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Original communication

Detectability and medico-legal value of the gunshot residues in the intracorporeal channel

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ABSTRACT

The application of the histochemical stain of sodium rhodizonate to the entrance wound for the detection of the lead (Pb) residues coming from the gunshot may be affected by false positive cases due to the contamination of the environmental Pb. The aim of the Authors is to histochemically search the Pb of GSR in a region which should be more protected by the contamination: the intracorporeal channel.

Two hundreds and eighteen serial histological specimens of the intracorporeal channels coming from 25 subjects (dead due to gunshots and being autopsied at the Section of Legal Medicine of the Milan University, in the years 2013–2014) were stained with the sodium rhodizonate and sodium rhodizonate in acid environment (HCl 5%), and then observed by the microscope.

The sodium rhodizonate showed a positivity for the Pb residues in the intracorporeal channel, with the detection of the particles within the first 2 cm beyond the entrance wound in 6 cases over the total number of 25 (24%). Victims were characterized by common features: short-barreled weapon; contact shots or short-distance shots; involvement of regions that were not covered by clothing; preservation of the microscopic structure of organs interested by the intracorporeal channel.

The searching of GSR in the intracorporeal channel, even in conditions securing a high sensitivity, could represent an important test for the discrimination between an environmental contamination of Pb and the presence of Pb residues by GSR: once confirmed the presence of GSR in the intracorporeal channel by the histochemical analysis, the diagnostic process should require the application of the SEM-EDX for the confirmation of the results. Although not yet studied, this combination could be applied to cadavers exposed to the environment, with advanced post-mortal phenomena permitting at least the suspects of the existence of gunshot wounds at the macroscopic autopsy evaluation. Indeed, in some cases, the putrefaction is so advanced that no suspect of gunshot injuries could be derived from the soft tissue.

1. Introduction

The search of GSR on the cadaver by the histochemical staining of sodium rhodizonate (Na-R) has been often studied to solve some medico-legal problems[1] concerning: the differential diagnosis of gunshot wounds in putrefied or badly preserved cadavers,[2] the identification of this type of injuries,[3] the distinction between entrance and exit wounds,[4] the definition of the shoot distance.[3,5–9] However, the results of these studies are currently under discussion in order to evaluate the medico-legal importance and strength in term of evidence contribution, as suggested by some Authors.[10,11]

The gunshot residues are produced during the explosion of the primer and propelling charge, and they are dropped off (in the form of particulates) on the killer's and victim's skin and clothes, as well as on all the objects present in the crime scene, in a pattern that is proportionally inversed with the shoot distance.[12] Regarding the primer, the typical composition is made of lead (Pb), barium (Ba) and antimony (Sb), although some primers do not contain lead.[13]

Concerning barium and antimony, no histochemical method is actually available for the observation of antimony, while a

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technique employing the sodium-rhodizonate was proposed for the barium.[14] However, this last methodology is not applied in the medico-legal literature and, in the Authors' experience it is characterized by a difficult microscopic interpretation as it is not supported by relevant comparison samples. The use of the Na-R for the detection of lead particles was contested due to the possibility of histological false positive cases,[15] that are attributable to the environmental lead coming from the fuel,[10] brake pads,[16,17] varnish,[18,19] batteries[10] and particular metallic alloys.[10] This difficult discrimination is even more important and relevant in case of decomposed cadavers after prolonged exposure to the external environment, and so to the risk of contamination. Furthermore, the use of the only Na-R method, like the other chromogenic techniques, can lead to false positive cases due to the binding of the pigment with other metallic deposits like Barium (Ba), Calcium (Ca) and Strontium (Sr).[20]

Therefore, multiple and more accurate methodologies were studied: one of these techniques is the Scansion Electronic Microscopy with Energy Dispersive X-ray spectroscopy (SEM-EDX) which allows the demonstration of the three main components of GSR (Pb, Sb e Ba) and its quantitative evaluation.[15] Despite its reliability, it is expensive, it is not available in all the centers and requires more time of execution and more specialization in the data interpretation (unlike the histochemical stain of Na-R, which is considered inexpensive, fast and easy to read by multiple Authors[5,21–26]).

On these basis, the Authors of the present article experimented the use of the Na-R stain completed by the following treatment with 5% hydrochloric acid[21] for the demonstration of Pb residues in the intracorporeal track; the Authors chose the Pb because it is the most used element in the primers and for the bullets.[13] The theoretical basis of the study lies in the hypothesis that the intracorporeal channel is more protected from environmental pollution by Pb, and its histochemical analysis could be more useful than the similar observations performed on the external skin, that definitely presents a higher risk of environmental contamination and false positive results.

2. Materials and methods

The study considered all the judicial autoptic cases of gunshots performed at the Section of Legal Medicine of the University of Milan, in the years 2013–2014: a total number of 25 cadavers of Caucasoid individuals (21 males and 4 females, age range between 20 and 83 years old). They presented gunshot wounds (13 cases of suicide, 11 cases of homicide and 1 accidental event) and they were in good condition of preservation. During the autopsies, for every case, an anatomical mapping of the intracorporeal way of the bullets was created by multiple serial histological specimens of the intracorporeal track, starting from the entrance wound, through all the anatomical structures (muscles, organs, serous membrans, etc.) involved by the bullet until to the exit wound, and measuring the distance of the specimen from the entrance wound, whenever possible. A total number of 218 samples were obtained (Table 1), with a minimum of 3 specimens for case, a maximum of 25 specimens for case and a mean value of 8.7 specimens for case; samples were fixed in 10% buffered formalin, included in paraffin and then cut. For each sample, three slides with different stains were prepared: a Hematoxylin-Eosin (HE), a Sodium Rhodizonate (Na-R) for the detection of deposits of Pb in the form of granulations of scarlet red and a Sodium Rhodizonate in acid environment (Na-R with 5% HCl)[21] for the confirmation of the presence of the residues, that show a purple-blue coloration. Na-R solution is an aqueous solution at 0.3% (in bidistilled water); the solution should be used within 15 min from its preparation, and it should be totally renewed at every utilisation.[27,28] The reactivity of the Pb is consolidated by the presence of a 0.1% tartaric acid solution (L-stereoisomer) that has to be mixed to the Na-R solution in a 5:1 ratio (5 drops of Na-R and 1 drop of tartaric acid). The 5% Na-R-HCl solution involves, after the precedent steps, that each slide has to be dipped in a 5% HCl solution for 1 min, until the granulations change its color from red to purple-blue. A total number of 654 slides were observed at the *Leica DMR* optic microscope (see Figs. 1–4).

Table 1
Schematization of the samples according to the anatomical district and to the localization.

District	Localization	No of samples
Head	Cranial bones	12
	Temporal muscle	9
	Masseter muscle	1
	Dura mater	25
	Brain	71
	Hard palate	6
	Auricular cartilage	1
Neck	Hyoid bone	1
Thorax	Sternum	1
	Ribs	9
	Intercostal muscles	6
	Pleura	1
	Lung	33
	Heart	21
Abdomen	Dyaphragm	1
	Stomach	8
	Liver	4
	Pancreas	3
	Spleen	3
	Kidney	2

3. Results

The histochemical search of Pb resulting from GSR in a region that is considered relatively protected from environmental pollution, like the intracorporeal channel, provided interesting result. At the standard Hematoxylin-Eosin stain, multiple homogeneous deposits of dark pigment were observed: they were attributable to combusted materials embedding the GSR, but they were indistinguishable from other residues. Furthermore, this typology of stain was useful for the detection (within the intracorporeal trajectory) of extraneous material (clothing fibers: cotton and synthetic fibers), fragments of skin, bone and cartilage that are thrown in the depth of the channel by the strength of the bullet.

The association of the histochemical techniques of Na-R and Na-R-HCl proved their specificity in the detection of residues of Pb, also in the intracorporeal channel: indeed the Pb particles were identified in the form of red-scarlet granules by the Na-R, and then confirmed by the Na-R-HCl as a result of their color change to blue-violet. The lead residues in the intracorporeal channel were detected in 6 cases out of 25 (24% of cases), dealing with 4 suicide, 2 homicide: the positivity was localized within the first 2 cm beyond the entrance wound (Table 2).

In the other 19 cases (76% of the cases, dealing with 9 suicides, 9 homicides and 1 accidental event) the search of lead residues was negative (Table 3).

The Graph number 1 depicts the number of samples for each distance interval (see Graph 1).

4. Discussion

As a first level tool, the presence of Pb residues from GSR in the bullet intracorporeal track (an anatomical region still unexplored) was analyzed by the application both of the Na-R histochemical staining, followed by the confirmation test of 5% Na-R-HCl.