated Stillbirth - Springer International Publishing (2015)*

Keywords: Gestational Age, Forensic Medicine, Imaging, Histology

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SUICIDE BY FENTANYL AND BUPRENORPHINE TRANSDERMAL PATCHES COMBINED WITH INTRAVENOUS APPLICATION OF GROUND BENZODIZEPINES TABLETS

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ABSTRACT

Background and Aims: Fentanyl and buprenorphine are potent opioids frequently used for chronic pain management. Fentanyl is approximately 100 times more potent than morphine, while buprenorphine, a partial agonist, is used for both analgesia and opioid dependency treatment. Despite their effectiveness, these medications pose significant risks of misuse and overdose, particularly when combined. This case report aims to highlight the dangers associated with the misuse of these medications and to emphasize the need for careful patient monitoring.

Methods: This case report describes a suicide involving a combination of fentanyl and buprenorphine patches, along with the intravenous injection of a solution made from crushed benzodiazepine tablets. The subject was a 31-year-old female with a complex medical history, including ulcerative colitis, hypothyroidism, Arnold-Chiari malformation, and post-traumatic stress disorder.

Results: The patient was found deceased in her apartment, with eight fentanyl patches (12 µg/h) and twenty-two buprenorphine patches (35 µg/h) applied to her body. A partially emptied 50 ml syringe containing a solution of crushed benzodiazepine tablets was attached to an intravenous line in her left arm. Toxicology analysis revealed lethal blood levels of fentanyl and buprenorphine, along with several other psychoactive substances.

Conclusions: This case underscores the critical need for recognizing the risks associated with opioid medications, the challenges in their regulation, and the importance of diligent patient monitoring, especially in individuals with a history of mental health issues or substance use disorders. The findings contribute to the ongoing discussion on opioid safety and the prevention of intentional and unintentional overdoses in clinical practice.

INTRODUCTION

Suicide by applying transdermal opioid patches is an unusual method of suicide which is only rarely described in the literature.

With increased use of synthetic opioids as illicit drugs the deaths associated with synthetic opioids are increasing. Deaths involving opioids can be associated with prescribed drugs as well, which can be intentionally accumulated and used for suicide.

CASE PRESENTATION

A 31-year-old female discharged herself from the hospital where she was being treated for a combination of somatic and mental health problems. She had a history of previous suicide attempt and a nurse was sent to check on her the next day as it was discovered that she sent suicidal text messages. She was discovered dead in her apartment. Police and medical examiner were notified and crime scene investigation was carried out. Several prescribed drugs were discovered at the scene and remnants of ground tablets were found in a mortar.

AUTOPSY FINDINGS

Autopsy was performed on the day following discovery of the body. No signs of recent injury or foul play were noted on external examination. Several scars from self injury were present on extremities.

There were in total 22 buprenorphine patches (TransteC 35 µg/h) applied to her thighs as well as 6 fentanyl patches (Durogesic 12 µg/h) applied to left arm and 2 to the right thigh. In addition to patches a half empty syringe filled with reddish liquid containing particles of ground material was connected to an IV line in the left cubital fossa.



Non specific autopsy findings of brain and pulmonary edema were found upon internal examination as well as previously known chronic diseases: ulcerative colitis, colloid nodular goiter and Arnold-Chiari malformation.

Microscopic examination of lung tissue samples revealed embolism with foreign material which was attributed to ground tablets which were injected from the attached syringe.

Samples of blood, urine and contents of the syringe were taken for toxicologic analysis.

Fatal blood concentrations of 0,003 mg/l fentanyl and 0,03 mg/l buprenorphine were detected as well as therapeutic concentrations of alprazolam, bromazepam and lorazepam which were determined to be present in the contents of syringe.



ALKOHOLOMETRIČNA PREISKAVA (KVANT. KROMATOGRAFSKA PREISKAVA)

Snov	Rezultat	Enota	Metoda	Datum	Analiz.
etanol	0,01 : 0,01	g/kg	PK3_A1:PK3_A2	3.6.2024	KS
etanol	0,01 : 0,01	g/kg	PK3_A1:PK3_A2	3.6.2024	KS

TOKSIKOLOŠKA PREISKAVA (KROMATOGRAFSKA PREISKAVA)

Snov	Rezultat	Enota	Metoda	Datum	Analiz.
7-aminonitrazepam	pozitivno		K GCMS1_00	20.6.2024	KS
alprazolam	pozitivno		K GCMS1_00	20.6.2024	KS
bromazepam	pozitivno		K GCMS1_00	20.6.2024	KS
buprenorfin	pozitivno		K GCMS1_00	20.6.2024	KS
desloratadin	pozitivno		K GCMS1_00	20.6.2024	KS
fentanyl	pozitivno		K GCMS1_00	20.6.2024	KS
omeprazol	pozitivno		K GCMS1_00	20.6.2024	KS
tietilperazin	pozitivno		K GCMS1_00	20.6.2024	KS
ivabradin	pozitivno		K LC-MS/MS	19.7.2024	AK
lorazepam	pozitivno		K LC-MS/MS	19.7.2024	AK
metoklopramid	pozitivno		K LC-MS/MS	19.7.2024	AK
alprazolam	0,02	mg/L	K LC-MS/MS	20.6.2024	NK
bromazepam	0,02	mg/L	K LC-MS/MS	20.6.2024	NK
buprenorfin	0,03	mg/L	K LC-MS/MS	1.7.2024	NK
fentanyl	0,003	mg/L	K LC-MS/MS	20.6.2024	NK
lorazepam	0,02	mg/L	K LC-MS/MS	20.6.2024	NK
nitrazepam	negativno		K LC-MS/MS	20.6.2024	NK

analgesics, especially in patients with history of mental health problems or substance use disorders, in connection of a possibility of intentional or unintentional overdose.





From Ecosystems to Crime Scenes; Environmental and AirDNA

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Background and aim : Technological advancements, particularly in DNA sequencing, have revolutionized many scientific fields, including forensic science. Massive parallel sequencing (MPS), also known as next-generation sequencing, allows researchers to efficiently analyze large-scale DNA data from various environmental sources such as soil, water, and air. This has facilitated the study of environmental DNA (eDNA), which includes genetic material shed by organisms into their surroundings. eDNA has traditionally been used to monitor ecosystems, track biodiversity, and study species distribution. Recently, however, its potential for forensic science has emerged, offering innovative ways to link crimes to perpetrators or locations.

eDNA can be collected from various environmental substrates and, through MPS, analyzed to identify organisms or humans that were present in a particular location. For instance, airborne DNA, known as "air DNA," or DNA recovered from soil and water, could potentially be used to link individuals to crime scenes. This study aims to compile existing research on eDNA and assess its potential applications and challenges in forensic investigations.

Methods: This study consists of a comprehensive review of articles that investigate eDNA, primarily in research contexts. The articles focus on the extraction, sequencing, and interpretation of eDNA data, with a specific interest in how these methods could support forensic analysis. Real-world examples and case studies were also included to illustrate how eDNA is currently being explored or could be utilized in forensic settings.

Results and Case Studies: The use of eDNA in forensic science presents both opportunities and challenges. Below are a few notable examples from the reviewed literature:

AirDNA: In one research, they studied how long it takes for a person to contaminate their surroundings by speaking while sitting, kneeling, or standing (30 seconds to 15 minutes). DNA profiles were obtained after just 2–30 seconds, with longer speaking times increasing detection. Contamination spread more from greater heights, with factors like air currents, wind speed, and ventilation also playing a role. In one study researchers tested how well protective clothing prevents DNA contamination at crime scenes. A participant made vigorous and normal movements for 15 minutes, both with and without protective gear (full-body suit). Without protection, cells shed from the participant's body and clothing generated full DNA profiles in the collection area. Even with protective clothing, vigorous movement caused contamination, likely from friction. Talking and coughing also produced detectable contamination, influenced by the person's position relative to the collection zone.

Another study assessed DNA contamination in air and dust from forensic labs, showing that cleaning and protective gear reduce but don't eliminate detectable DNA, suggesting aerosol DNA as a contamination risk.



Soil eDNA in Crime Scene Analysis: Soil from crime scenes has long been studied in forensic science, but with MPS, it's now possible to analyze the complex mixture of DNA within soil samples. In one case, soil samples taken from a crime scene were compared with samples from a suspect's shoes. The analysis revealed matching eDNA profiles, which included a unique microbiome signature from the crime scene environment, helping to confirm the suspect's presence at the location.

•**Airborne DNA and Human Traces:** Recent studies have begun investigating the collection of human DNA from the air. In a notable experiment conducted in a controlled indoor environment, scientists were able to collect and sequence human eDNA from air samples. The potential application in forensic investigations is significant, as air DNA could be collected from crime scenes to detect individuals who were present in the area without direct contact. While still in its infancy, this method could revolutionize how forensic teams collect trace evidence.

•**Water Bodies and Drowning Investigations:** eDNA has also been used in cases involving water bodies, such as identifying species present at drowning sites or determining whether a body has been in a particular body of water. In one instance, DNA from water samples was used to determine if the remains of a missing person had been transported in a certain river, adding crucial details to the investigation.

•For example pollen identification was used to investigate original and relocated grave sites in the former Yugoslavia after the Homeland War.

•Also in another case pollen and fungal spores from a suspect's shoes matched samples from the victim's body and deposition site, disproving the suspect's claim that he had never been near the location.



Wildlife Tracking: Air DNA can detect the presence of endangered species, aiding in conservation efforts and tracking wildlife populations.

1.In 2019, a study demonstrated how eDNA from smuggled wildlife could be used to identify illegally trafficked species. DNA from seized animal products, like scales, tusks, or feathers, can be sequenced using MPS, providing crucial evidence to combat poaching and wildlife trafficking. This highlights the use of eDNA in ecological crime investigations, where identifying species from tiny DNA samples is vital. AirDNA can detect the presence of humans or animals at a crime scene, providing valuable evidence for investigations.

Despite these promising applications, several challenges remain. For instance, eDNA degrades quickly in some environments, and external factors like weather conditions can significantly affect sample quality. Moreover, distinguishing between old and new DNA samples can be difficult, which might complicate criminal investigations.

Challenges and Limitations:

While the potential for eDNA in forensic applications is vast, it faces some limitations:

•**Lack of Standardization:** One of the most significant challenges is the absence of standardized protocols for collecting, analyzing, and interpreting eDNA in a forensic context. Unlike human DNA profiling, which follows strict guidelines, eDNA methods are still largely experimental.

•**Environmental Contamination:** Environmental samples are prone to contamination, which can complicate forensic analysis. For example, DNA from multiple organisms (including humans) can be mixed in soil or water samples, making it difficult to isolate relevant forensic information.

•**Degradation:** eDNA is sensitive to environmental factors such as UV light, temperature, and microbial activity, leading to degradation over time. In forensic investigations, this could result in the loss of crucial evidence if samples are not collected and preserved correctly.

•**Interpretation of Complex Data:** MPS generates vast amounts of data, often including DNA from various species. Accurately interpreting this data requires advanced bioinformatics tools, and the potential for errors in data analysis remains high without proper expertise and technology.



Conclusion: eDNA has made significant contributions to ecological studies, offering a wealth of information about species diversity, population dynamics, and ecosystem health. Its potential to be integrated into forensic science is equally promising, especially with the aid of advanced sequencing technologies like MPS. However, for eDNA to be widely adopted in forensic investigations, certain hurdles must be overcome. More extensive studies are needed to assess the reliability of eDNA in diverse forensic scenarios, databases must be developed for environmental samples, and standardized protocols must be established.

As research progresses and these challenges are addressed, the contribution of eDNA to forensic science is likely to grow, potentially transforming crime scene analysis by providing new avenues for detecting and linking criminals to specific locations or events.

Keywords: eDNA, air DNA, massive parallel sequencing (MPS), forensic science, wildlife crime, environmental DNA, soil eDNA



Are Trends Changing? The First Two Cases of Suicide Bags with Helium in Bulgaria

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Background and Aims:

Suicide rates continue to be a significant public health and social issue. Helium-induced asphyxiation with a suicide bag is among the most preferred methods of complex suicide due to the quick and painless death it provides. Such cases have not previously been reported in Bulgaria.

Methods:

The methods used included crime scene investigation, autopsy, blood analysis, rapid urine tests for drugs and opioids, and analysis of the collected data.

Results:

Case 1: A young man was found dead in his home, with a typical set of two disposable helium gas tanks, tubing, and a plastic bag with elastic laces placed over his head and face. After the crime scene examination (Fig. 1), an autopsy revealed minimal signs of asphyxiation and no evidence of violence or trauma. Notably, the blood and lungs exhibited a bright red color. Blood laboratory tests showed no significant abnormalities, and drug and opioid tests were negative. Due to technical limitations, it was not possible to test for helium concentration.

Case 2: A 37-year-old male was found decomposed in his home. A plastic bag was found over his head, secured at the neck, with two tubes connected to helium cylinders (Fig. 2). Post-mortem examination revealed advanced decomposition, with the absence of blood in the heart and vessels, no urine, and no fluids in the chest or abdominal cavities. No specific pathological changes or injuries were observed.

Discussion - Conclusions:

Crime scene investigation plays a crucial role in suspected cases of helium inhalation, as the presence of a typical suicide kit and related tools may be the only conclusive evidence when determining the cause of death. It is essential to rule out other potential contributing factors to the fatality. Additionally, investigators should ensure that no other hazardous chemicals are present at the scene, which could pose risks to the investigation team.

Keywords: Forensic Medicine, Helium inhalation, Suicide bag, Asphyxiation

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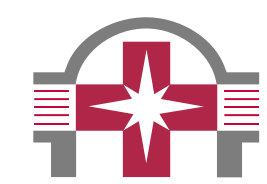
Fig. 1: The first local documented case is from Gabrovo, Bulgaria, in 2018. A young man, single and web-addicted introvert, was found dead in his home, with typical set of two helium disposable gas tanks, tubing and a plastic bag over the head/face. The autopsy revealed minimal signs of asphyxiation (subpleural petechial hemorrhages, edema of the brain and lungs and congestion in the internal organs). It is important to combine a detailed crime scene investigation with modern methods of detection of the gas in the body, when possible, to confirm the diagnosis.



Fig. 2: The second documented case is from Ruse, Bulgaria, in May 2024. A 37-year-old male was found in his home. He was well-educated, single and had a strong belief in the reincarnation cycle of life. Seeing himself as unsuccessful, years before he stated he was eager to move on to his next reincarnation. The apartment was locked and the keys were found where he typically kept them. Large quantities of food and water were found left for his pet. Upon inspection of the residence, letters to his relatives were found explaining his reasons for committing suicide. A note was found on his bedroom door which read "Relax, everything is fine (push very hard)". The edges of the door were found duct taped shut, as were the windows in the room. A sheet which had a detailed description and calculations of the quantities and pressures of gas required to achieve a rapid lethal outcome was found. This data was obtained via a suicide assistance website. A note for the police was found beside the body, on which was the date of the suicide, together with personal papers. There were no significant findings at autopsy. Again, detailed crime scene investigation is crucial for the diagnosis.

IDENTIFICATION STUDY OF 45 CHARRED CORPSES FOLLOWING A ROAD TRAFFIC ACCIDENT AND BUS FIRE IN BULGARIA

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Introduction

A review of statistical data on road traffic accidents (RTAs) in Europe over the past 30–40 years reveals that the incident we investigated, involving 45 fatalities, represents the deadliest event within this time frame. To carry out the forensic medical examination of the bodies, it was necessary to establish an appropriate organizational framework for activities, including the examination of the crime scene, dental status recording, autopsies, toxicological, anthropometric, and identification studies, utilizing fragmentary DNA analysis [1–10].

Case Report

On November 23, 2021, on the Struma Motorway, Lot "0," at high-speed road "20" near km 32, a traffic accident occurred involving a bus, resulting in the deaths of 45 individuals. A large-scale police investigation and forensic identification process were carried out, with the participation of investigators and experts from the National Investigation Service of Bulgaria, a team of forensic pathologists, chemists, and biologists primarily from the Department of Forensic Medicine and Deontology at the Alexandrovska University Hospital in Sofia, Bulgaria, as well as automotive experts, fire safety specialists, and many other professionals.

It was determined that the bus first collided with and broke through the safety barriers securing the outer section of the motorway. It then veered left, reached the central two guardrails, broke one of them, and became lodged between them [Image 1]. At this point, a fire erupted in the front part of the vehicle, which spread rapidly and engulfed the entire bus within minutes. Only a few passengers, who were awake at the time, managed to break the rear windows and escape the burning vehicle. Upon examination of the bodies, it was observed that many were clustered in the middle section of the bus and around the central door, which they were unable to open due to a mechanical obstruction caused by the guardrail pressing against it from the outside [Image 2].

The investigative authorities from the National Investigation Service (NIS) faced the challenge of effectively organizing the stages required for the thorough investigation of the incident, as well as the forensic examination of the bodies and the identification of the victims' identities.

Materials and Methods

Crime Scene Examination, Forensic Autopsy, and Additional Investigations

The forensic examination of the bodies of the victims involved in the incident commenced with the bus driver and proceeded towards the rear seats of the vehicle. Two forensic pathologists conducted the examinations, alternating with another pair every one to two hours. Each examined body was then transported by paramedics and firefighters, in some cases after cutting away metal sheets that had entrapped the bodies, and taken by ambulance to the Department of Forensic Medicine and Deontology (DFMD) at Alexandrovska University Hospital, Sofia. Simultaneously, teams were organized at DFMD to begin performing forensic autopsies as soon as the bodies arrived. Biological samples collected during the autopsies were immediately sent for analysis to the toxicological and DNA laboratories within the clinic, where work commenced without delay [Figure 1]. By the second and third days, comparative DNA samples were obtained from the presumed relatives of the deceased to conduct comparative analyses for identification. The identification of all 45 victims was completed by the seventh day following the incident. Toxicological reports were prepared by the sixth day and provided to the forensic pathologists, who finalized their forensic medical reports, submitting them to the investigators from the National Investigation Service (NIS) by the tenth day after the incident.

DNA Procedures

- DNA Extraction:** DNA was extracted from biological material, including blood samples from each of the deceased individuals and comparative cellular material from their identified relatives, using the AutoMate Express Forensic DNA Extraction System with the PrepFilter Express™ Forensic DNA Extraction Kit (Thermo Fisher Scientific).
- PCR (Polymerase Chain Reaction):** The PCR amplification of samples was initially performed in two stages using:
 - Real-Time PCR system 7500 (Life Technologies) with a PC Notebook for HID analysis, using the Quantifiler™ Trio DNA Quantification Kit for qualitative and quantitative assessment of DNA in the samples via HID Real-Time PCR Analysis Software v1.2.
 - PCR machine SimpliAmp™ Thermal Cycler, 96 x 0.2 ml (Life Technologies).
 - DNA profiles were generated for Y chromosome STR markers using the Yfiler™ Plus PCR Amplification Kit and for autosomal STRs using the NGM Detect™ PCR Amplification Kit.
- Fragment Analysis:** Fragment analysis was performed using 3500 Series Genetic Analyzers for Human Identification (Life Technologies), employing 8-capillary electrophoresis (with 3500 POP-4™ Polymer) and laser detection of fragments, followed by computer analysis using Gene Mapper™ v1.2 Full Software for HID analysis.
- Quality control and standardization of analyses** were performed using:
 - Positive control: DNA Control 007
 - Negative control: NC
 - Matrix standard: Matrix Standard Kit DS-37 (6-FAM™, VIC™, TED™, TAZ™, SID™, LIZ™ dyes) for the NGM Detect™ Kit (Applied Biosystems)
 - Matrix standard: Matrix Standard Kit DS-36 (6-FAM™, VIC™, NED™, TAZ™, SID™, LIZ™ dyes) for the Yfiler™ Plus Kit (Applied Biosystems)
 - Internal standard: GeneScan™ 600 LIZ™ Size Standard v2.0
 - Internal quality control markers: IQCS and IQCL
 - Allelic ladder (NGM Detect™ Allelic Ladder) for the respective STR markers, validated and included in the NGM Detect™ Kit (Applied Biosystems)
 - Allelic ladder (Yfiler™ Plus Allelic Ladder) for the respective Y-chromosome STR markers, validated and included in the Yfiler™ Plus PCR Amplification Kit (Applied Biosystems).

Toxicochemical Analysis

The examination for the presence of carboxyhemoglobin was performed using a UV/Vis spectrophotometer (Pharmacia Biotech Ultrospec 3000) in scanning mode within the 450–650 nm range. Quartz cuvettes with a range of 200–2500 nm were used. Absorbance readings of the samples were taken at 540 nm and 579 nm, and the carboxyhemoglobin content was calculated using the ratio of the two maxima.

The examination for the presence of methemoglobin was also performed using a UV/Vis spectrophotometer (Pharmacia Biotech Ultrospec 3000) in scanning mode within the 600–650 nm range. Quartz cuvettes with a range of 200–2500 nm were utilized. Absorbance readings were taken at 630 nm, and the methemoglobin concentration was calculated using the formula: $\text{MethHb}\% = ((A_1 - A_2) / (A_3 - A_4)) * 100$.

The examination for the presence of alcohol was conducted using a gas chromatograph (Thermo Scientific Trace 1310) equipped with an FID detector and a chromatographic column specialized for the analysis of alcohol and volatile substances (TRACE GOLD TG ALC 30m x 0.32 mm x 1.8 µm), as well as a headspace autosampler (Thermo Scientific TriPlus300) with an automatic magnetic feeder.

Results and Discussion

As a result of the professional work carried out by the formed teams and the timely execution of tasks by the respective specialists and laboratories within the Department of Forensic Medicine and Deontology (DFMD) in Sofia, the identities of all deceased individuals and the causes of their deaths were established within 7–10 days. The primary cause of death was determined to be thermal shock as a result of flame burns, a conclusion corroborated by the results of the toxicochemical analyses. The identification of the bodies through fragmentary DNA analysis was achieved by comparative testing of the DNA profiles of the deceased against the DNA profiles of their presumed relatives, utilizing both autosomal and Y-chromosome DNA markers.

The efficient organization of all necessary forensic examinations within a short timeframe allowed for the rapid identification, release, and transportation of the bodies for burial.

Conclusion

During the course of the investigations and forensic examinations, a robust new algorithm for action was developed. This protocol could be successfully applied in cases involving complex incidents with a large number of fatalities.

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Photo 1. Position of the burned bus in relation to the roadway, crime scene examination, and removal of bodies for transportation to conduct forensic autopsies.

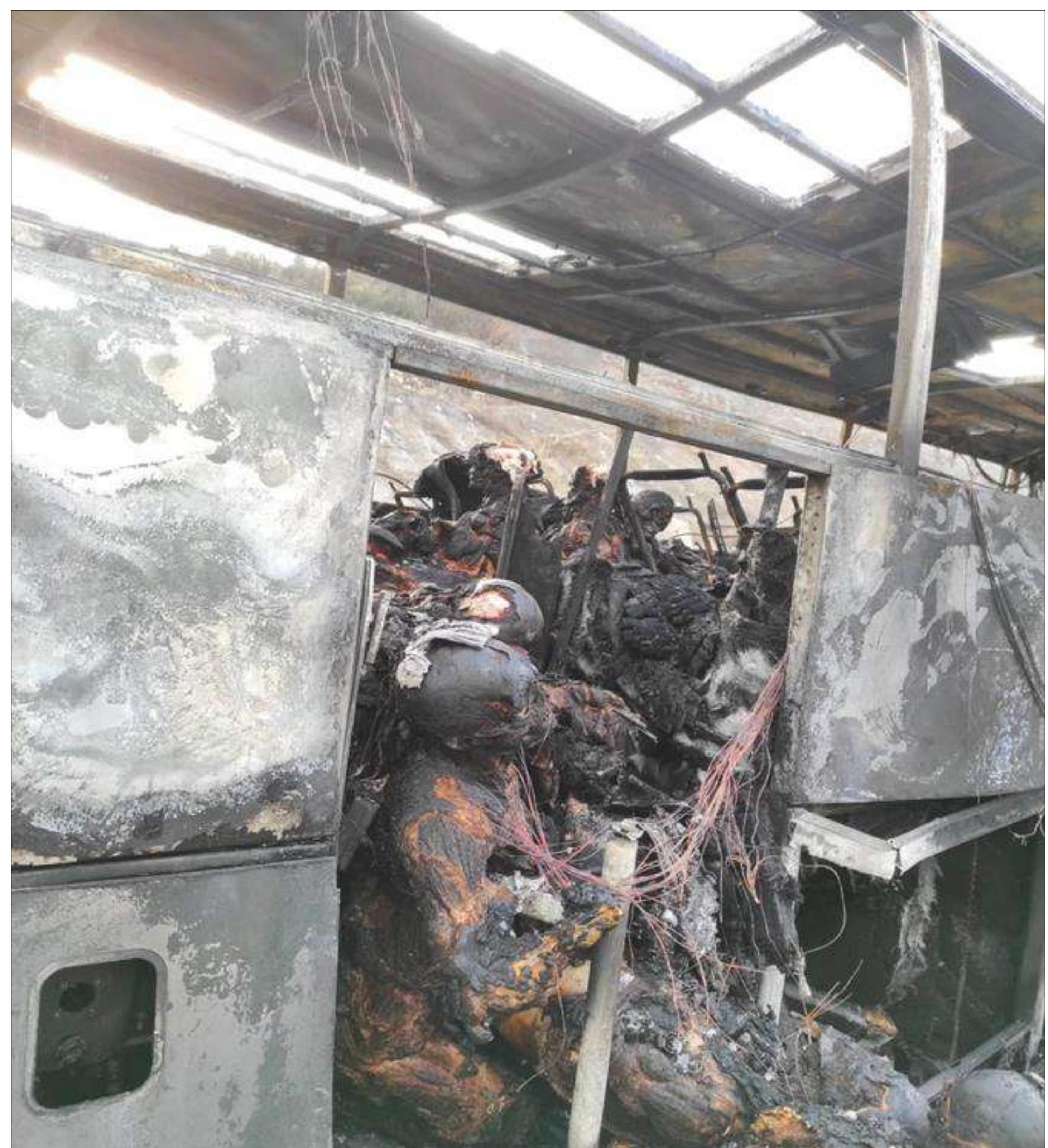


Photo 2. Position of some of the bodies near the middle door of the bus, which was blocked by a road guardrail from the outside, preventing passengers from exiting the vehicle.

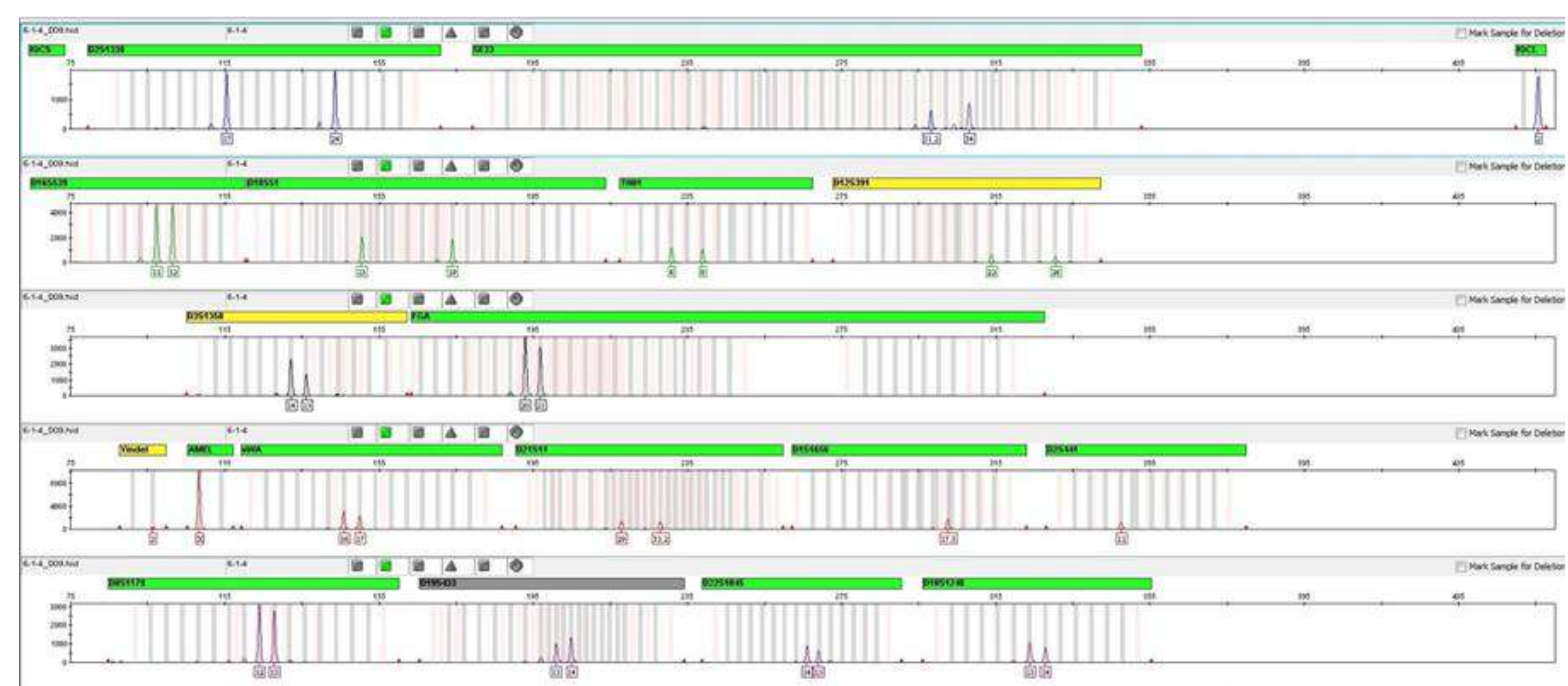


Figure 1. Full DNA profile extracted from one of the unidentified women found charred in the bus, which allowed her to be identified through comparative analysis with the DNA profiles of her mother and father.

DIFFERENTIAL DIAGNOSIS BETWEEN METOPISM AND SKULL FRACTURE – CASE REPORT

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Introduction: The metopic suture is one of the main sutures of the calvaria, premature closure is responsible for trigonocephaly, while persistence (metopism) is considered a physiological variant and could be considered the anterior continuation of the sagittal suture. Persistence of the metopic suture (PMS) is extremely rare in clinical practice, it is usually discovered accidentally, sometimes being associated with hypoplasia or aplasia of the frontal sinus. PMSs can be misdiagnosed as vertical skull fractures, so it is important to be aware of this anatomical variant.

Material and method: We present the case of a deceased female person, aged 31, found in a suitcase (trolley type). In June 2024, the autopsy was performed at SJML Bihor. The causes of death were: internal bleeding that occurred in the context of a thoraco-abdominal trauma with sternum fracture and multiple ribs fractures with visceral ruptures (lung, spleen). The necropsy revealed contusive wounds at the level of the cephalic extremity (left parietal, orbitopalpebral, oral cavity), multiple bone fractures (nasal pyramid, hyoid bone, sternum, ribs) with visceral tears and PSM. We aimed to establish the differential diagnosis between PMS and linear frontal fracture.



Photo1: Human corpse found in a suitcase



Photo 2: Human corpse found in a suitcase



Photo 3: Cephalic extremity - wound



Photo 4: Cephalic extremity - wound



Photo 5: Skull - metopic suture



Photo 6: Skull - metopic suture

Results: Metopism was generally reported as a postmortem finding in population studies. The particularity of the case consists in the fact that it was identified as a potential differential diagnostic with a skull fracture as a result of head trauma. The essential element that differentiates PMS from a fracture is the presence of jagged and sclerotic margins.

Conclusions: Knowing the possible presence of PMS serves to alert medical specialists (imaging, neurosurgeons, forensic doctors) to the fact that it is an anatomical variant and does not involve any harm to the health of the population. The finding of metopism is clinically important because it should be included in the differential diagnosis of a suspected skull fracture, especially if there is a history of head trauma.



THE GENETIC BASIS OF ADDICTION PSYCHIATRY

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Opioid and alcohol use disorders (OUD/AUD) are global public health crises, with increasing mortality rates from drug overdoses, particularly in the United States. The complexity of addiction necessitates a multifaceted approach involving the brain's reward system and its underlying neurotransmitter systems, such as the dopaminergic and serotonergic pathways. Genetic variations play a significant role in an individual's susceptibility to addiction. This paper explores the genetic underpinnings of addiction, focusing on key gene variants that affect neurotransmitter function and reward processing, such as *DRD2*, *ALDH2*, *OPRM1*, and *CHRNA5*. These insights may pave the way for targeted gene-based therapies that correct dysregulated pathways in addiction, providing individualized treatment options.

Introduction

Addiction is a complex, chronic disorder characterized by compulsive drug use, inability to control substance intake, and continued use despite adverse consequences. The prevalence of opioid use disorder (OUD) and alcohol use disorder (AUD) has risen dramatically in recent decades, leading to a surge in mortality rates from drug overdoses globally. In 2020 alone, the United States reported over 93,000 deaths from drug overdoses, making it the country with the highest rates of opioid-related fatalities. Globally, this growing crisis necessitates innovative therapeutic strategies.

The brain's reward system, particularly the dopaminergic and serotonergic pathways, plays a crucial role in addictive behaviors. Neurotransmitters like dopamine and serotonin mediate reward and punishment, influencing decision-making and reinforcing addictive behaviors. Genetic predispositions contribute significantly to the development of addiction by modulating the sensitivity of the brain's reward system. This paper reviews the role of specific gene variants in addiction and explores how these genetic insights may inform personalized therapies.

Methods

Understanding the biological underpinnings of addictive behaviors involves examining both the neurotransmitter systems and their genetic regulators. Several candidate gene studies have focused on identifying key variants linked to substance misuse and non-substance addictions, risk behaviors like impulsivity and novelty seeking, and stress-induced drinking.

- **Candidate Gene Studies** : Focused on identifying common genetic markers that affect neurotransmitter function, with particular attention to *DRD2*, *ALDH2*, *OPRM1*, and *CHRNA5*.
- **Gene-Environment Interaction Studies** : Investigated how specific genetic variants interact with environmental factors (e.g., stress) to influence addiction risk.
- **Neurotransmitter Pathway Analysis** : Explored how disruptions in dopaminergic and serotonergic pathways contribute to addictive behaviors and the potential for gene-based treatments to restore normal functioning.

Results

- ***DRD2* Gene and the A1 Allele** : The *DRD2* gene, which encodes the dopamine receptor D2, is a key player in reward processing. Individuals with the A1 allele have been consistently associated with higher rates of addiction to alcohol, cocaine, and opioids. The *DRD2/ANKK1* rs1800497 variant has been linked to increased marijuana use, suggesting its broader role in multiple forms of substance abuse.
- ***ALDH2* Gene Variant** : The *ALDH2* gene encodes aldehyde dehydrogenase, an enzyme involved in alcohol metabolism. Individuals with the *ALDH2* variant experience unpleasant symptoms such as nausea and rapid heartbeat when consuming alcohol, which can serve as a protective factor against heavy drinking. However, those with normal *ALDH2* function are at greater risk for alcohol dependence.
- ***OPRM1* Gene and Opioid Use Disorder** : The *OPRM1* gene, which encodes the mu-opioid receptor, is associated with opioid use disorder (OUD) and alcohol dependence. Variants in this gene affect receptor function, altering the brain's response to opioid and alcohol exposure. Individuals with specific *OPRM1* variants are more likely to develop substance dependence.
- ***CHRNA5* and Nicotine Dependence** : The *CHRNA5* gene, involved in the detection of nicotine, plays a critical role in smoking addiction. Individuals carrying certain variants of *CHRNA5* are twice as likely to become dependent on nicotine, making this gene a promising target for smoking cessation treatments.
- ***Per1* and *Per2* Gene Variations** : These genes, which are involved in circadian rhythms, are also implicated in addiction. Research has shown that individuals with *Per1* and *Per2* variations are more likely to increase alcohol consumption, especially during periods of stress, highlighting the interaction between genetics and environmental factors in the development of AUD.
- ***NFAT5*, *PLCB1*, and *KCTD20* Genes** : These genes have been implicated in the effects of cocaine and are associated with a predisposition to addiction. Variants in these genes may influence susceptibility to cocaine addiction, suggesting they could serve as potential targets for future therapies.

Discussion

The findings from genetic studies of addiction provide valuable insights into the molecular mechanisms that underlie OUD, AUD, and other substance use disorders. Each newly identified addiction gene offers a potential "drug target" for therapeutic intervention. For example, medications that modulate dopamine receptor function or enhance aldehyde dehydrogenase activity could reduce the rewarding effects of addictive substances and promote recovery.

Moreover, genetic screening could play a crucial role in developing personalized treatment strategies. By identifying specific genetic variants associated with addiction, healthcare providers could tailor interventions based on an individual's genetic profile. This precision medicine approach would allow for more effective treatments that target the root biological causes of addiction rather than just the symptoms.

- **Targeted Gene Therapy** : As researchers identify more addiction-related genes, the possibility of developing gene therapies to correct dysfunctional pathways becomes more feasible. For example, modifying the expression of the *DRD2* gene to enhance dopamine signaling or correcting *OPRM1* receptor function could restore balance in the brain's reward system.
- **Gene-Environment Interaction** : It is essential to consider how genetic predispositions interact with environmental factors such as stress, trauma, and social influences. Strategies that combine genetic insights with behavioral interventions (e.g., cognitive-behavioral therapy) may yield more comprehensive and effective treatments for addiction.



Conclusions

Addiction is a multifaceted disorder driven by both genetic and environmental factors. The identification of addiction-related gene variants provides a promising avenue for developing novel, targeted therapies. By focusing on the molecular and genetic mechanisms underlying addictive behaviors, researchers can design medications that modulate specific pathways involved in the brain's reward system, ultimately leading to more effective and personalized treatments for OUD, AUD, and other substance use disorders.

Future research should prioritize gene-environment interactions and explore the potential of gene therapy as a treatment for addiction. Additionally, genetic screening could become a valuable tool in predicting addiction risk and guiding treatment decisions, bringing us closer to a future where addiction treatment is not only more effective but also tailored to the individual.



**Balkan
Academy**
Forensic Sciences

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Aggression is influenced by both genetic and environmental factors, with a growing body of evidence pointing to the role of specific genes in regulating neurotransmitters and hormones that influence aggressive behavior. This paper explores the genetic factors involved in aggression, focusing on candidate gene association studies (CGAS) and genome-wide association studies (GWAS). Key genes involved in dopamine and serotonin regulation, such as MAOA, COMT, DRD4, and others, are associated with impulsivity, externalizing behaviors, and violence. The interaction between genetic predispositions and environmental influences is highlighted, providing a comprehensive understanding of the biological basis of aggression.

Introduction

Aggression is a complex behavioral trait influenced by multiple genetic and environmental factors. It manifests in various forms, from verbal hostility to physical violence, and plays a significant role in social, psychological, and clinical contexts. Research into the biological basis of aggression has focused on neurotransmitter systems, particularly dopamine and serotonin, as well as hormonal regulation. Genetic association studies, such as candidate gene (CGAS) and genome-wide association studies (GWAS), have identified several key genes that may contribute to aggressive behavior.

This paper examines the genetic underpinnings of aggression, with a focus on candidate genes involved in neurotransmitter regulation, hormone function, and other molecular pathways. By exploring these genetic factors, we aim to shed light on the biological mechanisms that drive aggressive behavior and the potential gene-environment interactions that modulate this trait.



Methods

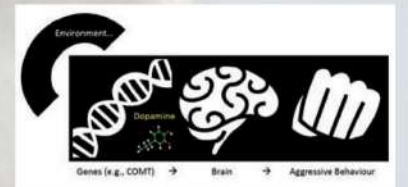
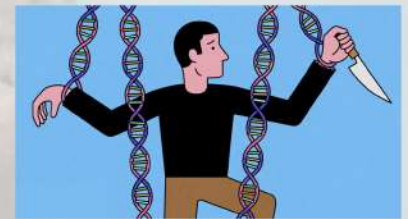
Understanding the genetic basis of aggression involves investigating both heritable and environmental influences. Twin and adoption studies have provided critical insights into the role of genetics in aggression, with meta-analyses suggesting that up to 50% of the variability in aggressive behavior can be attributed to inherited factors, while the remaining 50% is influenced by non-shared environmental factors.

- **Twin and Adoption Studies** : These studies have been instrumental in dissecting the heritability of aggression by comparing monozygotic (identical) and dizygotic (fraternal) twins, as well as individuals raised in adoptive environments.
- **Candidate Gene Association Studies (CGAS)** : These studies have focused on identifying specific genes involved in neurotransmitter regulation, particularly those related to dopamine and serotonin, which are critical to impulse control and aggression.
- **Genome-Wide Association Studies (GWAS)** : Unlike CGAS, which target specific genes, GWAS screen the entire genome for associations between genetic variants and aggressive behaviors. This method provides a broader perspective on the genetic architecture of aggression.

Results

Research into the genetic basis of aggression has identified several key genes involved in neurotransmitter regulation, hormone function, and molecular pathways related to aggression:

- The **MAOA** gene, located on the X chromosome, encodes monoamine oxidase A, an enzyme responsible for the breakdown of neurotransmitters such as dopamine, serotonin, and norepinephrine. A truncating mutation in the **MAOA** gene has been linked to increased aggression and violent behavior, particularly in males. This gene plays a crucial role in regulating the balance of neurotransmitters that influence mood and impulse control.
- The **COMT** (catechol-O-methyltransferase) gene is involved in the breakdown of dopamine, particularly in the prefrontal cortex. Variants in this gene have been associated with impulsivity and externalizing behaviors, contributing to aggressive tendencies.
- The **DRD4** gene encodes the dopamine receptor D4, which plays a key role in the brain's reward system. Variants of **DRD4** have been linked to novelty-seeking behavior, impulsivity, and externalizing behaviors, all of which are associated with aggression.
- Brain-derived neurotrophic factor (**BDNF**) is involved in the development and plasticity of neural circuits. Variants in the **BDNF** gene have been associated with aggression, anger, and impulsivity, highlighting the role of neuroplasticity in aggressive behavior.
- The **AVPR1A** gene encodes the vasopressin receptor 1A, while **AVP** encodes vasopressin, a hormone involved in social behaviors, including aggression. Both genes have been linked to aggressive behavior, with **AVPR1A** variants specifically associated with anger and impulsivity.
- The **AR** gene encodes the androgen receptor, which is critical for mediating the effects of testosterone. Variants in **AR** have been linked to violent behavior and aggression, particularly in males, underscoring the role of hormones in modulating aggressive tendencies.
- **CDH13** Gene : The **CDH13** gene, which encodes a protein involved in cell adhesion, has been associated with aggressive and violent behavior. This gene may influence the development of neural circuits that regulate social and aggressive behaviors.
- **NOS1** Gene : Nitric oxide synthase 1 (**NOS1**) is involved in the production of nitric oxide, a neurotransmitter that plays a role in neural communication. Variants in **NOS1** have been linked to impulsive aggression, suggesting its involvement in the regulation of aggression-related behaviors.



Discussion - Conclusions

The genetic factors influencing aggression are diverse and involve multiple pathways related to neurotransmission, hormone regulation, and neural plasticity. The most well-studied genes, such as MAOA, COMT, DRD4, and BDNF, are involved in the regulation of neurotransmitters like dopamine and serotonin, which play critical roles in mood, impulse control, and aggression. Hormonal genes, such as AR and AVPR1A, further modulate aggressive behavior, particularly in males, by influencing testosterone and vasopressin levels.

The role of genetic predispositions in aggression is not deterministic; rather, these genetic factors interact with environmental influences to shape aggressive behavior. Gene-environment interactions are critical in understanding the full spectrum of aggression, with non-shared environmental factors accounting for a significant portion of the variability in aggressive tendencies. For instance, individuals with certain genetic variants may be more prone to aggression when exposed to environmental stressors, such as trauma or adversity.

- **Gene-Environment Interactions** : While genetic factors contribute significantly to aggression, they often interact with environmental factors to influence behavior. For example, individuals with a variant in the **MAOA** gene are more likely to exhibit aggressive behavior if they have experienced childhood maltreatment.

- **Limited Effect Sizes** : Despite the identification of numerous candidate genes, the effect sizes of these genetic variants on aggression are generally small. This suggests that aggression is a polygenic trait influenced by the cumulative effects of multiple genes, each contributing a small amount to the overall risk.

Aggression is a complex trait influenced by a combination of genetic and environmental factors. Research has identified several key genes involved in the regulation of neurotransmitters and hormones that contribute to aggressive behavior. These genetic factors interact with environmental influences to shape the expression of aggression, with both CGAS and GWAS studies identifying numerous candidate genes.

The findings from genetic studies of aggression offer valuable insights into the biological mechanisms underlying aggressive behavior. By identifying the genetic factors involved in aggression, researchers can develop targeted interventions aimed at mitigating the risk of aggressive behaviors. Future research should continue to explore gene-environment interactions and investigate the potential for personalized treatment strategies based on an individual's genetic profile.



EVALUATION OF THE C1 VERTEBRA FOR THE ESTIMATION OF SEX IN UNKNOWN HUMAN REMAINS

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Introduction

- The vertebral column, especially the C1 vertebra, is crucial for sex estimation when traditional bones are unavailable [1][2][3][4].
- Cervical vertebrae are durable and resistant to decay, making them reliable for forensic analysis [5][6].
- The C1 vertebra exhibits significant sexual dimorphism, offering up to 84.5% accuracy in sex estimation [5][7].
- Louka et al. (2022) demonstrated strong correlations between C1 measurements and sex in Greek skeletal remains, providing a basis for studying Greek Cypriot populations [3].

Methods

Sample Size & Selection:

- Total: 128 skeletal individuals
- Males: 68; Females: 60
- Exclusion: Incomplete samples excluded
- Source: Cyprus Research Reference Collection, St. Nicholas Ossuary, Limassol, Cyprus



Tools: P54 waterproof stainless steel digital caliper (HL-150)

Parameters Measured:



Figure 1: C1 measurements: Maximum length of superior facets (LSF); Maximum width of inferior facets (WIF); Maximum distance between lateral edges, superior (MxDS) & inferior (MxDI); Vertebral foramen Length (LVF) *; Vertebral foramen Width (WVF); * Left & Right

Statistical Analysis: ○ R-Studio version 4.2.1

- Binary logistic regression with k-fold cross-validation

Conclusions

- ✓ Preliminary results show the C1 vertebra is a poor predictor of biological sex, with a classification accuracy of 75.23%.
- ✓ The reduced model demonstrates slight improvement but highlights the need for further statistical evaluation.
- ✓ Comparative studies yield mixed results:
 - Ekizoglu et al. (2021) found significant sexual dimorphism in the C1 vertebra in a Turkish population.
 - Rozendaal et al. (2020) reported 84.5% accuracy using cervical vertebrae in Greek and Portuguese populations.
 - Amores-Ampuero & Viciano (2020) showed vertebrae are useful for sex estimation when other elements are incomplete or fragmented.
- ✓ Combining C1 measurements with cranium or other vertebrae may enhance sex estimation accuracy.
- ✓ Further investigation is needed to evaluate the C1 vertebra's forensic potential.

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Objectives

- ❑ Apply Louka et al.'s (2022) osteological methods to refine sex estimation in the Greek Cypriot population.
- ❑ Focus on the C1 vertebra to improve forensic sex estimation with region-specific data for identifying unknown remains.
- ❑ Address challenges in identifying fragmented or commingled skeletal remains.
- ❑ Contribute a population-specific tool to enhance forensic anthropology practices.

Pre-liminary Results

Assumptions:

1. Binary response Variable: Sex
2. The observations are independent of one another.
3. There is little to no multicollinearity.
4. Linearity:

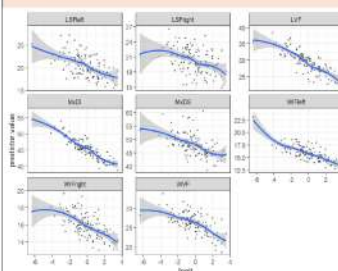


Figure 2: Shows the linear relationship between each predictor and its log odds.

5. There is a sufficiently large sample size.
 - Suggested minimum sample size:

$$N = 10 * \frac{k}{p} \text{ per or predictors} = 10 * \frac{8}{.47} = 170.2128$$

• p is the smallest proportion of negative or positive cases.

6. There are no influential points.

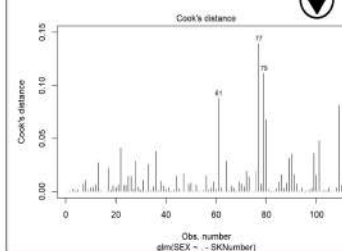


Figure 3: Cook's distance plot indicating no influential points (all values < 0.5), confirming the reliability of the regression model.

Binary Logistic Regression

Full Model: All eight measurements,

Reduced Model: Backward Stepwise Regression

- Only three measurements—[LVF], [WIFleft], and [MxDI]

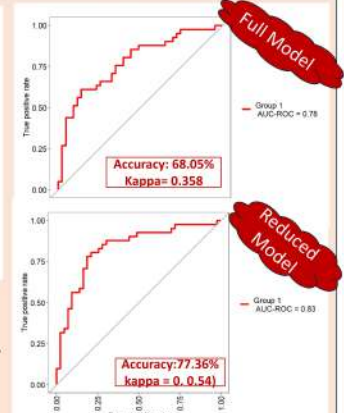


Figure 5: AUC-ROC Curve shows the model's performance in distinguishing between binary sex categories.

- The reduce model has higher accuracy but more variability.

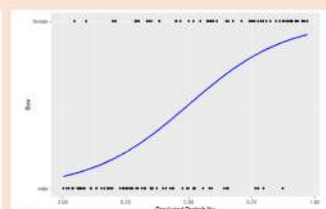


Figure 6: Predicted Probability Graph showing the correlation between actual sex and the likelihood of sex prediction based on bone measurements.

- Significant overlap between the two sexes.

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Abstract

As humanity's reach into space extends, the discipline of forensic science must adapt to the unique challenges posed by microgravity environments. This study explores the complexities of conducting forensic investigations in space, with a particular focus on bloodstain pattern analysis (BPA), the implications of legal frameworks such as the Outer Space Treaty (OST), and the potential for crimes in extraterrestrial settings. We emphasize the necessity for forensic techniques to evolve, combining traditional methods with knowledge from astrophysics, chemistry, and biology to ensure effective crime scene investigations in space. Microgravity refers to the condition in which objects appear to be weightless and experience very low gravitational forces.

Background and Aims

As human activity in space continues to grow with missions to the International Space Station, space tourism, and plans for lunar and Martian colonies, the potential for crimes occurring in microgravity environments has emerged as a critical area of study. Microgravity refers to the condition in which objects appear to be weightless and experience very low gravitational forces [1]. Traditional Earth-based forensic methods face unique challenges in space due to microgravity conditions, making it necessary to adapt these techniques for effective crime scene investigation [2]. This presentation aims to explore the implications of microgravity for forensic science, particularly focusing on bloodstain pattern analysis, crime scene procedures, and the legal frameworks needed for investigating crimes in space.

Methods

A review of existing literature highlights the necessary adaptations for conducting forensic investigations in microgravity. Key studies, including those focusing on bloodstain pattern dynamics in reduced gravity, have been conducted to better understand the behavior of fluids, particularly blood, in these environments [4]. Experimental setups involved parabolic flight maneuvers to simulate microgravity and assess fluid dynamics using synthetic blood analogs [4,8]. Additionally, research on cell death and cytokine production in microgravity has provided insights into biological factors that could impact forensic investigations [5]. Furthermore, an examination of emerging legal frameworks, including the Outer Space Treaty (OST), helps to understand the jurisdictional challenges associated with crimes that may occur in space [6].

Results

The findings reveal that blood behaves differently in microgravity compared to Earth. For instance, when an astronaut suffers a wound, blood forms a dome around the injury due to surface tension, complicating traditional forensic analyses of bloodstains [4]. The pilot studies conducted in microgravity showed that droplet formation is significantly influenced by surface tension and inertia, leading to unique patterns that need to be understood to reconstruct events accurately [4].



Credit: Zack Kowalske

Importantly, the Outer Space Treaty (OST) plays a pivotal role in establishing legal frameworks for handling crimes in space. This treaty emphasizes that activities in outer space must adhere to international law, including the principles of sovereignty and jurisdiction [6,7]. As the number of space missions increases, the OST will serve as a foundational document for determining how laws apply to potential crimes in space. Jurisdictional issues arise when crimes occur on spacecraft or celestial bodies, and the OST will help in developing appropriate legal responses [7].

Additionally, a research has shown that cytokine production, which is crucial for immune responses, may be diminished in microgravity environments, potentially impacting the handling of biological evidence during forensic investigations [5]. An intriguing aspect of firearms in space is highlighted by a study that discusses the implications of discharging a weapon in a vacuum environment. Modern ammunition contains its own oxidizer, allowing bullets to be fired without atmospheric oxygen [3]. When a bullet is shot, it will continue to travel indefinitely, demonstrating the unique physics of motion in space governed by Newton's third law of motion. The bullet's trajectory and the shooter's reaction due to the absence of intergalactic atoms can create scenarios that may also impact forensic analyses in case of a shooting incident in space [3].

Conclusion

In conclusion, the intersection of forensic science and space exploration is an emerging field that necessitates significant adaptations to traditional methods. The challenges posed by microgravity demand innovative approaches to evidence collection and analysis. The development of specialized training for forensic astronauts and the creation of robust legal frameworks, such as the OST, are essential to ensure effective crime scene investigations in space. As human presence in outer space continues to expand, understanding these dynamics will be critical for addressing the complexities of crime in microgravity environments.

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Commotio cordis – historical literature review and case-report

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INTRODUCTION

Commotio cordis (CC) is a recognized but rare and potentially fatal mechano-electric arrhythmogenic syndrome.

- It is caused by a sudden, seemingly minor blunt, nonpenetrating impact to the chest.
- The typical outcome is sudden death, most often due to ventricular fibrillation and cardiac arrest.
- This occurs in the absence of:
 1. Rib or sternum damage.
 2. Heart damage.
 3. Any underlying cardiovascular disease.

Diagnosing CC is a challenge for the forensic pathologist as it can have serious juridical implications, the most likely manner of death being homicide by imprudence. The Romanian medico-legal literature has only scarce mentions of this phenomenon, but its incidence in the world has been increasing starting with the last decade of the last century and the real incidence is still unknown.

Post-mortem diagnosis of CC is one of the most challenging issues in forensic pathology due to several reasons:

- Lack of specific necropsy signs.
- Incomplete medical history of the deceased.
- Insufficient data about the circumstances surrounding the death.

OBJECTIVES

Our purpose is to bring into the spotlight a severe condition which often affects young athletes, but not exclusively, and which is or could be an important health-care issue in the world.

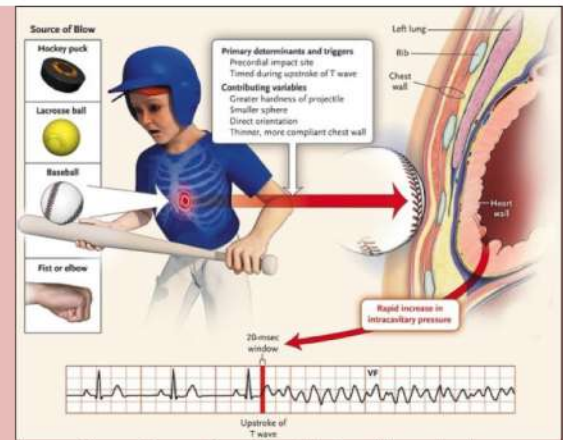
MATERIAL AND METHODS

Case Report #1

- Patient: 24-year-old male, soccer player.
- Incident: During a training session, the patient experienced an immediate loss of consciousness following an accidental, low-intensity blunt trauma to the chest.
- Initial Response: Witnesses initiated cardio-pulmonary resuscitation (CPR) immediately, CPR was continued by ambulance personnel upon arrival.
- Hospital Course: Despite medical efforts in the emergency room, the patient died 7 hours later. Throughout this time, the patient remained comatose with a Glasgow Coma Scale (GCS) score of 3-4. Initially, the pupils were intermediate, but soon after, fixed mydriasis was observed. The patient also had severe biventricular systolic dysfunction (20-25%). Electrocardiogram (EKG) results revealed ventricular fibrillation, ventricular tachycardia with narrow QRS complexes, and pulseless electrical activity.
- The medico-legal autopsy, performed approximately 48 hours later at the Institute of Legal Medicine Iasi, revealed: older abrasions in the cervical region and upper right thorax; generalized visceral stasis; several areas of acute myocardial necrosis and inflammation; no visible precordial or cardiac injuries.
- Given the patient's lack of relevant medical history and the findings from the clinical and medico-legal examination, the cause of death was determined to be a fatal cardiac arrhythmia, most likely as a consequence of **cardiac concussion**.

Case Report #2

- Patient: 22-year-old male.
- Incident: During a friendly soccer game, the patient suffered a sudden lipothymic episode after an accidental elbow strike to the base of the left hemithorax.
- Initial Response: The patient was transported to the emergency room by ambulance. During transport, CPR was performed, and the patient regained consciousness.
- Hospital Course: Upon admission to the emergency room, the patient's general condition was good: GCS score of 15, respiratory rate: 18 breaths per minute, cardiac frequency: 94 beats per minute, blood pressure: 127/80 mmHg. A cardiologic examination revealed no significant changes. The patient was subsequently discharged.
- The sudden loss of consciousness in a young patient with no significant medical history, following a low-intensity blunt trauma to the precordial region, strongly suggests the presence of **cardiac concussion**.



Commotio cordis – etiopathogenetic mechanism.

DISCUSSIONS

Although the first historic mentioning of "commotio cordis" ("disturbance of the heart") dates from the middle of the 18th century (Caspar, 1857), the mechanism behind this cause of sudden cardiac death has not been fully understood yet. In a 1996 case review, the term was used in reference to both lethal and non-lethal cardio-vascular disorders, as a consequence of blunt trauma to the chest, associated or not with cardiac bruising. Over time, the concept went through changes, and hence the distinction between blunt trauma to the chest associated with cardiac bruising (contusion) and the one without myocardium injury (concussion), mentioned by Riedinger in various publications between 1882 and 1903, with a proposed mechanism which involved autonomic nervous responses, following Meola's theory from the 1870s of a "profound vagal reflex". At the beginning of the 20th century, Schlomka's study on 600 anesthetized rabbits, cats and dogs, by means of recording ECG, respiration rate, arterial and venous pressures, identified 3 main factors which favor the induction of arrhythmia after a precordial impact of moderate intensity: type of impact, location of impact and force of impact. The following technological advancements identified a fourth factor with significant importance – the timing of impact relative to the cardiac cycle. The modern understanding of the concept of commotio cordis has been best defined by Maron et al's case reviews which presents the notion as a rare and dangerous condition with a poor response to CPR, induced by a thoracic trauma by small objects, with precordial impact.

One of the most complete definition in the literature, in our opinion, integrating both historical and contemporary views on commotio cordis was enounced by Nesbitt in 2007: "mechanical stimulation of the heart by non-penetrating, impulse-like impact to the precordium that, through intrinsic cardiac mechanisms, gives rise to disturbances of cardiac rhythm".

As far as the survival rate of the patients who suffer CC, the United States Commotio Cordis Registry mentions an increase from 15% in the 1990s to 35% in 2012, this depending on the rapid onset of the cardio-pulmonary resuscitation.

These two cases we presented fit into the definition of commotio cordis due to: the young age of the patients, the rapid appearance of dysrhythmia after a low-intensity blow to the chest during sport activity, with no significant underlying cardiac pathology could be identified. In both cases, the patients had no medical history of cardiac or any other kind of illness, and there was no sign (on EKG or during the autopsy) of myocardium injury. We encountered one other case of sudden death during sport activity at a 21 year old male patient, without any medical history, but the histopathological examination determined the presence of a arrhythmogenic right ventricular dysplasia, so the case can't be considered commotio cordis due to the predisposition to arrhythmia which accompanies this illness, although the intrinsic mechanism is similar.

CONCLUSION

The international literature has numerous mentions of commotio cordis, as a rare and underdiagnosed cause of sudden death.

The circumstances in which this pathological condition can be encountered are mostly sport related accidents, but there are also mentions of commotio cordis following horse kicks to the chest (Karakaya), traffic accidents (Michalodimitrakis) or intentional direct traumatic blunt force applied to the anterior chest (Jiao Mu).

The most alarming fact is that, the people most affected are young and in full health and this makes prevention an essential task. The pamphlets and information sheets on cardiac concussion and sudden cardiac death we found addressed to athletes, coaches and parents/guardians involved in soccer, american football, baseball and other sports and the awareness or acknowledgment forms the parents/guardians and athletes have to sign show the true importance of this issue. However, these safety precautions have been encountered only in American institutions. In our opinion, all countries should take up this model and increase the awareness degree of the population about this severe and, possibly deadly, condition.

Our literature review showed scarce mentioning of "commotio cordis" in the Romanian forensic literature, which can either mean that this illness is being severely underdiagnosed or that such cases are really very rare in Romania. Either way, being aware of its existence and implementing rigorous means of preventing and detecting it, is of utmost importance. Information sheets on this illness for athletes, coaches and parents (as the risk is higher during sports activities) should exist, in our opinion, in all countries and in every sport and, also, public presentations on the subject should be held so that the general public be made aware of it, and also, be ready to intervene, if necessary, with the appropriate actions.



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Introduction

Currently, there is a huge demand for stable and reliable person identification systems as well as for automatic diagnosis platforms. Our hypothesis was that the two desiderates could be combined and served by a single algorithm. Biometric features are amongst one of the most significant features in an individual's identification. Amongst all biometric features, human retina is the best attribute to consider both for identification and verification as well as for diagnosis of various conditions such as diabetes mellitus. Templates used for retina recognition and automatic diagnosis are quite small in size which makes the recognition and matching procedure quick.

We aimed to develop a fundus images classification model as a base for a person identification system and grading of diabetic retinopathy with no coding knowledge by using generative artificial intelligence (AI), a free image analysis tool, and an automated machine learning (ML) platform.

Materials and Methods

- 2961 good quality fundus images from the APTOS 2019 database belonging to patients with DR and graded by trained ophthalmologists as no DR, mild non-proliferative DR (NPDR), moderate NPDR, severe NPDR, and proliferative DR (PDR) (total of 5 classes) according to the International Clinical Diabetic Retinopathy Disease Severity Scale;
- batch segmentation of images using Contrast Limited Adaptive Histogram Equalization (CLAHE) filter implemented in Fiji ImageJ2 (free image analysis tool) and a Macro script provided by Copilot Pro (Microsoft™) generative AI to facilitate training and detection (figs. 1, 2);
- images were sorted by diagnosis using a Visual Basic Macro Script for Excel (Microsoft™) from Copilot Pro, and organized into subfolders according to the diagnoses listed in the accompanying APTOS database ground truth Excel file (fig. 3);
- pre-processed images were uploaded in Google Cloud™ and manually assigned to the 5 classes – No_DR (1371 images), Mild_NPDR (289 images), Moderate_NPDR (849 images), Severe_NPDR (181 images) and PDR (271 images);
- AutoML (Vertex AI) was chosen as model training method on the us-central1 (Iowa) server. Data was randomly assigned, 80% for training, 10% for validation and 10% for testing. Higher accuracy was selected for training options with a latency of 200 to 300 ms and a minimum of 8 node hours training.

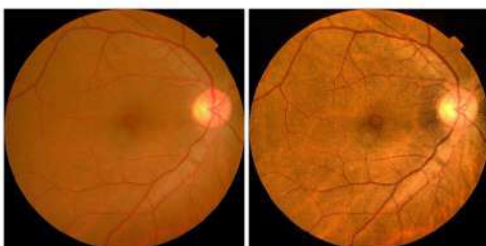


Fig. 1. CLAHE segmentation of fundus images: original image (left) and segmented image (right) (images from APTOS database)



Fig. 2. ImageJ batch CLAHE segmentation script generated by Copilot Pro

Results

Total training time -1h 38 minutes. The model area under the precision-recall tradeoff curve (AUPRC) for estimating severity grading precision was of 0.889 with a precision rate of 83.8% and a recall rate of 77% at 0.5 confidence threshold (fig. 4).

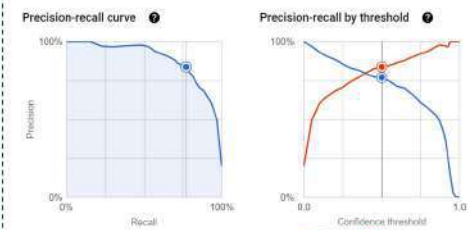


Fig. 4. Precision-recall curve and model features for grading DR

Precision and recall rates varied according to confidence thresholds:

Confidence threshold	Precision rate	Recall rate
0.25	70.7%	85.5%
0.5	83.8%	77%
0.75	92.3%	60.8%

The confusion matrix resulting from the model is detailed in the following table:

True label	Predicted label				
	No DR	Mild NPDR	Moderate NPDR	Severe NPDR	PDR
No DR	98%	2%	0%	0%	0%
Mild NPDR	7%	62%	28%	0%	3%
Moderate NPDR	1%	12%	80%	5%	2%
Severe NPDR	0%	6%	33%	56%	6%
PDR	0%	7%	37%	4%	52%

Based on the Confusion Matrix we asked Copilot Pro to provide formulas for calculating sensitivity, specificity, positive predictive value, negative predictive value, and accuracy in Excel. We applied provided formulas and obtained the following values:

	No DR	Mild NPDR	Moderate NPDR	Severe NPDR	PDR
True positive	1343	179	679	101	141
False positive	28	159	241	53	37
True negative	1563	2513	1872	2725	2653
False negative	27	110	169	82	130
Sensitivity	98%	62%	80%	55%	52%
Specificity	98%	94%	89%	98%	98%
Positive predictive value	98%	53%	74%	66%	79
Negative predictive value	98%	96%	92%	97%	95%
Accuracy	98%	91%	86%	95%	94%

Fig. 3. Visual Basic Macro script for separating images according to diagnosis

Discussions

- an "ideal" software would automatically extract the vascular map, identify the map in the provided database or detect and adequately grade disease severity with >90% sensitivity and specificity rates while also being capable of tracking changes over time. According to software output, a patient would then be referred for further evaluation / treatment;
- deep learning AI uses multi-layered neural networks to simulate human brain for image recognition and classification after being trained on large sets of images;
- major limitations of deep learning AI - tremendous amount of computing power required together with advanced programming knowledge;
- for a forensic pathologist or an ophthalmologist with no coding knowledge and limited computing power creating an image classification system in Matlab or Python is difficult and time consuming;
- we developed an automated DR classification system using digital tools that require no coding - generative AI, a free open-source software for medical images analysis (Fiji ImageJ2) and an automated ML platform developed by Google™ for general use. For generative AI we opted for Copilot Pro to generate the ImageJ segmentation script, an Excel macro script to split the APTOS 2019 database into separate folders according to DR severity and Excel formulas for calculating sensitivity, specificity, positive predictive value, negative predictive value, and accuracy rates to assess the classification model;
- classification model developed using automated ML implemented in Vertex AI. Our automated model proved comparable to other classification models developed using the same APTOS 19 dataset and various tools;
- compared to conventional ML model training that requires an adequate infrastructure and a strong foundation in statistics, algorithms, big data, and programming languages, AutoML can quickly and accurately select the best model for a given dataset and runs at small costs on virtual machines in Google's massive network. Even if AutoML offers limited customization options, it can be used by non-experts to develop person identification or image diagnosis tools.



Fig. 5. Potential use of Vertex AI in medicine:

- Medical imaging - detect abnormalities more accurately and quickly.
- Drug discovery - analysis of molecular structures and predict efficacy.
- Clinical decision Support - develop predictive models for informed decisions.
- Patient engagement - remote monitoring.

Limitations

- responsibility attribution : physician or software developer;
- increased specificity and sensitivity for affirming the presence of a certain condition and lower specificity and sensitivity when grading the condition;
- Compared to physicians, AI is more limited by image quality.

Conclusions

Generative AI implemented into Windows operating system together with a free imaging processing tool and Vertex AI allow forensic pathologists and ophthalmologists with no coding knowledge to benefit from publicly available image databases (thousands of cases) to develop accurate automated person identification and diagnostic tools. Such tools have the potential to equally facilitate person identification, screening, diagnosis, and therapeutic approach of various systemic and ophthalmologic diseases.

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Necrotizing fasciitis - forensic pathology perspectives

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Objectives

Described by Hippocrates in the 5th century BC as a complication of erysipelas, necrotizing fasciitis is an aggressive inflammatory condition affecting the skin and adjacent soft tissues, causing necrosis of the fascia muscle and subcutaneous tissue [1]. Gram-positive cocci (especially strains of *Staphylococcus aureus* and *Streptococci*) are involved in most cases of necrotizing fasciitis. However, *Klebsiella*, *Clostridium*, *E. coli*, *Proteus*, *Pseudomonas*, and *Aeromonas hydrophila* may also be involved. Polymicrobial infections have been also described [2, 3, 4]. Typically, the disease progresses rapidly along the poorly vascularized fascia, progressing to damage of the overlying and underlying layers, with massive lesions of the skin, connective tissue and muscle. It is a lethal condition in 15% of cases even if treated, particularly in patients with different comorbidities such as diabetes mellitus or cancer [5]. Our study aimed to describe the morphological features of the necrotizing fasciitis cases diagnosed in our department and their relationship with the comorbidities spectrum.

Material and Methods

The autopsy reports of our Department, from the last five years, have been reviewed, and 21 cases of necrotizing fasciitis have been selected. The autopsy exam has been associated with tissue specimen collection for microscopy. The collected specimens have been investigated by routine paraffin embedding, followed by haematoxylin-eosin (H&E) staining.

Results

The study group comprised 17 men and 14 women, ages ranging between 2 and 88 years old. Gross examen revealed shiny and tense skin areas up to 10 cm in diameter, associated with bullae, and red to purple and black areas of necrosis. The lesions have been located on the upper extremities in 17 cases and the abdominal wall in 2 cases. In 2 cases, the skin lesion could not be detected. The microscopic examination revealed an extensive polymorphonuclear infiltration of the dermis, subcutaneous fat, and muscle fascia associated with focal microhemorrhages (Figs. 1-4). Higher power magnification demonstrated extensive superficial fascial necrosis, small and medium-sized blood vessel thrombosis in all cases, and the absence of muscle involvement (Figs 5-6). Subepidermal cleft and bulla formation have been registered in 4 cases. A percentage of 66% of the total cases involved people with an average age of 75 years who had other associated comorbidities, such as diabetes mellitus (5 cases), kidney chronic disease (3 cases), liver chronic disease (2 cases), and obesity (7 cases).

Discussion

Necrotizing fasciitis arises in about 4 people per million per year in the U.S., and about 1 per 100,000 in Western Europe, and both sexes are equally affected [1, 5]. Despite its rarity, necrotizing fasciitis is a relatively common cause of death in forensic pathology. It typically occurs due to trauma, sometimes as trivial as a scratch or insect bite, to the skin of the extremities, abdomen, groin, or perineum. Despite the rapid treatment, necrotizing fasciitis is associated with a high mortality rate in some patients [6]. The main predisposing factors for NF are any immunosuppression conditions, such as advanced age, diabetes mellitus, chronic renal failure, alcoholism, childbirth, and the use of non-steroidal anti-inflammatory therapy [3, 7, 8]. These data are supported by our results, different comorbidities have been registered in 17 cases in our study group. Differential diagnosis of necrotizing fasciitis include paniculitis, neutrophilic dermatoses, and gas gangrene [5-8].

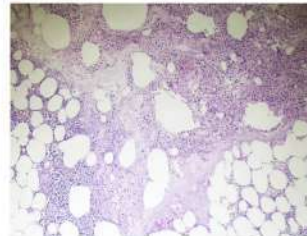


Fig. 1. General overview of a necrotizing fasciitis, H&E x4

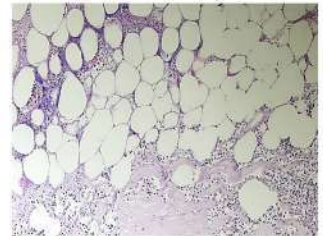


Fig. 2. Polymorphonuclear infiltration of the deep dermis and subcutaneous fat in a case of necrotizing fasciitis, H&E x10

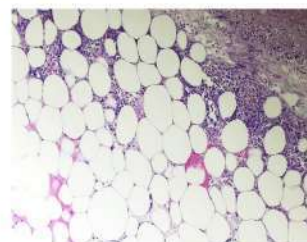


Fig. 3. Extensive polymorphonuclear infiltration of the subcutaneous fat and muscle fascia in a case of necrotizing fasciitis, H&E x10

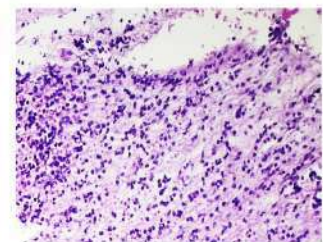


Fig. 4. Abundant acute inflammatory infiltrate and superficial fascial necrosis in a case of necrotizing fasciitis, H&E x20

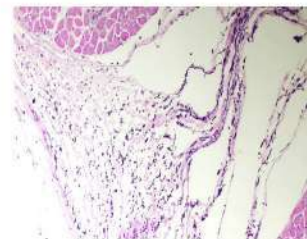


Fig. 5. Acute inflammatory infiltrate in muscular fascia without muscle involvement in a case of necrotizing fasciitis, H&E x20

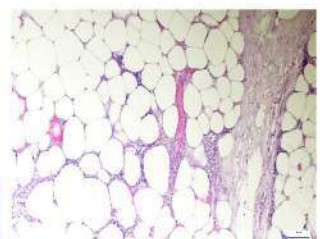


Fig. 6. Acute inflammatory infiltrate small sized blood vessel thrombosis in a case of necrotizing fasciitis, H&E x10

Conclusions

Necrotizing fasciitis is often a lethal condition involved in forensic pathology. It appears as a complication that should be considered not only in the presence of large, extensive, disfiguring wounds but also in minor trauma, phlebotomy, abrasions, and invasive procedures. The very rapid evolution to death has been linked to certain streptococcal strains, advanced age, localization of the primary lesion, immunosuppression state, and delayed surgery.

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AN UNCOMMON CASE OF SUICIDE: CASE REPORT

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Background and aims

- Multiple cavity-penetrating wounds are not specific for self-harming due to threshold of perception of pain stimuli.
- The purpose of this report is to reveal a case of suicide occurring on increased threshold of perception of pain stimuli by administer of psychoactive substances.



Figure 1.



Figure 2.

Results

A 40-year-old person who was found dead in his house with multiple cut wounds.

At the examination of the death scene where found different bloodstain pattern such as drops, flows, pools and contact stains, all of them where overlaying each other. It became quite difficult to work out exactly what happen there.

At the autopsy were traced non-penetrating cut wounds at the level of the neck and puncture-cut on the thorax, and penetrating puncture-cut wounds at the level of the anterior abdominal wall with evisceration of small intestine loops.

Death occurred as a result of external and internal bleeding.

Samples of soft tissues for laboratory findings were taken to appreciate the vitality and time of the injuries. It was confirmed that all the injuries were produced shortly before death.

Samples of blood, urine and internal organs for toxicology research were collected too. Methamphetamine, amphetamine and cocaine were found in samples of blood, urine and internal organs.

Police investigation showed that no other persons entered or exited the apartment.



Figure 3.



Figure 4.



Figure 5.

Discussion- Conclusions

- The cocktail from psychoactive substances leads to advanced changes in perception process, inclusively due to inhibition of pain stimuli.
- Although the injuries found on the dead body were not characteristic for suicide, the narcotic consumption explains their appearance as a result of the person's own actions.

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Fatal Consequences of Head Trauma: a Case Report of Venous Air Embolism Complicated by Substance Abuse and Liver Disease

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ABSTRACT

Purpose: Venous air embolism is a rare but potentially fatal complication from traumatic injuries or medical procedures, leading to gas emboli formation within the pulmonary vasculature and cardiac chambers. This case report aims to highlight the risks of venous air embolism following traumatic injury, especially in individuals with coexisting conditions, and to stress the need for timely medical intervention in such cases.

Methods: A case study of a 34-year-old female with a history of substance abuse and a head injury sustained during an altercation is presented. Despite initial refusal of medical care, she eventually received treatment. Postmortem examination was conducted, revealing a triangular wound on the scalp and air embolism in the venous and cardiac systems.

Results: The air embolism was discovered in the venous and cardiac systems, with the superficial temporal vein near the head wound being identified as the injury source. Contributing factors included mixed nodular cirrhosis, which led to coagulation disorders. The failure of veins to thrombose and the inadequate occlusion of venous lumens allowed air to enter the venous system, resulting in a fatal outcome.

Conclusion: This case emphasizes the importance of recognizing and managing head injuries in patients with substance abuse and medical comorbidities like liver disease. Awareness and education about the risks of delayed medical treatment for traumatic injuries are crucial for preventing similar fatalities in the future.

INTRODUCTION

Venous air embolism is a rare but serious condition caused by the entry of air into the bloodstream, forming gas emboli in the pulmonary vasculature and heart chambers. It often occurs due to traumatic vascular injuries or medical procedures, especially catheter insertions and surgeries like neurosurgery and heart surgery. Air enters through compromised veins, particularly when pressure differences trap it. Risk increases in surgeries performed with the patient in elevated positions. Symptoms vary from neurological to cardiovascular, making diagnosis difficult. Fatal air embolism can result from 75 to 300 cm³ of air. This case discusses a postmortem discovery of venous air embolism.



Figure 1. The wound following clamp removal.

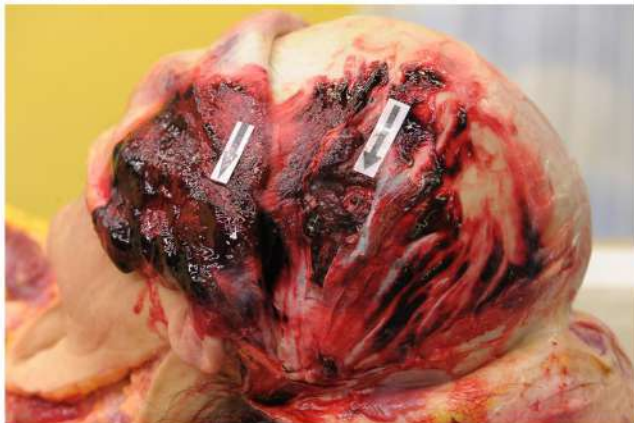


Figure 2. The depth of the injury.

DISCUSSION & CONCLUSIONS

- Damaged scalp veins failed to clot, allowing air entry due to negative pressure
- The patient's head injury, was compounded by delayed medical intervention
- Autopsy revealed a triangular scalp wound, significant trauma, and venous air embolism in the heart, linked to a superficial temporal vein injury
- An underlying coagulation disorder caused by cirrhosis likely had an effect on the delayed wound closure
- Air embolism, though recognized as a complication of head injuries, often goes undiagnosed
- Timely intervention, including positioning, oxygen, air aspiration, and hyperbaric therapy, could have improved survival
- Education on substance abuse risks and early injury treatment is crucial to prevent future fatal outcomes

CASE PRESENTATION

Emergency services were called in the evening, at approximately 10 pm, for a 34-year-old female patient with a history of substance abuse following a head injury. Amid a domestic dispute with her partner, she threw objects around the apartment while intoxicated. However, she inadvertently slipped and fell, hitting her head on a glass resting on the floor. When the emergency response team arrived, it was noted that the injured woman was experiencing significant bleeding from a wound on her head, and she was initially resistant to medical assistance due to her intoxicated state. Eventually, she agreed to have her head wound treated, but she refused transportation to the hospital for further care. At 2 am, another ambulance was requested because the head wound continued to bleed. Upon assessment in the emergency department, a wound was identified on the left parietal region of her head, with blood seepage and a surrounding hematoma. X-ray imaging was not conducted due to the patient's combative and restless behavior. The head wound was managed with staples. Upon admission to the hospital, the patient's blood pressure was recorded as 91/63 mmHg (heart rate: 93/min), gradually increasing during her hospitalization. The latest measurement at 5 am showed a blood pressure of 143/63 mmHg (heart rate: 61/min). Due to vessel occlusion, venous access could not be established, and the patient remained prone in the ward without receiving further treatment until she independently rose around 6 am to use the toilet. Shortly afterward, staff found her lying lifeless on the floor in front of the toilet door.

AUTOPSY FINDINGS

The autopsy was performed 28 hours after death, with the body having been moved to a refrigerator 2 hours after death. Neither external nor later internal examination revealed signs of putrefaction. The examination revealed a significant number of bruises and hematomas of various ages distributed across the body; however, the sole open wound was noted on the left parietal region of the head. Upon removal of surgical staples and shaving of surrounding hair, a triangular wound measuring 2.0 x 2.5 x 3.4 cm was identified (Fig. 1), penetrating through the total thickness of the scalp (skin, connective tissue, galea aponeurosis, loose areolar connective tissue, portion of the left temporal muscle, and the pericranium, which covers the outer cortical bone of the calvaria (Fig. 2). Subsequent examination of the soft tissues and skull revealed no evidence of fractures. Following the dissection of the neck muscles, it was noted that the jugular veins on both sides were distended with air (Fig. 3). Puncturing the heart in the water-immersed right atrium revealed the presence of a dense pocket of air bubbles (Fig. 4). Upon inspecting the brain, air bubbles arranged in a bead-like fashion were observed within the venous vessels of the soft meninges between the blood columns in the area of the circle of Willis (Fig. 5). Upon brain removal, clear cerebrospinal fluid drained out, and an examination of the skull base revealed no fractures. In addition to physical injuries, mixed nodular cirrhosis of the liver and cholecystolithiasis were identified as coexisting conditions. At the time of death, the deceased had 2.97 g/kg of ethyl alcohol in her blood and 4.04 g/kg in her urine, along with a confirmed presence of THC at an estimated concentration of 17 ng/ml in her blood. Based on the autopsy findings, it was concluded that the air embolism stemmed from damage to a vein near the head wound, most likely the superficial temporal vein—the only vascular structure in that area susceptible to such a condition. The delay in treating the head wound and potentially a clotting problem associated with liver disease could have played a role in this complication. Such an outcome, particularly with delayed wound treatment, would be improbable in a healthy individual without a clotting disorder.



Figure 3. Presence of air in the jugular veins.



Figure 5. Presence of air bubbles in the vertebral and basilar arteries.



Figure 4. Positive pericardial water test.

Simultaneous Natural Death of An Elderly Couple- Philemon and Baucis Syndrome: A Case Report

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BACKGROUND

The simultaneous or nearly simultaneous deaths of married couples are relatively uncommon, and such cases often raise concerns about unnatural causes. However, factors like forensic investigations, autopsy results, individuals' backgrounds have led to the consideration that certain couple deaths may be of natural origin. These cases have established a place in medicolegal terminology: "Philemon and Baucis Syndrome."

AIMS

This study contributes two additional cases to the literature. It highlights that acute stress can be a cause of death when considering individuals' pre-existing conditions, genetic foundations and interpersonal emotional connections.

CASES

The first case involves a 93-year-old man, and the second case an 81-year-old woman. They had been married for over 50 years. Their tenant found the man immobile at the location of the toilet and the woman immobile on the opposite side of the home. Both were fully clothed, and there were no signs of forced entry.



The cause of death for the individuals was determined to be natural, attributed to pre-existing heart and lung diseases.



In the autopsy of the decedent →

- Pleural adhesions in the upper parts of both lungs, which were edematous and contained bloody frothy fluid.
- Widespread atherosclerotic changes in the aorta and heart valves.
- The LAD is 80%, RCA is 95% occluded.
- In myocardial sections, old scar tissue is noted as white areas on the left ventricular free wall.
- Histopathology revealed notable findings, including severe acute pneumonia, bronchiolitis, intraalveolar hemorrhage.

In the autopsy of the decedent →

- Petechiae on the surface of the lungs, edema in the lung sections.
- Other specific findings were not encountered.

DISCUSSION-CONCLUSIONS

The simultaneous or near simultaneous natural deaths of two closely connected individuals in the same environment have been termed "Philemon and Baucis Syndrome(1). Witnessing the death of a closely connected individual can induce acute stress, activating sympathetic and parasympathetic pathways associated with the heart. Intense catecholaminergic activity can lead to myocardial infarction, malignant arrhythmias, left ventricular dysfunction, and myocarditis(2). In our cases, considering the individuals' pre-existing conditions and emotional ties, a psychogenic effect related to the death of the second spouse may be noted.

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A RARE COMPLICATION OF ANAPHYLACTIC SHOCK – A CASE REPORT

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INTRODUCTION

Anaphylaxis is a severe, life-threatening hypersensitivity reaction that can rapidly lead to cardiovascular and respiratory collapse if untreated^{1,2}. While extremely rare, complications such as intracerebral hematoma are documented only in case reports. The purpose of this presentation is to raise awareness regarding the complications of anaphylactic shock.

CASE PRESENTATION

48-year-old woman, with documented allergy to aspirin, and afflicted by multiple endocrinological comorbidities (radio treated pituitary microadenoma, bilateral adrenalectomy, replacement therapy) ➡ stung by wasps ➡ resuscitation team identified numerous wasp stings in the anterior cervical and thoracic regions ➡ cardiac arrest and cardiopulmonary resuscitation (30 minutes) ➡ sinus rhythm, comatose patient and in a very severe general condition. Hospital ➡ multiple consults and investigations, two cranial CT scans without any acute lesions ➡ right thoracic with peripheral cyanosis and absence of detectable pulse ischemia with conservative treatment ➡ multiple organ and system failure emerged with thrombocytopenia and prolonged coagulation times ➡ death by cardiac arrest 12 days after admission.

AUTOPSY FINDINGS

- **macroscopic:** massive cerebral edema, brainstem subarachnoid hemorrhage, right temporal intracerebral hematoma with invasion into the lateral and fourth ventricles, cystic pituitary gland, lower gastrointestinal hemorrhage and intestinal ischemia.

DISCUSSION

The rare connection between anaphylactic shock and intracerebral hematoma involves several mechanisms. Blood pressure instability, with initial hypotension followed by rebound hypertension after epinephrine, can rupture fragile cerebral vessels. Severe hypoxia during anaphylaxis may also weaken brain vessels, increasing the risk of rupture when blood flow is restored^{3,4}.

Additionally, epinephrine-induced hypertension, especially in individuals with pre-existing vascular conditions like aneurysms, can lead to intracerebral hemorrhage, making these cases clinically significant despite their rarity⁵.

CONCLUSION

Although intracerebral hematomas are rare in anaphylactic shock, their severity poses high risks of morbidity and mortality. Recognizing factors like blood pressure fluctuations, hypoxia, and medication-induced hypertension can help clinicians manage at-risk patients and prevent serious neurological outcomes.

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Fig 1 – massive cerebral edema



Fig 2 – right temporal intracerebral hematoma with invasion into the lateral ventricles



Fig 3 – invasion of cerebral hematoma into the fourth ventricle

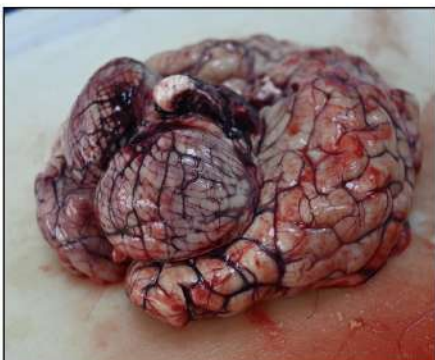


Fig 4 – brainstem subarachnoid hemorrhage



Fig 5,6 – lower gastrointestinal hemorrhage and intestinal ischemia



Copper staining in human skeletal remains.

A case report

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Background and aims

Skeletal remains were found partially buried in a forested area near the city of Varna, Bulgaria. A mostly complete skeleton was uncovered, the skull (Figure №1) of which showed green staining on the external sides of the left mastoid process (Figure №2) and on the left mandibular ramus (Figure №3).

As part of the police investigation the discovered bones were examined with the task of establishing their human specificity and creating a basic biological profile¹ of the person - sex, age at death, height, and post mortem interval.



Figure №1 – The skull after it was cleaned from dirt and other debris



Figure №2– The left mastoid process and the external auditory meatus

Methods

The methods used in the examination of the skeletal remains comprised of anthropometry and observation of the morphological features showing sexual dimorphism^{2,3}, and age-related changes. The anthropometry methods included statistical evaluation using 25 craniometric measurements and use of regression formulas⁴ to establish the height of the person based on the length of the long bones. In addition a stereomicroscope was used to observe the fine features of the green-stained parts.



Figure №3– The left mandibular ramus

Results

The examination of the skeletal remains concluded that they belonged to a young female between the 22 -24 years of age with a relatively low stature – between 153 and 159cm.

After careful observation of the green stained parts of the skull and comparison with similar examples found in various textbooks, it was concluded that the staining was caused by copper corrosion.

An other observed feature is that the stained parts of the bones seem to be smoother then the rest.

Discussion - Conclusions

In the presented case, the feature that has caused the most amount of speculation was the copper staining on the mandible and the mastoid process. Other authors attribute such stains to copper containing objects corroding near the bones. In the presented case due to the location of the stains, the object that caused them could be a piece of jewelry, like an earring. If that is the case, this would give cause to speculate about the age in which this woman lived in, since copper and its alloys are rarely used in modern jewelry.

Color variation of the corrosion products can be observed resulting from the environment and type of copper alloy⁵. The common green coloration observed on bone is the result of emerald/dark-green malachite, basic copper (II) carbonate ($\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$).

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THE THORACO-ABDOMINAL INJURIES PATTERN OF DRIVERS AND FRONT-SEAT PASSENGERS IN FATAL CAR CRASHES IN CLUJ COUNTY FROM 2016-2018

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Key words: thoraco-abdominal lesions, autopsy findings, car crashes, vehicle occupants.

Backgrounds and aims: Being in the second and third places, after the cranio-cerebral traumas, as causes of death in car crashes, thoraco-abdominal traumatic injuries associated with the death on spot, represent a challenge for forensic doctors both by the lesional polymorphism, but also by the multitude of their production mechanisms. Known by many authors as „la place du mort”, due to its positioning, the front-seat passenger presents a similar lesion pattern as the driver, but of different intensity. The purpose of this study is to discover a lesion pattern specific to the front-seat passenger, respectively the driver, but also to verify the hypothesis regarding the severity of the front-seat passenger's injuries.

Results: from 52 cases, 35 were represented by drivers and 17 by front-seat passengers. By demographic and epidemiological criteria (figure nr.1), all of the drivers were males and, in the front-seat passenger's category 5 were females and 12 males. The most affected age group in both categories was the age group 41-50 years one, followed by the age group 21-30 years. When it comes to the living environment, the victims from the urban environment were more affected.

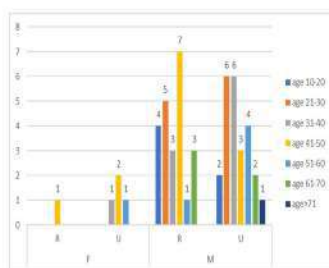


Figure nr. 1: The correlation between age, origin and gender.

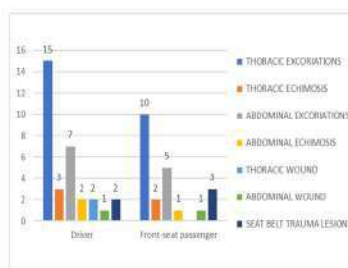


Figure nr. 2: External traumatic lesions and vehicle occupants.

Conclusions: Our results are in accordance with other international findings and it shows that:

- The male sex is the most affected (predominantly among drivers).
- The most affected age category is the 21-50 year one, in all categories the predominance is recorded in the 41-50 age group.
- Front-seat passengers present fractures of the sternum, ribs and hemothorax more frequently than drivers.
- Ruptures and contusions of the thoracic viscera (lung, heart) are more common in drivers than front-seat passenger.
- Ruptures of the portion of the aorta according to frequency: Descending aorta > Aortic arch > Ascending aorta > Abdominal Ao.
- Drivers present traumatic abdominal injuries more frequently compared to front-seat passengers.

Methods: This study is a retrospective one and involves the analysis and identification of thoraco-abdominal traumatic injuries from the autopsy reports between the years 2016-2018 from the archive of the Institute of Legal Medicine Cluj-Napoca. The following selection criteria were considered: road accident involving a vehicle, the passenger's place in the car (driver, front seat passenger), death on the spot and thoraco-abdominal traumatic lesions.

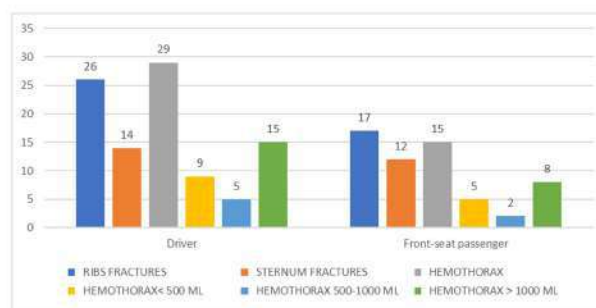


Figure nr. 3: The correlation between sternum, ribs fractures, the presence of hemothorax and the quantity of hemothorax.

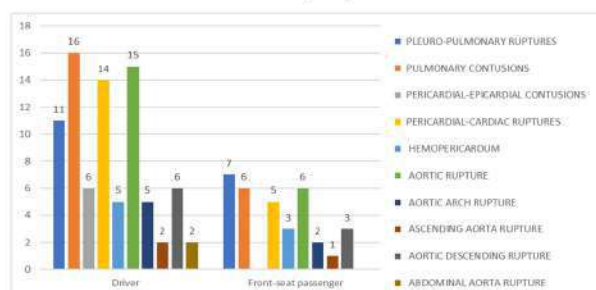


Figure nr. 4: The correlation between the traumatic injuries of the thoracic viscera and vehicle occupants.

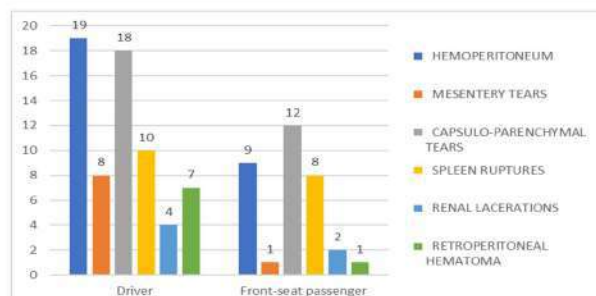


Figure nr. 5: The correlation between the traumatic injuries of the abdominal viscera and vehicle occupants.

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AUTOPSY TRENDS IN SPLIT-DALMATIA COUNTY FROM 2014.-2023.

Danijela Božinović-Karauz, Kristijan Bečić, Marija Definis

Background and aims: The aim was to prove the change in the number of autopsies from January 2014. to December 2023.

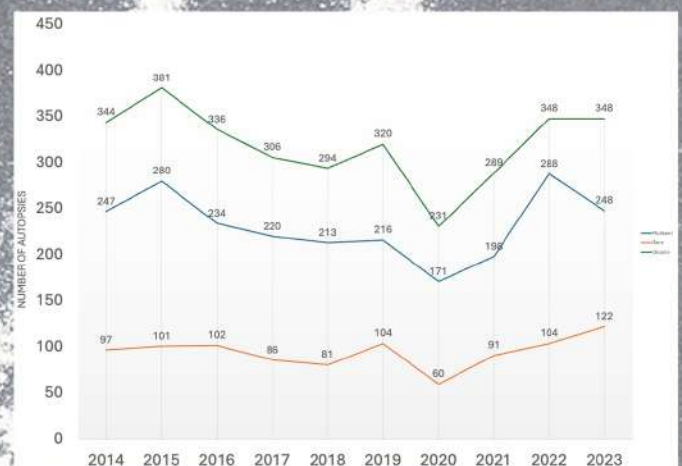
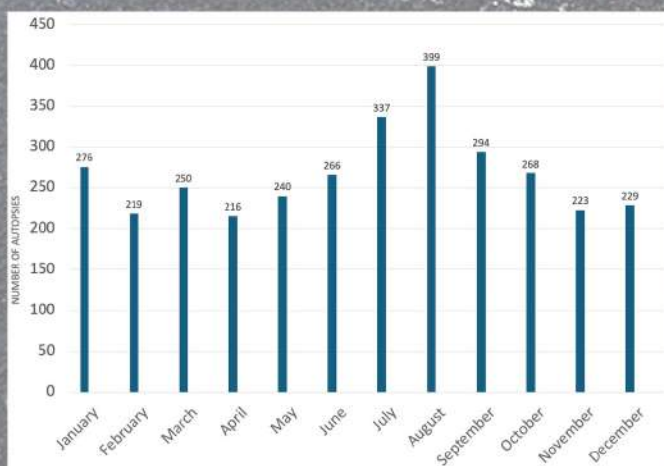
Materials and Methods: 3248 autopsy reports were analyzed in Split University Hospital, Departments of Forensic Medicine and Pathology. All the existing clinical research guidelines as well as Helsinki Declaration and GDPR regulations prescribed by the law in Croatia were followed. Descriptive statistics was used to analyze the data.

Results: In the period from 2014 to 2023, the year with the biggest number of autopsies was 2015. (385), and the smallest number was observed in 2020. (234). After 2015., the declining trend was evident with the new rise in the 2021. and the following years. The largest average number of autopsies was in August. Out of the total number, there were 2249 males and 944 females making it a ratio of 2,38 to 1. The age group with the most autopsies was above 65 years and the least were in the age group 0-17 years. When analyzing the cause of death, 2133 deaths were of natural causes, 1028 were unnatural and 87 deaths were undetermined. Violent deaths were further divided in three subgroups, murders, suicides and accidents. The accidents were the leading category with 776 deaths out of total 1028.

Discussion: Results correlated with the already existing literature. The number of autopsies declined from 2015. and the new rise could be explained with the post-COVID years. When comparing the month with largest number of autopsies, the previous studies found correlation between extreme weather and death toll. Considering hot weather and increased number of people in the analyzed county during summer months, the rise in numbers in August and July is understandable. Reduce in numbers of violent deaths is also observed.

Year	Forensic autopsies	Pathology autopsies	Total
2014.	187	157	344
2015.	198	187	385
2016.	202	135	337
2017.	202	112	314
2018.	194	104	298
2019.	222	100	322
2020.	154	80	234
2021.	191	99	290
2022.	230	123	353
2023.	291	80	371

Year	Natural deaths	Violent deaths	Unclear
2014	254	83	7
2015	266	107	12
2016	234	99	4
2017	207	95	12
2018	184	107	7
2019	195	119	8
2020	134	88	12
2021	188	97	5
2022	238	105	10
2023	233	128	10



Year	Homicides	Suicides	Accidents
2014	5	15	63
2015	9	15	83
2016	8	24	67
2017	7	19	69
2018	7	20	80
2019	11	11	97
2020	14	16	58
2021	6	24	67
2022	6	15	84
2023	5	15	108



MODAFINIL – TRENDING NOOTROPIC AMONG STUDENTS IN BULGARIA – CASE REPORT



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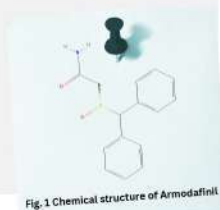
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INTRODUCTION

Modafinil is a nootropic drug registered in Bulgaria in 2011 under the brand name Aspandos®. It is indicated for the treatment of narcolepsy.

In recent years, modafinil and the enantiopure drug armodafinil - 2-[(diphenylmethyl)sulfinyl]acetamide (Fig. 1) are gaining popularity among university students especially those in the medical field. They are used as cognitive enhancers and stimulants with a low potential for abuse and dependence. [1]

Even though only one prescription drug is available in the Bulgarian pharmaceutical market, there are a lot of options to buy modafinil online. This creates a high risk of improper intake by patients with different diseases and medical conditions. Physicians should be familiar with this trend and stay vigilant.



CASE DESCRIPTION

A previously healthy 20-year-old male, first-year medical student, presented to the emergency department (ED) with altered mental status and psychomotor agitation. A few hours earlier he was found unresponsive but conscious by his parents who contacted the emergency medical service. Due to the risk of self-harm during the transportation, the patient received 10 mg of Diazepam intramuscularly.

In the ED the patient remained nonspeaking, noninformative, noncompliant and aggressive. After the initial examination (Fig. 2) was done, he was sedated with benzodiazepines, and further investigation was carried out. According to the parents the patient had no medical history and no prescriptions, but he would occasionally take armodafinil for studying (no certain dose was known) and he lost more than 13 kg in the last couple of months while being on a heavy ketogenic diet.

The diagnostic evaluation showed hypoglycemia, ketoacidosis and hyperammonemia. The toxicology screen confirmed presence of modafinil and metabolites in blood and urine samples. No other significant xenobiotics were found. The CT scan came back negative.

The patient was admitted to the Intensive Care Unit. He was started on continuous sedation with midazolam, rehydration, and nonspecific neuroprotective and hepatoprotective therapy. Hemodialysis was performed (two sessions in total). Exacerbation of the mental status led to intubation and artificial ventilation for 4 days.

During a psychiatric evaluation on the 5th day, the patient shared that he used armodafinil 1000 mg daily for a month. His mental status fluctuated in the following days with reported hallucinations. The patient was started on Risperidone 2 mg daily. MRT was done with a negative result.

The patient was discharged on the 14th day and was referred to a psychiatrist for further treatment and observation.

DISCUSSION

- There are few cases reported in the literature of modafinil-related psychosis after acute ingestion or prolonged use. [2,3,4,5]
- Daily doses of up to 250 mg of Armodafinil are said to be well-tolerated by naïve patients. [6]
- Effects of supratherapeutic exposures of modafinil are suggested to be minor and significant toxicity occurs rarely according to a retrospective study by Carstairs et al. The most common clinical effects included tachycardia, agitation, anxiety, headache, and hypertension. Hallucinations were reported in only 2,3% of the cases. [7]
- The hyperammonemia in the patient had no clear etiology. Non-hepatic and toxic origin was considered. No congenital disorders and enzymatic defects were researched in this case. [8]
- The non-diabetic ketoacidosis was assumed to derive from the low-carbohydrate diet of the patient. [9]
- Extracorporeal treatment was decided on due to the lack of specific therapeutical options for hyperammonemia, the presented ketoacidosis, and the fact that no plasma concentration of armodafinil could be acquired at the time.
- The complicated paraclinical constellation and the fluctuating mental disturbances observed throughout the hospitalization led to a 14-day long stay in an intensive care unit with regular psychiatric evaluations. Risperidone was the drug of choice although acute psychosis wasn't diagnostically confirmed.

CONCLUSIONS

There is a lack of current data about the use of modafinil and armodafinil in Bulgaria and Europe. More studies focused on their abuse potential are needed (European Medicines Agency recommends restricting the use of modafinil for treatment of narcolepsy only).

There are many readily accessible unregistered sellers online that create a real health risk, and they don't present enough scientific and medical information about the drugs. New regulations should be considered.

The acute illness of previously healthy young adults with no traumatic history requires the inclusion of toxicology testing and consulting in the diagnostic process. Thus more efforts should be put into the development of the National Poison Control Centre in Bulgaria.

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GCS - E(4)V(1)M(5)
BP - 140/90 mmHg
HR - 113 b/min
SpO2 - 98%
BT - 36.7°C

Table 1. Blood test results

pH	7.253	Acetone	1091 µg/ml*
pO2	141.0 mmHg	Ammonia	189.7 µmol/L (18-72)
pCO2	12.9 mmHg	ASAT/ALAT	68.5/33.8 U/L
HCO3S	11.8 mmol/L	Glucose	2.86 mmol/L
BE	-19.0 mmol/L	Creatinine	94.0 mmol/L

*toxic range of acetone plasma concentration - 100 - 400 µg/ml

Fig. 2 Initial examination

UNSEEN DANGERS: A FATAL CASE OF POSTPARTUM PSYCHOLOGICAL DISTRESS WITHOUT DIAGNOSED DEPRESSION

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¹Institute of Forensic Medicine, Medical Faculty, University of Ljubljana

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BACKGROUND AND AIMS

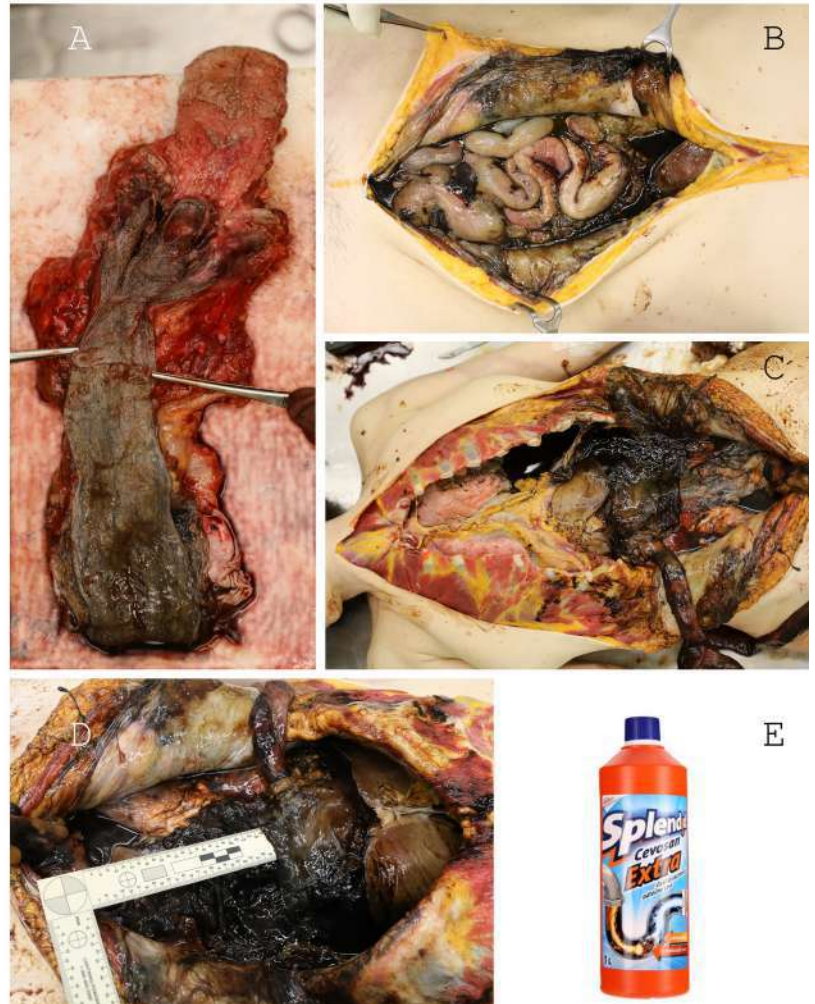
Suicidal ideation during pregnancy and after childbirth is a significant public health concern, prevalent in both industrialized and developing nations. While suicide attempts during this period are rare, the repercussions are severe for both the mother and the fetus/infant. This case report aims to highlight the critical need for awareness and early intervention in postpartum psychological distress, even in the absence of diagnosed postpartum depression.

METHODS

We present a case of a 34-year-old woman who died by suicide six months postpartum by ingesting 200 mL of sodium hydroxide (NaOH). Clinical history was obtained through family interviews, and autopsy findings were analyzed to understand the extent of internal injuries caused by the caustic substance.

RESULTS

The patient was presented restless and aphasic at the Emergency department with a progressive lactacidosis. Her husband reported no history of postpartum depression but noted low mood and migraine attacks coinciding with her recent menstrual period. The patient was discovered vomiting blood and in severe abdominal pain with a nearby bottle of NaOH. Despite comprehensive medical treatment, including an attempt of endoscopic gastric lavage that was not successful due to an extensive oedema of the esophagus, she died of sepsis the same day. The autopsy revealed extensive internal damage, including 700 mL of black fluid in the abdomen, bilateral pleural effusions with a perforation of the left hemidiaphragm by the caustic fluid, ulcerations of the esophageal mucosa, gangrene of the stomach, and diffuse hemorrhagic damage to the gastrointestinal tract.



The extent of caustic damage seen at autopsy with diffuse erosions and ulcerations of the mucosa of the tongue, the pharynx and the esophagus (A) with diffuse acute peritonitis (B) and necrosis of the left hemidiaphragm with perforation of the caustic fluid into the left hemithorax (C). The cause of peritonitis was perforation of the gangrenotic wall of the stomach after ingestion of the drain cleaner containing sodium hydroxide (E).

CONCLUSION

This case underscores the severe and fatal outcomes of postpartum psychological distress that may go undetected in the absence of overt postpartum depression. It highlights the importance of vigilance and timely intervention in postpartum women, particularly for those experiencing mood disturbances.



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THE USE OF FACIAL RECOGNITION AS URBAN CRIME'S PREVENTION: POSSIBLE USES AND CRITICALITIES IN THE ITALIAN CONTEXT

Christian Pallante

Background and aims: In recent times, crime in urban areas is increasing more and more exponentially. Whereas at first, national policies were concerned with the repression of crime, today the action is directed towards its prevention and neutralisation.

Therefore, new tools are emerging to help the police in this delicate task, including facial recognition, i.e. the set of AI applications that allow to perform analyses on the biometric features of a person's face and compare them with the subjects' features entered in a database, as well as to store them.

There are many critical issues, especially the lack of legislation to reconcile security requirements and fundamental rights, which lead to the opacity of the tool.

Methods: Analysis of EU, national and local regulatory sources and of practice to understand the state of the art.

Results: The analysis of regulatory sources shows that the current national legislative landscape does not offer guarantees for the use of facial recognition.

Although part of Italian doctrine has attempted to fill the legislative gap with systemic interpretations, this solution does not seem very feasible. Precisely on the front of new technologies - especially in the case of facial recognition - there appears to be a lack of a specific law perimitting their use.

Hence, there is a clear need to provide a regulatory provision fixing the an and quomodo of the use of these modern tools.

Discussion - Conclusions: The lack of a clear national legislative framework poses multiple problems for the circulation of this evidence in the EU context. In fact, the new Regulation modernising the Prüm System (Prüm II) has recently been approved, which seeks to provide common rules also for the exchange of face images, leaving room to national legislations. Only the drafting of specific legislation can fully guarantee the rights and freedoms involved.

Keywords: *Crime Prevention, Facial Recognition, Urban crimes, Prum Sistem, Italian Context*

Ammunition ingestion in a case of firearm suicide: A peculiar action instead of a note?

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INTRODUCTION

Suicide notes might have various contents, such as a farewell message or an explanation of the reason for the suicide and are thought to be common. However, they account for a minority, ranging between 3-45%. Furthermore, in suicides, bizarre findings can be observed, posing a controversial meaning for the forensic expert.

CASE PRESENTATION

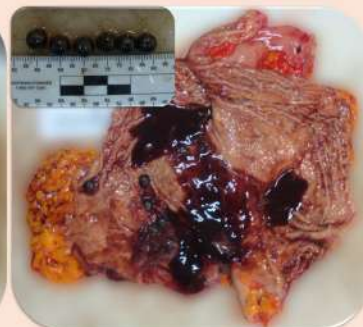
Scene investigation

- ✓ A 73-year-old man with a massive shotgun wound on the head.
- ✓ A superficial cut on the neck, and multiple cuts on the wrists and forearms (typical of self-inflicted injuries).
- ✓ Shotgun ammunition and used razors on a desk in front of the decedent.
- ✓ No suicide note. Suicide according to the police.



Postmortem examination

- ✓ A felted wad in the lumen of esophagus.
- ✓ Six lead pellets inside the stomach.



- ✓ Negative toxicological analysis.
- ✓ Cause of death: the gunshot wound.
- ✓ At first, it was alleged that he ingested ammunition as a suicide attempt by intoxication, but due to its presence in the upper gastrointestinal tract, we assumed that he was not expecting that.
- ✓ **Indication of his intention is a possible scenario.**

CONCLUSIONS

- ✓ This case demonstrates that peculiar actions may replace suicide notes, and their interpretation may be challenging.
- ✓ In suicide cases, multiple and various self-inflicted injuries can be observed (a sign of determination).
- ✓ A thorough postmortem examination should always be performed.
- ✓ Mental health disorders should be taken into consideration, even if they are not known or reported, as 90% of individuals who commit suicide have a psychiatric illness, expressed with complicated suicidal thoughts and related actions.

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ATTENTION: FENTANYL - ACUTE INTOXICATIONS

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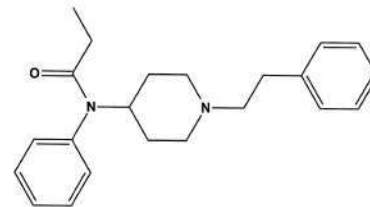
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BACKGROUND AND AIMS

Fentanyl was first synthesized in 1960 and after that became the most widely used synthetic opioid in medicine. In the recent years, there has been a steady increase of the number of acute intoxications with synthetic opioids, primarily fentanyl and its analogs. The rapid onset of poisoning requires timely administration of an antidote, since the Bulgarian citizens do not have access to a prevention programme for naloxone distribution to risk groups ("Take-home Naloxone"). This is a significant prerequisite for the large number of cases with pre-hospital mortality, which remain unidentified as to the cause of death. A special risk group are employees working with narcotic substances (police officers, customs officers, etc.).

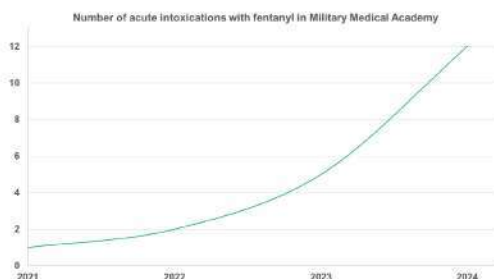


Fentanyl

METHODS



RESULTS



CASE #2

CASE HISTORY – 38 years old man, was found dead in his hotel room by staff. In addition, during the inspection of the crime scene, packages of medicines, syringes and aluminum foil with a brownish substance on it were found.

Sample	Immunoassay	GC/MS Screening	Quantitative analysis
Blood	BZD, FYL +/-	-	Fentanyl – 103.1 ng/mL Pregabalin – 0.92 µg/mL Diazepam – 0.03 µg/mL Nordazepam – 0.03 µg/mL
Urine	BZD, FYL +/-	Fentanyl, diazepam, pregabalin, paracetamol, caffeine	-
Aluminum foil	-	Fentanyl, paracetamol, caffeine	-

Fentanyl - [Therapeutic range: 0.3 – 300 ng/mL] [Comma-lethal range: > 3 ng/mL]

Pregabalin - [Therapeutic range: 2 – 8 µg/mL]

Diazepam - [Therapeutic range: 0.1 – 2.5 µg/mL]

Nordazepam - [Therapeutic range: 0.2 – 0.8 µg/mL]

Schulz M, Iversen-Bergmann S, Andresen H, Schmoldt A. Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics. Crit Care. 2012 Jul 26;16(4):R136. doi: 10.1186/cc11441.

CASE #1

MEDICAL HISTORY – 26 years old man, was admitted to the ER, brought by his friend. According to anamnestic data, he snorted white powdered drug. After 3-4 minutes, he became relaxed and lost consciousness. No accompanying diseases and/or allergies were reported. Inefficient vesicular respiration, cyanotic, SatO₂ 28%. The patient was intubated and switched to artificial medical ventilation in controlled regimens.

Biological sample	Fentanyl – 0 h	Fentanyl – 5 h	Fentanyl – 18 h
Blood concentration	4.2 ng/mL	1.8 ng/mL	0.9 ng/mL

[Therapeutic range: 0.3 – 300 ng/mL] [Comma-lethal range: > 3 ng/mL]

No other drugs were observed.

After 22 hours of treatment, the patient escaped from the hospital

CONCLUSION

The last report on Drug-induced deaths in Bulgaria (2022) shows that 92.3% of the cases are opioids' related. Thus, the analysis of biological samples for the presence of fentanyl (and other opioids) is crucial for the outcomes of the treatment of all poisoned patients and also for the needs of the forensic toxicology.

SUICIDE COMMITTED IN THE HOSPITAL ENVIRONMENT IN IASI COUNTY, ROMANIA. AN EPIDEMIOLOGICAL STUDY OVER A PERIOD OF 37 YEAR.

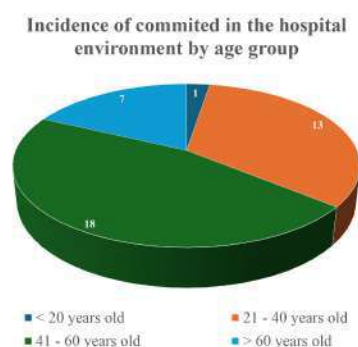
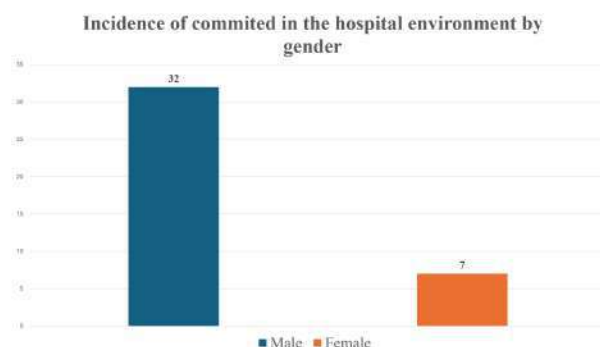
Authors: Scripcaru Andrei ^{1,2}, Scripcaru Veronica^{3,4}, Diac Mădălina^{1,2}, Hlescu Andreea^{1,2}, Scripcaru Călin ^{1,2}, Bulgaru-Iliescu Diana ^{1,2}

Affiliation: 1. Institute of Legal Medicine, Iași, Romania; 2. Forensic Medicine Sciences Department, University of Medicine and Pharmacy 'Grigore T. Popa', Iași, Romania; 3. Department of Morpho-functional Sciences I, University of Medicine and Pharmacy 'Grigore T. Popa' Iași, Romania; 4. Pathology Laboratory, Clinical Emergency Hospital 'Prof. Dr. N. Oblu' Iași, Romania.

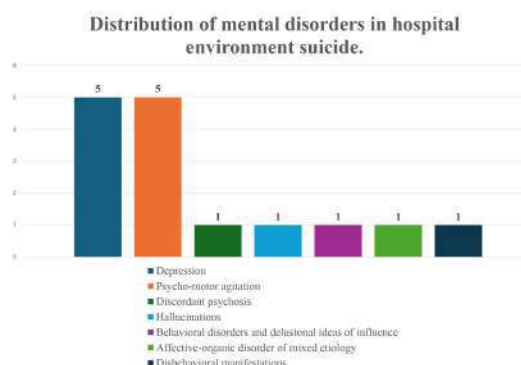
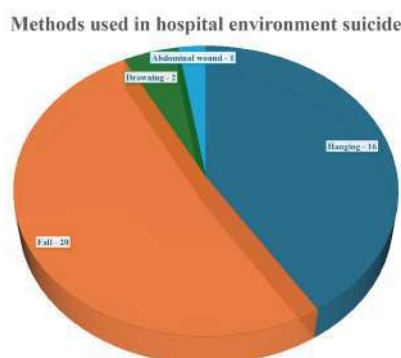
Background and aims: Suicide is a global health problem. The purpose of this study was to identify the deaths by suicide that occurred in the Hospitals of Iasi county.

Methods: The study was carried out through the retrospective analysis of all necropsied cases at the Institute of Forensic Medicine between 1982-2018 - over a period of 37 years, and the selection of suicide cases committed in the hospital environment. The obtained data were analyzed and processed statistically, descriptively.

Results: Our cohort consisted of 3696 suicide cases, of which 39 occurred in the hospital environment. 38 were patients and only one employee of the hospital. The incidence of suicide in the hospital environment was 4.5 times higher in males compared to females, with age ranged between 14 and 49 years.



The most used suicide methods were high-level falls (n=20) and hanging (n=16). The highest incidence was identified in the Socola Psychiatric Hospital, Iasi (n=16), followed by the Emergency Clinical Hospital "Prof. Dr. N. Oblu" Iasi (n=6) and the Emergency Hospital "Sf. Spiridon" Iasi (n=4). Of the 39 suicide cases, 5 presented depression, 5 psycho-motor agitation, one case with evolving discordant psychosis, one case with hallucinations, one case with behavioral disorders and delusional ideas of influence, one case with affective-organic disorder of mixed etiology and a case with disbehavioral manifestations. In 2 cases the patients presented neoplasias in terminal phases.



Conclusion: Patients hospitalized in neuro-psychiatric hospitals represented a group with a high risk of suicide, because the concentration of those with suicidal ideation and mood disorders is more evident here, and this can be a trigger for suicide.

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Domestic Violence On Children: A Forensic Point Of View

Radostina Miteva, MD, PhD

Department of General and Clinical Pathology, Forensic Medicine and Deontology,
Dermatovenereology, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria



Introduction



Source: <https://fajremind.com/tag/treatment/>

Domestic violence occurs globally and is a problem of major importance and significance. The reports of domestic violence show an increase in recent years. In 2023, the Bulgarian society was shaken by a case of a high-school student from the city of Stara Zagora, who reported that her intimate partner entered her home and severely beat her. This highlighted the need for gathering updated information on domestic violence on children. To this end, the aim of this study was to investigate the incidence of domestic violence on children in Bulgaria, utilizing voluntary surveys and patients examinations.

Methodology

This study was two-fold. We first conducted an anonymous voluntary survey among students in three high schools in 2023 using a questionnaire containing 142 questions (grouped in sexual, physical, and psychological categories, available upon request). A total of 164 students participated (137 completed surveys (83.5%) and 27 returned blank (16.5%)). We next analyzed the collected demographic data and results from 710 examinations (82 of which reported domestic violence) between 2021 and 2023 at the Forensic Medicine Department Office of University General Hospital "Prof. Dr. Stoyan Kirkovich" - Stara Zagora.



Source: <https://stock.adobe.com/images>

Results

Figure 1. A high rate of reported violence from the voluntary survey.

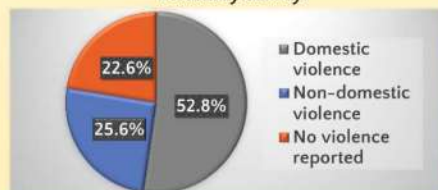


Figure 2. The prevalence of violence per category varies per victim gender.

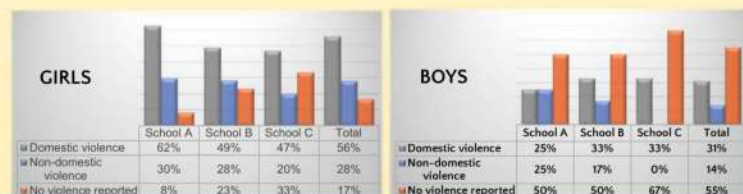


Figure 3. Prevalence of physical, psychological, and sexual violence.

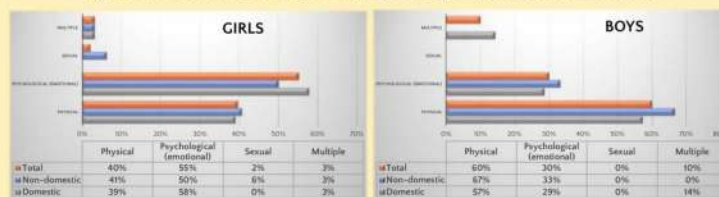


Figure 4. Distribution of violence based on perpetrator.

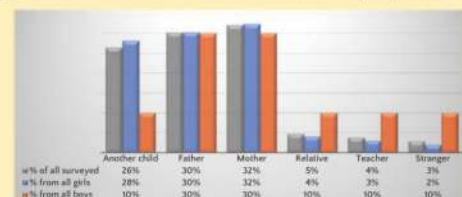


Figure 5. Distribution of age groups when violence occurred per survey self-reports.

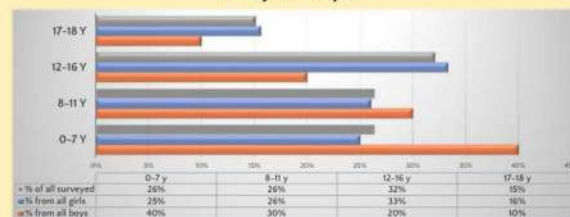


Table 1. Patients examined at the Forensic Medicine Department office and distribution of violence by gender and years.

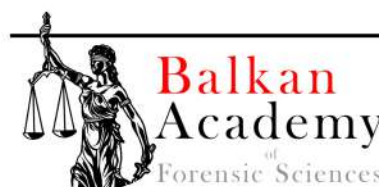
Year	Examined	With domestic violence	Female	Male	Children
		#	%	#	%
2021	249	29	11,7%	23	79,3%
2022	221	28	12,7%	20	71,4%
2023	240	25	10,4%	19	76%
Total	710	82	11,6%	62	75,6%

Conclusions

- Domestic violence in Bulgaria may be underreported as results from this survey (52.8% for domestic) show a higher prevalence compared to previous reports (47% for domestic + non-domestic).
- Physical violence occurs at a higher rate among boys and psychological (emotional) violence occurs at a higher rate among girls, but physical violence is still with high prevalence among girls.
- Boys reported significantly less incidents of violence.
- The highest rate of violence is received from a parent (no significant difference between father and mother), followed by another child.
- While violence rate among girls increased with age (until 16-year-old, then decreased), it consistently decreased among boys.
- Increased incidence of domestic violence on children was observed during 2021-2022 which may be related to pandemic quarantine.

Acknowledgements

The author gratefully acknowledges all colleagues from the Faculty of Medicine, the colleagues from the Forensic Medicine and Deontology Department of University General Hospital "Prof. Dr. Stoyan Kirkovich" - Stara Zagora, the high-school principals, and all students that participated in the survey.





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Introduction



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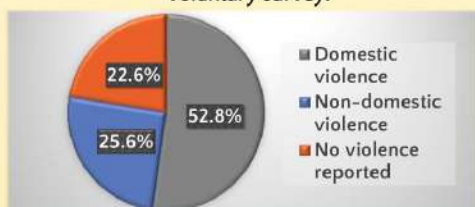


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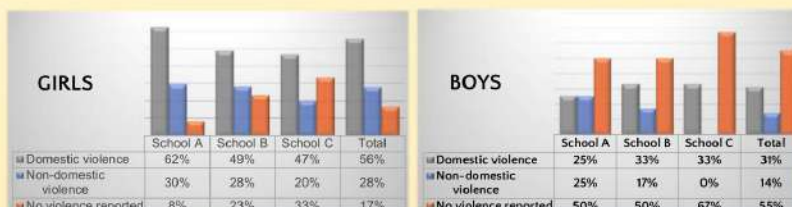


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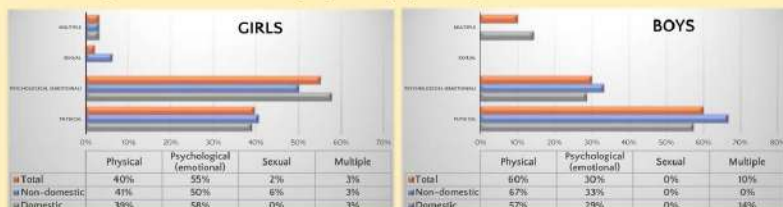


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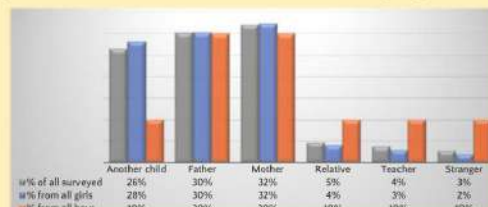


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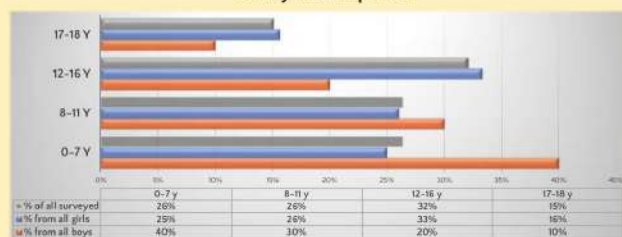


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Year	Examined	With domestic violence		Female		Male		Children	
		#	%	#	%	#	%	#	%
2021	249	29	11,7%	23	79,3%	2	6,9%	4	13,8%
2022	221	28	12,7%	20	71,4%	1	3,6%	7	25%
2023	240	25	10,4%	19	76%	4	16%	2	8%
Total	710	82	11,6%	62	75,6%	7	8,5%	13	15,9%

Conclusions

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THE ROLE OF SPECTRAL ANALYSIS - THE MODERN METHOD OF FORENSIC RESEARCH

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Background and aim: Spectral analysis is an essential tool in forensic medicine, providing precise information about the chemical composition of the analyzed materials and significantly contributing to the investigation of crimes. It facilitates the identification of toxic substances and the analysis of biological samples, such as blood, tissues, drugs, and poisons, with the aim of improving the quality of forensic expertise.

Methods: Spectral analysis employs several techniques, including mass spectrometry (MS), Fourier transform infrared spectroscopy (FTIR), ultraviolet and visible spectroscopy (UV-Vis), Raman spectroscopy, as well as hyperspectral analysis methods. These techniques allow for the identification and quantification of substances in complex samples. Additionally, recent technological advancements have increased the accessibility and efficiency of these methods, providing modern tools, including portable spectrometers, that can be used directly at crime scenes.



Figure 1: Liquid Chromatography Mass Spectrometry (LC-MS)



Figure 2: Fourier Transform Infrared Spectroscopy



Figure 3: Varian Cary 100 Bio UV-Visible Spectrophotometer



Figure 4: Handheld Raman Spectrometer

Results: Studies have demonstrated the effectiveness of spectral analysis in identifying drugs, toxins, and changes in biological tissues. For instance, mass spectrometry has been used to accurately identify toxic substances in the blood samples of overdose victims. Raman spectroscopy has provided rapid solutions for analyzing traces of accomplices at crime scenes, such as drugs or explosives. Furthermore, the integration of spectral analysis with other analytical techniques, such as chromatography, has led to improved accuracy and reliability.

Conclusions: Spectral analysis plays a critical role in forensic medicine, offering high precision, speed, and a non-destructive nature. However, there are limitations related to the cost of equipment, the need for trained personnel, and potential errors in result interpretation. The implementation of portable spectrometers and their integration with other analytical methods are recommended to enhance the efficiency of examinations and improve the quality of the obtained data. Additionally, future research should focus on the development of new biomarkers and the standardization of spectral analysis methods.

Retained intracerebral shrapnel following a bomb explosion – case report

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INTRODUCTION

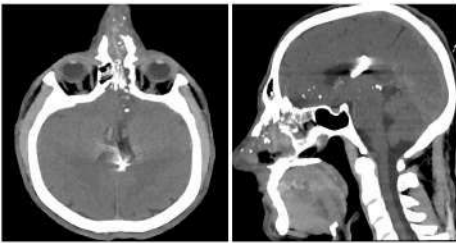
Traumatic brain injury (TBI) represents an increasing source of morbidity and mortality around the world. In the United States in 2020, there were approximately 214,110 TBI-related hospitalizations. In 2021, there were 59,473 TBI-related deaths¹.

Penetrating head trauma, characterized by the *breach of the skull by a projectile* (bullet, knife, other objects) that *enters the cranial cavity but does not exit*, are classified as a *severe form of TBI*^{2,3}. This report details a unique case involving such an injury.

CASE PRESENTATION

A 58-year-old male was admitted to the hospital following a bomb explosion in Africa, which occurred 48 hours prior to his admission. He had received initial medical care and undergone a CT scan revealing:

- ❖ fractures of the nasal pyramid and anterior cranial fossa;
- ❖ bilateral ethmoid-fronto-maxillary hemisinos;
- ❖ cerebral contusions;
- ❖ minimal intraventricular hemorrhage;
- ❖ and a metallic shrapnel lodged in the left frontal cerebral lobe.



Figures 1 & 2 – axial and sagittal CT scans showing the trajectory of the metallic shrapnel through the cranio-cerebral structures

Upon admission, he exhibited no neurological deficits (GCS 15) but presented with bilateral hearing loss, tinnitus, and anosmia. Examination revealed a perforated eardrum on both sides and a perforated nasal septum. **Relatives reported that the patient displayed behavioral changes indicative of frontal lobe syndrome.**

By the third day of admission, he developed a *persistent headache* and *rhinoliquorrhea*; however, a CT scan showed no changes in the lesions and normal positioning of midline structures. Two days later, a procedure was performed to repair the anterior cranial fossa and address the fistula, with no complications noted post-surgery.

The following morning, after standing up voluntarily, against medical advice, the patient experienced vertigo, dyspnea, and rapid desaturation, ultimately leading to cardiac arrest that did not respond to resuscitation efforts.

AUTOPSY FINDINGS

A penetrating shrapnel wound with:

- an entry point at the nasal level
- created a channel through the soft tissues of the nose, the naso-orbito-ethmoidal complex, and the fronto-basal region of the brain;
- there were also comminuted fractures of the anterior cranial fossa and nasal pyramid
- along with cerebral hemorrhage and laceration.

DISCUSSION AND CONCLUSIONS

The skull, orbits, nasal and oral cavities, can serve as entry points for foreign objects. Significant external force—such as that from a nail gun, reinforcing bar, etc.—is required for transcranial insertion. However, when it comes to penetrating the cavities, softer foreign objects like chopsticks or plants can penetrate with only slight external force.

In TBI cases involving the nasal cavity, there's a risk of shrapnel migration into the cranial cavity, potentially resulting in retained foreign bodies within the brain. While cranio-cerebral trauma is common in medical practice, it is also crucial to consider the patient's psycho-behavioral changes in managing such cases, alongside the morphology and topography of the lesions^{2,3}.



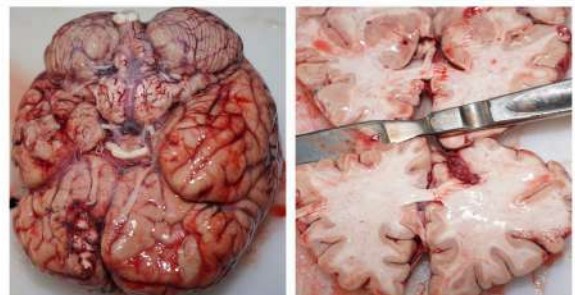
Figures 3 & 4 – the nasal entry point of the shrapnel, soft tissue laceration, comminuted fracture of the nasal pyramid



Figures 5 & 6 – 3D reconstruction of the cranium showing the comminuted fracture of the nasal pyramid, affecting the nasal bone itself, the medial walls of the orbits bilaterally



Figure 7 – comminuted fracture of the anterior cranial fossa; channel of shrapnel showed with a probe through the naso-orbito-ethmoidal complex (oblique direction from antero-inferior to postero-superior, making an angle of ~35° with the horizontal);



Figures 8 & 9 – channel through the fronto-basal region of the brain, cerebral hemorrhage and laceration

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**Balkan
Academy
of
Forensic Sciences**

FORENSIC NUCLEAR MEDICINE

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INTRODUCTION:

It is essential to distinguish between Forensic Nuclear Medicine and Nuclear Forensics. The second uses nuclear techniques to analyze samples related to crimes from drug trafficking to murders to art forgeries or others. While Forensic Nuclear Medicine investigates human body organs using radionuclides. Anatomical imaging may not reveal the whole picture of pathology incurred to a person. Here comes to help functional imaging represented by Nuclear Medicine.

SKELETAL TRAUMA:

Radioisotope bone scan has a significant role in investigating bone traumas, especially when multiple fractures are suspected, such as in child abuse or torture, as X-ray bone survey is time consuming and exposes the patient to high radiation dose.

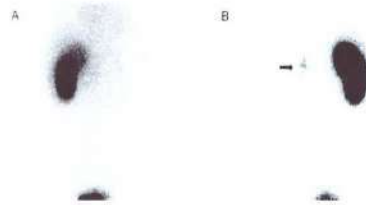


The image above shows

increased activity in the Rt 4th to 7th ribs posteriorly, due to rib fractures after compressive squeezing applied to the chest.

ABDOMINAL TRAUMA:

Blunt traumas to the abdomen may cause injuries to the internal organs like kidneys, which cannot be detected by anatomical imaging, while nuclear scintigraphy will show it clearly.



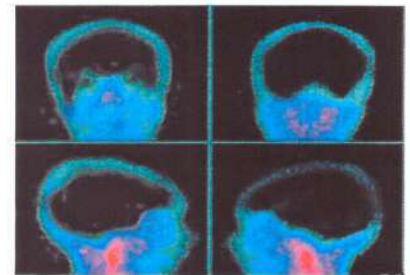
Anterior and posterior static renal images performed 2 months after blunt abdominal injury showed minimal activity of the Lt kidney posteriorly.



Hepatobiliary images of a 7-years old girl 3 days after a blunt abdominal trauma extrahepatic accumulation of tracer originating from the tip of the Lt lobe.

BRAIN DEATH:

Diagnosing clinical death is essential in organ transplants like cardiac, here nuclear medicine comes to help diagnose brain death, so the heart can be transplanted to a receptor.



Brain Death detected in Nuclear Medicine Gold Standard in Accuracy, using the Radiopharmaceutical ECD (Colloidal Tin) following the legal order for the release of organ donation.

SUMMARY:

Those are only a few examples of the use of nuclear medicine imaging in Forensic Medicine.

Nuclear medicine imaging can be used when there is a suspicion in stress fractures and spinal fractures that may not be detected on morphological imaging.

It can be used when shattered kidney, liver, or spleen is suspected.

And in many other conditions.

WATERHOUSE – FRIDERICHSEN SYNDROME IN A PEDIATRIC PATIENT WITH STREPTOCOCCUS SALIVARIUS SEPSIS: A CASE REPORT

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INTRODUCTION

Waterhouse-Friderichsen Syndrome (WFS), commonly known as purpura fulminans, is a life-threatening condition characterized by *adrenal hemorrhage, disseminated intravascular coagulation, and septic shock*. While *Neisseria meningitidis* accounts for the majority of WFS cases, a variety of other bacterial and viral pathogens can also be implicated. Among them, *Streptococcus salivarius*, typically regarded as a nonpathogenic inhabitant of the oral and gastrointestinal mucosa, has been recognized in rare instances as a cause of bacteremia leading to severe clinical outcomes^{1,2,3}. This case report aims to highlight a *rare etiology of WFS* in an 11-month-old pediatric patient, attributed to *Streptococcus salivarius*.



CASE PRESENTATION

An 11-month-old patient was admitted with a *presumptive diagnosis of purpura fulminans following a rapid clinical deterioration*. The patient initially exhibited symptoms the evening before admission, including fever. By 7:00 a.m. on the day of admission, she presented with purpuric and petechial rashes. At the emergency department, her clinical presentation included:

- ❖ *high fever;*
- ❖ *generalized petechial-purpuric rash;*
- ❖ *cyanosis of extremities;*
- ❖ *saburral tongue;*
- ❖ *hyperemic pharynx, with tonsils showing a purplish hue.*

The patient's condition rapidly worsened, leading to decreased reactivity to stimuli, necessitating orotracheal intubation, mechanical ventilation, and transfer to the intensive care unit (ICU). Despite aggressive management—including fluid resuscitation, broad-spectrum antibiotics, hemodynamic support, and blood product transfusions—her clinical status did not improve. Over the subsequent two hours, she experienced three cardiac arrests, culminating in a final arrest that was unresponsive to resuscitation efforts.



AUTOPSY AND LABORATORY RESULTS

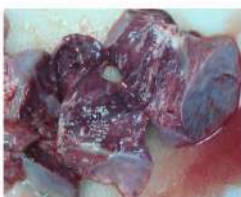
An autopsy was performed, revealing significant findings indicative of *severe infection and systemic failure*:

- *generalized petechial-purpuric rash;*
- *cyanosis of extremities;*
- *enlarged, erythematous palatine tonsils;*
- *pharyngitis;*
- *bilateral pneumonia;*
- *enlarged mediastinal and mesenteric lymph nodes;*
- *bilateral adrenal gland hemorrhage.*



Figure 1 – petechial-purpuric rash

✓ Bacteriological analysis of blood samples collected necroptically confirmed the presence of *Streptococcus salivarius*.



Figures 2 & 3 – bilateral pneumonia



Figure 4 – saburral tongue, pharyngitis

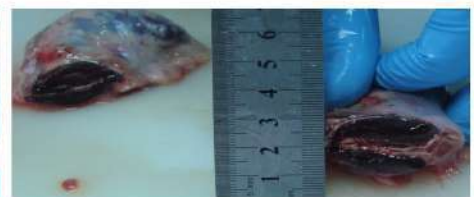


Figure 5 – bilateral adrenal gland hemorrhage



DISCUSSION - CONCLUSIONS

In the case presented above, the rapid clinical course and acute onset of symptoms underscored the urgency and severity of WFS, often making these cases subject to medico-legal scrutiny due to unexpected death in pediatric patients. By the time symptoms such as fever and hemorrhagic skin rashes are observed, it may be too late to save the child's life, due to coagulation abnormalities and extensive thrombosis in the microcirculation that lead to multiple organ dysfunction syndrome, along with adrenal hemorrhages that occur as a result of sepsis-associated disseminated intravascular coagulation. Further research and case studies are warranted to elucidate the mechanisms through which typically nonpathogenic organisms can precipitate such life-threatening conditions^{1,2,3}.

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