Tangsermkijsakul and Pipatchotitham
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ORIGINAL ARTICLE

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The comparative and correlation study of postmortem ethanol levels between axillosubclavian blood and femoral venous blood in forensic autopsy cases at Thammasat University Hospital, Thailand

Aphinan Tangsermkijsakul[1] and Tossanai Pipatchotitham[1*]

Abstract

Background Femoral venous blood (FVB) is accepted as a standard sample for postmortem ethanol analysis, but owing to the nature of unnatural death cases, FVB may not always be obtainable, and subclavian blood might be used for alternative specimens. This study investigated the correlation between postmortem ethanol concentrations in FVB and axillosubclavian blood (ASB) from autopsy cases in the tropical climate of Central Thailand. Two other factors potentially affecting the correlation, the time of death and ethanol absorption state, were also investigated. FVB, ASB, and vitreous humor were collected from 100 subjects between May 2021 and May 2022. Subjects with decomposition signs and large open wounds were excluded. ASB was drawn from the axillosubclavian vein at the thoracic outlet. Ethanol concentrations in all samples were measured by headspace GC-FID.

Results Ethanol concentrations in ASB were statistically identical to those in FVB ($p = 0.6761$) and their relationship was statistically correlated ($r = 0.9818$, $p < 0.001$). The correlation was not statistically influenced by time since death or absorptive statuses. The diagnostic study to assess the practical application of ASB instead of FVB at a cut-off concentration above 50 mg/dL yielded an area under the ROC curve of 0.96.

Conclusions Our study indicated that ASB can be used as an alternative specimen for postmortem ethanol analysis when FVB is unavailable in dead bodies that are in a tropical climate.

Keywords Ethanol, Femoral blood, Subclavian blood, Axillosubclavian blood, Postmortem toxicology, Forensic medicine

Background

Postmortem toxicological analysis plays an important role in the diagnosis of cause and manner of death. Substances detected in postmortem cases could be direct causes of death or contributing factors causing death. Specimen collection is an important step for the interpretation for toxicological results. Standard guidelines in forensic pathology state that peripheral venous blood, especially femoral venous blood (FVB) is a gold standard sample for postmortem toxicology, including for

*Correspondence:
Tossanai Pipatchotitham
tossanai.pip@gmail.com
[1] The Department of Forensic Medicine, Faculty of Medicine, Thammasat University, 99/209 Village No. 18, Phaholyothin Road, Khlong Nueng Sub-District, Khlong Luang District, Pathumthani 12120, Thailand

ethanol analysis, because its anatomic location is far from the center of the body and less affected by diffusion of drugs from the central compartment. Postmortem redistribution of drugs from the central compartment of the body results in higher drug concentrations in central blood samples than those in peripheral sites (Saukko and Knight 2015; Peterson et al. 2006; Dinis-Oliveira et al. 2016; Stimpfl et al. 1999).

Postmortem ethanol analysis is critical not only in terms of cause of death but also regarding legislation on ethanol concentrations. However, different blood sampling sites in postmortem cases can yield different ethanol concentrations. Pelissier-Alicot A-L et al. conducted a study in 30 autopsy cases and found that ethanol concentrations detected in the left cardiac blood were significantly different from the concentrations detected in the right cardiac blood, and the FVB (Pelissier-Alicot et al. 2006). Sylvester PA et al. studied the correlation of ethanol concentrations from five sampling sites in dead bodies with ethanol concentrations in the vitreous humor (VH) and found that blood from the left ventricle had the lowest correlation coefficient with VH (Sylvester et al. 1998). Owing to the nature of postmortem cases, FVB may not be available for toxicological analysis in every case because dead bodies may suffer from exsanguination or have severe injuries of lower extremities. Previous study from Sastre C et al. stated that subclavian blood is another option for postmortem ethanol analysis when FVB is not available because ethanol concentrations in subclavian blood and FVB were statistically correlated ($r = 0.961$, $p < 0.001$) (Sastre et al. 2013).

According to the standard procedure in forensic autopsy, the axillosubclavian vein can be accessed at the thoracic outlet during the opening of the thoracoabdominal cavity by subclavicular line incision (Connolly and Auchincloss 2021). Thus, this site can be a suitable option for blood collection for ethanol analysis when FVB is not available. Although there was a study from Sastre C et al. stating that subclavian blood could be an alternative sample for ethanol analysis (Sastre et al. 2013), there is no information about the relationship of ethanol concentrations between FVB and subclavian blood in Thai postmortem cases. Thus, this study aims to investigate the correlation between postmortem ethanol concentrations in FVB and axillosubclavian blood (ASB) in autopsy cases at Thammasat University Hospital (TUH) in Pathum Thani Province, Thailand. This location is in the Northeastern region of Central Thailand. This is the first study to be conducted in Mainland Southeast Asia with a tropical climate. Two other factors which may affect the correlation between these two sampling sites, the time of death and the state of ethanol absorption, are also investigated in this study.

Methods

FVB, ASB, and VH samples were collected from subjects who were sent for forensic autopsy at the TUH between May 2021 and May 2022. All subjects in this study were examined following the postmortem inquest under the Criminal Procedure Code of Thailand, Sect. 148–156 and the autopsy procedure was conducted under the standard recommendation from the Royal College of Pathologists of Thailand.

Inclusion criteria

Subjects dead from unnatural deaths in which ASB, FVB, and VH were available and ethanol concentrations were above 9.91 mg/dL (above the lower limit of quantitation) were included in the study. Unnatural deaths regarding the Criminal Procedure Code of Thailand are deaths resulting from an external cause and are classified into five categories as follows: (1) suicide, (2) death by the act of another person, (3) death caused by an animal, (4) death by accident, (5) death from a cause not yet known. The underlying causes and the circumstances of death are used to determine whether the death occurred naturally or unnaturally. When an unnatural death occurs, the physicians cannot sign a death certificate and notify an administrative or police official to initiate the postmortem inquest in the same way of the regulation in many countries (Madea and Argo 2014).

Exclusion criteria

Subjects with signs of decomposition, such as subjects with the presence of greenish discoloration on the lower abdomen, subjects with open wounds to the depth of muscle, and subjects with ethanol concentrations in the blood but not in the VH were excluded from this study.

Sample collection

In each case, after external examination, FVB was collected from the site of the femoral triangle by dissection. VH was collected by inserting a needle through the eye slightly lateral to the limbus. ASB was collected from the thoracic outlet.

To extract the ASB, skin and subcutaneous tissue were deflected laterally from the thoracic cavity site and the clavicular head of the pectoralis major muscle was dissected. Blood was then drawn from the axillosubclavian vein between the clavicle and the 1st rib in front of the anterior scalene muscle, targeting the tip of the needle towards the axillary vein as demonstrated in Fig. 1A, B. Fixation of the hypostasis in each subject was documented.

The subjects were refrigerated at 4 °C within 48 h prior to autopsy. This period is consistent with the routine work of many forensic pathology institutes in Thailand.

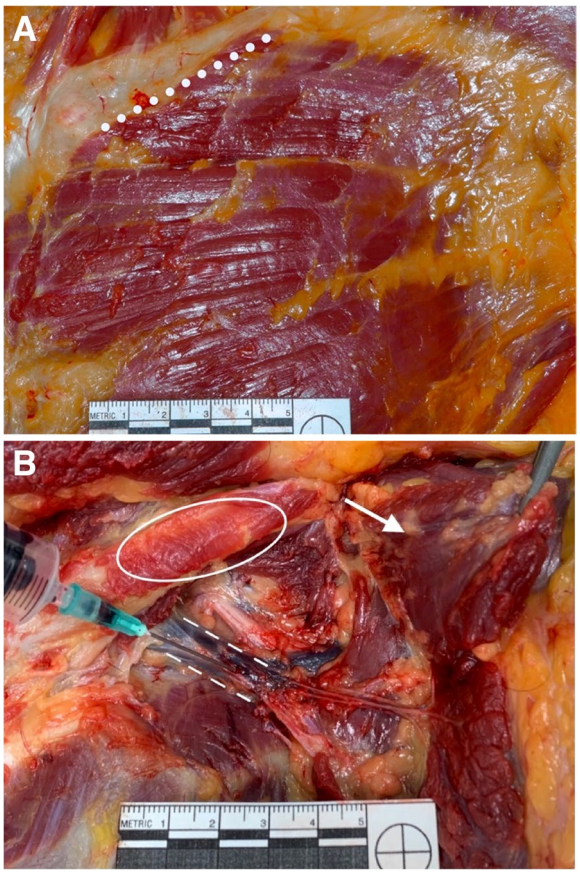

Fig. 1 **A** After skin and subcutaneous tissue of the anterior chest wall was opened laterally, the pectoralis major muscle was exposed. The dotted line indicated the incision site on the clavicle (left side). **B** After the clavicular head of the pectoralis major muscle was dissected (white ellipse) and raised (arrow), the axillosubclavian vein was exposed and blood sample was drawn. The dashed line indicated the anatomic location of the axillosubclavian vein

All specimens were stored in vacutainers containing sodium fluoride preservative at 4 °C promptly after being drawn.

Toxicological analysis

ASB, FVB, and VH from each subject were analyzed using the Agilent (California, USA) 7697A Headspace sampler with a 7890A series gas chromatography (GC) coupled with flame ionization detector (FID). Chromatographic separation was performed using HP-B ALC column (7.5 m×0.32 mm ID×20 µm film thickness) obtained from Agilent (CA, USA). The carrier gas was Helium with the flow rate at 20 mL/min and split injection mode was used. The GC oven (column temperature) was held at 120 °C for 2 min and ramped to 145 °C at a rate of 25 °C/min and held for 3 min. The injector temperature was set at 290 °C and the detector temperature was set at 300 °C. The FID hydrogen gas and air zero were employed at a flow rate of 30 mL/min and 400 mL/min, respectively. The sample injection volume was 1 µL. The analytical method was tested for suitability following forensic toxicology laboratories by ANSI/ASB standard 036, 1st Ed. 2109.

The linearity range of the calibration curve was 9.91 to 376.02 mg/dL. An internal standard solution was tert-butanol prepared at 100 mg/dL in deionized water. Each one hundred microliters of calibrators, controls, and case samples were placed into a 20-ml headspace vial and then mixed with 100 µm of internal standard. Then, the vials were crimp sealed and placed on the instrument for analysis.

Statistical analysis

The statistical analysis was performed using the program Stata 16. Descriptive statistics for ethanol concentrations in these three specimens were presented with mean, median, range, percentage, and 95% confidence intervals. The comparisons of ethanol concentrations in ASB and FVB were analyzed by Wilcoxon rank-sum test due to their non-normal distribution data, and their relationships were evaluated by the pairwise correlation coefficient. After analysis, all subjects were divided into four subgroups based on the development of lividity (fixed or unfixed) and absorption status, classified by using three different cut-off values of ethanol concentration ratio in VH/FVB including 1, 1.18, and 1.4. Subjects with the ratio of ethanol concentrations in VH/FVB less than 1, 1.18, and 1.4 were classified as the absorptive phase whereas subjects with the ratio of VH/FVB equal to or higher than 1, 1.18, and 1.4 were classified as the post-absorptive phase. The Wilcoxon rank-sum test and the pairwise correlation coefficient were also applied to these four sub-groups to evaluate their correlations. The diagnostic test (the receiver operating characteristics (ROC) curve) was analyzed for ethanol concentrations in ASB against FVB for the legal limit in Thailand (50 mg/dL). ROC curve parameters including sensitivity, specificity, positive and negative predictive values, and the area under the ROC curve were assessed. The statistical significance was set at the p-value less than 0.05.

Results

Three hundred specimens from 100 subjects were recruited for ethanol analysis in this study. The ethanol concentrations, the developments of livor mortis, and the ratios of VH/FVB for all samples are shown in Table 1. The mean ethanol concentrations in FVB, ASB, and VH were 157.08, 149.87, and 184.66 mg/dL, respectively. Of the total subjects, 51 cases were classified as unfixed lividity and 49 cases were classified as fixed lividity. Table 2

Table 1 Postmortem ethanol concentrations (mg/dL) in three types of studied samples, fixation of lividity, and the ratio of VH/FVB for all 100 subjects

Case no	Ethanol concentration			Lividity (unfixed = 1, fixed = 2)	Concentration ratio of VH/FVB	Case no	Ethanol concentration			Lividity (unfixed = 1, fixed = 2)	Concentration ratio of VH/FVB
	FVH	ASB	VH				FVH	ASB	VH		
1	213.45	181.47	214.18	1	1.003	26	203.51	179.68	328.87	2	1.616
2	209.26	172.97	198.62	1	0.949	27	11.46	11.91	10.58	2	0.923
3	13.41	21.35	33.78	1	2.519	28	168.01	159.19	218.58	2	1.301
4	33.8	34.18	52.4	2	1.550	29	333.71	311.4	324.64	1	0.973
5	269.91	295.81	286.93	2	1.063	30	163.14	143.29	184.15	2	1.129
6	251.24	289.94	360.51	2	1.435	31	10.24	14.61	13.73	2	1.341
7	106.18	115.62	146.2	2	1.377	32	29.98	36.83	46.19	2	1.541
8	44.33	55.6	35.62	2	0.804	33	398.05	406.39	475.59	2	1.195
9	204.56	197.74	232.82	2	1.138	34	38.04	40.49	44	2	1.157
10	144.53	135.53	140.86	2	0.975	35	132.33	147.43	134.61	2	1.017
11	241.93	244.48	276.35	2	1.142	36	235.18	192.41	272.28	1	1.158
12	18.23	28.86	29.42	2	1.614	37	268.78	253.62	326.59	2	1.215
13	19.03	11.71	18.41	2	0.967	38	67.27	46.47	71.4	1	1.061
14	12.35	12.99	15.87	2	1.285	39	54.69	46.61	80.85	2	1.478
15	13.68	22.25	26.02	2	1.902	40	43.59	42.14	68.94	2	1.582
16	300.17	292.54	370.85	2	1.235	41	137.63	118.05	143.04	2	1.039
17	137.49	104.01	154.04	1	1.120	42	142.24	108.39	166.91	1	1.173
18	139.92	131.69	145.08	2	1.037	43	301.09	251.15	298.03	1	0.990
19	31.39	27.23	12.38	2	0.394	44	179.61	171.16	157.87	2	0.879
20	71.72	95.76	119.53	2	1.667	45	231.62	206.36	233.83	1	1.010
21	312.38	296.12	459.38	2	1.471	46	120.63	107.33	154.78	1	1.283
22	281.65	248.95	333.39	2	1.184	47	235.1	197.59	187.23	2	0.796
23	17.54	13.26	47.99	2	2.736	48	97.35	97.16	143.77	2	1.477
24	452.8	407.09	478.47	2	1.057	49	11.78	12.18	11.16	2	0.947
25	183.63	150.16	210.14	2	1.144	50	211.51	205.36	137.46	1	0.650
51	145.3	136.85	194.48	1	1.338	76	153.89	152.72	162.54	1	1.056
52	283.75	297.87	354.56	1	1.250	77	252.17	282.09	365.93	1	1.451
53	49.06	47.24	66.59	1	1.357	78	116.83	126.15	164.62	1	1.409
54	262.55	281.57	300.9	1	1.146	79	56.96	64.17	65.01	1	1.141
55	247.47	246.12	252.52	1	1.020	80	24.15	19.57	42.12	1	1.744
56	108.06	157.1	206.03	2	1.907	81	127.21	106.71	132.73	1	1.043
57	14.19	36.68	11.32	2	0.798	82	14.87	14.13	12.71	1	0.855
58	267.4	245.39	351.34	2	1.314	83	61.94	44.49	56.74	1	0.916
59	325.68	369.09	454.69	2	1.396	84	17.72	20.39	16.34	2	0.922
60	205.5	180.61	214.21	1	1.042	85	179.94	166.79	185.55	1	1.031
61	197.85	222.45	241.55	1	1.221	86	21.27	20.9	31.39	1	1.476
62	12.09	22.81	11.77	2	0.974	87	177	150.64	191.15	2	1.080
63	315.74	268.94	341.1	1	1.080	88	187.62	181.76	184.42	1	0.983
64	236.99	204.58	277.42	1	1.171	89	248.15	255.61	265.33	1	1.069
65	10.43	10.54	10.51	2	1.008	90	133.72	125.46	211.54	1	1.582
66	328.23	324.25	396.54	1	1.208	91	123.1	110.85	150.57	1	1.223
67	160.01	129.48	142.32	1	0.889	92	366.25	360.03	583.73	2	1.594
68	170.81	136.14	169.73	1	0.994	93	234.56	226.17	412.34	1	1.758
69	165.45	147.88	192.33	2	1.162	94	254.26	253.64	329.79	1	1.297
70	20.09	16.89	21.64	1	1.077	95	155.67	154.64	127.7	1	0.820
71	240.34	234.87	276.84	1	1.152	96	233.61	247.07	335.6	1	1.437
72	316.26	306.29	351.41	1	1.111	97	77.73	78.84	105.36	1	1.355

Table 1 (continued)

Case no	Ethanol concentration			Lividity (unfixed = 1, fixed = 2)	Concentration ratio of VH/FVB	Case no	Ethanol concentration			Lividity (unfixed = 1, fixed = 2)	Concentration ratio of VH/FVB
	FVH	ASB	VH				FVH	ASB	VH		
73	10.46	10.51	10.27	1	0.982	98	18.23	17.57	31.36	1	1.720
74	338.39	250.94	313.64	1	0.927	99	99.09	91.21	123.21	2	1.243
75	168.64	156.05	163.67	1	0.971	100	220.5	179.33	216.09	1	0.980

Table 2 The summary of postmortem ethanol concentrations from all studied samples in each subgroup

Data set	*n*	FVB				ASB				VH			
		Mean	Median	Max	Min	Mean	Median	Max	Min	Mean	Median	Max	Min
Total	100	157.08	157.84	452.8	10.24	149.87	147.66	407.09	10.51	184.66	165.77	583.7	10.27
Subgroup													
Unfixed lividity case	51	172.75	179.94	338.39	10.46	160.57	166.79	324.25	10.51	194.88	185.55	412.34	10.27
Fixed lividity case	49	140.77	132.33	452.8	10.24	138.73	131.69	407.09	10.54	174.02	143.77	583.73	10.51
VH/FVB ratio < 1.00	25	130.63	155.67	338.39	10.46	116.05	135.53	311.40	10.51	118.78	137.46	324.64	10.27
VH/FVB ratio ≥ 1.00	75	165.90	163.14	452.80	10.24	161.13	150.16	407.09	10.54	206.61	192.33	583.73	10.51
VH/FVB ratio < 1.18	57	161.77	168.64	452.8	10.43	147.58	150.64	407.09	10.51	165.95	166.91	478.47	10.27
VH/FVB ratio ≥ 1.18	43	150.87	120.63	398.05	10.24	152.91	125.46	406.39	12.99	209.46	164.62	583.73	14.73

shows the number of cases and relevant statistics for each group classified by absorption status according to each of the three cutoff values of ethanol concentration in VH/FVB.

As the ethanol concentrations in this study were not normally distributed, the Wilcoxon rank-sum test was performed for the comparisons of ethanol concentrations among ASB and FVB. There was no statistical difference between ASB and FVB ($p = 0.6761$). Ethanol concentrations in these two sampling sites were significantly correlated ($p < 0.001$) with the pairwise correlation coefficient (r) as 0.9818. When the development of livor mortis was considered, there was also no statistical difference in ASB and FVB between unfixed and fixed lividity groups ($p = 0.4885$ and $p = 0.9688$, respectively). Ethanol concentrations in ASB were significantly correlated with those in FVB in both unfixed and fixed lividity groups ($r = 0.9751$, $p < 0.001$ and $\underline{r} = 0.9872$, $p < 0.001$, respectively). The comparison of ethanol concentrations between ASB and FVB among absorptive and postabsorptive phases also presented with no statistical difference and these two sampling sites were significantly correlated in all dividing ethanol concentration ratios in VH/FVB of 1, 1.18, and 1.4 as shown in Table 3.

In total, the diagnostic study was analyzed to assess the practical application of ASB as the alternative specimen for FVB at the cut-off concentration greater than 50 mg/dL. The ASB yielded sensitivity of 95.9%, specificity of 96.2%, area under the ROC curve of 0.96, positive predictive value of 98.6%, and negative predictive value of 89.3% as shown in Table 4.

Discussion

Ethanol concentrations in postmortem blood samples could differ between various sampling sites and this factor is crucial for the interpretation of blood ethanol concentrations. Postmortem blood ethanol concentrations might originate from ante-mortem ingestion, postmortem redistribution, or even from postmortem neogenesis by microbial activities during time since death (Kugelberg and Jones 2007). The availability and quality of blood samples are also related to the conditions of dead bodies. For example, deaths from exsanguination and severe burn might not provide sufficient blood samples for collection. Putrefied bodies might have blood of poor quality for samples due to postmortem neogenesis of ethanol from microbes. They might also have insufficient blood for sampling due to decomposition stages (Skopp 2010). Thus, alternative specimens that produce equivalent results to standard specimens, would be beneficial for postmortem toxicology.

Our study found that postmortem ethanol concentrations in the ASB were well correlated with the postmortem ethanol concentrations in FVB, which are the standard samples used for postmortem analysis. This finding is consistent with the study of Sastre C et al. who investigated the relationship of postmortem ethanol concentrations between subclavian blood and FVB

Table 3 Statistical comparison of ethanol concentrations between ASB and FVB

Comparative and correlation study (n)	Wilcoxon rank-sum test	Pairwise correlation coefficient
FVB-ASB (100)	0.6761	< 0.001 ($r = 0.9818$)
Subgroup analysis of FVB-ASB classified by fixation of lividity		
Unfixed (51)	0.4885	< 0.001 ($r = 0.9751$)
Fixed (49)	0.9688	< 0.001 ($r = 0.9872$)
Subgroup analysis of FVB-ASB classified by phases of absorption at different ethanol concentration ratios of VH/FVB		
VH/FVB ratio < 1.00	0.7052	< 0.001 ($r = 0.9872$)
VH/FVB ratio ≥ 1.00	0.8055	< 0.001 ($r = 0.9838$)
VH/FVB ratio < 1.18	0.4193	< 0.001 ($r = 0.9818$)
VH/FVB ratio ≥ 1.18	0.9140	< 0.001 ($r = 0.9751$)
VH/FVB ratio < 1.14	0.4958	< 0.001 ($r = 0.9815$)
VH/FVB ratio ≥ 1.14	0.8005	< 0.001 ($r = 0.9881$)

Table 4 The assessment of the application of ASB to determine driving under influence cases compared with FVB

Test characteristic	Result	95%CI
Sensitivity	95.9%	88.6,99.2
Specificity	96.2%	80.4,99.99
Positive predictive value	98.6%	92.5,100
Negative predictive value	89.3%	71.8,97.7
Area under the ROC curve	0.96	0.917,1

in Marseille, France, and found that ethanol concentrations in subclavian blood were not statistically different from those in FVB ($p = 0.948$) and ethanol concentrations from these two sites were significantly correlated ($r = 0.961$, $p < 0.001$) (Sastre et al. 2013). Our finding is also consistent with the study by DE Martinis BS et al. that was conducted in Sao Paulo, Brazil and revealed that subclavian blood was significantly correlated with femoral blood for postmortem ethanol analysis ($r = 0.98$) (Martinis et al. 2006).

Our study was conducted in the central region of Thailand where the tropical climate has 3 seasons including winter, rainy, and summer. The average temperature in Thailand in 2021 was 27.5 °C. The temperature in Central Thailand in 2021 ranged from a monthly average of 36.2 °C in May to a monthly average of 21.2 °C in December (Thai Meteorological Department (2022), Climatological Center (2022). Postmortem changes can progress more rapidly in a high temperature environment. The effect of the climate in Thailand on postmortem ethanol analysis has not yet been studied, but effects of climate on postmortem change resulting in distinct differences in decomposition stages from countries with temperate climates has been reported. Meewuttisom K et al. conducted a study on data from victims in the tsunami that hit the coast of southern Thailand on December 26, 2004, and found that full bloating, skin bleb, and skin-slippage of corpses could be observed approximately 48–72 h after death (Meewuttisom and Poriswanish 2014); whereas, it might take up to 2–3 weeks to develop these signs of decomposition in corpses in an indoor environment of 18–20 °C (Saukko and Knight 2015).

In different climate zones and in different periods after death, forensic pathologists obtain samples of blood with varying degree of postmortem coagulation, water content, hemolysis, or postmortem redistribution. Ethanol is an ambivalent molecule that usually diffuses into the water compartments of the body and readily crosses membranes. The decrease in water content of blood and the breakdown of vessels after death may affect the diffusion of ethanol in ASB and FVB (Skopp 2004), leading to changes of their correlation of ethanol concentrations. Hence, to determine the effect of the different periods of time since death before signs of decomposition began, the fixation of livor mortis was used as a representative finding for sub-group study. In general, livor mortis becomes fixed when the time since death has proceeded

to around 8 to 12 h (Mathur and Agrawal 2011). The results showed that each group showed good correlation coefficients.

This study excluded putrefactive bodies because the information about the postmortem ethanol production in Thai postmortem cases was scarce and the degree of ethanol produced from decomposition might have an impact on the correlation of ethanol concentrations in ASB and FVB. However, Sastre C et al. studied in the decomposed subjects with the degree of putrefaction from SMELLBAD score > 3, the putrefactive process did not significantly affect the relationship of ethanol concentrations in FVB and subclavian blood ($p = 0.419$) and these two sites still had a significant correlation ($r = 0.962$, $p < 0.001$) (Sastre et al. 2013; Zumwalt et al. 1982). Further research should be conducted both in terms of postmortem ethanol production and in terms of alternative specimens in corpses with varying degrees of decomposition in Thailand.

This study excluded subjects presented with ethanol that was positive only in blood samples but negative in VH, which indicated postmortem ethanol production (Lin et al. 2020; O'Neal and Poklis 1996; Caplan and Levine 1990). Thus, blood ethanol concentrations in this study were consumed before death and the phase of ethanol absorption could be determined by the ratio of VH/FVB. It was known that there was an arterial-venous difference in each phase of absorption. Ethanol concentrations detected in arterial blood are higher than in venous blood during the absorptive phase, but ethanol concentrations detected in venous blood are slightly higher than in arteries during the postabsorptive phase (Jones et al. 2004). Therefore, another subgroup analysis was conducted to determine the correlation of ethanol concentrations between ASB and FVB in each phase of absorption. However, the concentration ratios of VH/FVB had an individual variation. The median ratio of VH/FVB in the study of Caplan YH et al. was 1.18 and the mean concentration ratio from the study of Jones AW et al. was 1.19 (median, 1.18) with a standard deviation of 0.285 (Caplan and Levine 1990; Jones and Holmgren 2001). Therefore, our study also evaluated this correlation by using the concentration ratios of VH/FVB of 1, 1.18, and 1.4 which were comprehensive for the studied population. Our results showed a good correlation in both the absorptive phase and the postabsorptive phase for all different cut-off points.

According to statistical calculation for diagnostic test to determine the application of using ASB ethanol concentrations against FVB ethanol concentrations to detect driving under influence cases based on the legal limit of Thailand, the area under the ROC curve from our study suggested that ASB is an excellent alternative to use as a specimen when FVB is not available if corpses were found in tropical climates without large open wounds on the body and without signs of decomposition.

Conclusions

Our study indicated that ASB can be used as an alternative specimen for postmortem ethanol analysis when FVB is not available in dead bodies that are in a tropical climate and do not present with any signs of decomposition and large open wounds. This correlation could also be applied to all states of absorption. Additionally, our study illustrated the blood sampling site from the anatomic position at the thoracic outlet during the autopsy procedure using the subclavicular line incision. This showed the blood collection method after opening the thoracic cavity commonly used in general procedure. Thus, this blood collection procedure could be encouraged for postmortem ethanol analysis when there are limitations for FVB collection. Further studies for this correlation in corpses with varying degrees of putrefaction in countries with tropical climates are still required to compare ethanol concentrations in ASB samples with FVB samples.

Abbreviations

ASB	Axillosubclavian blood
FID	Flame ionization detector
FVB	Femoral venous blood
GC	Gas chromatography
ROC	Receiver operating characteristics
TUH	Thammasat University Hospital
VH	Vitreous humor

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Authors' contributions

AT initiated the study, applied for funding, and registered to ethical approval. TP is the corresponding author. Both authors have made a significant contribution to this study since the study design, specimen collection, data analyses, manuscript preparation, and the final manuscript approval. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

This study was approved by the Human Research Ethics Committee of Thammasat University No.1 (Faculty of Medicine) in compliance with the Declaration of Helsinki and International Conference on Harmonization of Good Clinical Practice which includes the informed consent obtaining from the corpse's relatives.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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