

Uncertainty in estimating blood ethanol concentrations by analysis of vitreous humour

A W Jones, P Holmgren

Abstract

Aims—To determine the concentrations of ethanol in femoral venous blood (FVB) and vitreous humour (VH) obtained during forensic necropsies. The ratios of ethanol concentrations in VH and FVB, the reference interval, and the associated confidence limits were calculated to provide information about the uncertainty in estimating FVB ethanol concentrations indirectly from that measured in VH.

Methods—Ethanol concentrations were determined in specimens of FVB and VH obtained from 706 forensic necropsies. The specimens were analysed in duplicate by headspace gas chromatography (HS-GC), with a precision (coefficient of variation) of 1.5% at a mean ethanol concentration of 500 mg/litre. The limit of detection of ethanol in body fluids by HS-GC in routine casework was 100 mg/litre.

Results—In 34 instances, ethanol was present in VH at a mean concentration of 154 mg/litre, whereas the FVB ethanol concentration was reported as negative (< 100 mg/litre). These cases were excluded from the statistical analysis. The concentration of ethanol in FVB was higher than in VH in 93 instances, with a mean difference of 160 mg/litre (range 0 to 900). The mean concentration of ethanol in FVB (n = 672) was 1340 mg/litre (SD, 990) compared with 1580 mg/litre (SD, 1190) in VH. The arithmetic mean VH/FVB ratio of ethanol was 1.19 (SD, 0.285) and the 95% range was 0.63 to 1.75. The mean and SD of the differences (log VH – log FVB) was 0.063 (SD, 0.109), which gives 95% limits of agreement (LOA) from –0.149 to 0.276. Transforming back to the original scale of measurement gives a geometric mean VH/FVB ratio of 1.16 and 95% LOA from 0.71 to 1.89. These parametric estimates are in good agreement, with a median VH/FVB ratio of 1.18 and 2.5th and 97.5th centiles of 0.63 and 1.92.

Conclusions—The ethanol distribution ratios (VH/FVB) show wide variation and this calls for caution when results of analysing VH at necropsy are used to estimate the concentration in FVB. Dividing the ethanol concentration in VH by 2.0 would provide a very conservative estimate of the ethanol content in FVB, being less than the true value, with a high degree of confidence.

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Vitreous humour (VH) is an important biological specimen in forensic toxicology for several reasons.^{1–3} First, VH is a relatively clean, watery fluid and is readily obtainable without the need to carry out a complete necropsy.⁴ Second, the location of the eyes relative to the gut minimises the risk of VH being contaminated by diffusion of ethanol, which can occur after death.^{2,3} Third, and most importantly, if the corpse has undergone decomposition, the risk of microbial synthesis of ethanol is much less likely to occur in VH compared with peripheral or central blood specimens.⁵ Under some circumstances—for example, when the body has been subjected to severe trauma and exsanguination—VH might be the only specimen available for toxicological analysis.^{5,6} Furthermore, if the blood ethanol concentration is considered suspect—for example, when putrefaction has obviously occurred—the concentration determined in VH might provide a more realistic estimate of the perimortem blood ethanol concentration.⁷

The aim of our present study was to establish a quantitative relation between the concentrations of ethanol in femoral venous blood (FVB) and VH from a large number of forensic necropsies. The results were used to establish VH/FVB reference limits that might be used to estimate FVB alcohol concentration from the VH content.

Materials and methods

BIOLOGICAL SPECIMENS

In Sweden (population ~ 8.7 million), about 5000 medicolegal necropsies are performed annually where a complete forensic toxicology service is requested. The biological specimens usually submitted for toxicological analysis are FVB, bladder urine, and VH. The primary reason for submitting VH for toxicological analysis is to corroborate the concentration of ethanol determined in samples of blood and urine,^{6,7} and sometimes for measuring glucose and lactate.⁸ The tubes used for collecting blood specimens contain 1–2% wt/vol potassium fluoride to block the enzymes involved in glycolysis.⁷ Specimens of VH (2–3 ml) were submitted for analysis in 5 ml vacutainer tubes containing 20 mg NaF and 120 units heparin.

From the case records at our laboratory, we extracted 706 postmortem reports in which both FVB and VH had been analysed and the concentrations of ethanol in one or both media exceeded 100 mg/litre.

DETERMINATION OF ETHANOL

Ethanol was determined in duplicate in both blood and VH by headspace gas chromatography (HS-GC) with t-butanol as the internal

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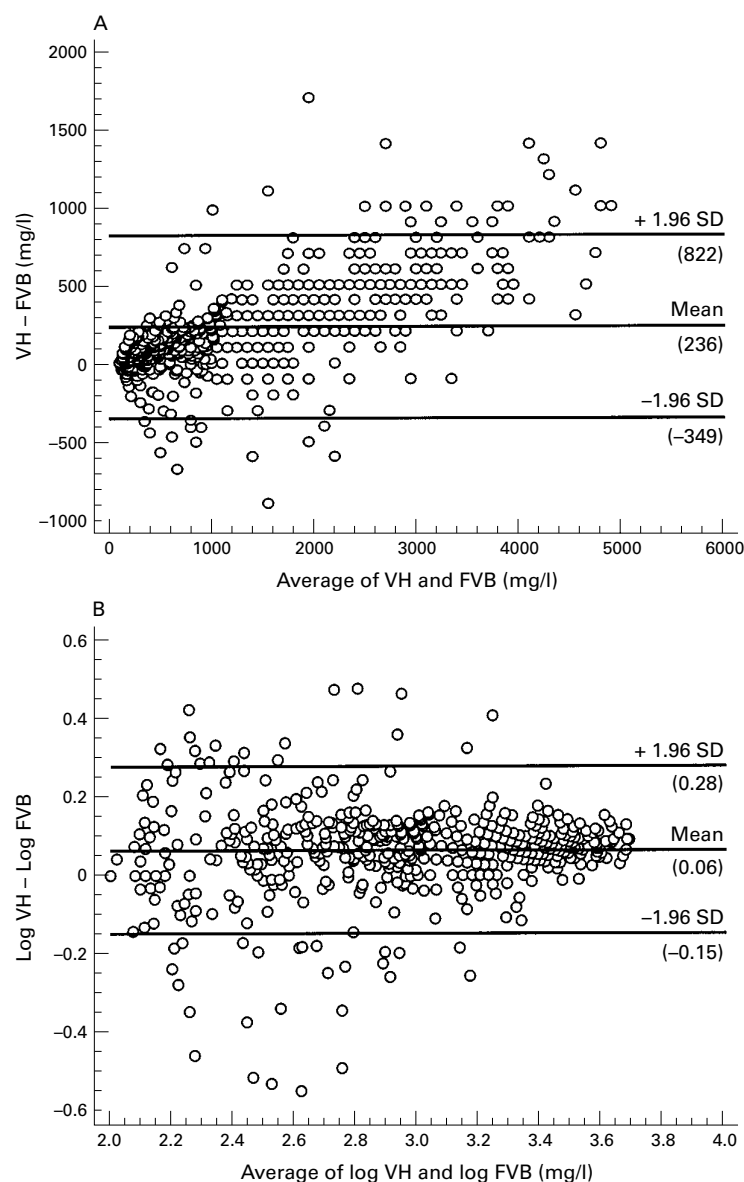


Figure 1 Bland and Altman plots of differences in ethanol concentration between vitreous humour (VH) and femoral venous blood (FVB) and mean concentration of ethanol (A) before and (B) after logarithmic transformation. The horizontal lines show mean difference (bias) and 95% reference limits of agreement.

standard. Two different stationary phases (Carbopak B and C) were used for chromatography, giving retention times of 1.00 minutes and 0.75 minutes for ethanol compared with 1.75 minutes and 1.65 minutes for t-butanol, respectively. Aliquots of blood and VH (100 μ l) were diluted 1/10 with t-butanol (50 mg/litre) in glass vials, which were immediately made airtight with crimped on aluminium caps,

Table 1 Summary statistics for ratios of ethanol concentrations in vitreous humour (VH) and femoral venous blood (FVB) derived from 672 postmortem examinations

Calculation	Mean (median)	SD	2.5th/97.5th reference limits
Ratio (VH/FVB)	1.19 (1.18)	0.285	0.63/1.75
Difference (log VH - log FVB)	0.063 (0.073)	0.109	0.63/1.92*
	1.16†		0.71/1.89‡

*Empirical centiles.

†Back transformed geometric mean VH/FVB ratio.

‡Back transformed 95% reference interval for VH/FVB ratio.

before equilibration and analysis with a Perkin-Elmer HS-100 headspace sampler and a Sigma 2000 gas chromatograph. The precision of this HS-GC method expressed as coefficient of variation was 1.5% at a mean ethanol concentration of 500 mg/litre. The limit of detection of ethanol in FVB and VH in routine casework was taken as 100 mg/litre. The mean concentrations of ethanol from duplicate determinations of VH and FVB were used in all statistical calculations.

Statistical analysis

The agreement between ethanol concentrations in VH and FVB was assessed by Bland and Altman's method.⁹ Because the VH - FVB differences increased as the concentration of ethanol increased, logarithmic transformations were made. The logarithmic differences were back transformed to give the geometric mean of the VH/FVB ratio and its 95% limits of agreement (LOA). The ratios of VH to FVB ethanol were also calculated and a reference interval determined using parametric and non-parametric methods. The concentration of ethanol in FVB was directly proportional to the concentration in VH so the VH/FVB ratio is the parameter of interest when ethanol in FVB is estimated from the VH content.¹⁰ A non-parametric reference interval for the VH/FVB ratio (2.5th and 97.5th centiles) was calculated as described by Bland.¹¹

Results

The ethanol concentrations in VH and FVB were highly correlated ($r = 0.979$; $p < 0.001$; $n = 672$). The mean concentration of ethanol in VH was 1580 mg/litre (median, 1300) compared with 1340 mg/litre (median, 1100) in FVB. The mean VH/FVB ratio of the ethanol concentration was 1.19 (median, 1.18), with SD of 0.285 and a 95% reference interval from 0.63 to 1.75. The corresponding non-parametric 2.5th and 97.5th centiles were 0.63 and 1.92.

Figure 1A shows the Bland and Altman plot and it can be seen that the differences (VH - FVB) correlate with the mean concentration of ethanol ($r = 0.68$; $p < 0.001$). Moreover, the variability of the differences seemed to increase as the concentration of ethanol increased. Figure 1B shows the log transformed data; the correlation coefficient was not significant ($r = 0.095$; $p > 0.05$) and the scatter of points (except at very high ethanol concentrations) was much more uniform over the range of measurements. The mean difference (log VH - log FVB) was 0.063 (SD, 0.109) and when back transformed to the original scale produced a geometric mean VH/FVB ratio of 1.16 with a 95% LOA of 0.71 to 1.89. This means that a measurement of ethanol in VH is likely to exceed the concentration in FVB by 1.16 times (16%) on average, but could be 1.89 times higher (89%) or 0.71 times lower (29%). These reference intervals have uncertainty and the 95% confidence intervals can be computed as described by Bland.¹¹ For the upper limit 1.89, the 95% confidence intervals are 1.83 and 1.95 and for

the lower limit 0.71 the 95% limits are 0.68 and 0.73. Table 1 summarises mean and median VH/FVB ratios and the reference intervals calculated by parametric and non-parametric methods.

Discussion

After absorption into the bloodstream, ethanol distributes into the total body water and binding to plasma proteins is negligible.¹² The amount of water in body fluids and tissues gives a good indication of the distribution of ethanol to be expected.^{7,12} However, an additional factor to consider is the ratio of blood flow to tissue mass, which impacts on the rate of ethanol equilibration, and considerable arteriovenous differences in ethanol concentrations might exist across some tissues (for example, skeletal muscle).¹³

In urine and cerebrospinal fluid, the blood–fluid distribution ratios depend to some extent on whether the absorption or postabsorptive stage of ethanol kinetics has been reached.^{7,8} Moreover, urine and cerebrospinal fluid are produced gradually over a period of time and stored in the bladder and lumbar space, during which time the blood ethanol concentration might change as a result of metabolism.^{7,14} These pharmacokinetic aspects influence the variation in urine–blood and CSF–blood ethanol relations determined at necropsy. VH is approximately 99% wt/vol water compared with a mean water content of 80% wt/wt for whole blood.¹⁵ This gives a theoretical VH/FVB ratio of 1.16:1, which agrees well with the experimental findings and speaks against any appreciable time lag for ethanol entering the blood and reaching the VH.

The postmortem reports with measurable amounts of ethanol in VH and negative FVB concentrations (< 100 mg/litre) probably reflect death occurring late during the elimination phase of ethanol metabolism or consumption of very small quantities of ethanol just before death occurred.^{7,12,16} Those specimens where concentrations of ethanol were higher in FVB than in VH (n = 94), with a mean difference of 160 mg/litre, suggest that some of the individuals might have been in the absorption phase, with alcohol still in the stomach when death occurred. Alternatively, ethanol might have been synthesised in the blood after death, probably as a result of microbial action on blood glucose, leading to abnormally low VH/FVB ratios.^{7,14} This phenomenon of post-mortem synthesis is more likely to have occurred when fairly low (< 500 mg/litre) blood ethanol concentrations are reported.³

The physiological principles governing the passage of non-electrolytes such as ethanol from blood into the fluids of the eye were described by Palm in 1946–1947,^{17,18} although it took until 1966 before the first comparisons were made between ethanol concentrations in the blood and VH specimens taken at necropsy.¹⁹ Many studies have since reported the usefulness of VH for alcohol analysis in postmortem toxicology.^{20–22} The concentration of ethanol in VH helps to corroborate the blood

ethanol concentration and gives further evidence that the person consumed ethanol before death.^{6,7,14} Indeed, VH is the fluid of choice whenever putrefaction is apparent, or if there is a risk of diffusion of ethanol from the gut or aspiration of vomit, both of which can compromise the results of ethanol determination in specimens of central or peripheral blood.^{1–3} Eye fluids are seemingly less prone to infiltration by bacteria, and VH is protected by the lens, making it an ideal fluid for ethanol analysis in post-mortem toxicology.^{5,6} However, contamination of the intraocular fluids with blood or vomit has been suggested as a remote possibility, depending on the circumstances surrounding death, and any unusual positioning of the corpse on discovery should be noted.²³

The variance associated with the method of analysis of ethanol (1.5% coefficient of variation) is small compared with the uncertainty in the VH/FVB relation. This points to other important factors influencing the residual variation, such as matrix effects, sampling variation, loss or gain of ethanol during transport and storage of specimens, and the absorption elimination status of ethanol at the time of death. Sousa *et al* found an excellent agreement between the concentrations of ethanol determined in VH specimens removed for right and left eyes (Sousa AP, Vieira DN, Liveira MMF, *et al*. Presented at the XXXV annual TIAFT meeting, University of Padova, Italy, 1998). In 40 cadavers with a postmortem interval of less than 48 hours no significant differences in ethanol concentration were found ($t = 0.657$; degrees of freedom, 39; dependent t test). The mean difference (bias) was only 1.5 mg/litre and the SD of the individual differences was 14.4 mg/litre, giving a 95% LOA of ± 29 mg/litre. This is an impressive result when one considers the combined influences of analytical and sampling variations.

Pounder and Kuroda²⁴ compared the concentration of ethanol in VH and in blood obtained from 349 necropsies. The blood specimens were “typically but not universally from a femoral vein”. The concentration of ethanol in VH ranged from 0–7050 mg/litre and the regression equation relating VH and blood ethanol concentration (BEC) was $BEC = 30 + 0.852 VH$. The residual SD was 260 mg/litre, and the authors warn about the large uncertainty associated with translating measurements made on VH into the concentration in a blood specimen. Our present study, based on 672 necropsies, confirms the large scatter and wide reference interval, which makes it unwise to translate the concentration of ethanol determined in VH into that existing in FVB with the aid of the population average VH/FVB ratio of 1.19 or a median 1.18.

In forensic toxicology, much depends on what the results of analysis are to be used for; that is, whether for scientific purposes or in criminal or civil litigation, where some threshold blood ethanol concentration is important—for example, statutory limits for driving such as 500 or 800 mg/litre. If the intention is to prove that a person’s blood ethanol concentration was above such a limit with a high degree of

certainty, then a conservative value for the VH/FVB ratio should be used. A probability of 50% (more likely than not) is achieved by dividing the concentration measured in VH by 1.18 (median value), whereas to be reasonably sure of not obtaining a FVB alcohol concentration more than the true value, it would be prudent to divide by 1.92, the 97.5th percentile. This would mean that 2.5% of cases might have a lower concentration than that given by VH/1.92. If the VH ethanol content is halved this could be considered, beyond a reasonable doubt, to provide an FVB result not exceeding the true concentration.

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