Reviewer’s report

Title: Methodologic Considerations in the Design and Analysis of Nested Case-Control Studies: Association between Cytokines and Postoperative Delirium

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Reviewer: Christina Dahm

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This methodological paper describing a clinical nested case-control study of postoperative delirium in relation to interleukin-6 (IL-6) levels has 4 aims: evaluating matching algorithms for matching cases to controls; determining the most appropriate measures of association; comparing the associations to a simulated cohort study; and considering overmatching.

The argument given for using a nested case-control study design is to use expensive measures of exposure most efficiently, which is quite fair. IL-6 is here evaluated at 4 time points, both before and after incident delirium. However, in a case-control study the appropriate exposures to investigate are those preceding the incidence of case status, which in this study is defined as delirium on postoperative day 2 or day 1 plus residual effects on day 2. Thus the IL-6 measures determined in blood samples taken after delirium cannot be evaluated appropriately within this study design. This is because delirium is now the exposure, but the unexposed group, the matched controls from the nested study, is not representative of all unexposed operated persons from the original cohort. Similarly, it is problematic to reject determining the OR for exposure within the nested study in favor of median paired differences (MPD), as the controls do not necessarily have the same distribution as the cohort from which they have been sampled. Naturally the statistical significance level is approximately the same whether OR or MPD are computed - the same data are being analyzed. But the MPD determined using these controls cannot be assumed to represent the MPD that would be determined in the full cohort, were data available. An option would instead be to transform IL-6 levels to achieve normally distributed data, and compute the OR per unit change. (eg. log2 would allow the computation of the OR per doubling of IL-6 levels).

When discussing the matching algorithms, no mention was made of the 10 cases that were not selected. How can we be sure that the selected cases are representative of all cases, and thus an unbiased sample of cases? It appears to particularly be vascular comorbidity that is unevenly distributed among cases and controls pre-matching. If this is associated with delirium, it is likely that the controls are overmatched to the cases.

Many decisions in generating the simulated cohort used to evaluate bias in the nested design are made without reference to any literature. I am not convinced, given the striking differences between the pre-sampling and post-sampling characteristics shown in Table 1, that the chosen method to generate the simulated cohort from the IL-6 values determined in the matched case-control study can be expected to represent the results that could have been determined had actual data from the entire cohort been available.
As the controls should represent the source population from which the delirium cases arise, the general linear models investigating the matching factors vs IL-6 levels should be performed only in the control group. Whether this was the case is not clear.

In general, the statistical methods are not clearly described. For example: Which analysis generated the beta coefficients in Table 3? Which statistical package was used for analyses?

If the research question has to include post-delirium IL-6 measures, a case-cohort design would be more appropriate. In this design, a random sample of all cohort participants is sampled, and compared to cases. This would allow for a follow-up design, and to use delirium as an exposure in relation to later IL-6 levels.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

No

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

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If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

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