produce changes in its concentration and in bone markers over the 3 days immediately post fracture. There is an extension to this theory that other researchers may find worthy of investigation. Vitamin B_6 is an essential cofactor for the enzyme ornithine decarboxylase, the rate-limiting enzyme in the formation of putrescine, which in turn regulates osteoblast glucose-6-phosphate dehydrogenase activity and thus osteoblast NADPH concentrations *(3–6)*. NADPH is essential for the vitamin K cycle, in which the epoxide form of vitamin K is converted back to the naphthoquinone form, which is required for γ-carboxylation of osteocalcin *(2)*. It is therefore possible that vitamin B_6 status could modulate the effects of vitamin K on bone metabolism.

Although the chain of events described above may seem excessively complicated, there is evidence that vitamin B_6 deficiency in rats reduces bone healing *(7)* and that vitamin B_6 assayed by HPLC *(8)* is statistically significantly lower in patients who fracture their hips in low-energy falls than in patients whose hip fractures are elective procedures *(9)*. Further research into the interaction of these two vitamins may be indicated.

References

1. Takahashi M, Kushida K, Hoshino H, Aoshima H, Ohishi T, Inoue T. Acute effects of fracture on bone markers and vitamin K. Clin Chem 1998;44:1583–4.
2. Stenflo J, Suttie J. Vitamin K dependent formation of γ-carboxyglutamic acid. Annu Rev Biochem 1977;46:157–72.
3. Bachrach U. Physiological aspects of ornithine decarboxylase. Cell Biochem Funct 1984;2: 6–10.
4. Mamony PS, Bohlen P, McCann PP, Bey P, Schuber F, Tardif C. Alpha methyl ornithine, a potent competitive inhibitor of ornithine decarboxylase blocks proliferation of rat hepatoma cells in culture. Proc Natl Acad Sci U S A 1976;73:1626–30.
5. Raina A, Janne J. Physiology of the natural polyamines putrescine, spermidine and spermine. Med Biol 1975;53:121–47.
6. Dodds RA, Dunham J, Bitensky L, Chayen J. Putrescine may be a natural stimulator of glucose-6-phosphatase dehydrogenase. FEBS Lett 1986;201:105–8.
7. Dodds RA, Dunham J, Bitensky L, Chayen J. Abnormalities in fracture healing induced by vitamin B_6 deficiency in rats. Bone 1986;35: 241–5.
8. Reynolds T, Brain A. A simple internally standardised isocratic HPLC assay for vitamin B_6 in human serum. J Liq Chromatogr 1992;15:897–914.
9. Reynolds T, Marshall P, Brain A. Patients with hip fracture may be vitamin B_6 deficient. Acta Orthop Scand 1992;63:635–8.

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Serum Cystatin C, a New Marker of Glomerular Filtration Rate, Is Increased during Malignant Progression

Cystatin C has recently been shown to be an accurate marker of glomerular filtration rate with advantages over serum creatinine *(1, 2)*. Cystatin C, a potent inhibitor of cysteine proteases, is found mainly in extracellular fluids such as blood, cerebrospinal fluid, and seminal plasma. Its low molecular weight and stable production rate indicate that the blood concentration of cystatin C is determined mainly by glomerular filtration. The production rate of cystatin C is less altered by nonrenal factors than is the production of creatinine, and it has been reported that circulating cystatin C concentrations are not affected by inflammatory conditions or malignancy *(3)*. Our observations, however, have revealed a significant correlation between increased serum cystatin C and malignant progression in melanoma and colorectal cancer.

In malignancy, an imbalance between cysteine proteases and their inhibitors, associated with a metastatic tumor cell phenotype, is thought to facilitate tumor cell invasion and metastasis *(4)*. Numerous studies have provided evidence of substantial increases in mRNA, protein, and the activity of tumor cysteine proteases, accompanied by only moderately increased or unchanged concentrations of intracellular inhibitors *(5)*. Enhanced extracellular secretion of cysteine proteases is another feature associated with tumor cell phenotype. We recently published evidence that high serum concentrations of the cysteine proteases cathepsins B and H are of prognostic importance in predicting the rate of death in colorectal *(6)* and melanoma cancer *(7)*. These high concentrations

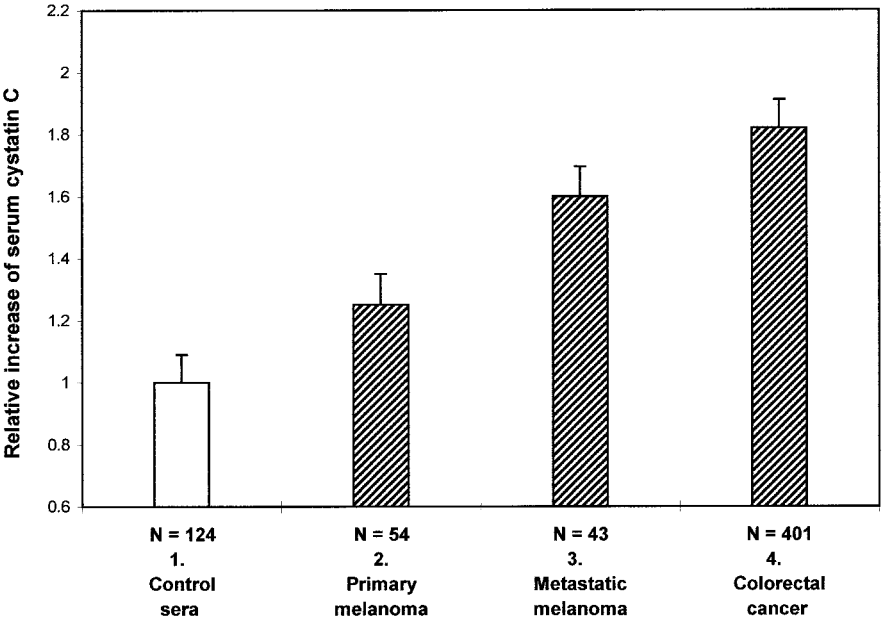

Fig. 1. Determination of cystatin C in sera of healthy controls and cancer patients, using ELISA as described by Kos et al. *(7)*.

Ratio 1:2, not significant; ratio 1:3, $P = 0.02$; ratio 1:4, $P < 0.0001$. *Error bars* represent mean + 2 SE.

were balanced by increased serum cystatin C, which in addition to kininogens and α_2-macroglobulin is the most important inhibitor for controlling the proteolytic activity of extracellular cysteine proteases. In melanoma we found significant increases ($P = 0.02$) in the cystatin C concentration among patients with metastatic disease and smaller increases in patients with primary melanoma (Fig. 1), indicating the up-regulation of cystatin C in later events of tumor progression. In colorectal cancer, serum concentrations of cystatin C were significantly increased ($P < 0.0001$) in patients at all Dukes stages, correlating weakly with patient age and gender (unpublished data). The correlation between cystatin C and creatinine serum values *(7)*, however, was much weaker in cancer patients than that reported for healthy controls *(3)*, suggesting the influence of nonrenal factors on the concentration of cystatin C in malignant sera. The creatinine values, not significantly changed in cancer patients, suggest that patients' renal function had not been altered at the time of sample collection.

In our opinion the number of patients included in previous studies was too low to provide relevant information about changes in the cystatin C serum concentration during malignant progression. The results of our studies, which involved 401 patients with colorectal cancer, 97 patients with melanoma, and 124 healthy controls, strongly support the need for further evaluation of cystatin C as a marker for glomerular filtration rate determination, at least in cancer patients, to determine its potential for use in clinical practice.

References

1. Kyhse-Andersen J, Schmidt C, Nordin G, Andersson B, Nilsson-Ehle P, Lindstrom V, Grubb A. Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate. Clin Chem 1994;40:1921–6.
2. Finney H, Newman DJ, Gruber W, Merle P, Price CP. Initial evaluation of cystatin C measurement by particle-enhanced immunonephelometry on the Behring nephelometer systems (BNA, BN II). Clin Chem 1997;43: 1016–22.
3. Newman DJ, Thakkar H, Edwards RD, Wilkie M, White T, Grubb AO, Price CP. Serum cystatin C measured by automated immunoassay: a more sensitive marker of changes in GFR than serum creatinine. Kidney Int 1995; 47:312–8.
4. Sloane BF. Suicidal tumor proteases. Nat Biotech 1996;14:826–7.
5. Lah T, Kos J. Cysteine proteinases in cancer progression and their clinical relevance for prognosis. Biol Chem 1998;379:125–30.
6. Kos J, Nielsen HJ, Krašovec M, Christensen IJ, Cimerman N, Stephens RW, Brünner N. Prognostic values of cathepsin B and carcinoembryonic antigen in sera of patients with colorectal cancer. Clin Cancer Res 1998;4: 1511–6.
7. Kos J, Štabuc B, Schweiger A, Krašovec M, Cimerman N, Kopitar-Jerala N, Vrhovec I. Cathepsins B, H, L, their inhibitors stefin A and cystatin C in sera of melanoma patients. Clin Cancer Res 1997;3:1815–22.

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Anti-Thyrotropin Antibody Interference in Thyrotropin Assays

To the Editor:

We read with interest the paper by Després and Grant *(1)* on antibody interference in thyroid assays. Thyroid hormone autoantibodies, heterophile antibodies, and rheumatoid factors are certainly the main sources of artifacts. As mentioned by the authors, anti-thyrotropin (anti-TSH) antibodies are more uncommon but may nevertheless deserve additional comments.

The existence of anti-TSH antibodies in patient sera has been reported after injections of bovine TSH *(2, 3)*. The antibodies also appear in autoimmune thyroid diseases such as Graves disease, Hashimoto thyroiditis, silent thyroiditis, and subacute thyroiditis *(4–7)*, and nonthyroid autoimmune disease *(6)*. In sera from patients with Graves disease, the possibility that thyrotropin receptor antibodies (TRAbs) may be anti-idiotype antibodies against anti-TSH antibodies or that anti-TSH antibodies may be anti-idiotype antibodies against TRAbs is controversial *(8–12)*. Most of the reported anti-TSH antibodies reacted against bovine TSH; however, some also reacted against human TSH *(4, 12–14)*.

The results of published studies on anti-TSH antibody interference in TSH assays concerned mainly RIAs. In those cases, depending on the assay design and the antibody specificity, interference may yield lower or increased values. Increased results were found with the double antibody techniques *(4, 5, 13, 15–17)*. Single antibody techniques with polyethylene glycol (PEG) precipitation yielded low values *(14, 15)*. Fewer results have been reported with the widely used, "sandwich" immunometric assays (IMAs). IMA results have been found to be lower *(5)* or similar to double antibody results *(6)*. Moreover, different IMA kits may yield discrepant values *(14)*.

We previously reported *(18)* TSH concentrations that we measure *(19)* with eight different third-generation IMAs in four serum samples that contained anti-TSH antibodies as determined by increased precipitation of protein-bound ^{125}I bovine or human TSH. Two samples from patients with autoimmune thyroid disorders (Graves disease and postpartum thyroiditis) contained only anti-bovine TSH antibodies. The results of the different TSH kits were not grossly discrepant, ranging from 0.36 to 0.60 mIU/L and from 2.9 to 4.7 mIU/L for the two samples, respectively. The other two sera contained both anti-bovine and anti-human TSH antibodies. In the first case, our suspicion was aroused because the high serum TSH contrasted with an apparently healthy clinical picture. The second case was from a euthyroid woman who had given birth to two children with tran-