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ORIGINAL ARTICLE

Estimation of the firing distance through micro-CT analysis of gunshot wounds

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Abstract Estimation of the firing range is often critical for reconstructing gunshot fatalities, where the main measurable evidence is the gunshot residue (GSR). In the present study intermediate-range gunshot wounds have been analysed by means of a micro-computed tomography (micro-CT) coupled to an image analysis software in order to quantify the powder particles and to determine the firing distance. A total of 50 shootings were performed on skin sections obtained from human legs surgically amputated for medical reasons. For each tested distance (5, 15, 23, 30 and 40 cm), firing was carried out perpendicularly at the samples using a 7.65-mm pistol loaded with jacketed bullets. Uninjured skin sections were used as controls. By increasing the firing distance, micro-CT analysis demonstrated a clear decreasing trend in the mean GSR percentage, particularly for shots fired from more than 15 cm. For distances under 23 cm, the powder particles were concentrated on the epidermis and dermis around the hole, and inside the cavity; while, at greater distances, they were deposited only on the skin surface. Statistical analysis showed a nonlinear relationship between the amount of GSR deposits and the firing range, well explained by a Gaussian-like function. The proposed method allowed a good discrimination for all the tested distances, proving to be an objective, rapid and inexpensive tool for estimating the firing range in intermediate-range gunshot wounds.

Keywords Terminal ballistics · Firing range · Gunshot residue · Micro-CT

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Introduction

The evaluation of the firing range has often a critical importance in gunshot fatalities [1, 2]. When the issue of suicide, homicide and accidental firing is in question, the investigation of gunshot residue (GSR) particles gains extensive forensic significance [3]. These products of firearm discharge are principally composed of burnt and unburnt particles from the propellant, as well as components from the primer, the bullet and its jacket, and from the cartridge case [4, 5]. The amount and distribution of GSR reaching the target surface are generally considered to be related to the firing range [6].

In the past, forensic scientists have studied the distribution of GSR at the entrance wound using a variety of methods, including visual inspection [1], as well as chemical (Walker test, Greiss test, etc.) [7, 8] and histochemical techniques (Alizarin Red S [6, 9, 10] or sodium rhodizonate [11, 12] stainings). Other methods for characterising GSR include atomic absorption spectroscopy [13], neutron activation analysis [14, 15], autoradiography [14], anodic stripping voltammetry [16], scanning electron microscopy [17] and 3-dimensional computed tomography [18, 19].

Recently, micro-computed tomography (micro-CT), a tomographic technique with a spatial resolution of a few microns, has opened the possibility of performing tri-dimensional histological investigations on a virtual representation of the sample [20]. The aim of our investigation was to test this novel application coupled to an image analysis software for the determination of the firing distance in intermediate-range gunshot wounds, experimentally produced on human leg sections.

Materials and methods

Samples

After approval of the Ethical Committee of the University-Hospital of Padua (Protocol n. 2013P), 50 sections of approximately 6 cm in length were obtained from human legs, surgically amputated for medical reasons (circulatory disorders, road or workplace trauma). Inclusion criteria were male sex, and age between 20 and 50 years. Exclusion criteria were neoplastic and/or inflammatory and/or infective diseases of the skin, scars and traumatic skin wounds. Uninjured skin sections were used as controls ($n=10$). Each sample was cleaned of dried blood and any other contaminants before the firing trials.

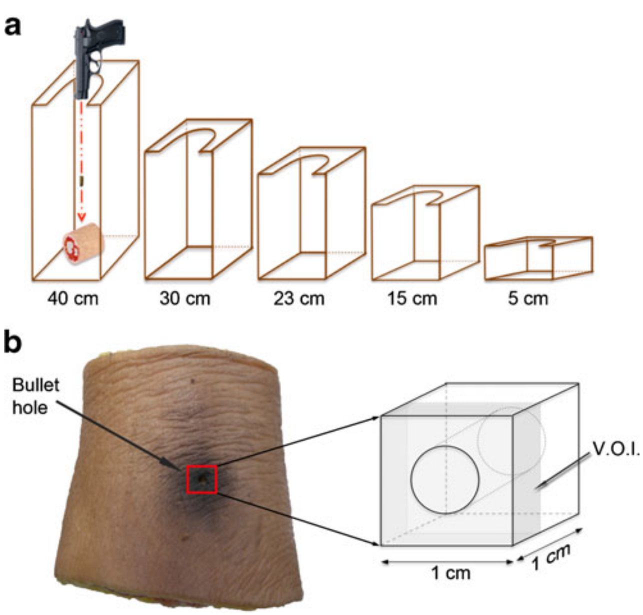

Fig. 1 **a** Sketch of the stands used to perform the firing trials (40, 30, 23, 15, and 5 cm); **b** diagram showing the preparation of a skin sample (a cube with a edge length of 1 cm, comprising the epidermidis, dermis and subcutaneous fat) for micro-CT analysis; the volume of interest (*VOI*) has a side of 1 cm and a height of 3.8 mm

Firing trials

Shooting experiments into the leg sections were carried out in a ballistic laboratory at distances of 5, 15, 23, 30 and 40 cm, using a 7.65-mm semi-automatic pistol (Beretta mod. 81) loaded with 7.65×17 mm Browning SR and full metal-jacketed bullets. The firearm was placed on a fixed stand and the surface of the skin sample was kept perpendicular to the muzzle of the gun (Fig. 1a). All the ammunitions came from the same production lot. For each firing distance, ten shots were performed to determine the variability in the amount of GSR particles within and around the entrance hole at each firing range.

Micro-CT analysis

The skin specimens, comprising the epidermis, dermis and subcutaneous fat, were cut into parallelepipeds (height 1 cm, side 1 cm) with a lancet and fixed in formalin (4%, pH 7.4) for 3 days (Fig. 1b). After vertical positioning in a cylindrical polyethylene container (1.1-cm-diameter) with formalin, the samples were scanned using a Skyscan 1172 HR Micro-CT (Skyscan, Aartselaar, Belgium). Micro-CT settings were as follows: 100 kV of voltage; 100 μA of current; filter of aluminium with 1 mm of thickness to reduce the beam hardening artefact; 1,280×1,024 pixel of Field of View (FOV); 13 μm of isotropic voxel size. All samples underwent a 360° rotation, with a rotation step of 0.4° and a frame averaging of two. The acquired raw data were reconstructed with a N-Recon software (Skyscan, Aartselaar, Belgium) which uses the back-projection algorithm to reconstruct axial subsequent images saved as bitmap format. To calibrate the Hounsfield Units (HU), one water phantom was acquired and reconstructed at the same sample settings.

The bitmap images were analysed by a CT-An Software (Skyscan, Aartselaar, Belgium): the selected volume of interest (VOI side of 1 cm and height of 3.8 mm) was focused into the centre of the specimen in order to position the entire entry wound in the middle. All the samples were binarized using the same parameters.

Since GSR deposits are mainly formed of heavy metal particles (such as barium, antimony, lead, etc.), their percentage (considered as the proportion of the VOI occupied by binarised solid objects) was calculated analysing all particles with a density higher than 1,000 HU (particles with a density lower than 1,000 HU were excluded to reduce iron artefacts). The 3D images were reconstructed through a CT-Vox Software (Skyscan, Aartselaar, Belgium).

Statistical analysis

Statistical analysis comprised two main phases. In phase 1, through a curve fitting process, the mathematical model

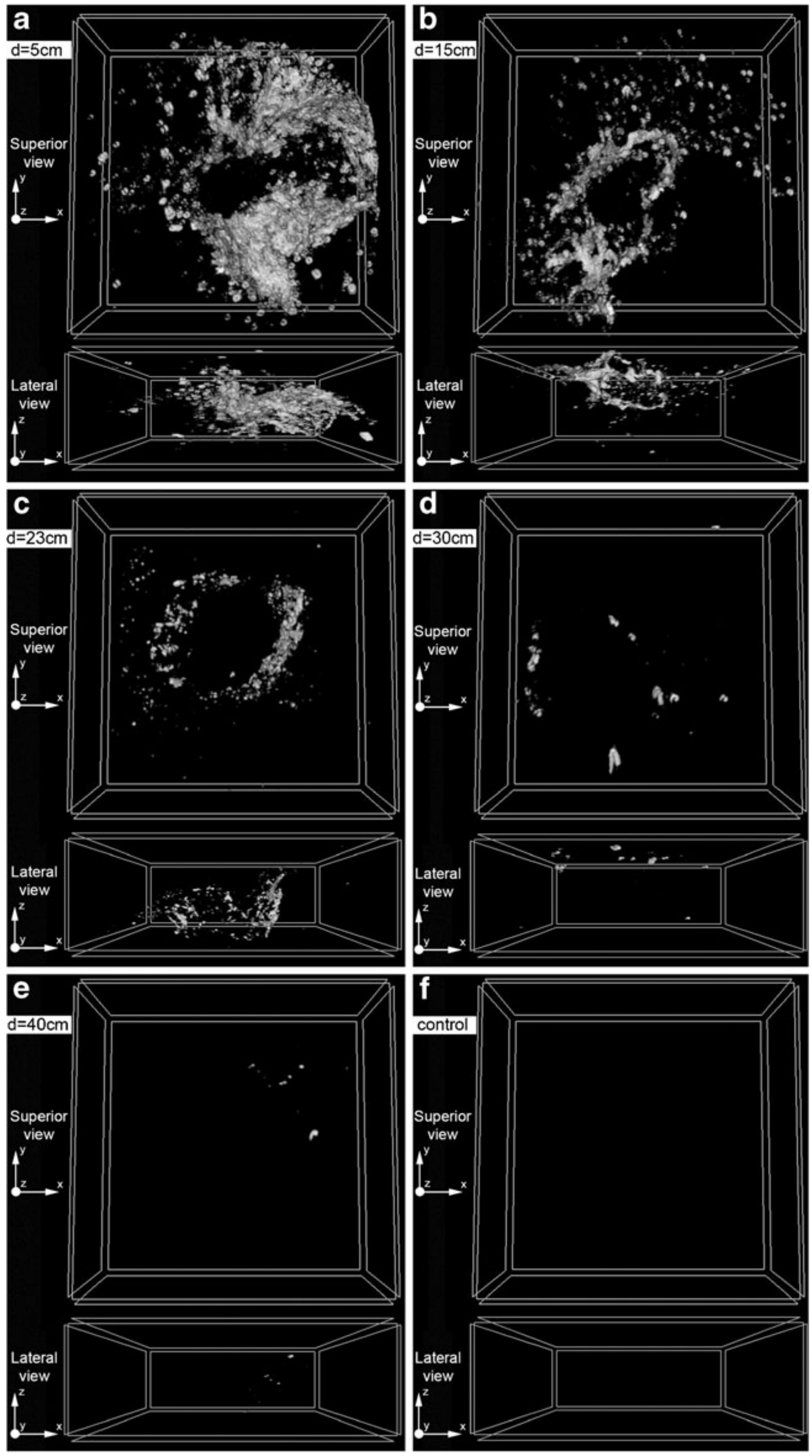

Fig. 2 3D reconstructions of the gunshot wounds at different firing distances. Only particles of density higher than 1,000 HU are shown. For the 5 cm (**a**), 15 cm (**b**) and 23 cm (**c**) distances, the GSR was concentrated on the skin around the hole, in the epidermis and dermis layers, and inside the cavity. For the 30 cm (**d**) and 40 cm (**e**) the GSR was deposited only on the skin surface. No GSR particles were detected in control specimens (**f**)

which best described the relationship between GSR percentages and the firing distances was chosen, and the R^2 index was calculated.

The statistical estimation of the model applied to our data was performed through a PROC NLIN procedure with the software SAS®9.2 (SAS Institute INC, Cary, North Carolina). Subsequently, the 95% confidence interval (95% CI) of the interpolation function was calculated.

In phase 2, the target was the estimation of the firing distance based on a given GSR percentage. Thus, a mathematical model derived from the inversion of the interpolation function was calculated. The parameters of the

Table 1 Results of the shooting trials. The mean percentages of GSR particles, standard deviation (SD), minimum and maximum values of GSR deposition, are reported for each firing distance (5, 15, 23, 30 and 40 cm)

Analysis variable (GSR percentage)					
Firing range	Number of shots	Mean	SD	Minimum	Maximum
5 cm	10	0.420	0.038	0.370	0.470
15 cm	10	0.211	0.021	0.180	0.250
23 cm	10	0.044	0.011	0.030	0.060
30 cm	10	0.028	0.018	0.010	0.050
40 cm	10	0.002	0.001	0.001	0.003
Controls	10	0.000	0.000	0.000	0.000

model were estimated using a PROC NLIN procedure with the software SAS®9.2 (SAS Institute INC, Cary, North Carolina). The 95% CI was calculated for a single firing distance.

Results

At micro-CT analysis, a clearly decreasing trend in the mean GSR area was observed by increasing the firing distance (Fig. 2). In particular, the amount of GSR particles (density higher than 1,000 HU) was noticeably reduced when the shot was fired from more than 30 cm, compared with firing ranges of less than 23 cm (Fig. 2). For the 5-, 15- and 23-cm distances, the GSR were concentrated on the skin around the hole, in the epidermis and dermis layers, and inside the cavity (Fig. 2a–c); while, at greater distances, they were deposited only on the skin surface (Fig. 2d–e). No GSR particles were detected in control specimens (Fig. 2f). The mean percentages of GSR deposits for each tested firing distance are listed in Table 1.

Statistical analysis showed a nonlinear relationship between the amount of GSR deposits and the firing range. A negative exponential model (R^2=0.9749), and a second order polynomial model (R^2=0.9725) were fit to the experimental data appearing, however, poorly performing. A Gaussian model (Fig. 3a) was found to better describe the relationship between the firing distance (independent variable 'x') and the GSR percentage (dependent variable 'y'; F [2; 48]=1,919.7, P<0.0001; R^2=0.9876; Fig. 3b). The estimated function of the model is:

$$y = 0.4646 \times \exp\left[-(x/16.3134)^2\right]$$

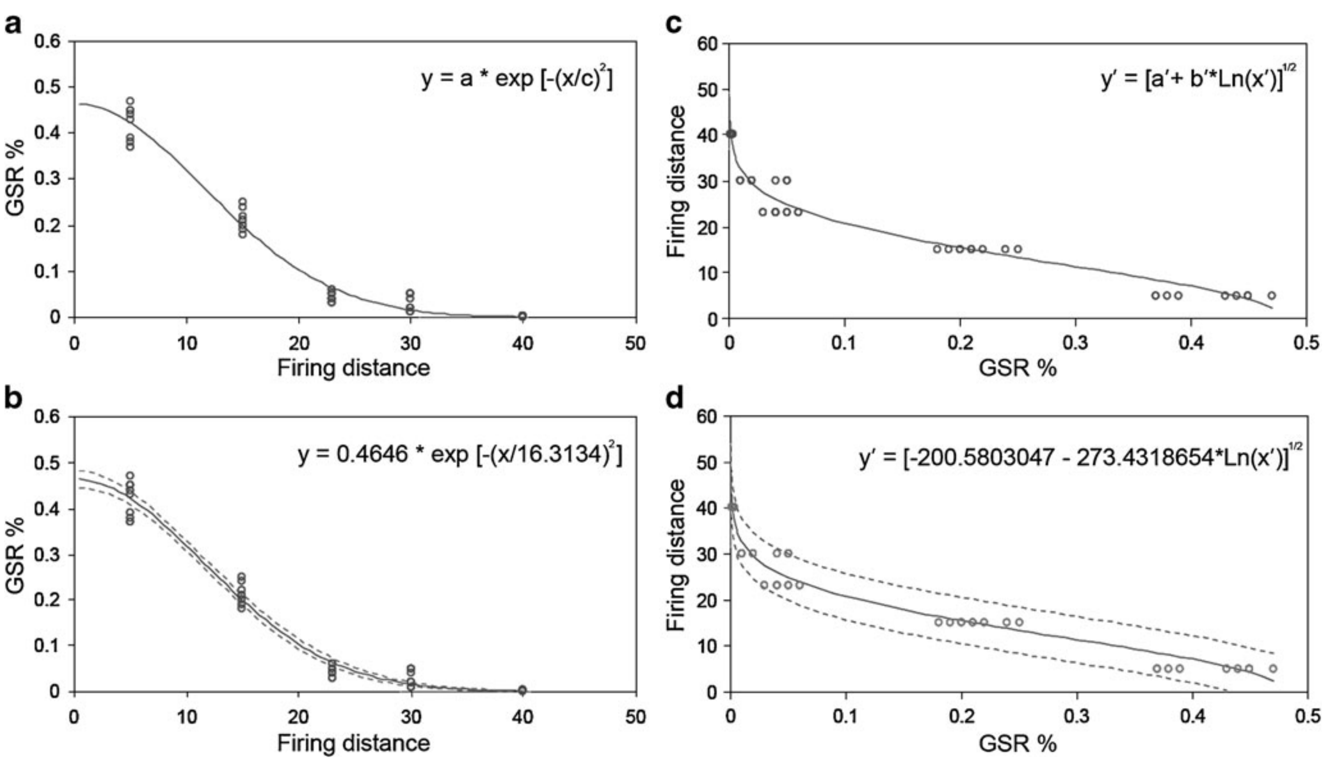

Fig. 3 **a** Graphs showing the Gaussian-like curve and its function, which were found to best fit the experimental data; **b** graphs showing the regression function estimated through a nonlinear regression analysis

Table 2 Estimation of the firing distance and its 95% CI given a known value of the GSR percentage (several calculation examples are reported)

GSR%	Firing distance	Lower 95% CI	Upper 95% CI
0.0001	48.1437	42.9363	53.3512
0.0005	43.3330	38.1632	48.5028
0.0010	41.0880	35.9343	46.2416
0.0050	35.3291	30.2130	40.4453
0.0100	32.5364	27.4362	37.6366
0.0500	24.8706	19.8059	29.9354
0.1000	20.7128	15.6612	25.7644
0.1500	17.8368	12.7909	22.8828
0.2000	15.4755	10.4312	20.5199
0.2500	13.3595	8.3132	18.4059
0.3000	11.3413	6.2880	16.3945
0.3500	9.2992	4.2300	14.3684
0.4000	7.0684	1.9588	12.1781
0.4500	4.2139	0	9.5046
0.4600	3.4274	0	8.8590
0.4700	2.4222	0	8.2522

The algebraic inversion (Fig. 3c) of the above-reported function and the statistical evaluation of its parameters permitted to estimate the relationship between GSR percentages (independent variable 'x') and the firing distances (dependent variable 'y'; F [2; 48]=2,609.03, $P<0.0001$; R^2=0.9909; Fig. 3d):

$$y' = [-200.5803047 - 273.4318654 \times \mathrm{Ln}(x')]^{1/2}$$

The distance values and their 95% CI estimated on the basis of a number of GSR percentages are reported in Table 2.

Discussion

GSR analysis has been widely studied in the forensic literature for the estimation of the firing range [6–19]. Although animal models (pig [19, 21, 22] or goat [6, 10]) have been mainly used in experimental investigations of firearm injuries, wounds in animals are not directly comparable to those in human beings because of the differences in the thickness and structure of the skin and the subcutaneous tissue, especially, in terms of retracting properties [23]. Furthermore, as the gross anatomy of the animal differs from the human, the entrance and exit holes may have a different morphology [24].

To avoid these limitations, we have elaborated a standardised experimental protocol using human skin samples derived from surgically amputated legs, the same weapon and fixed stands with heights from 5 to 40 cm (Fig. 1a). Only intermediate-range gunshot wounds (5–40 cm) were investigated because when present they might offer critical information for reconstructing the dynamics of the event, above all when the issue of suicide, homicide and accidental firing is in question [25].

In the last decades, several techniques have been used for the detection and identification of GSR particles in intermediate-range gunshot wounds [6–19]. These experiments have clarified that the amount and spatial distribution of GSR on the skin surrounding the entrance wound is strictly correlated to the shooting distance [10, 12].

Noteworthy are the results obtained by Brown et al. [6, 10] who performed a statistical analysis of the relationship between the firing range and the amount and distribution of GSR deposits in firings below 45 cm performed on pig skin. Using Alizarin Red S staining and an automated image analysis software the authors showed a decreasing trend in the mean GSR area by increasing the firing distance. In addition they found that GSR particles were heavily concentrated in the wound tract only for contact and close-range shots at 2.5 cm, while the particle distribution was more uniform between the wound tract and the skin surfaces for shots fired from more than 2.5 cm.

Our results, obtained scanning the entire entrance wound by means of a micro-CT system coupled to an image-analysis software, confirm the findings by Brown et al., clearly showing that, in intermediate-range gunshot wounds, the amount of GSR resulting from the discharge of the firearm is dependent in a non-linear fashion on the distance from which the gun was fired. Indeed, using a curve fitting process, a Gaussian model was found to best describe the relationship between the firing distance (independent variable 'x') and the GSR percentage (dependent variable 'y'), with R^2=0.9876 (Fig. 3b).

Although histochemistry [9–12] and electron microscopy methods [17] are accurate and precise for estimating the firing distance, they require a labour intensive exercise, because each specimen has to be cut into several sections, and each section requires a microscopic analysis.

On the contrary, in our work, the entrance wound was cut into a single cube (edge length=1 cm), formalin fixed and scanned with a micro-CT system. This procedure, which is less time-consuming and expensive than histochemistry or electron microscopy, allowed to simultaneously analyse the epidermidis and dermis around and inside the entrance hole and to perform tri-dimensional reconstructions of the spatial distribution of the GSR particles, permitting a gross optical discrimination between shots fired from distances greater than 30 cm and those from 23 cm or less; moreover, statistical analysis found significant differences in the amount of powder residue deposited on the skin at each tested firing distance (Fig. 3a, b). Based on the Gaussian function $\left(y' = [-200.5803047 - 273.4318654 \times \mathrm{Ln}(x')]^{1/2}\right)$ it was possible to estimate the firing distance (expressed as a 95% CI of values) given a known percentage of the GSR deposit. For example, a GSR% of 0.15% corresponds to a firing distance of 17.8 cm (95% CI 12.8–22.9 cm; Table 2). Obviously, to reduce the size of the firing range corresponding to a single GSR deposit, the shootings experiments for each distance and the number of tested distances should be increased.

The potential effect of the material picked up by the bullet during its passage through the bore and left on the skin of the entrance hole (bullet wipe) on the variability of the GSR amount for a specific firing distance has been described also for full metal-jacketed bullets [26, 27]. In our study, however, no specific experiments were carried out in order to quantify the percentage of deposits referable to the bullet wipe mark and its influence on the standard deviation.

Beyond that, the present method exhibits the limits discussed below. First, micro-CT analysis gives an indirect identification of GSR particles; thereby, substances with the same density of powder particles might be erroneously tagged as GSR, generating a false positive result. Considering that micro-CT is a non-destructive technique [20], a possible solution for the above-mentioned problem could be the identification of the chemical composition of the metal residues by means of an environmental scanning electron microscope coupled to an X-ray fluorescence energy dispersive spectroscope [28], thus strongly reducing the possibility of a misinterpretation of the results.

Another limit might be the decision to calculate the percentage of GSR excluding all the particles with a density lower than 1,000 HU; this implies that a range of light metal alloys could not be detected as GSR deposits. However, in order to determine the firing distance, a precise quantification of the real amount of the particles is probably not as important as the measurements of the relative variations in the amount of GSR between different firing distances.

Finally, since the relationship between the distribution and the amount of firearm discharge residue associated with a gunshot wound depends on several variables [22, 29], micro-CT analysis might be of practical use only if experiments are conducted scanning both the victim's gunshot entrance holes, and a minimum number of experimental wounds produced on human skin samples (i.e. ten replicates for each firing distance). Moreover, when the weapon is not found during the crime investigation, or the bullets are not collected at the autopsy, it is fundamental that an equivalent type of firearm and ammunition (or at least a gun of the same calibre) are used during the experimental shootings, in order to control the variables related to the type of firearm [22, 29].

In conclusion, our work, which is the first application of micro-CT analysis in the field of forensic ballistics, demonstrates that micro-CT is an objective, rapid and inexpensive tool for estimating the firing range in firearm fatalities. In the next future, further work will be necessary to test the proposed method on forensic casework, as well as on samples altered by the effects of fire, water or by post-mortem decomposition, for estimating its sensitivity and specificity.

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