Author’s response to reviews

Title: Preventing misdiagnosis of diabetes in the elderly: Age-dependent HbA1c reference intervals derived from two population-based study cohorts

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Point-by-point response to editorial and reviewer comments

Technical Comments:

Consent for publication - Seeing as this is not applicable to your manuscript please state “Not Applicable” in this section.

Answer: This statement was already included in the originally submitted manuscript in the section Declarations on page 12. In case that we should place this statement elsewhere, please give us a short information on where exactly this should be written.

Reviewer reports:

Baqiyyah Conway (Reviewer 1): This was a very enlightening article on the age-dependency of HbA1C, with important clinical implications. I have only a few minor comments and some concerns that need clarification.

Answer: We are grateful for these very kind words and will try to address all issues raised.
1. The Background section was quite informative, but missing was whether the RBC lifespan changes with age. While the authors provide some information about change in RBC lifespan with age, this needs to be included in the introduction as well as it is important to the rationale of why HbA1c changes with age should be considered (otherwise the So What? is missing—people may simply have a deterioration of metabolic glycemic homeostasis with age).

Answer: We thank you, Dr. Conway, for this advice and included this notion also in the introduction section on page 4 line 78: “In line with this notion, also RBC lifespan appears to be affected by several aging-associated changes, e.g. alterations of the hematopoietic system [23], compromising either RBC production or clearance ultimately influencing HbA1c measures.”

2. In the Methods section, please provide more information about the SHIP and SHIP-Trend cohort studies. What were/are the purposes of those cohort studies.

Answer: Thank you very much for the opportunity to extend the description of the study populations and the SHIP project in general. We included the following description within the methods section on page 4 line 94: “SHIP (Study of Health in Pomerania) was designed to assess prevalence and incidence of common risk factors, subclinical disorders and clinical diseases; and, in addition, to investigate the complex associations among risk factors, subclinical disorders and clinical diseases. SHIP does not specifically address one selected disease but attempts to describe health-related conditions covering a wide focus to address the issue of overall less life-expectancy in this region in Germany [26]. The first cohort (SHIP-0) is based on representative samples of the population aged 20-79 years living in West Pomerania, a rural region in northeast Germany. The sampling was based on official data from population registries in the Federal State of Mecklenburg/West Pomerania. The baseline examinations in the SHIP cohort were performed between October 1997 and May 2001. Baseline examinations of the second independent cohort SHIP-Trend were conducted between September 2008 and September 2012. Participation in SHIP was exclusion criterion for SHIP-Trend. A second stratified (age, sex and city/county of residence) random sample of adults aged 20-79 years was drawn from population registries covering essentially the same area as SHIP-0 with only minor deviations. The rationale to perform a second study within the same region was to analyze the secular trend of subclinical and overt diseases and their determinants in a high-risk population and also to assess the prevalence of subclinical findings defined by highly innovative non-invasive methods (only within SHIP-Trend). The study design and sampling methods have been previously described in detail [26]. In the baseline examinations of the SHIP cohort, 4308 men and women from a representative population sample of 7008 subjects were examined. An additional 4420 men and women from a representative sample of 8016 adults participated in the baseline examinations of the independent SHIP-Trend cohort.”

3. Why were there different exclusion criteria for the SHIP and SHIP-Trend (Figure 1)?

Answer: Thank you very much for this question. Actually, the exclusion criteria as presented in the figure 1 were applied to both populations which is also stated in the methods section. However, the figure is obviously not designed unambiguously. Therefore, we changed figure 1 making it more intuitively, we hope, also in order to comply with the requirements as asked in the following questions.

4. First paragraph of the Discussion section and Table 1: related to the previous question, it is not clear whether obese subjects were included in the current study. The methods section says they were excluded, Figure 1 says they were excluded from the SHIP-0 study, but Table 1 indicates they were included in both cohorts where approximately 25% of the subjects were obese. Please make this much
Answer: We agree, that this issue is not explained clearly enough throughout the manuscript. Indeed, all statistical model were applied in the first instance on the whole populations (SHIP-0: 2109 men, 2154 women; SHIP-Trend: 2140 men, 2262 women) without exclusion of subjects with obesity, selected medication or diseases. These results are given in Fig. 2 as white boxplots and in Fig. 3A as solid lines. In the next step, to account for potential influences of obesity, selected medication or diseases as mentioned in Fig. 1, all calculations were also performed on the healthy subpopulations ((SHIP-0: 382 men, 572 women; SHIP-Trend: 499 men, 535 women) presented as grey boxplots in Fig. 2 and as dashed lines in Fig. 3A. Results were basically not changed when only the healthy subpopulation was investigated. This procedure is only mentioned in a sentence in the methods section in Statistical analyses on page 7 line 150: “All models were performed in the whole study populations as well as in subpopulations of healthy subjects (see figure 1).”

Therefore, we additionally changed the following expressions throughout the methods section to clarify this issue and hope to alleviate your concerns:

Page 5 line 115: “For the present analyses all pregnant women and subjects without HbA1c measurement were excluded resulting in 4263 SHIP-0 and 4402 SHIP-Trend participants.” We add here: “These populations formed the basis for our analyses.”

Page 5 line 122: we add: “The healthy subpopulations were used for confirmatory reasons to exclude influences of obesity, selected medication or diseases as outlined in Fig. 1 on the observed associations.”

We add in the caption of figure 1: “All statistical models were applied to the total population as well as the healthy subpopulation. HbA1c reference values were derived from the combined healthy subpopulations.”

In the results section, the differentiation is made already between total and healthy sub-/populations. We believe, that the change of figure 1 and expressions in the methods section makes the results section more intuitively.

5. Page 11, lines 254-255: the authors imply that HbA1C is the only biomarker being used for the diagnosis of diabetes. This is not the case.

Answer: We absolutely agree, that HbA1c is not the only biomarker to diagnose diabetes and we did not intend to claim so. Therefore, we changed the sentence as follows: “In addition, in regard of the numerous factors that may affect HbA1c levels independent of glycemia, it becomes increasingly clear that special care needs to be taken for HbA1c as a biomarker for the diagnosis of diabetes.” (page 11, line 270ff)

6. Page 11, line 266: insert "age-related" before "increase" where it says "...the increase in HbA1C..."

Answer: Thank you, we made the respective amendment.

7. Figure 2. This figure needs to be better labeled. There appears to be four panels, not two. So labeling the panes "a" "b" "c" "d" might be more appropriate than simply "A" "B". Also, the figure legend for this figure should also clearly specify all four figures, otherwise the reader is left to guess that the two top figures belong together and the two bottom figures belong together.

Answer: Thank you very much for this advice. We changed the figure and caption according to your suggestions.