

CASE REPORT

The importance of the histopathological examination in establishing the diagnosis of delayed splenic rupture. Report of a case and literature review

CAMELIA LIANA BUHAŞ^{1,2)}, GABRIEL CONSTANTIN MIHALACHE^{1,2)}, CLAUDIA TEODORA JUDEA-PUŞTA^{1,2)}, LUCIA GEORGETA DAINA³⁾, GABRIELA MUŢIU¹⁾, BOGDAN ADRIAN BUHAŞ⁴⁾, AMORIN REMUS POPA⁵⁾, MARIA CLAUDIA JURCĂ⁶⁾, NICOLAE DUMITRU NICOARĂ¹⁾, ADRIAN MARIUS MAGHIAR⁷⁾

¹⁾Department of Morphological Disciplines, Faculty of Medicine and Pharmacy, University of Oradea, Romania

²⁾Forensic Service of Bihor County, Oradea, Romania

³⁾Department of Psycho-Neuroscience and Rehabilitation, Faculty of Medicine and Pharmacy, University of Oradea, Romania

⁴⁾Department of Urology, Emergency County Hospital, Oradea, Romania

⁵⁾Department of Medical Disciplines, Faculty of Medicine and Pharmacy, University of Oradea, Romania

⁶⁾Department of Preclinical Disciplines, Faculty of Medicine and Pharmacy, University of Oradea, Romania

⁷⁾Department of Surgical Disciplines, Faculty of Medicine and Pharmacy, University of Oradea, Romania

Abstract

In forensic activity, splenic ruptures occur frequently in cases involving abdominal trauma due to road traffic collision, strokes, falls and work accidents. Splenic lesions can occur either immediately after the trauma or within variable timeframes after it, the last scenario being the case of a delayed splenic rupture. Delayed splenic rupture is ranked third in abdominal traumatology frequency, after liver and intestine rupture. Delayed splenic rupture is more frequently the result of abdominal contusions or compressions, rather than the result of direct wounds located in the left flank. In the first stage, an intraparenchymatous hematoma is formed. The second stage occurs within a variable timeframe (between two days and one month), either spontaneously or following a second trauma, when the capsule ruptures leading to secondary peritoneal hemorrhage. In order to correctly determine the chronology of splenic lesions, histopathological (HP) investigations have an important role to play. The presence of hemosiderin highlighted by Hematoxylin–Eosin (HE) staining or special stainings, confirms the diagnosis of delayed splenic rupture. This study will present the case of a 55-year-old male from the countryside, a chronic alcohol consumer, animal care provider, who suffered multiple traumatic injuries four days prior to his death. He was found lying on the ground, presenting an open craniocerebral trauma (CCT) with a bleeding wound on the scalp. The autopsy showed CCT with meningeal hemorrhage and subdural hematoma, along with a splenic rupture presumably produced in two stages. The HP investigations did not reveal the presence of hemosiderin in the tested specimen samples. Using this scientific diagnostic criterion, it was thus confirmed that the splenic rupture occurred after the aggression, in another traumatic stage, the same one where the other thanatogenerator lesions occurred, as a result of the victim's collapse from the same level and hitting the planes with irregular surfaces.

Keywords: hemosiderin, Pearls staining, splenic rupture, delayed splenic rupture.

Introduction

Splenic rupture occurs frequently post-traumatically, rarely spontaneously [1], under pathological conditions, as a result of coughing, sneezing, vomiting, laughter, or even after examination of the abdominal region in patients suffering from malaria, tuberculosis located in the splenic area, Gaucher disease, leukemia, infectious mononucleosis, etc.; anamnestic data have an essential role in establishing the diagnosis along with the clinical examination and paraclinical investigations [2–6].

Post-traumatic splenic rupture is the result of blunt abdominal traumas of variable intensity that targets the left flank, in car accidents, work accidents, falls or injuries [7]. It is frequently encountered due to the anatomical position of the spleen in the abdominal region. It is sometimes accompanied by fractures of the bones that form the left coastal rib cage. Spleen lesion can produce post-traumatic shock immediately or within variable

timeframes after the trauma. In the last scenario, we are talking about delayed spleen rupture [8, 9].

Delayed splenic rupture was first described by Baudet, in 1907 [10]. It occurs most frequently as a result of variable intensity trauma due to abdominal contusions or compressions, and less frequently due to wounds affecting the spleen parenchyma [7, 9]. It is placed on the third place in frequency in abdominal traumatology, after the rupture the liver and intestine [1, 9]. It rarely occurs spontaneously, when it does underlying pathological conditions can be found as predisposing factors [1].

The frequency of delayed spleen rupture is reported in literature between 0.3% and 24% [10].

According to literature reviews, the pathophysiology of delayed spleen rupture is not fully elucidated. There are two theories: one theory argues that when the trauma occurs, there is a rupture in the splenic capsule and a clot is formed which is buffered by the neighboring organs, the rupture in the free abdominal cavity appearing at a

later stage; the second theory supports the formation of a sub-capsular (intraparenchymatous) hematoma, which subsequently breaks into the abdominal free cavity either spontaneously or after a second trauma, when the splenic capsule yields and the intraperitoneal hemorrhage occurs [11, 12].

The timeframe between the actual trauma and the time of the splenic rupture varies within fairly wide ranges, between two days and a month [8, 9].

Clinically, when the spleen capsule breaks, the signs and symptoms of severe abdominal pain occur. The diagnosis is confirmed by a computed tomography (CT) scan, which reveals hypo- or hyperdense outbreaks in the parenchyma, which do not increase after the administration of i.v. contrast substance, perisplenic and/or intraperitoneal collection, but not always the absence of imagistic lesions excludes a delayed spleen rupture [13]. For diagnosis, abdominal ultrasound, barium transit, irigoscopy, selective arteriography can be made to determine the topography and the extent of vascular arterial lesions and splenic scintigraphy that may reveal lacunae areas in the parenchyma [4, 5, 14–18].

In the case of delayed spleen rupture, the mortality rate is between 5–15%, while in the case of blunt spleen rupture this is 1% [3, 19, 20].

From forensic point of view, in the case of delayed post-traumatic rupture followed by death, it is important to establish the traumatic moment related both to the splenic capsule rupture and to death of the individual [7]. The chronology of events in forensic practice in such cases is difficult to establish, especially in the absence of clinical and paraclinical investigations prior to death [21, 22]. Histopathological (HP) investigations with common stainings [Hematoxylin–Eosin (HE) staining] and special stainings (*e.g.*, Pearls staining), which highlight hemosiderin [23–25] proved to have an important role. Its presence confirms HP diagnosis of delayed spleen rupture. It is thus possible to set the elapsed timeframe from the moment of the initial trauma up to the moment of the spleen rupture [22].

Aim

We present a particular case of post-traumatic splenic rupture. The specificity of the case is given by: the existence of multiple traumatic injuries four days before death; the absence of the traumatic mark in the abdominal region; atypical evolution after the initial traumatic moment, with the absence of specific acute surgical abdominal symptoms after the trauma until death; absence of clinical and paraclinical examinations of the victim prior to death; death in full apparent health status; mixed thanatogenerator mechanism established following necropsy, splenic rupture in a two-time mechanism being seen as one of the causes of death. Determining the age of splenic lesion and implicitly the causal/non-causal relationship between death – aggression could only be done with HP investigations.

Case presentation

N.M., 55-year-old, male, from countryside, animal care provider, alcoholic, no known medical history. On September 10, 2013, he suffered multiple traumas due

to an aggression, with minor injuries to his head, chest, upper and lower limbs. The following days, he continued to consume alcohol, look after the animals and accompany them to the pasture. He did not report thoraco-abdominal symptoms in relation to the trauma suffered and did not appear for clinical or paraclinical investigations.

On September 13, 2013, around 6 p.m., N.M. was found dead in his house, lying on the ground in a pool of blood, in a room furnished with wooden objects (bed, table, wardrobe), by another animal caretaker. His death was considered suspicious. Investigators requested a forensic autopsy to determine the cause and conditions of the death.

Forensic autopsy

Injuries indicating signs of violence identified at the external corpse examination: excoriation plaque covered with brown, thick crust, stretched over an area of about 6/1 cm, situated on a purple ecchymosed base in the left subscapular region; linear excoriation of about 12 cm long in the upper left arm on the back; 0.5/0.2 cm brown incision, with pink halo around, on the left elbow; wound with irregular edges in the form of an inverted T-shape, deep-looking, with sides of 1.5 cm and 2.5 cm in the left occipital region and on the right side another wound of 1.8 cm long, in the form of an arcuate line, vertically placed, slightly deep. Both wounds present 8/1.5 cm red-purple ecchymoses, with small excoriations at the upper and lateral poles, in the upper 1/3 of the right posterior thigh; purple ecchymosis at the left eye. The corpse did not show a traumatic mark in the left abdominal flank, in the lower 1/3 of the left anterior left chest or the left lumbar region.

Injuries indicating signs of violence found in the internal corpse examination: blood infiltration at the scalp region (corresponding to the plaques described in the external exam); a subdural hematoma consisting of fresh blood and a area of 1 cm thick violet blood clot of 7/5 cm, located fronto-temporo-parietal right; meningeal hemorrhage spread across the entire brain surface; cerebral edema; pulmonary anthrax; pulmonary emphysema; hemoperitoneum formed from about 700 mL fluid blood and blood clots, one of the large clots containing about one liter of blood located in the spleen vessel; rupture of the splenic capsule located near the splenic hilum with dimensions of about 1.3/0.5 cm; hepatic steatosis; left perirenal blood infiltration in the mesenteric region; anemic aspect of internal organs.

During the autopsy, organ fragments were collected for HP examination. HE staining was used. The HP examination revealed: brain with pericelular and perivascular edema; hemorrhage of meninges; lung with pulmonary emphysema; liver with hepatocytes with vacuolar dystrophy; kidney with periglomerular edema and hematic infiltration in the subcapsular region; spleen with hematic infiltrate in the parenchyma, rupture of the splenic capsule (Figures 1–6).

The autopsy and HP examination (HE staining) concluded: the death of the N.M. was due to mixed cardio-respiratory insufficiency, as consequence of internal hemorrhage of spleen rupture and a cerebrovascular trauma with compressive subdural hematoma. The main

thanatogenerator lesions were those generated from the spleen (delayed spleen rupture with massive hemoperitoneum) and from the cranio-cerebral level (meningeal hemorrhage, subdural hematoma, cerebral edema).

HP examinations using Pearls staining were made for diagnostic certainty.

Pearls staining in this case study, on pieces made from fragments taken from the spleen, denied a hemosiderosis-type pathology, hemochromatosis (negative Pearls reaction). It was also used for kidney fragments due to the left perirenal blood infiltrate found on autopsy. The result was negative as well (Figure 7, a-c).

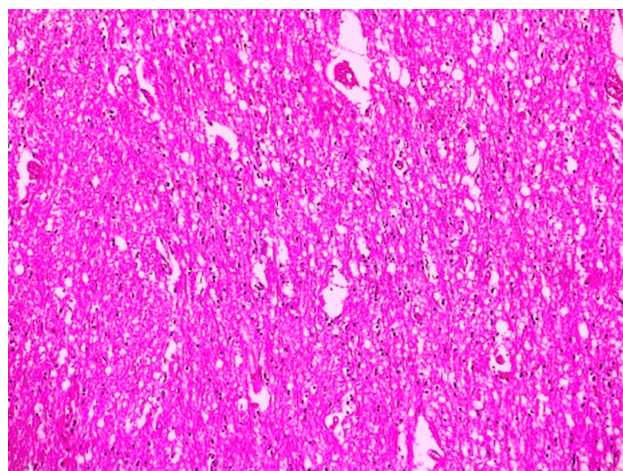


Figure 1 – Image showing an area of cerebral parenchyma affected by perineuronal and perivascular edema (HE staining, $\times 100$).

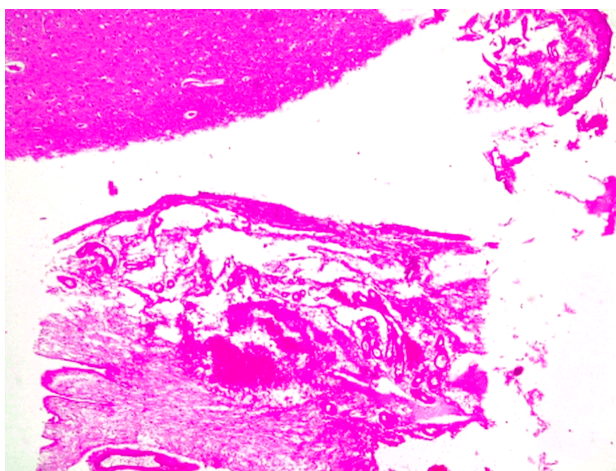


Figure 2 – Image showing an area of meninges suffering of meningeal hemorrhage (HE staining, $\times 100$).

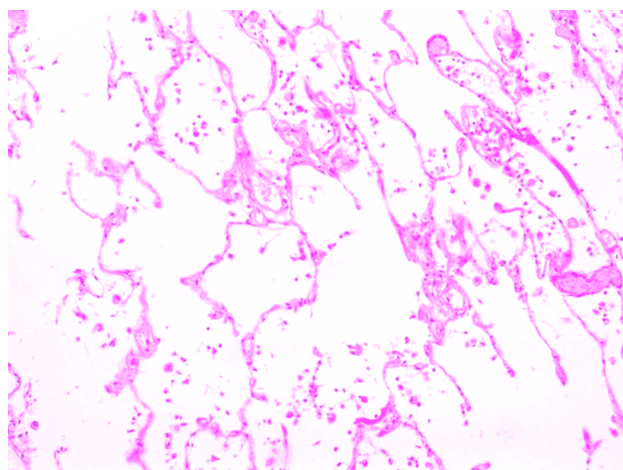


Figure 3 – Image showing an area of lung parenchyma with pulmonary emphysema (HE staining, $\times 40$).

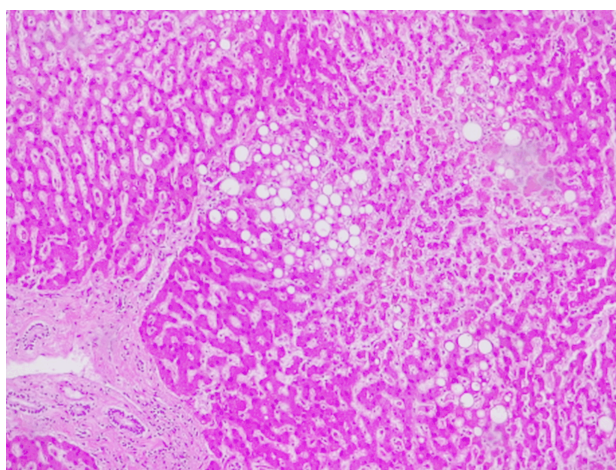


Figure 4 – Image of the liver with hepatocytes affected by vacuolar dystrophy (HE staining, $\times 100$).

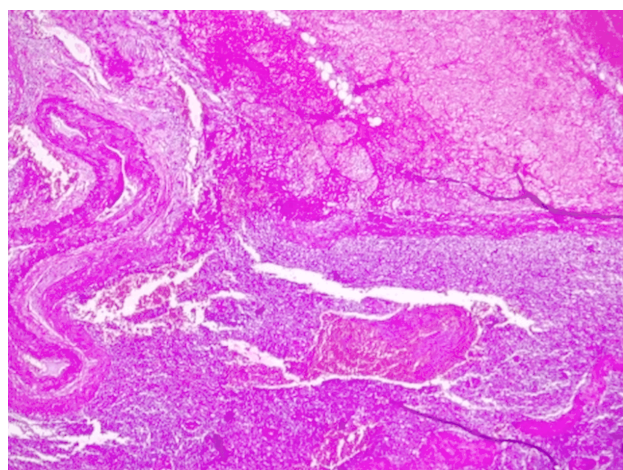


Figure 5 – Image showing an area of the spleen with zones of hematic infiltration localized in the parenchyma (HE staining, $\times 20$).

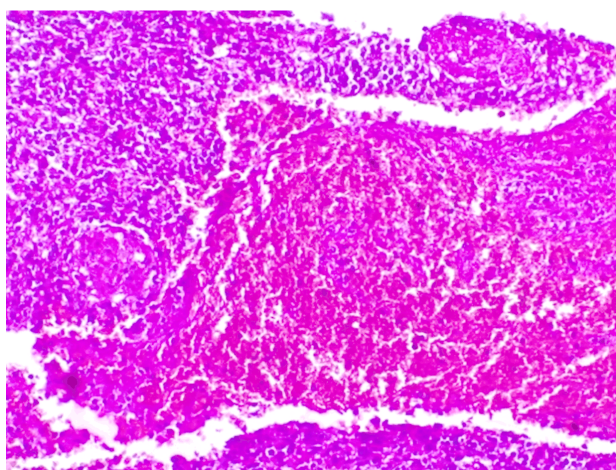


Figure 6 – Image showing an area of the spleen with zones of hematic infiltration in the parenchyma (HE staining, $\times 40$).

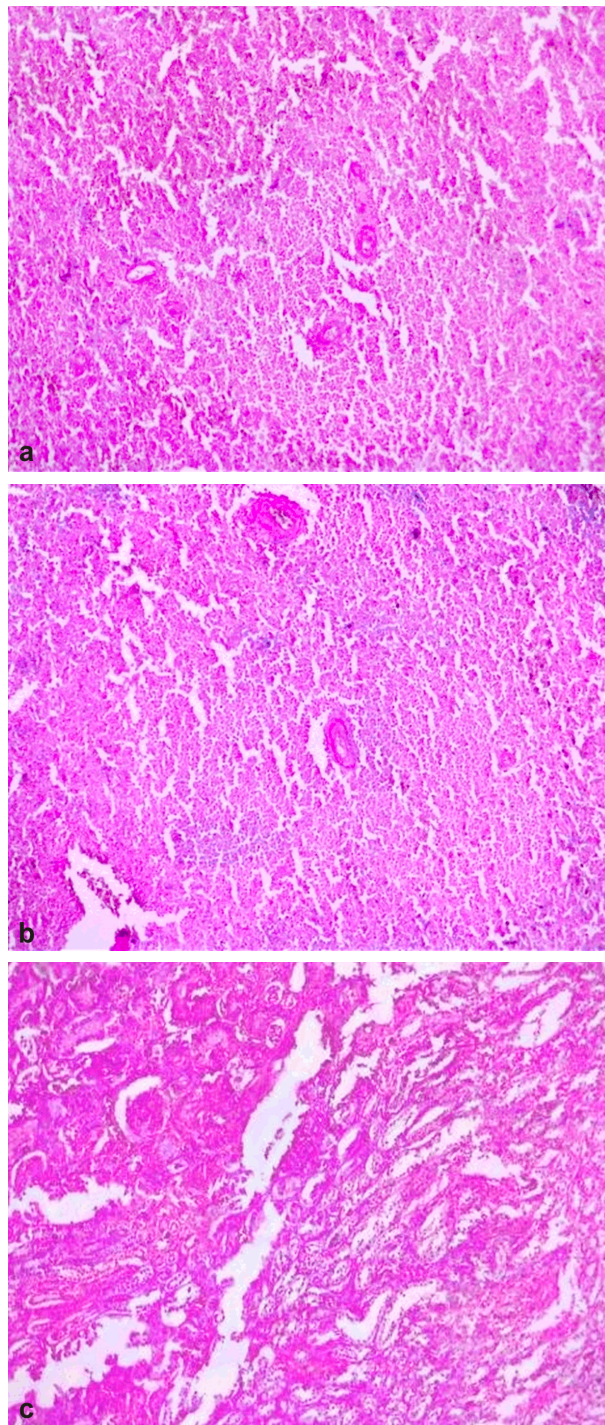


Figure 7 – (a–c) Images showing areas of the spleen: negative Papanicolaou reaction. Papanicolaou staining: (a and c) $\times 100$; (b) $\times 200$.

Discussions

Following the autopsy and HP examination of organ fragments from the corpse, where HE staining method was used, it was not possible to tell with certainty whether the lesions that caused the death were the result of the aggression from September 10, 2013. It thus raises questions upon the diagnosis of delayed splenic rupture.

The HE staining confirmed the hematic infiltrate in the splenic parenchyma and rupture of the capsule but did not specify the age of the hematic infiltrate, *i.e.*, whether

it was a delayed splenic rupture and the consequence of the aggression from September 10, 2013.

Due to these uncertainties, the investigators have requested a forensic expertise to clarify whether or not there is a causal link between the actual death and the aggression from September 10, 2013. It had to be established if there was only one traumatic moment or multiple ones for the two large thanatogenic lesions and if there were different traumatic moments, whether the spleen rupture was or not a result of the trauma from September 10, 2013. The case was reassessed. In such cases (uncertainties, incomplete diagnosis, etc.), the investigation data, autopsy findings, HP investigations, literature reviews [9, 22, 24–27] are revised. The purpose of re-evaluating the case was to state when the lesion involving the spleen actually took place.

According to the literature reviews, this most probably occurred on the 13th day after the trauma, on a two days and 30 days post-traumatic timeframe [19, 20]. In the case presented, the individual suffered multiple traumatic injuries four days before death, thus falling within the timeframe mentioned in the literature reviews. This justifies the re-evaluation of the initial HP examination and additional HP investigations to determine when the hematic infiltrate in the splenic parenchyma occurred.

Literature reviews state also that clinically, in the case of delayed splenic rupture, after a syncopal state with nausea and vomiting or even acute abdomen symptoms, the patient recovers almost completely in the timeframe from when the initial trauma and dramatic symptomatology given by the massive hemoperitoneum with hypovolemic shock occurred, interposing a latency period in which a number of clinical signs persist: a degree of paleness, tachycardia, tendency to transient loss of consciousness, 38°C fever, spontaneous pain and palpation in the left hypochondrium, nausea, vomiting, abdominal meteorism, chest pain or acute pain in the upper left abdomen [11]. The physiopathological substrate responsible for this frustrating symptomatology may be: secondary rupture of an intrasplenic hematoma, delayed rupture of a perisplenic hematoma initially blocked by adhesions around the organ, or secondary occlusion of the clot obstructing a dry vascular plaque from its pedicle or in a juxta-capillary position. Bleeding can reappear after two–three days, or in the first two weeks up to one month due to coughing, vomiting, physiological Valsalva maneuver, etc. However, there are also relatively asymptomatic cases that may develop with jaundice [13].

In the above-mentioned case, there was no symptom within the timeframe from when the trauma occurred until the death of the individual. The patient did not require a clinical or paraclinical examination in a medical unit during that timeframe. This clinical development pleads for a recent spleen rupture instead of a delayed with a four-day splenic contusion.

According to the autopsy, the internal corpse examination showed a 7/5 cm subdural hematoma of fresh blood and a 1 cm thick violet blood clot, located fronto-temporo-parietal left and hemoperitoneum of about 700 mL fluid blood and blood clots, one of the large clots located in the spleen vessel containing about one liter of blood. According to literature reviews, in the absence of a known lesion-

causing context, the certainty level in determining with accuracy when the blood clot appeared is difficult and limited to the differentiation between a recent and an old clot. For an old, organized clot, it advocates: the tendency to form a wall of its own, the lax adhesions of the surrounding organs, especially the epiploon that tends to block, localize the intrusion of an organ, the presence of fibrin membranes, brown spots on the surrounding tissues due to hemosiderin impregnation [2, 11]. Both the blood clot (hematoma) in the subdural, fronto-temporo-parietal right and the peritoneal cavity (spleen lobe) found at autopsy does not match the macroscopic characteristics of an old blood clot.

Based on the result of the autopsy on one hand and the clinical data from the literature reviews on the other hand, new HP investigations were required to reveal the presence/absence of hemosiderin in the splenic lesion.

According to the data from literature reviews, hemosiderin is produced by the disintegration of hemoglobin (Hb) from post-traumatic or senescent [1, 22, 24–26]. The disintegration of Hb is important in post-traumatic rupture of blood vessels with the occurrence of bleeding, as it proves their age [28]. The process of transforming Hb is important from a forensic point of view and is considered a vital reaction of the body [29]. The most studied Hb disintegration product in cases involving trauma is hemosiderin [30]. The occurrence of hemosiderin in blood-impregnated tissue occurs after a minimum of 2–3 days [7, 23]. This appears in tissues, at the level of macrophages degrading Hb [22, 31].

Experimental animal models show that hemosiderin formation requires at least three days [32]. Another study showed that hemosiderin occurs in macrophages at the earliest after 50 hours as pale blue inclusions, with a more pronounced color after 72 hours. It has also been demonstrated that the occurrence of hemosiderin is faster *in vivo* than *in vitro* [33]. The presence of hemosiderin in traumatized tissues can be highlighted by HP examination using common stainings (HE staining) or special stains (Pearls staining) [34, 35].

In the case study, the HE staining performed *per primam* as well as in the re-assessment of the case did not reveal the presence of hemosiderin in the fragments collected from the spleen. Its existence would have been observed in the form of brown granules in macrophages [36].

In Pearls staining, hemosiderin is released by acid hydrolysis using hydrochloric acid. Then, potassium ferrocyanide detects hemosiderin and leads to the formation of dense blue precipitates. The forming precipitate is insoluble in acid and therefore acid solutions are used as counterstainings [37].

The negative result obtained from the two used stainings (HE and Pearls) confirmed the lesions in the splenic parenchyma after 2–3 days, before death. This allowed therefore to exclude the connection between the delayed splenic rupture and the trauma from September 10, 2013. By gathering all the data, it was concluded that both thanatogenerator lesions [spleen rupture and craniocerebral trauma (CCT) with meningeal hemorrhage and subdural hematoma] occurred in the same traumatic moment, after the aggression occurred on September 10,

2013 by the victim's fall from the same level and hitting plans with irregular surfaces.

In was the HP examination that ultimately contributed to the resolution of the case, a particular case due to the lack of imagistic and clinical diagnosis both after the first traumatic episode and after the second trauma, followed by the person's death. In this case, the pathophysiological history of the patient was left to the forensic physician, who had to establish the existence or not of the causal link between the two traumas and death. HP investigations, which indicate/exclude the presence of hemosiderin, have been used by the physician through which a direct causality with the first trauma was excluded.

Conclusions

Splenic rupture with hemoperitoneum is a clinical reality with thanatogenerator potential. Determining the type of splenic rupture (blunt spleen rupture or delayed spleen rupture) is very important in forensic traumatology, allowing the legal classification of the act generating the case. Clinical examination and paraclinical examinations are not always conclusive in these cases. In such situations, only the HP examination can be considered reliable. Special stainings, which highlight hemosiderin, are the ones that can establish with certainly the actual time of the splenic rupture.

Conflict of interests

The authors declare that they have no conflict of interests.

All authors read and approved the final manuscript.

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Corresponding authors

Claudia Teodora Judea-Pusta, Lecturer, MD, PhD, Department of Morphological Disciplines, Faculty of Medicine and Pharmacy, University of Oradea, 1 Universității Street, 410087 Oradea, Romania; Phone +40742–756 540, e-mail: claupustaml@yahoo.com

Lucia Georgeta Daina, Assistant Professor, MD, PhD, Department of Psycho-Neuroscience and Rehabilitation, Faculty of Medicine and Pharmacy, University of Oradea, 1 Universității Street, 410087 Oradea, Romania; Phone +40771–255 673, e-mail: lucidaina@gmail.com

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