However, our findings are in agreement with our previously published studies demonstrating cTnT expression in skeletal muscle from muscular dystrophy and polymyositis patients [2]. Our discussion [1] cites these findings as “a possible source of abnormally elevated cTnT” in renal disease patients. What needs to be determined is whether the cTnT form expressed in these adults’ diseased muscles is the adult cardiac, fetal cardiac, or regenerative cardiac isoform [3]. To help clarify these concerns, we would need access to the monoclonal anti-cTnT antibodies used in the second-generation Boehringer Mannheim cTnT immunoassay so we could perform similar Western blot experiments on our stored blots.

Finally, we do not disagree that the possibility does exist that subclinical myocardial injury may account for the increased serum cTnT concentrations. In our renal disease population with several cardiac risk factors, we plan to continue to follow these patients over the next several years.

References


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Effect of Plasma Replacement Therapy on Determinations of Urine Protein Concentration

To the Editor:

Total protein concentration in urine is currently analyzed in our laboratory by a colorimetric method—Pyrogallol Red (PR)–molybdate complex [1]—adapted to a Cobas Integra analyzer (Cobas Integra Total Protein Urine/CSF 07.5723.3; Roche). We quantify specific urinary proteins by the following immunochemical methods: turbidimetry for urinary albumin (Cobas Integra) and nephelometry for IgG, α1-microglobulin, and light chains of immunoglobulins (BNAII; Behring).

We recently observed discrepancies between results obtained for some urine samples, in that the total protein concentration was increased and did not agree with the sum of the concentrations of individual urine proteins such as albumin, IgG, α1-microglobulin, and light chains of immunoglobulins. These urines also tested negative for myoglobin and hemoglobin. When we then determined the urine total protein concentration of the discrepant samples with the Coomassie Brilliant Blue (CBB) method [2] adapted to a Cobas Fara II (Roche) analyzer, this total protein concentration (CBB) did agree with the sum of the specific proteins, for a given urine. We thus thought it likely a substance or drug was present that positively interfered with determination of total protein by the PR method but had no effect on the CBB method.

A careful review of the clinical amnoses revealed that the discrepant urines came from patients receiving replacement plasma therapy for hemodynamic instability. Interestingly, all of these patients were administered a plasma substitute commonly used in Spain: Hemocer® (Behringwerke). Hemocer contains polypeptide polymers (average molecular mass 35 kDa) obtained from gelatin. Supplied as a 35 g/L colloidal solution, it is mostly (75%) excreted through the kidneys via glomerular filtration.

Subsequently, several dilutions of the Hemocer preparation were analyzed by various methods: the biuret method in a Hitachi 747 analyzer, the PR method in a Cobas Integra analyzer, a modified biuret method [3] in a Vitros 250 analyzer, and the CBB method in a Cobas Fara analyzer.

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Fig. 1. Protein concentrations of Hemoce dilutions measured by different methods.
Transferrin Saturation and Screening of Genetic Hemochromatosis

To the Editor:

Genetic hemochromatosis is the most frequent inherited disease in Caucasian populations. The gene located on the short arm of chromosome 6 was cloned in 1996 [1] and unfortunately named HLA-H. It encodes a protein that is very similar to HLA class I proteins. Two mutations have been described, but the missense mutation (C 282 Y) is observed in >90% of homozygous patients [2]. The fact that the percentage of mutations is variable from one population to another implies the existence of other genes, and makes a genotypic screening impossible.

In 1997 a consensus conference met in Atlanta to define the best procedure to be followed in screening for genetic iron overload. The assay of serum iron and total iron-binding capacity (TIBC) has been recommended as the first step of a phenotypic diagnosis. The ratio between serum iron and TIBC gives the percentage of transferrin saturation with iron.

TIBC is an indirect measurement of the transferrin concentration. The first method described by Ramsay [3] was recently modified by the same author [4]; Excess iron is added to serum to saturate both binding sites of transferrin, and non-bound iron is removed by adsorption on “light” magnesium carbonate. The adsorption capacity of MgCO₃ is well known to vary from one preparation to another [4, 5]. Checking the ability of each brand to adsorb iron is rather cumbersome. There is no reference method for TIBC; nevertheless, a selected method has been published [5]. In 1990 the NCCLS proposed a method for the assay of serum iron and TIBC and will probably finalize it this year. In between-laboratory comparisons, different results were obtained, depending on the analytical procedure [6].

In some pathological disorders, molecules such as ferritin or desferrioxamine bind added iron [7]; TIBC is falsely increased and does not correspond to the binding capacity of transferrin. In iron overload, non-transferrin-bound iron is often present in serum and leads to inaccurate results in transferrin saturation. From a practical point of view the determination of TIBC is tedious, and the step of saturation and removal of excess iron requires technical skillfulness. Few lyophilized control sera are suitable for assessing the quality of TIBC results [8].

The best approach to measure transferrin saturation is to assay the protein itself by an immunological method. Transferrin is a well-known protein that is easily purified. Immunonephelometric or immunoturbidimetric methods are widely used in clinical chemistry laboratories. Their precision and accuracy have been greatly improved by the availability of a new international reference preparation (RPPHS-CRM 470) with assigned values for 14 proteins [9, 10]. This reference material was certified by the Bureau Communautaire de Référence (BCR, Bruxelles) and approved by the US Food and Drug Administration. The standardization of commercial calibrators against it contributes to the improvement of quality in control surveys [11]. For this reason, immunonephelometric assay of serum transferrin should be preferred to the “old” TIBC. The TIBC of transferrin is easily calculated from molecular mass and binding capacity of transferrin [7].

It must be pointed out that the use of CRM 470 triggers significant changes in the standardized results of some proteins, these being lower by 14% for transferrin [9]. Two important consequences must be borne in mind. First of all, new reference ranges should be determined on a world-wide scale. This is the task of the Committee for Plasma Protein Standardization of the IFCC, until available “consensus” reference ranges have been published [12]. Meanwhile, it is probably advisable that reference ranges be determined in each country. Second, new cutoff levels of transferrin saturation should be applied in the laboratory assessment of iron status, either in depletion or in overload.

References