CLIN. CHEM. 39/11, 2288–2292 (1993)

Relation between Serum and Whole-Blood Ethanol Concentrations

Petrie M. Rainey

An algorithm is suggested for interpretation of serum ethanol concentrations under legal statutes that specify whole-blood alcohol concentrations. The algorithm uses the distribution of individual serum:whole-blood alcohol concentration ratios to allow calculations at various levels of confidence that can be related to legal standards of evidence. Serum:whole-blood alcohol concentration ratios were determined for 211 patients. The ratios ranged from 0.88 to 1.59 (median 1.15). The distribution of ratios was positively skewed, but the logarithms of the ratios were normally distributed. This allowed the parametric calculation of a range of ratios of 0.90–1.49 for the central 99% of the population and a range of 0.95–1.40 for the central 95%. The serum:whole-blood alcohol concentration ratio was independent of both the serum alcohol concentration (R^2 = 0.005) and the hematocrit (R^2 = 0.03).

Indexing Term: *forensic medicine*

Legal statutes define driving while intoxicated in terms of whole-blood alcohol (ethanol) concentrations (*1*). A whole-blood alcohol concentration determined in a forensic laboratory is the proper measurement for legal purposes, but may not always be available. In such cases, serum alcohol measurements obtained for medical purposes have been used as surrogates. A common misconception is that serum and whole-blood alcohol concentrations are equivalent (*2*). Another is that the two are related by a simple conversion factor.

The alcohol content of whole blood is a weighted average of the alcohol concentrations in plasma, erythrocytes, leukocytes, and platelets. The plasma alcohol concentration is essentially identical to the serum alcohol concentration (*3*), but the alcohol concentrations in the blood cells are lower than in the plasma (*4–10*). Consequently, the whole-blood alcohol concentration should always be lower than the serum alcohol concentration.

The exact ratio of serum alcohol to whole-blood alcohol is variable and depends on several factors, including hematocrit, erythrocyte water content, and plasma water content (*7–10*). Use of an average ratio to convert a serum alcohol measurement to a whole-blood alcohol value is inappropriate for most individuals, who will have ratios that differ from the average. To fully interpret a serum alcohol within a legal context, one also should know the range of possible whole-blood alcohol concentrations that may correspond to a given serum concentration.

Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT 06510. Fax 203-785-7340.

Received April 21, 1993; accepted July 28, 1993.

To provide a basis for interpreting serum alcohol concentrations measured in our clinical laboratory, we determined serum and whole-blood alcohol concentrations in samples from patients in a large, urban emergency department. These data have been incorporated into an algorithm for interpretation of serum alcohol concentrations for legal statutes that specify whole-blood concentrations.

Materials and Methods

Blood samples were obtained in the emergency department from 211 patients. For each patient a blood specimen was collected without anticoagulant for serum alcohol testing; results exceeded 10 mg/dL (100 mg/L; 2.2 mmol/L). An anticoagulated specimen was simultaneously drawn for another test (usually hematocrit). The tests were ordered for medical purposes by emergency department physicians who were unaware of the study. Specimens were analyzed and data anonymously recorded under a protocol approved by the Human Investigation Committee of Yale University School of Medicine.

Samples were held in sealed tubes at ambient temperature until analyzed. Serum specimens were analyzed within 30 min of receipt; whole-blood specimens (anticoagulated with lithium heparin or EDTA) were analyzed within 2 h of receipt. Serum and whole-blood alcohol concentrations were measured in an essentially identical fashion. A 100-μL sample of serum or well-mixed whole blood was diluted with 500 μL of water containing 0.25 μL of *n*-propanol as an internal standard. Dilutions of whole blood were centrifuged briefly in a microcentrifuge to remove insoluble stroma (30 s at 10 000 × *g*). A 1.0-μL aliquot of the resulting solution or supernate was then injected into a Sigma 4 gas chromatograph (Perkin-Elmer, Norwalk, CT) with a flame ionization detector. Injector temperature was 175 °C and column temperature was 100 °C. A 183 × 0.3 cm column packed with 0.2% Carbowax 1500 on Carbopack-C (Alltech, Deerfield, IL) was used, with a helium flow rate of 30 mL/min. The alcohol concentration was determined from the height ratio between the ethanol peak (retention time, 0.8 min) and the *n*-propanol peak (retention time, 1.9 min); a standard curve was generated once per shift. The assay is linear up to a concentration of 500 mg/dL (5.00 g/L). Specimens with greater concentrations were reanalyzed after dilution with an equal volume of water. The in-practice precision, determined from replicate control specimens analyzed once each shift during the study, indicated an overall CV of 7.4% at a mean concentration of 89.6 mg/dL (896 mg/L; 19.4 mmol/L).

Data were analyzed with the Statview 4.0 statistics program (Abacus Concepts, Berkeley, CA).

Results

The range of serum alcohol values in the 211 patients was 15–622 mg/dL (150–6220 mg/L; 3.3–135 mmol/L); the range of whole-blood values was 12–522 mg/dL (120–5220 mg/L; 2.6–113 mmol/L). The serum:whole-blood concentration ratios ranged from 0.88 to 1.59. The central 99% of the ratios ranged from 0.92 to 1.54, the mean ratio was 1.16 (95% confidence interval 1.14–1.17), and the median ratio was 1.15. The distribution of the ratios was positively skewed (Figure 1*A*), as is expected for ratios of two normally distributed variables (*16*); the skewness was 0.45 and the kurtosis was 0.68. The distribution of the logarithms of the ratios was approximately gaussian (Figure 1*B*), with a skewness of 0.12 and a kurtosis of 0.19. The mean log ratio was 0.063 (SD 0.043). The corresponding geometric mean was 1.15 (95% confidence interval, 1.14–1.17), and the range of serum:whole-blood alcohol ratios corresponding to the mean ± 2.576 SD (central 99%) of the log distribution was 0.90–1.49.

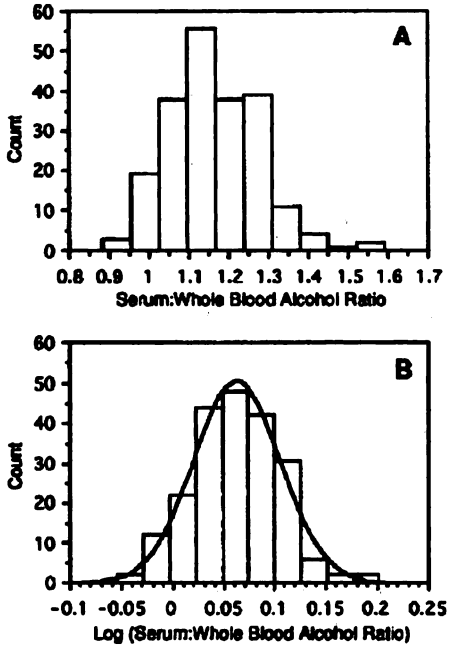

Fig. 1. Distribution histogram of serum:whole-blood alcohol concentration ratios (*A*) and the logarithmic transformation of the distribution (*B*)

The superimposed *curve* in *B* is the normal distribution, defined by the mean and SD of the data comprising the histogram

Correlation of the serum:whole-blood alcohol ratio vs the serum alcohol concentration gave $R^2 = 0.005$ (Figure 2*A*), indicating no dependence of the ratio on the underlying alcohol concentration. Hematocrits were determined for 177 of the subjects (mean ± SD, 0.428 ± 0.0595). When serum:whole-blood alcohol ratios were correlated with the hematocrit, the R^2 value was 0.03 (Figure 2*B*).

Discussion

Clinical laboratories have traditionally measured ethanol concentrations in serum or plasma. All state laws that define driving while intoxicated are written in terms of whole-blood concentrations (*1*). Because treatment of injuries takes precedence over collection of evidence, alcohol concentrations obtained in the emergency department are often the only measurements available on injured motorists. These measurements may be used as legal evidence in both civil and criminal proceedings. However, differences between serum and whole-blood alcohol concentrations have created difficulty in interpreting serum concentrations under legal statutes.

Because of individual variations in blood makeup, any given serum alcohol concentration reflects a range of possible whole-blood alcohol concentrations. Determining the median as well as the expected range of possible whole-blood concentrations allows an interpretation of the serum alcohol concentration that is both scientifically sound and compatible with legal standards of evidence.

The median concentration is most useful in civil proceedings, where the standard of proof is typically the "preponderance of evidence" (i.e., more likely than not). If x percent of a population has the median value, then $50 - (x/2)$ percent will have a value greater than the median, and $50 - (x/2) + x$, or $50 + (x/2)$, percent will have a value greater than or equal to the median. Similarly, $50 + (x/2)$ percent will have a value less than or equal to the median. For example, 11 patients (5.2%) had the median serum:whole-blood ratio of 1.15. One

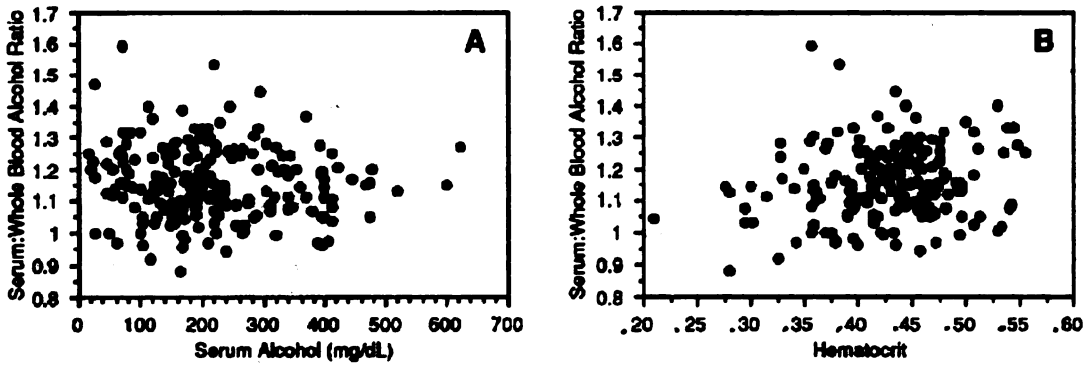

Fig. 2. Scatterplots of serum:whole-blood alcohol concentration ratio vs (*A*) serum alcohol concentration ($R^2 = 0.005$) and (*B*) hematocrit ($R^2 = 0.03$)

hundred patients (47.4%) had ratios >1.15, and 100 (47.4%) had ratios <1.15. Accordingly, 111 patients (52.6%) had ratios ≥1.15 and 111 patients (52.6%) had ratios ≤1.15. For a given serum alcohol concentration, there is a 52.6% probability (i.e., more likely than not) that the actual whole-blood alcohol concentration would be greater than or equal to the value obtained by dividing the serum concentration by 1.15. It is also more likely than not (52.6% probability) that the whole-blood alcohol concentration would be less than or equal to the value obtained by dividing the serum concentration by 1.15. Because those individuals whose blood alcohol concentration is equal to the serum alcohol concentration divided by 1.15 are in both the "greater than or equal to" population and the "less than or equal to" population, both populations include >50% of the total. Because distributions of ratios are positively skewed (*16*), mean values will not bisect the population into equal halves and are thus less useful than median values.

The range of possible whole-blood alcohol concentrations is of greatest utility in criminal proceedings, where the usual standard of evidence is "beyond a reasonable doubt." Exactly what constitutes reasonable doubt is not well defined in the law. However, if the distribution of serum:whole-blood alcohol concentration ratios can be described parametrically (e.g., if the distribution can be normalized and defined in terms of a mean and standard deviation), then the range of possible whole-blood values can be specified with any level of probability desired. A certainty of >99% (P <0.01 of an erroneous conclusion) is a well-accepted standard for scientific evidence and would seem appropriate in this context.

In some cases, the standard of evidence may be "reasonable medical certainty." Because >95% certainty (P <0.05) is the most common standard of proof in testing medical hypotheses, it would seem an appropriate benchmark for establishing reasonable medical certainty.

The literature does not offer a good basis for determining the median value and range of whole-blood alcohol concentrations for a given serum concentration. Most previous studies involved small sample sizes and used methods that are no longer in widespread use (see Table 1); a normal distribution of ratios was assumed, and medians were not given. The study of Winek and Carfagna (*3*) provides the most useful data. This study was designed to achieve very high precision, with preparation of dilutions in duplicate and analysis of each duplicate in triplicate. Although the results should closely reflect the underlying true ratios, they are less useful in defining the range of ratios that might be obtained from measurements from a busy clinical laboratory, where fast turnaround is more important than pinpoint precision and where multiple analysts may be involved.

The present study was carried out to conservatively determine the range of possible serum:whole-blood ratios that might be encountered under real clinical laboratory conditions. Single measurements of specimens were made at the time of receipt during the course of regular business of the laboratory; multiple analysts were involved, and measurements were made at all times of the day and night.

Table 1. Previous Reports of Plasma:Whole-Blood and Serum:Whole-Blood Alcohol Concentration Ratios

Mean ± SD (range)	No.	Method	Ref.
*(1.20–1.25)[a]	10	Dichromate, titrimetric	*12*
1.17 ± 0.06 (1.05–1.25)	10	Dichromate, titrimetric	*13*
1.21 ± * (1.12–1.31)	*	Dichromate, titrimetric	*4*
1.12 ± 0.06 (*)	6	Dichromate, titrimetric	*14*
1.16 ± * (*)	10	Dichromate, colorimetric	*5*
1.17 ± 0.06 (0.98–1.37)	42	Dichromate, titrimetric	*6*
1.13 ± 0.06 (0.94–1.32)	42	Alcohol dehydrogenase	*6*
1.15 ± 0.03 (1.12–1.20)	5	Dichromate, titrimetric	*7*
1.18 ± 0.06 (1.10–1.35)	20	Dichromate, titrimetric	*8*
1.11 ± 0.02 (1.08–1.16)	4	Gas chromatography	*15*
1.14 ± 0.02 (1.09–1.18)	50	Gas chromatography	*3*
1.10 ± 0.03 (1.03–1.24)	17	Gas chromatography	*11*

[a] Asterisk (*) indicates that data were not reported.

The range of serum:whole-blood alcohol concentration ratios found in this study population was 0.88–1.59—much wider than the range of 1.09–1.18 reported by Winek and Carfagna (*3*) and reflecting the effects of the greater analytical variability in our measurements. The finding of ratios <1.0, which violates the theoretical premise that the serum alcohol concentration should always exceed the whole-blood alcohol concentration, is also presumed to result from analytical variation in both measurements involved in the ratio.

Eliminating the highest and lowest ratios to obtain the central 99% of the sample yielded a range of 0.92–1.54. The sample was not sufficiently large for accurate nonparametric analysis of the tails of the distribution, which included only a few measurements. Parametric analysis includes the full sample in defining the distribution and can provide precise values throughout the distribution. The distribution of the log ratios was most appropriate for parametric analysis, because it is theoretically expected to be normally distributed (*16*) and did indeed conform very well to a normal distribution (Figure 1*B*). This distribution can be accurately described in terms of its mean and SD: 0.063 ± 0.043. The central 99% of the log ratios will be included in the range defined by the mean ± 2.576 SD, or −0.048 to 0.174. This corresponds to a range of serum:whole-blood ratios from 0.90 to 1.49.

Dividing a given serum alcohol concentration by these limiting ratios will determine a range of corresponding whole-blood concentrations. The resulting range of whole-blood concentrations will comprise a 99% confidence interval; i.e., there is a 99% certainty that a simultaneously obtained whole-blood alcohol measurement would have fallen within this range. For example, for a serum specimen with an alcohol concentration of 125 mg/dL (1250 mg/L; 27.2 mmol/L), there is at least a 99% certainty that a simultaneously obtained whole-blood specimen would have yielded an alcohol concen-

tration between 84 and 139 mg/dL (839–1389 mg/L; 18.2–30.2 mmol/L). The 95% confidence interval, obtained by using the limiting ratios corresponding to the mean ± 1.96 SD of the log ratio distribution, corresponds to a range of serum:whole-blood ratios of 0.95 to 1.40.

Alcohol concentrations measured for forensic purposes are usually expressed in weight percent, or grams of alcohol per 100 mL of blood. Dividing by 1000 will convert values in mg/dL to values in weight percent (to convert values in mmol/L to weight percent, divide by 217). According to legal precedent, the resulting numbers should always be rounded down to the nearest one hundredth. A serum alcohol of 125 mg/dL (1250 mg/L; 27.2 mmol/L) would therefore correspond to a whole-blood alcohol concentration that has a 99% certainty of being between 0.08% and 0.13% by weight.

The median ratio for the sample was 1.15, which agrees well with a median of 1.14 determined from the data of Winek and Carfagna (*3*). Agreement on the median ratio is expected, despite differences in the precision of the methods of the two studies, because the effects on the median of random analytical variability average out in large samples. The median whole-blood alcohol concentration can be calculated by dividing the serum alcohol by 1.15 for a result in mg/dL or by 1150 for a result in weight percent.

In most jurisdictions, the percent by weight of alcohol is defined as the grams of alcohol in 100 mL of blood ("w/v"). In some jurisdictions, however, the percent by weight is defined as the grams of alcohol in 100 g of blood ("w/w"). Because 100 mL of blood weighs more than 100 g, the standards are not interchangeable. In this study, we measured whole-blood concentrations in mg/dL (mg/100 mL). To use these serum:whole-blood ratios to determine weight percent (w/w) of alcohol, one must correct for the weight of blood. Given that 100 mL of blood weighs 105.0–106.4 g (*17*) [median 105.8 g (*18*)], applying an adjustment for the median weight results in a division factor of 1217 to convert serum alcohol in mg/dL to the median expected whole-blood alcohol concentration in grams per 100 g of blood. Adjusting the division factors for the confidence intervals for the low and high extremes of the blood weight yields respective division factors of 945 and 1585 for the 99% confidence interval and 998 and 1490 for the 95% confidence interval.

The factors derived above can be used to determine serum alcohol concentrations above which intoxication under legal statutes is more likely than not, >95% certain, and >99% certain. These values have been summarized in Table 2, assuming a whole-blood concentration of 0.1% (w/v or w/w) as the standard for intoxication.

One can argue that whole-blood alcohol concentrations in this study should have been measured under forensic laboratory conditions, rather than clinical laboratory conditions: use of the more precise forensic techniques would have yielded narrower ranges. In an ideal situation, each laboratory would determine its own range of serum:whole-blood alcohol ratios, much as each laboratory determines its own reference ranges. Serum alcohol concentrations would be measured under the same conditions as routine patient specimens and whole-blood alcohol concentrations would be measured under conditions comparable with those in the local police or forensic laboratory.

Table 2. Minimum Serum Alcohol Concentrations Corresponding to Legal Intoxication at Various Probabilities

	Legal standard of 0.1 g of alcohol in	
Probability	100 mL of blood (w/v)	100 g of blood (w/w)
>50% (more likely than not)	115[a]	122
>95% (reasonable medical certainty)	140	149
>99% (beyond a reasonable doubt)	149	159

[a] Concentrations (mg/dL) based on the data reported here. To convert values in mg/dL to mmol/L, multiply by 0.217.

However, many laboratories do not have ready access to forensic technology. In such cases, the use of clinical laboratory methods to measure the whole-blood alcohol concentration is acceptable, although the confidence intervals may be considerably wider. The use of the wider intervals will reduce the probability of a false allegation of legal intoxication when none was present. No error is made in claiming a certainty of >99% when the certainty is also >99.5%. As in testing for drugs of abuse, an occasional false negative can be tolerated in the interest of avoiding false positives.

An important requirement for applying population-based conversion factors to individual serum alcohol concentrations is that the serum:whole-blood concentration ratio must be independent of the underlying serum alcohol concentration. This has been assumed, but not explicitly tested, in previous studies. In this study, regression of serum:whole-blood ratios against serum concentrations gave an R^2 value of 0.005 (Figure 2*A*). A similar regression of the data of Winek and Carfagna (*3*) yielded $R^2 = 0.008$. Thus, the serum:whole-blood alcohol ratio does not vary significantly over the range of serum concentrations investigated.

If individuals could be categorized into subpopulations with narrower ranges of possible serum:whole-blood alcohol ratios, more precise interpretations of serum alcohol concentrations could be made. Given that whole-blood alcohol concentrations are weighted averages of plasma and blood cell alcohol concentrations, an obvious approach would be to use hematocrit-adjusted ranges. However, the attempt to correlate serum:whole-blood alcohol ratios with hematocrit gave an R^2 value of only 0.03 (Figure 2*B*). Calculation with the data of Winek and Carfagna (*3*) gave $R^2 = 0.002$, confirming the lack of correlation. These findings suggest that no improvement will be achieved by adjusting for hematocrit.

The approach described here may serve as an algorithm for interpreting serum alcohol concentrations un-

der statutes written in terms of whole-blood concentrations. This algorithm will have the greatest accuracy when used with laboratory-specific serum:whole-blood alcohol concentration ratios. In case it is necessary to interpret a serum alcohol concentration from a laboratory that has not determined its own range of ratios, the limiting ratios determined in this study may be useful. Because these ratio are very conservative, confidence intervals derived from them are unlikely to overestimate the level of certainty when applied to serum alcohol concentrations measured in most clinical laboratories.

I thank Max Levy and the Toxicology technologists in the Yale–New Haven Hospital Clinical Chemistry Laboratory for technical support and W. L. Roberts for careful review of the manuscript.

References

1. National Highway Traffic Safety Administration. Digest of state alcohol–highway safety related legislation, 11th ed. Washington, DC: US Department of Transportation, 1993:536pp.
2. Rainey PM. Drunk drivers and medical inquiry [Letter]. N Engl J Med 1988;318:1277.
3. Winek CL, Carfagna M. Comparison of plasma, serum and whole blood ethanol concentrations. J Anal Toxicol 1987;11:267–8.
4. Künkele F. Zur Blutalkoholbestimmung. (Über die Verteilung des Alkohols in geronnenem Blut). Dtsch Z Gesamte Gerichtl Med 1936;26:241–4.
5. Grüner O. Die Verteilung des Alkohols im Blut. Dtsch Z Gesamte Gerichtl Med 1957;46:10–7.
6. Illchmann-Christ A. Untersuchungen über die Relationen von Blutkuchen- Vollblut-(Serum-) Alkoholwerten. Zugleich ein Beitrag zur Umrechnung von Serum- auf Vollblut-Alkoholkonzentrationen. Dtsch Z Gesamte Gerichtl Med 1959;49:113–29.
7. Payne JP, Foster DV, Hill DW, Wood DGL. Observations on interpretation of blood alcohol levels derived from analysis of urine. Br Med J 1967;3:819–23.
8. Payne JP, Hill DW, Wood DGL. Distribution of ethanol between plasma and erythrocytes and whole blood. Nature 1968;217: 963–4.
9. Grüner O. Die Bedeutung des Körperwassers für die Verteilung des Alkohols im Organismus. Dtsch Z Gesamte Gerichtl Med 1957;46:744–60.
10. Brettel F. Blutalkohol and Blutwassergehalt. Lubeck, Germany: Verlag Max Schmidt-Komhild, 1972:97pp.
11. Jones AW, Hahn RG, Stalberg HP. Distribution of ethanol and water between plasma and whole blood: inter- and intra-individual variations after administration of ethanol by intravenous infusion. Scand J Clin Lab Invest 1990; 50:775–80.
12. Miles WR. The comparative concentrations of alcohol in human blood and urine at intervals after ingestion. J Pharmacol Exp Ther 1922; 20:265–319.
13. Elbel H. Untersuchungen zur Verwertbarkeit der Blutalkoholbestimmungen nach Widmark. Dtsch Z Gesamte Gerichtl Med 1935; 25:124–9.
14. Dotzauer D, Redetzki H, Johannsmeier K, Bücher T. Erprobung einer spezifischen Fermentmethode zur Mikrobestimmung von Äthylalkohol. Dtsch Z Gesamte Gerichtl Med 1952;41: 15–29.
15. Hodgson BT, Shajani NK. Distribution of ethanol: plasma to whole blood ratios. Can Soc Forensic Sci J 1985;18:73–7.
16. Atchley WR, Gaskins CT, Anderson D. Statistical properties of ratios. I. Empirical results. Syst Zool 1976;25:137–48.
17. Altman PL, Dittmer DS. Blood and other body fluids. Washington, DC:FASEB, 1961:540pp.
18. Muirhead EE, Grow MH, Walker AT. Practical observations on the copper sulfate method for determining the specific gravities of whole blood and serum. Surg Gynecol Obstet 1946;82:405–13.