Author's response to reviews

Title: Hepatitis B virus vaccination booster does not provide additional protection in adolescents: A cross-sectional school-based study

Authors:

Yung-Chieh Chang (tonyijane@hotmail.com)
Jen-Hung Wang (paulwang@tzuchi.com.tw)
Yu-Sheng Chen (lister3813401@yahoo.com.tw)
Jun-Song Lin (8887241@ms21.url.com.tw)
Ching-Feng Cheng (chengcf@mail.tcu.edu.tw)
Chia-Hsiang Chu (chuchia@ms6.hinet.net)

Version: 5 Date: 30 July 2014

Author's response to reviews: see over
Dear Editor:

We thank the reviewers for the insightful and helpful comments. We have revised our manuscript and changed the title to “Hepatitis B virus vaccination booster does not provide additional protection in adolescents: A cross-sectional school-based study” according to reviewer’s suggestions. We have explained in details to all comments raised by the reviewers and rewritten our manuscript in Methods and Results. We hope this revised manuscript had answered all questions asked by the reviewers and will be acceptable for publication. Points to points reply to the reviewer’s comments were attached in the following pages.

Best Regards,

Yung-Chieh Chang, M.D.
Fellow of Pediatric Gastroenteroloy
Department of Pediatrics
Hualien Tzu-Chi Medical Center, Taiwan
Author’s response to reviewers

Title: Booster Vaccination of Adolescents Does Not Prevent Hepatitis B Infection: A Cross-sectional School-based Study

Authors:
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Ching-Feng Cheng (chengcf@mail.tcu.edu.tw)
Chia-Hsiang Chu (chuchia@ms6.hinet.net)
Reviewer’s report

Title: Booster Vaccination of Adolescents Does Not Prevent Hepatitis B Infection: A Cross-sectional School-based Study

Version: 3 Date: 28 April 2014

Reviewer: Thomas Peto

Reviewer’s report:

Major Compulsory Revisions
1. The Methods and Results section needs to be rewritten so that it is clearer what was done, and a table reporting data on those who did and didn’t receive booster doses is needed.
   
   A flow chart of our study design was added as Figure 1 and Methods were divided into several sections such as vaccination program in Taiwan, study population, epidemiological study, subgroup analysis (comparison of HBV markers by different vaccine types, booster effects in subjects double seronegative for hepatitis B markers at 15 years of age, and longitudinal study).
   
   We had included one table (Table 3) to clarify the numbers of booster recipients, the booster response rate about 6-week after one HBV booster dose which was only available from 2007 to 2012 (birth cohort 1992-1997) were identified in 15-year age group.

Minor Essential Revisions
1. The tables needed to be rewritten for clarity
   
   Tables and Figures were rewritten and rearranged according to the results of each section.

Discretionary Revisions
1. The title does not reflect the data-it is not that booster doses don’t prevent chronic infection in teens, it is that there is no evidence that these infections are acquired by people who have not receive boosters
   
   We were able to find the quantitative results of anti-HBs titers in our database, but it was only available after year 2007. Therefore, we did some analysis based on the time frame related to booster event. We
clarify the list of booster recipients from 2007-2012 (birth cohort 1992-1997 at 15 years of age). We focused on the booster response and longitudinal study (referral to our table 3, table 4 and figure 4) this time. We changed our title to “Hepatitis B virus vaccination booster does not provide additional protection in adolescents: A cross-sectional school-based study”.

Further comments on the MS are in the pdf attached

1. Is the question posed by the authors well defined?
   Partly. They wish to report the prevalence of antibody to HBsAg (anti-HBs-implying immunity), and HBV surface antigen (HBsAg implying chronic infection with HBV); and also to assess the efficacy of a booster dose of the HB vaccine, among Taiwanese high-school students born since the introduction universal infant HBV vaccination.
   We still aimed to provide a booster strategy for adolescents in our daily practice. We had verified the immunogenicity response in booster recipients and clarify the need of booster in teenagers (table 3 and figure 3) who had received primary HBV infant vaccination here in Taiwan. Current suggestion for HBV booster was only for high-risk persons. Taiwan is still not considered as low endemic area yet. Our teenagers exposed themselves under the risks of hepatitis B infection as a result of their changes in lifestyle and sexual activity.
   Therefore, we want to verify the duration of protection with HBV vaccine.

2. Are the methods appropriate and well described?
   The methods are okay (taking advantage of a reasonably sized sample of blood routinely collected from students), but it is difficult to follow exactly what was done and how the data was assembled. It seems that 68 people were given a booster dose (selected because they had no detectable antibodies to HBsAg), but how many doses, whether this was an appropriate group, and how the comparator group was selected is not easy to follow.
   Flow chart of our study design was added as Figure 1 and Methods were divided into several sections based on our flow chart. We simplified our study design and comparator group to one. We must apologize for misleading by just stating the group into “booster”
versus “non-booster” group. There were two improvements in our data gathering. First, the quantitative data of anti-HBs titers was available in our database after year 2007 analysis. Also, we got the list of booster recipients from 2007-2012 (birth cohort 1992-1997) at 15 years of age group from the school nurses. No booster list or quantitative titers of anti-HBs were available in 12-year age group.

We decided to follow the subjects of birth cohort 1993–1994 in the 18-year age group who had received the recombinant HBV vaccine in infancy. In this way, the effects of different vaccine type were eliminated. If the subjects previously studied in our senior high school (15-year age group, birth cohort 1993–1994), they were grouped as “the same school”. Their serum data of both anti-HBs and HBsAg were analyzed longitudinally to trace the pattern of seroconversion and post-booster change in this 3-year interval. The other subjects of the 18-year age group from the 1993–1994 birth cohort were used as the comparison group, which was labeled as “the others”. At this time, “the same school” versus “the others” was the only comparison group we arranged.

If the endpoint was change in anti-HBs then was it suitable as a marker for efficacy? If the endpoint was HBsAg, then so few infections would be expected that there is no power to detect a difference (notwithstanding that a small or moderate effect against chronic infection would be worth knowing about)

Immunity against HBV provided protection against infection as well as against disease. Protection against infection is associated with antibody persistence, which is directly related to the peak production of anti-HBs after primary vaccination. Protection against disease (i.e., acute hepatitis, prolonged viraemia, carriership, and chronic infection) is associated with immune memory that persists beyond the time at which anti-HBs disappears Therefore, the change of anti-HBs is not a suitable marker for efficacy even we got anti-HBs titers before and after booster. We assumed subjects with positive for HBsAg as hepatitis B carriers. To compare the differences of HBsAg carrier rate was a better option to monitor the efficacy of protection. In our study, we didn’t have accurate data for booster rate in comparison group “the others”. We could only compare two subgroups of same birth
cohort at 18 years of age if there was any difference of seropositive rate of HBsAg (carrier rate) to monitor the efficacy of protection which would point to how effective the anti-HBs and immune memory clean up the acute viral infection.

3. Are the data sound?
Only anti-HBs (binary-not quantified) and HBsAg data were collected, and this was available for nearly all the students. There are no obvious inconsistencies and the tests are standard, so the data is probably sound. However, the tables and results need to be reported more clearly and numbers as well as percentages used throughout. Negative results need to be included in the tables so that the totals add up to 100%. There is no single figure or flow chart that explains what went on with the boosters.
Quantified anti-HBs titers were available from year 2007-2012 for all age groups except age-12 group in our study now. We had added on a flow chart (Figure 1) to clarify how we analyzed the data, and conducted for subgroup analysis. Numbers and percentages (including negative results) were both reported in our tables and results now. A table (Table 3) for all booster subjects in age-15 group was recorded in our revised paper.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition? No sure.
We verified our process of data analysis by a clear flow chart, clarify the booster list, some quantitative titers of anti-HBs were available, follow up some subjects from senior high school to university stage. We hope we had made our study design, results, and conclusion much clear this time.

5. Are the discussion and conclusions well balanced and adequately supported by the data? Yes, but with the caveat that this does need to be written more clearly. The statement that there is no need for HBV booster doses rests primarily from the strong evidence that the protective efficacy of infant vaccination against HBV persists, with no signs that infections among vaccines that are acquired after childhood become chronic.
Our conclusion was clear this time. We stated that booster vaccination didn’t provide additional protection since the immune memory response was still intact among 92.5% of our booster recipients at 15
years of age and no increase of seropositive rate of HBsAg in “the others” subgroup if compared with subjects from “the same school”. The “the others” group got much lower median titer of anti-HBs (24.7 v.s. 4.2mIU/mL) and seropositive rate of anti-HBs (76.3% v.s. 39.7%) if compared with “the same school” group who got 96% booster rate among their anti-HBs seronegative subjects.

6. Are limitations of the work clearly stated?
Partly, reflections on the same number of people who received a booster dose and any biases or are missing. There is some difficulty in interpreting whether changes in anti-HBs and HBsAg prevalence among people of different ages reflect time since vaccination, other risk factors, or differences in vaccination coverage when they were infants (as individual vaccination records were not obtained).

Figure 2 represented the trend of anti-HBs seropositive rate and titers of anti-HBs by age and year to reflect the change in time. The average vaccine coverage rate of complete HBV vaccination were 88.8% to 97.7% based on the data of birth cohort 1984-2013 from Taiwan National Immunization Information System (NIIS). After July 1991 (birth cohort 1986), all new enrolled first graders of elementary school were mandatory to provide their vaccination cards for check-up and those children with incomplete vaccination records were given catch-up HBV vaccine before enrolment.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
Yes, as far as I can comment.

8. Do the title and abstract accurately convey what had been found? In my opinion the title should be changed. “Booster Vaccination of Adolescents Does Not Prevent Hepatitis B Infection” The title is confusing-it somewhat implies that infections continue to occur despite booster doses, whereas, actually the article reports few new infections and therefore, no need for booster doses.

We changed our title and abstract based on our revised methods, results and conclusion. Our title was changed to “Hepatitis B virus vaccination booster does not provide additional protection in adolescents: A cross-sectional school-based study”. Immunogenicity
response in booster recipients was most solid evidence in our study. Although, we didn’t have perfect comparison group which could label as “non-booster” group, “the others” group represented the subjects with low proportion of booster recipients among their subjects based on their low seropositive rate and anti-HBs titers if compared with those data in 15 years of age.

9. Is the writing acceptable?
   Yes. This MS requires rewriting for clarity in the Methods and Results sections but the English is fine.
   We rewrite our methods and results sections, or even the discussion and conclusion sections based on the new data. Flow chart of our study design was provided in our revised paper too. Our manuscript had been submitted for professional language editing service.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics

**Declaration of competing interests:**
I declare that I have no competing interests
The manuscript "Booster Vaccination of Adolescents Does Not Prevent Hepatitis B Infection: A Cross-sectional School-based Study" presents results of whether we need to booster vaccination in adolescence in order to prevent HBV infection. The study is an interesting topic, but the research results seem not enough to support their key conclusion.

We provided more conclusive evidences (Table 3 and 4, Figure 3) in our revised papers and clarify our study designs with flow chart (Figure 1). Hope you will notice the results seem more solid to support our key conclusion this time.

Title
This result sounds to tell us whether or not we need to booster vaccination, not direct to prove "does not prevent hepatitis B infection"
We changed our title based on the findings of immunogenicity response in booster recipients since the booster list and response rates were very clear. High booster response rate upto 92.5% in booster recipients (subjects with double seronegative anti-HBs and HBsAg) confirmed the immune memory was intact in most subjects 15 years after infant HBV vaccination. Our title was changed to “Hepatitis B virus vaccination booster does not provide additional protection in adolescents: A cross-sectional school-based study”.

Methods
- Line 101-102: There is an obvious different in the type of HB vaccine, vaccination procedure and immunization coverage rates before and after 1992, which would affect the conclusion.
  The reason to exclude the subjects born before Jan 1st 1987 was due to none of vaccination record was reviewed in our database and the universal infant HBV vaccination was conducted after July, 1986 in Taiwan. We hypothesized that all of our subjects received HBV vaccination within about 6 months following the introduction of the new vaccination policy at that time. Based on the statistical records of Taiwan National Immunization Information System (NIIS), the immunization coverage rates of complete HBV vaccination were 88.8–97.7% (birth cohort 1984–2013), and the complete rate of HBV vaccination among elementary school enrollments was above 99% after the 1997 birth cohort. When we did subgroup analysis or longitudinal study, we only follow up the subjects born after recombinant HBV vaccines were provided based on their birth cohort ex birth cohort 1993-1994 in 15 and 18 years of age.

- The data collection methods should be supplement, such as vaccination after birth, type of vaccine, booster vaccination in adolescence and other related data.
  Vaccination type and schedule: Prior to July 1992, infants were given four doses of plasma-derived vaccine at birth, one, two and 12 months of age. After July 1992, three doses of recombinant vaccine were administered at the age of less than one week, one month and six months.
  Booster schedule at adolescence: we only got the booster list of subjects with birth cohort 1992-1997 in age-15 age group. But possible
booster schedule at 12 years group was also noted when we traced subjects who studied in our junior high school and senior high school. We also noted those subjects who previously studied in our junior high school got higher seropositive rate of anti-HBs in senior high school age (27.3% v.s 80.9%, respectively). Booster effects were highly suspected but we didn’t have any evidence to prove these findings.

- Design scheme for “Booster Vaccination of Adolescents Does Not Prevent Hepatitis B Infection Disgine” should be supplement.
  Flow chart of our study design was supplied as Figure 1 in revised paper.

**Results**

- Please make a flow diagram for inclusion and exclusion subjects to reduce word in text
  Flow chart of our study design was provided as Figure 1. The exclusion criteria were as the followings—subjects without records of paired HBV markers (anti-HBs/HBsAg), subjects born before 1987/01/01. In our previous data, no quantitative value of anti-HBs titer was available at that time. We also excluded the subjects with “equivocal” results of anti-HBs. We were able to verify some titer status of subjects whose anti-HBs status were labeled as “equivocal” initially since anti-HBs titers were available from 2007 to 2012.

- Part I: Epidemiological Study: The 95% CI of the rate should be given, not just the point estimation, especial in Table 1.
  95% CI was provided in Table 1, Table 2 and Table 4.

- Line 135-138: Those results were sum of 2004-2012, how these indicators changed with time?
  Trends of seropositive rate of anti-HBs by age and study year were provided in Figure 2.

- Line 153-155: Those students are all born after 1992, they should use the same type of HB vaccine, how to minimize the effects of different types of vaccine? These sample size selection is based on what?
  We only used the subjects born after recombinant HBV vaccine era in longitudinal subgroup analysis (birth cohort 1993-1994) in both age-15
and age-18 age group. The 38 subjects selected in previous edition was based on their birth cohort 1993-1994 and studied both in our senior high school and university. Therefore, we could follow up change of anti-HBs and HBsAg in 3-year interval. In our revised edition, subjects from birth cohort 1993-1994 at both 15 and 18 years of age were selected and labeled as “same school” including 38 subjects studied from senior high school to university (3-year interval), and 7 subjects studied from junior, senior high school and university (6-year interval).

- Line 175-177: Why the students in other school didn’t get booster vaccination?
  We had to apologize for our mis-description in the previous edition. According to the booster policy from Taiwan ACIP (Advisory Committee on Immunization Practices), it was possible for subjects from the other school had received booster dose of HBV vaccine after junior or senior high school enrolment. But we didn’t have accurate booster lists in the subjects in age-12 and age-18 group (except the subjects were traced from birth cohort 1992-1997 at age-15 group).

Discussion
- Line 212-216: HB vaccine booster is major factor, breakthrough infections and natural boosters are only account for <2%. What does this phenomenon suggest?
  The phenomenon that the seropositivity rate of anti-HBs decayed from 63.4% (age 6 years) to 31.6% (age 12 years) and then increased from 12 to 18 years of age was most likely contributed by booster effects. Based on one study conducted in 2004 in Taiwan, breakthrough infection rate among children younger than 18 years was 1% (ref 7 in our paper). Natural infection rate was also low due to low seropositive rate of HBsAg or anti-HBc rate (3.5% in eastern Taiwan, ref 16 in our paper).

- Line 248-251 and 289-293: Although there was no statistical difference in HBV carrier rate between booster group and non-booster group, 10 students among 1256 were HBsAg(+) in the others, and no case among 31 was detected in the same school. The conclusion, “the booster strategy in adolescents does not show any cost-benefit effects toward preventing
hepatitis B infection”, sounds lack of enough evidence. For example, small sample size in booster group, and maybe less chance to contact infection source.

The relatively small sample size in “the same school” group could be one of the factors, affect the conclusion—no statistically difference in seroprevalence rate of HBsAg (carrier rate). In our revised paper, we were able to follow up the immunogenicity response in booster recipients in 15-year age group. 92.5% of booster recipients had seropositive response at 6-week post-booster interval. At the same time, the carrier rate among two groups—“the same school” versus “the others” still showed no statistically significance. We also compare the median anti-HBs titers in age-15 (birth cohort 1993-1994, pre-booster titers) and subjects of “the others” in age-18 group (birth cohort 1993-1994) which showed some low proportion of booster effects were present within “the others” group. Therefore, we could only conclude that the booster of HBV vaccine didn’t provide additional protection in adolescents.

Tables
- Table 1: What does “Total” mean?
  “Total” column means the total numbers of each subgroup. Total numbers of subjects: 6861, total numbers of male subjects: 2870. Please refer to our new Table 1 for further information.

- Tables (Tab 3) and terms are lack of specification, for example: “(+ )HBsAg”, “(+ )anti-HBs” should be “HBsAg(+ )”, “anti-HBs(+)”
  Thanks for suggestions. We will correct them. The contents of former Table 3 were removed due to it was confusing to be presented in this way. We were unable to trace the booster list among the 12-year age group therefore we made a new figure 3 which only showed the change of serological status of anti-HBs among 38 subjects who could be followed up for 3-year interval.
Reviewer’s report

Title: Booster Vaccination of Adolescents Does Not Prevent Hepatitis B Infection: A Cross-sectional School-based Study

Version: 3 Date: 18 June 2014

Reviewer: Noele Nelson

Reviewer’s report:

Major Compulsory Revisions
- Table 2, school year 2008/2009 (15 year old), (+)HBsAg, chi-square is not the appropriate test since at least 20% of the expected frequencies are less than 5. Please clarify if the Yates correction was employed for this analysis. If not, the analysis should be repeated using the appropriate statistics.
  
  Yates correction was employed for this analysis

- In addition to the overall results for Table 1(age), include a subanalysis (chi-square test results) for 6-12 years old and 15-18 years old separately. Update the results section accordingly (line 38-43)
  
  According to your suggestions, we further pooled the two younger and two older age groups (6–12 vs 15–18 years) for comparison in Table 1, and the results showed a similar pattern to the four age group comparison.

Minor Essential Revisions

- Methods, paragraph 2, line 101, it is stated, “Therefore, we excluded 906 individuals born before January 1st, 1987 from our study due to no vaccination record being available to confirm their vaccination status.” Line 277-278, “Although we didn’t have vaccination records for all individuals, we are confident that most of them had completed their vaccination schedule due to the high vaccine coverage rate in Taiwan.” Please clarify how it was determined if an individual was excluded, and what percent of subjects had available vaccination records and how the results might have been effected by lack of vaccination confirmation in some individuals.
  
  The universal HBV infant vaccination was launched in July, 1986 in
Taiwan and the vaccination records was arranged to be checked before elementary enrolment since Sep 1991. If our study population started with birth cohort 1987, they would become enrolled in elementary school in school year 1992. No vaccination record was reviewed in our study population. It’s our weak point. In this way, we hypothesize that most of them had received complete HBV vaccination in infancy or even catch-up vaccine before elementary enrolment due to high HBV vaccine complete rate.

- Methods, paragraph 2, line 101-10, State that recombinant vaccine administration (3 dose series) was not confirmed for all subjects.
  We hypothesize the subjects received different vaccine types based on their birth cohort. For subjects born before July 1992, four doses (5 µg/dose) of plasma-derived vaccine were given at birth, 1, 2, and 12 months of age. For those born after July 1992, three doses of recombinant vaccine were given at the age of less than 1 week, 1 month, and 6 months.

- Methods, paragraph 3, line 116, Clarify what is meant by “unlinked study”
  “Unlinked study” design means that we didn’t have complete Identify Numbers (Personal ID) and full name of the subjects in our study.

- Methods, paragraph 3, line 116-117, “Due to our retrospective study.” should be moved to the paragraph above or clarify that “these study subjects” refers to the pooled subjects who enrolled twice.
  In our revised paper, we decided to follow the subjects of birth cohort 1993–1994 in the 18-year age group who had received the recombinant HBV vaccine in infancy. In this way, the effects of different vaccine type were eliminated. If the subjects previously studied in our senior high school (15-year age group, birth cohort 1993–1994), they were grouped as “the same school”. Their serum data of both anti-HBs and HBsAg were analyzed longitudinally to trace the pattern of seroconversion and post-booster change in this 3-year interval. The other subjects of the 18-year age group from the 1993–1994 birth cohort were used as the comparison group, which was labeled as “the others”.

- Methods, paragraph 3, line 117-125, Add a description of the longitudinal
subgroup analysis.

In the sections of Methods, we had added a description of the longitudinal subgroup analysis from line 149 to 168 in new edition.

- Results, Part II: Longitudinal Subgroup Assessment, line 167, “Taiwan” should be inserted before ACIP to distinguish the Taiwan ACIP recommendations from the United States ACIP recommendations.
  
  Thank you very much for reminding us of this insertion to clarify our statement.

Discretionary Revisions

- Consider breaking the methods into "epidemiological study" and "longitudinal subgroup" for better clarity and consistency with the results.
  
  Methods were divided into several sections such as vaccination program in Taiwan, study population, epidemiological study, subgroup analysis (comparison of HBV markers by different vaccine types, booster effects in subjects double seronegative for hepatitis B markers at 15 years of age, and longitudinal study). We had tried our best to make the results clear and consistent. Flow chart of our study design was also provided as Figure 1.

- Consider adding a sub-title for the sections describing the subjects who entered into study more than once for better clarity.
  
  Thanks for valuable suggestions. We had added subtitles for the sections in Method and Results to clarify our design and findings better.

- Consider mentioning and citing a recent related publication: Middleman AB, Baker CJ, Kozinetz CA, Kamili S, Nguyen C, Hu DJ, Spradling PR.
  
  
  This updated and relevant paper supports our findings in the immunogenicity response in booster recipients, therefore, it was cited as our reference (Ref 23) as well.

- Table 1 and 2: Consider adding the frequency (percent) of anti-HBs (−) and
HBsAg(-) for ease of reader calculation of the Chi-square test.

Frequency (percent) and 95% CI (confidence interval) were both added in our tables including Table 1, 2 and 4.

Minor issues not for publication

- Abstract, Results, line 46-47: change wording to “a marked increase” instead of “markedly increase”, and insert words in this phrase, “among the subjects who received a booster dose”, and “HBV booster contributed to a marked increase in seropositive rates of anti-HBs among subjects who received a booster dose either at 12 years of age or 15 years of age.”
  
  Thanks for this kindly help. We rewrote our abstract according to our current results. This sentence had been removed in our paragraphs.

- Table 2: Remove “or” from caption.
  
  Thanks for reminding. Does “or” refer to “of” instead in the caption of Table 2.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published.

  
  Our revised manuscript had been sent for professional language editing before resubmission.

**Statistical review:** Yes, and I have assessed the statistics in my report

**Declaration of competing interests:**

I declare that I have no competing interests