Which Types of Alcohol-Use Disorder Will Asialotransferrin Detect?

To the Editor:

Approximately 20% of patients seen in clinical practice have an underlying alcohol-use disorder (1). In the last 20 years, specialist work on alcohol misuse has focused mainly on diagnosis at the dependence stage. However, there is also a need to direct attention to alcohol abuse, the long, little-studied, and insufficiently treated disease during which processes damaging to health and social functioning are initiated. Recently, Legros et al. (2) concluded that as a biomarker, asialotransferrin offered the best differentiation between moderate and abusive alcohol consumption.

From a methodologic point of view, clinical investigations concerning biomarkers of alcohol abuse or alcohol dependence have to define their study populations according to internationally accepted clinical categories of alcohol misuse (DSM IV and CIM 10) or to patient populations well defined by alcohol intake (e.g., 0–10, 10–20 g/day, and so forth). This is the basic requirement to make these investigations reproducible, comparable, and interpretable by doctors working in care.

Legros et al. (2), in their excellent work on the efficacy of asialotransferrin and disialotransferrin, recommended the “analysis of the asialo-Tf [asialotransferrin] isof orm, which will be present in 92% of alcohol abusers and absent in 95% of moderate alcohol consumers”, and Arndt (3) cited numerous advantages, including high specificity, simple standardization of the analytical definition, and the possibility of producing specific antibodies for direct assay, as strong arguments in favor of asialotransferrin. In this sense, the results of the study by Legros et al. (2) are very promising.

Nevertheless, we found in the study by Legros et al. the methodologic problem of definition of the study population. The “alcohol abusers” in that study were probably poorly identified. No inclusion criteria according to alcohol abuse defined by DSM IV (F305) and checked by the corresponding MINI questions were used (4). One criterion for inclusion in the study was an AUDIT score >11. Patients with AUDIT scores between 7 and 11 were excluded. The mean AUDIT score for the included patients was, in fact, 27. However, the internationally validated cutoff for the AUDIT questionnaire is 8; higher AUDIT scores seem associated with greater severity of alcohol misuse, and scores >12 are characteristic of alcohol dependence (5).

Another inclusion criterion of this study was a daily ethanol intake >50 g. In fact, the alcohol abusers included drank, on average, 166 g/day, with a range from 70 to 310 g/day. In a recent study determining the nutritional intake of alcohol-dependent patients, Manari et al. (6) reported that the dependent patients included had mean ethanol consumption of 162 g/day. Nicolas et al. (7) reported a mean ethanol intake in alcohol-dependent patients of 177 g/day. Legros et al. (2) may therefore not have fully differentiated between alcohol abuse and alcohol dependence according to the DSM IV criteria. Alcohol-dependent patients may thus have been unwittingly included in that study. Testing patients with dependence but using the term “abuse” to classify them will tend to overestimate test sensitivity, producing serious consequences in screening practice.

References

cysteine (Hcy) values were largely <15 μmol/L in the participants in their case–control study. The cases were born at their institution over a 2-year period and had birthweights below the 10th percentiles for gestational age and sex. The control group included babies born at or above the 10th percentiles. The mothers were also included in the study. These investigators initiated their research based on an assumption that higher maternal and newborn Hcy concentrations in plasma would increase the risk of intrauterine growth restriction through placental thrombosis. In contrast to their proposed hypothesis, however, they concluded that mothers with small babies had lower Hcy concentrations than those giving birth to larger infants.

There are several issues with the conclusion that the authors made in their article that I would like to address. The first is that the maternal total homocysteine (t-Hcy) was measured within 48 h postpartum. This measurement does not reflect the actual t-Hcy during pregnancy, because t-Hcy may have been decreased or increased during the 48 h after labor. A proper investigation should include specimen collection at various points during pregnancy (first, second, and third trimesters), immediately after labor, at least 1 week postpartum, and ideally even before pregnancy to determine whether the difference between the obtained t-Hcy values is meaningful and statistically significant. Without these data, conclusions and arguments such as those made by Infante-Rivard et al. (1) in their report are not valid. These investigators previously published a reference range study (2) for t-Hcy for maternal blood; however, that study suffers from the same problem of inappropriate sampling time.

Infante-Rivard et al. (1) indicated that, contrary to their hypothesis, the probability of a mother giving birth to a baby with growth restriction decreased with increasing t-Hcy; i.e., birthweight increased with t-Hcy concentrations. There were no conclusive statistical data included in their communication showing that the values for t-Hcy were significantly different between control infants and cases or between their mothers. Although the mean t-Hcy of 5.11 μmol/L [confidence interval (CI), 4.95–5.26 μmol/L; range, 1.76–14.03 μmol/L] for case mothers in Table 2 of their report (1) seems different from the mean t-Hcy of 5.59 μmol/L (CI, 5.41–5.76 μmol/L; range, 1.92–15.98 μmol/L) for control mothers, it does not say much about the individuals at risk. It would have been more interesting to see the distribution of the results and their quartiles in boxplots so that the reader could see whether there is a significant overlap between the data in the two groups. The confidence interval for the mean is calculated based on “mean ± 2 SE,” and although this conveys that the two populations are different, it does not provide additional information on the individuals at risk. In the first paragraph of their discussion, Infante-Rivard et al. (1) claimed that the results for Hcy in the newborns were in the same direction as in the mothers, but it is hard to believe that the mean of 4.99 μmol/L (CI, 4.84–5.15 μmol/L; range, 1.03–17.94 μmol/L) reported in Table 2 for newborn cases is statistically different from the mean of 5.06 μmol/L (CI, 4.92–5.21 μmol/L; range, 0.73–13.62 μmol/L) for the newborn controls, particularly when the analytical CV was 10%. Again, presentation of the data in a boxplot would have been of great help in illustrating the trends. The authors raised the question of what factors could explain their unexpected findings. They indicated that because the range of t-Hcy values fell below the cutoff for mild hyperhomocysteinemia, those values should be considered as within the reference interval. However, individuals with a t-Hcy value closer to the upper reference limit may have had a better diet during pregnancy. Hcy is derived from methionine, an essential amino acid (5), and this could qualify it as a novel nutritional biomarker for risk assessment of intrauterine growth restriction during pregnancy if their conclusion is valid. Obviously, this needs to be substantiated further and requires appropriate statistical analysis of the t-Hcy concentrations for the cases and controls. To do this, an appropriate reference range study for t-Hcy is needed in pregnant and nonpregnant women in conjunction with a study of weight gain during pregnancy. This may further support the conclusions of Zappacosta et al. (4) because better nutrition may provide sufficient t-Hcy to work as an antioxidant. Therefore, a higher t-Hcy concentration may mean that the mother of an infant with a higher birthweight had a better diet, which in turn means that there were more nutrients available in the maternal blood to be transferred to the fetus. On the other hand, Haulrik et al. (6) showed that a high-protein, high-methionine diet did not lead to increased Hcy concentrations compared with a low-protein, low-methionine diet in overweight adults. They also showed that Hcy concentrations after a 3-month intervention were inversely associated with vitamin B₁₂ intake and with weight change.

Infante-Rivard et al. (1) claim that their results were comparable with other North American results (7–9). I did not see any comparability between the results obtained by these authors and the cited references (7–9). Walker et al. (7) measured Hcy in nonpregnant control women and in healthy pregnant women during the first, second, and third semesters. However, they did not measure postpartum Hcy. Walker et al. (7) also found that Hcy decreased during pregnancy. This is evident from Fig. 2 of their publication (7). Therefore, the results reported by Infante-
Rivard et al. (1) are not comparable to those reported by Walker et al. (7), especially because the latter group did not measure Hcy in women after delivery. Malinow et al. (8) hypothesized in their publication that Hcy, an amino acid that is not a constituent of proteins, crosses the maternal-placental-fetal interphases and is taken up by the fetus. To test this hypothesis, Malinow et al. (8) determined the concentration of plasma Hcy in the maternal vein and neonatal umbilical vessels at the time of delivery. Their findings demonstrated a progressive decrease in the concentration of plasma Hcy going from the maternal vein to the umbilical vein and to the umbilical artery. Their data support the hypothesis that Hcy is sequestered by the fetus. In my view this study had a proper design for the time of specimen collection. They sampled the blood at the time of delivery but not 48 h postpartum. Pagan et al. (9) studied serum Hcy in smoking and non-smoking pregnant women (18–30 weeks of gestation only). They found that the mean (SD) Hcy in smokers [5.7 (3.4) μmol/L] was not different from that in the nonsmokers [4.9 (1.6) μmol/L]. Interestingly, the difference in Hcy concentrations between the two groups was almost the same as the one reported by Infante-Rivard et al. (1); however, Pagan et al. (9) did not report the difference as significant.

In conclusion, additional data are required to support the unexpected relationship observed by Infante-Rivard et al. (1) between plasma Hcy and intrauterine growth restriction. In addition, the roles of vitamin B₁₂ and folic acid should also be investigated.

References


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DOI: 10.1373/clinchem.2003.028944

Drs. Infante-Rivard and Rivard respond:

To the Editor:

In his letter, Moridani raises a few points about our most recent report (1) on the relationship between maternal and newborn plasma total homocysteine (tHcy) and intrauterine growth restriction (IUGR), and about a previous report showing perinatal references values for tHcy (2). It is not clear to us why, as stated by Moridani, “specimen collection at various points during pregnancy . . . [T]o determine whether the difference between the obtained t-Hcy values is meaningful and statistically significant” is more proper than what we have done. Our comparison between cases and controls, whose measures of tHcy were taken at the same time, is completely proper and valid. Our conclusions were about perinatal measures of tHcy; if others want to measure and compare tHcy at other times during, or even before, pregnancy, that is another matter. Validity of results has nothing to do with timing of measurements but with quality of measures and appropriateness of the comparisons. Our study meets both.

Another point of Moridani’s letter is confusing to us. It seems to oppose the description of means, or of data, with the notion of “individuals at risk”. As a simple descriptive analysis, Table 2 of our report (1) shows mean tHcy values and their confidence intervals. For the comparison of means, elementary statistical theory informs us that confidence intervals that do not overlap are equivalent to a statistically significant difference. As stated in our report, the mean tHcy was different between case and control mothers, but not between case and control newborns. However, the study’s main objective was not to compare tHcy between cases and controls, but to determine whether tHcy is a risk factor for IUGR, accounting for established IUGR risk factors. Moridani seems to have missed this perspective. Our statistical analysis, in which we used unconditional logistic regression and linear regression, which may not be familiar to the author, was completely appropriate for the study’s goal. A box plot is not, to our knowledge, a way to estimate risk, whereas contribution to the probability of disease, i.e., risk, is readily estimated by odds ratios in a multivariable model.

Finally, Moridani proposes an apparently new mechanism for our findings, which relates to a better diet, to be studied along with weight gain during pregnancy. We have already shown in our previous report that a better diet (folate-rich foods) reduces the tHcy concentration (2), and in the present study (1), we measured and adjusted for weight gain during pregnancy. Although Moridani’s suggestion does not seem fruitful, we agree that additional data are required to support the observation of an inverse relationship between tHcy and IUGR, including the roles of vitamin B₁₂ and folic acid.