trolled by the tube manufacturer (specific gravity, yield stress, viscosity, density, and tube material), some by the hospital laboratory (centrifugation speed, temperature, acceleration and deceleration conditions, and storage conditions), and some of which are patient specific [heparin therapy, low hematocrits, increased plasma proteins (1), and the use of iodinated blood contrast media (2)].

A retrospective analysis in our clinical chemistry laboratory of total protein requests showed that during a 5.5-year period, 5 of 13,221 patients (0.04%) had a total protein concentration $\leq 135$ g/L. Therefore, we anticipate that our laboratory should observe this phenomenon several times a year. This number may vary depending on the degree of oncology-related patients visiting the hospital. To our knowledge, only one single case report has been published on this topic for a blood collection system from a different manufacturer (1).

Laboratories, in which preanalytical steps include automatic centrifugation and sample transport to on-line chemistry analyzers, are particularly vulnerable for occlusion of sample probes from inappropriately separated blood samples. Visual checks to determine the adequacy of barrier formation after centrifugation should prevent the inappropriately separated samples from being transferred to the analyzer, although labels on tubes can often prevent rapid visual inspection.

Despite the fact that inappropriate barrier formation is occurring at a low frequency, the impact on costs (sample probe replacement and downtime of the analyzer causing discontinuation of the workflow process) and patient outcomes (e.g., potential danger of reporting falsely low results when no sample is aspirated) can be substantial. Laboratories and tube manufacturers should be aware of the limitation of using any tubes containing gel-separator in patients with high plasma viscosity because of the presence of high total protein concentrations. In these particular cases, subsequent blood drawings should be collected in non–separator-based blood collection tubes. We will conduct further with the tube manufacturer to assess the amount of total protein at which gel barrier formation is compromised. Our observation contributes to the increasing awareness of the impact on patient outcomes and the costs of laboratory errors occurring in the preanalytical phase (3).

References

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Survival Related to Plasma C-Reactive-Protein in Nonagenarians Is Modified by Apolipoprotein E Genotype

To the Editor:
In the present study, we combined 2 thoroughly studied markers of inflammation and lipid metabolism—sensitive C-reactive protein (hsCRP) and apolipoprotein E genotype (apoE)—to predict mortality in the elderly (1, 2). Plasma hsCRP concentration (1) and apoE genotype (2) are both important predictors of coronary artery disease (CAD) and stroke (1, 2). The risk of myocardial infarction in people with hsCRP concentrations in the highest third of the population range is twice that of those with hsCRP in the lowest third (1). ApoE genotype is a key regulator of lipoprotein metabolism (2, 3), and the e4-allele has been associated with ini-
creased plasma LDL cholesterol concentration and CAD (2, 3). These 2 factors are biologically linked; low hsCRP concentrations have been measured in apoE e4-allele carriers, indicating that variation in plasma hsCRP concentration is partially determined by the common apoE (e2, e3 and e4) gene polymorphism (3, 4).

ApoE e4 allele carrier status has been modified to predict the valuable of hsCRP for CAD (3). Therefore we hypothesized that apoE e4 status may also modify the clinical value of plasma hsCRP in predicting total mortality among nonagenarians.

The present study included 291 nonagenarian volunteers who were participants in the population-based Vitality 90+ study, which included all nonagenarians born in 1909–1910 and living in the city of Tampere and its surroundings in southern Finland in 2000. At the beginning of the study, we determined apoE gene polymorphisms and hsCRP concentrations in 285 persons (67 men, 218 women) (4). Four years later, in April 2004, we extracted mortality data from the Finnish Register of Causes of Death.

As hypothesized, multinomial regression analysis revealed a statistically significant interaction between apoE e4-allele group and hsCRP group (low/high) (P = 0.040) in relation to 4-year survival status. Therefore, we analyzed the apoE e4-allele carriers and noncarriers separately.

Cox regression analysis showed that in noncarriers of the apoE e4-allele, after adjustment for sex and baseline LDL cholesterol concentration, high plasma hsCRP concentrations (≥3.3 mg/L, upper third) were associated with increased mortality [odds ratio (OR) 1.95, 95% confidence interval (CI) 1.39–2.73, P < 0.001] but lower plasma hsCRP concentrations (<3.3 mg/L) were not. In e4-allele carriers no similar relationship between plasma hsCRP groups and mortality was found (OR 0.78, 95% CI 0.32–1.90, P = 0.589). The statistical power to detect the observed differences at the α level of 0.05 was >0.95 for noncarriers and <0.5 for apoE e4-carriers. The number of deaths according to apoE genotype and hsCRP groups is shown in Table 1. In all study participants, the mean (SD) plasma hsCRP concentrations of those who did not survive were higher than for those who did survive [6.62 (15.64) vs 3.39 (8.38) mg/L, P = 0.04]. ApoE allele or genotype distributions did not differ between survivors (n = 114) and nonsurvivors (n = 171).

Our findings suggest that the relationship between plasma hsCRP concentration and mortality in Finnish nonagenarians is dependent on apoE e4-allele carrier status.

In Finland in 2004 the main causes of death in persons older than 90 years were cardiovascular system diseases (51%) and mental/behavioral disturbances and neurological diseases (21%). Several of these diseases involve vascular pathology, in which CRP has been localized within atheromatous plaques, where it precedes and mediates monocyte recruitment (5). Moreover, in vitro studies have shown that CRP can bind to LDL, which in turn can enhance foam cell formation, stimulate tissue factor production in macrophages, and start coagulation (5). Therefore the relatively lower hsCRP concentration of apoE e4-allele carriers (4) may lead to slower recruitment of monocytes to atheroma, decreased binding of CRP to LDL (5), and slower foam cell and atheroma formation. In the case of low plasma LDL concentrations (e4-noncarriers) (4), high hsCRP concentration might be the rate-limiting step in the CRP-LDL complex and foam-cell formation in the arterial wall. This hypothesis is in line with findings indicating that persons with low LDL cholesterol and high plasma hsCRP can develop CAD (1).

One limitation of the study was that data were available on all-cause mortality, but not cardiovascular mortality. The small number of participants who were e4-allele carriers leads to low statistical power, making it difficult to rule out an effect of hsCRP in that group, and the data cannot be generalized to other age groups.

Nevertheless, our study results indicate that the prognostic value of hsCRP in predicting total mortality of Finnish nonagenarians is dependent on the apoE polymorphism.

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References


<table>
<thead>
<tr>
<th>Table 1. The number of deaths according to apoE genotype and hsCRP concentration during 4-year follow up.</th>
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<tbody>
<tr>
<td>hsCRP-concentration, mg/L</td>
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<td>Noa</td>
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<td>e3/e2</td>
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The numbers in parentheses indicate the total number of study participants.

Statistics: a χ2-test, difference between high and low hsCRP groups in the occurrence of deaths in apoE e4-noncarriers; P = 0.001; b in apoE e4 carriers, P = 0.489.
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Haptocorrin (Transcobalamin I) and Cobalamin Deficiencies

To the Editor:

Based on analyses in cobalamin-deficient patients before and after therapy, Morkbak and colleagues (1) confirm our finding that cobalamin concentrations correlate with haptocorrin (HC; transcobalamin I) (2) but propose that HC concentrations are regulated by cobalamin status rather than being genetically deter-

mined, which they mistake as my view. In fact, no exclusive theory of HC regulation is likely. Many things affect HC synthesis, release, and clearance, and HC concentrations are altered in many varied disorders (3). Moreover, cobalamin changes often follow, rather than precede, such HC changes because HC’s long half-life disproportionately influences retention of its attached cobalamin (holo-

HC). In addition, highly variable release of leukocytic apo-HC (cobal-
amin-free HC) frequently occurs whenever serum is tested instead of plasma (4); critical effects of this arti-

fact on Morkbak’s vegan serum data cannot be dismissed merely because leukocyte counts did not change after therapy.

Statistical associations between cobalamin and HC concentrations require no complicated theories. The 75% or greater identity between circul-

ating cobalamin and holo-HC, which in turn also constitutes 80% or more of total HC, guarantees signif-

icant associations and renders most alternative interpretations speculative. Nor should too much be made of the probably nonindependent sta-

tistical associations of methylmalonic acid and homocysteine with HC, given HC’s confounding near-iden-

tity with cobalamin.

Morkbak’s claims that HC was “decreased” in cobalamin deficiency and that cobalamin deficiency may explain much HC deficiency are under-
cut by her data: most patients with low cobalamin (<200 pmol/L) actually had total HC concentrations well within the reference interval (>240 pmol/L). Closer study of those few exceptions with total HC <240 pmol/L [see Fig. 1A in (1)] might have proved enlightening; posttreatment values in Table 1 of (1) imply that some very low HC concentrations persisted after cobalamin therapy, casting doubt on their relation to cobalamin status. Inattention to individually important patients, especially those who do not quite conform to group expectations, is unfortunately commonplace in contemporary studies of cobalamin status, which too often focus exclusively on overall group statistics.

Further weakening Morkbak’s thesis is the likelihood that the disparity in posttherapy HC changes between cobalamin-deficient and nondeficient patients had much more to do with the grossly disparate cobalamin reg-

imens the 3 study groups received than with differences in their cobal-

amin status. Excessive cobalamin doses were given to the cobalamin-

deficient vegan group (5 mg orally daily) and the group suspected of deficiency (1 mg intramuscularly every week). As a result, mean cobalamin concentrations rose mas-

sively from 97 to 1016 pmol/L in the first group (947% increase) and from 281 to 960 pmol/L in the second (242% increase). Compare these with the nondeficient group, who received only 0.4 mg orally daily and whose mean cobalamin therefore rose just 51%, from 350 to 527 pmol/L. Small wonder that the first group showed significant increases in serum holo-HC and total HC—holo-HC be-

cause of apo-HC saturation by massive cobalamin doses and total HC possibly from leukocytic HC release because serum was tested instead of plasma—and the second group showed only the holo-HC increase in plasma as massive cobalamin injections con-

verted apo-HC to holo-HC, whereas the third group showed neither plasma HC saturation nor increase because relatively modest amounts of new cobalamin entered the bloodstream. Nor do HC data stratified by MMA response to therapy prove the claimed influence of metabolic cobalamin status on HC concentrations. The 2 groups whose MMA concentrations responded to therapy were those also confounded by massive cobalamin doses and serum testing, unlike the nonrespon-

sive controls. Proof of HC dependence on cobalamin status awaits studies with uniform treatment regi-

mens and uniform testing of plasma.

To dispel potential diagnostic confu-

sion and Morkbak’s concerns about assuming HC deficiency simply from low circulating cobalamin concentra-

tions, the apparently underappreci-

ated diagnostic criteria for primary HC deficiency (as fulfilled in all our published cases save one unusually