archiwum medycyny sądowej i kryminologii

Praca oryginalna
Original paper

Marcin Tomsia[1], Joanna Nowicka[1], Rafał Skowronek[1], Gulnaz T. Javan[2], Elżbieta Chełmecka[3]

Concentrations of volatile substances in costal cartilage in relation to blood and urine – preliminary studies

[1]Department of Forensic Medicine and Forensic Toxicology, Faculty of Medical Sciences in Katowice, Medical University of Silesia in Katowice, Katowice, Poland
[2]Department of Physical Sciences and Forensic Science Programs, Alabama State University, Montgomery, AL, USA
[3]Department of Statistics, Department of Instrumental Analysis, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, Katowice, Poland

Abstract

Aim: The study aimed to examine whether volatile substances (ethanol, isopropanol, and acetone) can be detected in costal cartilage and also if concentrations of detected substances reliably reflect their concentrations in the peripheral blood – the standard forensic material for toxicological analyses. Such knowledge can be useful in cases when a cadaver's blood is unavailable or contaminated.

Material and methods: Ethanol, isopropanol, and acetone concentrations were determined in samples of unground costal cartilage (UCC), ground costal cartilage (GCC), femoral venous blood, and urine. The samples were analysed by gas chromatography (GC) with a flame ionization detector using headspace analysis.

Results: Volatile substances were detected in 12 out of 100 analysed samples. There was a strong positive correlation between ethanol concentration in the blood and urine ($r = 0.899$, $p < 0.001$), UCC ($r = 0.809$, $p < 0.01$), and GCC ($r = 0.749$, $p < 0.01$). A similar strong correlation was found for isopropanol concentration in the blood and urine ($r = 0.979$, $p < 0.001$), UCC ($r = 0.866$, $p < 0.001$), and GCC ($r = 0.942$, $p < 0.001$). Acetone concentration in the blood strongly correlated only with its concentration in urine ($r = 0.960$, $p < 0.001$).

Conclusions: We demonstrated for the first time the possibility of detecting volatile substances: ethanol, isopropanol and acetone in a human costal cartilage. Also, the study showed that higher volatiles concentrations were better determined in ground samples.

Key words: ethanol, acetone, costal cartilage, volatile substance, isopropanol, gas chromatography with a flame ionization detector.

Introduction

Cases of poisoning with non-consumable alcohol are rare in Poland, but it can be detected during forensic ethanol analyses [1, 2]. Autopsy reports, containing gas chromatography (GC) results of routine ethyl alcohol tests, sometimes state the presence of acetone and its minor metabolite isopropanol [3, 4]. Both compounds were reported in cases of cadavers with suspected diabetes [5, 6], death from hypothermia [7, 8], starvation [9], dehydration [10], and chronic ethanol overuse [11]. A high isopropanol/acetone ratio in the tested samples strongly indicates the exogenous origin of isopropanol, particularly

Copyright © 2021 by PTMSiK

when it is accompanied by high levels of ethanol [12]. In such cases, the victim most likely ingested isopropanol by drinking alcohol of dubious origin [1]. Isopropanol (2-propanol; rubbing alcohol), besides being a component of various household solutions, such as antifreeze or window cleaner [13, 14], is also commonly used as antiseptic and disinfectant agent [15].

Several papers describe the post-mortem distribution of volatile substances in different biological fluids and tissues, e.g. vitreous humour [16], pericardial fluid and bone marrow aspirate [17], kidney [18], liver [19], muscle [20], brain [21], and even haematoma [22]. Because blood and other soft tissues are prone to rapid post-mortem degradation, the search for alternative post-mortem sampling materials is extremely important.

Cartilage is a specialized type of connective tissue formed by chondrocytes lying in an amorphous matrix rich in collagen and elastic fibres [23]. Costal cartilage connects the ribs to the sternum and allows for rib cage flexibility. Unlike other types of cartilages, which are only a few millimetres thick, the costal cartilage can reach up to approximately 1 cm in diameter [24]. Its extracellular matrix contains collagen IIAI (COL2A1) type, aggrecan, decorin, and biglycan [25]. In clinical procedures, sometimes it is used for the autologous ear reconstruction [26, 27], mentoplasty [28] and rhinotherapy [29, 30] and in cadaveric allografts [31].

Considering the characteristics of both costal cartilage and volatile substances, we assume that costal cartilage could be treated as a new alternative material for post-mortem volatile substance analysis, especially in cases where blood is unavailable or contaminated. The main purpose of this study was to establish correlations between volatile substances levels in the blood, urine and costal cartilage.

Materials and methods

The study was approved by the local Bioethical Commission (decision no. KNW/0022/KB/206/18).

The samples of the costal cartilage, femoral blood, and urine were collected during medico-legal autopsies. The study group was selected from 100 cadavers with volatile substances detected in their blood and urine (positive results for ethanol, isopropanol, and acetone in their body fluids). The study group consisted of 12 corpses of middle-aged

Table I. Descriptive statistics of the concentrations of non-consumable volatile substances in the blood, urine, and unground and ground costal cartilage samples collected from human cadavers

Non-consumable substances concentration [unit]	N	x_{min}	x_{max}	M	SD	Me	IQR
Ethanol							
Blood (mg/mL)	12	0.00	4.93	2.55	1.39	2.72	1.91
Urine (mg/mL)	12	0.06	4.59	2.69	1.44	2.95	1.93
UCC (mg/g)	12	0.16	1.43	0.55	0.33	0.47	0.32
GCC (mg/g)	12	0.00	4.59	1.54	0.17	1.00	0.62
Isopropanol							
Blood (mg/mL)	12	0.010	0.800	0.300	0.277	0.270	0.430
Urine (mg/mL)	12	0.020	0.810	0.295	0.282	0.290	0.380
UCC (mg/g)	12	0.000	0.156	0.046	0.047	0.036	0.066
GCC (mg/g)	12	0.006	0.234	0.110	0.093	0.122	0.180
Acetone							
Blood (mg/mL)	12	0.020	0.410	0.157	0.131	0.140	0.215
Urine (mg/mL)	12	0.001	0.460	0.218	0.177	0.215	0.350
UCC (mg/g)	12	0.000	0.006	0.002	0.003	0.000	0.005
GCC (mg/g)	12	0.001	0.036	0.013	0.011	0.015	0.019

GCC – ground costal cartilage, IQR – inter quartile range, M – mean, Me – median, SD – standard deviation, UCC – unground costal cartilage

people 48.8 ± 13.0 years of age (mean ± standard deviation) and with time from death of 6.3 (4.3–8.3) days (median (lower quartile – upper quartile). Other descriptive characteristics of the study group are presented in Table I. The costal cartilage samples (n = 12) were collected from each of the cadavers.

Sample preparation

Femoral blood and urine samples were collected as described in forensic guidelines [32]. Analyses of ethanol, acetone, and isopropanol levels in these samples were estimated using procedures developed by Tomsia *et al.* [33].

The costal cartilage fragments (dimensions 5 × 8 cm) were taken from the rib arches. Each sample was divided into two subsamples that were processed differently. For each cadaver (n = 100) we obtained costal cartilage samples differing in the degree of putrefaction: 1) ground costal cartilage (GCC) and 2) unground costal cartilage (UCC). GCC was ground in a cryogenic mill in the presence of liquid nitrogen (Cryogenic Mill 6770 SpexSamplePrep with 3 min precooling and 1 cycle of 2 min grinding at 12 CPS). UCC was obtained by manual scalpel fragmentation. In each case 0.2 g of costal cartilage was analysed.

Volatile substance analysis

The headspace analysis was performed on a Focus GC gas chromatograph equipped with a Triplus autosampler, flame ionization detector (Thermo Fisher Scientific, Inc., USA) and Rtx®–BAC2 column (30 m × 0.53 mm ID × 2.0 μm) (Restek Corp., USA). The oven temperature sequence program was 45°C (5 min), 45–80°C (10°C/min), 80°C (1 min). The injector and detector temperatures were 200°C and 250°C, respectively. The carrier gas was helium (5.0 mL/min), and t-butyl alcohol was used as an internal standard. The validation procedures for isopropanol and acetone analysis in GCC and UCC were done the same way as the validation procedure for ethanol presented in the study by Tomsia *et al.* [33].

Statistical analysis

Distribution of variables was evaluated by the Shapiro-Wilk test and quantile-quantile plot. The interval data were expressed as a mean value ± standard deviation in the case of a normal distribution or as a median (lower – upper quartiles; Me [Q_1; Q_3]) in the case of a skewed or non-normal data distribution. To determine the relationship between quantitative variables, ordinary least square regression was carried out. Statistical significance was set at $p < 0.05$, and all tests were 2-tailed. The agreement between the 2 methods of costal cartilage preparation (GCC, UCC) was analysed using Bland-Altman analysis. The results are presented as the mean differences between the 2 compared methods, and the 95% confidence intervals (95% CI) for these differences. Statistical analysis was performed using Statistica v. 13.3.0 (TIBCO Software Inc.).

Results

We detected the presence of isopropanol and acetone in 12 out of 100 samples analysed for the purposes of this study. The detailed descriptive statistics of ethanol, isopropanol, and acetone concentrations in various sampling materials are presented in Table I.

The results of concentrations of volatile substances obtained for blood samples were treated as a 'gold standard', according to all available guidelines, so we could easily compare the results obtained for other forensic materials.

The ethanol concentration in the blood positively correlated with its concentration in other forensic material (urine: $r = 0.899$, $p < 0.001$; UCC: $r = 0.809$, $p < 0.01$; and GCC: $r = 0.749$, $p < 0.01$) (Table II, Fig. 1 A, B). A 1 mg/mL increase in blood ethanol content corresponded to 0.936 mg/mL increase in urinary ethanol concentration, whereas the corresponding values for UCC and GCC were lower: 0.193 mg/g and 0.634 mg/g, respectively.

Ordinary least square regression explained up to 80.8% of the change in ethanol concentration in urine and up to 65.4% and 56.1% of the ethanol concentration in UCC and GCC, respectively.

The isopropanol concentration in the blood also positively correlated with its concentration in other tested forensic samples (urine: $r = 0.979$, $p < 0.001$; UCC: $r = 0.866$, $p < 0.001$; GCC: $r = 0.942$, $p < 0.001$) (Table II, Fig. 2 A, B). A 1 mg/mL increase in blood isopropanol content corresponded to a 0.996 mg/mL increase in urinary isopropanol concentration, but only to a 0.146 mg/g and 0.315 mg/g increase in

Table II. Analysis of univariable ordinary least square regression for ethanol, acetone, and isopropanol concentrations in the blood vs. their concentrations in urine and costal cartilage prepared with unground costal cartilage and ground costal cartilage methods

Concentrations of blood non-consumable volatile substances	Biological matrices	b	SE (β)	*r*	*p*
Ethanol	Urine	0.936	0.143	0.899	< 0.001
	UCC	0.193	0.044	0.809	< 0.01
	GCC	0.634	0.177	0.749	< 0.01
Isopropanol	Urine	0.996	0.065	0.979	< 0.001
	UCC	0.146	0.027	0.866	< 0.001
	GCC	0.315	0.035	0.942	< 0.001
Acetone	Urine	1.299	0.119	0.960	< 0.001
	UCC	0.011	0.005	0.549	0.064
	GCC	0.045	0.023	0.524	0.080

β – regression coefficient, SE (β) – standard error for the regression coefficient, r – Pearson's linear correlation coefficient, UCC – unground costal cartilage, GCC – ground costal cartilage

UCC and GCC, respectively. The linear regression method explained up to 95.8% of the change in isopropanol concentration in urine, up to 75.0% and in UCC, and up to 88.7% in GCC.

Regarding the acetone concentration in different forensic materials, its concentration in the blood correlated only with its concentration in urine ($r = 0.960$, $p < 0.001$) (Table II). For the UCC and GCC method, we only observed a tendency toward statistical significance: $p = 0.064$ and $p = 0.080$, respectively (Fig. 1 E, F).

The ordinary least square regression model showed that for the costal cartilage ethanol concentration (UCC and GCC), the blood ethanol concentration was the only factor that was statistically important. The same was confirmed for isopropanol. We found that no statistically significant correlation exists between acetone concentration in blood and GCC and UCC costal cartilage. The only statistically significant variable reflecting acetone concentration in the blood was its concentration in urine (Table II).

The results of linear regression and agreement analysis for ethanol, isopropanol, and acetone concentrations in costal cartilage samples ($n = 12$) prepared using 2 different methods are presented in Figure 2.

The linear regression analysis showed that the results of ethanol and isopropanol concentrations obtained using the GCC method closely correlated with the results obtained with the UCC method (ethanol: $r^2 = 0.905$ and isopropanol: $r^2 = 0.705$). For acetone, the linear regression analysis showed the average correlation between the results obtained with GCC and UCC method ($r^2 = 0.310$).

The ethanol, isopropanol, and acetone concentrations measured in GCC samples were higher than those measured in UCC samples by 0.6064 mg/g, 0.0638 mg/g, and 0.0110 mg/g, respectively.

The Bland-Altman analysis showed that the mean difference for UCC-GCC for ethanol concentration analysis is statistically different from the 0 value ($p < 0.05$) (Fig. 2 B). However, the one data point outside of the 95% confidence interval in Figure 2 B draws attention. The difference it represents probably does not result from a measurement error but represents instead a random factor. After repeating the analysis without this extreme value, the mean difference for UCC-GCC was –0.374 ± 0.122 (mean ± SD), which was different from the 0 value ($p < 0.001$). The shape of the correlation indicates that the ethanol concentrations obtained using the GCC method are higher than those obtained using the UCC method. Additionally, the higher the ethanol concentration in the sample, the greater the difference in results obtained using GCC and UCC methods. The uniformly scattered data on Figure 2 D indicate no correlation between mean isopropanol concentration measured using UCC and GCC methods and the difference between UCC and GCC methods. It shows that the designated confidence interval limits (–0.1795, 0.0519) are uniform for the entire measurement range. All data points lie within

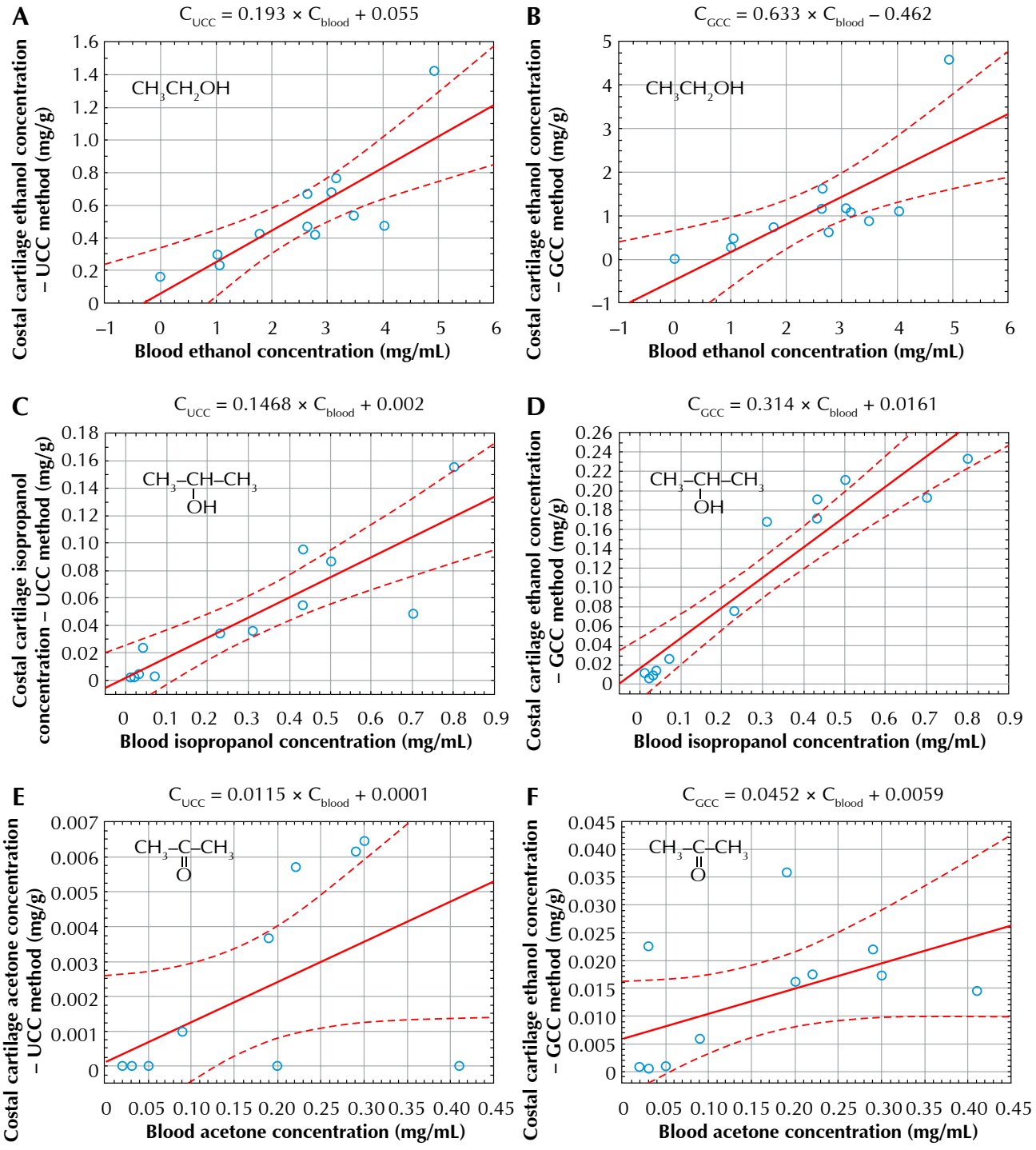

Fig. 1. The ordinary least square regression model for the relationship between the concentration of volatile substances in the blood and their concentration in costal cartilage samples prepared using 2 different methods (unground costal cartilage – UCC, ground costal cartilage – GCC): **A**, **B** – ethanol, **C**, **D** – isopropanol, **E**, **F** – acetone. Legend: the red line represents regression line, dashed lines indicate 95% confidence intervals

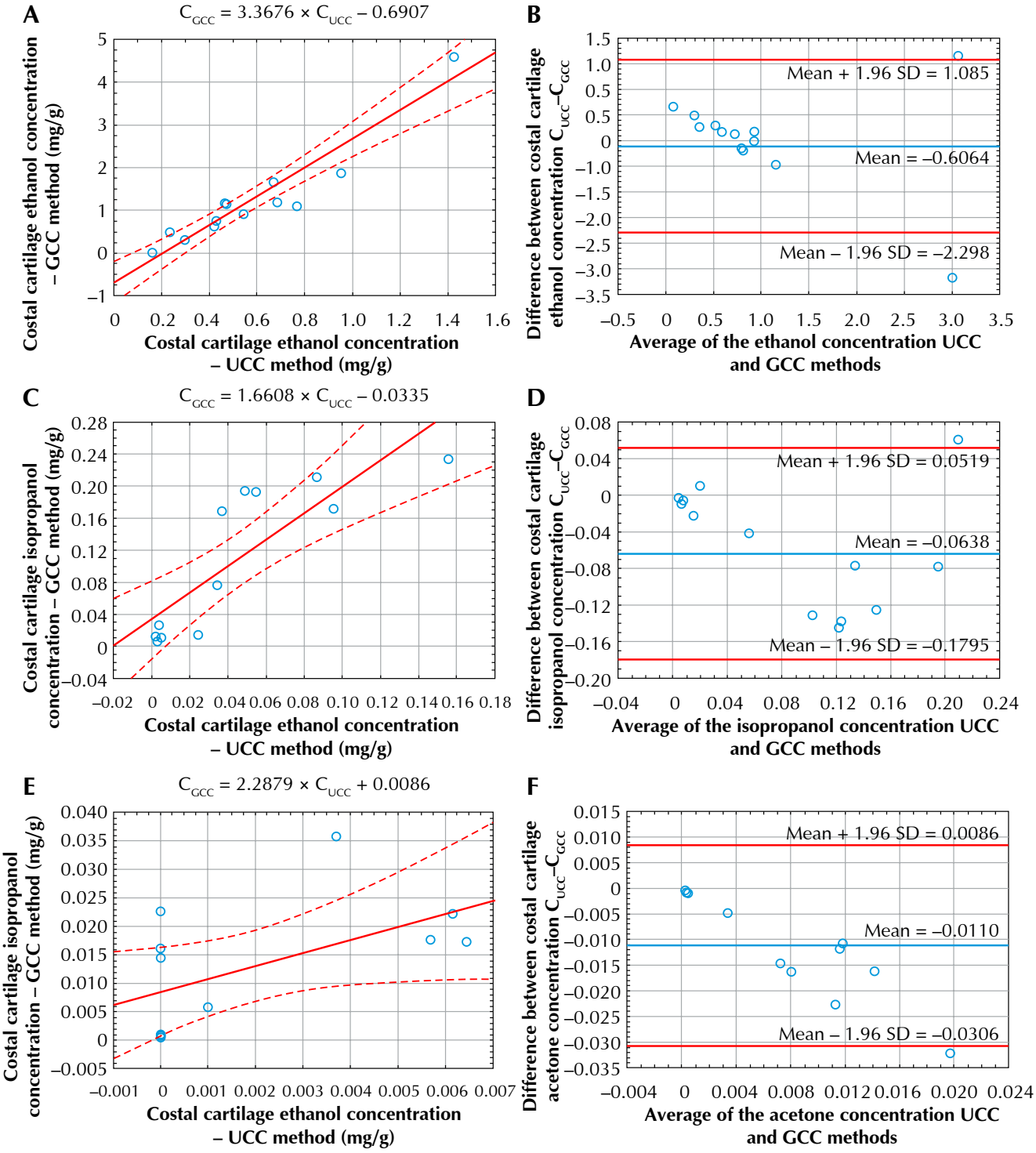

Fig. 2. The ordinary least square regression model (**A**, **C**, **E**) and Bland Altman plot (**B**, **D**, **F**) for the concentration of volatile substances in costal cartilage samples prepared using 2 different methods (unground costal cartilage – UCC, ground costal cartilage – GCC): **A**, **B** – ethanol, **C**, **D** – isopropanol, **E**, **F** – acetone. Legend: **A**, **C**, **E** – the red line represents regression line, dashed lines indicate 95% confidence intervals, **B**, **D**, **F** – the blue line represents the mean difference between volatile substance concentration measured using the UCC and GCC method, and the red lines indicate 95% confidence interval for the mean difference

the 95% CI range, which means that the observed differences result from the measurement errors. The same situation was observed for acetone concentration measurements (Fig. 2 F). The mean difference between the results for the UCC and GCC methods does not statistically differ from the 0 value both for isopropanol and acetone measurements.

Discussion

Post-mortem analyses performed by the academic department of forensic medicine show that the numbers of tests positive for the presence of isopropanol, classified as an ethanol substitute, have substantially increased in recent years. This phenomenon is probably related to the large quantities of spirits contaminated with isopropanol that have been sold illegally [34]. Forensic reports prepared for legal authorities sometimes state that not only ethyl alcohol but also toxic levels of other impurities may be responsible for sudden death. The medicolegal practice also indicates that prolonged exposure to relatively small amounts of volatiles, like isopropanol, may be equally dangerous [35]. That is why researching different volatile substances in biological material, both standard and alternative, is so important.

Based on the analysed literature, or rather the lack of it compared to other tissues, costal cartilage is one of the neglected and underestimated alternative materials in forensic science. Usually it is used to predict a cadaver's age, either by using computed tomography [36], estimating the ossification rate [37], or by using differences in its pigmentation [38]. New research has attempted to use costal cartilage to predict post-mortem interval basing on the extracellular matrix macromolecules degradation, but it seems to require further investigation [39]. More successfully, Siriboonpiputtana *et al*. have demonstrated that costal cartilage can serve as an alternative source for DNA typing in personal identification, and it enables faster and more cost-effective DNA isolation than hard tissues [40].

So far, the costal cartilage has not been included in forensic medical and toxicological guidelines for ethanol and volatile level analysis, even though it is confirmed that the penetration of xenobiotics into cartilage occurs (most probably through the perichondrium and intercostal veins) [41]. Our statistical analysis indicates a strong positive correlation between ethanol levels in the blood and UCC samples ($r = 0.809$, $p < 0.01$) or GCC samples ($r = 0.749$, $p < 0.01$). We found a similar strong correlation for isopropanol (blood vs. UCC: $r = 0.866$, $p < 0.001$; blood vs. GCC: $r = 0.942$, $p < 0.001$). Regarding acetone, no statistically significant correlation was found for acetone concentration in the blood and costal cartilage in both forms. It is probably related to the low concentrations and the small number of samples tested. The Bland-Altman analysis showed a high similarity between the UCC and GCC methods, which suggests that it would be possible to develop reliable analytical methods for analysis of volatile compounds in unground and GCC samples.

The major self-limitation of this study is the low number of tested samples, so it should be treated only as a preliminary report, and further studies are required. Considering all of the above, we conclude that it is possible to successfully and reliably determine ethanol and isopropanol levels in both unground and GCC samples. We also think that costal cartilage may serve as a reliable biological material for forensic toxicology and may allow reliable determination of the concentration of volatile substances. We are aware that costal cartilage sampling has its limitations because, in the case of fresh corpses, material preparation is time-consuming and requires the use of a cryogenic mill. However, we support the idea that costal cartilage may be used in post-mortem cases where blood is unavailable or contaminated, and that this possibility expands sampling options in forensic science.

Conclusions

In this study, we demonstrated for the first time the possibility of detecting volatile compounds: ethanol, isopropanol, and acetone, in human costal cartilage. The comparison of the 2 methods of costal cartilage preparation showed a high similarity of the results obtained using the UCC and GCC method. However, our results showed that high concentrations of volatile substances were better determined in GCC samples. We suggest that further investigations on post-mortem redistribution of volatile substances in costal cartilage might be important for future diagnostic applications, especially in cases where costal cartilage is the only material available for forensic toxicological analysis.

Acknowledgements

Samples from cadavers were taken with the consent of the local Bioethical Commission (decision number KNW/0022/KB/206/18).

This work was supported by the institutional grants for young scientists, from the Medical University of Silesia in Katowice (Poland), grant no. KNW-2-O18/N/9/N and partially by grant no. PCN-1-119/K/0/Z and by the National Science Foundation (NSF) grant no. HRD 2011764.

The authors declare no conflict of interest.

References

1. Borowska-Solonynko A, Siwińska-Ziółkowska A, Piotrkowicz M, Wysmołek M, Demkow M. Analysis of the origin and importance of acetone and isopropanol levels in the blood of the deceased for medico-legal testimony. Arch Med Sadowej Kryminol 2014; 64: 230-234.
2. Boumba VA, Ziavrou KS, Vougiouklakis T. Biochemical pathways generating post-mortem volatile compounds co-detected during forensic ethanol analyses. Forensic Sci Int 2008; 174: 133-151.
3. Wille SM, Lambert WE. Volatile substance abuse-post-mortem diagnosis. Forensic Sci Int 2004; 142: 135-156.
4. Vujasinovic M, Kocar M, Kramer K, Bunc M, Brvar M. Poisoning with 1-propanol and 2-propanol. Hum Exp Toxicol 2007; 26: 975-978.
5. Palmiere C. Postmortem diagnosis of *diabetes mellitus* and its complications. Croat Med J 2015; 56: 181-193.
6. Jones AE, Summers RL. Detection of isopropyl alcohol in a patient with diabetic ketoacidosis. J Emerg Med 2000; 19: 165-168.
7. Palmiere C, Bardy D, Letovanec I, et al. Biochemical markers of fatal hypothermia. Forensic Sci Int 2013; 226: 54-61.
8. Palmiere C, Mangin P. Postmortem biochemical investigations in hypothermia fatalities. Int J Legal Med 2013; 127: 267-276.
9. Palmiere C, Tettamanti C, Augsburger M, et al. Postmortem biochemistry in suspected starvation-induced ketoacidosis. J Forensic Leg Med 2016; 42: 51-55.
10. Davis PL, Dal Cortivo LA, Maturo J. Endogenous isopropanol: forensic and biochemical implications. J Anal Toxicol 1984; 8: 209-212.
11. Dwyer JB, Tamama K. Ketoacidosis and trace amounts of isopropanol in a chronic alcoholic patient. Clin Chim Acta 2013; 415: 245-249.
12. Chan KM, Wong ET, Matthews WS. Severe isopropanolemia without acetonemia or clinical manifestations of isopropanol intoxication. Clin Chem 1993; 39: 1922-1925.
13. Molina DK. A characterization of sources of isopropanol detected on postmortem toxicologic analysis. J Forensic Sci 2010; 55: 998-1002.
14. Wu X, Lu G, Qi B, Wang R, Guo D, Liu X. Antifreeze poisoning: a case report. Exp Ther Med 2017; 13: 701-704.
15. Slaughter RJ, Mason RW, Beasley DM, Vale JA, Schep LJ. Isopropanol poisoning. Clin Toxicol (Phila.) 2014; 52: 470-478.
16. Bévalot F, Cartiser N, Bottinelli C, Fanton L, Guitton J. Vitreous humor analysis for the detection of xenobiotics in forensic toxicology: a review. Forensic Toxicol 2016; 34: 12-40.
17. Tominaga M, Ishikawa T, Michiue T, et al. Postmortem analyses of gaseous and volatile substances in pericardial fluid and bone marrow aspirate. J Anal Toxicol 2013; 37: 147-151.
18. Lewis RJ, Johnson RD, Angier MK, Vu NT. Ethanol formation in unadulterated postmortem tissues. Forensic Sci Int 2004; 146: 17-24.
19. Jenkins AJ, Levine BS, Smialek JE. Distribution of ethanol in postmortem liver. J Forensic Sci 1995; 40: 611-613.
20. Garriott JC. Skeletal muscle as an alternative specimen for alcohol and drug analysis. J Forensic Sci 1991; 36: 60-69.
21. Chun HJ, Poklis JL, Poklis A, Wolf CE. Development and validation of a method for alcohol analysis in brain tissue by headspace gas chromatography with flame ionization detector. J Anal Toxicol 2016; 40: 653-658.
22. Boonyoung S, Narongchai P, Junkuy A. The relationship of alcohol concentration in epidural or acute subdural hematoma compared with vitreous humor and femoral blood. J Med Assoc Thai 2008; 91: 754-758.
23. Huwe LW, Brown WE, Hu JC, Athanasiou KA. Characterization of costal cartilage and its suitability as a cell source for articular cartilage tissue engineering. J Tissue Eng Regen Med 2018; 12: 1163-1176.
24. Stacey MW. Biochemical and histological differences between costal and articular cartilages. In: Saxena AK (ed.). Chest wall deformities. Springer, London 2017, 81-99.
25. Grogan SP, Chen X, Sovani S, et al. Influence of cartilage extracellular matrix molecules on cell phenotype and neocartilage formation. Tissue Eng Part A 2014; 20: 264-274.
26. Yotsuyanagi T, Yamashita K, Yamauchi M, et al. Establishment of a standardized technique for concha-type microtia-how to incorporate the cartilage frame into the remnant ear. Plast Reconstr Surg Glob Open 2019; 26: e2337.

27. Mao X, Li X, Jia J, et al. Validity and reliability of three-dimensional costal cartilage imaging for donor-site assessment and clinical application in microtia reconstruction patients: A prospective study of 22 cases. Clin Otolaryngol 2020; 45: 204-210.
28. Zhang L, Ma WS, Bai JP, Li XX, Li HD, Zhu T. Comprehensive application of autologous costal cartilage grafts in rhino- and mentoplasty. J Craniofac Surg 2019; 30: 2174-2177.
29. Mohan R, Shanmuga Krishnan RR, Rohrich RJ. Role of fresh frozen cartilage in revision rhinoplasty. Plast Reconstr Surg 2019; 144: 614-622.
30. Talaat WM, Ghoneim MM, El-Shikh YM, Elkashty SI, Ismail MAG, Keshk TFA. Anthropometric analysis of secondary cleft lip rhinoplasty using costal cartilage graft. J Craniofac Surg 2019; 30: 2464-2468.
31. Saadi R, Loloi J, Schaefer E, Lighthall JG. Outcomes of cadaveric allograft versus autologous cartilage graft in functional septorhinoplasty. Otolaryngol Head Neck Surg 2019; 161: 779-786.
32. Dinis-Oliveira RJ, Vieira, DN, Magalhães, T. Guidelines for collection of biological samples for clinical and forensic toxicological analysis. Forensic Sci Res 2017; 1: 42-51.
33. Tomsia M, Nowicka J, Skowronek R, et al. A comparative study of ethanol concentration in costal cartilage in relation to blood and urine. Processes 2020; 8: 1637.
34. Nowicka J, Kulikowska J, Chowaniec C, et al. Medicolegal and toxicological aspects of isopropanol levels in post-mortem material. Problems Forensic Sci 2010; 82: 191-199.
35. Drela E, Rosół M, Trnka J. Poisonings with iso-propanol and acetone as the substitutes of ethyl alcohol. Problems Forensic Sci 2004; 58: 58-69.
36. Zhang K, Fan F, Tu M, et al. The role of multislice computed tomography of the costal cartilage in adult age estimation. Int J Legal Med 2018; 132: 791-798.
37. Ikeda T. Estimating age at death based on costal cartilage calcification. Tohoku J Exp Med 2017; 243: 237-246.
38. Meng H, Zhang M, Xiao B, Chen X, Yan J, Zhao Z. Forensic age estimation based on the pigmentation in the costal cartilage from human mortal remains. Leg Med (Tokyo) 2019; 40: 32-36.
39. Alibegović A, Blagus R, Martinez IZ. Safranin O without fast green is the best staining method for testing the degradation of macromolecules in a cartilage extracellular matrix for the determination of the postmortem interval. Forensic Sci Med Pathol 2020; 16: 252-258.
40. Siriboonpiputtana T, Rinthachai T, Shotivaranon J, Peonim V, Rerkamnuaychoke B. Forensic genetic analysis of bone remain samples. Forensic Sci Int 2018; 284: 167-175.
41. Redouane F, Lambert M, De Greef J, Malghem J, Lecouvet FE. Primary infectious costochondritis due to *Prevotella nigrescens* in an immunocompetent patient: clinical and imaging findings. Skeletal Radiol 2019; 48: 1305-1309.

Address for correspondence
Dr Marcin Tomsia
Department of Forensic Medicine and Forensic Toxicology
Faculty of Medical Sciences in Katowice
Medical University of Silesia in Katowice
18 Medyków St.
40-752 Katowice, Poland
e-mail: marcint87@gmail.com

Submitted: 15.01.2021
Accepted: 4.03.2021