BLOOD ALCOHOL CONTENT ASSESSMENT IN DROWNING: CASE REPORT AND REVIEW OF LITERATURE

Martina Focardi¹, Beatrice Defraia¹, Amalia Rizzo^{2,*}, Ilenia Bianchi³, Antonello Grippo⁴, Fabio Vaiano⁵

¹Careggi University Hospital, Legal Medicine Unit, ²University of Florence, Forensic Pathology Section, Department of Health Sciences, ³University of Florence, Section of Forensic Medical Sciences, Department of Health Sciences, ⁴AOU Careggi, Neuropsychopathology Unit, Neuromuscular Department, ⁵University of Florence, Forensic Toxicology Unit, Department of Health Science, Florence, Italy

Abstract: Blood alcohol content (BAC) assessment in post-mortem (PM) analysis is essential in accessing cause of death. Many factors influence BAC leading to either a decrease or possibly even an increase in its levels. Consequently, discriminating between antemortem ingestion and PM alteration is the primary goal for forensic scientists. In cases of drowning, this evaluation is further hampered by the environmental conditions and by the lack of specific studies on this topic. In this paper, we described the interpretation of BAC in the drowning case of a young man whose corpse remained afloat in the Arno river for 48 days. Alcohol was the only psychoactive substance found in his body and it was quantified in the peripheral and cardiac blood, the gastric content, and in urine in the following concentrations: 3.96. 3.18, 7.21, and 2.34 g/L respectively. Neither n-propanol or iso-propanol were detected. The ratio between urine and blood concentrations (< 1) suggested an absorptive phase and the absence of a PM neoformation. This phenomenon could be explained by the water temperature (approximately < 5-8 °C). Thus, we concluded that the BAC of 3.96 g/L in the femoral vein was a result of antemortem ingestion. The significance of this case is the outcome of a protracted period of immersion and is a fine example of how the study of multiple matrices and the adoption of a toxicological-forensic methodology are necessary for reliable data analysis.

Keywords: drowning, BAC, toxicological assessment, postmortem neoformation.

INTRODUCTION

Assessment of Blood Alcohol Concentration (BAC) in cadaveric specimens continues to be a challenge for forensic scientists. Beyond the interindividual variability, several other post-mortems (PM) phenomena may also modify the levels. These include PM redistribution, putrefaction, and PM neoformation. However, these factors should be taken into account as an effective and reliable estimation of antemortem (AM) alcohol consumption and might play a key role when investigating the cause of death. Discriminating between AM ingestion and PM formation is the primary goal in such cases. Further challenges occur when the PM BAC study involves cases of drowning. Reviewing the literature, we find few articles on PM production of alcohol in drowning cases thus underlining the

importance of proper data analysis based on accurate diagnostic procedures and matrices [1–4]. Nonetheless, research on PM neoformation in aquatic environments is ongoing and shared guidelines are far from finalized. In this paper, we described the toxicological findings in a drowning case of a young man whose body was removed from the Arno River 48 days after death. The novelty lies in the excessive immersion period thus it could offer useful data for interpreting PM BAC evaluation in drowning deaths.

MATERIALS AND METHODS

The toxicological investigation was performed on peripheral and cardiac blood, urine, and gastric content, following the analytical method described in a previous article [5]. Briefly, 1 mL of biological fluid was

*Correspondence to: Amalia Rizzo MD, University of Florence, Forensic Pathology Section, Department of Health Sciences, Florence, Italy, Email: amaliarizzo11@gmail.com

added with 2-butanol (internal standard) and analyzed by a headspace-gas chromatograph coupled to a flame ionization detector. We decided not to proceed with the toxicological analysis of the vitreous humor because of the advanced putrefactive phenomena. In particular, alcohol determination was performed by means of a headspace sampler Agilent 7697A Headspace (Agilent Technologies, Palo Alto, CA, USA); the oven was set at 60 °C. After 30 min of incubation, the gas was injected in an Agilent 7890 B GC system (Agilent Technologies) equipped with a flame ionization detector. The column was an Alltech Superox II, 10 m length, 0.54 mm i.d. and 1.2 mm film thickness (Alltech Associates INC., Deerfield, IL,USA). Chromatographic run was carried out at 60 °C for 4.5 min.

CASE HISTORY

The corpse of a 30-year-old man was found afloat in the Arno River in the city of Florence, Italy in the month of February. The river has a length of 240 km and an average depth of 3.9 meters. The water temperature ranged from 1 to 7 °C, with a mean value of 5 °C; the air temperature range was 1-11 °C (mean: 6°C).

The victim was last seen 48 days before, drinking beers with his friends. The friends reported having been with him until 03:00 a.m. the day he disappeared. With the exception of the macerated feet and hands, the body appeared sufficiently preserved.

Immediately after recovery from the river was transferred and stored in a cell refrigerator at 0°C until the forensic examination could take place, 24 hours later. The autopsy revealed the first signs of putrefaction (venous reticulum spread throughout the abdomen and upper and lower limbs), as well as the first phase of the emphysematous period (globose abdomen, negroid faces). No external injuries were detected. Lungs

Table 1. Alcohol concentrations found in the analyzed specimens

weighted 610 g (left) and 690 g (right) with the presence of water in the alimentary canal (as well as in the stomach and the duodenum). Petechiae on the anterior pulmonary surface, and airways flushed away by water, were also noted. Although the presence of putrefactive processes was detected on external examination, the internal organs appeared intact for the most part, with the exception of the brain, and the presence of a small amount of putrefactive liquid near the pleural cavity.

The histological examination showed the presence of plant material in the lungs, and the death was attributed to drowning. The anamnestic data determined the victim consumed more alcohol than the beers his friends mentioned.

RESULT AND DISCUSSION

Alcohol was the only psychoactive substance found in the specimens and concentrations are reported in Table 1; neither isopropanol nor n-propanol was detected.

This is a good case to begin examining the relevant features of PM BAC analyses in drowning. Forensic pathologists and toxicologists are often asked to discriminate between AM alcohol consumption and PM neoformation. Although several articles on PM BAC in drowning have been published, most of them lack the necessary scientific and methodological specifics. Additionally, immersion times were not as extreme (Table 2) as in this case. Some authors reported endogenous ethanol production not exceeding 50 mg/ dL, even though there are reports of much higher endogenous production [1,6]. Given the interpretation issues, many authors suggested the adoption of a careful, specific forensic methodology in order to obtain the proper and, consequently, more effective analysis, Kubelberg and Jones [7]. Apart from the neoformation, PM BAC alterations might be a result of a number of

| Specimen | Alcohol concentration (g/L) |
|------------------|-----------------------------|
| Peripheral blood | 3.96 |
| Cardiac blood | 3.18 |
| Gastric content | 7.21 |
| Urine | 2.34 |

Table 2. Reported studies on BAC evaluation cases in drowning

| Reference | N° of Cases | PM submersion time | BAC 0mg/dL (%) | 0< BAC < 50mg/dL (%) | BAC≥50 mg/dL (%) |
|-----------|-------------|--------------------|----------------|----------------------|------------------|
| [3] | 1394 | Indeterminate | - | - | 861 (61,8%) |
| [5] | 52 | >7 days | 11 (21.1) | 8(15.4) | 33(63.5) |
| [11] | 1 | 31 | - | - | 260mg/100ml |
| [11] | 1 | 14 | - | - | 280mg/100ml |

other phenomena i.e.the passive diffusion of alcohol across the mucous membrane. This may involve bacterial flora activity or post-mortem diffusion of the alcohol, depending on the degree of concentration. An example of that would come from the stomach on the sections to the left of the heart [8]. On the other hand, it could come from the diffusion of ethanol to sections to the right of the heart (however, data from the left is generally more reliable), coming from the vena cava, ethanol being produced from the fermentation of glycogen in the liver [9]. The choice of matrices is crucial. The accepted worldwide gold standard is the femoral venous blood for a low PM modification rate. Yet, several studies have suggested the importance of other matrices, bladder urine, and vitreous humor. These specimens are less affected by PM modification because they are either sterile with low amounts of glucose and enclosed within very stable tissues or have a very low bacterial load [10]. That is why urine (UAC) and vitreous humor (VAC) alcohol contents can be useful in confirming positive BAC. Indeed, negative UAC and VAC, and positive BAC could suggest PM neoformation [6,11]. However, some caution is necessary when interpreting the BAC-positive and UAC-negative results. In these cases, the subject may have ingested alcohol shortly before drowning [12]. Overall, UAC/BAC ratios < 1 may suggest dying during the absorptive phase or the production of PM ethanol in the urine or even a passive diffusion from the gut to the bladder [7,13].

An interesting study by Hadley [14], posits that endogenous BAC increases linearly in proportion to immersion time and that at one week the maximum adjustment is 40 mg/dl (cases of corpses immersed in water for longer than this period were excluded) this adjustment is not applicable in cases with a maximum immersion time of 12 hours. The authors have developed an equation that is applicable in all cases where the immersion time does not exceed 168 hours.

For cases of longer periods PMI, when the suggested formula isn't suitable, there are other parameters to consider, which, when integrated with each other, can lead to either a clearer or at least more plausible forensic toxicological diagnosis. We are reminded of a case by Nanikawa dating back to 1974 in which alcohol was detected in a body extracted from the ocean after 2-3 months [15]. Because the relationship between the concentration of ethanol (in the pleural cavity and in the urine) and n-propanol (in the same matrices) was 20:1, he concluded that this concentration of ethanol was attributable to post-

mortem production.

In fact, in the literature it is frequently reported that the PM production of ethanol when compared to other volatiles, such as n-propanol, is 15 - 20 times higher. Thus, the presence of these volatile substances could be a red flag regarding alcohol PM synthesis.

Based on these observations, once the data is obtained, it might be possible to extrapolate the antemortem quota from that which was produced postmortem. Indeed, given the scarcity of data and scientific input from similar cases it is nearly impossible to link these results from the studies of such very different samples.

O'Neal and Poklis [16] have advanced a methodology with a number of points that are worth sharing: 1) obtaining anamnestic data; 2) look for signs of decomposition to the body, in these cases, if the concentration of ethanol in different matrices is high (greater than 0.20%), consider an ante-mortem ingestion; 3) collect different matrices, especially peripheral blood, urine and vitreous humor. If the BAC is detectable in the blood, but not in the urine or vitreous humor then in all likelihood the production is post-mortem 4) do not use n -propanol as an internal standard in gas chromatography because this is one of the volatiles produced by a decomposing body, the dosage of which, however, must always be evaluated with caution 5) good conservation of the matrices 7) a BAC of 0.03% should be considered irrelevant.

In our case, we observed very high alcohol concentration from blood in both the femoral (3.96 g/L) and right heart section (3.18 g/L). UAC was 2.34 g/L, with a UAC/BAC ratio < 1, suggesting an absorptivephase and the absence of a PM neoformation. Thus, we concluded that the BAC from the femoral vein was likely the AM BAC. This interpretation was also supported by the non-detection of volatile compounds, especially n-propanol and iso-propanol. Moreover, we took into account that the corpse remained in water between the coldest months, of January and February, for 48 days, when the temperatures were very low (approximately < 5-8 °C). The relationship between water temperature and PM alcohol production is conditioned by several factors, including pre-drowning activities, exposure to water, patterns of alcohol consumption in aquatic settings, and circumstances of drowning. In the investigation by Pajunen et al., it was demonstrated that the highest percentage of drownings with BAC < 50 mg/ dL occurred during the summer months and in bodies with prolonged PM immersion time, confirming the relationship between temperature- and time-dependent putrefaction and PM alcohol production [4].

As proven by *in vitro* studies, PM endogenous alcohol formation generally does not occur below 4-5°C [17].

Circumstantial data confirmed ingestion of high amounts of ethanol on the evening he disappeared; therefore, the man was known to be a chronic alcohol consumer.

In conclusion, we presented BAC evaluation in a case of drowning characterized by a prolonged period of immersion (48 days). A so long immersion period is not common in drowning cases reported in literature. Thus, this paper could be a very useful tool for interpretation in this circumstances. Moreover, we underline further how the study of multiple matrices and the adoption of a toxicological-forensic methodology are necessary for reliable and effective data analysis.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- 1. Lunetta P, Smith GS, Penttilä A, Sajantila A. Unintentional drowning in Finland 1970-2000: A population-based study. International Journal of Epidemiology. 2004;33:1053-1063.
- 2. Ahlm K, Saveman BI, Björnstig Ü. Drowning deaths in Sweden with emphasis on the presence of alcohol and drugs–a retrospective study, 1992–2009. BMC Public Health. 2013;13:216-225.
- 3. Pajunen T, Vuori E, Vincenzi FF, Lillsunde P, Smith G, Lunetta P. Unintentional drowning: Role of medicinal drugs and alcohol. BMC Public Health. 2017;17:388-397.

- 4. Pajunen T, Vuori E, Lunetta P. Epidemiology of alcohol-related unintentional drowning: is post-mortem ethanol production a real challenge? Inj Epidemiol. 2018;5:39-43.
- 5. Bertol E, Di Milia MG, Fioravanti A, Mari F, Palumbo D, Pascali JP, Vaiano F. Proactive drugs in DFSA cases: Toxicological findings in an eight-years study. Forensic Science International. 2018;291:207-215.
- 6. Levine B, Smith ML, Smialek JE, Caplan YH. Interpretation of low postmortem concentrations of ethanol. J Forensic Sci. 1993;38:663-667.
- 7. Kugelberg FC, Jones AW. Interpreting results of ethanol analysis in postmortem specimens: a review of the literature. Forensic Sci Int. 2007;165:10-29.
- 8. Chikasue F, Yashiki M, Miyazaki T, Okamoto I, Kojima T. Abnormally high alcohol concentration in the heart blood. Forensic Sci Int. 1988;39:189-195.
- 9. Pelissier-Alicot AL, Coste N,Bartoli C, Piercecchi-Marti MD, Sanvoisin A, Gouvernet J, Leonetti G. Comparison of ethanol concentrations in right cardiac blood, left cardiac blood and peripheral blood in a series of 30 cases. Forensic Sci Int. 2006;156:35-39.
- 10. Belsey SL, Flanagan RJ. Postmortem biochemistry: Current applications. J Forensic Leg Med. 2016;41:49-57.
- 11. Singer PP, Jones GR, Lewis R, Johnson R. Loss of ethanol from vitreous humor in drowning death. J Anal Toxicol. 2007;31:522-525. 12. Jones AW. Urine as a biological specimen for forensic analysis of alcohol and variability in the urine-to-blood relationship. Toxicol Rev. 2006;25:15-35.
- 13. Jones AW. Ethanol distribution ratios between urine and capillary blood in controlled experiments and in apprehended drinking drivers. J Forensic Sci. 1992;37:21-34.
- 14. Hadley JA, Smith GS. Evidence for an early onset of endogenous alcohol production in bodies recovered from the water: implications for studying alcohol and drowning. Accid Anal Prev. 2003;35:763-769.
- 15. Nanikawa R, Kotoku S, Medicolegal observations on a dead body drawn up from the sea bed, with special reference to ethanol and diatoms. Forensic Sci. 1974;3:225-232.
- 16. O'Neal CL, Poklis A. Postmortem production of ethanol and factors that influence interpretation: a critical review. Am J Forensic Med Pathol. 1996;17:8-20.
- 17. Vuori E, Renkonen OV, Lindbohm R. Validity of post mortem blood alcohol values. Lancet. 1983;1:761-762.