Pilot Application of SEM/EDX Analysis on Suspected Cigarette Burns in a Forensic Autopsy Case of Child Abuse

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Abstract: Cigarette burn lesions present forensic scenarios that are often difficult to investigate, both from a morphological diagnostic point of view and with regard to the mode of infliction, especially if the victim is unable to speak or has died. Although there may be the suspicion for a lesion to be produced by a lit cigarette, to date one can only rely on the morphological aspects that characterize it, and there is a lack of tools to reach the most evidence-based diagnosis possible. This limitation arose when managing a forensic autopsy case of possible child abuse that resulted in the death of the child, characterized by the presence of 3 suspicious cigarette burn lesions. We therefore decided to perform scanning electron microscopy/energy-dispersive x-ray (SEM/EDX) spectrometry analysis on these lesions and on the cigarette butt found at the crime scene. At the same time, SEM/EDX was applied to the analysis of an unlit cigarette in its entirety (obtained from the same source package as the cigarette butt), a positive control skin sample with an iatrogenic cigarette burn injury, and a negative control skin sample. Among the various compounds highlighted on compositional analysis, only sulfuric anhydride (SO_3) and phosphoric anhydride (P_2O_5) showed a highly significant distribution pattern by being found in the autopsy samples, the cigarette butt, the tobacco of the unlit cigarette, and the positive skin control. Considering this, cigarette burns appear to follow Locard's principle as well, and similarly to other lesions, SEM/EDX allowed the diagnosis of cigarette burn lesions, already suspected morphologically, to be corroborated. Therefore, SEM/EDX is confirmed as a helpful tool in forensic pathology investigations.

Key Words: SEM/EDX analysis, cigarette burns, child abuse, forensic pathology, autopsy

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Injuries that can be observed on a body can vary greatly, differing in morphological features and mode of onset. Sometimes, macroscopic evaluation alone does not provide diriment elements for identifying the type of injury and the injurious means that produced it.[1] This includes, for example, cigarette burn lesions, as they may present with different morphological appearances.[2] In fact, their characteristics can vary based on the severity and time of production.[3] Typically, immediately after their formation, they appear round or oval in shape, about 0.5 to 1 cm in diameter,[4] with a moist or dry base,[5] sometimes covered by a blister or with more raised edges.[6] Thereafter, a brown, firm eschar develops, which diminishes as healing progresses, until it disappears within a few weeks. After healing, the lesion typically leaves behind a silvery scar resembling tissue paper with radial wrinkles, which a brownish border may surround.[2] Many of these features are also shared by dermatologic diseases, with which cigarette burns must necessarily enter into a differential diagnosis.[7,8] In association with the already mentioned wide variability in the presentation of such lesions, this aspect makes cigarette burns very challenging and requires careful consideration in the forensic setting. This difficulty is even more significant when minors are involved, in whom, unfortunately, cigarette burns are a frequent finding in cases of abuse, estimated at 5% to 22%.[9] In fact, very often in daily forensic practice, there is a strong suspicion that a lesion may have been caused by a lit cigarette, but there is no way to prove it with certainty, especially if the victim is unable to speak or has died.[2] This limitation is also and above all a consequence of the fact that studies related to this issue are very few, and to date, the histological examination of the lesion is limited to highlighting diagnostic elements of heat injury. Other methods even more technologically advanced have never been applied to this type of injury in the forensic field.

In this challenging situation, we found ourselves managing a forensic autopsy case of possible child abuse that culminated in the victim's death. We macroscopically documented skin lesions that were very highly suggestive of cigarette burns on the body of the victim. Given the extreme legal significance of this aspect, we opted to use scanning electron microscopy/energy-dispersive x-ray (SEM/EDX) spectrometry analysis on the observed lesions and the cigarette butt found at the scene. At the same time, a pilot study was initiated aimed at applying SEM/EDX to the complete analysis of a cigarette of the same brand, as well as the study of a proven cigarette burn lesion, to compare the results obtained from our autopsy case.

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CASE REPORT

One morning, a man alerted emergency services as his 2-year-old son was not breathing. Upon arrival, the rescuers found the child dead, without clothes, and characterized by multiple bruises over his entire body. The child's mother accused the man of violently beating the child in a fit of rage and while under the influence of hashish. The man claimed instead to have only pushed the child as he was disturbed during his sleep. The man was arrested, and the victim's body was transported to the Institute of Legal Medicine, where a judicial autopsy was carried out 2 days later.

Gross Examination

Upon external examination, the body appeared to be in good preservation condition (length 90 cm, weight 12 kg). There were multiple red-violaceous bruises of different shapes and sizes all over the body, especially on the face and trunk. Some bite marks were also observed, mainly on the upper limbs and buttocks, as well as some abrasions. However, apart from these lesions, further evidence emerged. In fact, 3 very similar lesions were observed in the left zygomatic region, left clavicular region, and proximal phalanx of the third finger of the right hand. They appeared rounded, with a diameter between 0.7 and 1 cm, with a homogeneous reddish background, mostly dried in appearance and with slightly raised edges (Fig. 1).

During the dissection, a fracture of the cranial vault, subdural and subarachnoid hemorrhage, as well as a fracture of the sternal manubrium and the 10th right rib with perilesional hemorrhagic infiltration were documented. Moreover, left renal laceration with hemorrhagic infiltration and blood in the abdominal cavity, as well as a fracture of the right sacral wing, were found.

At the end of the autopsy, the cause of death was identified as blunt trauma producing cranioencephalic and abdominal injuries.

Histological Examination

During the autopsy, in addition to the usual samples of organs, skin fragments were also taken from the suspected cigarette burn lesions previously described. Histological examination was conducted using postfixative techniques, and the sections were stained with hematoxylin-eosin and Masson's trichrome staining according to Goldner. The preparations obtained were observed with a Leica DMR optical microscope, and the most significant images were acquired with a Leica DC300F digital camera. Massive acute hemorrhagic foci were evident in the brain, thymus, pancreas, and left kidney, confirming a significant blunt trauma and corroborating the cause of death.

Very interesting findings emerged from the analysis of the skin lesions in the left zygomatic region, left clavicular region, and right hand third finger. All these lesions were characterized by the presence of a large skin ulcer in their central area, lined by necrotic and hyperbasophilic material that extended down to the deep dermis and subcutaneous adipose tissue. The ulcer, at the transition point with the adjacent skin epithelium, had fairly sharp peripheral margins, in which remnants of epithelium showing marked coagulative alterations of the cytoplasm and marked nuclear pyknosis were recognized. The dermis underlying the ulcer showed marked coagulative alterations, with homogenization of the connective tissue structures and hypereosinophilia. In this area, a moderate inflammatory infiltrate was found, consisting of neutrophil granulocytes associated with multifocal hemorrhagic infiltrates partially compressed between the dermal structures. Minimal foci of inversion of dermal staining affinity[10] were observed with Masson's trichrome reaction. All these findings appeared to be unequivocally convergent in highlighting the occurrence of heat damage producing third-degree burns. Furthermore, from a chronological standpoint, based on the evidence from the literature currently available,[11–14] the inflammatory cellular infiltrate found was indicative of a recent lesion that had occurred approximately 24 hours before death.

PILOT STUDY WITH APPLICATION OF SEM/EDX

At the end of the autopsy, although the cause of death was clear, the skin lesions observed in the left zygomatic region, left clavicular region, and right hand third finger still left some questions unanswered. In fact, histological examination had shown clear signs of heat damage occurring a few hours before death. At the same time, the morphological characteristic (rounded shape and reddish background) were highly suggestive of cigarette burns, although this could not be proven with certainty. The presence of a cigarette butt in the bedroom could point in this direction. However, for legal purposes, it was necessary to pursue a diagnosis that was as evidence-based as possible. This was even more necessary in this case, as there could be a charge of child abuse preceding the homicide.

Given the great versatility of SEM/EDX in forensic investigations, we thought it appropriate to try to analyze the skin lesions in question and the cigarette butt seized from the scene with a JSM-ITS00LV-JEOL electron microscope equipped with EDX detectors. In detail, it was used as a "standardless" EDX system, in which no standards are needed, as the system reads and measures the unknown sample, comparing it with internal references, created specifically using a WDS microprobe.

The skin lesions were collected before the body was washed, using a Kyocera Ishi Ba ceramic blade knife, and thin sections were obtained with the same instrument. The sections were mounted on glass slides and coated with graphite (conductive coating) for SEM/EDX analysis. All the SEM/EDX analyses were performed using the high vacuum mode, which was preferred because it allows images with the highest possible resolution to be obtained.

FIGURE 1. Macroscopic views of 2 of the suspected cigarette burn lesions (left zygomatic region and right hand third finger), observed at autopsy; multiple violaceous bruises and a bite mark between the third and the fifth fingers of the right hand were clearly visible.

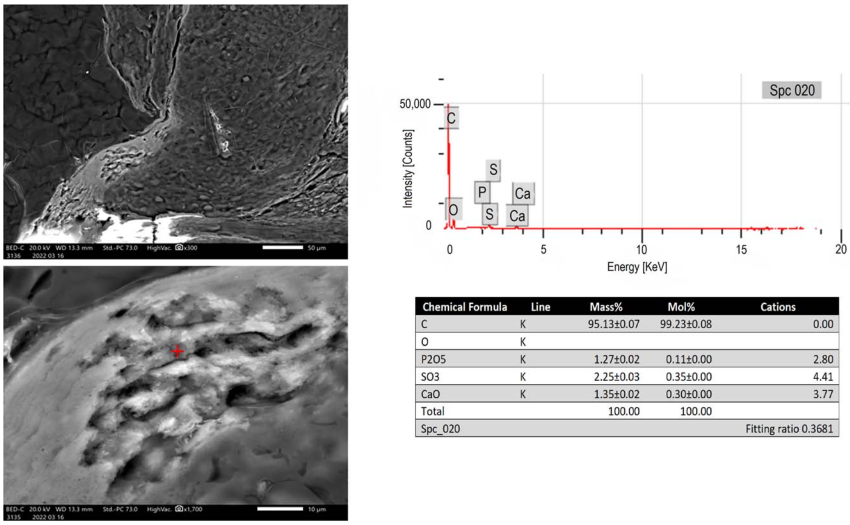


FIGURE 2. SEM/EDX analysis of one of the suspected cigarette burn lesions (on the right hand third finger), with a morphological view of the lesion and a close-up of the point subjected to compositional analysis (on the left) as well as its corresponding compositional spectrum and percentage values (on the right).

Moreover, in this mode, morphological or elemental information can be obtained with secondary electrons for detailed surface analysis, elemental mapping, x-ray analysis, and back-scattered electron detection for elemental analysis.

In parallel, we set up a pilot study with SEM/EDX for 3 purposes: (i) to analyze an unlit cigarette of the same brand as the one seized (from the same pack) to study the structure and composition of all its main parts that could be lost during combustion; (ii) to analyze a positive control sample, consisting of an undamaged abdominal skin flap sampled along the margin of the autopsy incision, on which a cigarette burn was produced using one of the same brand; and (iii) to analyze a negative control sample, consisting of an undamaged abdominal skin flap sampled along the margin of the autopsy incision. All these analyses have been expressly authorized by the prosecutor, who has ordered all necessary investigations to pursue the correct classification of the case for the purpose of justice. The skin samples taken and the analyses carried out have been minimally invasive (not exceeding 1 cm in diameter) and always in accordance with the respect for the deceased individual.

SEM/EDX Analysis of Skin Lesions Collected during Autopsy

Analysis of the injured skin areas revealed the skin structure in all its components (epithelium, dermis, and subcutaneous adipose tissue) within which a concavity of crateriform appearance was highlighted, with a heterogeneous structure and the presence

TABLE 1. Schematic Representation of the Different Components that Emerged at EDX Analysis and Their Percentage Values

			Compositional Analysis and Percentage Values (Mass%)									
			C	CaO	SO_3	P_2O_5	Cl	K_2O	SiO_2	Al_2O_3	MgO	Total
Skin lesions	Left zygomatic		95.22	1.37	2.18	1.23						100
	Left clavicular		95.32	1.33	2.22	1.13						100
	Right hand		95.13	1.35	2.25	1.27						100
Cigarette butt	Outer edge of the residual paper near the burnt end		67.11	24.94	0.53		1.13	6.29				100
	Central portion of the burnt end	Tendrils	63.21	30.37	0.50		0.82	5.10				100
		Particles	57.50	35.13	0.58		1.77	5.02				100
	Filter at the interface with the burnt part	Darker-colored aggregates	82.84	3.71	1.19	0.91	1.03	7.74			2.58	100
		Lighter-colored aggregates	58.56	14.38	1.19	2.16	5.57	12.79	0.24	0.20	4.91	100
		Filter	92.08	1.61			1.55	4.76				100
	Ash		53.85	6.05	2.17	1.62	8.13	22.19	0.45	0.23	5.31	100
Virgin cigarette	Outer paper layer		49.77	48.88				1.35				100
	Central portion with unburnt tobacco		70.30	10.36	1.40	0.64	0.73	10.84	1.35	1.02	3.36	100
Positive control			94.03	1.55	4.42	0 2.81						100
			77.54	18.74	0.91							100
Negative control			99.29	0.71								100

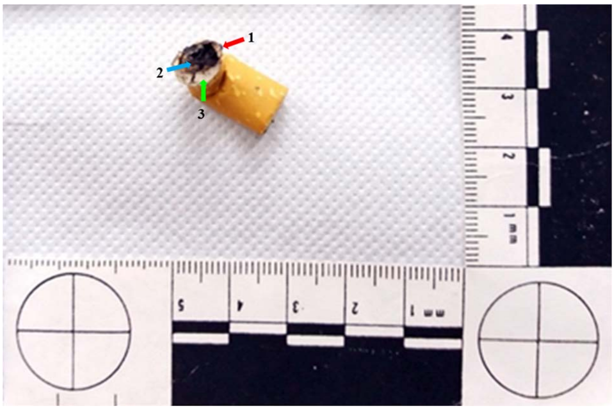


FIGURE 3. Cigarette butt seized from the crime scene with details of the different portions analyzed using SEM/EDX.

of protein coagulation and microvesiculations. Compositional analysis with EDX at the bottom of the ulcer in all 3 lesions subjected to analysis revealed the presence, in descending order, of sulfuric anhydride (SO_3), calcium oxide (CaO), and phosphoric anhydride (P_2O_5) (Fig. 2) with overlapping percentage values (Table 1).

SEM/EDX Analysis of the Cigarette Butt Seized from the Scene

From the bedside table in the bedroom, a partially burnt cigarette butt of the Marlboro® brand was seized (Fig. 3). The following areas of the cigarette butt were analyzed: (i) the outer edge of the residual paper near the burnt end (red arrow, no. 1); (ii) the central portion of the burnt end (blue arrow, no. 2); and (iii) the portion of the filter at the interface with the burnt part (green arrow, no. 3). In addition, a separate analysis was performed on the ash that had detached from the cigarette butt. The outer edge of the residual paper near the burnt end (red arrow) appeared heterogeneous (Fig. 4A) and was found to be mainly composed of calcium oxide (CaO), potassium oxide (K_2O), chlorine (Cl), and sulfuric anhydride (SO_3). The central portion of the burnt end (blue arrow) was found to have a nestlike structure with tendrils, within which lighter-colored particles were located (Fig. 4B); compositional analysis of both the tendrils and particles revealed the presence of calcium oxide, potassium oxide, chlorine, and sulfuric anhydride (Table 1).

Finally, regarding the portion of the filter at the interface with the burnt part (green arrow), a homogeneous background surface was observed on which a number of raised structures stood out, some darker in color and some white (Fig. 5). Compositional analysis revealed that the darker-colored aggregates were mainly composed of potassium oxide, calcium oxide, magnesium oxide (MgO), sulfuric anhydride, and phosphoric anhydride (P_2O_5). The brighter-colored particles, on the other hand, were predominantly composed of calcium oxide, potassium oxide, chlorine, magnesium oxide, phosphoric anhydride, and sulfuric anhydride; aluminum oxide (Al_2O_3) and silicon dioxide (SiO_2) were also present in traces. As for the background surface (belonging to the filter), it was found to be composed of potassium oxide, calcium oxide, and chlorine; sulfuric anhydride and phosphoric anhydride were not present (Table 1).

For completeness, we also analyzed the ash that had detached from the seized cigarette butt using SEM/EDX. From a compositional perspective, the presence of calcium oxide, potassium oxide, chlorine, and magnesium oxide as well as sulfuric anhydride and phosphoric anhydride was found (Table 1).

SEM/EDX Analysis of Whole Unlit Cigarette

An unlit cigarette recovered from the same pack as the seized burnt one was analyzed using SEM/EDX technique. Specifically, the outer paper layer and the central portion with unburnt tobacco were assessed (Fig. 6). The outer paper layer was found to be composed solely of calcium oxide and potassium oxide. The unburnt tobacco, on the other hand, contained potassium oxide, calcium oxide, magnesium oxide, sulfuric anhydride, and phosphoric anhydride with traces of aluminum oxide, silicon dioxide, magnesium oxide, and chlorine (Table 1).

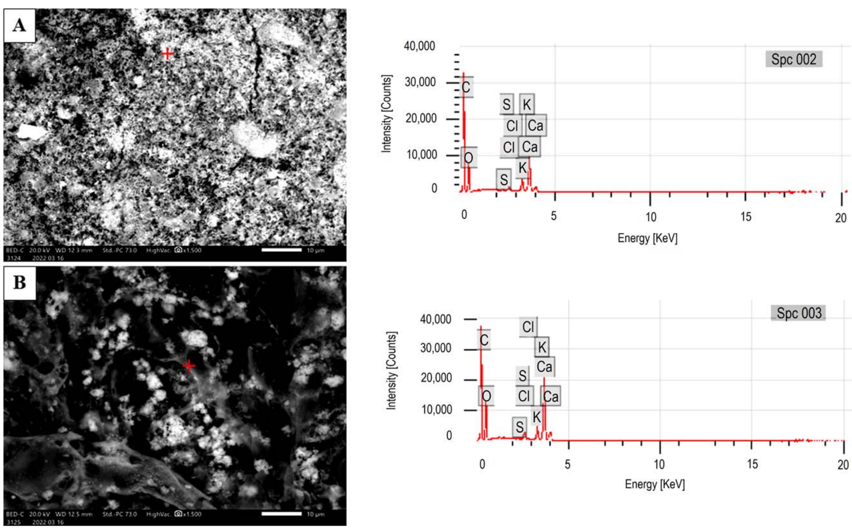


FIGURE 4. SEM/EDX analysis of the seized cigarette butt, at the outer edge of the residual paper near the burnt end (A), and the central portion of the burnt end (B), with details of the points subjected to compositional analysis as well as their corresponding compositional spectra.

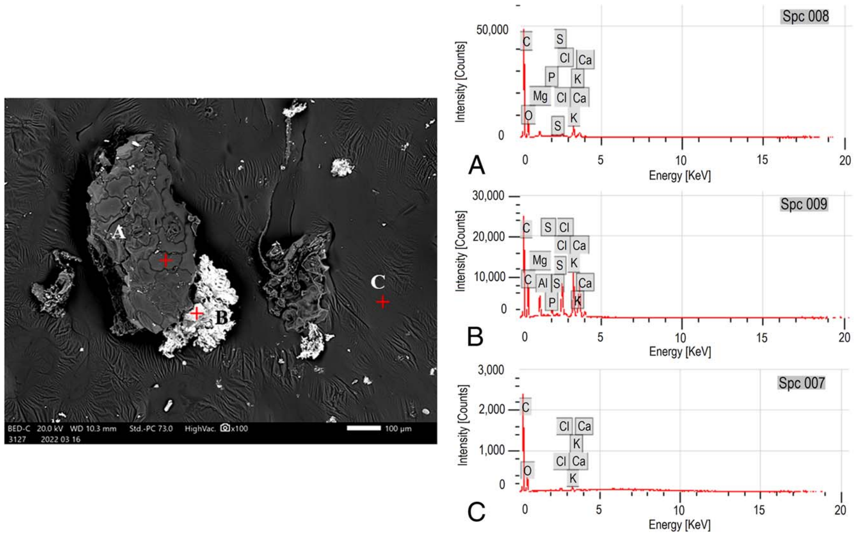


FIGURE 5. SEM/EDX analysis of the seized cigarette butt at the interface between the burnt part and the filter, with details of the points subjected to compositional analysis (a darker-colored aggregate in panel A, a brighter-colored particle in panel B, homogeneous background surface in panel C) and their corresponding compositional spectra.

SEM/EDX Analysis of the Positive and Negative Control Sample

Using a cigarette of the same brand as the previous ones, a burn lesion on a flap of skin was created in the laboratory. For this purpose, the cigarette was held stationary and applied perpendicularly to the skin for a duration of 10 seconds. Subsequently, this lesion was subjected to SEM/EDX analysis, which confirmed the visualization of the skin structure in all its components. In particular, an ulcerated and depressed lesion with a crateriform appearance and heterogeneous structure was observed (Fig. 7). Compositional analysis with EDX, performed in 2 different locations on the ulcerated lesion, revealed the presence of sulfuric anhydride in multiple points, associated with calcium oxide and phosphoric anhydride (Table 1).

Finally, analysis of a sample of intact skin without any lesions (negative control), also from the child, revealed skin integrity and only the presence of traces of calcium oxide (Fig. 8) (Table 1).

Table 1 summarizes and schematically reports the compositional findings of the individual substrates and their respective percentages in the SEM/EDX analysis. Table 2 shows the basic settings of all the SEM/EDX analyses conducted and reported in the manuscript.

DISCUSSION

Cigarette burn lesions present many critical interpretive issues in the forensic field. In fact, in addition to the variability of morphological characteristics, particular attention is required to correctly establish the mode of infliction.[2] Indeed, whether on

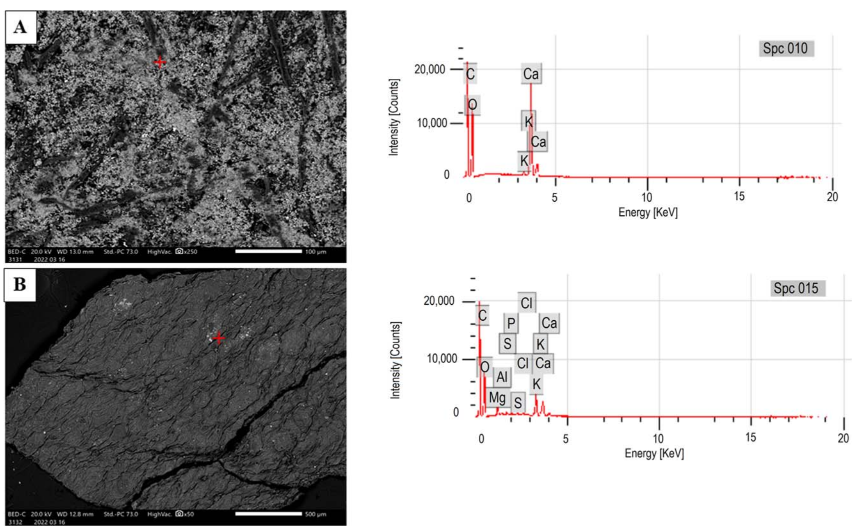
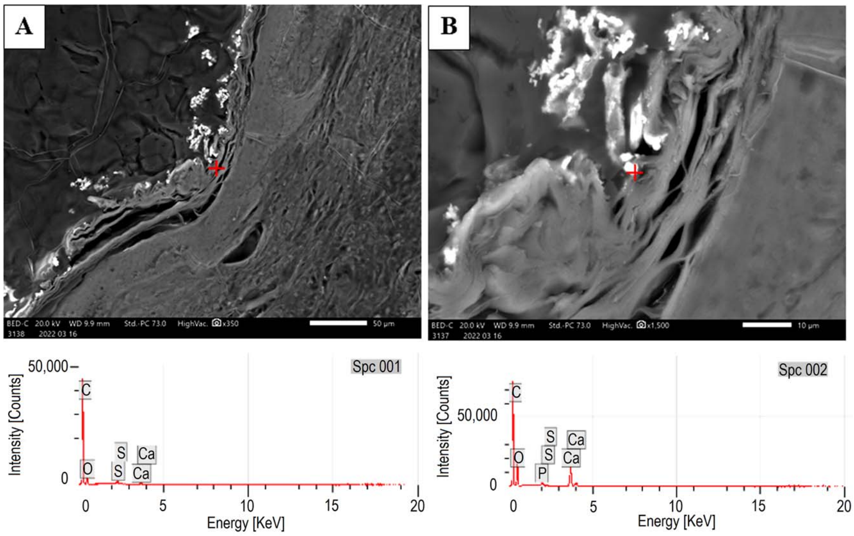


FIGURE 6. SEM/EDX analysis of the unlit cigarette: (A) the outer unburnt paper; (B) the central portion with unburnt tobacco, with details of the points subjected to compositional analysis as well as their corresponding compositional spectra.

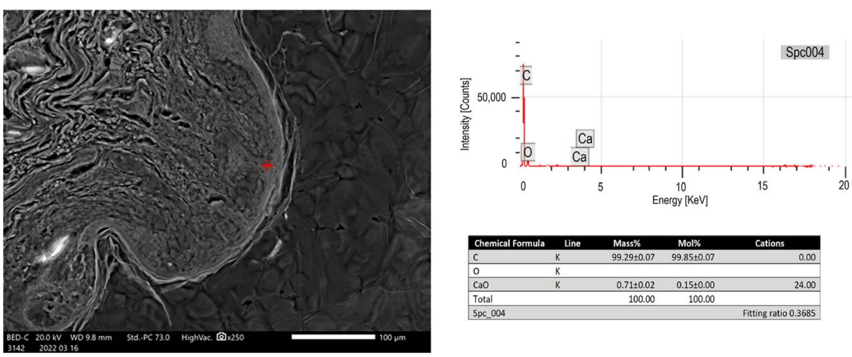


Chemical Formula	Line	Mass%	Mol%	Cations
C	K	94.03±0.09	98.95±0.09	0.00
O	K			
SO3	K	4.42±0.04	0.70±0.01	6.86
CaO	K	1.55±0.03	0.35±0.01	3.42
Total		100.00	100.00	
Spc_001				Fitting ratio 0.3611

Chemical Formula	Line	Mass%	Mol%	Cations
C	K	77.54±0.06	94.64±0.07	0.00
O	K			
P2O5	K	2.81±0.02	0.29±0.00	2.03
SO3	K	0.91±0.02	0.17±0.00	0.58
CaO	K	18.74±0.05	4.90±0.01	17.17
Total		100.00	100.00	
Spc_002				Fitting ratio 0.2870

FIGURE 7. SEM/EDX analysis of a skin sample as a positive control with details of the 2 different points subjected to compositional analysis as well as their corresponding compositional spectra.

living individuals or bodies, even if a diagnosis of a cigarette burn lesion is posed, it becomes urgent to answer the question of whether the injury is accidental, self-inflicted, or caused by a third party. In the latter context, however, it is necessary to further investigate and evaluate whether the cigarette burn is a consequence of an accidental or intentional event. In general, accidental cigarette burn injuries caused by another person are most often observed in children who are near smoking adults and are inadvertently touched with the lit cigarette. In this case, the lesions are localized on areas of the body not covered by clothing, such as the face, neck, and hands. They are typically superficial, irregularly or oval-shaped, ill-defined, and heterogeneous.[5] These characteristics are a consequence of the very brief contact with the skin, as the pain reflex causes an instinctive withdrawal of the affected body part.[8] Accidental cigarette burn injuries can also occur in adult smokers. In these individuals, deeper second- or third-degree burns can occur on the fingers[15] or other body parts,[16] especially when they are in a state of impaired consciousness or altered pain perception due to intoxication.[2] Concerning self-inflicted lesions, they are typically observed in the context of self-injurious behavior[17] in individuals with borderline personality disorder,[18] low self-esteem, emotional and personality disorders,[3] and anorexia nervosa[19] or schizophrenia.[20] The lesions are usually located in easily accessible parts of the body,[2] and their location reflects the handedness of the subject who presents them,[2] with the forearms being the most frequently involved site. In this category of self-inflicted injuries, there are also those intentionally produced to simulate criminal assault.[21] There may also be injuries that occur with mutual consent in so-called act of bravery and initiation rituals of gangs.[22] These raise many interpretive problems, but analyzing the personalities of the individuals involved, their living conditions, and evaluating the consistency between the reported narrative and the injuries on the body can help clarify what really happened. However, cigarette burn injuries often reflect intentional actions and occur in the context of child abuse,[8] rape,[23] cases of torture,[24,25] and violence among prisoners.[3] The lesions are mostly localized in regions of the body such as the forehead,[26] neck, anterior and posterior chest,[5,8] hands,[4] soles of the feet,[27] genitals,[28] and buttocks.[29] Both single burns located in regions of the body usually protected by clothing



Chemical Formula	Line	Mass%	Mol%	Cations
C	K	99.29±0.07	99.85±0.07	0.00
O	K			
CaO	K	0.71±0.02	0.15±0.00	24.00
Total		100.00	100.00	
Spc_004				Fitting ratio 0.3685

FIGURE 8. SEM/EDX analysis of a skin sample as a negative control with details of the point subjected to compositional analysis as well as its corresponding compositional spectrum.

TABLE 2. Basic Settings Used for the SEM/EDX Analysis Conducted

Figure	Spectra	Vacuum Mode	Magnification	Accelerating Voltage (kV)	Live Time (Seconds)	Real Time (Seconds)	Dead Time (%)	Count Rate (CPS)
Figure 2	Spc 020	High	×1800	20.00	60.00	63.68	6.00	11156.00
Figure 4	Spc 002	High	×1500	20.00	60.00	63.78	6.00	11260.00
	Spc 003	High	×1800	20.00	60.00	65.31	8.00	16482.00
Figure 5	Spc 008	High	×300	20.00	60.00	63.86	6.00	11569.00
	Spc 009	High	×1500	20.00	60.00	65.18	8.00	15793.00
	Spc 007	High	×300	20.00	60.00	62.89	5.00	1407.00
Figure 6	Spc 010	High	×1500	20.00	60.00	64.05	6.00	11856.00
	Spc 015	High	×1500	20.00	60.00	63.04	5.00	7906.00
Figure 7	Spc 001	High	×1100	20.00	60.00	63.08	5.00	8684.00
	Spc 002	High	×2200	20.00	60.00	65.74	9.00	18000.00
Figure 8	Spc 004	High	×550	20.00	60.00	63.90	6.00	11744.00

or inaccessible[30] and multiple burns arranged in a geometric pattern[26] are considered particularly indicative of abuse. In these cases, the firm contact of the lit cigarette on the skin typically produces third-degree burns that are round, with a diameter of 5 to 10 mm, and have well-defined edges. The longer the contact is prolonged, until the cigarette is extinguished, the deeper the lesions will be, with a craterlike appearance and raised edges.[2,6]

It clearly emerges that cigarette burns can be found in many different contexts and with varying forensic relevance. Further burdening this aspect is the fact that people accused of inflicting the cigarette burn often provide false explanations for the origin of the injury, or deny that it was intentionally inflicted. In cases where the victim is unable to speak (such as children), unwilling to speak, or has died, the diagnosis of cigarette burn injuries, the time of production, and the method of infliction can be extremely challenging and currently relies solely on the evaluation of morphological criteria.[2] However, this represent a major limitation, especially for lesions found in deceased individuals during autopsy. Moreover, to date, this type of injury has been poorly investigated using more advanced techniques, and there is a lack of adequate tools to reach the most evidence-based as possible diagnosis when needed. We became aware of this deficiency when managing an autopsy case that came to our attention. It involved a child found dead with obvious signs of physical abuse (bruises, abrasions, and bites), which were also confirmed during the autopsy as the cause of death due to the visceral injuries suffered. However, on external examination, 3 lesions with a peculiar appearance were also found, the morphology of which was suggestive of cigarette burn injuries. Histological examination confirmed the application of a heat injury, resulting in a third-degree burn several hours before death. It therefore became more essential than ever to provide scientific evidence to confirm that these were cigarette burn injuries, as the possible offense of child abuse preceding the homicide was of great legal relevance in this case. That is why specific analyses were requested by the prosecutor. Because there was no evidence in the literature, due to the great versatility demonstrated by SEM/EDX in forensic science, we applied this technique to the study of the observed injuries. The electron microscopy image clearly showed the existence of a crateriform, irregular lesion, entirely consistent with the ulcer observed both macroscopically and histologically. From a compositional point of view, it was surprising to find the presence of sulfuric anhydride and phosphoric anhydride in significant percentages in all the 3 suspected cigarette burn injuries, respectively ranging from 2.18% to 2.25% and from 1.13% to 1.27%. To make a comparison, we also analyzed the cigarette butt seized at the crime scene with SEM/EDX. Specifically, it emerged that both the outer paper and the central portion of the burnt end were characterized by the presence of microtraces of sulfuric anhydride (<1%). The analysis of the interface between the filter and the burnt end confirmed the presence of sulfuric anhydride in the agglomerates of burnt material and also highlighted the presence of phosphoric anhydride (highest value of 2.16%). These compounds were completely absent inside the unburnt filter, being present instead in the compositional analysis of the ash detached from the analyzed cigarette butt (SO_3, 2.17%; P_2O_5, 1.62%). Once we established that both the suspected cigarette burn injuries and the burnt end of the cigarette butt seized at the crime scene were characterized by the presence of sulfuric anhydride and phosphoric anhydride, we conducted a pilot study aimed at investigating the significance of these findings. We first analyzed an unlit cigarette of the same brand to investigate its composition before the combustion process. We found no trace of either sulfuric anhydride or phosphoric anhydride in the outer paper or filter; by contrast, there were traces of both of these compounds in the unburnt tobacco. Subsequently, using another cigarette from the same pack, we produced a burn lesion on a cadaveric skin flap that was analyzed with SEM/EDX. We found an ulcerative lesion entirely similar to that found in the victim's injured skin samples, and we detected a significant presence of sulfuric anhydride (4.42%) and phosphoric anhydride (2.81%). Finally, upon analysis of a negative control skin sample, no microtraces of either sulfuric anhydride or phosphoric anhydride were found. Therefore, only sulfuric anhydride and phosphoric anhydride showed such a peculiar distribution pattern: in detail, (i) presence in the suspected cigarette burn lesions, (ii) presence in the burnt cigarette butt found at the crime scene, (iii) presence in the ash detached from the cigarette butt, (iv) presence in the unburnt tobacco of a cigarette of the same brand, (v) presence in the positive control sample, and finally (vi) absence in the negative control sample. This is consistent with the fact that phosphate and sulfuric compounds and are known ingredients in cigarette tobacco.[31] In particular, phosphoric anhydride is one of the main fertilizers used in tobacco plant cultivation, which is absorbed in significant amounts by the leaves and remains present in subsequent processing stages.[32] This fact justifies the presence of P_2O_5 in the unburned tobacco of the unlit cigarette. As for sulfuric anhydride, it is also produced by the combustion of sulfur with atmospheric oxygen at high temperatures, which occurs precisely in a burning cigarette, where temperatures can reach values of 600°C to 900°C.[2] Of course, the compositional analysis of the different substrates analyzed also showed presence of other elements, but none of them had a significant pattern like sulfuric anhydride and phosphoric anhydride. In fact, the other elements or compounds detected were ubiquitously distributed in the environment or known components of cigarettes.[31] Unfortunately, any kind of comparison of our data with the literature is not possible because there is a lack of any SEM/EDX analysis of cigarette burn injuries. Likewise, there is no report (not even from a forensic point of view) in which a cigarette has been systematically analyzed for all its main components with this technique, both from an ultrastructural and compositional point of view. In this context, the analyses we conducted and reported here can be considered pioneering and begin to fill a gap in the literature. The lack of published data also does not allow us to make any considerations regarding the percentage values we detected and the differences observed among the various substrates analyzed. In our case, these differences may at least partly be explained by the fact that, unlike the lesions on the child's body (produced a few hours before death and exposed to air), the positive control sample was analyzed immediately after the production of the lesion. However, as a consequence of this, a higher percentage presence of all compounds in the positive skin control sample appears entirely consistent. Furthermore, it appears that compositional variations occur within a cigarette before and after combustion. Additional variables that may come into play include the contact time between the cigarette and the skin, the force and direction of pressure, the temperature of the cigarette, the level of combustion, and its specific composition.

Despite these limitations arising from a lack of information in the literature, we argue that what we observed in the case of sulfuric and phosphoric anhydride reflects what is enunciated by Locard's principle,[33] which states that when 2 substrates come into contact, an exchange of microtraces takes place. This has been observed to occur in other types of injuries,[34] such as blunt force trauma,[35] sharp force trauma,[36,37] ligature strangulation,[38] and electrocution.[39] In light of our findings, it appears that this can also be true for cigarette burns. In this sense, SEM/EDX has provided scientific evidence that, in the case at hand, allowed us to confirm the diagnosis of cigarette burns, which was already suspected morphologically. Consequently, for the first time, it has been possible to observe how a cigarette burn appears under SEM/EDX, which made it possible to observe the typical characteristics of cigarette burn lesion from an ultrastructural point of view. An aspect that requires particular consideration concerns the possibility of uniquely correlating a cigarette burn with the specific cigarette that produced it. In our case, we were facilitated because the only cigarette butt present at the crime scene was seized and analyzed, and its composition was consistent with the microtraces documented in the skin lesions. Therefore, we were able to conclude that with a high probability, the lesions were produced with that cigarette. However, at present, there is no evidence which enables to generalize such a statement, as all cigarettes from the same pack (as well as those of the same brand likely) may share the same composition. Therefore, it appears that SEM/EDX can be more decisive in proving that a lesion was produced by a cigarette by highlighting the microtraces of combustion (sulfuric and phosphoric anhydride in our case) rather than correlating a cigarette burn with a specific cigarette. Overall, our findings confirm the great importance that SEM/EDX can have in forensics investigations, even in unusual and peculiar applications.[40]

Finally, if SEM/EDX can provide evidence that a burn is caused by a cigarette, to assess the mode of infliction (accidental or intentional, self-inflicted, or caused by a third party), one must necessarily rely on the morphological characteristics, as described in the literature. In our case, the morphology and severity of the burns led us to believe that the injuries were the result of intentional harm, thus constituting a crime of child abuse, which, thanks to the histological findings, was chronologically placed before the murder. The fatal outcome of this case allowed us to analyze the suspicious injuries with SEM/EDX, an approach that is not possible in living subjects, for which alternative investigative methods should be found. However, our preliminary findings, fully applicable to autopsy forensic contexts, can provide inspiration for different applications in clinical forensic medicine.

In conclusion, cigarette burns are highly insidious injuries to investigate and correctly frame from a forensic perspective. This pilot approach to a suspected cigarette burn with SEM/EDX has highlighted promising results that seem to make this technique suitable for the study of these lesions and, therefore, worthy of further investigation. We do believe that advances in the study of cigarette burns can only be effectively made by raising the bar, resorting to more sophisticated investigative techniques.

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