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TECHNICAL NOTE

PATHOLOGY/BIOLOGY

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A Minimally Invasive Technique for the Detection and Analysis of Pulmonary Fat Embolism: A Feasibility Study*

ABSTRACT: We investigated the feasibility of postmortem percutaneous needle biopsy (PNB) for obtaining pulmonary samples adequate for the study of pulmonary fat embolism (PFE). Samples of both lungs were obtained from 26 cadavers via two different methods: (i) PNB and (ii) the double-edged knife technique, the gold standard at our institute. After water storage and Sudan III staining, six forensic pathologists independently examined all samples for the presence and severity of PFE. The results were compared and analyzed in each case regarding the vitality of the PFE and its relationship to the cause of death. The results showed that PFE was almost identically diagnosed and graded on the samples obtained via both methods. The discrepancies between the two techniques did not affect the diagnoses of vitality or cause of death related to PFE. This study demonstrates the feasibility of the PNB sampling method for the diagnosis and interpretation of PFE in the postmortem setting.

KEYWORDS: forensic science, forensic pathology, virtual autopsy, pulmonary fat embolism, percutaneous needle biopsy, double-edged knife

One of the most important issues in forensic pathology is the investigation of vital signs to assess whether an injury occurred before or after death. Vital reactions primarily depend on the presence of intact respiration, circulation, metabolic processes, and consciousness after a damaging event. One of the most commonly investigated signs of vitality is fat embolism (FE).

FE is usually asymptomatic, but its clinical manifestation, the so-called fat embolism syndrome (FES), consists of an identifiable pattern of signs and symptoms as a result of the dysfunction of several organs, mainly the lungs, brain, and skin (1–5). Otherwise, the most common presentation of FES is via respiratory symptoms, with variable severity, including death. In fact, the lungs are usually the first organ involved and the most severely affected during FE. Two pathophysiological mechanisms involving the lungs have been suggested to explain fatal cases of FE (1,6). In an early mechanical phase, fat droplets occlude lung capillaries, impairing gas exchange. Then, toxic and biochemical effects associated with chemical pneumonitis, vasculitis, and the related perivascular hemorrhage and edema may result in acute respiratory distress, and consequent hypoxia can lead to death.

The postmortem diagnosis and grading of pulmonary fat embolism (PFE) are traditionally based on the histological demonstration and analysis of fat droplets within the lung microcirculation. A fervent debate exists in the literature regarding the etiology, pathogenesis, diagnostic, and forensic value of PFE. The most frequently reported causes of PFE are major fractures, particularly of long bones, the pelvis, or the spine, and the related soft tissue damage because of trauma, with an incidence ranging between 47 and 100% following major trauma (7–12). PFE is also reported to be associated with cardiopulmonary resuscitation maneuvers, advanced putrefaction, inflammation of bone and adipose tissue, extensive cutaneous burns, poisoning, steroid therapy, alcoholic fatty liver, acute pancreatitis, diabetes, barotrauma, surgical operations on fatty tissues, sickle cell disease, transplanted lungs from a traumatized donor, and blast injuries (1,6,13–17).

The documentation and grading of FE are crucial medico-legal issues, primarily for two reasons. First, the detection of PFE could be interpreted as a sign of antemortem violence, although this conclusion may be confounded by energetic attempts at resuscitation. In trauma cases with attempted resuscitation, where there is potential for low-grade PFE, it is very difficult to distinguish whether it occurred as the result of antemortem violence or whether it happened as the result of resuscitation maneuvers, or a combination of the two (6,17). Moreover, at advanced states of decomposition, or in patients who died a nontraumatic death, including burns and the natural causes described above, the postmortem evidence of PFE cannot be used as evidence in favor of the antemortem nature of fractures found at autopsy (1,13–16). Second, the documentation of moderate or massive embolism, together with the consideration

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of other data (6), could suggest to the forensic pathologist a hypothesis regarding the cause of death.

Although postmortem imaging is a useful tool in many fields of forensic investigation, these methods fail to completely document histopathological findings because their image resolution is not high enough to demonstrate cellular changes at the microscopic level. As reported by Thali et al. (18), one example of forensic investigation in which imaging falls short is the detection of FE. Thus, both traditional autopsy and virtual autopsy currently rely on a histopathological analysis for the definitive diagnosis of PFE. Prior studies from the Virtopsy Group (19–22) demonstrated that postmortem biopsy can provide tissue specimens of a sufficient size and quality for histological examination and forensic conclusions, even for PFE (23), but, at this time, no systematic evaluations of minimally invasive methods for obtaining samples for PFE analysis have been documented in the literature. Such methods would be desirable in the performance of a virtual autopsy for the evaluation of PFE in cases where autopsy is refused, or simply as an alternative to the double-edged knife (DEK) technique or frozen sections used in traditional autopsy.

Our goal was to investigate the feasibility of minimally invasive postmortem percutaneous needle biopsy (PNB) for providing adequate samples for the diagnosis and grading of PFE compared to our institute's gold standard, the DEK. We also compared the assessment of vitality of the PFE and the relationship of the PFE to the cause of death using the two techniques.

Materials and Methods

Between September and November 2008, 52 corpses were delivered to the Institute of Forensic Medicine, University of Bern, for forensic evaluation and autopsy. In each case, samples were obtained using PNB prior to autopsy and DEK during autopsy. Twenty-six cases were excluded from the study according to the following criteria: documentation of osteomyelitis, pancreatitis, and significant decomposition (1,13–16), yielding a final study population of 26. In the final study population, 19 were men and seven were women. The average age was 55.1 years, with a standard deviation of 15.9 years. Table 1 illustrates an overview of the study population.

Samples from both lungs were obtained using both PNB and DEK. Prior to the autopsy, multiple PNB samples from the mediobasal and apical regions of both lungs were taken blindly, using clinically approved and postmortem-tested ACN-III biopsy core needles (14 gauge; 160 mm) with an automatic pistol device (Bard Magnum, Medical Device Technologies, Stenløse, Denmark). During autopsy of the same cases, thin slices of both lungs were taken using DEK. The DEK, which is routinely employed for the analysis of FE at our institute, uses a knife consisting of a blade sharpened on one or both sides, to which a second blade, similar in size and shape, is added on the side. The blades can be folded out by means of a joint. A knurled nut regulates the distance between the blades and thus the layer thickness.

After storage in water for 5–10 min to obtain hemolysis, both the PNB and DEK samples were stained with Sudan III without sectioning and were then squeezed directly between cover slips. In each case, to prevent the recognition of the collecting tool by the shape of the sample, histological photographs (×25) of each slide were acquired by a forensic pathologist at microscopy; then, a PowerPoint file was created with the photographs coded separately and nonconsecutively by mixing the cases, collection methods, and lungs (right and left). Then, six forensic pathologists, blinded to the method of biopsy and case identity, independently analyzed all of the pictures. The observers were requested first (i) to identify the presence of PFE and then (ii) to assign a grade of severity to the positive cases. For a semiquantitative analysis of the PFE, the method of Falzi et al. (24) was adopted (Table 2).

TABLE 1—*Overview of the study population.*

Case No.	Age	Sex	Autopsy-Based Cause of Death	Blunt Trauma/ Fractures*
1	57	F	Myocardial infarction	No
2	52	F	Pulmonary thromboembolism	Yes
3	20	F	Cerebral lesions	Yes
4	55	M	Myocardial infarction	Yes*
5	70	M	Myocardial infarction	Yes
6	54	M	Myocardial infarction	Yes*
7	60	F	Myocardial infarction	Yes
8	54	M	Hemorrhage	Yes
9	54	M	Myocardial infarction	Yes*
10	50	M	Cerebral lesions	Yes
11	67	M	Brainstem laceration	Yes
12	79	M	Cerebral lesions	No
13	39	M	Myocardial infarction	Yes*
14	63	M	Aortic rupture	Yes*
15	44	M	Cerebral hemorrhage	No
16	43	M	Mechanical asphyxia	Yes
17	80	M	Heart failure, fat embolism	Yes
18	60	M	Myocardial infarction	No
19	40	F	Myocardial infarction	No
20	34	M	Myocardial arrhythmia	Yes*
21	47	M	Acute intoxication	Yes*
22	92	F	Heart failure	Yes*
23	31	M	Myocardial arrhythmia	No
24	60	F	Pulmonary thromboembolism	Yes*
25	63	M	Myocardial infarction	No
26	65	M	Choking	No

The fractures indicated by an asterisk (*) in the table were rib fractures caused by cardiopulmonary resuscitation maneuvers, the only traumatic injuries detected in the related cases.

TABLE 2—*Grading of pulmonary fat emboli (PFE). (Modified from Falzi et al. 1964) (24).*

Grading Scale	Shape of Emboli	Localization
I = light PFE	Drop-shaped	Sporadic in every microscopic field*
II = moderate PFE	Sausage-shaped, or rounded	Multiple, disseminated in every microscopic field*
III = massive PFE	Antler-shaped	Numerous everywhere, in every microscopic field*
0 = no PFE	Punctiform	Sporadic, not in every field*

*Enlargement ×25.

Two statistical analyses were then performed to compare PNB and DEK in the diagnosis of PFE and the grading of PFE. Regarding the diagnosis of PFE, the differences between the two lungs and the different examiners were investigated by applying contingency tables and the chi-square test. Then, a rank order test was used to compare PNB and DEK.

The analysis of PFE grading was also performed in two steps. First, a two-way factorial analysis of variance test was used to examine the differences between the two lungs and the different examiners and, second, PNB and DEK were compared by applying a *t*-test.

Finally, the results obtained by examining the PNB and DEK slides were separately analyzed in the context of the autopsy findings and case circumstances to assess in each single case PFE as proof of a vital reaction and as a primary or contributing cause of death. Then, the PNB sample-based diagnoses were compared with those obtained from the DEK slides, the latter being considered as the gold standard for our institution and our study.

Results

In total, PFE was identified in 21 cases, using one or both of the sampling techniques. The results of the microscopic analysis of the DEK and PNB samples are presented in Table 3. There were no statistically significant inter-lung or inter-observer differences in the detection of PFE using either PNB or DEK. Regarding the comparison of the two techniques, in 20 cases, PFE seen by DEK was

TABLE 3—*Pulmonary fat embolism grading of specimens by six forensic pathologists at independent microscopic analyses of Sudan III-stained samples of left (LL) and right lungs (RL) sampled using the double-edged knife (DEK) and percutaneous needle biopsy (PNB) techniques. The grades were assigned according to the scale by Falzi et al. (24), but the ordinal grades of the original scale were transformed into numeral grades and also half grades (0.5; 1.5; 2.5) to provide data that would be useful for the statistical analysis.*

	Forensic Pathologist 1				Forensic Pathologist 2				Forensic Pathologist 3			
	DEK		PNB		DEK		PNB		DEK		PNB	
Case No.	LL	RL	LL	RL	LL	RL	LL	RL	LL	RL	LL	RL
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0.5	0.5	0.5	0.5	0	0	0.5	0	0.5	0.5	0.5	0.5
3	1.5	2	2	2	1	1.5	1.5	2	2	2	2	2
4	0.5	0.5	1	0	0	0	0	0	0.5	0.5	0.5	0
5	1.5	1.5	2	2	1.5	0.5	3	2.5	2	2	2.5	2.5
6	1.5	1.5	1.5	1	1	1	0.5	0.5	1.5	1	1	0.5
7	2.5	2.5	2.5	2.5	3	2.5	3	3	3	3	3	3
8	1	1	1	1	0	0.5	0.5	0	0.5	0.5	0.5	0.5
9	0.5	0.5	0.5	0.5	0.5	0	0	0.5	0.5	0.5	0.5	0.5
10	1	1	1	1	0	0.5	0	0.5	0.5	0.5	0.5	0.5
11	0	0.5	1	1	0	0	0	0	0	0	0	0.5
12	3	3	3	3	3	2.5	3	3	3	2.5	2.5	3
13	1.5	1.5	2	1.5	0.5	1	1.5	0.5	1.5	1.5	1.5	1.5
14	1.5	1.5	1.5	1.5	1	0.5	1	1	1.5	1.5	1	1.5
15	0	0	0	0	0	0	0	0	0	0	0	0
16	2	1.5	2	2	2	1	3	2	2	2	2	2.5
17	2.5	2.5	2.5	2.5	2.5	2.5	2	2	2.5	2.5	2.5	2.5
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0.5	0.5	1	1	0.5	1	0	0	1	1	1	1
21	0.5	0.5	0.5	0.5	1	0.5	0	0.5	0.5	0.5	0.5	0.5
22	1	1.5	1	1.5	1	1	0.5	0.5	1.5	2	1.5	1.5
23	0	0	0.5	0.5	0	0	0	0	0	0	0.5	0.5
24	0.5	0.5	0.5	0.5	1	0	0.5	0.5	0.5	0.5	0.5	0.5
25	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0
	Forensic Pathologist 4				Forensic Pathologist 5				Forensic Pathologist 6			
	DEK		PNB		DEK		PNB		DEK		PNB	
Case No.	LL	RL	LL	RL	LL	RL	LL	RL	LL	RL	LL	RL
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0.5	0	1	0.5	0	0	0	0
3	1	2	1.5	1.5	1.5	2	2	2	1	1	1	1.5
4	0	0	0	0	0	0.5	0.5	0	0	0	0	0
5	2	1	2.5	2.5	2	1.5	2	2	1	0.5	2	1.5
6	1.5	1	1	0.5	1.5	1	0.5	0.5	1	0.5	0.5	0
7	2.5	2.5	2.5	3	2	2.5	2.5	3	1.5	2	2	2.5
8	0.5	0.5	0	0.5	0.5	0.5	0.5	0.5	0	0	0	0
9	0.5	0	0	0	0.5	0.5	0.5	0	0	0	0	0
10	0.5	1	1	0.5	0.5	0.5	0.5	0.5	0	0	0	0
11	0.5	0	0	0	0.5	0	0	0.5	0	0	0	0.5
12	3	3	3	3	3	2.5	2.5	3	2.5	2.5	2.5	2.5
13	1	1	1.5	0.5	1	1	1.5	1	0.5	1	0.5	0.5
14	1	1	1	1	1.5	1	1	1.5	1	1	1	1
15	0	0	0	0	0	0	0	0	0	0	0	0
16	2	1.5	2.5	2	1.5	1.5	2	2.5	1.5	1	1.5	1.5
17	3	3	2.5	2	2.5	2.5	2.5	2	2	2.5	1.5	1.5
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0.5	1	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
21	1	1	0	0.5	1.5	1.5	0.5	1	0.5	1	0	0.5
22	1	1.5	0.5	1	1.5	1.5	1.5	1.5	0.5	1	0.5	0.5
23	0	0	0.5	0.5	0	0	1	0.5	0	0	0	0
24	0	0.5	1	0	0.5	0.5	1	1	0.5	0	0.5	0.5
25	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0

correctly identified by PNB (Figs 1 and 2). For case no. 23, PNB showed the presence of a low-grade PFE (0–I) (Figs 1 and 2) (Table 3), whereas DEK did not. Figure 3 illustrates two cases, one negative and one positive, that were found to be in agreement by both DEK and PNB.

Regarding the PFE grade attributed by the different pathologists to the samples of both lungs, no significant inter-lung or inter-observer differences were observed. Moreover, the grading of PFE for samples obtained via the two techniques was uniformly attributed by the six examiners (Fig. 4). There were, however, statistically relevant discrepancies found in six cases (case nos. 5, 6, 16, 17, 21, 23) (Fig. 5). As shown in Fig. 5, in three out of the six discrepant cases, PNB yielded a higher score (more severe grade) than DEK. In the remaining three discrepant cases, PNB yielded a lower score (less severe grade) than DEK.

TABLE 4—*Diagnoses of pulmonary fat embolism as a vital sign and as the primary/contributing cause of death based on double-edged knife (DEK) technique and percutaneous needle biopsy (PNB) samples, analyzed separately, after a consideration of the autopsy findings and case circumstances.*

	Vital Sign		Cause of Death	
Case No.	DEK	PNB	DEK	PNB
1	–	–	–	–
2	No	No	No	No
3	Yes	Yes	No	No
4	No	No	No	No
5	Yes	Yes	No	No
6	No	No	No	No
7	Yes	Yes	No	No
8	Yes	Yes	No	No
9	No	No	No	No
10	Yes	Yes	No	No
11	Yes	Yes	No	No
12	Yes	Yes	No	No
13	No	No	No	No
14	No	No	No	No
15	–	–	–	–
16	Yes	Yes	No	No
17	Yes	Yes	Yes	Yes
18	–	–	–	–
19	–	–	–	–
20	No	No	No	No
21	No	No	No	No
22	No	No	No	No
23	No	No	No	No
24	No	No	No	No
25	–	–	–	–
26	–	–	–	–

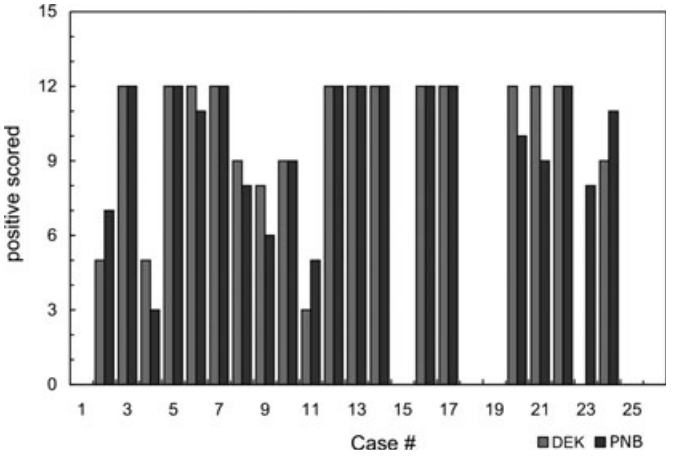

FIG. 1—*This figure shows the total number of times pulmonary fat embolism was diagnosed, as scored by all pathologists in both lungs (maximal 12), for the double-edged knife (DEK) technique and percutaneous needle biopsy (PNB) samples. This allows a direct comparison between the two methods.*

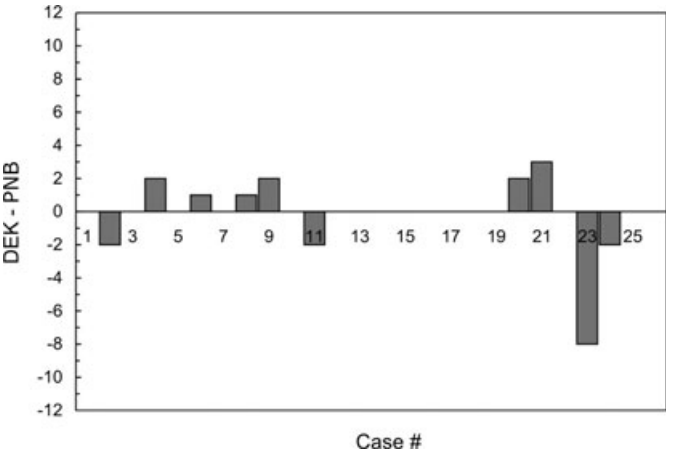

FIG. 2—*Difference between the total number of times pulmonary fat embolism was diagnosed in double-edged knife (DEK) technique and percutaneous needle biopsy (PNB) samples (see Fig. 1). The rank order test showed that all of these differences were not significant except in case no. 23.*

Regarding the final decisions of vitality and relationship to the cause of death, there was 100% agreement between the decisions based on PNB and those based on DEK samples (Table 4).

Discussion

This study demonstrated the feasibility of the PNB sampling method in the diagnosis and interpretation of PFE in the postmortem setting. Regarding the diagnosis or detection of PFE, all cases of PFE identified by the gold standard, in this study the DEK technique, were also detected by PNB. One diagnostic discrepancy was found (case no. 23) in which PFE was detected by PNB but not by DEK. This case involved a young man who died from a myocardial arrhythmia and had a history of aggressive resuscitation attempts. Because of the low grade of FE reported by all the six readers on the examination of the PNB samples (Table 3), and after a consideration of the autopsy findings, especially the absence of traumatic injuries, and after analyzing the case circumstances, the positive detection of PFE in this case was not interpreted as proof of antemortem violence, being most likely caused by heart massage (6,17) (Table 4). Equally, in the nine cases (Table 1) where resuscitation-related fractures occurred, PFE was detected by both techniques, but in absence of other traumatic injuries, it was attributed to resuscitation maneuvers and was not considered as proof of antemortem violence (6,17) (Table 4).

Similarly, there was no discrepancy in the determination of the relationship of PFE to the cause of death between PNB and DEK. The PFE grade, or severity, significantly differed between PNB and DEK in six cases. One case (case no. 23) reflected an instance in which a positive diagnosis was rendered by PNB but not by DEK. However, a systematic discrepancy in the attribution of severity to samples via the two techniques cannot be assessed, given that in three out of six cases the PNB sample analysis gave a higher score than the DEK samples, and in the other three cases the grading was lower (Fig. 5). Importantly, cases with a discrepancy in severity did not affect the ultimate assignment of the cause of death (Table 4). In fact, in case nos. 6, 21, and 23, PFE was equally evaluated as being absent or of a low grade in DEK and

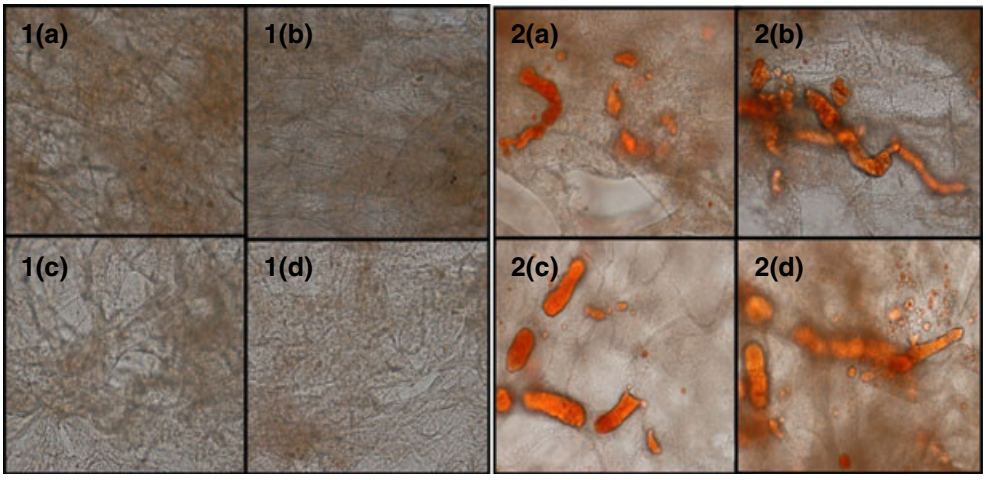

FIG. 3—*Histological images (×25) of two cases investigated for fat embolism with Sudan III staining: The images 1(a-d) and 2(a-d) are percutaneous needle biopsy samples from left (a) and right (b) lungs, and double-edged knife technique samples from left (c) and right (d) lungs in cases without (image 1.) and with (image 2) pulmonary fat embolism. Note the absence of Sudan III staining in all 1(a-d) images. Images 2(a-d) show the presence of Sudan III-stained fat emboli.*

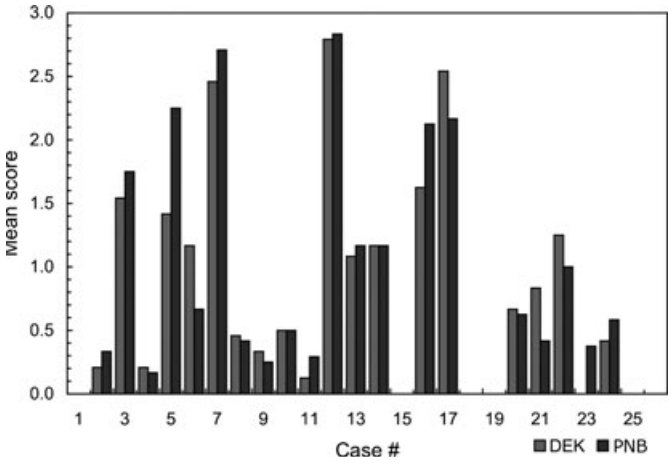

FIG. 4—*This figure shows the mean score per case attributed by all pathologists for both lungs in double-edged knife (DEK) technique and percutaneous needle biopsy (PNB) samples, respectively. This allows a direct comparison between the two methods.*

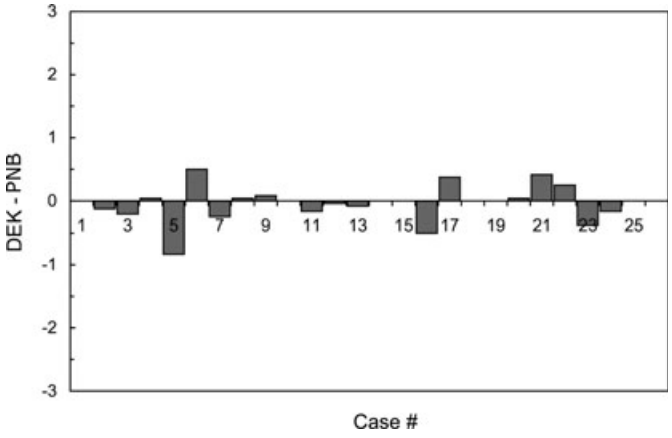

FIG. 5—*Differences between the means per case of the pulmonary fat embolism grade for double-edged knife (DEK) technique and percutaneous needle biopsy (PNB) samples (see Fig. 4). The* t*-test showed that all of these differences were not significant except in case nos. 5, 6, 16, 17, 21, and 23.*

PNB slides and presumed not to be related to the cause of death, but more likely to the cardiopulmonary resuscitation maneuvers performed in the three cases (6,17). In case nos. 5 and 16, the presence of higher grades of PFE in the PNB samples was attributed to multiple rib fractures caused by severe blunt trauma and did not affect the determination of cause of death. After a consideration of the autopsy findings and case circumstances, as well as the analysis of PNB samples, the cause of death was ascribed to myocardial infarction (case no. 5) and to mechanical asphyxia (case no. 16) (Table 1). Finally, case no. 17 involved an 80-year-old man with a clinical history of heart infarction and coronary bypass, who underwent an orthopedic intervention and died the day after. After a consideration of the history and the evidence of grade II-III FE on the DEK samples, the cause of death was attributed to heart failure and FE. Although the PFE grade was evaluated to be of lesser grade by PNB than DEK slides (Fig. 5), the medical history and the clinical scenario before death yielded the same conclusions.

In the remaining 20 cases, where statistically relevant discrepancies on grading were not seen, the contribution of PFE to death was uniformly assessed via the two techniques (Tables 1 and 4).

Although the feasibility of the PNB approach for the study of PFE as a vital reaction and as a cause of death was demonstrated by this study, the discrepancies found deserve some further considerations.

The reasons behind these discrepancies could be explained by the fact that, because this study was performed within the routine practice of the Institute of Forensic Medicine of Bern, five different operators performed the DEK cuts, possibly resulting in cuts of variable thickness. Furthermore, the correct use of DEK requires a certain expertise of the operator to obtain effective samples. In this work, all of the five operators who executed the cuts had long or sufficient experience regarding the use of DEK. However, a comparison of the quality of the DEK cuts made by the different operators is technically impossible, as the tissue samples were squeezed between glass slides, thus making an assessment of the most important quality criterion—namely the slice thickness—impossible.

In contrast, because of the defined diameter of the needle, the thickness of the samples obtained via PNB can be assumed to be constant or at least less variable than those acquired via DEK.

Additionally, it is possible that our modification of the grading scheme for statistical analysis introduced greater variability in the

grading than would have been present if the observers were required to restrict their grading to whole integer values from 0 to 3.

There are some important advantages of the PNB technique compared to DEK and traditional biopsy sampling techniques during autopsy. Performing PNB requires less time, *c.* 10 min, compared to DEK and traditional sampling techniques, which are performed after autopsy excision and examination of the lungs, which take at least 30 min. The DEK technique is the method of choice for the detection of PFE at our institute because it is fast, inexpensive, and may offer advantages in diagnostic accuracy based on the visualization of fat droplets in vascular ramifications (25). Presumably, the time savings in comparing PNB to the evaluation of a frozen sample would be even greater, although frozen sections were not evaluated as a part of this study.

Additionally, PNB is a simpler technique, not as dependent on operator expertise, with the added benefit of decreased operator exposure to potentially infectious bodily tissues and fluids (19–23,26–31). The minimally invasive approach not only minimizes the risk of infection, but it might also be critical in certain forensic cases where an autopsy is rejected by relatives for religious or cultural reasons, or where it is refused by the practices of a court system (19–23).

However, some drawbacks have to be considered, particularly if a traditional autopsy is not performed. As highlighted by Bolliger et al. (21), the information that can be gained from the macroscopic appearance of an organ, which could guide the forensic examiner in tissue sampling for histological examination and could provide important information for forensic conclusions, is lost when invasive autopsy is not performed. The minimally invasive approach involves blind biopsies of the target organs. For this reason, some pathological alterations, possible expressions of the primary or contributing cause of death, or changes too tiny and localized to be detected by imaging, could be lost to chance biopsies, although given the diffuse nature of PFE this is not expected to effect the diagnosis of PFE by PNB. Moreover, an adequate minimally invasive assessment of the cause of death requires more than simply PNB of the lungs; a full minimally invasive autopsy with full body imaging and sampling of all major organs could actually require more time than a traditional autopsy. Finally, given that the frozen sections are not routinely used for the diagnosis of PFE in our institute, it is impossible to say what the diagnostic performance of PNB would be in comparison with frozen techniques.

In conclusion, the possibility of detecting and interpreting PFE as a vital reaction and as a primary or contributing factor in the cause of death using the minimally invasive approach of PNB was proven, and a previous limitation of the Virtopsy approach (18) can be considered as having been overcome. The usefulness of the PNB sampling method for PFE goes beyond the performance of virtual autopsy and could be applied to analysis of PFE in cases where autopsy is refused and virtual autopsy is not available, or simply as an alternative to DEK or frozen sections in traditional autopsy. It is possible that in the future, new sophisticated imaging techniques might allow the identification of the different biochemical tissue components and may allow for a diagnosis and grading of FE with the same diagnostic value as traditional histopathological techniques. Until such techniques are developed, PNB offers a simple method for obtaining samples for the diagnosis and interpretation of PFE in the postmortem setting.

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