

C. Herder et al. Circulating levels of interleukin 1-receptor antagonist and risk of cardiovascular disease: meta-analysis of 6 population-based cohorts

SUPPLEMENTAL MATERIAL

Supplemental Table I. Baseline Characteristics of the MONICA/KORA Study Population

Characteristic	Incident CVD Cases	Non-Cases	<i>P</i>
N	803	1,942	
Age (years)	61 (54; 66)	51 (43; 60)	<0.001
Male sex (%)	69.1	46.5	<0.001
Body mass index (kg/m ²)	28.0 (25.7; 30.7)	26.5 (24.1; 29.5)	<0.001
Systolic blood pressure (mmHg)	141 (130; 156)	130 (120; 143)	<0.001
Diastolic blood pressure (mmHg)	83 (75; 91)	81 (74; 88)	<0.001
Hypertension (% yes)*	65.4	40.4	<0.001
Ratio total/HDL cholesterol	5.1 (4.2; 6.5)	4.2 (3.4; 5.2)	<0.001
Prevalent diabetes (% yes)	15.4	4.2	<0.001
Parental history of CHD (%)			0.001
Negative	51.9	59.7	
Positive	22.0	19.8	
Unknown	26.0	20.5	
Physical activity (% inactive)	69.6	61.4	<0.001
Smoking status (%)			<0.001
Never smoker	35.9	48.7	
Former smoker	33.3	27.1	
Current smoker	30.9	24.2	
Alcohol consumption (%) [†]			0.796
0 g/day	30.3	31.5	
>0 to <40/20 g/day	43.1	41.9	
≥40/20 g/day	26.7	26.5	
hsCRP (mg/l)	2.3 (1.2; 5.2)	1.4 (0.6; 3.1)	<0.001
IL-1 receptor antagonist (pg/ml)	364 (297; 476)	338 (273; 438)	<0.001

Descriptive analyses of baseline characteristics were carried out for cases and non-cases. Weighting was performed using the survey- and sex-specific sampling weights. Missing values were imputed using 20-fold multiple imputation by chained equations (MICE).¹ We imputed a total of 59 variables including all biomarkers measured as well as important covariables with missing values. Depending on the individual measurement level, different imputation methods were applied. For binary variables, logistic regression imputation was applied. For categorical variables, such as smoking status (current/former/never) or paternal history of diabetes (yes/no/unknown), polytomous regression imputation was used. Continuous variables were, if appropriate, transformed to approximate normal distribution (choosing from natural log, square root and cubic root). Then they were imputed using

predictive mean matching and were afterwards transformed back to their original scale. All variables relevant for later analyses were included to impute the missing values to preserve correlations among variables, also comprising outcome variables and the respective times to event.

Data are given as median (25th percentile; 75th percentile) for continuous variables and as percentages for categorical variables. For continuous variables participants with and without incident CVD were compared using t-tests on regression coefficients based on the SAS procedure SURVEYREG and using Rubin's rules for multiple imputation.^{2,3} In case of non-normality, tests were carried out with log-transformed variables and results were presented as geometric means with antilogs of standard errors of the adjusted log-means. Categorical variables were compared using Wald chi-square test based on the SAS procedure SURVEYFREQ and imputation was accounted for using a method proposed by Schafer to combine multiple results of chi-square statistics.^{4,5}

*Hypertension is defined based on 1999 ISH-WHO guidelines or medical treatment given that study participants were aware of being hypertensive.

†Alcohol consumption was classified for men as 0, >0 and <40 or ≥40 g per day, and for women as 0, >0 and <20 or ≥20 g per day.

Supplemental Table II. Hazard Ratios (HR) and 95% Confidence Intervals (CI) for the Association between Serum IL-1RA Quarters and Incident CVD in the MONICA/KORA study

	Serum IL-1RA for men*	Serum IL-1RA for women*	N cases / non-cases	Model 1	Model 2
Quarter 1	233.0 (85.2; 268.6)	238.1 (85.2; 279.9)	143 / 523	1.00	1.00
Quarter 2	303.1 (268.8; 331.9)	312.9 (280.0; 351.0)	169 / 490	1.17 (0.83; 1.63)	1.04 (0.73; 1.48)
Quarter 3	367.8 (332.0; 426.9)	396.8 (351.1; 465.3)	230 / 485	1.51 (1.12; 2.03)	1.25 (0.90; 1.73)
Quarter 4	528.6 (427.0; 2312.4)	581.1 (465.4; 2312.4)	261 / 444	2.13 (1.58; 2.86)	1.42 (1.02; 1.97)
P_{trend}	-	-	-	<0.0001	0.0109

*Sex-specific serum IL-1RA levels in pg/ml for quarters 1 to 4 are given as median (lower limit; upper limit).

Cox proportional hazard (PH) regression was applied. For the estimation of hazard ratios (HRs) per quarter of IL-1RA, sex-specific quarters of IL-1RA (based on the random sample subcohort) were calculated with the bottom quarter as the reference category. The case-cohort design required correction of the variance estimation based on the sampling weights to give standard error estimates for the parameter estimates. Correction for standard errors was made using the method by Barlow.⁶ Incorporation of the additional variation due to imputation was performed using Rubin's rules for multiple imputation.² Tests for trend were conducted assigning the median value within each quarter to the corresponding quarter and including this variable in the Cox regression model.

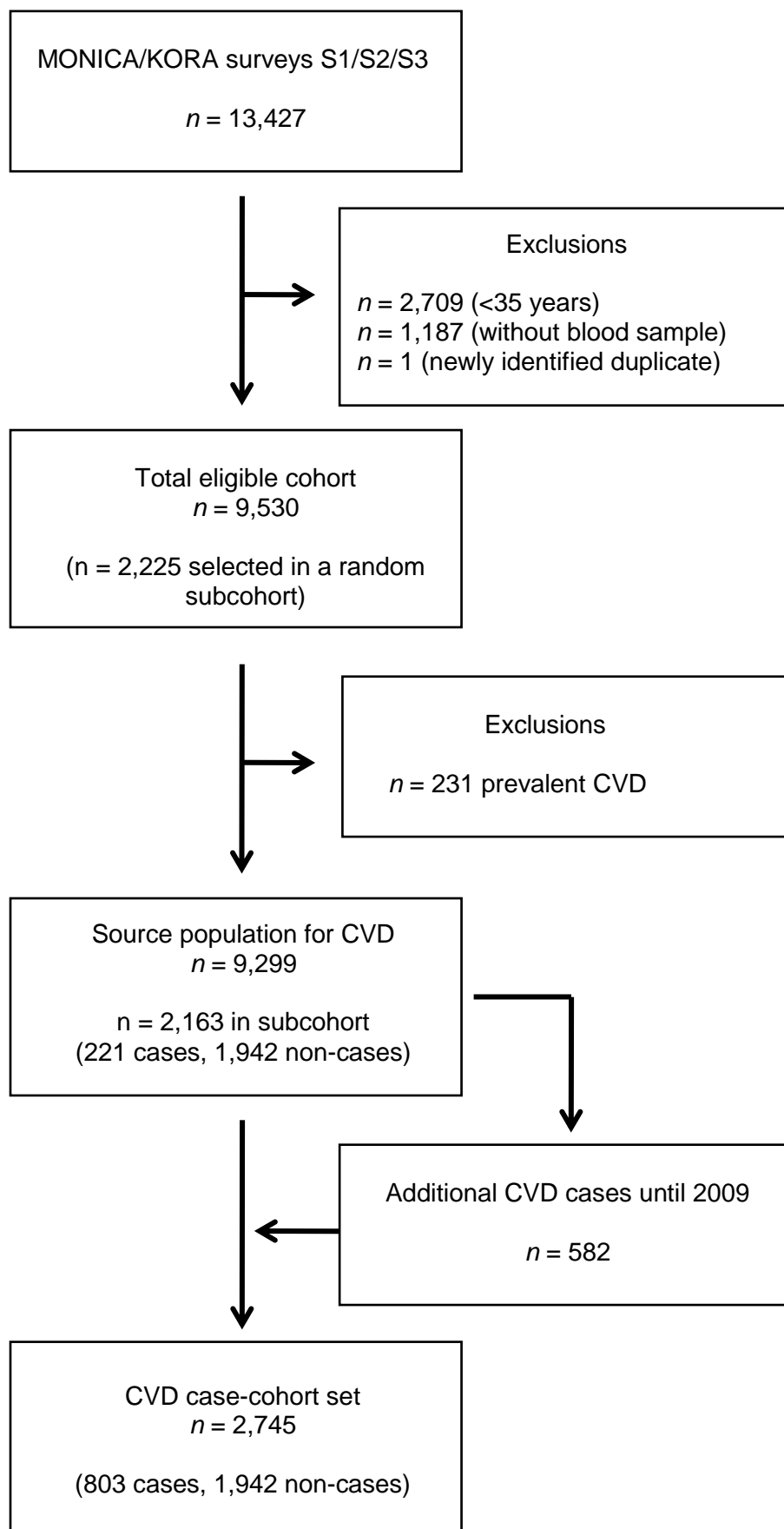
Model 1: adjusted for age, sex and survey.

Model 2: model 1 + BMI, systolic blood pressure, total/HDL cholesterol, smoking, alcohol consumption, physical activity, parental history of CHD, prevalent diabetes.

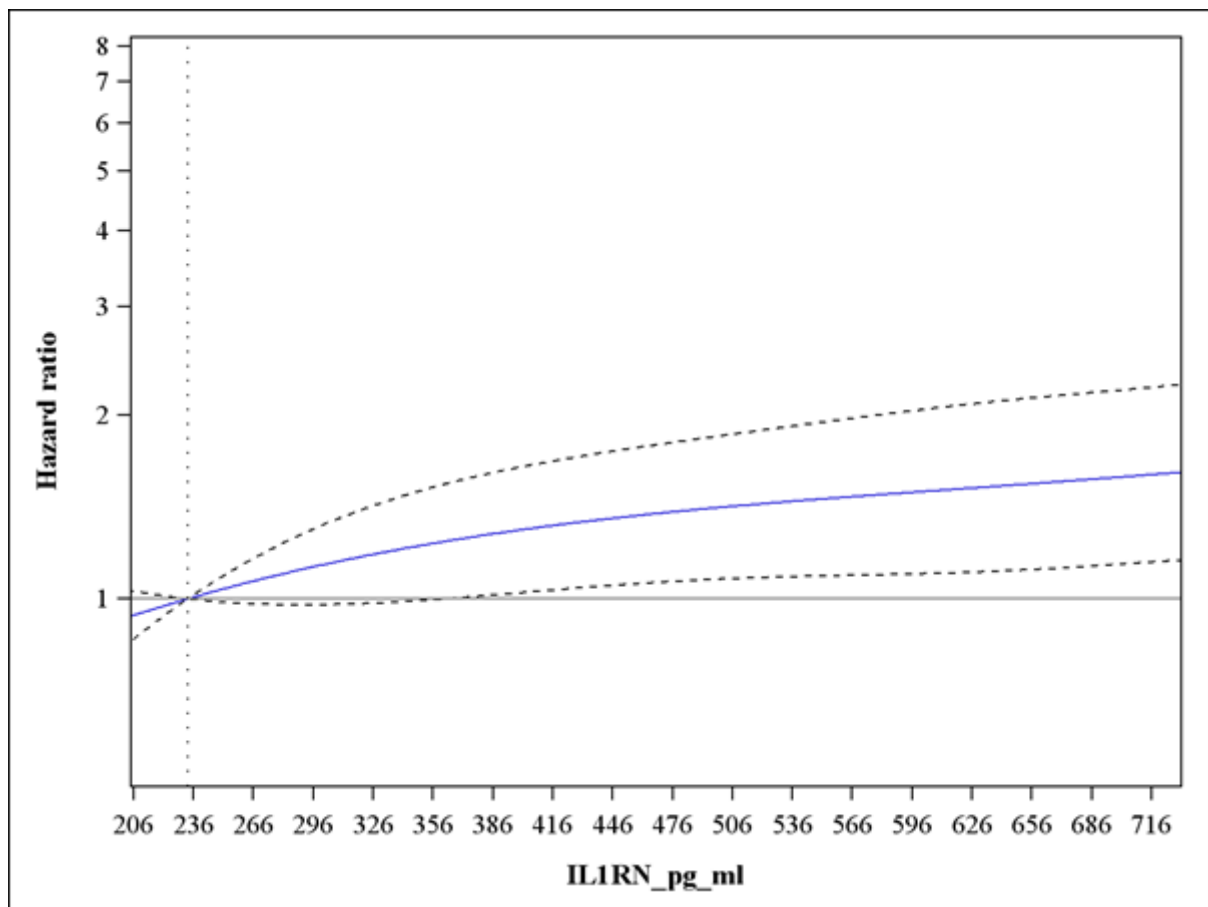
Supplemental Table III. Associations Between IL-1RA and Incident CVD in Six Prospective Cohort Studies

Study population	Duration of follow-up	Covariates	HR per SD (95% CI) and <i>P</i> value
MONICA/KORA Population-based case-cohort 803 cases, 1942 non-cases	16.0 years	Age, sex, survey, BMI, systolic blood pressure, ratio of total cholesterol to HDL cholesterol, smoking, alcohol consumption, physical activity, parental history of CHD, prevalent diabetes	1.165 (1.039; 1.306) <i>P</i> = 0.009
FINRISK 1997 Population-based cohort 394 cases, 6099 non-cases	14.8 years	Age, sex, BMI, systolic blood pressure, smoking, alcohol, ratio of total cholesterol to HDL cholesterol, physical activity, prevalent diabetes	1.101 (1.008; 1.203) <i>P</i> = 0.034
FINRISK 2007 Population-based cohort 65 cases, 4226 non-cases	4.9 years	Age, sex, BMI, systolic blood pressure, smoking, alcohol, ratio of total cholesterol to HDL cholesterol, physical activity, prevalent diabetes	1.057 (0.899; 1.242) <i>P</i> = 0.503
HEALTH 2000 Population-based cohort 267 cases, 5585 non-cases	11.1 years	Age, sex, BMI, systolic blood pressure, smoking, alcohol, ratio of total cholesterol to HDL cholesterol, physical activity, prevalent diabetes	1.104 (0.969; 1.202) <i>P</i> = 0.169
Belfast PRIME Men Cohort Population-based cohort 227 cases, 2008 non-cases	10 years	Age, BMI, systolic blood pressure, smoking, non-HDL cholesterol, HDL cholesterol, anti-hypertensive or lipid-lowering medication, prevalent diabetes	1.149 (1.043; 1.266) <i>P</i> = 0.005
Rotterdam Study Population-based cohort 99 cases, 740 non-cases	10.6 years	Age, sex, BMI, systolic blood pressure, ratio of total cholesterol to HDL cholesterol, smoking, prevalent diabetes, anti-hypertensive medication	1.109 (0.887; 1.386) <i>P</i> = 0.364

Supplemental Figure I. MONICA/KORA Case-Cohort Study Population

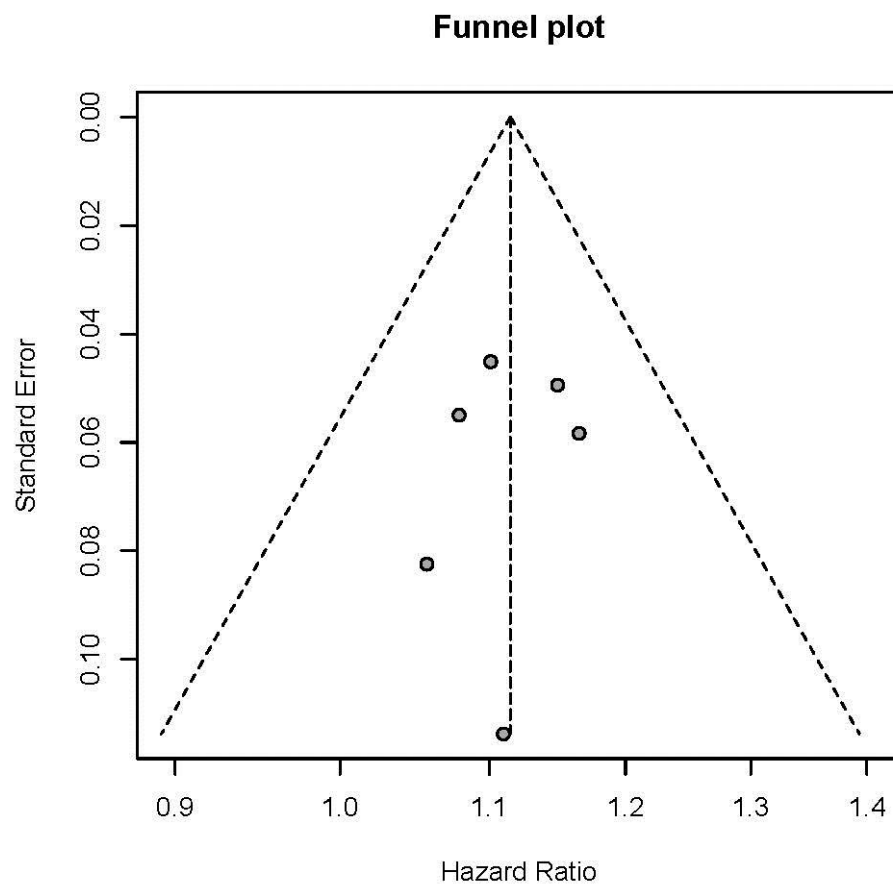


Supplemental Figure II. Dose-Response Relationship of the Association Between Serum IL-1RA and Incident CVD in the MONICA/KORA Study



The figure shows the HR (blue line) and 95% CI (dotted black lines) for the association between IL-1RA levels and incident CVD in model 2. The figure is limited to the IL-1RA range between the 5th and 95th percentiles (206 to 728 pg/ml) with the median of the first IL-1RA quarter (233 pg/ml) as reference (shown for one imputation).

Supplemental Figure III. Funnel Plot to Assess Publication Bias



Egger's linear regression test of funnel plot asymmetry yielded a P value of 0.67, so that the hypothesis that the funnel plot is symmetric cannot be rejected (i.e. no evidence for publication bias).

Supplemental References

1. van Buuren S, Boshuizen HC, Knook DL. Multiple imputation of missing blood pressure covariates in survival analysis. *Stat Med*. 1999;18:681--694.
2. Rubin DB. Multiple imputation for nonresponse in surveys. New York: J. Wiley & Sons, Inc.; 1987.
3. Raghunathan T, Lepkowski J, Hoewyk J, Solenberger P. A multivariate technique for multiply imputing missing values using a sequence of regression models. *Survey Methodology*. 2001;27:85--96.
4. Schafer JL. Analysis of incomplete multivariate data: Chapman & Hall; 1997.
5. Li KH, Meng XL, Raghunathan TE, Rubin DB. Significance levels from repeated p-values with multiply-imputed data. *Statistica Sinica*. 1991;1:65--92.
6. Barlow WE. Robust variance estimation for the case-cohort design. *Biometrics*. 1994;50:1064--1072.