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PAPER

PATHOLOGY/BIOLOGY

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Increased Adrenaline to Noradrenaline Ratio Is a Superior Indicator of Antemortem Hypothermia Compared with Separate Catecholamine Concentrations

ABSTRACT: The significance of urinary catecholamines and small gastric mucosal bleedings, Wischnewsky's spots, in postmortem diagnosis of hypothermia deaths was evaluated. Autopsy cases (n = 358) were divided into hypothermia, suspected hypothermia, and control groups. The catecholamine levels did not correlate with the length of the postmortem period. The adrenaline to noradrenaline ratio was most effective in detecting hypothermia (68.9% sensitivity, 78.1% specificity). The median adrenaline concentrations were significantly higher in hypothermia than in control groups. The control group containing mostly sudden cardiac deaths with no cold exposure had a noradrenaline level comparable to the hypothermia groups. The sensitivity and specificity of determining Wischnewsky's spots in hypothermia deaths were 63.9% and 88.3%, respectively. The adrenaline to noradrenaline ratio is more suitable in proving antemortem cold stress than either of these independently, and its diagnostic value is comparable to that of Wischnewsky's spots.

KEYWORDS: forensic science, forensic pathology, hypothermia, postmortem diagnosis, catecholamines, gastric mucosa, urine

Cold is a significant factor in many deaths. However, there are no specific markers that could exclusively indicate the role of cold in a death. This makes the postmortem diagnosis of hypothermia very challenging in forensic death investigation. Some findings, such as high levels of catecholamines, adrenaline, and noradrenaline, in the cadaver's urine, as well as small gastric mucosal bleedings ranging from <5 mm to over 10 mm in diameter (1), known as Wischnewsky's spots, are considered to be indications of antemortem cold stress. Urinary catecholamine concentrations have been shown to increase in hypothermic animals (2,3). The few studies conducted on human cadavers (4–6) have produced similar results, but with some controversy as to which of the catecholamines is more prominently secreted. Since Wischnewsky's spots were discovered in the late 19th century, the presence of them in hypothermia deaths has been studied on a somewhat larger scale. Their frequency among hypothermia death cases has varied from <50% almost to 90% (1,4,7,8).

The number of autopsy cases in most of the previous studies has been very limited, making it difficult to draw any firm conclusions about the significance of catecholamine and Wischnewsky's spots' assays in diagnosing hypothermia in forensic death investigation. We studied a larger material of forensic autopsies including a comprehensive group of control cases in addition to hypothermia deaths to gain a better understanding of the usefulness of these assays in diagnosing hypothermia as the primary or a contributory cause of death.

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Materials and Methods

Autopsy Cases

The medico-legal autopsies had been performed in the Institute of Diagnostics, Department of Forensic Medicine, University of Oulu, Finland, during the years 1990–2008, and all the subjects had died in the province of Oulu. Of all 358 cases, 283 (79.1%) were men and 75 (20.9%) were women. The cases were divided into five different groups (Table 1). All cases with hypothermia as the main cause of death constituted group I (n = 145). Group II (n = 59) included cases in which hypothermia or exposure to cold had been determined as a contributory factor. Group III (n = 204) contained the cases in both groups I and II combined. Group IV (n = 39) included cases in which the person was found dead in a cold environment, raising suspicion of hypothermia-related death, but in the final forensic examination, hypothermia was not regarded as either the main or a contributory cause of death. Finally, group V (n = 115) was a control group with no hypothermia, consisting mostly of sudden cardiac deaths and some other unexpected deaths caused, for example, by head injuries and alcohol and drug intoxication, solely or in combination with one another. The grouping was made according to the main and contributory causes of death (summarized in Table 2), which were concluded by forensic

TABLE 1—*Description and characteristics of groups.*

Group	Description of Cases	Cases *n*	Male/Female *n*	Age (Years) (Mean ± SD)	Median PM-Time (Days)
I	Hypothermia as primary cause of death	145	110/35	55 ± 16	4
II	Hypothermia or cold exposure as a contributory cause of death	59	42/17	60 ± 17	5
III	Groups I and II combined	204	152/52	56 ± 17	5
IV	Suspicion of hypothermia in the beginning of investigation, but no evidence supporting it	39	30/9	52 ± 15	5
V	No hypothermia, mostly sudden cardiac deaths	115	101/14	62 ± 12	4

SD, standard deviation; PM, postmortem.

TABLE 2—*Primary and contributory causes of death.*

	Group										
	I		II		III		IV		V		
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	Total
Primary Cause of Death											
Hypothermia	145	100	0	0.0	145	71.1	0	0.0	0	0.0	145
Intoxication											
Ethanol	0	0.0	6	10.2	6	2.9	4	10.3	3	2.6	13
Drugs	0	0.0	3	5.1	3	1.5	2	5.1	1	0.9	6
Combination	0	0.0	7	11.9	7	3.4	1	2.6	1	0.9	9
Other	0	0.0	0	0.0	0	0.0	3	7.7	1	0.9	4
Cardiovascular diseases	0	0.0	30	50.8	30	14.7	15	38.5	103	89.6	148
Injuries and their sequelae	0	0.0	4	6.8	4	2.0	5	12.8	6	5.2	15
Drowning	0	0.0	7	11.9	7	3.4	3	7.7	0	0.0	10
Other	0	0.0	2	3.4	2	1.0	6	15.4	0	0.0	8
Contributory factors											
Hypothermia	0	0.0	59	100	59	28.4	0	0.0	0	0.0	59
Acute or chronic use of											
Ethanol	98	67.6	19	32.2	117	57.4	14	35.9	11	9.6	142
p-value	<0.001 (I vs. V)*				0.014 (III vs. IV)*				<0.001 (III vs. V)*		
Drugs/narcotics	18	12.4	1	1.7	19	9.3	0	0.0	0	0.0	19
Intoxication											
Ethanol	3	2.1	0	0.0	3	1.5	0	0.0	0	0.0	3
Drugs	10	6.9	1	1.7	11	5.4	0	0.0	0	0.0	11
Diabetes mellitus	5	3.4	1	1.7	6	2.9	4	10.3	13	11.3	23
Degenerative brain diseases and/or dementia	13	9.0	1	1.7	14	6.9	1	2.6	1	0.9	16
p-value	0.004 (I vs. V)*				0.477 (III vs. IV)†				0.015 (III vs. V)*		
Other mental and neurological diseases	12	8.3	4	6.8	16	7.8	3	7.7	4	3.5	23
Cardiovascular diseases	61	42.1	18	30.5	79	38.7	10	25.6	47	40.9	136
Pulmonary diseases	8	5.5	4	6.8	12	5.9	1	2.6	6	5.2	19
Injuries and their sequelae	10	6.9	5	8.5	15	7.4	6	15.4	2	1.7	23
p-value	0.049 (I vs. V)*				0.119 (III vs. IV)*				0.032 (III vs. V)*		
Other	10	6.9	4	6.8	14	6.9	4	10.3	20	17.4	38

*χ^2-test.
†Fisher's exact test.

pathologists. The diagnosis of hypothermia was based on overall evidence consisting of the course of events and circumstances, external and internal autopsy findings, microscopic examinations, toxicological and biochemical analyses, as well as the exclusion of other causes of death. When the overall evidence indicated that cold had had an impact but some other cause of death than hypothermia was evident, hypothermia was diagnosed as a contributory cause.

Death was classified as accidental in 130 (89.7%) cases in group I. In two (1.4%) cases, the mode of death was suicide, and the remaining 13 (9.0%) were undetermined because of lack of sufficient background information. In group II, more than half of the people had died naturally, and around one-third of the deaths were accidental. There were also four suicides, one homicide, and two unclear deaths. In groups IV and V, the majority of cases were natural deaths (53.8% and 89.6%, respectively). Twelve (30.8%) cases in group IV and 7 (6.1%) cases in group V were classified as accidental deaths. Both groups had 3 (7.7% and 2.6%) suicides and 2 (5.1% and 1.7%) unclear deaths. One (2.6%) homicide was also included in group IV.

Catecholamine Measurements and the Examination of Gastric Mucosa

After sample collection, hydrochloric acid (6 N) was added to cadaveric urine to adjust pH around 1. Samples were stored at −70°C. The isolation of catecholamines from the urine samples was performed by aluminum oxide extraction (9). The noradrenaline and adrenaline levels were measured by high-pressure liquid chromatography (HPLC) with electrochemical detector (10). The adrenaline to noradrenaline ratio was also calculated for each case.

The macromorphological examination of gastric mucosa was carried out by forensic pathologists as a routine step of autopsy. Comments concerning gastric mucosa were recorded in three categories:

spotted hemorrhages, actual erosions or ulcerations, and no particular abnormalities.

Statistics

The collected data were saved and analyzed using SPSS Statistics 17.0. Statistical significance of the differences in the catecholamine levels between groups was calculated using the Mann–Whitney nonparametric test for each pair and the Benjamini–Hochberg correction for multiple comparisons. Other statistical analysis tests included Pearson's chi-square test, Fisher's exact test, independent samples *t*-test, and Kendall's correlation test.

Results

Urine Catecholamine Levels in Hypothermia and Control Cases

Median values of adrenaline were 53.5, 39.5, 46.5, 20.0, and 12.0 ng/mL for groups I–V, respectively (Fig. 1*a*, Table 3). The adrenaline levels in hypothermia groups I and III were significantly higher than in control groups IV and V ($p < 0.001$ for all comparisons). There was also a statistical significance between groups II and V ($p < 0.001$), but not between groups II and IV ($p = 0.057$). There was no notable difference ($p = 0.068$) in the adrenaline values between primary hypothermia cases (group I) and cases with hypothermia or cold exposure as a contributory cause of death (group II).

Median values for noradrenaline were 165.0, 157.0, 159.5, 105.0, and 193.0 ng/mL for groups I–V, respectively (Fig. 1*b*, Table 3). The noradrenaline values did not differ significantly between groups I, II, and V. The median value of group IV was the lowest (105.0 ng/mL), differing significantly between groups I, II, and III with *p*-values 0.037, 0.040, and 0.035, respectively. The highest median concentration was seen in group V, but the differences between hypothermia and control cases were not statistically significant.

The adrenaline values were proportionally more elevated than noradrenaline values in all hypothermia cases, so the adrenaline to noradrenaline ratio (Fig. 1*c*, Table 3) was also higher in group III (0.3063) than in groups IV (0.1876; $p = 0.010$) and V (0.0670; $p < 0.001$). Thus, the adrenaline to noradrenaline ratio was more sensitive in finding differences between groups. Even groups I (hypothermia as primary cause of death; 0.3177) and II (hypothermia or cold exposure as a contributory cause of death; 0.2226) had significantly differing index values ($p = 0.033$).

Here, the values for basal and elevated levels of catecholamines in Table 4 are based on the median values of group IV. This control group was chosen as the basis for cutoff values as it consisted of suspected but not hypothermic cases. Furthermore, group IV was more heterogeneous in terms of death cases, contrary to group V where cardiovascular deaths dominated. Group-specific distribution of cases according to the cutoff values is shown in Fig. 2. The sensitivity of adrenaline as hypothermia marker was 73.7% and the specificity 65.8% (Table 4). The sensitivity of noradrenaline to detect hypothermia was 76.3%, but specificity only 30.3%. Using the adrenaline to noradrenaline ratio, the sensitivity was 68.9% and the specificity 78.1%.

The Relationship Between Postmortem Period and Urine Catecholamines

The relationship between the postmortem period and urine catecholamine levels was analyzed by Kendall's correlation test. The

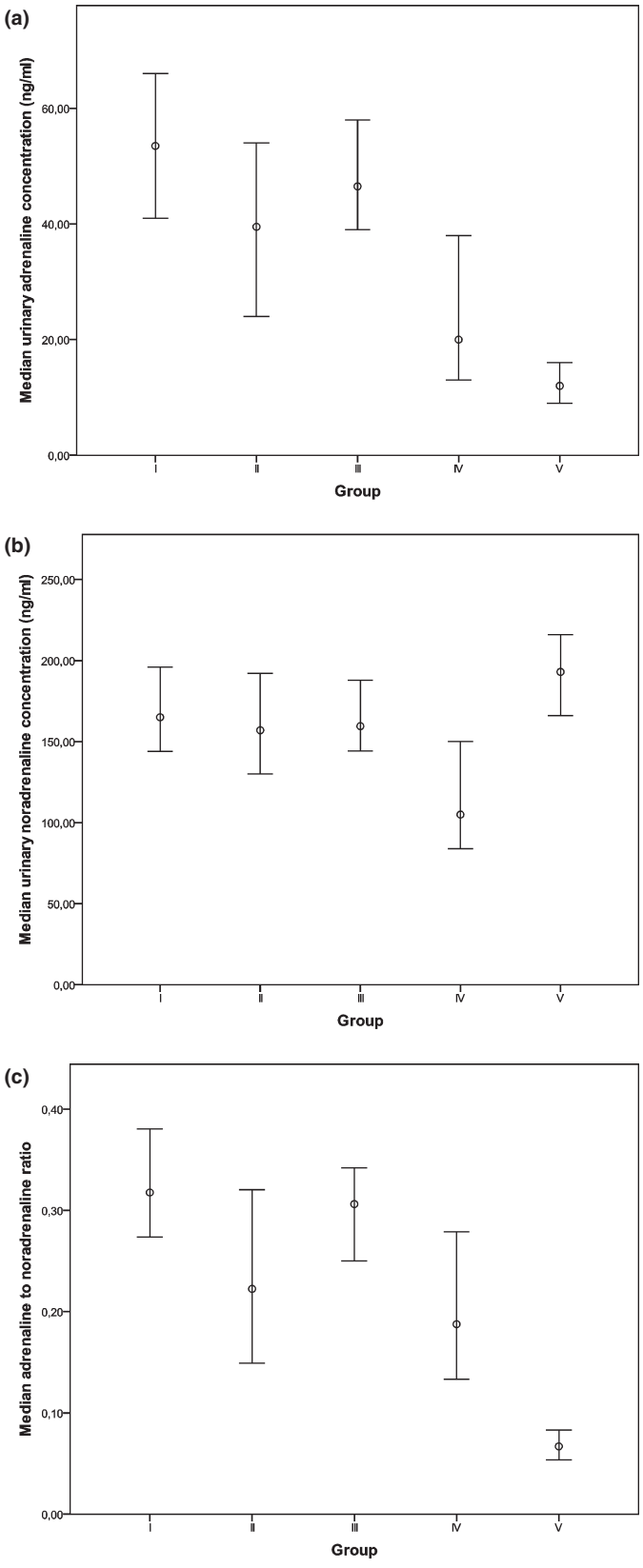

FIG. 1—*Median concentration and ratio of urinary catecholamines within groups. The error bars represent 95% confidence interval. (a) adrenaline, (b) noradrenaline, (c) adrenaline to noradrenaline ratio.*

postmortem period ranged from 0 to 195 days, with a median of 4 days and a mean of 7.31 ± 19.16 days. The correlation coefficient values were 0.115 for adrenaline, 0.135 for noradrenaline, and

TABLE 3—*Urine catecholamine concentrations, adrenaline to noradrenaline ratio, median (range), and statistical analysis.**

	Group				
	I	II	III	IV	V
n	134/145	56/59	190/204	37/39	115/115
Adrenaline, A (ng/mL)	53.5 (0–655)	39.5 (0–346)	46.5 (0–655)	20.0 (0–345)	12.0 (0–319)
p-value (vs. I)	–	0.068	0.422	<0.001	<0.001
p-value (vs. III)	0.422	0.171	–	<0.001	<0.001
Noradrenaline, Na (ng/mL)	165.0 (26.8–822)	157.0 (23–890)	159.5 (23–890)	105.0 (29–849)	193.0 (37–1283)
p-value (vs. I)	–	1.243	0.998	0.037	0.388
p-value (vs. III)	0.998	1.107	–	0.035	0.404
Adrenaline to noradrenaline ratio	0.3177 (0–2.58)	0.2226 (0–2.04)	0.3063 (0–2.58)	0.1876 (0–0.97)	0.0670 (0–1.25)
p-value (vs. I)	–	0.033	0.345	0.002	<0.001
p-value (vs. III)	0.345	0.118	–	0.010	<0.001

*Mann–Whitney nonparametric test with the Benjamini–Hochberg correction for multiple comparisons.

TABLE 4—*Distribution of hypothermia and control cases according to urinary catecholamine levels.*

	Hypothermia Cases (group III) *n*	Control Cases (groups IV and V) *n*
Adrenaline		
Basal (≤20 ng/mL)	50 (26.3%)	100 (65.8%†)
Increased (>20 ng/mL)	140 (73.7%*)	52 (34.2%)
Noradrenaline		
Basal (≤105 ng/mL)	45 (23.7%)	46 (30.3%†)
Increased (>105 ng/mL)	145 (76.3%*)	106 (69.7%)
Adrenaline to noradrenaline ratio		
Basal (≤0.1876)	59 (31.1%)	118 (78.1%†)
Increased (>0.1876)	131 (68.9%*)	33 (21.9%)

*Sensitivity of the assay.
†Specificity of the assay.

0.055 for adrenaline to noradrenaline ratio, indicating a lack of correlation between the postmortem period and urine catecholamine levels.

Wischnewsky's Spots

Small bleedings or hemorrhage-like lesions in the gastric mucosa could be identified in 95 (66.4%) cases in group I and 34 (57.6%) cases in group II (Table 5). Similar changes were also seen in a few cases with no cold exposure: 13 (33.3%) in group IV and 5 (4.3%) in group V. The frequencies in hypothermia death groups I and III were, however, significantly higher in comparison ($p < 0.001$ in all comparisons). Groups IV and V differed also from each other ($p < 0.001$). The difference between groups I and II was not significant ($p = 0.236$). The sensitivity of determining Wischnewsky's spots in hypothermia deaths was 63.9%. Specificity was 66.7% in group IV, 95.7% in group V, and 88.3% in both these groups combined.

False Negative and Positive Cases

Although the sensitivity of the adrenaline to noradrenaline ratio to detect hypothermia is at a reasonable level, there were several negative (low ratio) cases in the hypothermia-related groups. To clarify the reasons behind this, we compared urine alcohol levels, the presence of drugs, and mean ages between negative and positive (increased ratio) cases. Significant differences were not detected in any of the variables analyzed (results not shown). Interestingly, proportions as high as 83% (30/36) in group I and 61% (14/23) in group II of the false-negative cases were positive regarding Wischnewsky's spots.

As the medians of group IV were used as cutoff values for catecholamines, half of the cases were classified as false positives in this group. According to the presence of Wischnewsky's spots, the proportion of false positives in group IV was, however, lower (33.3%; Table 5).

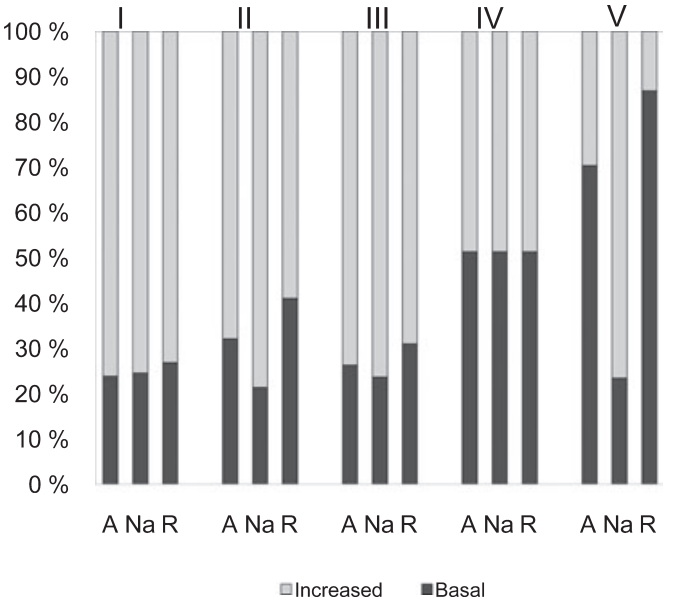

FIG. 2—*The distribution of cases within groups according to adrenaline (A) and noradrenaline (Na) concentrations and adrenaline to noradrenaline ratio (R). Basal levels were considered to be ≤20 ng/mL for A, ≤105 ng/mL for Na, and ≤0.1876 for R.*

TABLE 5—*The frequency of gastric mucosal Wischnewsky's spots.*

		Positive for Spots	
Group	*n*	*n* (%)	*p*-Value
I	143	95 (66.4)	<0.001 (I vs. V)*
II	59	34 (57.6)	0.236 (I vs. II)*
III	202	129 (63.9)	<0.001 (III vs. V)*
IV	39	13 (33.3)	<0.001 (III vs. IV)*
V	115	5 (4.3)	<0.001 (IV vs. V)†
Total	356	147 (41.3)	

*χ^2-test.
†Fisher's exact test.

Aiming to improve the postmortem diagnosis of hypothermia or cold exposure, we tried to combine the high sensitivity of catecholamines and the high specificity of Wischnewsky's spots, but the sensitivity of both of these assays combined was at most only 50.0% (noradrenaline and Wischnewsky's spots) in group III. However, high adrenaline to noradrenaline ratio (>0.1876) accompanied by Wischnewsky's spots on the gastric mucosa detected hypothermia with a specificity of 94.7% in groups IV and V combined.

Predisposing Factors

As seen in Table 2, some medical conditions were more frequent in hypothermia deaths than in the control cases. Significant differences were found in consumption of alcohol ($p = 0.014$ III vs. IV, $p < 0.001$ I vs. V and III vs. V), degenerative brain diseases, and dementia ($p = 0.004$ I vs. V, $p = 0.015$ III vs. V), as well as sustained injuries and their complications ($p = 0.049$ I vs. V, $p = 0.032$ III vs. V).

Discussion

The catecholamines were shown to be stable in the cadaveric urine, as there was no significant change in the concentrations in relation to the postmortem period. A previous experiment on guinea pigs confirms that there is no significant increase in the urinary catecholamines because of autolysis (11). Thus, measuring urinary catecholamines should be a reliable method for assessing the amount of antemortem stress. Plasma, serum, and cellular catecholamines have also been used to study stress in hypothermia and other deaths (2,3,12–16). Although there are some data showing that catecholamines in blood remain stable in cadavers for a limited time when stored appropriately (17), their levels seem prone to increase in the postmortem time, as catecholamines diffuse from sympathetic nerve endings and adrenal glands into the blood (18). Blood catecholamine levels vary also greatly depending on physical and emotional factors as well as numerous drugs and foods, which naturally affects postmortem levels and makes blood testing difficult. In addition, there seems to be variation in catecholamine concentrations depending on the site of blood sampling (19).

In this study, adrenaline was found to be a better marker of hypothermia death than noradrenaline, which could be as a result of its more important role in thermogenesis enhancing glucose metabolism, as suggested by Hirvonen and Huttunen (5). On the other hand, it has been reported that during core cooling below the normothermic baseline, adrenomedullary activation occurs after sympathoneural activation in mild hypothermia (20). This finding seems to gain support from our studies, if groups I and II are considered to represent more severe and milder cold exposure, respectively: compared with control group IV, the levels of noradrenaline were significantly higher in groups I and II, whereas in the case of adrenaline, this was only seen in group I.

The adrenaline to noradrenaline ratio was shown to be basically the best indicator of cold exposure. The use of the ratio eliminates the significance of any possible variation in cadaveric urine concentrations. It was slightly less sensitive (68.9%) than the adrenaline concentration, but the specificity was clearly better, 78.1%. Although both reasonable sensitivity and specificity for catecholamines as hypothermia markers were achieved, their diagnostic value in the challenging group IV, which consisted of cases with suspected hypothermia, was limited with the cutoff values applied. The sensitivity of determining Wischnewsky's spots in hypothermia deaths was a little lower than that of the adrenaline to noradrenaline ratio, with a somewhat better specificity even in group IV. It became clear, however, that a high adrenaline to noradrenaline ratio (>0.1876) accompanied by Wischnewsky's spots on the gastric mucosa is a strong indication of antemortem cold stress with a specificity of almost 95%.

Increased serum concentrations of both adrenaline and noradrenaline have been reported in myocardial infarction deaths (21). As for noradrenaline, this also seemed to be the situation here concerning urinary concentrations in cases with no cold exposure, consisting mainly of cardiovascular deaths (group V). On the other hand, the adrenaline concentrations were very low, which is why the adrenaline to noradrenaline ratio was also the lowest in group V (0.067). The ratio was even lower than that previously reported for deaths with a short agonal period (0.13) (14). The highest adrenaline to noradrenaline ratios were seen in hypothermia groups I (0.32) and II (0.22), being comparable to the ratio reported for long agony deaths (0.21) (14). Interestingly, long-distance swimming in ice-cold water has induced a ratio of 0.21 according to the reported average adrenaline and noradrenaline levels (22).

Our studies concerning catecholamine assays as well as Wischnewsky's spots indicate that these traditional markers are useful in postmortem diagnosis of hypothermia. Recently, several other factors have also been used as markers for cold exposure, including chromogranin A (15), sodium concentration of skeletal muscle (23), heat shock protein 70 (24), and adrenocorticotropic hormone (25). However, most targets used for the purpose are involved in common stress reactions, and more specific markers for hypothermia remain to be identified.

As opposed to previous studies (4,5), ethanol concentrations were similar in hypothermia death cases with both normal and increased catecholamine concentrations. Thus, high doses of alcohol did not explain the low response to cold stress in this study. There was no difference in the presence of drugs in hypothermia death cases with high or low catecholamine concentrations, either. We also tested whether the false-negative cases had a higher mean age, as stress reactions in general are thought to be dampened in the elderly. There was, however, no difference in the mean ages.

There were some differences in the contributory factors between hypothermia deaths and other deaths. The use of alcohol was more frequent in the hypothermia death groups than in the control groups. This is understandable, as alcohol is known to disrupt both endocrine and behavioral thermoregulation (26), making the individual more vulnerable to cold. Degenerative brain diseases and dementia as well as acute injuries and their sequelae were also more often contributing to hypothermia deaths than to deaths with no hypothermia. The influence of these medical conditions is understandable. Individuals with dementia are more likely to go outdoors without proper clothing in cold weather. In addition, they are often elderly people, which can in itself be a risk factor for accidental hypothermia (27). Severe injuries caused by traffic accidents, for example, can cause a person to be exposed to a cold environment for a long time until rescue arrives. Heavy bleeding can contribute to the loss of heat, accelerating the development of hypothermia.

In conclusion, as practice and many studies have previously shown, the postmortem diagnosis of a hypothermia death can be very difficult. The findings can vary considerably between cadavers, partly depending on the factors and outer circumstances contributing to the progression of death. However, we have shown here that the adrenaline to noradrenaline ratio is valuable in detecting antemortem cold stress, while Wischnewsky's spots further support the postmortem diagnosis of hypothermia, particularly because of their high specificity.

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