Acute-Phase Proteins in Acute Appendicitis

To the Editor:

After tissue injury or inflammation there may be increased synthesis of several "acute-phase" proteins. Although this response is nonspecific, increases in these proteins in serum are commonly interpreted as a sign of inflammation. Assessment of the diagnostic value of acute-phase proteins in this setting is hampered by the lack of reliable, definitive criteria to establish the presence of inflammation.

We chose to evaluate the diagnostic value of measuring five acute-phase proteins (haptoglobin, α_1 -antitrypsin, α_1 -acid glycoprotein, ceruloplasmin, and C-reactive protein) for the detection of acute appendicitis. By utilizing defined histological criteria to establish the presence of appendicitis after surgery, the sensitivity and specificity of each protein for the diagnosis of acute appendicitis could be evaluated with reasonable certainty.

We studied 98 patients admitted to the emergency room with the clinical designation of "rule-out" appendicitis who subsequently underwent surgery. Blood was sampled on admission to the emergency room. During the period of study, no patient who was discharged without surgery was subsequently readmitted for an appendectomy. The diagnosis of acute appendicitis, made by an anatomical pathologist, was based on standard criteria (1). If initial sectioning failed to show inflammation, the remainder of the specimen was sectioned and microscopically examined *in toto*.

α_1 -Antitrypsin, α_1 -acid glycoprotein, haptoglobin, ceruloplasmin, and C-reactive protein were determined by nephelometry with an ICS Analyzer (Beckman Instruments, Fullerton, CA 92008), according to the manufacturer's procedure.

The diagnostic sensitivity and specificity (2) of each acute-phase protein for the detection of appendicitis were determined at multiple discrimination levels. The decision levels tested ranged from the lower limit of the suggested reference interval to five-fold the upper limit. These data were evaluated by generating a family of receiver operating characteristic curves (3), shown in Figure 1. These curves clearly demonstrate that none of the proteins evaluated exhibited a high sensitivity at specificities greater than 20%. C-reactive protein and α_1 -antitrypsin were more sensitive than the other proteins at specificities from 20 to 90%, but remained less than 90% sensitive for acute appendicitis. At maximal levels of sensitivity, no pro-

Fig. 1. Receiver operating characteristic curves for acute-phase proteins in the diagnosis of appendicitis

True positive ratio (sensitivity) is plotted against false positive ratio (1 - specificity) at various decision levels. Proteins evaluated were C-reactive protein (closed circles), α_1 -antitrypsin (open circles), α_1 -acid glycoprotein (open triangles), haptoglobin (closed triangles), and ceruloplasmin (closed squares)

tein exhibited a positive predictive value of greater than 90%.

Acute appendicitis without rupture is a relatively focal inflammatory disease of unclear etiology. It is a surgically correctable condition, which incurs a significant morbidity if left untreated. The surgical approach to acute appendicitis has resulted in a reported diagnostic error rate (removal of a normal appendix) of 15 to 25% (4, 5). This error is weighed against the risk of delayed surgery and appendiceal perforation. In this study, the clinical diagnostic error was 18% and the perforation rate 8%. We found no evidence of clinical false-negative (missed case) workups. Therefore, the "clinical" diagnostic sensitivity was apparently 100% with a positive predictive value of 82%.

To provide additional diagnostic information, laboratory tests for acute appendicitis should possess 100% sensitivity (no false negatives) and 100% negative predictive value, and a significant increase in positive predictive value above that found without testing. Any significant false-negative ratio is unacceptable, owing to the potential complications of appendiceal rupture if surgery is delayed, especially when the clinical diagnosis is made with such high sensitivity. The acute-phase proteins we tested fail to demonstrate this required sensitivity. Lowering the decision levels to a point where sensitivities approached 90% resulted in a marked loss of specificity and in positive predictive values similar to those seen without testing. We conclude that determination of individual acute-phase proteins does not provide the clinician with any additional information that could decrease the proportion of normal appendices surgically removed while ensuring that all true cases of appendiceal inflammation received prompt surgical treatment.

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Frederick Van Lente
Robert S. Galen

Dept. of Biochem.
Cleveland Clinic Found.
Cleveland, OH 44106

Anthony Marchand

Dept. of Pathol.
Overlook Hosp.
Summit, NJ 07901

Supplier Misidentified

To the Editor:

In a review of hemostatic disease markers (1), Table 4 incorrectly states that New England Nuclear offers a kit for measurement of leukotrienes. Ours is the only company currently manufacturing and marketing kits for quantification of lipooxygenase products. Currently we have kits for 5-HETE, 12-HETE, and 15-HETE. Additionally Seragen's kits for measurement of thromboxane B₂ and 6-ketoPGF_{1 α} (both ³H-RIA or ¹²⁵I-RIA) were not mentioned. In fact, Seragen has the world's most extensive line of kits for prostaglandin measurement.

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Sheila G. Magil

Seragen Inc.
Boston, MA 02122

Improved Liquid-Chromatographic Determination of 5-Hydroxyindoles

To the Editor:

Recently, we described a liquid-chromatographic method for determining indole-3-acetic acid and 5-hydroxyindole-3-acetic acid (5HIAA) in human plasma (1). The procedure involves addition of 40 μ L of a 20 g/L solution of

Fig. 1. Chromatogram corresponding to human plasma treated as described (7), except that 50 μ L of 1 g/L ascorbic acid was added. Peaks corresponding to serotonin (5HT) and 5-hydroxyindole-3-acetic acid (5HIAA) are indicated. The sample injected corresponds to 20 μ L of plasma

ascorbic acid to 200- μ L plasma samples to avoid possible losses due to oxidative degradation. Such a high concentration of ascorbic acid results in a gross peak at the beginning of the chromatogram and precludes the electrochemical detection of any other components eluting ahead of 5HIAA.

We have now tested several different concentrations of ascorbic acid to improve this determination. Addition of 20 to 50 μ L of a 1 g/L solution of ascorbic acid not only fulfills the antioxidant requirement, it also results in liquid-chromatographic profiles in which the peak corresponding to serotonin (5HT) is also present and well resolved from other components (Figure 1). Thus, this parent amine of 5HIAA can also be measured.

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Francesc Artigas
Emili Martinez
José M. Tusell
Cristina Suñol
Emilio Gelpí

Anal. Neurochem. Unit
Instit. de Química Bio-Organica, CSIC
Jorge Girona Salgado s/n
Barcelona-34, Spain

Further Discussion of a Urine Specific Gravity Test Strip

To the Editor:

Opinions differ (1-4) concerning the value of "N-Multistix SG" strips (Ames Division, Miles Laboratories, Elkhart,

IN) for the colorimetric estimation of urine specific gravity. Sources of concern are the insensitivity of the test to non-ionic solutes present in urine and the color-suppressing effect of increasing urine pH. Therefore, little is to be learned from analyzing correlation coefficients reported in surveys, because the results are so easily affected by patient selection.

Patient populations for whom agreement between methods will predictably be poor include: elderly patients with a high frequency of obstructive uropathy and recurrent infections which raise urine pH; patients with chronic renal disease, proteinuria, and tubular acidification defects; patients on unusual sodium chloride and protein intakes whose urinary solute excretion is largely non-ionic; and diabetic patients with glycosuria, with or without proteinuria and renal insufficiency. Because glycosuria constitutes a solute diuresis that blunts the concentrating mechanism, N-Multistix will not accurately portray renal concentrating ability in the glycosuric patient despite its insensitivity to glucose.

Nurses, physicians, and medical trainees are likely to use N-Multistix strips to obtain immediate information on a patient's status in settings where quality control is not practiced. They will probably use the same rules of interpretation that apply to more standard methods of specific gravity measurement. As a result, misleading information may be generated. In most situations involving patient care, no information is preferable to misinformation.

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Barry B. Kirschbaum

Dept. of Med.
Med. College of Virginia
Box 160
Richmond, VA 23298-0001

Increase in Serum Ferritin Concentration Induced by Fasting

To the Editor:

Caballero et al. (1) reported normal

and unchanged serum ferritin concentrations before and after one week of refeeding of children with severe protein-energy malnutrition. This is surprising, because serum ferritin concentration usually increases rapidly after iron is administered to patients deficient in this element (2-5). Although it was not stated in their report, we assume that they administered iron as part of the nutritional program. We present here some observations showing that serum ferritin homeostasis is even more complex than they indicated in their review of the literature. Although the ferritin concentration in serum usually reflects the body iron stores, disproportionately high values may be found in patients with liver disease, malignant disorders, or inflammatory disease. Intra-individual variation in serum ferritin concentration is usually small (5 and references therein), but large variations have been observed in patients with hemochromatosis by some (6, 7) but not all (8) investigators.

We measured serum ferritin (as well as thyroid hormones and several other serum components) during a short-term fast in eight healthy members of the laboratory staff in March, April, and September 1983: five women, three men, all with normal body mass. One woman was studied on two different occasions. The fast lasted two days or longer. The caloric intake was estimated to be not more than a few hundred kilocalories per day (in a single case up to 600 kcal/day). Food intake was water and (or) coffee, tea, "herb-tea" (only six of the eight volunteers), dilute juices from fruits or vegetables. No alcoholic beverages were consumed. No subject received medicinal iron. All individuals remained in good health during the study.

Venous blood was sampled before and during fasting and, in one individual, after one day of refeeding. Ferritin was measured with reagents from Diagnostic Products Corp. (DPC), Los Angeles, CA.

Our quality-assurance program involved analysis of three serum pools and three controls (DPC) at the start and at the end of each assay run (up to 50 patients' samples per run). From six consecutive assay series done during this time we calculated the total between-assay CV to be, for the three serum pools, 6.4% and 6.8% at the beginning and end of the run, respectively (means, 14.5 and 15.1 μ g/L, 3.9 and 4.3% (means, 43.4 and 43.4 μ g/L), and 4.7 and 4.1% (means, 383 and 377 μ g/L). For the commercial control sera the respective values for total inter-assay imprecision were 2.4 and 9.0% (means, 23.1 and 23.7 μ g/L), 2.9 and 3.5% (means, 53.0 and 52.5 μ g/L), and