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Physiological matrix metalloproteinase (MMP) concentrations: comparison of serum and plasma specimens

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The authors appreciate the critical comments of Professor K. Jung, and agree with his demonstration, here and elsewhere (1,2), that differences between serum and plasma samples exist for measurement of MMP-9. We also agree, and have cautioned in our original manuscript, that plasma is the preferred clinical specimen for measurement of both MMP-8 and MMP-9 (3). Serum levels of these MMPs are very likely influenced by release of MMPs following degranulation of leukocytes and platelets during the ex vivo blood clotting process in the specimen collection tube (4). Finally, we agree that clinical research studies reporting on circulating MMP concentrations should clearly specify the sample collection methodology. Serum samples utilized in our analyses were prepared using “gold top” BD Vacutainers® (with clot activators and silicon coating, Becton Dickinson and Company, Franklin Lakes, USA) #367382; by centrifugation at 3400 rpm (2000×g) for 5 min.

We would challenge, however, the inference that serum is never “appropriate” for the physiological ascertainment of MMP concentrations or that conclusions relative to MMP-9 can be generalized to all MMP measurements. For disease states characterized by cellular inflammatory responses, such as multiple sclerosis (5,6) and coronary artery disease (7–9), or malignant proliferation of inflammatory cell types such as multiple myeloma (10,11) and chronic lymphocytic leukemia (12,13), differences in serum measurements of MMPs have been detected and may provide a marker of pertinent intracellular protease activity. Alternatively, elevated concentrations of MMPs in serum might emanate from a circulating cell-type associated with a pathological condition. Moreover, recognizing that serum concentrations of MMPs have been assayed in disease states to identify clinically relevant or prognostic biomarkers of disease activation (10), we would suggest that both age-appropriate and specimen-appropriate reference ranges (or control data) be used in the interpretation of MMP concentrations in biological fluids. Finally, in our laboratory, Fluorokine® MultiAnalyte Profiling (F-MAP) methods have been used effectively for the simultaneous measurement of multiple MMPs in various biological fluids, including plasma, serum and urine (data not shown) and these measurements and their interrelationships may provide useful information.

To demonstrate that differences in plasma vs. serum MMP-9 concentrations cannot necessarily be generalized to all MMPs, we offer some additional clinical data. We have recently assayed paired plasma (potassium EDTA BD Vacutainers®) and serum (“redtop” BD Vacutainers® with clot activator and silicon coated) specimens for MMP-1, -2, -3, -8 and -9 using F-MAP

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kits (3). Specimens were collected during morning hours in the fasting state from 29 healthy volunteers. The experimental protocol governing collection of these specimens was approved by the Institutional Review Board of the University of Arkansas for Medical Sciences, and informed written consent was obtained from all participants. Samples were stored at -20°C until batch analysis was performed. The relationship between paired plasma and serum values was examined using Pearson linear correlation and linear regression analysis. The computation of a confidence interval for Pearson's correlation coefficient (r) was conducted using Fisher's z transformation since the sampling distribution of r was not normally distributed. Student's t -test was used to compare differences between group means and such data are presented as the mean $\pm$ SEM.

Subjects in this cohort ranged in age from 14 to 41 years, with a mean of 21.7 ± 6.4 years. The study population included 59% males and 90% Caucasian individuals. As noted in Table 1, plasma values were significantly related to serum values for MMP-2, MMP-3, MMP-8 and MMP-9. The correlation was strongest for MMP-2 and MMP-3. In addition, these relationships were strongest when the analysis was restricted to subjects ≥ 18 years of age, consistent with our original report that MMP-2 and MMP-3 values are age-dependent (3) (Table 1). Only for MMP-1 were plasma and serum values not related. For MMP-2, there was also no statistically significant difference between the group mean values for MMP-2 in plasma and MMP-2 in serum ($140,100.2\pm 21,721.8$ vs. $159,974.4\pm 14,772.5$ pg/mL, respectively; $p=0.45$). For MMP-3, -8, and -9, mean serum values were consistently between two- and four-fold higher than plasma values. Mean values of MMP-1 in serum were approximately 14-fold higher than plasma MMP-1 concentrations. These data suggest that with the relatively high correlation for plasma and serum MMP-2 and MMP-3 (using a scaling factor for MMP-3), analysis of serum samples may provide similarly pertinent clinical information to the analysis of plasma samples.

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Table 1

Correlation of plasma and serum values for MMPs.

Log MMP	All (n=29)		≥ 18 years (n=18)	
	Correlation		Correlation	
	Lower 95% limit	Upper 95% limit	Lower 95% limit	Upper 95% limit
Ln (MMP-2)	0.69	0.44	0.80	0.53
Ln (MMP-3)	0.83	0.66	0.82	0.56
Ln (MMP-8)	0.42	0.07	0.66	0.27
Ln (MMP-9)	0.47	0.12	0.76	0.46