Reviewer's report

Title: Frequency of apnea and bradycardia following first diphtheria-tetanus-pertussis-inactivated polio-Haemophilus influenzae type B immunization in hospitalized preterm infants

Version: 2 Date: 14 February 2006

Reviewer: Jukka Jokinen

Reviewer's report:

General

The authors have revised the manuscript by making a more elaborate description of the characteristics of the control group, which was criticized in my previous report. It is now more clear that some of the assumed differences according to the previous version between the two populations are nonexistent, since the children in the "control" group were still in neonatal intensive care unit. In my previous report, I also suggested using the child itself as a control, where the frequencies 72 hours pre and post vaccination would be compared. The authors suggest that since multiple factors (sepsis, hypothermia, worsening of a cardiac lesion, or increased observation because of change of nursing staff) can contribute to the apparent increase in adverse events, the use of control group of approximately the same age is justified. They also note that this is supported by the fact that an increase of adverse events were observed for 25% of the infants in the control group in observation period #2 compared to #1. The way how the adverse events were recorded is also clarified and the possible bias caused by it is discussed.

In spite of these clarifications, two major concerns still arise from this response

1. The choice of the control group
2. Definition of an adverse event

1. The two populations from which the "case" and "control" groups are collected may not differ that much as could have been assumed from the previous version. However, there is one serious problem distinguishing these children that, in the context of current evaluation, seriously undermines this comparison. I elaborated in the previous report that the setting in this study is clearly not a case-controlled study. Therefore, all references to case-controlled studies should be removed and different names for the groups should be used. The more appropriate wording that points out the differences in these two groups is the vaccinated and the unvaccinated group. The "control" group is the unvaccinated group, where also children that have received some other vaccination during the time in question were excluded. When the vaccination coverage is high, the unvaccinated typically represent a very different group of children, and thus the direct comparison between the vaccinated and unvaccinated is likely to be biased. Therefore, the standard practise in the analysis of vaccine adverse event studies is that the adverse events are evaluated only for the vaccinated, where different periods with respect to the vaccination are compared (see, for example, Farrington et al, 1996, and references therein). This approach was also suggested in my previous report. If the authors insist on using the unvaccinated as a control group, an elaborate discussion should be included where the reasons for deviating from well-founded and clearly established way of analysis are stated. In my mind, the current response cannot be seen as adequate; see further considerations below.

In addition, the authors claim that since there are multiple factors that can contribute to the increased frequency of adverse events in time, the use of control group is justified. However, the ages of 55
children in the unvaccinated group are adjusted up to +/- 7 days from the age of the vaccinated, thus representing more variability than possible comparison between pre and post vaccination ages +/- 3 days. It is also hard to see how the multiple factors stated in the reply would more likely be increased in the age of ~10 weeks+3 days compared to ~10 weeks-3 days. The stated support for this claim is that 25% of the unvaccinated experienced increase in adverse events during period #2. However, this is based on asymmetric definition of an adverse event; see concern 2.

2. The definition of an adverse event has been defined as 1 if there is an increase during observation period #2 compared to observation period #1, and zero otherwise. This definition is asymmetric, i.e. only focused on increase during period #2. For example, in the unvaccinated group, 25% of children experienced increase during period #2. The reader is left wondering what is the proportion out of 75% of children that have experienced increase during period #1 compared to period #2. This is the reason why, in the previous report, I suggested using a difference (or a ratio) of events as the response variable. This would account for changes also in another direction. This asymmetry of the measure is also the reason why using the unvaccinated group as a control cannot be justified from the 25% increase in one direction only.

Furthermore, as was stated in my previous report, if the adverse events were recorded only by observation, an increased concern after vaccination by the study nurses is likely to cause an increased charting of adverse events during the post-vaccination period. The authors now confirm that the recording was indeed done only by observation. The authors also seem to agree on this possible bias but state in the discussion that the use of matched controls should decrease this as a confounding factor. However, since the "control", i.e. the unvaccinated group did not receive the vaccination, there is no reason why there would be an increased concern during period #2 for these children, and therefore the unvaccinated (control) group cannot remove this as a confounding factor. As the authors state in their report, a more detailed monitoring would be impractical, and therefore this source of bias cannot be removed.

Reference:

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

In summary, since the 1) comparison between the vaccinated and unvaccinated, as well as 2) comparison between pre- and post-vaccination periods present apparent and clearly established sources of bias, most of the currently hypotesized effects of the vaccination cannot be adequately addressed from these data. Therefore, I cannot but to conclude that these data is only suitable for comparison of the effects of whole-cell and acellular pertussis vaccination, where the bias due to vaccination is removed by discarding the unvaccinated (i.e. "control") group from the analysis (as I suggested in my previous report), and the asymmetry from the definition of adverse event is removed by taking into account the changes into another direction (either by using difference or ratio, or similar definition also into another direction). As was stated in General discussion, the apparent bias caused by increased charting cannot be removed, but the bias will most likely be the same for children with whole-cell and acellular pertussis vaccinations. Therefore the absolute increase is rather meaningless number from these data, but the difference in the increase between the whole-cell and acellular pertussis vaccines can provide answers about the differences of these two vaccinations.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
Discretionary Revisions (which the author can choose to ignore)

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

I declare that I have no competing interests