Reviewer’s report

Title: Effects of long-term low-level radiation exposure after the Chernobyl catastrophe on immunoglobulins in children residing in contaminated areas: cohort and cross-sectional studies

Version: 1
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Reviewer: Suminori Akiba

Reviewer’s report:

The information presented in this manuscript seems important.

In the analyses of data for 1993-98 data and for 2008-2010, authors divided study subjects into five and three groups, respectively, based on soil 137Cs contamination levels, which are largely determined by geography. Since authors did (could) not use individual radiation doses when evaluating the relationship between radiation exposure and serum immunoglobulin levels, this study should be regarded as a kind of an ecological study.

Authors should present the results of the wholebody counting of study subjects even if the data are not available for all of them. (when doing so, authors should present its median and range, at least.)

In the previous paper (Stepanova et al. 2008 Environ Health PMID: 8513393), authors used five groups of residential exposure (<116, 116-, 165-, 266-, 350+ kBq/m2), which appear to be the same dose grouping as the one used in the present study. The individual effective equivalent doses for those exposure categories were 13.6, 11.7, 16.7, 34.9, 19.6 mSv, respectively. Also in the present study, authors should present the average (or median) individual effective equivalent dose for each exposure category of soil Cs-137 contamination (if they are different from the previous study).

The relationship of this study with the one reported by Svendsen et al. (Svendsen ER et al. 137Cesium exposure and spirometry measures in Ukrainian children affected by the Chernobyl nuclear incident. Environ Health Perspect. 2010;118:720-5. PMID: 20100677) is not entirely clear. In the study of Svendsen et al., the soil 137Cs contamination levels were divided into five categories. (The mean radiation levels of five categories were 90.7, 128.2, 195.5, 308.6 and 355.5. kBq/m2. The range of radiation level for each category was not reported.) Then, they investigated the association of soil contamination levels with spirometry measures for 415 children of the contaminated Narodichesky region who were examined from 1993 to 1998. The frequencies of respiratory problems were determined based on repeated measurement data obtained from their longitudinal study. It is desirable to examine the association of immunoglobulin levels with the frequency of respiratory problems, which were identified by Svendsen et al.
Fig 3: IgA showed no clear dose response in any single year during 1993-1998. The significant interactive effect between time and radiation exposure levels on IgA levels appears to stem from the observation that the second highest exposure group showed a time trend different from the other groups. Authors failed to identify the factors involved in this trend over time.

Fig 3: IgM and IgG levels increased evidently during 1993-98. Are there any possibilities that their increases were due to technical problems? Another factor possibly involved in this observation is nutrition since blood counts of RBCs, platelets and WBCs were also increased during this period (as reported by their previous paper: Sepanova et al 2008). Authors should discuss the factors involved in this time trend, including technical problems and nutrition.

Fig 3: In the case of IgM, the secular changes are much larger than the differences among exposure groups if the second highest exposure group (266-310 kBq/km²) is excluded. The IgM levels of this group (the second highest exposure group) were evidently higher than other groups. Authors did not discuss the possibility that the IgM levels in this group may be affected by factors other than radiation. Authors should discuss this problem.

In the previous paper (Stepanova et al. 2008 Environ Health PMID: 8513393), authors reported that the counts of RBCs, platelets and WBCs were lowest in the second highest exposure group (266-310 kBq/km²). Authors did not make any discussions regarding the similarity (the direction was opposite but the pattern was similar) in the results of IgM in the present study and blood cell counts in their previous report.

Fig 3: In the case of IgG, the secular changes are larger than the differences among exposure groups if the highest exposure group is excluded. The IgG levels of this group were evidently lower than other groups. Authors did not discuss the possibility that the IgG levels in this group may be affected by factors other than radiation.

In the present study, the highest and second highest groups of soil 137Cs contamination had immunoglobulin levels different from the other groups. Authors should divide the highest group into several areas, and examine area differences of immunoglobulin levels. Authors should conduct a similar analysis for the second highest group. If area differences are found, authors should present the results, creating a new table.

In page 16, authors wrote as follows: “In addition, we tested whether IgA, IgG, or IgM serum concentration co-varied with antibiotic use in the last 12 months and found no significant or suggestive association (data not shown). Therefore we discarded antibiotic use as a potential confounder.”

Comments: I understand that factors without statistical significance (and factors without suggestive significance) can confound the results. Therefore, I am afraid the decision made by authors was not correct. According to the most famous textbook of modern epidemiology (Modern Epidemiology, 2nd edition, edited by
Rothman and Greenland p256), many epidemiologists think that adjustment for a variable should be made if the estimate of parameter of association is changed by 50% or larger by the adjustment for that variable.

In ll20-22, p13, authors wrote as follows: “For the 2008-2010 data, we additionally adjusted for exposure to environmental tobacco smoke (ETS), active smoking, antibiotic use, exposure to cats and dogs, and coal, coke, wood or gas used as a fuel in stoves for heating and cooking.”

Comments: the criteria for selecting those covariables are not clear.

In the discussion (pp18-19), authors wrote as follows: “There is no clear linear pattern, but various convex-curve-like associations.”

However, Models 1 and 2 in Table 4 (cross-sectional study 2008-2010) apparently showed different shapes of dose-response for association between radiation levels and Ig A. It was U-shaped in Model 1 and linear in Model 2.

IgM levels in the dynamic cohort (N=617) increased during the period 1993-1998. In the longitudinal analysis (N=25) from time 1 (1998-1997) to time 2 (2008-2010), IgM levels decreased. Authors did not make any discussions regarding those observations. Anyway, authors should present average IgA, IgM and IgG levels for all the children (N=650) and those aged 8-17 (n=523) examined during 2008-2010 so that readers can understand the time trend from the 1990s to 2000s.

Authors found that IgE specific against indoor allergens, but not outdoor allergens, were statistically significantly less frequent in children residing in villages with higher soil concentrations. They suspect that 137Cs radiation reduces the population of mite and mold species, reducing the indoor allergen but not outdoor allergen burden. The IgE assay was conducted using two mixtures of allergens: indoor (Dermatophagoides pteronyssinus, Dermatophagoides farina, Aspergillus fumigates, Aspergillus niger, cat and dog dander) and outdoor (seasonal weeds and flowers). It is not entirely clear why they selected those antigens. If they conducted the CAP-RAST tests (or similar tests) to examine various antigens, including food antigens, more insightful information could have obtained.

The amount of radioactive fallout in a particular area is affected by weather and terrain (wind, rain, the pass of wind, etc), and the extent of soil contamination can be affected by the nature of soil, drifted dust deposits, wind and waterfall. Those factors might have also affected the daily lives of residents (before and after the accident) that are related to various determinants of serum immunoglobulin levels. Therefore, it seems difficult to imagine that study subjects were randomly exposed to radiation. --Authors wrote that they conducted a random sampling of study subjects.

In page 5, authors wrote as follows: “Another study linked serum immunoglobulins to internal doses of 137Cs in children accumulated over 14 years after the Chernobyl incident [14].”
Comments: I think this statement is important to understand the backgrounds of the study made by authors. However, since the reference 14 is written in Russian, it is not easy for readers to read it. Authors should describe more details about this paper. In particular, authors should describe how internal doses were measured or estimated. The unit of internal dose should also be presented. If available, authors should state the median and the range of the dose.

Minor points
In the analysis of 1993-98 data, authors divided study subjects into five groups based on soil contamination levels. The means (or medians) of those five categories (<116, 116-, 165-, 266-, 350+ kBq/m²) should be presented. In the analysis of 2008-2010 data, authors divided study subjects into three groups based on soil contamination levels. The means (or medians) of those three categories (<116, 116-164, 165-265 kBq/m²) should also be presented.

The definition of dynamic cohort is not entirely clear. In a broad sense, a cohort is the population followed for a certain period. Therefore, I guess a “dynamic cohort” is an “open population” followed for a certain period. However, if a part of population is not followed at all, that subpopulation is not a cohort. In this study, were the newly-added members followed for a certain period? –Authors might have planned to follow them-up. However, that is irrelevant to the study reported in this manuscript.

The definition of “linear mixed models” is not entirely clear. Is it a mixed effect model? (it appears to be merely a linear model with an interaction term).

Results described in the abstract section are not clear enough.

In Abstract, authors wrote as follows: “Residential soil contamination in 2008 highly correlated with the individual body burden of 137Cs.”

Comments: In the abstract, authors should use a term much more easily understood instead of “the individual body burden”

In Abstract, authors wrote as follows: “Serum IgG and IgM concentrations increased between 1993 and 1998. Children with higher 137Cs soil exposure had lower serum IgG levels, which, however, increased in the small cohort assessed between 1997 and 2008.”

Comments: Isn’t it 1997-2010?

In Abstract, authors wrote as follows: “Children with higher 137Cs soil exposure had lower serum IgG levels, which, however, increased in the small cohort assessed between 1997 and 2008.”

Comments: I guess that “between 1997 and 2008” is “between 1997 and 2010”. (or, I may be confused)

Line 4, page 5: The meaning of “incident” is not clear.

The bottom line, page 5: the meaning of “suppressed-increased” is not entirely
In page 6, authors wrote as follows: “During the first 1.5 months after the Chernobyl accident IgM serum levels in children 1-14 years 16 residing in Braginsky region of Belarus with density of soil contamination 137Cs 3Ci/km² (111 kBq/m²), seemed to be slightly increased [4, 9].”

Comments: I am afraid that there are typographical errors in the underlined part of this sentence.

In page 6, authors wrote as follows:

“Hence, it seems that long term exposure to ionizing radiation after the incident results in higher IgE levels in children residing in contaminated areas.”

Comments: In the introduction, authors did not make any discussion regarding potential confounders and biases. Therefore, the arguments made by authors do not seem convincing. BTW, the meaning of “incident” is not clear, either.

In page 15, authors wrote as follows:

“In addition, using individual measurements, after adjusting for age, gender, weight, and month of measurement, we determined whether the residuals of the individual radiation levels (not explained by residential exposure) correlated with the area soil contamination level.”

Comments-1: Authors should clearly state the meaning of individual measurements. (Are they the results of wholebody counting?) It is necessary to present the unit of radiation levels.

Comments-2: Authors should explain how the adjustment was made. --In the statistical model, individual measurements were log-transformed? Age and weight, month of measurements were categorized? If so, were they used as categorical variables or continuous variables? Were any interaction term (for example, gender*age) included in the model?

In page 15, authors wrote as follows: “In the dynamic cohort, 617 children had repeated measurements between 1993-1998, providing 2,407 measurements of IgA, IgG and IgM. The analyses focused on soil contamination (quintiles) and adjusted for gender and age of the child, year of the exam, and the interaction between year and soil contamination.”

Comments: The statistical model used by authors is unclear.

In page 15, authors wrote as follows: “For IgA, the main effect of soil radiation and its interaction with the year of the exam were statistically significant (p=0.039, Table 2).”

Comments: This sentence is not clear. Is the P value of 0.039 for the main effect or for the interaction term? – I guess that it is for the interaction between the main effects of soil radiation and the year of exam.

In page 16, authors wrote as follows: “In addition, active smoking was significantly higher in older children residing in areas with the lowest 137Cs soil...”
contamination.”

Comments: Authors should present a p value for the observed association

In p17, authors wrote as follows: “Controlling for age, gender, dog and cat exposure, IgE responses to indoor allergens were statistically significantly lower in children residing in the 116-265 kBq/m2 area (p=0.013, Table 20 5). There was no statistically significant difference for IgE specific to outdoor allergens.”

Comments: the statistical analysis used for calculating the p value of 0.013 is not clear. If authors conducted a t-test or a similar test, authors should describe whether the p value was adjusted for multiple comparisons or not.

P values of 0.0067 for 137Cs in soil in Table 4: The method used for this p value is not entirely clear. Since the footnote says “t-test in linear regression models”, it is considered to be a sort of a p for trend. However, I am not sure whether such a small p value for trend can be obtained from such a U-shaped dose response.

In the footnote of Table 4, authors wrote as follows: “Multivariable generalized linear model adjusted for gender, age and ETS.”

Comments: It is not clear whether this description is for model 1 or model 2 or both. This footnote seems to be at variance with the description in li20-22 in p13.

Authors should present a figure.

“overtime” should be “over time”.