

Table 1. Detection results for 11 patients with acute myeloid leukemia (M2a).^a

Sample	BSA	CD13	CD14	CD15	CD45	CD33	CD34	HLA-DR
1	0	263	1	126	292	371	488	305
2	3	182	0	108	228	292	356	246
3	1	195	3	1	343	385	502	352
4	2	383	1	235	420	478	3	390
5	0	168	2	0	256	1	282	295
6	0	252	1	308	337	350	304	318
7	1	172	0	272	316	0	1	294
8	0	234	2	179	285	1	279	218
9	2	262	2	272	214	268	327	254
10	0	330	1	0	307	331	352	298
11	2	234	0	302	181	242	276	202

^a Results are given as the number of leukocytes/spot.

To increase leukocyte capture, various experimental conditions, such as the antibody fixing time, incubation time, and leukocyte concentration, were optimized. The results for the CD45 immobilization experiments showed that various experimental conditions, such as antibody fixing time, incubation time, and leukocyte concentration, affected the leukocyte capture efficiencies. We identified the following optimized conditions: 37 °C, 16-h antibody fixation, 120-min incubation time, and 5.0×10^5 leukocytes/mL, as determined by hemocytometer counting. Seven monoclonal antibodies for a set of leukocyte surface antigens (including CD13, CD15, CD33, CD34, HLA-DR, CD14, and CD45) have been immobilized on a modified slide (left side of Fig. 1). On the slide we spotted CD45 as the positive control spot and bovine serum albumin (BSA) as the negative control spot. CD13, CD15, CD33, CD34, and HLA-DR are positive for M2a leukemia, and CD14 is negative for M2a leukemia (9). Leukocytes from 11 patients with leukemia M2a were incubated with the slides. Four spots (CD13, CD15, CD34, and CD14) are shown in the image on the right in Fig. 1. The spots with leukocytes indicate the existence of associated cell surface antigens, and the cell counts on these spots could be used to obtain the phenotype of the leukemia. Table 1 shows the total experimental results of all 8 spots for 11 patients. The results are in agreement with the flow cytometric results.

Acute leukemia displays characteristic patterns of surface antigens (CD antigens), which facilitate their identification and proper classification and hence play an important role in instituting proper treatment plans (1). In current practice, the surface antigens of cells in acute leukemia are analyzed by two-, four-, or six-color flow cytometry (10). However, cell immuno-chip technology can also detect cell surface antigens and might be convenient for identifying and confirming the patterns of a large number of surface antigens simultaneously and, perhaps, achieving high throughput.

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Hormonal Regulation of Circulating C-Reactive Protein in Men, Alexander Kovacs,^{1,2} Peter Henriksson,³ Anders Hamsten,^{1,2} Håkan Wallén,³ Johan Björkegren,² and Per Tornvall^{1,2*} (¹ Department of Cardiology and ² Atherosclerosis Research Unit, King Gustaf V Research Institute, Karolinska University Hospital, and ³ Department of Medicine, Danderyd Hospital, Karolinska Institute, Stockholm, Sweden; * address correspondence to this author at: Department of Cardiology, Karolinska University Hospital, S-17176 Stockholm, Sweden; fax 46-8-311044, e-mail per.tornvall@karolinska.se)

The plasma concentration of C-reactive protein (CRP), measured by high-sensitivity methods, is a reliable marker of risk of future coronary heart disease (CHD) in healthy individuals (1, 2) and of CHD death in patients with unstable angina pectoris or non-Q-wave myocardial infarction (3). The possibility of hormonal regulation of circulating CRP is of great interest because male gender is considered an independent risk factor for CHD and estrogen has many beneficial cardiovascular effects in women (4).

Both in vivo and in vitro data support the hypothesis that interleukin-6 (IL-6) is the main inducer of the acute-phase CRP response, but other factors such as IL-1 β might potentiate IL-6 stimulation of CRP (5). The factors determining unstimulated CRP expression are less known.

Because of the strong association between CRP and CHD and the fact that previous studies have shown that

CRP to a large extent is regulated on the transcriptional level (5), it is of great interest to identify factors that regulate CRP production. A study of randomized treatment of prostate cancer by orchidectomy or estrogen allowed us to investigate the hormonal influence on circulating CRP concentrations in middle-aged and elderly men.

A total of 100 consecutive patients with prostate cancer suitable for hormonal therapy were enrolled in the study. Patients were randomized to either orchidectomy or estrogen treatment. The estrogen regimen was 160 mg of polyestradiol phosphate intramuscularly every month during the first 3 months, then 80 mg monthly, plus 1 mg of ethinylestradiol orally for 2 weeks, followed by 150 μ g daily. For different reasons, only 83 patients completed the study (orchidectomy, $n = 41$; estrogen, $n = 42$). Blood samples were taken before and 6 months after surgery or start of estrogen treatment. The study group has been described previously (6). All patients gave their informed consent to participate in the study, the protocol of which had been approved by the local ethics committee.

The high-sensitivity CRP concentration was measured in serum by a nephelometric method using a particle-enhanced reagent (Dade Behring) on a Behring BN ProSpec analyzer (Dade Behring). Serum concentrations of IL-6 and IL-1 β were measured in duplicate by ELISA (R&D Systems). Serum concentrations of IL-6 and IL-1 β were below the detection limits in 3 and 40 patients, respectively, on either the first or second or both occasions of blood sampling.

Values are expressed as the numbers (%), mean (SD), or median (interquartile range). Statistical analysis using analysis of covariance was performed on logarithmically transformed values with the SAS 8.2 program. Multivariate analysis was performed taking into account CRP concentrations before treatment, smoking status, and IL-6 concentrations after treatment. P values were corrected for multiple testing.

The basic characteristics of the study groups are presented in Table 1 of the Data Supplement that accompanies the online version of this Technical Brief at <http://www.clinchem.org/content/vol51/issue5/>.

Univariate analysis showed that orchidectomy tended to decrease circulating CRP concentrations ($P = 0.45$), whereas estrogen treatment tended to increase CRP ($P > 0.5$). There was no difference in CRP concentrations between the groups before treatment, whereas after treat-

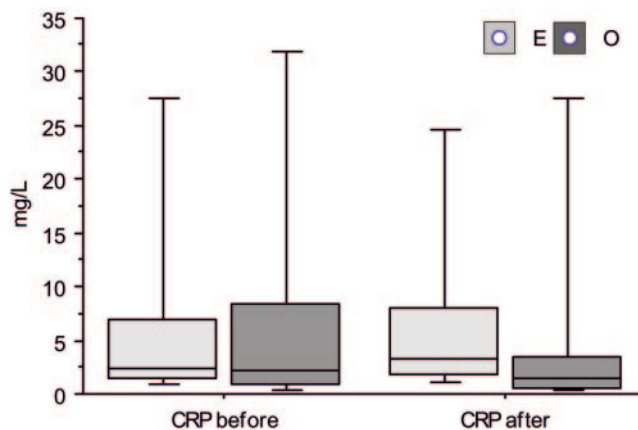


Fig. 1. Box-plots of CRP concentrations grouped by treatment and time. The error bars encompass 95% of the values, and the boxes encompass 50%. O, orchidectomy; E, estrogen.

ment, patients treated with estrogen had twofold higher CRP concentrations than patients who underwent orchidectomy ($P = 0.0003$; Fig. 1 and Table 1). Multivariate analysis showed that the difference in CRP concentrations after treatment was highly significant ($P = 0.0008$; Fig. 1 and Table 1).

There were no differences between the orchidectomy and estrogen groups regarding serum IL-1 β and IL-6 concentrations either before or after treatment (Table 1).

The major finding that estrogen treatment had a pronounced effect on circulating CRP concentrations in men extends the results of previous studies of oral contraceptives and hormone replacement therapy in women (7). Because treatments varied with respect to conjugates of estrogen, estradiol, and their metabolites, it is difficult to compare results between the different studies. Furthermore, the route of administration is of great importance because oral administration leads to increased circulating CRP concentrations whereas transdermal administration does not appear to have any effect on circulating CRP. The authors of one recent study using increasing doses of oral estrogen came to the conclusion that the effect of estrogen was dose-dependent (8). The oral dose used in the present study was higher than the highest dose given in the study by Prestwood et al. (8). The results of our study therefore support the conclusion that high doses of estrogen are associated with increased circulating CRP concentrations.

Table 1. IL-1 β , IL-6, and CRP concentrations.

	IL-1 β , ng/L		IL-6, ng/L		CRP, mg/L	
	Orchidectomy	Estrogen	Orchidectomy	Estrogen	Orchidectomy	Estrogen
Before treatment						
Median	0.28	0.16	5.43	5.17	2.14	2.32
Interquartile range	0.08–0.43	0.07–0.24	3.79–11.20	3.90–7.88	0.86–8.46	1.38–6.97
n	23	20	39	41	41	42
After treatment						
Median	0.12	0.18	4.61	5.02	1.51	3.32
Interquartile range	0.05–0.33	0.08–0.52	3.08–8.04	3.16–7.93	0.61–3.50	1.77–7.96
n	23	20	39	41	41	42

The mechanism behind the pronounced effect of estrogen on CRP is most likely an effect on gene expression in the liver. Strong support for this assumption that this effect is mediated by estrogen itself and not by a factor stimulated by estrogen comes from the observations that the effect is dose-dependent, is seen in both women and men, and occurs only after oral treatment. It is not clear whether the effect of estrogen on gene expression is mediated by transcriptional activation or RNA stabilization. No hormone response elements have been discovered in the promoter of the CRP gene, but one study has shown that estrogen can stimulate the transcription factor C/EBP- β , which is involved in CRP transcription (9).

Our data contradict findings in mice transgenic for human CRP, in which testosterone but not estrogen was essential for expression of both unstimulated and acute-phase CRP (10). This discrepancy could be explained by the fact that the transgenic mice had the human CRP gene integrated into another chromosomal context. Because of study design, we cannot exclude that testosterone has an effect on expression of the CRP gene.

The findings of the present study might be of major relevance because CRP is associated with an increased risk of CHD, and hormone replacement therapy has been shown to increase the risk of CHD (11). In addition, in the present study, patients who underwent estrogen therapy had more cardiovascular events (6).

One limitation of the present study is that participants suffered from prostate cancer, which could possibly, through some unknown mechanism, trigger CRP expression independently of IL-6. However, the randomized design of the study, which produced treatment groups that were balanced regarding severity of disease, should minimize this risk. In this context, it cannot be excluded that estrogen might have pro-inflammatory effects on the prostate itself. However, the correction for IL-6 stimulation of CRP is likely to exclude this reason for the difference in circulating CRP concentrations between the two groups. Another limitation is that the analyses were performed on blood samples that had been frozen for more than 20 years. Such long-term storage might lead to concentration of samples as a result of freeze drying. Speaking against such an effect is the fact that serum CRP concentrations were within the reference interval. Furthermore, freeze-drying effects would most likely affect both treatment groups similarly.

In conclusion, we have shown that estrogen treatment in middle-aged and elderly men is associated with increased circulating CRP concentrations, indicating a role for estrogen in the regulation of unstimulated CRP. The results clearly emphasize the need for further molecular studies of hormonal effects on the regulation of CRP expression.

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N-Terminal Pro-B-Type Natriuretic Peptide Concentrations Are Markedly Higher in the Umbilical Cord Blood of Newborns than in Their Mothers, Angelika Hammerer-Lercher,^{1*} Johannes Mair,² Gernot Tews,³ Bernd Puschendorf,¹ and Rudolf Sommer⁴ (¹ Biocenter, Division of Clinical Biochemistry, and ² Department of Internal Medicine, Clinical Division of Cardiology, Innsbruck Medical University, Austria; ³ Gynecologic and Paediatric Hospital Linz, Linz, Austria; ⁴ Institute of Medical and Chemical Laboratory Diagnostics, Gynecologic and Paediatric Hospital Linz, Linz, Austria; * address correspondence to this author at: Biocenter, Division of Clinical Biochemistry, Innsbruck Medical University, Fritz-Pregl-Strasse 3, A-6020 Innsbruck, Austria; fax 43-512-507-2876, e-mail Angelika.Lercher@uibk.ac.at)

Natriuretic peptides are well-established markers in adult heart failure patients (1) and may also be useful for identifying neonates or children with cardiac diseases. Recent studies demonstrated high N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations in healthy neonates with a subsequent rapid decrease within several days (2, 3). However, in the transition from fetal to neonatal life, the physiologic role of natriuretic peptides is not fully understood. Furthermore, it is currently not known whether natriuretic peptides in the fetal circulation derive from the fetus itself or whether there is a placental exchange of maternal natriuretic peptides. The aim of this study, therefore, was to determine the NT-proBNP concentrations in healthy neonates and to compare their concentrations with the values for their respective mothers to indirectly demonstrate a possible placental NT-proBNP exchange.

From 100 neonates delivered consecutively between