Author’s response to reviews

Title: The association between urinary kidney injury molecule 1 and urinary cadmium in elderly during long-term, low-dose cadmium exposure: a pilot study

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Author’s response to reviews: see over
Concerning revision manuscript Environmental Health

Dear reviewer,

Please find attached the point-by-point response to the concerns on our manuscript entitled “The association between urinary kidney injury molecule 1 and urinary cadmium in elderly during long-term, low-dose cadmium exposure: a pilot study”. We adapted the manuscript to the best of our knowledge and hope it now meets your expectations.

Kind regards,

Valérie Pennemans
Reviewer 1:

The manuscript “The association between urinary kidney injury molecule 1 and urinary cadmium in elderly during long-term, low-dose cadmium exposure” by Pennemans et al. highlights the diagnostic potential of KIM-1 in subjects exposed to a nephrotoxic metallic element (cadmium), owing to its correlation with urinary cadmium concentration in an elderly population after long-term, low dose exposure to cadmium in an area allegedly polluted by this metallic element. The comparison among markers is only possible if ALL markers are measured relying on the most sensitive and reliable technique, which is not the case of this study, using a very sensitive sandwich ELISA for KIM-1 and much less sensitive methods for other renal markers.

The purpose of this exploratory study was to investigate a possible association between long term low dose cadmium intoxication and urinary KIM-1 levels (cfr title). We did not intend to compare different biomarker measurements, but we assessed other renal markers in order to draw well-documented conclusions. We agree with the reviewer that the technique to measure b2-microglobulin was less sensitive and therefore all discussions concerning this marker were removed upon receiving the same comments after a previous submission.

Owing to its cross-sectional design, the present study has limited value to draw any statistical inference and the novel finding (very high correlation between urinary Cd and KIM-1 in the absence of any significant correlation with traditional markers of cadmium nephropathy) may be due to:

1. High cysteine content of KIM-1, suggesting a possible reverse correlation (high Cd binding capacity of excreted KIM-1) not necessarily due to nephrotoxicity; The possible role for high cysteine content is added to the discussion section.

2. Major methodological issues in the measurement of urinary markers: procedures to avoid the possible degradation of b2-microglobulin in acidic urine samples are missing (details should be given on urine collection, buffering and storage prior to analysis); In the current version of the manuscript, all analyses and discussions on the b2-microglobulin measurements are removed from the manuscript since the techniques used to measure b2-microglobulin were not sensitive enough and most of the samples were below the detection limit. However, samples were buffered to a neutral pH (which is also necessary for the KIM-1 measurements) and details on this are provided in the methods section.

3. the statement “of the 140 urine samples that were tested for #2M-U, 118 were below the limit of detection of 0.206 mg/l” is not acceptable, because it suggests the inadequacy of the methodology to address the problem at hand. Methods to reliably measure for #2M-U in a physiological range have been developed in the late seventies – early eighties This statement has already been removed in the current version of the manuscript due to earlier revision, so this comment is no longer relevant.

The statement “as expected, the #2M-U levels exceeded the detection limit of 0.206 mg/l of the automated assay we used only in a few urine samples” in not
acceptable. It is well known that the vast majority of reference values fall below the detection limit of the “automated assay” used by the authors, clearly unsuitable for the purpose of measuring this marker in the general population. This statement has already been removed in the current version of the manuscript due to earlier revision, so this comment is no longer relevant.

Nor is it acceptable the inference “the #2M-U assay does not detect any cell stress yet in case of low-level Cd intoxication and is therefore less sensitive than KIM-1.”: whereas cadmium exposure cannot be avoided (Cd is an element of the earth crust), defining “low-level Cd intoxication” exposures leading to Cd-U below 1 mg/g creatinine is a challenging concept deserving attention, but requiring support by a more consistent body of evidence. This statement has already been removed in the current version of the manuscript due to earlier revision, so this comment is no longer relevant.

Minor issues
Please note that expressing urinary concentrations as a function of creatinine is neither a correction nor an adjustment (which would occur if the unit were the same, e.g. g/l, normalizing the numerical values to a fixed density, e.g. 1020), but a change of unit (the denominator being no longer the liter, but the gram of creatinine). I suggest to reword “correction, adjustment, etc.” throughout the text: “as a function of creatinine” or “normalized to the g of creatinine” are preferable expressions. The expressions ‘creatinine correction’ and ‘creatinine adjustment’ have already been changed to ‘as a function of creatinine’ due to earlier revision, so this comment is no longer relevant to the current version of the manuscript.

Additional points need to be clarified:
1. Urinary BUN (or urinary creatinine) is not a used and reliable biomarker for renal injury. Please, explain why it was used. BUN (blood urea nitrogen) is no urinary renal marker but a serum renal marker, which has been used to assess kidney function in previous studies (e.g. Vaidya et al. 2010, Nature biotechnology). Although this marker does encounter shortcomings concerning specificity, we choose to use this biomarker because its measurement was concluded in the routine analyses.
2. How was selected the sample size? The sample size was selected based on previous studies, that proved that -for a visibility study- a sample size of 150-200 provides enough power (Staessen et al. 2001, Lancet)
3. How were selected the participating individuals? Consecutively? They should be selected at random in the houses close to the pollution source. Please, explain. The participating individuals were selected from a representative patient databank of a general practice in Genk. Although patients were consecutively selected after meeting the inclusion criteria, a participation rate of 99% was reached.
4. The results were not controlled for time living in the area and distance to the sources of pollution. Please, explain. The purpose of our study was to investigate a possible association between long term low dose cadmium intoxication and KIM-1. The source of the pollution was of lesser importance. However we looked at a possible correlation between the time living in the area and the distance to the sources of pollution on the one hand and urinary cadmium and KIM-1 on the other hand, but no significant correlation was observed.
5. How be sure that this level of KIM1 is not associated with aging? The study must have a paired control group living in an area free from cadmium contamination. The mean urinary cadmium concentration in our study is 0.76µg/g creat, with a range of 0.05µg/g creat to 5.03µg/g creat. We therefore believe there is enough contrast among the population of the currently used area. Nevertheless, since aging was indeed a determinant for both urinary KIM-1 and cadmium concentrations, reported associations between those compounds were statistically corrected for age and other possible determinants. Details on the determinants are reported in the manuscript.

Reviewer 2:
Urine samples were collected from 153 non-smoking men and women aged 60 or more living in an area with moderate cadmium pollution from a metal plant. Urinary cadmium and KIM-1 as well as alpha1-microglobulin, beta2-microglobulin, blood urea nitrogen, urinary proteins and microalbumin were measured. KIM-1 levels were correlated with urinary cadmium concentration. No significant association was found among any of the other studied renal biomarkers with urinary cadmium. The authors conclude that “…urinary KIM-1 might be considered as a biomarker for early-stage metal induced kidney injury by cadmium.”

1. 153 of 154 individuals asked to participate agreed to do so. This is an important feature of the study.
We thank the reviewer for this comment; this is indeed a strength of our study.

2. Urinary Kim-1 correlated positively with urinary cadmium levels below 1 µg cadmium per gram creatinine, lower than it has been possible to see biochemical changes with cadmium using other approaches.

3. The authors should be more clear about why they plot the data the way they do, plotting the residuals log of Kim-1 or the other biomarkers vs the residuals of cadmium. By use of residuals we removed potential confounding by factors as age, sex, … . This is an accepted method, which is used in other studies (Kuznetsova et al. 2010, American Journal of Epidemiology). Nevertheless we attach to this letter the plots of the crude associations between cadmium and the different biomarkers (see below).