I read with interest the analysis of the heart rate variability and mortality in the Zutphen Study (1). One potentially confounding factor which was not addressed is alcohol consumption. Alcohol has been traditionally associated with smoking and increased mortality as a result of a number of causes, including cancer.

It is known that heart rate variability is affected by alcohol (2). Chronic alcohol consumption decreases heart rate variability; this decreased heart rate variability returns to normal after a number of days of abstinence (3). Because of this, alcohol induced decreased heart rate variability could be an important confounding factor, accounting for the apparent association of cancer with heart rate variability in this study.

REFERENCES

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Editor's note: In accordance with Journal policy, Dr. Dekker and her coauthors were given the opportunity to reply to the above letter, but they chose not to do so.

RE: "p53 PROTEIN OVEREXPRESSION IN RELATION TO RISK FACTORS FOR BREAST CANCER"

We read with great interest the recent article by van der Kooy and colleagues (1) examining the association between epidemiologic risk factors and p53 protein overexpression. Tumor markers, such as p53, may help define more homogeneous case groups yielding clearer patterns with risk factors. While tumor markers have mainly been used to subdivide cases for prognostic purposes, researchers are also increasingly using markers for etiologic investigation.

A key methodological difference between using tumor markers for prognostic studies and for etiologic studies is that the former involves case-case comparisons while the latter often involves a control group. Use of a control group means that the outcome variable in the regression model is no longer binary but tertiary, e.g., p53+ cases, p53− cases, and controls. Misclassification patterns for all outcome categories need to be considered when interpreting measurement error in tumor markers. As we are considering odds ratios, we can directly apply knowledge from the literature on nondifferential misclassification of polytomous exposure variables (2–7).

Specifically, when there are three outcome categories, and the "lowest" category is the referent category (controls), comparisons with the intermediate category (p53− cases) may be biased away from the null. This occurs even if the misclassification of the p53 status is nondifferential with respect to exposure if 1) there is a true monotonic trend in risk, 2) there is misclassification from the highest category to the intermediate category, and 3) there is no misclassification between cases and controls. The last assumption stems from the fact that tumor markers are absent for all controls and, therefore, there is no likelihood of misclassification of controls into either case category. The second assumption is supported by data that suggest that protein expressivity may be misclassified because of tumor heterogeneity, storage, or fixative issues (1). The first assumption of monotonicity follows from the expectation that p53+ cases may reveal higher risk estimates than p53− cases, compared with controls. Thus, any misclassification of p53+ cases to p53− cases means that the higher exposure odds associated with the misclassified p53+ cases will mix with the lower exposure odds associated with the true p53− cases, resulting in an overall bias away from the null for comparisons with the control group. Misclassification of p53− cases into p53+ cases (false positives), while less likely, may be possible. However, under the assumption of monotonicity, such misclassification of the p53− cases into p53+ cases will result in comparisons of the p53+ cases with controls being biased toward the null. As a result, the overall likelihood of seeing heterogeneity in risk factor patterns by p53 status is reduced.

In discussing potential biases of their study, van der Kooy et al. (1) state the focus of their study is case-case comparisons and, therefore, correctly recognize "...misclassification of the p53 status that may have biased our risk estimates toward unity" (p. 930). However, as they report odds ratios with respect to a control group, it might also be helpful to discuss misclassification for the two case groups relative to the control group. Given the likely scenario of higher risk estimates for p53+ cases compared with controls than p53− cases compared with controls, and the absence of misclassification between cases and controls, we believe it is clearer to state that the misclassification of the p53 status may likely bias the comparisons of the p53+ cases compared with controls toward unity. However, comparisons of the p53− cases with controls may likely be biased away from unity, resulting in a reduced likelihood of seeing any heterogeneity in risk patterns by p53 status.