In the realm of forensic pathology, ethanol is one of the most frequently encountered xenobiotics. The determination of ethanol concentration in blood after death is of great interest in forensic settings. It is important to be able to determine the level of intoxication of the deceased at the time of death, which is directly correlated to the ability to act prior to death, especially when a suicide is suspected. This estimation is not always easy to establish owing to various artifacts that are important to know for a proper ethanol blood level interpretation, among them postmortem (PM) diffusion. We describe here a case of unusual ethanol distribution in body compartments and discuss the importance of PM diffusion and redistribution while performing complementary toxicological analysis, especially when the blood and urine samples seemed to be inconsistent after the first results.

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2. Toxicological and biochemical analysis and results

Toxicological analysis included blood ethanol, and other volatile compound determination, as well as general screening for common drugs and illegal substances by gas chromatography-mass spectrometry (GC-MS) using commercial mass spectrum libraries (MPW2016, Wiley10, Designer Drugs 2014, NIST17 and Pragst UV-Spectra 2007), high-performance liquid chromatography with diode-array detection (HPLC-DAD) and headspace-gas chromatography-flame ionization detection (HS-CG-FID) [1]. Ethanol concentration was determined using two HS-CG-FID methods (Perkin Elmer Clarus 590 equipped with two HS, two columns and two detectors). The considered value corresponds to the average of the values obtained with 2 replicates for each method (n = 4). Elite-BAC-2 Advatage column (30 m x 0.32 mm ID x 0.6 µm film thickness) and J&W HP-Innowax column (30 m x 0.32 mm x 0.5 µm film thickness) were used. 0.2 g of sample were weighed in HS vial, as well as 1 g of ammonium sulfate 1 M containing dioxane 0.04%, respectively acetoneitrile 0.04% as internal standards. Six commercial solutions (Lipomed, Switzerland) were used for calibration (0.25, 0.5, 1, 1.5, 2, and 3 g/kg). For concentrations > 4 g/kg, samples were reanalyzed after water dilution.

First toxicology results showed high ethanol concentration in peripheral blood from the femoral vein (2.04 g/kg) but negative ethanol concentration in urine; these results led to measure ethanol concentration in all available biological samples (brain, cerebrospinal fluid, vitreous humor, cardiac blood, cardiac muscle, pericardial fluid, stomach content, liver (external part of right lobe), bile, skeletal muscle (m. psoas), urine (2 samples), peripheral blood (5 samples from the femoral vein) and peripheral serum from the femoral vein) (results are shown in Fig. 2). In addition, ethyl glucuronide (EtG) and ethyl sulfate (EtS) were also measured in all samples and a hair sample (results are shown in Table 1), phosphatidylethanol (PEth) was measured in a blood sample, CDT, ASAT, ALAT and GGT were also measured in blood serum, and ethanol congeners were measured in stomach content (results are shown in Table 2). Concentrations of congeners, such as acetone, 1-propanol, and isobutanol, were determined using HS-GC-FID methods (Agilent 6850 Network GC system coupled to Agilent G1888 Network HS sampler). HP-Innowax column (30 m x 0.535 mm x 1 µm film thickness) was used. Six commercial solutions (Medidrug BGS W-cal, Medichem, Germany) were used for calibration.

Based on the result of all investigations, the cause of death was attributed to mechanical asphyxia by hanging. Considering the unusual discrepancy between the ethanol concentrations in our samples, which was very high in stomach content, high in femoral blood and negative in urine and vitreous humor, we assumed that femoral blood ethanol concentration measured in our samples was the result of postmortem (PM) diffusion and did not reflect the femoral blood ethanol concentration at the time of death, which should have been negative, as shown by urine and vitreous humor concentrations. Therefore, the man had the ability to act when he hung himself.

3. Discussion

3.1. PM ethanol diffusion

In the realm of forensic pathology, ethanol is one of the most frequently encountered xenobiotics and its consumption is a common precursor to suicide [2]. PM blood ethanol estimation must take in account antemortem (AM) factors, such as ingested quantity, route of consumption and pharmacokinetic state at the time of death, but also PM factors, such as PM production (through putrefactive process) or post-sampling production (fermentation related to microbial contamination of samples), and PM ethanol diffusion and/or redistribution.

PM drugs diffusion and redistribution artifacts have been long described [3]; the main mechanism seems to be a diffusion along a concentration gradient from solids organs (e.g. liver or lung) into the blood (e.g. pulmonary artery, pulmonary vein and inferior vena cava) and then in cardiac chambers and other vessels. This means that
drug concentration in PM blood may not reflect AM concentration. “Redistribution” has been typically used to describe movement of drugs along a concentration gradient through blood vessels; on the other hand, “diffusion” has been typically used to describe diffusion of drugs across organs, such as from the stomach to the liver [4].

PM ethanol diffusion is a debated issue [5]. Some authors found substantial site-dependence blood ethanol concentration (mainly related to diffusion of ethanol from the stomach in the heart chambers between time of death and sample collection, even with intact gastric wall) with significant difference between heart and peripheral blood concentrations. Turkel & Gifford described higher ethanol concentration in heart than in femoral blood in 35 out of 75 autopsies [6]; however, heart blood sampling had been made by transecting the major vessels in the mediastinum and collecting the blood which pooled in the pericardial sac, which is currently not recommended, because of contamination risk. Another described diffusion route could be increased pulmonary vein blood ethanol through agonal regurgitation of gastric content and aspiration of ethanol-rich gastric fluid in the blood-rich pulmonary system [7]; they even suggested that even without agonal vomiting, gastric fluid might enter the airways as a result of PM relaxation of the esophageal sphincter and passive regurgitation. On the other hand, other authors found only relatively small differences in the heart and femoral blood ethanol concentrations; Plueckhahn even concluded that under normal circumstances and with proper attention to procedure, PM diffusion of ethanol from intact stomach to heart cavities was unlikely even with a high ethanol concentration in stomach content [8].

Even today some aspects remain unclear, such as which sampling site would be most appropriate, or whether or not the water content of blood samples should be considered (mainly because of PM desiccation and putrefactive process) [9]. PM diffusion could be also related to AM factors such as time of last drink before death, quantity and strength of the beverage and dilution with food in the stomach. Estimating blood ethanol concentration based on concentration from another sample (urine, vitreous humor) is quite hazardous.

PM ethanol diffusion from the stomach to surrounding tissues has already been described [10, 11]. The interpretation of the ethanol concentrations in the case described by Singer & Jones was limited by a state of mild decomposition and by an uncertainty of interpretation of ethanol value in femoral blood sample due to potential mislabeling [10]. To our knowledge, no case of PM ethanol diffusion has been described in a case of suicide by hanging; this position could prevent or reduce agonal regurgitation and contamination of lung tissue by ethanol-rich gastric content. By comparison, Table 3

![Fig. 2. Ethanol concentration in all samples (each value comes from a different sample).](image_url)
shows ethanol values in biological samples in our case and in the scientific literature. In addition, ethanol urine/blood ratio measured in 12 other “standard” hanging cases in our center were from 0.77 to 2.07.

Since we don’t use ethanol in the autopsy room, no sampling contamination could have occurred. Sampling technique had been performed as a routine from the femoral vein following proximal cross-clamping and from the bladder; blood had been stored in ad hoc conditions (fluorinated tubes, stored at 4 °C), therefore excluding post-sampling ethanol production. Ethanol measurement had been conducted following up-to-date recommendations and results were reliable.

3.2. Current case interpretation

In our case, gastric content ethanol concentration showed that the deceased ingested a fair quantity of alcoholic beverage before death; according to Bonte, ethanol congeners would be congruent with composition of rum [12]. Negative vitreous humor and urine concentrations would be congruent with the absence of AM ethanol absorption and distribution to organs. Skeletal muscle concentration is usually thought to reflect the blood concentration; in our case, its ethanol concentration (coming from either right or left m. psaas lying in close proximity with the stomach) would also be consistent with PM diffusion rather than AM absorption and in vivo distribution. Ethanol concentration measured in the other matrices (pericardial fluid, cardiac blood, cardiac muscle, bile, liver (from right lobe)) would thus reflect passive PM diffusion of ethanol through organ walls; furthermore, relatively low results in cerebrospinal fluid, which had been sampled after opening the skull, and brain tissue support even more the theory of passive PM diffusion rather than active AM absorption and distribution. Unfortunately, we did not sample lung fragment for toxicological analysis; this could have been useful in order to exclude contamination such as described previously [3,7]. Although, since we only noticed a small amount of brownish material in the esophagus and no evidence of gastric material in the pharynx and in the respiratory tract, this diffusion pathway was not considered significant in that particular case. Considering these results, the fixed aspect of the gastric wall and the strong aromatic odor at the opening of the abdominal cavity, the possibility of ethanol diffusion through the intact gastric wall seems highly plausible. Jungmann and al. previously reported a case of suicide involving the consumption of a highly concentrated alcoholic beverage, with a fixed aspect of the gastric wall visible at the autopsy after a PM period of five days [13].

Regarding ethanol consumption habits of the deceased, PEth concentration in peripheral blood and CDT concentration in peripheral serum were in favor of regular and important ethanol consumption during days and weeks preceding death, and EtG concentration in hair spoke for regular ethanol consumption during the 5–6 month preceding death [14,15]. As ASA T and ALA T values are likely to increase after death, we did not interpret these values as chronic ethanol consumption signs; regarding GGT level, which had been described as stable or instable in PM peripheral serum depending on authors, the postmortem GGT value was interpreted as within normal range [15].

Ethanol diffusion through organ walls has been described as broadly proportional to the concentration gradient and to time lapse; it has been described as inhibited by refrigeration at 4 °C [16]; furthermore, membrane permeability, quantity and strength of the alcoholic beverage and possible dilution with stomach content play a role as well [9]. In our case, a maximum of 14 h elapsed between the time of death and the discovery of the body (with the body in upright position), and 24 more hours between death scene examination and autopsy (with the body lying, refrigerated at 4 °C); a maximum of 39 h elapsed between death and blood and urine sampling.

Regarding ethanol concentration in abdominal cavities, organs and vessels, this raises a question about the negative ethanol concentration in urine: why ethanol won’t be capable of crossing the bladder wall? To our knowledge, only one case report described PM diffusion of drugs (diphenhydramine and dihydrocodeine) from the bladder into venous blood [4], but no case has been described with diffusion from peripheral blood or from abdominal cavity into the bladder. In our case, diffusion of ethanol through the bladder wall may have been prevented by inhibiting factors such as refrigeration, and the absence of thinning of the bladder wall, because the bladder was relatively empty (only 45 ml of urine).

In this case, we considered that blood ethanol concentration at the time of death must have been insignificant, due to the short time interval between alcohol consumption and death (as shown by negatives results in vitreous humor and in urine) and concentrations measured in other samples were the result of PM diffusion. Factors contributing to such an artifact would be the high concentration gradient as described by Pounder & Jones [3]. The role of the upright position could also have had an influence on the distribution pattern.

4. Conclusion

In case of forensic necessity of investigating vitality and ability to act at the time of death, toxicological analysis is mandatory. Concerning ethanol measurement on PM samples, peripheral blood and urine taken by needle puncturing are of choice.
When contradictory results show up in PM analysis, the first step is to exclude a technical error (sampling, storage, measure, analysis, interpretation). Then, it could be worth analyzing complementary samples. The sole PM blood ethanol concentration should not always be considered as equal to AM value due to several phenomenon known to happen after death (e.g. putrefactive process and fermentation related to microbial activity). In this case, even with high ethanol concentration in peripheral blood, we finally concluded that those values did not reflect the degree of ethanol intoxication at the time of death; we rather think that a certain amount of highly concentrated alcoholic beverage has been ingested shortly before death, with virtually no vital ethanol absorption.

When interpreting PM ethanol concentration in fluids and tissues, diffusion and redistribution artifacts must be taken into account. For this reason, we recommend analysis of fluids from different sample sites. Many diffusion pathways have been described in literature, such as diffusion through intact gastric wall, active and passive contamination of lungs via gastric content, or even diffusion from the bladder into femoral blood.

CRedit authorship contribution statement

Valentin Marti: Conceptualization, Writing – original draft, Investigation, Data curation, Visualization, Project administration.
Marc Augsburger: Validation, Writing – review & editing.
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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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