Plasma Lead and Risk of Late Kidney Allograft Failure: Findings From the TransplantLines Biobank and Cohort Studies

**Major Finding**

**CONCLUSION:** Plasma lead is independently associated with increased risk of late kidney graft failure.

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<td>With a functioning graft ≥1 year</td>
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Plasma Lead Concentration and Risk of Late Kidney Allograft Failure: Findings From the TransplantLines Biobank and Cohort Studies

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ABSTRACT

Rationale & Objective: Heavy metals are known to induce kidney damage and recent studies have linked minor exposures to cadmium and arsenic with increased risk of kidney allograft failure, yet the potential association of lead (Pb) with late graft failure in kidney transplant recipients (KTR) remains unknown.

Study Design: Prospective cohort study in the Netherlands.

Setting & Participants: We studied outpatient KTR (n=670) with a functioning graft for ≥1 year recruited at a university setting (2008–2011, NCT02811835) and followed, on average, for 4.9 (IQR, 3.4–5.5) years. Additionally, end-stage kidney disease patients (n=46) enrolled in the ongoing TransplantLines Cohort and Biobank Study (2016–2017, NCT03272841) were studied at admission for transplantation and at 3, 6, 12, and 24 months after transplantation.

Exposure: Plasma Pb was log₂ transformed to estimate the association with outcomes per doubling of plasma Pb concentration and also considered categorically as tertiles of the Pb distribution.

Outcome: Kidney graft failure (restart of dialysis or re-transplantation) with the competing event of death with a functioning graft.

Analytical Approach: Multivariable-adjusted cause-specific hazards models where follow-up of KTR who died with a functioning graft was censored.

Results: Median baseline plasma Pb was 0.31 (IQR, 0.22–0.45) µg/L among all KTRs. During follow-up, 78 (12%) KTR developed graft failure. Higher plasma Pb was associated with increased risk of graft failure (HR 1.59, 95% CI 1.14–2.21 per doubling; P=0.006) independent of age, sex, transplant characteristics, eGFR, proteinuria, smoking status, alcohol intake, and
plasma concentrations of cadmium and arsenic. These findings remained materially unchanged after additional adjustment for dietary intake and were consistent with those of analyses examining Pb categorically. In serial measurements, plasma Pb was significantly higher at admission for transplantation than at 3-months post-transplant ($P=0.001$), after which it remained stable over 2 years of follow-up ($P=0.2$).

**Limitations:** Observational study design.

**Conclusions:** Pretransplant plasma Pb concentrations, which fall after transplantation, are associated with increased risk of late kidney allograft failure. These findings warrant further studies to evaluate whether preventive or therapeutic interventions to decrease plasma Pb may represent novel risk-management strategies to decrease the rate of kidney allograft failure.

**Index words:** Lead; oxidative stress; nephrotoxicity; kidney transplant recipient; late graft failure.

**Plain language summary**

Heavy metals are known to induce kidney damage and transplanted kidneys may be particularly susceptible. Recent evidence showed that plasma concentrations of the heavy metals cadmium (Cd) and arsenic (As) are associated with increased the risk of kidney graft failure. It is unknown if this association is also true for plasma lead (Pb) concentrations. We measured plasma Pb in 670 kidney transplant recipients with a functioning graft for ≥1 year, who were followed for approximately 5-years at our outpatient clinic (Groningen, the Netherlands). Plasma Pb concentrations were independently associated with an increased risk of late kidney graft failure, suggesting that Pb-targeted interventions could be examined in future research as novel strategies to decrease the burden of kidney allograft failure.
INTRODUCTION

Chronic kidney disease (CKD) is a major global public health concern and kidney transplantation is the gold standard treatment for end-stage kidney disease. Extensive research over the last decades has made it possible to significantly improve 1-year graft survival rates, while long-term graft survival continues to lag behind.\(^1\) The need for improving kidney allograft survival is demonstrated by the fact that late graft failure is an increasingly important indication for dialysis or re-transplantation.\(^2\) In the past few decades, the number of patients returning to dialysis after graft failure has increased,\(^3\) and graft failure is one of the most frequent indications to start dialysis treatment in the United States.\(^4\)

Graft failure is multifactorial, and can be caused by both immune and non-immune mechanisms against a background of various donor and recipient risk factors.\(^5\) There is great need for identifying potentially modifiable, yet otherwise overlooked, risk factors. Heavy metal exposure may be such a risk factor, since it is an established cause of kidney damage in native kidneys.\(^6\) In recent studies, we have shown that plasma cadmium (Cd) and arsenic (As) levels are each associated with increased risk of graft failure in kidney transplant recipients (KTR).\(^7,8\) Another toxic heavy metal, lead (Pb), can be found in construction sites, paint, children’s jewelry, folk remedies, glazed pottery, and even candy.\(^9\) While occupational exposure is especially relevant in developing countries,\(^10\) in developed countries, such as The Netherlands, significant amounts of Pb can be found in topsoil from construction works, disposal of coal ashes and fertilization of land with city waste, from where it can end up in food.\(^11\) Cereals, milk, fruits, vegetables and non-alcoholic beverages (including tea and fruit juices) have been shown to contribute the most to total Pb intake from food.\(^11\) Cigarette smoking, alcoholic beverages, and urban drinking water have also been identified as important sources.\(^12–14\) Interestingly, it has
been suggested that, even though the calculated intake of Pb in the Dutch population lies below the European Food Safety Authority’s proposed limits of exposure for augmented risk of developing systemic diseases, detrimental health effects cannot be excluded. In fact, the Dutch Health Council recently identified Pb-containing water service pipes as a relevant source of over-exposure to Pb, and recommended avoidance in vulnerable groups such as pregnant women, infants and young children.

In adults, among the organs most affected by Pb burden are the kidneys. Chronic exposure results in glomerular dysfunction and chronic tubulo-interstitial nephritis, ultimately leading to fibrosis. Oxidative stress has been suggested to be the main mechanism underlying Pb-associated toxicity. Pb inactivates functional thiol (SH) groups in antioxidant enzymes and molecules, which can also enhance the toxicity of other metals, leading to lipid peroxidation and loss of membrane integrity in kidney cells.

Minor exposures to Pb can have nephrotoxic effects, especially in patients with hypertension, diabetes, or existing CKD. KTR are especially susceptible to oxidative agents due to chronic exposure to oxidative challenges, including a large burden of the aforementioned concomitant conditions, but also due to maintenance immunosuppressive therapy and decreased kidney function. We hypothesize that Pb exposure represents an as yet overlooked risk for decreased long-term graft function, thereby representing a potentially modifiable risk factor, open for clinical monitoring and non-toxic therapeutic interventions.

In the current study we determined plasma Pb concentrations in a large cohort of KTR from the TransplantLines Food and Nutrition Biobank and Cohort Study and investigated the association with late kidney graft failure. We additionally studied changes in plasma Pb
concentration prior to and after transplantation in patients from the ongoing TransplantLines Biobank and Cohort Study.\textsuperscript{24}

**METHODS**

**Study Population**

Between November 2008 and March 2011, all adult KTR with a functioning allograft for \( \geq 1 \) year, visiting the outpatient clinic of the University Medical Center Groningen (UMCG, the Netherlands) were invited to participate in the TransplantLines Food and Nutrition Biobank and Cohort Study (NCT02811835), as described previously.\textsuperscript{7} A total of 707 of 817 (87\%) eligible KTR signed informed consent. Pancreas transplant patients \((n=1)\) and patients missing plasma Pb measurements \((n=36)\) were excluded from the current analyses, resulting in 670 KTR (Figure S1) at a median of 5.4 (IQR, 1.9–11.8) years post-transplantation. The study protocol has been approved by the institutional review board (METc 2008/186) and was conducted in accordance with the Declaration of Helsinki.

To investigate intra-individual variability of plasma Pb levels at pre-transplant and over time post-transplantation, we requested follow-up plasma samples (at admission for transplantation, and at 3-, 6-, 12-, and 24-months post-kidney transplantation) from 46 KTR consecutively enrolled between February 2016 and May 2017 in the ongoing TransplantLines Prospective Cohort and Biobank Study, NCT03272841 (Figure S2).\textsuperscript{24} To additionally investigate whole blood Pb compared to plasma Pb, we also collected both plasma and whole blood samples of 122 KTR (Figure S3) at a median of 4.9 (IQR 1.4–10.9) years post-transplantation \((i.e.,\) with a comparable transplant vintage to baseline measurement of plasma Pb in the 670 KTR of the main patient cohort of the current study).

**Data Collection and Definitions**
All patients received transplants at the UMCG and were treated with standard immunosuppressive therapy (described in Supplemental Materials, Item S1), as detailed elsewhere.\textsuperscript{25} Medical and transplantation history as well as medication use were extracted from electronic patient records. Patients were asked to collect a 24-hour urine specimen during the day before their outpatient clinic. Blood was drawn the morning after completion of the 24-hour urine collection. The measurement of clinical and laboratory parameters has been previously described.\textsuperscript{7} To investigate whether dietary exposure is associated with plasma Pb levels,\textsuperscript{11} dietary intake was assessed using a validated semi-quantitative food frequency questionnaire (FFQ) developed and updated at Wageningen University.\textsuperscript{26} The questionnaire consisted of 177 food items to record intake during the last month, while taking seasonal variations into account. For each item, the frequency was expressed in times per day, week, or month. The number of servings was recorded in natural units (\textit{e.g.}, slice of bread or apple) or household measures (\textit{e.g.}, cup or spoon). The FFQ was self-administered and then checked by a trained researcher on the day of visit to the outpatient clinic. Inconsistent answers were verified with the patients. The results of the FFQ were converted into total energy and nutrient intake per day by using the Dutch Food Composition Table of 2006. Information on alcohol consumption and smoking behavior was obtained by questionnaires.\textsuperscript{26} History of diabetes was defined as the use of antidiabetic medication or a fasting blood glucose $\geq 7.0$ mmol/L. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.\textsuperscript{27}

**Pb, Cd and As Analyses**

Whole blood and plasma Pb concentrations were determined with use of an inductively coupled plasma mass spectrometer (ICP-MS, Varian 820-MS; Varian, Palo Alto, USA) with a validated
method for the measurement of heavy metals, as previously reported.\textsuperscript{7,8} Standards were made by addition to blank blood or plasma of known amounts of Pb to obtain added concentrations of: 2.5; 5; 10; 15; 20 and 25 $\mu$g/L. Control samples were made by spiking blank blood or plasma with known amounts of Pb to obtain added concentrations of respectively: 7.5 (low); 25.0 (medium) and 45.0 $\mu$g/L (high). Sample preparation consisted of diluting 100 $\mu$L sample with 1.0 mL dilution reagent. The dilution reagent contained 0.005\% Triton X100, 0.005\% EDTA and 0.1 mg/L Yttrium as internal standard. Characteristics of this method are summarized in Table S1. Plasma Cd and As were determined as detailed previously.\textsuperscript{7,8}

**Clinical End-Points**

The primary end-point of this study was graft failure, defined as the requirement of dialysis or re-transplantation, in adherence with current recommendations and state of the art in the field.\textsuperscript{28} Death with a functioning graft ($n=112$) was a competing event. The surveillance system of the outpatient program at our university hospital ensures updated information on patient status and events of graft failure as assessed by a nephrologist. Within this system, patients visit the outpatient clinic with decreasing frequency, in accordance with the guidelines of the American Society of Transplantation. End-points were recorded until September, 2015. General practitioners or referring nephrologists were contacted in case the status of a patient was unknown. No patients were lost to follow-up.

**Statistical Analyses**

Data analyses were performed by using SPSS 27.0 for Windows (IBM, Chicago, Illinois, USA) and R version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria). Baseline characteristics of study subjects were described by subgroups of patients according to tertiles of plasma Pb distribution. Normally distributed variables are described as mean (standard deviation,
SD), and skewed variables as median (interquartile range, IQR). Categorical variables are expressed as $n$ (number) with percentage (%). Differences were studied using the chi-squared test or Fisher's exact test for categorical variables and using linear regression analyses for continuous variables. Residuals of linear regression were checked. Variables were log$_2$-transformed when appropriate. A two-sided $P$ value <0.05 was considered significant.

Box plots were used to illustrate medians (IQR) of plasma Pb levels at admission for transplantation and at post-transplant follow-up visits. Significance of potential difference between plasma Pb at admission for transplantation and 3-months post-transplant was tested using the Wilcoxon matched-pairs signed rank test, and significance of potential change during post-kidney transplant follow-up visits was tested using the one-way repeated measure Anova test. To investigate post-transplant intra-individual variability of log$_2$-transformed plasma Pb concentrations, we calculated the intra-individual coefficient of variation (CV) for post-kidney transplant follow-up plasma Pb levels using the formula $CV=(SD/mean) \times 100$, in which SD is the standard deviation and mean is the mean value of log$_2$-transformed plasma Pb concentrations. The associations between plasma Pb and plasma cadmium and plasma arsenic were studied by means of linear regression analyses. Residuals were checked for normality and log$_2$-transformed when appropriate.

**Prospective analyses**

In prospective analyses of the primary end-point graft failure, the association of baseline Pb concentration (which was assessed from samples taken at a median of 5.4 (IQR, 1.9–11.8) years post-transplantation) with risk of graft failure was examined incorporating time to event by means of cause-specific hazards models. For these analyses, the competing risk of death with a functioning graft was accounted by censoring at time of death. Schoenfeld residuals were
calculated to assess whether proportionality assumptions were satisfied. The association of Pb with risk of graft failure was analyzed both as continuous and categorical variable. In cause-specific hazards models with continuous variables plasma Pb was log2 transformed to estimate regression coefficients per doubling of plasma Pb concentration. For categorical analyses, subjects were divided according to tertiles of plasma Pb. To account for potential confounders, several multivariable-adjusted cause-specific hazards models were fitted to the data. We adjusted for demographics, kidney transplant characteristics and lifestyle-related exposure to Pb (age, sex, transplant vintage, warm ischemia time, donor type, eGFR, proteinuria, and smoking status, and alcohol intake) in model 1. Further models were performed with additional adjustments to model 1 (primary model). Thus, subsequently, we additively adjusted for co-occurring pro-oxidant conditions (history of hypertension and diabetes) in model 2; history of cardiovascular disease and dyslipidemia (triglycerides and high-density lipoprotein cholesterol, and use of statins) in model 3; cereals, vegetables, fish and seafood intake in model 4; plasma cadmium and plasma arsenic (model 5). Covariates were handled as linear variables unless they were primarily collected as categorical variables (i.e., history of hypertension, diabetes, use of statins). To illustrate the association of plasma Pb with risk of graft failure, data were fitted using median plasma Pb concentration (0.31 µg/L) as reference value (HR 1.00) to estimate and plot regression coefficients.

Potential effect-modification by age, sex, systolic blood pressure, eGFR, calcium, parathyroid hormone, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase, triglycerides, diabetes and cadmium were tested by fitting models containing both main effects and their cross-product terms. The Bonferroni-adjusted significance threshold ($P_{interaction} < 0.004$) was considered to indicate presence of significant effect-
modification, after which was proceeded with further evaluation through stratified prospective analyses.

RESULTS

Baseline Characteristics
We included 670 KTR (mean age 53±13 years-old, 58% male) at a median of 5.4 (IQR, 1.9–11.8) years post-transplantation. Mean eGFR was 52±20 mL/min/1.73 m². Median Pb concentration was 0.31 (IQR, 0.22–0.45) µg/L. Detailed description of baseline characteristics by tertiles of the study population according to plasma Pb distribution is shown in Table 1. Age, frequency of male sex, frequency of former smoking status, intake of potatoes, plasma calcium concentration, plasma parathyroid hormone concentration, plasma alkaline phosphatase, plasma triglycerides and plasma Cd concentration increased significantly across tertiles of plasma Pb concentration. Transplant vintage and frequency of transplantation with a kidney from a living donor decreased significantly across tertiles of plasma Pb concentration. Linear regression analyses of log₂-transformed plasma Pb versus other potentially co-occurring heavy metals exposures, i.e., log₂-transformed plasma Cd and log₂-transformed plasma As, are shown in Figure 1. We found that higher plasma Cd is associated with higher plasma Pb levels, while this was not so for plasma As. This may be due to overlapping or usually co-occurring sources of exposure for Cd and Pb (smoking and alcohol intake, respectively).14

Prospective Analyses of the Association Between Pb and Risk of Graft Failure
During a median follow-up of 4.9 (IQR, 3.4–5.5) years, 78 KTR developed graft failure (12%; event rate 78/3270 patient-years). Higher plasma Pb concentrations were associated with increased risk of graft failure (HR 1.59, 95% CI 1.14–2.21 per doubling of plasma Pb concentration; P=0.006), independent of adjustment for age, sex, transplant vintage, donor type,
warm ischemia time, smoking status, alcohol intake, eGFR and proteinuria (Table 2; Figure 2). Similarly, in categorical analyses according to tertiles of plasma Pb distribution, higher plasma Pb was significantly associated with increased risk of graft failure ($P_{\text{trend}}=0.01$). These findings remained materially unchanged in further multivariable-adjusted analyses.

**Analyses for Potential Effect-Modification**

Results of analyses for assessment of potential effect-modification of the association between plasma Pb and risk of graft failure are shown in Table S2. We did not find evidence of effect-modification.

**Serial Plasma Pb Levels in Kidney Transplant Recipients of the TransplantLines Cohort and Biobank Study**

Plasma Pb at admission for transplantation and at different follow-up visits post-transplantation was investigated in 46 KTR (mean age 52±14 years-old, eGFR 43±28 mL/min/1.73 m$^2$) from the ongoing TransplantLines Prospective Cohort and Biobank Study. In Figure 3 we show that (A) plasma Pb at admission for transplantation was significantly different from plasma Pb at 3-months post-transplantation (median 2.34 (IQR, 1.81–2.95) versus 2.11 (IQR, 1.52–2.62) µg/L, respectively; $P=0.001$), and that (B) plasma Pb at transplantation was significantly associated (Std. $\beta=0.61$, $P<0.001$) with plasma Pb at 3-months post-transplantation ($R^2=0.37$). In Figure 4 we show box plots with medians (IQR) of plasma Pb concentrations at different follow-up visits post-transplantation (2.11 (1.53–2.62), 2.01 (1.55–2.28), 2.19 (1.48–2.52), 2.09 (1.64–2.39) ng/L, at 3-, 6-, 12-, and 24-months post-transplantation, respectively). Median (IQR) intra-individual coefficient of variation post-transplantation was 15% (6–32%), and we did not find signs of a significant change in plasma Pb levels post-transplantation ($P=0.2$, one-way repeated
measure Anova). The distribution of the intra-individual coefficient of variation is shown in Figure S4.

**Blood Versus Plasma Pb Levels in Kidney Transplant Recipients of the TransplantLines Cohort and Biobank Study**

In Figure S5, we show the association of whole blood Pb (mean 29.82, median 21.50, IQR 15.18–37.18, range 7.10–114.0 µg/L) with plasma Pb (mean 0.60, median 0.40, IQR 0.30–0.70, range 0.20–3.10 µg/L) concentration (Std. β=0.68; P<0.001) in 122 KTR of the TransplantLines Prospective Cohort and Biobank Study.

**DISCUSSION**

In a large cohort of KTR, this study shows that plasma Pb is associated with increased risk of late kidney graft failure. Our results were independent of adjustment for age, sex, transplant characteristics, eGFR, proteinuria, smoking status, history of hypertension and diabetes mellitus, dietary items including cereals, vegetables, fish and seafood intake, and plasma concentrations of Cd and As. These results suggest that Pb exposure may be a potentially modifiable, yet previously overlooked, risk factor for late graft failure in KTR, underscoring the question whether plasma Pb monitoring and non-toxic therapeutic interventions to decrease its levels might diminish the burden of late graft failure in KTR.

We found lower plasma and whole blood Pb concentrations than previous studies in the general population (e.g., mean Pb 0.54 and 119 µg/L, respectively), and occupational cohorts (e.g., geometric mean 0.57 and 227 µg/L, respectively). In a large (n=15,211) cohort of representative sample of the civilian noninstitutionalized United States population with and without hypertension, participating in the Third National Health and Nutrition Examination Survey, mean blood Pb was 42.1 and 33.0 µg/L, respectively, which are higher than blood Pb
levels than in our study. Evaluating the relationship between plasma and blood Pb concentrations, Smith *et al.* 30 described a curvilinear relationship with the mean plasma/whole blood Pb in the 0.308 and 0.291% range. The median baseline plasma Pb in the current series of 670 KTR was 0.31 μg/L. Using the ratio of 0.3% reported by Smith *et al.*, this value would correspond to a whole blood Pb concentration of 103 μg/L, which is approximately 5 times higher than the whole blood Pb concentration we found. This indicates a much higher plasma Pb over whole blood Pb ratio in the KTR population of our study, than in subjects of the general population. For this it may be interesting to realize that Pb is known as a “bone-seeking” element, with Pb from blood first being incorporated in bone, to later on be released from it, at rates depending on bone turnover rates. 31 This notion is of particular interest if it is appraised in the context of the fact that plasma Pb over whole blood Pb ratios have consistently found to be more strongly associated with bone Pb levels than whole blood Pb concentrations, 30 which could indicate that plasma Pb concentration are more closely related to bone Pb levels than whole blood Pb concentrations. If one realizes that secondary and tertiary hyperparathyroidism leading to high bone turnover are very common in KTR, 32 while these conditions are very uncommon in the general population, it is conceivable this could play a role in a higher plasma Pb over whole blood Pb ratio in KTR than in the general population. Interestingly, we also found that the group of KTR with serial plasma Pb measurements *(n=46)* had approximately 10-fold higher plasma Pb concentrations than the 670 KTR of the main patient cohort in this study. Of note, the KTR with the 10-fold higher plasma Pb concentrations were studied at a rather short transplant vintage (3 to 24 months post-transplantation), while the 670 patients of the main cohort were studied at a later transplant vintage (median of 5.4, IQR 1.9–11.8 years post-transplantation). It is possibly that the high plasma Pb concentrations in the early phase after transplantation are also reflective
of the fact that Pb is known as a “bone-seeking” element,\textsuperscript{31} since it is widely acknowledged that post-kidney transplantation osteodystrophy is a special entity, with most rapid net bone loss in the first year after transplantation, followed by more mitigated, but continued loss, thereafter.\textsuperscript{32} The high rate bone loss in the early phase after transplantation may set more Pb free from bone, and this itself, or circumstances accompanying it, like e.g. low phosphate concentrations or acidosis,\textsuperscript{33} may shift the equilibrium between plasma Pb and whole blood Pb towards relatively high concentrations of the former. It would be of interest if future studies could investigate the association of plasma Pb and whole blood Pb with metabolic milieu and bone turnover early and late after transplantation.

Previous literature has linked Pb exposure to impaired kidney function,\textsuperscript{22,34} contributing to deterioration of kidney function both in the general population\textsuperscript{35} and in CKD patients.\textsuperscript{23,36} Our findings are in agreement with the evidence pointing towards the kidney as a relevant site of Pb toxicity\textsuperscript{37} with chronic exposure inducing progressive proximal tubular atrophy, interstitial fibrosis and vascular changes.\textsuperscript{18,22,38} Since higher blood Pb levels are associated with increased risk of hypertension,\textsuperscript{39} it could be hypothesized that at least part of the Pb-associated risk of graft failure is attributable to an intermediary role of increased blood pressure in KTR.\textsuperscript{4} We observed a non-significant trend towards higher systolic blood pressure over increasing tertiles of plasma Pb distribution, and a borderline higher use of antihypertension medication in patients with higher plasma Pb levels. Yet, the association between Pb and graft failure was independent of hypertension, which may suggest that plasma Pb is associated with risk of late graft failure mainly by direct mechanisms of nephrotoxicity.

Food, tobacco and alcohol consumption are the most relevant sources of Pb exposure in the general population.\textsuperscript{12–14} In the Netherlands, particularly, water service pipes have been identified
as a relevant source of over-exposure to Pb.\textsuperscript{16} Pb is available in organic and inorganic forms. Inorganic Pb is not metabolized, but distributed, and deposited in soft tissues and bones.\textsuperscript{17} The fact that we found plasma Pb concentrations to be positively associated with plasma calcium concentration, plasma concentrations of PTH and alkaline phosphatase may be appreciated as a sign of the affinity of Pb to the bone and its acknowledged adverse effect on bone mineralization. After absorption, Pb enters the bloodstream, where it is predominantly bound to erythrocyte proteins\textsuperscript{40} with an approximately 35-days half-life.\textsuperscript{41} Clearance from circulation occurs through distribution into soft tissues and bone as well as excretion. A small amount of Pb is excreted in feces, sweat, hair, and nails, while the main excretion is through kidney filtration and elimination in urine.\textsuperscript{40} In human kidney cells, Pb-binding proteins (PbBPs) have been identified, which are supposedly endocytosed, entering proximal tubular epithelial cells.\textsuperscript{42} At toxic levels, once inside the cells, these proteins tend to form inclusion bodies in the cytoplasm, which has a temporal correlation with the onset of tubular dysfunction.\textsuperscript{43} It has been suggested that these inclusion bodies reduce cytoplasmic Pb concentrations, allowing renal tubular epithelium to remain viable, albeit at a reduced functional level.\textsuperscript{44} Plasma Pb levels reflect exposure from exogenous sources plus the release of endogenous Pb from bone. Plasma rather than blood levels reflect the fraction of circulatory Pb that is more freely available for exchange with tissues,\textsuperscript{45} and that in the kidney is filtered to from the ultrafiltrate to which the kidney tubular epithelial cells are exposed, thus more closely signaling Pb kidney burden for estimation of kidney function risk.\textsuperscript{30}

Our findings are relevant for informing clinical follow-up of outpatientKTR. Our findings may underscore the need for asking about occupation and hobbies with chemical exposures. Next, chelation therapy, used in heavy metal poisoning, may warrant further studies as a potential interventional approach to reduce the burden of long-term graft failure in KTR. Of
Note, it has been repeatedly shown that the urinary excretion of Pb can be increased by using Ca-
EDTA (calcium ethylenediaminetetraacetic acid) chelation, which in turn has proven to lessen
progression rates of diabetic\textsuperscript{46} and non-diabetic\textsuperscript{47} nephropathy in patients with high-normal body
Pb burden, as well as progression of CKD in patients with increased body Pb burden.\textsuperscript{36}

It is worth noting that, our study was conducted in a population from the northeastern region
of The Netherlands, an area with known low Pb environmental exposure, when compared to
developing countries\textsuperscript{48} or industrial countries such as China, where child Pb intoxication has
been a much more severe health concern. Our data underscore that mildly elevated plasma Pb
concentrations (higher than approximately 0.30 µg/L, but much lower than 5 µg/L as previously
indicated by Ekong \textit{et al.}\textsuperscript{22}) may be a risk factor associated with impaired long-term graft
function in KTR. We acknowledge that our predominantly Caucasian study population derived
from a single center from the northern part of The Netherlands, and may not be generalizable to
other populations with different environmental contamination and exposure to Pb. Since point
estimates of hazard ratios in the prospective analyses remained materially unchanged after
adjustment for food items, it may be suggested that food sources were not a major route of
exposure. We also acknowledge potential confounding effects of low socioeconomic status,
which is linked at least in the US to high lead exposure due to lead-based paint and lead pipes,
faucets, and plumbing fixtures.\textsuperscript{49} Further studies are needed to better determine exposure routes
and the association between exposure and circulating Pb levels. In our study of serial plasma Pb
levels in a sample population of the TransplantLines Cohort and Biobank Study,\textsuperscript{24} we found low
intra-individual variability, indicative of relatively stable plasma Pb levels over time post-
transplantation. It should be noted that we used post-transplantation plasma Pb concentrations as
baseline Pb concentration for the prospective analyses of the association with graft failure, which
assumes that the plasma Pb concentrations did not change over time in the patients included in these analyses. Although we found no evidence for changes over time in plasma Pb concentration post-transplantation, this remains a rather strong assumption, which requires confirmation in further studies. Although several investigators have suggested that plasma Pb represents a more relevant index of exposure to health risks associated with Pb than does whole blood Pb, because plasma Pb may better reflect the fraction of circulatory Pb that is more freely available for exchange with tissues,\textsuperscript{30,31} it is also true that research on associations between plasma Pb and toxicologic outcomes is still sparse, and a significant gap in knowledge remains.\textsuperscript{31} It has been suggested that plasma Pb is too imprecise to be useful in individuals with low level exposure, while whole blood Pb concentration was a useful biomarker in this situation.\textsuperscript{50} We, however, found a strong association between plasma Pb and long-term outcome, which suggests that plasma Pb concentrations are a meaningful biomarker, at least in KTR. Further studies are needed to determine whether plasma Pb detected with newest and most sensitive ICP-MS equipment also serves as a meaningful biomarker in other populations and whether it can be used as an alternative to whole blood Pb concentrations or even outperform it as a biomarker. Finally, due to its observational nature, the current study does not prove causality. Residual confounding may occur despite adjustment for potential confounders.

Our results show that plasma Pb is independently associated with risk of late kidney graft failure, indicating the need for future studies to confirm our results and externally validate our findings among different populations of KTR. Pb exposure may be a potentially modifiable risk factor for adverse long-term kidney graft outcomes. Whether clinical monitoring of Pb concentrations, reduction of environmental exposure, and non-toxic therapeutic interventions
(e.g., chelation) to decrease system Pb in KTR may represent novel risk management strategies to decrease the burden of long-term kidney graft failure remains to be investigated.

SUPPLEMENTARY MATERIAL

<table>
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</tr>
</tbody>
</table>

ARTICLE INFORMATION
TRANSPLANTLINES INVESTIGATORS


AUTHORSHIP CONTRIBUTIONS

Data acquisition: DJT, SJLB; data analysis: CGS; data interpretation: CGS, FG, DG, CF, IMN, GJN, AWG-N, DK, TJK, MFE, RR, DJT, and SJLB; cohort design: SJLB. Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual’s own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work, even one in which the author was not directly involved, are appropriately investigated and resolved, including with documentation in the literature if appropriate.

SUPPORT

This study was based on the TransplantLines Food and Nutrition Biobank and Cohort Study (NCT02811835), which was funded by the Top Institute Food and Nutrition of the Netherlands (grant A-1003), and TransplantLines Biobank and Cohort Study (NCT03272841), which is supported by a grant from Astellas BV. Dr. Sotomayor was supported by a doctorate studies
grant from CONICYT (F 72190118). The funders had no role in study design, data collection, analysis, reporting, or the decision to submit for publication.

FINANCIAL DISCLOSURES

CGS declares that he has no other relevant financial interests. The other authors declare that they have no relevant financial interests.

ACKNOWLEDGMENTS

We thank Jan IJmker for the provided support with laboratory measurements. We thank FONDECYT 1211988 for the provided support with human resources.

Peer Review: Received May 4, 2021. Evaluated by 3 external peer reviewers, with direct editorial input from a Statistics/Methods Editor, an Associate Editor, and the Editor-in-Chief. Accepted in revised form October 15, 2021.

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Table 1. Baseline characteristics of 670 kidney transplant recipients

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Tertiles of plasma lead concentrations</th>
<th>( P_{\text{trend}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \leq 0.24 \mu g/L (n=224) )</td>
<td>( 0.24–0.38 \mu g/L (n=224) )</td>
</tr>
<tr>
<td><strong>Lead, ( \mu g/L, ) median (IQR)</strong></td>
<td>0.19 (0.16–0.22)</td>
<td>0.31 (0.27–0.35)</td>
</tr>
<tr>
<td><strong>Demographics and anthropometrics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years, mean (SD)</td>
<td>50 (13)</td>
<td>53 (13)</td>
</tr>
<tr>
<td>Sex (male), ( n ) (%)</td>
<td>107 (48)</td>
<td>145 (65)</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2), mean (SD)</td>
<td>26.3 (5.1)</td>
<td>26.8 (4.2)</td>
</tr>
<tr>
<td>Diabetes, ( n ) (%)</td>
<td>57 (25)</td>
<td>52 (23)</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never, ( n ) (%)</td>
<td>107 (48)</td>
<td>87 (39)</td>
</tr>
<tr>
<td>Former, ( n ) (%)</td>
<td>81 (36)</td>
<td>96 (43)</td>
</tr>
<tr>
<td>Current, ( n ) (%)</td>
<td>25 (11)</td>
<td>27 (12)</td>
</tr>
<tr>
<td><strong>Alcohol use</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( 0 ) g/d, ( n ) (%)</td>
<td>23 (10)</td>
<td>22 (10)</td>
</tr>
<tr>
<td>( 0–10 ) g/d, ( n ) (%)</td>
<td>134 (60)</td>
<td>121 (54)</td>
</tr>
<tr>
<td>( 0–30 ) g/d, ( n ) (%)</td>
<td>37 (17)</td>
<td>53 (24)</td>
</tr>
<tr>
<td>( &gt;30 ) g/d, ( n ) (%)</td>
<td>5 (2)</td>
<td>12 (5)</td>
</tr>
<tr>
<td>History of cardiovascular disease, ( n ) (%)</td>
<td>93 (42)</td>
<td>102 (46)</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg, mean (SD)</td>
<td>134 (17)</td>
<td>136 (17)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg, mean (SD)</td>
<td>82 (11)</td>
<td>83 (11)</td>
</tr>
<tr>
<td>Use of antihypertensive medication, ( n ) (%)</td>
<td>192 (86)</td>
<td>193 (86)</td>
</tr>
<tr>
<td><strong>Kidney function and transplant history</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m(^2), mean (SD)</td>
<td>55 (21)</td>
<td>51 (20)</td>
</tr>
<tr>
<td>Proteinuria, ( n ) (%)</td>
<td>47 (21)</td>
<td>54 (24)</td>
</tr>
<tr>
<td>Transplant vintage, years, median (IQR)</td>
<td>6 (2–14)</td>
<td>7 (3–12)</td>
</tr>
<tr>
<td>Acute rejection, ( n ) (%)</td>
<td>65 (29)</td>
<td>65 (29)</td>
</tr>
</tbody>
</table>
Cold ischemia time, hrs, median (IQR) | 13 (3–21) | 15 (3–21) | 15 (3–21) | 0.2
Warm ischemia time, minutes, mean (SD) | 43 (15) | 42 (14) | 45 (17) | 0.04
HLA mismatches, n, mean (SD) | 2.2 (1.6) | 2.1 (1.5) | 2.4 (1.6) | 0.7
Living donor, n (%) | 93 (42) | 80 (36) | 58 (26) | 0.003

Primary kidney disease

| Glomerulosclerosis, n (%) | 66 (30) | 72 (32) | 52 (23) |
| Glomerulonephritis, n (%) | 21 (9) | 15 (7) | 15 (7) |
| Tubulointerstitial nephritis, n (%) | 25 (11) | 28 (13) | 22 (1) |
| Polycystic kidney disease, n (%) | 42 (19) | 45 (20) | 54 (24) |
| Kidney hypo/dysplasia, n (%) | 14 (6) | 8 (4) | 7 (3) |
| Renovascular disease, n (%) | 10 (5) | 12 (5) | 17 (8) |
| Diabetes, n (%) | 9 (4) | 11 (5) | 12 (5) |
| Other/miscellaneous, n (%) | 37 (17) | 33 (15) | 43 (19) |

Records of Immunosuppressive Therapy Use

| Use of calcineurin inhibitor, n (%) | 124 (55) | 127 (57) | 133 (60) | 0.6
| Use of proliferation inhibitor, n (%) | 185 (83) | 187 (84) | 186 (84) | 0.9
| Corticosteroids dose <10 mg/24 h, n (%) | 101 (45) | 92 (41) | 81 (37) | 0.2

Dietary intake

| Cereals, g/day, median (IQR) | 182 (138–227) | 176 (125–225) | 172 (134–218) | 0.9
| Potatoes, g/day, median (IQR) | 100 (60–150) | 119 (60–158) | 119 (60–160) | 0.04
| Vegetables, g/day, median (IQR) | 81 (55–121) | 79 (56–119) | 75 (48–115) | 0.7
| Fruits, g/day, median (IQR) | 104 (47–189) | 107 (51–199) | 107 (48–178) | 0.5
| Nuts, g/day, median (IQR) | 4.8 (0.0–9.6) | 3.0 (0.7–9.4) | 3.5 (0.0–8.9) | 0.3
| Fish and seafood, g/day, median (IQR) | 10.5 (4.0–18.7) | 11.7 (4.7–23.0) | 11.5 (3.9–20.0) | 0.2
| Meat, g/day, median (IQR) | 94 (69–114) | 91 (71–118) | 97 (73–119) | 0.2
| Milk and dairy products, g/day, median (IQR) | 377 (251–498) | 347 (229–488) | 369 (235–536) | 0.5

Laboratory measurements

| Calcium, mmol/L, mean (SD) | 2.38 (0.14) | 2.39 (0.15) | 2.43 (0.15) | <0.001
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Median (IQR)</th>
<th>Mean (SD)</th>
<th>Median (IQR)</th>
<th>Mean (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathyroid hormone, pmol/L</td>
<td></td>
<td>8.4 (5.9‒13.8)</td>
<td>9.8 (6.4‒15.0)</td>
<td>10.2 (6.8‒17.0)</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>FGF-23, RU/mL, median (IQR)</td>
<td></td>
<td>58 (42‒98)</td>
<td>66 (44‒98)</td>
<td>62 (43‒99)</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L, mean (SD)</td>
<td></td>
<td>5.0 (1.1)</td>
<td>5.2 (1.1)</td>
<td>5.1 (1.1)</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L, mean (SD)</td>
<td></td>
<td>1.3 (0.4)</td>
<td>1.4 (0.5)</td>
<td>1.4 (0.5)</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L, median (IQR)</td>
<td></td>
<td>2.8 (2.2‒3.6)</td>
<td>2.9 (2.4‒3.5)</td>
<td>2.9 (2.3‒3.5)</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mmol/L, median (IQR)</td>
<td></td>
<td>1.7 (1.2‒2.2)</td>
<td>1.7 (1.2‒2.3)</td>
<td>1.8 (1.3‒2.5)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/L, median (IQR)</td>
<td></td>
<td>5.3 (4.7‒6.1)</td>
<td>5.2 (4.7‒6.0)</td>
<td>5.3 (4.9‒6.2)</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>HbA1C, %, mean (SD)</td>
<td></td>
<td>6.0 (0.9)</td>
<td>5.9 (0.7)</td>
<td>6.1 (0.9)</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Cadmium, μg/L, median (IQR)</td>
<td></td>
<td>0.05 (0.04‒0.06)</td>
<td>0.06 (0.04‒0.07)</td>
<td>0.07 (0.05‒0.09)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Arsenic, μg/L, median (IQR)</td>
<td></td>
<td>1.23 (1.04‒1.86)</td>
<td>1.31 (1.05‒2.23)</td>
<td>1.24 (1.02‒2.01)</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

Values presented as mean (SD) unless stated otherwise. Differences among tertiles of the plasma lead distribution (tertile 1: ≤0.24 μg/L; tertile 2: 0.24‒0.38 μg/L; tertile 3: ≥0.38 μg/L) were studied by means of analysis of variance or the linear regression test for continuous variables and by means of the chi-squared test for categorical variables. Abbreviations: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; GGT, gamma glutamyl transferase; HDL, high-density lipoprotein cholesterol; HLA, human leukocyte antigens; LDL, low-density lipoprotein cholesterol.
Table 2. Association of lead with risk of graft failure

<table>
<thead>
<tr>
<th>Models</th>
<th>Lead, per log₂ (μg/L)</th>
<th>Lead, per tertiles</th>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P</td>
<td>Reference</td>
<td>HR (95% CI)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.59 (1.14‒2.21)</td>
<td>0.006</td>
<td>1.00</td>
<td>0.95 (0.46‒1.96)</td>
<td>2.11 (1.03‒4.33)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.60 (1.15‒2.24)</td>
<td>0.006</td>
<td>1.00</td>
<td>0.91 (0.43‒1.90)</td>
<td>2.12 (1.03‒4.35)</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.60 (1.14‒2.26)</td>
<td>0.007</td>
<td>1.00</td>
<td>1.03 (0.48‒2.20)</td>
<td>2.18 (1.03‒4.57)</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.54 (1.10‒2.16)</td>
<td>0.013</td>
<td>1.00</td>
<td>0.94 (0.45‒1.95)</td>
<td>1.94 (0.93‒4.03)</td>
</tr>
<tr>
<td>Model 5</td>
<td>1.56 (1.11–2.18)</td>
<td>0.010</td>
<td>1.00</td>
<td>0.94 (0.45–1.93)</td>
<td>1.94 (0.94–4.02)</td>
</tr>
</tbody>
</table>

Cause-specific hazards models were performed to assess the association of plasma lead concentration with death-censored graft failure (n<sub>events</sub>=78). Associations are shown with plasma lead concentration as a continuous variable and according to tertiles of the plasma lead distribution (Tertile 1: ≤0.24 μg/L; Tertile 2: 0.25–0.38 μg/L; Tertile 3: ≥38 μg/L). Models were adjusted for age, sex, transplant vintage, donor type, warm ischemia time, smoking status, alcohol intake, eGFR and proteinuria (model 1). Further models were performed with additional adjustments to model 1 (primary model), as follows: history of hypertension and diabetes mellitus (model 2); history of cardiovascular disease and triglycerides, high-density lipoprotein cholesterol and use of statins (model 3); cereals, vegetables, fish and seafood intake (model 4); plasma cadmium and plasma arsenic (model 5).
FIGURE LEGENDS

**Figure 1.** Linear regression analyses of the association between plasma lead and (A) plasma cadmium (Std. β=0.27, P<0.001) and (B) plasma arsenic (Std. β=0.04, P=0.3).

**Figure 2.** Association of plasma lead concentration with risk of graft failure in kidney transplant recipients. Data were fitted by cause-specific hazards models using median lead (0.31 µg/L) as reference value. The black line represents the hazard ratio and the gray area represents the 95% confidence interval.

**Figure 3.** Plasma lead concentrations at admission before transplantation (Tx) and at 3-months post-transplantation (3m post-tx) in 46 kidney transplant recipients of the TransplantLines Prospective Cohort and Biobank Study. (A) Plasma lead at 3m post-tx was significantly different from plasma lead at admission for transplantation (median (interquartile range), 2.11 (1.52–2.62) and 2.34 (1.81–2.95) µg/L, respectively; P=0.001). Box plots show medians (interquartile range). Significance of potential difference between transplantation and 3m post-tx plasma lead was tested using the Wilcoxon matched-pairs signed rank test. (B) Plasma lead at transplantation was significantly associated (Std. β=0.61, P<0.001) with plasma lead at 3m post-tx (R²=0.37).

**Figure 4.** Plasma lead concentrations in 46 kidney transplant recipients of the TransplantLines Prospective Cohort and Biobank Study, at different follow-up visits post-transplantation. Box plots show medians (interquartile range). Significance of potential change during follow-up visits was tested using the one-way repeated measure Anova test, which indicated no significant change over time (P=0.2). Median (interquartile range) intra-individual coefficient of variation of plasma lead concentrations was 15% (6–32%). The distribution of the intra-individual coefficient of variation is shown in Figure S3.
Log$_2$-transformed plasma lead (µg/L) at admission for transplantation

Log$_2$-transformed plasma lead (µg/L) at 3-months post-transplantation

A

B

C

Plasma lead, µg/L

Plasma lead, µg/L

Log$_2$-transformed plasma lead (µg/L) at admission for transplantation

Change

0 2 4 6 8 10

0 2 4 6

0 2 4 6 8

0 2 4 6 8