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To the editor:

Use of desmopressin (DDAVP) during early pregnancy in factor VIII–deficient women

Desmopressin (DDAVP) is being used with caution in the first 2 trimesters of pregnancy in women with bleeding disorders[1,2] because there is concern that the compound may cause placental insufficiency due to arterial vasoconstriction and increase the risks of miscarriage due to an oxytocic effect and to maternal and/or neonatal hyponatremia.[3] The latter concern is in principle justified because desmopressin is a potent antidiuretic nonapeptide that acts thorough V2 vasopressin receptors.[4] On the other hand, its potential for vasoconstriction and uterus contraction is negligible because the compound is practically devoid of these biologic activities related to the activation of V1 vasopressin receptors.[4] Evidence of its safety during pregnancy in women with diabetes insipidus is available.[3] Since the original description of its use for the treatment of mild hemophilia and von Willebrand disease (VWD),[5] between 1988 and 2002 we used desmopressin in 32 pregnant women with low factor VIII levels in order to improve hemostasis at the time of invasive procedures.

Of 32 pregnant women, 27 were obligatory carriers of hemophilia with factor VIII deficiency, presumably due to extreme random inactivation of their normal X chromosome (lyonization), and 5 had type 1 VWD. Twenty women underwent chorionic villus sampling at gestational weeks 11 to 12 for prenatal diagnosis of hemophilia, the remaining 12 underwent amniocentesis at weeks 16 to 18 for prenatal diagnosis (7 hemophilia carriers) or karyotyping (5 women with type 1 VWD). In all women, factor VIII levels were low enough to engender an increased risk of bleeding (median value, 18 U/dL; range, 10-35 U/dL) and hence to justify the coverage of the procedure with a hemostatic agent. In 22 cases the treatment schedule was based upon the use of a single intravenous infusion of 0.3 μg/kg before the procedure because factor VIII levels attained after infusion were judged sufficient to avoid secondary bleeding. In the remaining 10 cases the same dose was repeated 2 to 3 times at intervals of 18 to 24 hours because factor VIII levels were not sufficient for hemostasis. No laboratory test other than factor VIII assays was carried out after desmopressin because this is the protocol routinely followed in our treated patients with mild hemophilia and VWD. As usual, however, women were advised to avoid excessive fluid intake and to control that their body weights did not increase. Sixty minutes after the infusion, factor VIII levels increased in average 3 times (median, 60 U/dL; range, 40-121 U/dL). In all 32 women there was no abnormal bleeding and in 20 of them pregnancies went successfully to term with the delivery of healthy newborns. In the remaining 12 cases, male fetuses affected by hemophilia on genotyping were aborted under coverage with additional doses of desmopressin. There was no side effect in the treated women other than mild facial flushing and headache and no significant increase in body weight.

This series shows that desmopressin can be used during the first and second trimester of pregnancy and that it is safe during invasive procedures that increase per se the risk of miscarriage. Electrolytes and osmolality were not measured because this is our routine protocol. However, there was no clinical sign of water intoxication or body weight increase in these women, who were warned to restrict fluid intake.

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To the editor:

Hyperhomocysteinemia: cause or effect of disease?

We read with interest the article on the *methylenetetrahydrofolate reductase (MTHFR)* C677T polymorphism by Frederiksen et al.[1] We do not agree with the hypothesis of the authors that testing C677T *MTHFR* is an alternative approach to establish if hyperhomocysteinemia is a cause or simply an effect of the disease. Indeed, hyperhomocysteinemia is not a monogenic condition, but a number of acquired and inherited risk factors contribute to its development. Several polymorphisms in the

genes coding for the enzymes involved in the methionine metabolism are associated with elevated homocysteine levels. Furthermore, vitamin status and renal function play a crucial role in affecting homocysteine levels.[2]

The conclusion of Frederiksen et al on the role of homocysteine levels does not appear evidence based for these reasons: (1) they determined homocysteine levels 2 to 4 years after the end of follow-up in only a subgroup of subjects (we do not know the criteria used to select this subgroup), so it is possible that some selection biases were introduced; (2) the authors do not report any information on the vitamin status of subjects enrolled, whereas it is well documented that the effect of *MTHFR* polymorphism on homocysteine levels is modulated by vitamin status; (3) the authors performed a multivariate analysis adjusting for several parameters, but not for the main determinant of homocysteine levels such as creatinine levels. They concluded that hyperhomocysteinemia associated with *MTHFR* homozygosity is not causally related to vascular disease based on the fact that carriers of *MTHFR* 677TT had 25% higher homocysteine levels without an increased vascular risk. This subgroup of patients had mean homocysteine levels of 14.7 ± 0.5 μM, but we do not know how many subjects were hyperhomocysteinemic. Carriers of *MTHFR* homozygosity had 25% higher homocysteine levels with respect to the other genotypes, but it is unlikely that they were all hyperhomocysteinemic. On this basis, it does not appear reasonable to conclude that homocysteine is an effect of disease.

As homocysteine seems to be implicated in several diseases, it might be plausible that it is simply a marker of disease or of a metabolic derangement in which the leading actor is another parameter still unknown, but the study of Frederiksen et al does not provide the demonstration.

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Response:

Mendelian randomization suggests that vascular events cause hyperhomocysteinemia, rather than vice versa

We appreciate the comments by Marcucci et al to our recent article.[1] In that study, we examined 9238 individuals from the Danish general population of which 1374 and 208 during 23 years of follow-up developed ischemic cardiovascular disease (ICD) and venous thromboembolism (VTE), respectively; there were an additional 2961 Danes with ICD also enrolled. Plasma homocysteine was elevated 25% in *methylenetetrahydrofolate reductase (MTHFR)* 677T homozygotes versus noncarriers and 19% in ICD/VTE cases versus controls; however, hazard ratios and odds ratios for ICD and VTE for homozygotes versus noncarriers did not differ from 1.0, either in prospective or in case-control studies. We therefore concluded that ICD and VTE might cause hyperhomocysteinemia, rather than vice versa. Although Marcucci et al accept that this conclusion may indeed be true, they do not believe our data provide this demonstration. We disagree.

Our conclusion is based on the concept of Mendelian randomization, that is, the use of genotype-disease associations to make inferences about environmentally modifiable causes of disease.[2,3] Mendelian randomization is the term applied to the random assortment of alleles at the time of gamete formation, which results in population distributions of genotypes that are generally independent of environmental factors.[2,3] This is exactly what we observed in our study for 11 classical cardiovascular risk factors, that is, that these risk factors were equally common in individuals with either of *MTHFR* C677T noncarrier, heterozygote, and homozygote genotypes (Frederiksen et al[1] (Tab1)). Likewise, among noncarriers, heterozygotes, and homozygotes, 59%, 58%, and 59%, respectively, were daily taking vitamins (chi-square: $P = .74$), while 1.8%, 1.4%, and 0.8%, respectively, had creatinine levels higher than 120 μM ($P = .15$). Finally, other polymorphisms in genes involved in methionine metabolism most likely will also be equally distributed between individuals with the 3 different *MTHFR* C677T genotypes, like we observed for *factor V Leiden* genotype.[1] Therefore, our data clearly demonstrate the key advantage of Mendelian randomization, that is, avoidance of confounding.[2,3]

Marcucci et al have a number of questions that we are happy to answer: (1) The subgroup of individuals in which homocysteine levels were measured was simply those attending the 2001 to 2003 examination of the Copenhagen City Heart Study. Any selection bias introduced between this and former examinations is unlikely to influence relative homocysteine levels between genotypes. (2) As the fraction daily taking vitamins did not differ between genotype groups (previous paragraph), vitamin status is unlikely to have confounded our results. (3) As creatinine levels did not differ between genotype groups (previous paragraph), differences in renal function also are unlikely to have confounded our results. (4) Finally, among those with the *MTHFR* C677T noncarrier, heterozygote, and homozygote genotypes, 15%, 15%, and 30%, respectively, had plasma homocysteine levels higher than 15 μM (Refsum et al[4(p19)]) (chi-square: $P < .0001$).

In conclusion, therefore, because of Mendelian randomization generally avoiding confounding,[2,3] we believe that our data suggest that ICD/VTE causes hyperhomocysteinemia, rather than vice versa.

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To the editor:

Serum C-reactive protein parallels secretory phospholipase A_2 in sickle cell disease patients with vasoocclusive crisis or acute chest syndrome

Previous studies from our institution have shown that serum secretory phospholipase A_2 ($sPLA_2$) levels are markedly increased in sickle cell disease (SCD) patients with acute chest syndrome (ACS)[1] and that sequential measurements of $sPLA_2$ are useful in predicting the subsequent development of ACS in patients hospitalized for vasoocclusive crisis (VOC).[2] However, $sPLA_2$ assays are not widely available in clinical laboratories. Thus, we sought a surrogate marker that might be measured instead of $sPLA_2$. Because $sPLA_2$ is an acute-phase protein,[3] we hypothesized that serum concentrations of another acute-phase reactant, C-reactive protein (CRP), might change in concert with $sPLA_2$. CRP and $sPLA_2$ have been shown to correlate well in patients with septic fever.[4,5] In addition, CRP values are comparable whether measured in serum or plasma, after delayed sample processing or prolonged storage, or after up to 7 freeze-thaw cycles.[6]

We performed assays for $sPLA_2$ and CRP on 139 serum samples from 20 hospitalized SCD patients; 13 patients had VOC and 7 had ACS. Between 5 and 10 serum samples collected during the course of hospitalization were analyzed for each patient. Serum samples were assayed for $sPLA_2$ on the day of collection, prior to freezing. Enzyme activity was determined with a fluorometric assay[7] and expressed as arbitrary units (AU), where 1 AU was the activity associated with a serum immunoreactive $sPLA_2$ concentration of 100 ng/mL.[7] CRP concentrations were measured with an in-house sandwich enzyme-linked immunosorbent assay (ELISA). To validate the assay, we measured CRP levels in serum obtained from 17 healthy individuals. Serum CRP (mean ± SEM) for this group of donors was 0.81 ± 0.26 mg/L. This concentration is similar to previously published CRP values for healthy donors.[6,8]

Daily mean serum CRP concentrations and $sPLA_2$ activity during 10 days of hospitalization are shown in Figure 1 and Table 1. Mean CRP values paralleled changes in $sPLA_2$ very closely for the first 5 days of hospitalization, after which they decreased somewhat more slowly than did $sPLA_2$. Examination of plots from individual patients showed that the day of peak CRP concentration coincided with the day of peak $sPLA_2$ concentration in 9 cases, was one day earlier in 5 instances, and one day later in 5 instances. Thus, based on the pattern of changes in concentration, CRP appeared to be a good surrogate for $sPLA_2$.

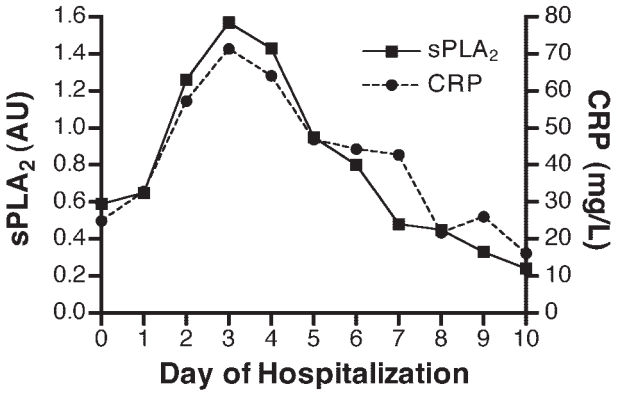

Figure 1. Day-by-day comparison of mean $sPLA_2$ and CRP values in SCD patients with VOC or ACS. Mean serum $sPLA_2$ activity (AU) and immunoreactive CRP values (mg/L) are shown on a day-by-day basis for 139 serum specimens. Numeric data for each day of hospitalization are shown in Table 1, with mean and SEM $sPLA_2$ and CRP values for the indicated number (N) of serum specimens.

In this analysis, we wished only to determine whether CRP could serve as a suitable substitute for $sPLA_2$ in serum samples from SCD patients, and our sole criterion for selecting patients was the availability of several daily serum samples for assay. Therefore, we can draw no conclusions regarding the ability of CRP to predict the likelihood that ACS will develop after hospitalization with VOC. However, changes over time in CRP closely paralleled changes in $sPLA_2$, and values for the 2 analytes were correlated (Spearman rank correlation coefficient = 0.641; $P < .0001$; data not shown). Overall, these results suggest that further studies should be performed to determine whether CRP could be used as an alternative to $sPLA_2$ to predict the development of ACS in VOC patients.

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Table 1. Numerical data for results shown in Figure 1

Day	0	1	2	3	4	5	6	7	8	9	10
N	18	16	17	17	18	13	13	10	8	6	3
$sPLA_2$ mean	0.59	0.65	1.26	1.57	1.43	0.95	0.80	0.48	0.45	0.33	0.24
$sPLA_2$ SEM	0.13	0.14	0.30	0.44	0.62	0.48	0.20	0.11	0.12	0.07	0.05
CRP mean	24.91	32.87	57.31	71.42	64.13	46.85	44.30	42.69	21.65	26.02	16.14
CRP SEM	10.16	12.60	21.03	19.40	18.44	13.92	15.47	16.04	9.13	9.63	9.38