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LUNGE'S REAGENT FOR THE DETECTION OF NITRITES FROM GUNSHOT RESIDUE – AN OUTDATED METHOD?

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Abstract: ***Purpose.*** The aim of the study was to investigate the reliability of Lunge's reagent for the detection of nitrites from entry and exit gunshot wounds.

Methods. This was a prospective autopsy cohort study conducted at the University Hospital Ostrava. During the study period, all persons who died after gunshot injury were assessed for the study eligibility.

Results. In total, 80 cases of perforating gunshot wounds were included in the study and underwent further analysis. The most commonly used firearm was a handgun (85.0% of cases). Gunshot wounds were located on the head in 90.0% of cases, on the neck in 7.5% of cases and on the chest in 2.5% of study cases.

Detection of nitrites from entry wounds was positive in 41 (51.3%) cases and negative in 39 (48,7%) cases. The Lunge test was positive in 21 (26.3%) exit wounds. Analysis of the 21 cases with a positive Lunge's test from exit wounds showed that only 12 (57.1%) of these individuals also had a positive Lunge's test from entry wounds.

Conclusion. Lunge test for the detection of nitrites from gunshot wounds has a very low accuracy/reliability. Therefore, the Lunge test should no longer be used in common practice by forensic pathologists.

Keywords: gunshot residue, nitrites, Lunge test, dermal nitrate test, autopsy study, entry and exit gunshot wounds.

INTRODUCTION

When a firearm is fired, the bullet is followed by a complex mixture of chemical debris (organic and inorganic materials) that is expelled through the barrel. This chemical debris is known as gunshot residue (GSR) and plays an important role in the forensic investigation of gun-related violence. The detection and analysis of the GSR can be very valuable in identifying those involved in the shooting, differentiating between entry and exit wounds, estimating the shooting distance, and determining the angle of the gun to the target [1,2].

There are many different methods/techniques used to detect GSR – colour tests, instrument-based methods, and electrochemical sensor-based methods. The traditional technique for detecting GSR is the chromogenic test, which uses reagents that target a specific chemical entity within the GSR; the chemical interaction produces a specific colour. The colour tests are inexpensive, simple and usually consist of rapid sets of procedures [1,3].

The presence of nitrite derivatives in GSR can be confirmed by the Lunge test, Griess test or its modifications. The Lunge test has been used in forensic practice since 1933, when Teodoro Gonzalez (Mexico City Police Laboratory) introduced the dermal nitrate or paraffin test. Lunge's reagent (diphenylamine and concentrated sulphuric acid) produces pink/red coloration with nitro compounds from GSR (Fig. 1) [4,5]. False-positive results of the dermal nitrate test have been reported from reactions with compounds found in tobacco, fertilisers, pharmaceuticals, leguminous plants, tyres, pesticides, dyes or fingernail polish [1].

The Lunge test is the traditional and the most commonly used method of GSR detection by forensic pathologists in the Czech Republic. During autopsy, it is mainly used to differentiate between entry and exit gunshot wounds. No other colour test for GSR detection has been used by forensic pathologists in the Czech Republic for the last 20 years.

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However, several authors have suggested that the accuracy/reliability of the Lunge test may be quite low [1-5]. That's why we conducted a cohort study to assess the results of the Lunge test in forensic pathologists' practice. The aim of the present study was to investigate the reliability of Lunge's reagent for the detection of nitrites from entry and exit gunshot wounds in the experience of our institution.

MATERIAL AND METHODS

Design and Setting

This was a prospective autopsy cohort study conducted at the University Hospital Ostrava, Czech Republic. The study was conducted in accordance with the ethical standards of the Declaration of Helsinki (1964) and its subsequent amendments. During the study period (January 2010 – December 2019), all deceased persons with gunshot wounds who were brought to the Department of Forensic Medicine were assessed for the study eligibility.

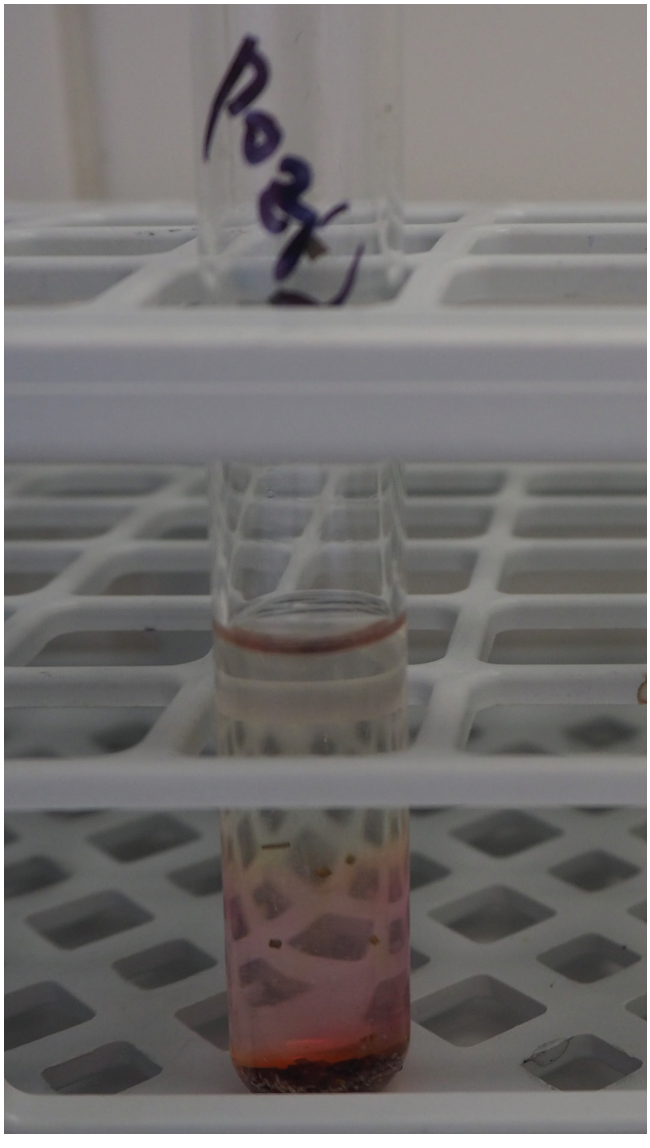

Figure 1. Positive Lunge test (dermal nitrate test) for the presence of nitrites in gunshot. Residue from an entry gunshot wound.

The primary objective of the study was to investigate the reliability of Lunge's reagent for the detection of nitrites from entry and exit gunshot wounds. Inclusion criteria were age ≥ 18 years, death after gunshot injury and autopsy performed in our department. Deceased who suffered gunshot injury from long or medium range (more than 1metre) were excluded from the study. Deaths with incompletely penetrating gunshot wounds (the bullet did not penetrate the body, so the person only had an entry wound) were also excluded. These exclusion criteria were established in an effort to create a homogeneous cohort study group with gunshot wounds from contact or near-contact range (in these individuals, entry wounds should potentially contain maximum contamination with GSR). All data were collected prospectively and analysed using descriptive statistics.

Autopsy and histopathology examination

All autopsies were performed in accordance with the Recommendation (99) 3 on the Harmonisation of Medico-Legal Autopsy Rules, adopted by the European Council in 1999 [6]. Two experienced forensic pathologists were involved in all autopsies, which have been included in the study. A standardized autopsy consisted of external and internal examination of the body. The autopsy findings were supplemented with standard histopathological samples from all major body organs.

Special attention was given to the examination of gunshot wounds by forensic pathologists. The differentiation between entry and exit wounds in each study case was based on the macroscopic findings of the gunshot wounds, the orientation of the bone fractures caused by the penetrating bullet and data from the crime scene investigation/reconstruction.

GSR collection and testing

The "ethanol extraction" technique was used to obtain inorganic GSR residues from the skin surfaces around each gunshot wound (entry and exit wounds). Samples of skin around the gunshot wound (each sample weighing approximately 5g) were immersed in an extraction solution (5g of potassium hydroxide in 100% ethanol) for 12 hours. The extraction solution was then tested for GSR using Lunge's reagent.

RESULTS

The Department of Forensic Medicine (University Hospital Ostrava, Czech Republic) provides autopsy services for the Moravian-Silesian Region with a population of 1.19 million people. During the study period, our department performed 136 autopsies (127 men, 9 women) on deceased with gunshot wounds. With regard to the study design, 56 cases were excluded from the study (29 cases with non-penetrating gunshot wounds and 27 cases with long or medium range gunshot wounds).

Figure 2. Entry gunshot wound (right temporal region on the head).

Figure 3. Exit gunshot wound (left parietal region on the head).

A total of 80 cases of perforating gunshot wounds were included in the study and underwent further analysis. There were 74 (92.5%) males and 6 (7.5%) females in the study group. The mean age was 62.3±14.1 (mean±SD), the mean height 173.1±9.3 cm, the mean weight 81.8± 12.5 kg and the mean BMI (body mass index) was 28.7± 4.7 kg/m^2.

All study cases committed suicide (individuals with gunshot wounds from medium and long range were excluded from the study because of study design). The most commonly used firearm was a handgun (85.0% of cases), with 12 (15.0%) individuals using a rifle. Gunshot wounds were located on the head in 72 (90.0%) cases, on the neck in 6 (7.5%) cases and on the chest in 2 (2.5%) study cases. Fig. 2 and 3 show an example of entry and exit gunshot wounds on the head (in this particular case, entry wound was tested positive, exit wound negative using the Lunge test).

With regard to the study design, all deceased in our study had two gunshot wounds (entry and exit wound). In all our study cases, the correct determination of entry and exit wounds was based on the macroscopic findings of the gunshot wounds, the orientation of the bone fractures caused by the penetrating bullet and data from the crime scene investigation.

Detection of nitrites from entry wounds (using the Lunge test) was positive in 41 (51.3%) cases and negative in 39 (48,7%) cases. The Lunge test was positive in 21 (26.3%) exit wounds. Analysis of the 21 cases with a positive Lunge's test from exit wounds showed that only 12 (57.1%) of these individuals also had a positive Lunge's test from entry wounds.

DISCUSSION

In firearm-related criminal cases, ballistics usually covers three different parts of the projectile trajectory – internal ballistics, external ballistics and terminal ballistics. The different aspects of ballistics are dealt with by different scientific disciplines, with internal and external ballistics being dealt with by specialists from the police or from scientific fields such as physics or mathematics [7,8]. Terminal ballistics is primarily the domain of forensic pathology, where the detection/analysis of GSR plays an important role.

The forensic pathologist focuses on the careful examination of the body, determining the number and characteristics of the gunshot wounds, the distance

of the shooting and the angle of entry of the bullet. In the vast majority of close-range gunshot wounds, GSRs are found primarily around the entry wounds. In some cases of short-range wounds, depending on the type of firearm, gunpowder is even "tattooing" the skin around entry wound [9,10]. The size, intensity and appearance of GSR patterns depend on a number of factors including ammunition type, firearm type, barrel length, firing angle, atmospheric conditions, target composition and, most importantly, firing distance [2,11].

The detection of GSR has been extensively described in the available literature as an aid to differentiation between entry and exit gunshot wounds [9-12]. It has even been postulated that an exclusion criterion for an exit wound is the presence of GSRs. However, there is a growing body of evidence showing that GSRs can also be found in exit gunshot wounds [3,12-14]. Grosse Perdekamp *et al.* published a very interesting experimental study showing that the presence of GSR can be detected along the entire shot channel. The highest amount of GSR was found around the entry gunshot wound, the amount decreased along the shot channel and it increased again in the region of the exit wound [14].

The colour/chromogenic tests have been used for GSR detection in many forensic laboratories for almost a century [15]. These methods are simple, quick and inexpensive. However, the reliability of colour tests for distinguishing entry and exit wounds and for determining the shooting distance is very questionable [1-3,5]. The Lunge test is the most commonly used colour/chromogenic test for GSR detection by forensic pathologists in the Czech Republic.

Our study data clearly demonstrated that the Lunge test is an outdated method for detecting nitrites in GSR. In only 51.3% of our study cases were the entry gunshot wounds positive using Lunge's reagent. We find this low level of reliability unacceptable (especially considering that the study design was created with the aim of creating a homogeneous group of close-range gunshot wounds). We also find it very disappointing that almost a third of the exit gunshot wounds (26.3%) in our study tested positive for nitrites. In addition, almost half of these cases had negative Lunge test results on the entry wounds. We therefore conclude that there is no longer any justification for the use of the Lunge test in forensic pathology practice.

Despite being cost-effective, colour/chromogenic tests lack reliability and specificity because of the vast number of false-negative and false-positive findings. The instrumental techniques (SEM/EDX technique, neutron activation analysis technique, atomic absorption spectroscopy or X-ray microfluorescence) have proved to be more reliable and accurate for analysis of GSR and therefore have endeavoured to replace the chromogenic test procedures [1-3,15,16].

In conclusion, Lunge test for the detection of nitrites from gunshot wounds has a very low accuracy/reliability. The distinction between entry and exit wounds by determining the presence or absence of nitrites is outdated; ballistic principles are much more complex. Therefore, the Lunge test should no longer be used in common practice by forensic pathologists.

Conflict of interest

The authors declare that they have no conflict of interest.

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