The ability to use DBS to measure ASA protein and activity will simplify procedures for collection, handling, and storage of patient samples. The ease of transporting DBS, combined with the diagnostic sensitivity and specificity of these assays, provide a powerful approach to the diagnosis of MLD. After further investigation and validation, these assays may be used for newborn screening for MLD and other lysosomal storage disorders. Importantly, the inability to differentiate ASA-PD from ASA by the activity assay alone suggests that ASA protein determination from DBS may be the most appropriate screening approach for newborns, as has recently been proposed in a report of a multiplex immune-quantification assay (5).

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Circulating Matrix Metalloproteinase-7: An Early or Metastatic Marker for Renal Cell Carcinoma?

To the Editor:

We read with great interest the article by Sarkissian et al. (1) reporting the identification of serum matrix metalloproteinase-7 (MMP-7) as a marker for renal cell carcinoma (RCC). For the purpose of identification, the authors used sera from healthy individuals and RCC patients and combined 2-dimensional electrophoresis of an RCC cell-line supernatant and an immunoblotting procedure. Subsequently the authors measured serum MMP-7 concentrations with a self-developed immunoassay. Higher concentrations were found in RCC patients than in healthy individuals (7.56 vs 2.13 μg/L), whereas serum MMP-7 concentrations in patients with localized RCC did not differ from concentrations in patients with metastatic RCC (7.26 vs 7.87 μg/L). These results suggest that MMP-7 could be a useful marker for detecting RCC during the early stages of the disease. These findings are of utmost importance because at present no blood marker is known for the early detection of RCC.

Continuing our studies on the diagnostic performance of MPPs, we investigated circulating MMP-7 in RCC patients, but our results (2) differed from those of Sarkissian et al. We measured MMP-7 in heparin-plasma samples using Fluorokine multianalyte profiling assays (R&D Systems). In our study MMP-7 concentrations (Fig. 1) in healthy controls were not found to be different from those in RCC patients with localized cancer

References

1. Nonstandard abbreviations: MMP-7, matrix metalloproteinase-7; RCC, renal cell carcinoma.
(N0M0 stage; TNM-1997 classification) (1.64 μg/L, n = 50 vs 1.85 μg/L, n = 39), but were markedly higher in patients with lymph-node involvement (N1M0 stage, n = 13; 3.82 μg/L) or distant metastases (M1 stage, n = 45; 3.34 μg/L). These data do not support the use of circulating MMP-7 as a marker for detecting early stages of RCC.

The clinical importance of these data makes it worthwhile to consider possible reasons for the disparities between these 2 studies regarding the clinical validity of serum MMP-7 measurements. Pre-analytical and analytical factors as well as aspects of study design should be considered. One reason may be differences in blood sampling, which is well known to be a significant preanalytical determinant of MMP measurements (3). Sarkissian et al. (1) used sera for their measurements, whereas we used plasma. Circulating MMP-7 values, however, are not greatly affected by the blood collection procedure (3); therefore blood sampling seems an unlikely explanation for the discrepant findings. Analytical variables seem more likely to be causes of the discrepant findings. The 2 studies used different antibodies in the MMP-7 assays. In consequence, different epitopes of MMP-7 may have been recognized, leading to differing detection of the various forms of MMP-7. MMP-7 exists in human serum as polymers or complexed with serum proteins (4). According to the information from the manufacturer, the assay that we used measures pro- and active forms of MMP-7 as well as MMP-7 complexed with tissue inhibitor of MMP 1. The assay used by Sarkissian et al. (1) detected only pro- and active MMP-7, but the detection of MMP-7 forms complexed with serum proteins cannot be excluded. Sarkissian et al. observed higher MMP-7 concentrations in their patients (1) than we did in ours, and more importantly, they found increased MMP-7 concentrations in both the patients with localized cancer and the patients with metastases. In contrast, the 95th percentile they observed in their control group (3.29 μg/L vs our 3.35 μg/L) suggested that these concentration differences did not exist in the healthy participants of the 2 studies. Because the same recombinant human MMP-7 was used as a calibrator in both assays, it can be assumed that MMP-7 forms possibly typical for RCC patients are more sensitively detected by the assay of Sarkissian et al. (1) than by the R&D MMP-7 assay we used. Different assay designs including the use of the mentioned specific antibodies could affect the assay sensitivity, but autoantibodies to pro–MMP-7 occurring in sera of RCC patients (1) could also interfere with measurement of MMP-7. The differences in study results may also be attributable to different approaches used for selection of study participants and the sizes of study groups. The study of Sarkissian et al. (1) was designed to identify proteins related to RCC rather than to validate the clinical utility of the analyte. In contrast, our study focused on the potential diagnostic and prognostic performance of MMP-7 in somewhat larger and well-classified RCC groups. Sarkissian et al. (1) may have been unable to demonstrate an association of MMP-7 with advanced RCC owing to the limited number of RCC patients in their study population. Finally, MMP-7, although predominantly expressed by tumor cells, may also be released from other cell types (2, 5). For this reason, various clinical conditions may influence the concentrations of circulating MMP-7 in study populations and contribute to such variation in data. Prospective studies will be required to clarify the clinical applicability of circulating MMP-7 as an early or metastatic marker in RCC patients.

![Fig. 1. Scatterplots of MMP-7 concentrations in plasma of healthy controls and patients with renal cell carcinoma.](image-url)

Short horizontal lines with respective figures indicate the medians of the groups and the dotted horizontal line represents the cutoff point of 3.35 μg/L calculated as the 95th percentile of the control group by use of the nonparametric approach for establishing reference limits. We found significant differences between the study groups by using the Kruskal–Wallis test ($P < 0.0001$) with Dunn post hoc comparisons indicated by the following symbols: a, compared to controls; b, compared to the N0M0 group (with localized cancer); c, compared to the N1M0 group (with lymph-node involvement); and d, compared to the M1 group (with distant metastases) (all $P < 0.05$). Adapted from (2).
Dear Editor,

We investigated the phenomenon, we compared the novel recombinant Neoplastin R PT reagent (ISI 1.08; Roche Diagnostics GmbH) with the tissue extract PT reagent Neoplastin Plus (ISI 1.29; Roche Diagnostics GmbH) in both stable oral anticoagulant patients and patients recently started with acenocoumarol. We collected citrate samples from 25 patients starting with a standard loading dose of acenocoumarol (day 1, 6 mg; day 2, 4 mg; day 3, 2 mg) between 3 and 7 days after initiating oral anticoagulation and from 20 patients on stable acenocoumarol therapy (therapeutic INR range 2.0–4.0). We measured PT using a STA-R analyzer (Roche Diagnostics GmbH).

We found excellent linear correlation \( r^2 = 0.94; \) slope 1.31; intercept –0.51 between recombinant Neoplastin R and tissue extract Neoplastin Plus for patients on stable oral anticoagulant therapy. The INR differences are plot-

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Strongly Increased International Normalized Ratio with Recombinant Neoplastin R* Compared with Tissue Extract Neoplastin Plus* in Patients Initiating Oral Anticoagulant Therapy: Implications for Anticoagulation Dosage

To the Editor:

High-accuracy determination of the international normalized ratio (INR)1 is of importance in controlling treatment of patients with oral anticoagulants. The INR is defined according to a calibration model adopted by the WHO (1) as the normalized prothrombin clotting time (PT/MNPT)ISI, where MNPT is mean normal prothrombin time of 20 healthy individuals. The factor ISI (international sensitivity index) compensates for the differing sensitivities of thromboplastin reagents used to measure prothrombin clotting time. Thromboplastin reagents can vary widely in their sensitivities to clotting factor deficits (induced by coumarin treatment), resulting in INR differences (2). Recombinant thromboplastins have been discussed as being more sensitive to variation in coagulation factor VII (FVII) than tissue extract thromboplastins (2–4). FVII has a relatively short plasma half-life of 6 h compared to the half-lives of factor X (45 h) and prothrombin (60 h). The increased sensitivity for FVII may be observed during unstable anticoagulation, such as in the initial phase of oral anticoagulant therapy, and may lead to differences in observed INRs that have implications for subsequent oral anticoagulant dosing advice.

To investigate this phenomenon, we compared the novel recombinant Neoplastin R PT reagent (ISI 1.08; Roche Diagnostics GmbH) with the tissue extract PT reagent Neoplastin Plus (ISI 1.29; Roche Diagnostics GmbH) in both stable oral anticoagulant patients and patients recently started with acenocoumarol. We collected citrate samples from 25 patients starting with a standard loading dose of acenocoumarol (day 1, 6 mg; day 2, 4 mg; day 3, 2 mg) between 3 and 7 days after initiating oral anticoagulation and from 20 patients on stable acenocoumarol therapy (therapeutic INR range 2.0–4.0). We measured PT using a STA-R analyzer (Roche Diagnostics GmbH).

We found excellent linear correlation \( r^2 = 0.94; \) slope 1.31; intercept –0.51 between recombinant Neoplastin R and tissue extract Neoplastin Plus for patients on stable oral anticoagulant therapy. The INR differences are plot-