

## **Reviewer's report**

**Title:** Mobile and cordless phones, serum transthyretin and the blood-cerebrospinal fluid barrier: a cross-sectional study

**Version:** 1 **Date:** 28 February 2009

**Reviewer:** Michael Kundi

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The article addresses interesting questions: (1) whether blood concentration of transthyretin (TTR) as a marker of the integrity of the blood-CSF barrier (BCSFB) is suitable for epidemiological investigations; (2) whether "chronic" and/or "acute" mobile phone use is associated with serum concentrations of TTR.

In the last years it has been recommended to explore the feasibility of early markers of developing diseases for epidemiological studies of environmental factors instead of waiting until illness or death has occurred in a significant proportion of the exposed population (e.g. Carbone M, Klein G, Gruber J, Wong M. 2004. Modern criteria to establish human cancer etiology. *Cancer Res* 64:5518–5524). In line with this recommendation, authors have analyzed serum levels of S100B as a potential marker of BBB integrity in a previous paper and present a similar analysis for TTR in the present manuscript.

From a theoretical point of view, using TTR for the investigation of a potential effect of microwave exposure from mobile phone use seems not too promising. First, TTR is predominantly produced in the liver and, therefore, serum concentrations are mainly from this source. TTR from production in the choroid plexus and reaching the blood stream via CSF is estimated to contribute to the variance of serum TTR at a level of less than 10%. Therefore, only rather strong effects on BCSFB could be detected. Second, TTR varies with several factors most notably with nutritional status. Malnutrition results in a reduction of blood levels of TTR by 50% and more. But also within normal ranges significant correlation with BMI have been established. This aspect must be considered because intensity of mobile phone use and BMI might also be correlated. Furthermore, TTR varies with sex and age. Therefore, these variables need to be considered as potential confounders in the analyses, which has been done by the authors.

Major compulsory revisions:

There is a fundamental problem of statistical analysis of the different types of wireless phone use (NMT, GSM, UMTS, DECT). The reason is the consecutive marketing of these types (NMT->GSM->UMTS). The data show that almost all individuals that used a NMT later switched to GSM and/or UMTS and those using an UMTS phone previously used another type. The majority of DECT users were also mobile phone users. This overlap makes separate analyses of these types subject to confounding and, therefore, the results on UMTS are likely artifacts and do not indicate a specific difference to other phone types. There are several

possible solutions to this problem. I suggest the following procedure. Because the main interest is the overall wireless phone use the main independent variables should be total hours of wireless phone use and total years of wireless phone use. Further variables for a multivariate analysis should be the fraction of that duration that the subject used a NMT, GSM, UMTS and DECT phone. In order to avoid multicollinearity one of these variables must be omitted. Because GSM was likely the most frequent it is the best candidate for omission. The fraction for NMT, UMTS and DECT could be arcsine transformed ( $\arcsin(\sqrt{\text{fraction}})$ ) if residuals turn out to deviate from normality. Hence the result would be an effect estimate for cumulative use and effect modifiers for NMT, UMTS and DECT use depending on their fraction of use.

Results of linear regression seem to be presented as unstandardized coefficients which makes no sense without reporting all other coefficients and the regression constant. Therefore, present results as standardized coefficients. This must be stated in the table or column header.

Minor compulsory revisions:

Abstract: Omit reference to the reagents and analyzer used. It suffices to say that TTR has been determined by standard immunonephelometric techniques. Note that the correct analyzer name is BN ProSpec. Add ® after ProSpec because it is a registered name and state the manufacturer (Siemens Healthcare Diagnostics).

Abstract: State that logistic regression of dichotomized TTR serum levels with a cutpoint of 0.31 g/l on wireless phone use yielded increased odds-ratios that were statistically not significant.

Abstract: Report of linear regression results is too technical. There is no need to speak of  $\beta$  coefficients. Report that results of linear regression of log TTR concentration on cumulative duration of use overall and on the day of blood withdrawal gave different results for males and females. The term 'latency' in this context is inappropriate (this should be changed throughout the manuscript and especially in the methods section) because there is no disease that is studied for which a latency period can be defined. Either use 'years since first use' or 'years of use' as appropriate.

Page 6: Replace 'Chemical analysis' with 'Analytical methods' because measurement is not done by a chemical but a physical method. Change the manufacturer name because as much as I know BN ProSpec is manufactured by Siemens and not Dade Behring (although it is also sold by Dade Behring).

Page 6, 'Statistical methods': I have found no case where the Kruskal-Wallis test has been applied. Therefore, omit this test.

Page 6, 'Statistical methods': For logistic regression analyses state clearly the cutpoint used to define the exposed (this should also be mentioned in the footnote to table 3).

Page 7, 'Descriptive analysis': For comparison of age a Chi-Square is reported. Change to Wilcoxon rank-sum.

Table 2: Change  $\mu\text{g/l}$  to g/l. Instead of 'median' state 'cutpoint at median'.

Footnotes for tables 3 to 6: Clarify that age has been adjusted for in all analyses, while adjustment for sex was applied only in analyses of total samples. In tables 3 and 5 include the unit g/l after the cut-off of 0.31 g/l.

**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I have no competing interests in relation to this paper