

serum than in citrate plasma [mean (SD), 59 (7) vs 8 (2)  $\mu\text{g/L}$ , respectively;  $P < 0.01$ ], but the active/latent isoform proportions were similar (Fig. 1A). Platelet aggregation during clotting (6) may have caused these differences.

MMP expression was lower in  $\text{K}_2\text{E}$  plasma than in LH plasma [14 (3) vs 25 (4)  $\mu\text{g/L}$ ;  $P < 0.05$ ; Fig. 1A, lane 4 vs lane 5]. The concentrations of MMP-9 forms decreased significantly with increasing amounts of  $\text{K}_2\text{E}$  during PB collection, whereas MMP-2 was increased ( $P < 0.01$ ; Fig. 1B). When we added anticoagulants to the zymography buffer (to mimic the conditions in Vacutainer Tubes), only  $\text{K}_2\text{E}$  inhibited the gelatinolytic activities (data not shown). Although EDTA may alter MMP expression (7), the reasons for the contrasting  $\text{K}_2\text{E}$  effects remain unknown.

To minimize interindividual variability, we collected PB from the same individual into different buffers. We found mainly proMMP-2 in the buffered/acidic citrate plasma [202 (15)  $\mu\text{g/L}$ ], whereas there were no statistically significant differences among the 9NC, ACD, and CPDA plasmas. We found additional proMMP-9 in the  $\text{K}_2\text{E}$ , LH, and NaF/KOx plasmas (Fig. 1A, lanes 1–3 vs lanes 4–6). Our observations revealed that anticoagulants can act as preanalytical determinants of PB MMPs.

LH and 9NC plasmas collected after Lympholyte gradient (Fig. 1C, lanes 3 and 4 vs lanes 1 and 2), as well as after 9.65% sodium diatrizoate alone (Fig. 1C, lanes 5 and 6 vs lanes 3 and 4), showed increased concentrations of all MMPs. Polysucrose 400 alone did not affect MMP concentrations (data not shown) or isoform profiles.

Cytometric analysis revealed differences in MMP composition between leukocytes from LH PB vs leukocytes from 9NC PB (data not shown). Physiologic buffy coats from 9NC PB showed only MMP-9 forms, had lower gelatinase activity, and had a different zymographic profile with respect to LH PB (Fig. 1C, lane 7 vs lane 8). The MMP differences between LH vs 9NC plasma could be caused by differential release of MMPs from, e.g., platelets and leukocytes, with a changed MMP con-

centration/profile depending on the anticoagulant used (6, 8).

Although previous reports suggested heparin as the anticoagulant of choice to study circulating MMPs (2, 3), to optimize the diagnostic validity of PB MMPs as cancer biomarkers (1), we recommend the use of buffered/acidic citrate (9NC, ACD, and CPDA), whereas LH,  $\text{K}_2\text{E}$ , and NaF/KOx, which affect the MMP content and zymographic profiles of plasma and leukocytes, should be avoided.

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#### Measurement of Immunoglobulin Free Light Chains in Serum

To the Editor:

In a recent issue of this journal, Tate et al. (1) reported studies using assays for free immunoglobulin light chains (FLCs) that we have been instrumental in developing (2). Although in general agreement with their findings, we would like to highlight some additional data that are pertinent to several of their comments.

Tate et al. (1) concluded that more clinical data were required before the assays are adopted for routine clinical use. Since the acceptance of their report, however, several relevant studies have been published. Bradwell et al. (3) presented data showing that 224 of 224 patients with Bence Jones myeloma could be identified based on abnormal serum concentrations of FLCs at presentation, without the requirement for urine testing. They also demonstrated that serum FLC measurements were more sensitive than urine, presumably because of the kidney's high capacity for protein catabolism. For the diagnosis of AL amyloidosis, Lachmann et al. (4) reported that serum FLC assays were more sensitive than electrophoresis or immunofixation of both serum and urine. Of 262 patients with primary amyloidosis, 257 had abnormal serum FLC concentrations, whereas only 207 had monoclonal proteins in their serum and/or urine detectable by immunofixation electrophoresis or protein electrophoresis ( $\chi^2 = 45.19$ ;  $P < 0.0001$ ). Changes in the concentrations of serum FLCs were also found to be the best marker for disease monitoring and prognosis. Preliminary results from a study at the Mayo Clinic indicate the existence of "free light chain monoclonal gammopathy of undetermined significance (MGUS)", which can be identified only by use of serum FLC assays (5).

Additional confirmation of all of these results is appropriate, and many additional studies are underway. All patients entered in the Med-

ical Research Council, Myeloma IX trial and the Leukemia Research Fund/Cancer Research UK, MERIT trial, will have FLC measurements at entry and each follow-up attendance. However, current data are sufficiently compelling that FLC assays are used routinely in many centers worldwide for diagnosis and monitoring of AL amyloidosis, nonsecretory myeloma, and Bence Jones myeloma. The assays are in routine use here at Birmingham, with 277 non-trial patients currently being monitored.

We agree with Tate et al. (1) that the FLC assays should not be used in isolation for the diagnosis of myeloma. Preliminary analysis of data from our laboratories has indicated that FLC concentrations and ratios are within reference values in ~4% of patients with intact immunoglobulin multiple myeloma and in 40% of individuals with MGUS at the time of presentation (6). Our recommendation is for FLC assays to be run alongside serum electrophoresis. However, serum FLC assays may well be of value for monitoring multiple myeloma patients who secrete intact monoclonal immunoglobulins. The serum half-life of FLCs is much shorter than that of IgG (2–6 h vs 20 days); therefore, changes in FLC concentrations could provide an earlier measure of remission and a more rapid indication of the efficacy of treatment (6). In patients posttreatment, normalization of FLC concentrations and bone marrow biopsy results correlate, even when persisting intact immunoglobulin M-proteins suggest residual disease. This was also noted by Tate et al. (1).

Tate et al. (1) presented data regarding the quantitative performance of the FLC assays, and the results are broadly similar to our own assessments. However, any analysis of the performance of the FLC assays should be considered not only in the context of their demonstrated clinical utility but also in the context of the inadequacies of the current alternative methods for FLC quantification (7).

Drs. Mead and Carr-Smith are employed by The Binding Site, Professor Bradwell is a shareholder and chairman of the company, and The Binding Site developed, manufactures, and markets the FLC assays.

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*Some of the authors of the article cited above respond:*

*To the Editor:*

We agree with Mead et al. that recent clinical studies support the routine clinical use of serum free light chain (FLC) measurements. The data suggest the use of laboratory FLC testing

for the diagnosis and monitoring of nonsecretory myeloma and AL amyloidosis, as a prognostic marker for AL amyloidosis, and as a more sensitive marker of light-chain myeloma than urine Bence Jones protein. Serum FLC assays may offer clinical and technical advantages over current testing for monoclonal light-chain diseases. The current methods for quantification of Bence Jones protein concentration are inaccurate and may not reflect tumor mass, and immunofixation electrophoresis cannot quantify low concentrations of monoclonal light-chain proteins.

The evidence supporting use of serum FLC assays for the monitoring of myeloma patients who secrete intact monoclonal immunoglobulin, however, is less clear. In myeloma patients after autologous stem cell transplants, monoclonal serum immunoglobulins may decrease and persist at a low concentration despite normalized FLC concentrations and ratios. In the posttransplantation setting, we have also observed increasing monoclonal serum immunoglobulin and clinical disease progression despite FLC concentrations and ratios remaining unchanged. Both false-negative and false-positive FLC results have been observed (1). The clinical utility of serum FLC to monitor myeloma patients with intact monoclonal immunoglobulin remains to be demonstrated.

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