Clinical Chemistry 56:5
861–864 (2010)

Brief Communications

Reproducibility in Serial C-Reactive Protein and Interleukin-6 Measurements in Post–Myocardial Infarction Patients: Results from the AIRGENE Study

Mahir Karakas,[1] Jens Baumert,[2] Sonja Greven,[3] Regina Rückerl,[2] Annette Peters,[2] and Wolfgang Koenig[1]*
[1] Department of Internal Medicine II – Cardiology, University of Ulm Medical Center, Ulm, Germany; [2] Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; [3] Department of Biostatistics, Johns Hopkins University, Baltimore, MD; * address correspondence to this author at: Department of Internal Medicine II – Cardiology, University of Ulm Medical Center, Albert-Einstein-Allee 23, D-89081 Ulm, Germany. Fax +49-731-500-45021; e-mail wolfgang.koenig@uniklinik-ulm.de.

BACKGROUND: Among the numerous emerging biomarkers, high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6) have received widespread interest, and a large database has been accumulated on their potential role as predictors of cardiovascular risk. The concentrations of inflammatory biomarkers, however, are influenced, among other things, by physiological variation, which is the natural within-individual variation occurring over time. Implementation of hsCRP and IL-6 measurement into clinical practice requires data on the reliability of such measurements.

METHODS: We serially measured hsCRP and IL-6 concentrations in up to 6 blood samples taken at monthly intervals from 200 post–myocardial infarction patients who participated in the AIRGENE study.

RESULTS: The mean (SD) of the ln-transformed plasma concentrations (in milligrams per liter for hsCRP and nanograms per liter for IL-6) for all participants over all samples was 0.16 (1.04) for hsCRP and 0.76 (0.57) for IL-6, with no significant differences between men and women. The within-individual and analytical variance component for the ln-transformed hsCRP data was 0.37, and the between-individual variance component was 0.73. For the ln-transformed IL-6 data, these values were 0.11 and 0.22, respectively. A substantial part of the total variation in plasma hsCRP and IL-6 concentrations was explained by the between-individual variation (as a percentage of the total variance, 66.1% for the ln-transformed hsCRP data and 66.2% for the ln-transformed IL-6 data). For both markers, 2 measurements were needed to reach a sufficient reliability.

CONCLUSIONS: Our results demonstrate considerable stability and good reproducibility for serial hsCRP and IL-6 measurements. Thus, there should be no major concern about misclassification in clinical practice if at least 2 subsequent measurements are taken.

Among the numerous emerging biomarkers, high-sensitivity C-reactive protein (hsCRP)[4] and interleukin-6 (IL-6) have received widespread interest *(1)*. In a recent metaanalysis of 54 prospective studies, the multivariable-adjusted risk ratio for coronary heart disease per 1-SD increase in the ln-transformed hsCRP concentration was 1.37 (95% CI, 1.27–1.48), a magnitude of effect larger than that observed in the same studies for cholesterol or blood pressure *(2)*. Several recent studies have shown a statistically significant contribution of hsCRP to coronary-risk prediction that is independent of the Framingham risk score, with better discrimination, better calibration, and improved reclassification of individuals at risk *(3, 4)*. The JUPITER trial (Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin) demonstrated that the use of hsCRP as a biomarker to screen for low-grade inflammation may be an important tool for identifying individuals at increased risk who would benefit most from targeted preventive interventions *(5)*.

IL-6 is an "upstream" proinflammatory cytokine that stimulates hepatocytes to synthesize acute-phase response proteins such as CRP *(6)*. A systematic review of 17 prospective studies demonstrated a pooled odds ratio of coronary heart disease per 1-SD increase in the baseline IL-6 concentration of 1.26 (95% CI, 1.19–1.35) after multivariable adjustment, suggesting a moderate role in cardiovascular risk assessment *(7)*.

The concentrations of inflammatory biomarkers, however, are influenced by analytical and physiological variation *(8)*. The implementation of hsCRP and IL-6 measurements into clinical practice requires sound data on the reliability of such measurements. Although some data on variation in healthy individuals have been published, data are still scarce for the medium-term analytical and physiological variation of hsCRP and IL-6 measurements in patients with cardiovascular

[4] Nonstandard abbreviations: hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; JUPITER, Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin; MI, myocardial infarction; AIRGENE, Air Pollution and Inflammatory Response in Myocardial Infarction Survivors: Gene-Environment Interaction in a High Risk Group; VC_b, between-individual variance component; VC_{w+a}, combined within-individual and analytical variance component; ICC, intraclass correlation coefficient of reliability; IoI, index of individuality; CARE, Cholesterol and Recurrent Events.

disease *(9)*. Therefore, we investigated analytical imprecision and intra- and interindividual physiological variation in serial hsCRP and IL-6 measurements conducted over a 6-month period in a cohort of stable post–myocardial infarction (MI) patients.

The design and general protocol of the multicenter AIRGENE (Air Pollution and Inflammatory Response in Myocardial Infarction Survivors: Gene-Environment Interaction in a High Risk Group) study have recently been described in detail *(10)*. The present analysis was restricted to study participants from Augsburg, Germany, and included 200 post-MI patients (39–76 years), from whom approximately 6 blood samples were collected at monthly intervals between May 2003 and March 2004. Cholesterol parameters, triglycerides, and blood pressure were measured only at baseline. Individuals with disorders associated with an acute-phase reaction were excluded *(10)*.

Serum IL-6 concentrations were measured by ELISA *(11)*, and hsCRP concentrations were measured with a high-sensitivity latex-enhanced nephelometric assay on a BN II analyzer (Siemens) *(12)*. All analyses were run in a blinded fashion.

All statistical analyses have been described in detail elsewhere *(10)* and followed concepts described by Fleiss *(13)*, Bland and Altman *(14)*, and Fraser and Harris *(15)*. hsCRP and IL-6 concentrations were ln-transformed to achieve an approximately normal distribution. For the analysis of physiological variation, we computed estimates for the between-individual variance component (VC_b) and a combined within-individual and analytical variance component (VC_{w+a}), assuming a nested normal random-effects model and using the SAS MIXED procedure (restricted maximum-likelihood method, RANDOM statement). Moreover, we evaluated within-individual and analytical variation by assessing critical differences. The repeatability of measurements for comparing individuals or groups of individuals was characterized by calculating the intraclass coefficient (ICC). We also calculated an index of individuality (IoI), with reference values ≤ 0.6 indicating little utility of the test for detecting unusual individual results and values ≥ 1.4 indicating the usefulness of test results in many settings. The number of measurements required to achieve a reliability of 75% was computed with the Spearman–Brown prediction formula. Finally, to assess differences between various subgroups in within-individual and analytical variation, we tested the homogeneity of variances with the asymptotic χ^2 distribution of the likelihood ratio test statistic and compared the models with equal and unequal variances.

Nonfasting blood samples were collected during a mean follow-up period of 7.3 months. The mean number of samples per individual was 5.7, and at least 6 blood samples were drawn from 179 study participants (89.5%), for a total of 1144 blood samples. The mean age of the study participants was 61.9 (9.0) years, with 82% being men (Table 1). The mean time since the last MI was 2.1 (0.9) years, and 12.5% of the study participants had experienced recurrent MI.

Table 1. Demographic, clinical and laboratory characteristics of study participants at baseline visit (n = 200).[a]

Characteristic	Post-MI patients
Sex, %	
Men	82.0
Women	18.0
Age, years	61.9 (9.0)
Median (range)	63 (39–76)
MI, %	
First MI	87.5
Recurrent MI	12.5
Body mass index, kg/m^2	28.8 (4.0)[b]
Systolic BP,[c] mmHg	128.4 (19.9)
Diastolic BP, mmHg	78.2 (10.6)
Physical activity, %	
Low or moderate	70.0
High	30.0
Smoking status, %	
Never smoker	31.0
Ex- or occasional smoker	69.0
Current smoker	0.0
Alcohol intake, %[d]	
None	14.7
Moderate	67.9
Heavy	17.7
History of diabetes, %	17.5
History of hypertension, %	51.0
Statin intake, %	86.5
Total cholesterol, mmol/L	4.68 (1.00)
HDL cholesterol, mmol/L	1.24 (0.31)
hsCRP, mg/L	1.0 (2.6)[e]
Median (range)	1.0 (0.2–16.9)
IL-6, ng/L	2.2 (1.6)[e]
Median (range)	2.2 (0.5–9.6)

[a] Data are presented as the mean (SD) unless otherwise indicated.
[b] BP, blood pressure.
[c] One missing value.
[d] Two missing values.
[e] Data are presented as the geometric mean and geometric SD.

Table 2 shows the values calculated for the measures of variation for ln-transformed hsCRP and IL-6

Table 2. Medium-term variation of hsCRP and IL-6 measurements.[a]

Statistic	Ln hsCRP	Ln IL-6
No. of individuals	200	200
No. of measurements	1144	1144
Mean ln concentration (SD)	0.16 (1.04)	0.76 (0.57)
Variance components		
VC_b	0.73	0.22
VC_{w+a}	0.37	0.11
Proportion of total variance, %		
Between individuals	66.1	66.2
Within individuals plus analytical	33.9	33.8
Within-individual plus analytical variation		
95% repeatability coefficient	1.69	0.92
ICC	0.66	0.66
No. of measurements needed[b]	2	2
IoI	0.72	0.71

[a] All measures relate to ln-transformation of the hsCRP and IL-6 concentrations (in milligrams per liter for hsCRP and nanograms per liter for IL-6).
[b] Number of samples required for an ICC of 0.75.

concentrations. The mean (SD) of ln-transformed plasma concentrations (in milligrams per liter for hsCRP and nanograms per liter for IL-6) for all individuals over all samples was 0.16 (1.04) for hsCRP and 0.76 (0.57) for IL-6.

The VC_{w+a} for ln-transformed hsCRP data was 0.37, and the VC_b was 0.73. For ln-transformed IL-6 data, these values were 0.11 and 0.22, respectively. An important part of the total variance in plasma hsCRP and IL-6 concentrations was explained by between-individual variance, which was 66.1% of the total hsCRP variance, whereas the within-individual and analytical variance was 33.9% of the total variance (66.2% and 33.8%, respectively, for IL-6). ICC values calculated for hsCRP and IL-6 measurements (0.66 for both) for the within-individual and analytical reproducibility indicated a good correlation of serial measurements from the same individual during follow-up. We also calculated the required number of measurements for the present study population and found that 2 serial hsCRP and IL-6 measurements were required to achieve an ICC of 0.75. Finally, our calculation of IoI, which describes the relationship between within-individual variation and between-individual variation, yielded a value of 0.72 for hsCRP and 0.71 for IL-6.

As shown in Table 1 in the Data Supplement that accompanies the online version of this Brief Communication (http://www.clinchem.org/content/vol56/issue5), male sex, increasing age, and a lower body mass index were determinants of increased variation in hsCRP concentration, whereas male sex, decreasing age, a lower body mass index, and the absence of statin intake determined greater variation in IL-6 concentration.

Our data demonstrate considerable stability in and good reproducibility of serial hsCRP and IL-6 measurements. The precision of the assays was good. Only 33.9% of the total variation in the ln-transformed hsCRP data and 33.8% of the variation in the ln-transformed IL-6 data were explained by the within-individual variation or by analytical issues. Interestingly, a previous large meta-analysis showed that the reproducibility of hsCRP measurements over time was comparable to that of cholesterol and blood pressure, demonstrating good reproducibility not only in the secondary prevention setting but also in the primary prevention setting *(2)*. The ICC values of 0.66 further demonstrate an acceptable within-individual reproducibility of serial hsCRP and IL-6 measurements. To achieve an ICC of 0.75, however, we recommend 2 serial measurements of hsCRP. A similar number of measurements was found to be needed for total cholesterol, and the National Cholesterol Educational Program guidelines therefore have recommended collecting 2 samples a week apart before deciding on a therapeutic strategy *(9, 16)*. Our within-individual variation is generally consistent with estimates from previous studies of primary prevention. Ockene et al. reported a within-individual SD of 1.66 mg/L and, similar to our study, an ICC of 0.66 *(9)*. Similarly, Glynn et al. reported comparable reproducibility data for >34 000 assessments of hsCRP over 4 years within the JUPITER trial's placebo arm *(17)*. The estimated ICC for the ln-transformed hsCRP data was 0.54 and remained unchanged after the investigators controlled for time. In the Cholesterol and Recurrent Events (CARE) trial, hsCRP concentrations were also measured over time in stable post-MI patients *(18)*. Blood samples were taken for measurement twice, during prerandomization and at the 60-month visit after randomization. The absolute magnitude of the correlation coefficient for hsCRP in these data was similar to that observed for total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides over the 5-year follow-up period.

hsCRP and IL-6 differed with respect to differences in the variation of specific subgroups. Male sex and a lower body mass index were determinants of increased variation in hsCRP and IL-6, whereas the absence of statin intake determined greater variation only in IL-6 concentration. Increasing age was associated with higher hsCRP variation but lower IL-6 variation. In contrast, a previous analysis including all AIRGENE participants showed a U-shaped function for the influence of age on hsCRP variation *(19)*.

The strengths of our study are the use of the well-phenotyped AIRGENE cohort as a registry-based sample of post-MI patients, the fairly large sample size for this kind of study, and 6 repeat measurements taken at monthly intervals under well-standardized conditions. Nearly all samples were collected at the same time of day (98%) and on the same weekday (95%) to minimize the impact of circadian and day-to-day variation.

One limitation that should be kept in mind is that extrapolation of results from the AIRGENE cohort to healthy individuals may not be justified, because the physiological variation in cases of pathologic conditions may be different from that in apparently healthy individuals. The fact that samples were not collected under fasting conditions may have contributed to the observed variation. Moreover, only a small number of female participants (approximately 20%) were recruited in the present study, and the participants were mainly middle-aged individuals. Finally, we note that 87% of our study participants were receiving statin therapy, which has been shown to lower hsCRP concentrations *(5)*. This feature may have been associated with the lower variation in hsCRP concentration observed in our population; however, the fact that we investigated stable post-MI patients may have counterbalanced this concern.

Our results demonstrate considerable stability and good reproducibility of serial hsCRP and IL-6 measurements over the medium term. Thus, there should be no concern about misclassification in clinical practice, provided that 2 subsequent blood samples are taken for measurements.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures of Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: W. Koenig, Steering Committee of the JUPITER trial.
Stock Ownership: None declared.
Honoraria: W. Koenig, AstraZeneca.
Research Funding: The AIRGENE study was funded as part of the European Union's 5th Framework Program, key action number 4: "Environment and Health," contract number QLRT-2002–02236.
Expert Testimony: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

Acknowledgments: The authors are indebted to the AIRGENE study group. We also thank Gerlinde Trischler (University of Ulm) for excellent technical assistance and Prof. Peter Kern, University of Ulm, for providing access to the BN II analyzer. Finally, we express our appreciation to all study participants.

References

1. Koenig W, Khuseyinova N. Biomarkers of atherosclerotic plaque instability and rupture. Arterioscler Thromb Vasc Biol 2007;27:15–26.
2. Emerging Risk Factors Collaboration. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 2010;375:132–40.
3. Ridker PM, Buring JE, Rifai N, Cook NR. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score. JAMA 2007; 297:611–9.
4. Wilson PW, Pencina M, Jacques P, Selhub J, D'Agostino R Sr, O'Donnell CJ. C-reactive protein and reclassification of cardiovascular risk in the Framingham Heart Study. Circ Cardiovasc Qual Outcomes 2008;1:92–7.
5. Ridker PM, Danielson E, Fonseca F, Genest J, Gotto AM Jr, Kastelein JJ, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008; 359:2195–207.
6. Le JM, Vilcek J. Interleukin 6: a multifunctional cytokine regulating immune reactions and the acute phase protein response. Lab Invest 1989; 61:588–602.
7. Danesh J, Kaptoge S, Mann A, Sarwar N, Wood A, Angleman SB, et al. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. PLoS Med 2008;5:e78.
8. Rudez G, Meijer P, Spronk HM, Leebeek FW, ten Cate H, Kluft C, de Maat MP. Biological variation in inflammatory and hemostatic markers. J Thromb Haemost 2009;7:1247–55.
9. Ockene IS, Matthews CE, Rifai N, Ridker PM, Reed G, Stanek E. Variability and classification accuracy of serial high-sensitivity C-reactive protein measurements in healthy adults. Clin Chem 2001;47:444–50.
10. Peters A, Schneider A, Greven S, Bellander T, Forastiere F, Ibald-Mulli A, et al. Air pollution and inflammatory response in myocardial infarction survivors: gene-environment interactions in a high-risk group. Inhal Toxicol 2007;19(Suppl 1): 161–75.
11. Ljungman P, Bellander T, Nyberg F, Lampa E, Jacquemin B, Kolz M, et al. DNA variants, plasma levels and variability of interleukin-6 in myocardial infarction survivors: results from the AIRGENE study. Thromb Res 2009;124:57–64.
12. Kolz M, Koenig W, Müller M, Andreani M, Greven S, Illig T, et al. DNA variants, plasma levels and variability of C-reactive protein in myocardial infarction survivors: results from the AIRGENE study. Eur Heart J 2008;29:1250–8.
13. Fleiss JL. The design and analysis of clinical experiments. New York: Wiley; 1986.
14. Bland JM, Altman DG. Measuring agreement in method comparison studies. Stat Methods Med Res 1999;8:135–60.
15. Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. Crit Rev Clin Lab Sci 1989;27:409–37.
16. Summary of the second report of the National Cholesterol Educational Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). JAMA 1993;269:3015–23.
17. Glynn RJ, MacFadyen JG, Ridker PM. Tracking of high-sensitivity C-reactive protein after an initially elevated concentration: the JUPITER study. Clin Chem 2009;55:305–12.
18. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E, for the Cholesterol and Recurrent Events (CARE) investigators. Long-term effects of pravastatin on plasma concentration of C-reactive protein. Circulation 1999;100:230–5.
19. Rückerl R, Peters A, Khuseyinova N, Andreani M, Koenig W, Meisinger C, et al. Determinants of the acute-phase protein C-reactive protein in myocardial infarction survivors: the role of comorbidities and environmental factors. Clin Chem 2009;55:322–35.

Previously published online at
DOI: 10.1373/clinchem.2010.143719