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The detection of metallic residues in skin stab wounds by means of SEM-EDS: A pilot study

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ABSTRACT

The morphological analysis of stab wounds may often not be accurate enough to link it with the type of wounding weapon, but a further evaluation may be performed with the search for metallic residues left during the contact between the instrument and the skin. In this study, Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS) was applied to the study of cadaveric stab wounds performed with kitchen knives composed of iron, chromium and nickel, in order to verify the presence of metallic residues on the wound's edge. Two groups of 10 corpses were selected: group A, including victims of stab wounds and a control group B (died of natural causes). Samplings were performed on the lesions and in intact areas of group A, whereas in group B sampling were performed in non-exposed intact skin. Samples were then analysed with optical microscopy and SEM-EDS. In group A, optical microscopic analysis showed the presence of vital haemorrhagic infiltration, while SEM-EDS showed evidence of microscopic metal traces, isolated or clustered, consisting of iron, chromium and nickel. Moreover, in two cases organic residues of calcium and phosphate were detected, as a probable sign of bone lesion. Control samples (group A in intact areas and group B), were negative for the search of exogenous material to optical microscopy and SEM-EDS. The results show the utility and possible application of the SEM-EDS in theidentification of metallic residues from sharp weapons on the skin.

1. Introduction

The macroscopic and microscopic characterization of stab wounds is of utmost importance in forensic cases, in order to distinguish between different types of wounding mechanisms and to identify the characteristics of the wounding weapon and methods of production [1]. Among the analysis of skin stab wounds, the wounding weapon may be hardly identified, especially when nor even a “suspect” weapon is found at the crime scene. In addition, if the weapon (or a suspect weapon) is present, in terms of compatibility between tool and wounds, if the evaluation is limited to a macroscopic assessment, the match between skin injuries and wounding weapon can be tough and expressed only in terms of “compatibility” [2,3].

For these reasons, several studies have been performed with the aim of evaluating, by means of scanning electron microscopy (SEM), the possibility of finding metallic residues left by the wounding tools. The great opportunities provided by SEM concern the large depth of focus with the possibility to perform three-dimensional evaluations along with elemental analysis.

Among this crucial topics of interest within forensic pathology and anthropology, SEM analysis also enabled the characterization of the chemical composition of metallic residues. The potential of scanning electron microscopy has been deeply analysed, acknowledging the help that this method has to offer toward a thorough analysis of wounds.

Several experimental studies demonstrated the possibility of detecting metallic residues in bone left by blunt or sharp objects [4–6], mainly on bone: it is intuitive that the impact between two hard surfaces (bone and metallic wounding tool) can be effective in determining the release of metallic fragments. On the other hand, if we consider skin as target tissue, the impact between the wounding weapon and the surface is completely different. Therefore, the question arises on the possibility that the same residues may be left on the skin and detected with the SEM.

If we take a look back in literature, the possibility of recognizing metal fragments with SEM on cadaver skin has been demonstrated in a real forensic case concerning blunt force trauma [7]. For what concerns

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Table 1
Summary of group A (cases).

Case	Sex	Age	Place of death	Wounding weapon	Location of stab wounds	Num. of wounds	Location of the sampling
1	M	35	Courtyard	Knife (smooth blade)	Face	2	Right neck
					Neck	3	
					Abdomen	2	
2	F	38	Public street	Knife (smooth blade)	Head	1	Right thorax
					Thorax	1	
3	M	32	Public street	Knife (smooth blade)	Face	1	Left thorax
					Thorax	1	
4	F	42	Home kitchen	Knife (smooth blade)	Face	1	Right thorax
					Thorax	5	
					Abdomen	1	
5	M	20	Public street	Knife (serrated blade)	Head	1	Right thorax
					Thorax	18	
					Abdomen	2	
6	M	29	Hotel's corridor	Knife (serrated blade)	Neck	1	Right neck
7	F	34	Home bedroom	Knife (smooth balde)	Face	2	Right hypochondrium
					Neck	2	
					Thorax	3	
					Abdomen	17	
8	F	70	Home bedroom	Knife (smooth blade)	Neck	4	Left neck
					Thorax	14	
					Abdomen	11	
9	M	54	Public street	Knife (smooth blade)	Thorax	1	Lef thorax
10	M	52	Home kitchen	Knife (smooth blade)	Thorax	1	Precordium

experimental studies with sharp instruments, the same ability has been demonstrated in a study performed on pig skin [8,9]. However, a systematic study developed from real cases, thus under real conditions in a forensic scenario, has never been previously performed (i.e., how much can the presence of blood influence the deposition of residues and their possibility to be detected?).

Therefore, the present study was performed on stab wounds taken from cadaveric skin in real forensic cases. The aim of the study was to examine the presence of metal particles on skin wounds inflicted by kitchen knives, with regard to chemical composition, and possible forensic implications of these findings.

2. Materials and methods

The study was performed on a group of judicial autopsies performed in 2016 at the Institute of Legal Medicine of Milan, Italy, selecting two groups of 10 cases: group "A" regarded murder victims with multiple stab injuries (summarized in Table 1) performed with kitchen knives (with blades between 10 cm and 18 cm in length). The scene of crime regarded an open environment in 5 cases and an indoor environment in the other 5.

In all the selected cases the wounding knife was identified with certainty, since it was found at the crime scene, or confirmed by DNA analysis, or indicated by the self-confessed, as well as recorded by video-surveillance systems. Therefore, the wounding weapon was well known in all cases. To standardize them, only wounds produced by kitchen knives were selected, of which 2 with serrated blade and 8 with un-serratedblade. In all the cases, blades were chemically composed of iron (Fe), chromium (Cr), silicon (Si), manganese (Mn) and nickel (Ni).

Only one stab wound on the skin was sampled for each case. For each lesion a skin sample of 2.0 × 1.0 × 0.5 cm was sampled with a ceramic knife *Kyocera Ishi Ba-*, including 2 cm of the entire margin and approximately 1 cm lateral to the wound's margin. The sampling was performed after the wounds had been photographed and a macroscopic analysis of the margins by means of plastic tweezers was performed, avoiding any probe with metallic tools.

The blade of the ceramic knife was composed of zirconium (Zr) and hafnium (Hf), so the contamination by sampling procedures has been avoided.

Moreover, in order to confirm the specificity of any metal traces found on the wound's margins, a similar skin lozenge was sampled from the anterior part of the thigh, far from any wounded area.

As additional control cases, a total of ten subjects was chosen (group B), in good state of preservation, died of natural causes. For each cadaver, a skin lozenge in the anteriorpart of the thigh was sampled with the ceramic knife, in an area covered by clothing, to exclude any environmental metal contamination. The choice of using real cases and not an experimental set (i.e. animal skin) was determined by the will to evaluate real scenarios and their variables: therefore, structure and elasticity of the skin was that of real human bodies, and the presence of residues was surveyed even in the presence of bleeding. In fact, in previous experimental setting the possibility that residues could be "washed" from bleeding associated was not taken into account. Finally, even human skin debris may affect deposition and detection of metallic particles on the surface [10]. Samples were collected according to the Italian mortuary regulations and laws.

All skin samples were divided in two: one half was fixed in buffered 10% formalin, dehydrated in a scale of increasing ethanol coloured with basic hematoxylin-eosin and Masson's trichrome staining (specific for the vitality of the lesions) and finally, observed with an optical microscope Leica DMR.

The other half was dehydrated and coated with graphite and analysed by means of SEM-EDS with a microscope Cambridge Stereoscan 360 and Link Isis energy dispersive x-ray system, in order to search for the presence and characterization (elemental composition) of metallic residues.

3. Results

3.1. Optical microscopy

In all the skin samples taken from the 10 corpses of group A (forensic cases), there was a microscopic evidence of massive acute hemorrhagic infiltration, with well-defined and stored red cells, dissociating the fibers and placed within the deepest layers. Such evidence confirmed the "vitality" of the injuries, all inflicted when the victim was alive. Samples taken from the same corpses at the thigh (away from wounded areas), and all group B controls were free of any microscopic haemorrhagic infiltration.

Table 2
Results concerning the search for metallic residues with SEM-EDS in group A.

Case	Place of sampling	Amount of metallic residues	Composition of metallic residues	Other residues	Shape of metallic residues
1	Right neck	7	Fe - Cr – Ni Fe		Irregular
2	Right thorax	> 10	Fe	Ca P	Irregular
3	Left thorax	> 10	Fe – Cr Fe – Ni Fe	P	Irregular
4	Right thorax	1	Fe		Irregular
5	Right thorax	1	Fe	Ca P	Oval-shaped
6	Left neck	6	Fe – Cr Fe Cr		Irregular Oval-shaped Round-shaped
7	Right hypochondrium	8	Fe Cr		Irregular
8	Left neck	1	Fe		Irregular
9	Left thorax	3	Fe		Irregular
10	Precordium	1	Fe - Cr		“Comma” - shaped

3.2. *Scanning electron microscopy equipped with energy dispersive X-ray spectrometer (SEM-EDS)*

Experimental description is summarized in Table 2. Metallic residues were detected in all the samples of group A (Fig.1: visualized by backscattered electron images and x-ray spectra showing the qualitative analysis), placed in the context of the soft tissues of the sample and mainly consisting, in iron (Fe), iron and chromium (Fe-Cr) (Fig.1), iron, chromium, and nickel (Fe-Cr-Ni).

As shown, in all the samples almost 1 single metallic residues was found, and in 6 cases out of 10 residues were multiple (between 3 and 8 residues, and in 2 cases over 10 residues), whereas in 4 cases only 1 single residue was detected. A concordance between the composition of the tool (kitchen knife) and the metallic composition of the detected particles was present in all the cases. In most cases the shape of the residues was irregular, although in 2 cases (cases 5 and 6) a round or oval shape was detected. In case 10, a single residue of combined iron and chromium with a shape of “comma” was detected (Fig.2). Moreover, residues of calcium (Ca) and phosphorus (P) were detected in 2 cases, while only phosphorus was detected in one case.

The samples taken (at the thigh) from the same victims of group A and the control group B, showed the absence of metal particles.

4. Discussion

The crucial role of the application of the SEM-EDS technique in the detection of metallic residues has been widely highlighted in the forensic practice, especially in the field of the qualitative and quantitative assessment of gunshot residues [11–14].Further areas of research include toxicology [15,16], anthropology [17,18], search for inorganic and organic material [19]. Moreover, the potential of SEM has been widely applied to the microscopic analysis of morphology and size of bone lesions [20–24].

However, to our best knowledge, only few experimental surveys have been performed so far with the aim of assessing the possibility of detecting metallic residues left by blunt or sharp objects [4–6] and mainly on the bone: the reasoning behind these studies is that any metallic tool impacting the bone surface may leave residues which may hence be detected by the great sensitivity of SEM-EDS. This type of capability on sharp and blunt injuries on bone was investigated in forensic cases [25,26].

However, no studies have been carried out on real cases, but only in an experimental setting on pig skin [8], where the possibility of findings such residues with SEM-EDS was demonstrated. An interesting challenge is to assess whether a similar chance may be present performing the same analyses on the skin.

Although cutting tools may be variable in type and shape, forensic cases frequently involve knives. However, tools like kitchen knives of the present study, were uniformly composed of iron, chromium, silicon, manganese and nickel. Nevertheless, the morphology of stab and incised wounds on the skin can be manifold and a source of pitfalls [27,28], hence morphological assessments may often be not enough to link the wounds to the weapon used. Therefore, the analysis of residues left by the wounding tool may be of help.

Based on these observations, this study aimed at verifying, with SEM-EDS, the presence, quantity and nature of microscopic metallic residues left behind by the wounding tool (kitchen knives) in stab wounds on the skin taken from autopsies of homicide cases. The study was carried out directly on forensic cases, thus considering the characteristics of the human skin and, above all, the presence of bleeding, major limitation of the experimental studies previously carried out on animal skin. The chemical composition of the weapon was kept constant.

Firstly, the viability of the lesions was demonstrated by light microscopy, allowing to exclude that sampling procedures had been carried out in non-vital areas. Therefore, it was assumed that all injuries had been made on a living person and thus associated with a certain amount of bleeding. The results showed the real possibility of detecting, where stab wounds were present, metal residues left by the weapon. In

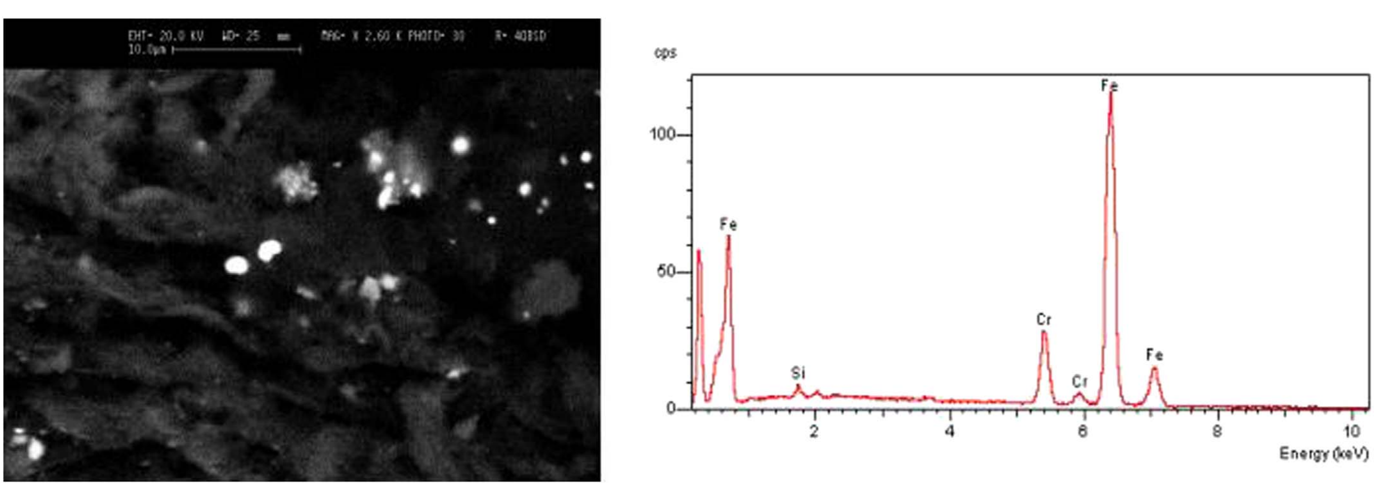

Fig. 1. Case 6 – SEM picture of many irregular-shaped metallic fragments (bright areas) of iron and chromium, as shown in the spectrum on the right.

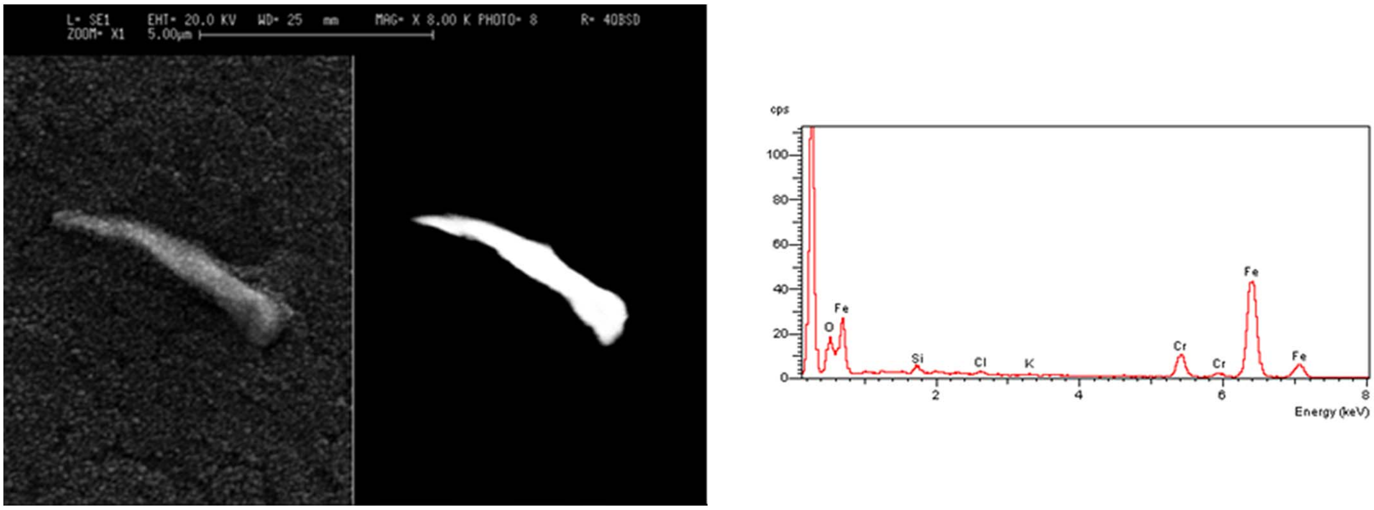

Fig. 2. Case 10 - SEM picture of metallic fragment with a "comma"-shape (5,5 μ × 0,7 μ) of iron and chromium, as shown in the spectrum on the right.

all 10 cases analysed, the constituent elements of the wounding weapon were detected: iron, chromium, nickel or a composition of these elements (in association with iron, as typically occurs in metal alloys). The documented presence of micro-particles consisting of chromium has to be linked to media a plating process of steel, where an outer coating made with chromium in order to impart to the weapon greater hardness and protection to corrosion.

Residues consisting of calcium (Ca) and phosphorus (P), were considered of bone origin, according to the penetrating action of the weapon, which has fought against a hard surface, with enough force to determine the fragmentation of the bone and thus the projection of micro-fragments toward the outer layers of the skin. This possible reconstruction is suggested by the fact that where organic residues were detected (cases 3 and 5), wound was placed in the thorax and the knife perforated the costal bone below: it is hence conceivable that the wounding effect of the tool facilitated the contact between skin and bone.

Therefore, the presence of micro-metallic traces of the wounding weapon was detected with SEM-EDS, while on the contrary no evidence of such residues was detected in controls (group B) and in the samples performed in group A in intact areas, away from stab wounds.

As demonstrated by the study, SEM-EDS analysis provides reliable qualitative, and not quantitative analysis, so that its possible application in forensic practice may help differentiating between two (or more) wounding knives, especially if they are consisted of same or similar chemical compounds. This is due to the qualitative chance provided by this analysis. On the other hand, the number of metal residues in the stab wounds may be related to the characteristics of the weapon (hardness, integrity, shape) or tissue type, but no further information can be obtained so the number of residues may not be considered useless and reliable to draw any further element. This may be an aspect worth deepening in future studies. Another interesting point concerns a possible environmental contamination: literature describes chromium, iron and nickel as possible environmental contaminants, since chromium may be present into different environmental matrices (air, water, and soil) from a wide variety of natural and anthropogenic sources, high concentrations of iron may be found in freshwater, nickel may be a common contaminant of soil [29,30]. Nevertheless, the absence of these residues both in samples performed in the same cadavers of group A far from wounds, as well as in the control group, makes reason to believe that these metallic elements were not present as environmental contaminants, thus supporting the hypothesis that metal residues may be directly linked to the release from the blade of the wounding weapon. Furthermore, results suggest that these residues may be embedded in the skin and not "washed out" by the action of bleeding.

A limitation of the study is represented by the small number of samples, even if the results seem promising. However, the analysis performed starting from real cases, supplies to the present study (which can be considered a pilot study) survey's conditions as real as possible, thus opening the possibility of application of these methods even in real cases, in an attempt to reach an evidence that can be discussed even within trials. Moreover, the qualitative nature of the method may be used in order to perform a comparison between different weapons or to exclude some given weapon as the one which inflicted the wound, but not to exactly confirm some given weapon as the weapon that inflicted the wound. In other words, at the state of the art and on the basis of the study, its use can be "indicative" and not "diagnostic".

In the present cases the chemical composition of the blades was constant and determined by SEM-EDS investigation, but a possible limitations in real forensic practice is that in most cases, these studies are not required by the judicial authority and the forensic pathologist may not further deepen the level of investigations to be performed. However, the importance of this type of analysis may be crucial to answer the questions put by the Magistrate.

In conclusion, in forensic cases the sole morphological analysis of stab wounds on the skin may not be sufficient to accurately identify the wounding tool, thus it is necessary to carry out a step forward: this could be represented by the analysis with the SEM-EDS technique. The study highlighted that metallic residues may be left on the skin as well as on the bone, hence SEM-EDS may provide indications on the compatibility between weapons and residues, on the comparison of different weapons (albeit based on a qualitative and non-quantitative assessment, since additional information cannot be drawn by the number of residues). Other limitations may be the influence of pollutant elements on the skin, which is worth investigating with future studies. It can be thus inferred the usefulness of a systematic use of this method in the analysis of stab wounds, both on the skin and on the bone, combining elemental and morphological analysis with the purpose of identifying the wounding weapon.

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