J Forensic Sci, May 2009, Vol. 54, No. 3
doi: 10.1111/j.1556-4029.2009.01023.x
Available online at: www.blackwell-synergy.com

TECHNICAL NOTE

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Morphometric Investigation of Death by Asphyxia

ABSTRACT: The aim of this study was to investigate the possibility of distinguishing deaths by asphyxia from those due to natural causes by comparing morphometric measurements in lungs. The study population comprised 27 subjects: 14 cases of death by asphyxia (hanging or drowning) and 13 cases of sudden natural death. Eighty parenchyma sections were used for each subject. Data were collected by computerized image analysis. Measurements aimed at quantifying, as percentages, pulmonary parenchyma (mean values of positive-fraction areas [PFA] and their standard deviations). Subjects who died of natural causes and of drowning showed a relative internal homogeneity compared to those who hanged. Results show significant discrimination between drowned subjects and those dying of natural causes (mean of PFA $p = 0.01$) and between hanged and drowned subjects (SD of PFA $p = 0.04$). Attention must be paid to the possible role played by senile emphysema. The method is proposed as a complementary tool in forensic cases.

KEYWORDS: forensic science, forensic pathology, asphyxia, morphometry, quantitative pathology, differential diagnosis, hanging, drowning

Postmortem diagnosis of death by asphyxia involves not only assessment of circumstantial, documentary, and anatomo-pathological data, but also histological examination. However, in practical forensic work, the finding of pulmonary emphysema (also of senile type) is frequently found in subjects who died a natural death. This aspect may complicate definite establishment of the cause of death.

This pilot study aims at identifying an objective parameter, measurable and comparable in histological specimens of lung samples, for use as a differential diagnostic aid in cases of doubt regarding death from asphyxia or a natural event.

The application of analytical morphometry, mainly in the anatomo-pathological sphere, has been proposed in the past, when technological means were far from the potential offered by present-day methods (1–3). Later, also in the forensic field, semi-quantitative or morphometric techniques were more extensively proposed (image superimposition, manual point counting, semi-automated histomorphometry using a manual digitizer, automated image analysis) (4–7), with proposed applications in cases of asphyxial deaths (8–10).

Methods

Our sample population was composed of 27 subjects, selected from routine autopsy cases according to the following criteria of exclusion: age at death >75 years, signs of general or pulmonary putrefaction, and occasional pulmonary pathology, such as pneumonia or severe chronic bronchitis. The mean interval between death and autopsy (in many cases rated integrating all available data) was 50 h (range 24–80). Subjects' characteristics (14 deaths due to mechanical asphyxia, 13 due to natural causes of cardiac origin, hereafter called natural deaths) are listed in Table 1. All subjects who died from myocardial infarction or myocarditis were not hospitalized and death, unexpected, occurred suddenly, unwitnessed in some cases.

Choice and Treatment of Samples

Four cube-shaped fragments, approximately 2.5 × 2.5 cm, were taken from the costal surface (including pleura) of upper and lower lobes of the lung. Each fragment was routinely processed for optical microscope evaluation: fixing in formalin 4% (each fragment in one container filled with the same volume; change of formalin after 4 days); embedding in paraffin; microtome sections (thickness of 8 μm); hematoxylin-eosin staining.

Image Analysis

Images of all fields were evaluated on a CIRES workstation image analysis system (Zeiss, Jena, Germany), consisting of a conventional light microscope (Axioskop, Zeiss) connected to a 3CCD color video camera (KY-F55BE, Victor Company of Japan [JVC], Yokohama, Japan). The images were captured by a frame grabber (Kontron, Eching, Germany) and then analyzed. The frame grabber and the image analysis (IA) program, operating on-line with the camera, were hosted by a PC. During all measurement sessions, illumination was kept constant at a fixed value and the stray light effect was reduced using Koehler's illumination setting. The on-line segmentation and measurement routine allowed rejection of artefacts and revision of all selected areas after measurements. Red-green-blue (RGB) grabbed pixels corresponding to the images were processed and coded by the software in a gray intensity scale according to luminance ($y = 0.3R + 0.6G + 0.1B$) from 0 to 256 gray values (8-bit coding) in our measurements (11–13).

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Received 26 Jan. 2008; and in revised form 7 May 2008; accepted 9 Aug. 2008.

TABLE 1—*Distribution of sample population by cause of death.*

Deaths Because of Asphyxia (n = 14)		Deaths Because of Natural Causes (n = 13)	
Female: 7	Male: 7	Female: 7	Male: 6
Hanging: 9 (2 complete)	Drowning (freshwater): 5	Myocardiac infarct: 12	Myocarditis: 1
Mean age: 47 years (range 42–55)		Mean age: 69 years (range 59–75)	

Each pulmonary section was examined with 2.5× objective. Twenty fields from each histological specimen, for a total of 80 fields per subject, were evaluated, moving randomly along the slide.

Segmentation and measurement: an adaptive threshold was applied, based on levels of gray, of the parenchymal component of each image, with automatic binarization. These operations involved: skipping fields containing large vasal or bronchial components; considering the percentage of pixels of pulmonary parenchyma with respect to total framed area (corresponding to the parameter positive-fraction area: PFA); excluding any edema (manually, according to previous method) (4) from calculation of the PFA, with the aim to consider only parenchymal modifications; obtaining the derived parameter standard deviation (SD) of the PFA. Observation was carried out according to a single blind, random distribution design from an experimenter not involved in autopsy cases performing and selection.

Statistical Analysis

The following three groups were distinguished: (a) deaths due to hanging; (b) deaths because of drowning; (c) natural deaths. Mean and SD of PFA were calculated for each group. Comparative studies using one-sided *t*-test, corrected for differences in variances, were applied as follows:

(a) subjects who died by hanging versus those who died of natural causes;
(b) subjects who died by drowning versus those who died of natural causes;
(c) subjects who died by drowning versus those who died by hanging;
(d) subjects who died by asphyxia (drowning and hanging) versus those who died of natural causes.

In view of the size of the sample and the absolute variations found, we considered as significant values of $p < 0.05$ and $p < 0.20$ as indicating possible differences to be verified by enlarging the study population. Values of $p > 0.20$ were not considered significant.

Results

Figures 1 and 2 show examples of the histological specimens from a subject who died of drowning, with images processed to calculate the parenchymatous fraction. Values obtained for each subject (Mean of PFA, SD of PFA, and age) are listed in Table 2.

Mean and SD of PFA for each group are listed in Tables 3 and 4. Figure 3 illustrates the clustering of the three groups according to the mean of PFA. Figure 4 describes the clustering of the three groups according to the SD of PFA. Figure 5 shows the scatterplot of the PFA as a function of age. The results of statistical analysis for group comparisons are shown in Tables 5–8.

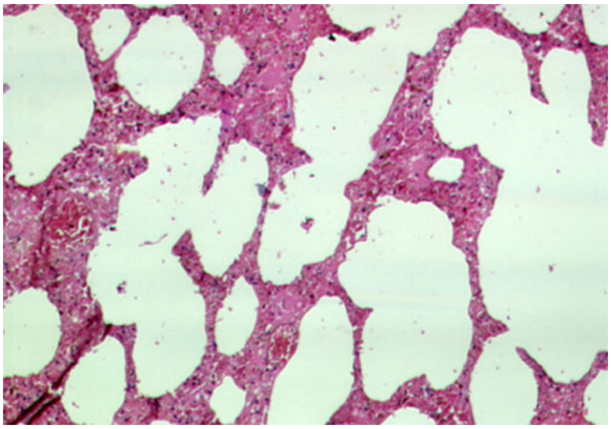

FIG. 1—*Histological specimen from a subject who died of drowning. Hematoxylin-eosin staining (2.5×).*

FIG. 2—*Binary image of the previous sample: white denotes parenchymal regions. No deletion of intra-alveolar structures or edema needed.*

Discussion and Conclusions

The same operator noted substantial morphological homogeneity in the four samples from each subject. Preliminary analyses by morphometric parameters did not reveal significant differences in the same subject, according to site of sampling. This led us to consider all measurements from every single subject together.

Results demonstrated two significant variations, and some differences were found. The type and extent of the discrimination among the three groups according to the parameters' mean of PFA and SD of PFA, which express the different degrees of pulmonary emphysema, allow us to make the following considerations.

The data from the two groups of subjects who died of natural causes and of drowning both showed internal homogeneity of values, as clearly illustrated in the corresponding box plots. Instead, those of subjects who died of hanging had widely ranging morphometric values, from 25.44 to 61.70 (mean of PFA) and from 9.33 to 15.56 (SD of PFA).

This means that, contrary to expectations, the degree of emphysema is neither typical nor greater in subjects who died of asphyxia. Therefore, in many cases of death due to natural causes, a conspicuous if not prevalent degree of emphysema exists. We report the possible role, within data variability, played by senile emphysema, which is very often although not exclusively found in long-term smokers. The considerable difference in age between the

TABLE 2—*Results of morphometric analysis of all cases.*

Subject (Cause of Death)	Positive Fraction Area (Mean)	SD of PFA	Age
Hanging			
1	35.82	11.09	42
2	25.44	9.33	43
3	27.98	10.5	44
4	61.36	15.56	45
5	30.16	14.42	46
6	29.96	13.02	44
7	49.43	14.78	44
8	61.7	13.74	49
9	52.61	10.41	50
Drowning			
10	42.76	13.16	51
11	36.7	9.83	52
12	31.12	10.29	48
13	27.12	9.12	48
14	34.86	10.8	55
Natural causes			
15	32.29	11.04	59
16	38.3	12.98	71
17	46.09	14.74	68
18	51.82	11.59	66
19	42.33	10.5	67
20	51.27	11.61	66
21	44.54	12.13	70
22	29.34	9.06	69
23	46.84	13.57	72
24	45.8	12.04	69
25	48.94	12.34	69
26	48.73	12.93	73
27	39.55	11.47	75

TABLE 3—*Results according to parameter means of PFA.*

PFA	Hanging (9)	Drowning (5)	Natural Causes (13)
Mean	41.60	34.51	43.52
Median	35.82	34.86	45.80
SD	14.67	5.89	6.95
Minimum value	25.44	27.12	29.34
Maximum value	61.70	42.76	51.82

TABLE 4—*Results according to parameter SD of PFA.*

SD of PFA	Hanging (9)	Drowning (5)	Natural Causes (13)
Mean	12.53	10.64	12.00
Median	13.02	10.29	12.04
SD	2.24	1.53	1.42
Minimum value	9.33	9.12	9.06
Maximum value	15.56	13.16	14.74

three groups studied here must be noted. In any case, the results indicate that the group with the greatest inter-individual variability, i.e., the most dishomogeneous, was that of subjects who died of hanging.

After preliminary data evaluation, when the number of cases was smaller, this group showed a far lower degree of emphysema than those who died of natural causes. It was therefore hypothesized that the physiopathological mechanism of death is mainly of nervous origin, and that its rapid onset prevents the manifestation of the typical anatomic alterations.

When the number of cases was increased, this hypothesis was refuted, and the internal heterogeneity of the subjects who died of

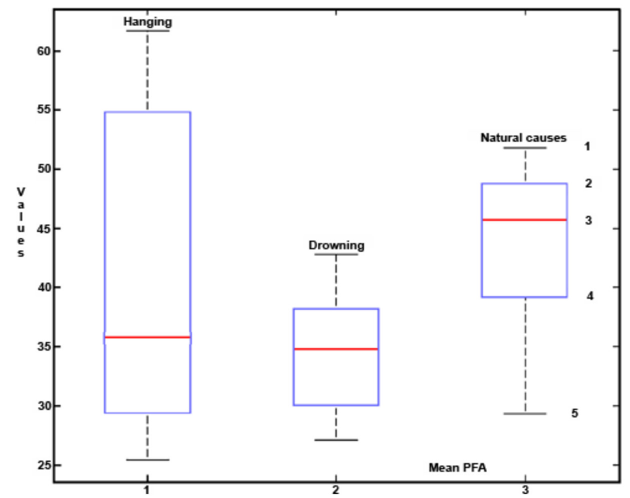

FIG. 3—*Box plot of the three different groups according to the parameter "mean of Positive Fraction Area (PFA)." 1, Maximum value; 2, third quartile; 3, median; 4, first quartile; 5, minimum value.*

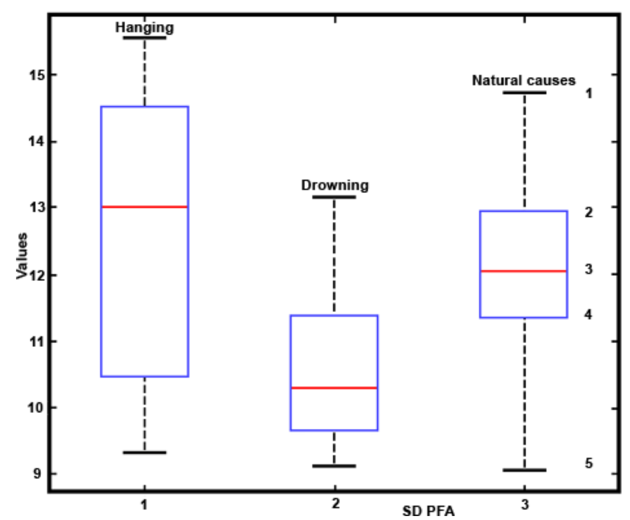

FIG. 4—*Box plot of the three different groups according to the parameter SD of PFA. 1, Maximum value; 2, third quartile; 3, median; 4, first quartile; 5, minimum value.*

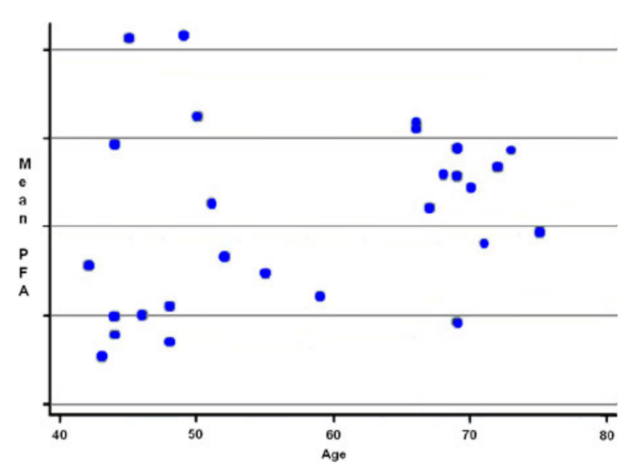

FIG. 5—*Scatter plot of the mean of PFA and age of each subject.*

TABLE 5—*Comparison between hanging and natural causes.*

Comparison Hanging/Natural Causes	SD of PFA $p = 0.26$	Not significant
	Mean (PFA) $p = 0.36$	Not significant

TABLE 6—*Comparison between drowning and natural causes.*

Comparison Drowning/Natural Causes	SD of PFA $p = 0.06$	Indicative
	Mean (PFA) $p = 0.01$	Significant

TABLE 7—*Comparison between hanging and drowning.*

Comparison Hanging/Drowning	SD of PFA $p = 0.04$	Significant
	Mean (PFA) $p = 0.11$	Indicative

TABLE 8—*Comparison between asphyxia and natural causes.*

Comparison Asphyxia/Natural Causes	SD of PFA $p = 0.42$	Not Significant
	Mean (PFA) $p = 0.13$	Indicative

hanging required a different interpretation. The well-known combinations of physiopathological mechanisms responsible for death by hanging (nervous, vascular and asphyxial) and their prevalence in each single case may explain the heterogeneity of our results.

As regards comparative statistics, a good level of data significance was reached, demonstrating the applicative potential of morphometric measurements in helping differential diagnosis of cause of death. The three groups examined here were compared in pairs, and the variables mean and SD of PFA were examined each time.

Comparisons between subjects who died of drowning or of natural causes by *t*-test, revealed a significant value, clearly discriminating two groups ($p < 0.01$). Comparisons between subjects who died of hanging and those who died of natural causes yielded quite different results, with no group discrimination.

Values with statistical significance or indicative of discrimination, to be verified after increasing the number of the sample population, were obtained by comparisons between drowned and hanged subjects ($p = 0.04$ for SD of PFA and $p = 0.11$ for mean).

Only one group of subjects who died of asphyxia (drowned) could be discriminated from that of subjects who died of natural causes. This explains why the entire group of asphyxial deaths, overall considered, could only be slightly differentiated from the group of natural deaths. For this kind of comparison, the most encouraging results came from analysis of the mean ($p = 0.13$, Table 8), with the clearcut absence of significance of the comparisons between SDs.

Because of the possible interference of age on results (scatter diagram in Fig. 5), we performed a Spearman rank test that showed a not significant value for both parameters: mean of PFA ($p = 0.13$) and SD of PFA ($p = 0.71$). According to this experimental study, we conclude that:

- the method proposed can be helpful (used together with well-established methods) to discriminate between death due to sudden cardiac arrest and that resulting from drowning, using the parameters' mean of the PFA, which express the degree of emphysema;
- the parameter SD of PFA (i.e., variability of emphysema in different fields of the lungs) can help to distinguish hanged subjects from drowned ones;
- death because of asphyxia presents an intrinsic heterogeneity in quantitative data. This heterogeneity is definitely greater in the group of subjects who died of hanging, and is probably due to the complex mechanism of death, which is not simply asphytic.

In our view, the results of this pilot study are of interest. Larger population samples, comorbidities consideration, agony lasting evaluation, and weighting of other confounding parameters will be necessary to better clarify the found variability. At the moment results suggest that, with respect to differential diagnosis between some natural death with sudden cardiac arrest and death by asphyxia (which is sometimes difficult, and may lead to serious penal consequences), morphometric study of routine histological preparations represents an additional technical and objective element aiding pathologists in their judgment.

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