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Distributions of C-reactive Protein Measured by High-Sensitivity Assays in Apparently Healthy Men and Women from Different Populations in Europe, Armin Imhof,¹ Margit Fröhlich,¹ Hannelore Loewel,² Nicole Helbecque,³ Mark Woodward,⁴ Phillipe Amouyel,³ Gordon D.O. Lowe,⁵ and Wolfgang Koenig^{1*} (¹ Department of Internal Medicine II, Cardiology, University of Ulm, D-89081 Ulm, Germany; ² GSF-National Research Center for Environmental and Health, Institute for Epidemiology, 85764 Neuherberg, Germany; ³ Inserm U508, Institut Pasteur de Lille, 59079 Lille Cedex, France; ⁴ Institute for International Health, University of Sydney, Sydney NSW 2042, Australia; ⁵ Department of Medicine, Royal Infirmary, Glasgow G31 2ER, United Kingdom; * address correspondence to this author at: Department of Internal Medicine II, Cardiology University of Ulm, Robert-Koch-Strasse 8, D-89081 Ulm, Germany; fax 49-731-500-33872, e-mail wolfgang.koenig@medizin.uni-ulm.de)

C-reactive protein (CRP), the classic marker of acute-phase response, is an indicator of a variety of pathologic processes, including infections, tissue damage, and chronic inflammatory diseases (1,2). The majority of more than 15 well-conducted prospective studies in initially healthy individuals have shown a strong and independent association between concentrations of CRP within the reference interval (<5 mg/L) and future major cardiovascular events (3), although in some of them, no such association could be established (4-7). The summary estimate of the relative risk in formal metaanalysis was 2.0 (95% confidence interval, 1.6-2.5) (3). Furthermore, CRP has been shown to add to risk prediction beyond and above established cardiovascular risk factors (8). On the basis of data from the Physicians' Health Study and the Nurses' Health Study, an algorithm for risk assessment of future coronary events that combines both CRP concentration and the ratio of total cholesterol to HDL-cholesterol has recently been proposed (9).

Because atherosclerosis represents a low-grade inflammatory process in the vascular bed, high-sensitivity (hs) assays are needed when using circulating CRP concentrations for risk prediction in cardiovascular diseases. Such assays have been developed and are now commercially available (10,11). However, before screening of individuals at risk can be recommended, CRP distributions in apparently healthy adults in the general population must be known. Such information is scarce. Furthermore, in previous reports, women using oral contraceptives or receiving hormone replacement therapy (HRT), both of

which have been shown to significantly increase CRP concentrations, had not been excluded (12–15). In this report, we describe the frequency distribution of CRP concentrations in 13 527 adult men and women from different representative populations in Western Europe. Furthermore, for one area [the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) project in Augsburg], data from four surveys at 5-year intervals were available, thus providing information on potential CRP changes in the same population over time.

Seven cross-sectional samples, each randomly drawn from the general population of four different geographic areas in former West Germany, France, and Scotland (total of 16 945 men and women), were examined. The VERA (Verbundstudie Ernährungserhebung und Risikofaktoren Analytik) sample, representative of former West Germany in 1987–1988, consisted of 862 men and 1144 women 18–88 years of age. Also in Germany, the Augsburg surveys were performed in 1984–1985 (1074 men; ages 45–64 years), 1989–1990 (1550 men; 45–74 years), 1994–1995 (2450 men and 2451 women; 25–74 years), and, in the framework of the KORA (Kooperative Gesundheitsforschung in der Region Augsburg) program, in 1999–2000 (2090 men and 2171 women; 25–74 years). The MONICA Lille (France) survey of 1994–1995 consisted of 601 men and 594 women, ages 35–65 years, randomly selected from the general population. Finally, the MONICA north Glasgow survey of 1992 consisted of 928 men and 1030 women, ages 25–74 years. After the exclusion of women on oral contraceptives or HRT and of individuals with missing values for any variable, 13 527 participants remained for distribution analyses. Additional study details have been published elsewhere (10, 16, 17). All serum/plasma samples were immediately stored after collection and frozen at -70 to -90 °C until analysis.

CRP was determined in these populations by two different methods. A sensitive solid-phase monoclonal-polyclonal IRMA with a range of 0.05–10.0 mg/L was used for all samples except for the MONICA/KORA Augsburg survey 1999–2000 and for the Glasgow MONICA survey 1992. Method details have been described elsewhere (10). The intraassay CVs ranged from 5.6% for the 0.50 mg/L calibrator to 1.4% for the 0.10 mg/L calibrator; the interassay CVs ranged between 8.0% for the 0.5 mg/L calibrator and 0.5% for the 0.10 mg/L calibrator. All samples with values exceeding the upper limit of the assay range, i.e., 10 mg/L, were reassayed at appropriately higher sample dilutions. CRP concentrations in the MONICA/KORA Augsburg sample 1999–2000 and in the Glasgow MONICA sample 1992 were measured by a particle-enhanced immunonephelometric assay performed on a BN II Analyzer (Dade Behring) (11). The interassay CVs were $<6\%$ for both samples. There was no difference in CRP measurements between serum and plasma samples. Validation analyses between the two methods have been performed in a sample of 792 men and women from a case-control study (18). Comparisons according to Bland and Altman (19) after log-transforma-

tion of CRP concentrations gave a mean difference between the IRMA and the BN II of -0.11 with 95% limits of agreement of -1.17 and 0.95 , indicating excellent agreement between the two methods. The Spearman rank correlation coefficient was 0.87 (unpublished data).

In all samples, CRP concentrations were highly skewed to the right among both men and women. There was a trend to higher CRP values with increasing age in all samples. Median CRP values for men and women, the latter not taking oral contraceptives or HRT, up to 44 years of age were 0.6–1.1 mg/L among the seven samples, and among those 45 years of age and older, the median CRP values were 1.2–1.7 mg/L (Table 1). Slight differences between CRP medians in the samples could mainly be explained by the somewhat different age ranges covered, above and below the cutpoints of 45 years. Distributions of CRP were comparable between men and women among the different geographic areas studied and were stable over time in the MONICA Augsburg samples (men 45 years and above, 1.6–1.7 mg/L). Fewer than 5% of men and women had CRP >10 mg/L, indicating an ongoing high-grade inflammatory process, such as acute infection. However, a remarkable subsample of up to one-third of individuals had CRP concentrations >3 mg/L, which has been shown in several studies to be associated with an increased risk for future cardiovascular events (20).

We have described frequency distributions of hs-CRP in seven cross-sectional samples randomly selected from the adult general population of three European countries (Germany, France, and Scotland). Data sets included both genders, excluded women on oral contraceptives or HRT, and covered a wide age range. Furthermore, we looked for potential changes of CRP over time in the MONICA Augsburg samples covering a time period of ~ 15 years. In all samples, CRP distributions were highly skewed to the right, increased with age, and were similar in adult men and in women who neither used oral contraceptives nor received HRT. In addition, CRP concentrations in the general population did not substantially differ among the geographic areas studied and were stable over time.

On the basis of the risk prediction for cardiovascular endpoints associated with increased CRP (3, 21, 22), measuring this biomarker has recently been suggested as an additional tool for risk stratification (9). However, a new risk marker of disease needs to fulfill several requirements (23). It should show high sensitivity and specificity and low intraindividual variability but high interindividual variation. For CRP, sensitive and robust methods are commercially available (11). However, although CRP represents an extremely sensitive marker of the acute-phase response, it is completely unspecific and is influenced by a variety of disease states, including infections, rheumatic diseases, and malignancies among others (24).

Frequency distributions of a marker designated for risk assessment in the general population are inevitably required for identifying individuals at increased risk. For CRP, such reports are scarce, and, to the best of our knowledge, no data from European countries exist from

Table 1. CRP distributions in various European populations.

	Gender	Age, years	n	Percentile												
				5	10	20	25	33.3	40	50	60	66.6	75	80	90	95
MONICA Augsburg Survey 1984–1985	Men	45–64	990	0.3	0.4	0.6	0.7	0.9	1.1	1.6	2.2	2.8	3.8	4.6	6.8	10.1
MONICA Augsburg Survey 1989–1990	Men	45–74	1379	0.3	0.4	0.6	0.8	1.0	1.3	1.7	2.3	2.8	3.8	4.5	7.3	10.4
MONICA Augsburg Survey 1994–1995	Men	25–44	869	0.2	0.2	0.4	0.4	0.6	0.7	0.9	1.3	1.5	2.1	2.5	3.9	5.6
		45–74	1424	0.3	0.4	0.7	0.8	1.0	1.2	1.6	2.0	2.4	3.3	4.0	6.5	8.6
	Women ^a	25–44	624	0.1	0.2	0.3	0.4	0.5	0.5	0.7	1.0	1.2	1.7	2.2	4.1	6.9
		45–74	1031	0.3	0.4	0.7	0.9	1.1	1.3	1.7	2.2	2.7	3.4	4.2	6.2	8.8
MONICA/KORA Survey 1999–2000	Men	25–44	800	0.2	0.2	0.3	0.4	0.5	0.6	0.7	1.0	1.2	1.6	2.0	3.4	5.5
		45–74	1236	0.3	0.4	0.6	0.8	1.0	1.2	1.6	2.2	2.8	3.5	4.3	6.9	9.5
	Women ^a	25–44	645	0.2	0.2	0.3	0.4	0.5	0.6	0.7	1.0	1.3	1.8	2.2	4.6	8.0
		45–74	865	0.2	0.4	0.6	0.8	1.0	1.2	1.6	2.2	2.8	3.5	4.3	6.9	9.5
MONICA Glasgow Survey 1992	Men	25–44	158	0.2	0.2	0.2	0.2	0.4	0.5	0.8	0.9	1.1	1.4	1.7	3.2	6.3
		45–74	256	0.3	0.4	0.5	0.7	0.9	1.1	1.4	1.9	2.3	3.3	3.8	6.2	8.6
	Women	24–44	168	0.2	0.2	0.2	0.3	0.4	0.5	0.6	0.9	1.0	1.4	1.7	3.4	5.5
		45–74	297	0.2	0.2	0.4	0.6	0.8	0.9	1.2	1.6	1.9	2.6	3.2	6.4	9.8
MONICA Lille Survey 1994–1995	Men	35–44	162	0.2	0.3	0.5	0.6	0.7	0.8	1.1	1.4	1.7	2.5	2.8	4.8	8.9
		45–65	439	0.3	0.4	0.6	0.7	0.9	1.1	1.4	1.9	2.4	3.5	3.9	8.0	9.4
	Women ^a	35–44	98	0.1	0.3	0.4	0.4	0.5	0.6	0.8	1.0	1.4	1.8	2.9	5.3	7.8
		45–65	303	0.1	0.3	0.6	0.7	0.8	1.1	1.5	2.0	2.6	3.7	4.4	6.7	9.7
VERA Survey 1987–1988	Men	18–44	426	0.2	0.2	0.3	0.4	0.5	0.5	0.7	1.0	1.2	1.9	2.5	5.5	9.1
		45–88	380	0.3	0.4	0.6	0.6	0.8	1.1	1.7	2.2	2.9	4.1	4.9	7.3	9.8
	Women ^a	18–44	547	0.2	0.3	0.4	0.5	0.6	0.8	1.0	1.6	2.4	3.4	4.4	7.3	9.9
		45–80	430	0.3	0.4	0.5	0.6	0.8	1.0	1.4	1.9	2.5	3.3	4.1	6.5	8.4

^a Women on oral contraceptives or HRT were excluded.

which women on oral contraceptives or HRT have been excluded. Median CRP concentrations in these samples are comparable to those reported from other populations (25, 26). Furthermore, CRP distributions among men and women not receiving hormones are very similar; thus, sex-specific cutpoints for risk stratification are not needed. Moreover, CRP concentrations in regions from three European countries with different social status and lifestyle habits are remarkably consistent.

The present analysis has several limitations. Only single measurements were performed, which may not reflect the true long-term values for the respective individuals; and we studied only Caucasians. We did not exclude individuals with diseases that might have affected CRP concentrations because this would have led to a selection bias that could have affected the generalizability of our results. However, the numbers of such persons were small in all of the samples. Serum/plasma samples from all participants had been stored immediately after collection at -70 to -90 °C for up to 12 years. CRP is known to be a very stable protein, and in one study (27), no significant change was seen in CRP concentrations of samples stored at 4 °C for 5 months and at -20 °C for up to 25 months.

Our own data from measurements of pooled samples over a period of 5 years also showed no systematic shift (unpublished data). Our analyses also have several strengths. The two hs-CRP assays that we used showed excellent agreement and were standardized according to the WHO reference standard (85/506) (28). Finally, the examined populations were representative of the general population in three Western European countries.

Unsolved issues remain relating to the fact that, despite similarities in its distribution, CRP has been shown to be predictive for future cardiovascular events in various populations with greatly differing absolute risks (3, 29, 30). In addition, CRP distributions are comparable between men and women, but cardiovascular risk is clearly different between them. Finally, CRP concentrations were stable over time although, e.g., data from the MONICA project indicate a decrease in coronary heart disease morbidity and mortality in several centers during a 10-year period.

In summary, our data describing CRP concentrations in representative populations may be useful for the clinician and the general practitioner in assessing the risk of future cardiovascular events by means of hs-CRP.

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Simultaneous Genotyping of Seven Single-Nucleotide Polymorphisms in the *MDR1* Gene by Single-Tube Multiplex Minisequencing, Pai-Chung Gwee,¹ Kun Tang,¹ John M.Z. Chua,^{1,2} Edmund J.D. Lee,³ Samuel S. Chong,^{4,5} and Caroline G.L. Lee^{1,2,6*} (Departments of ¹Biochemistry, ³Pharmacology, and ⁴Pediatrics, National University of Singapore, Singapore 117597, Singapore; ²Division of Medical Sciences, National Cancer Center, Singapore 169610, Singapore; Departments of ⁵Pediatrics and ⁶Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD 21205; *address correspondence to this author at: Division of Medical Sciences, National Cancer Center, Level 6, Lab 5, 11 Hospital Dr., Singapore 169610, Singapore; fax 65-6224-1778, e-mail bchleec@nus.edu.sg)

Responses to different drugs can vary widely among different individuals as a result of genetic variations in drug-metabolizing enzymes, transporters, receptors, and/or other cofactors. The multidrug resistance 1 (*MDR1*) transporter, a well-characterized member of the ATP-binding cassette superfamily, was shown to efflux a wide variety of structurally and functionally unrelated drugs, including anticancer, antiarrhythmic, antidepressant, antipsychotic, and antiviral agents. The pharmacogenetics of the *MDR1* multidrug transporter have recently received much scientific attention. Several single-nucleotide polymorphisms (SNPs) have been identified in the *MDR1* gene; some occur only in specific ethnic groups, whereas others occur in all ethnic groups but at significantly different allele frequencies among the different races [see Ref. (1) and references therein]. Nonetheless,