# Measurement of Total Protein Is a Useful Inclusion in Liver Function Test Profiles

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**2 authors:**

Katharine Hayden
Manchester University NHS Foundation Trust
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Charles Van Heyningen
The Royal College of Pathologists
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### Measurement of Total Protein Is a Useful Inclusion in Liver Function Test Profiles

*To the Editor:*

The letter by Watts et al. *(1)* indicated that the routine inclusion of total protein in liver function tests does not appear to contribute to patient management. We undertook a retrospective audit to assess the value of the addition of serum total protein to liver and bone profiles in detecting new patients with paraproteins. Requests for liver and bone profiles received by our laboratory during a 6-month period in 1999 were examined. A search of the computer system (William Woodard Associates) was made to select all cases with a total protein measurement >80 g/L, the upper limit of our reference interval. Each of these patient requests was analyzed to determine whether laboratory-initiated electrophoresis should be carried out or whether clinical details or other results suggested that this was not required.

Total protein was measured by the Biuret method *(2)* on a Dax 72 analyzer (Bayer plc, Diagnostics Division). Our liver profile included total protein, albumin, calculated globulin, bilirubin, alkaline phosphatase, and aspartate aminotransferase. Our bone profile included total protein, albumin, calculated globulin, calcium, adjusted calcium, phosphate, and alkaline phosphatase. In our laboratory practice, all total protein measurements >80 g/L fail validation procedures after analysis. Electrophoresis is initiated by the clinical biochemist when the procedure is considered necessary from the clinical details and other results.

There were 36 102 serum total protein investigations performed as part of liver and bone profiles in this period, of which 32 226 were liver profiles and 3876 were bone profiles. We found that 1419 samples had a total protein result >80 g/L. Ten samples were sent for laboratory-initiated electrophoresis. The majority of the requests for which electrophoresis was not required, as listed in Table 1, had clinical details or results suggesting other causes of an increased total protein, such as known liver disease or rheumatoid arthritis. From the 10 samples sent for laboratory-initiated electrophoresis, 2 new serum paraproteins were detected; 1 was determined as IgG-κ and the other was determined as IgG-λ by immunofixation. The two patients were referred for a bone marrow biopsy, which subsequently confirmed the diagnosis of multiple myeloma in both patients. Although the 10 samples sent for laboratory-initiated electrophoresis originated from both liver and bone profiles, both samples found to have paraproteins originated from liver profiles alone.

The identification of 2 new paraproteins in 36 102 requests gives an incidence of ~1 in 18 000. Because Watts et al. *(1)* examined only 15 000 requests, it is not surprising that they did not discover any new diagnoses of myeloma or other conditions associated with paraproteinemia in their study. Our findings indicate that the size of the group they studied was not large enough to enable an assessment of the value of the inclusion of total protein in liver function test profiles. Although the upper reference limit for total protein used by Watts et al. was higher—83 g/L compared with 80 g/L in our study—both paraproteins found by our laboratory had total protein measurements >83 g/L (86 and 108 g/L).

The inclusion of total protein in liver and bone profiles has been in place in our laboratory since January 1997 after an independent investigation into a complaint made by a patient about a delay of several months in making a diagnosis of myeloma after presenting with unexplained anemia and renal impairment. An independent professional review recommended that globulin (derived from total protein and albumin) should be included in all liver function test profiles. However, we could find no literature in the last 30 years that supported the use of total protein for this purpose. Indeed, the significance of total protein estimation in the detection and characterization of paraproteins has been described as doubtful *(3)*. Despite this, a national survey indicated that 73% of hospital laboratories in the United Kingdom include total protein in liver function test profiles and 44% in bone profiles *(4)*.

The cost of including total protein measurement as part of liver and bone profiles in our laboratory is estimated as £8300 per year (~0.18% of the total laboratory budget), with some marginal expenditure for the follow-up electrophoretic investigations. The lower threshold of 80 g/L [instead of the 83 g/L used by Watts et al. *(1)*] used for reviewing liver and bone profile results is therefore unlikely to substantially influence the laboratory costs involved. These reagent costs could be outweighed by the potential high cost of litigation if a case of myeloma is missed because of the exclusion of these tests. In addition, the early diagnosis of myeloma is clinically important when dealing with rapidly progressive cases or hyperviscosity. We conclude that the routine inclusion of total protein in liver function test profiles is useful for the detection of new patients with paraproteins.

**Table 1. Liver and bone profile requests with total protein >80 g/L.**

| Investigations | Number of requests | % |
|---|---|---|
| Laboratory-initiated electrophoresis | 10 | 0.7 |
| Electrophoresis already requested by clinician | 73 | 5 |
| Previous electrophoresis result | 26 | 2 |
| Known myeloma patient/paraproteinemia | 138 | 10 |
| External quality assessment specimen | 9 | 0.6 |
| Insufficient sample | 3 | 0.2 |
| Clinical details/results suggest electrophoresis not required | 1009 | 71 |
| No action taken; no reason determined | 151 | 11 |
| Total | 1419 | |

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**Katharine Hayden*
Charles van Heyningen**

*Department of Clinical Biochemistry
University Hospital Aintree
Lower Lane
Liverpool, L9 7AL
United Kingdom*

*Author for correspondence. Fax 44-151-529-3310; e-mail katharine.hayden@aht.nwest.nhs.uk.

## Effects of Blood-Collection Systems and Tubes on Hematologic, Chemical, and Coagulation Tests and on Plasma Hemoglobin

*To the Editor:*

Numerous preanalytical variables may affect the outcome of clinical laboratory tests *(1–3)*. Blood-collection procedures are considered an important impact factor because they are associated with several possible sampling problems, including the use of a tourniquet with hand-clenching, the site of venipuncture, hemolysis because of venipuncture, and the inappropriate use of sample tubes.

In our hospital, two systems are used for venous blood collection: the Becton Dickinson (BD) Vacutainer™ system and the Greiner Vacuette® system (Greiner Labortechnik GmbH). The BD product includes a specific blood-collection needle, a holder into which the needle is assembled before phlebotomy, and evacuated blood collection tubes. The Greiner product consists of a sterile holder (Holdex®) with a luer adapter to fit regular needles and evacuated blood collection tubes. The design of the BD needle holder enables direct linear flow of blood from the venipuncture site into the blood collection tubes. The Greiner Holdex was designed with an offset luer adapter to enable a convenient puncture angle. Therefore, the straight path within the Holdex is interrupted twice by angles of 90°, thus forming three consecutive flow segments. These changes in the linear flow of blood might impose mechanical strain on blood cells, affecting membrane integrity, which may cause efflux of intracellular constituents into the serum.

We compared the Greiner system to the BD system by evaluating within-subject variations in the results of blood analyses.

Fifty-five healthy individuals participated in our study (mean age ± SD, 26.7 ± 4.3 years). Seventeen volunteers were women (age, 25.8 ± 2.1 years) and 38 were men (age, 27.2 ± 5.0 years). All resided in Jerusalem and its environs. The study protocol was approved by the institutional research ethics board, and all subjects gave their informed consent.

The study participants were seated for 5 min before the tourniquet was applied. Hand-clenching was avoided. A 21-gauge needle attached to its specific holder (BD or Greiner) was inserted into the antecubital vein, and blood was collected into evacuated tubes (into the serum separation tube, followed by the citrate tube, and then into the EDTA tube). The tourniquet was released when blood began flowing into the first tube. The tubes were inverted six times after withdrawal from the holder to ensure proper mixing of the blood with anticoagulants. Phlebotomy was performed twice in each patient. By random assignment, the BD blood-collection system was used in one arm and the Greiner blood collection system was used in the opposite arm.

Serum and plasma were prepared by centrifugation of blood (1200*g* for 10 min) at room temperature within 30 min of collection. Each serum sample was analyzed for sodium and potassium using ion-selective electrodes (Cobas Integra chemistry analyzer; Hoffmann-La Roche). Additionally, aspartate aminotransferase, alanine aminotransferase, amylase, alkaline phosphatase, lactate dehydrogenase, bilirubin, HDL-cholesterol, magnesium ($Mg^{2+}$), and calcium were determined by specific colorimetric assays (Cobas Integra analyzer). Differential blood counts were carried out using the Vega blood cell counter (ABX Hematologie). Coagulation assays [prothrombin time (PT), activated partial thromboplastin time, and fibrinogen] were performed with the ACL 1000 coagulation analyzer (Instrumentation Laboratory). Hemoglobin in plasma was measured by spectrophotometric scanning technique as described previously *(4)*.

Statistical analyses were performed with the use of SPSS statistical software (Ver. 7.5.21). Differences in quantitative variables were assessed for statistical significance with the Student paired *t*-test.

In blood samples obtained by the nonlinear blood collection system (Greiner), significant increases were observed in serum $Mg^{2+}$ concentration, plasma hemoglobin, and PT (Table 1). All other analytes were essentially unchanged compared with the direct-flow system (BD). The 31% increase in hemoglobin was not accompanied by changes in serum potassium concentrations and lactate dehydrogenase activity. This indicates that the extent of erythrocytolysis was relatively low and the leakage of intracellular constituents

**Table 1. Within-subject comparison of the effect of the BD and Greiner blood-collection systems on blood tests.**

| Analyte | BD | Greiner | *P* |
|---|---|---|---|
| Hemoglobin in plasma, g/L | 0.16 ± 0.16 | 0.25 ± 0.21 | <0.025 |
| $Mg^{2+}$, mmol/L | 0.83 ± 0.057 | 0.85 ± 0.054 | <0.001 |
| PT, s | 11.86 ± 0.9 | 12.86 ± 3.2 | <0.030 |