tions (n = 7), systemic autoimmune disease (n = 8), AMI (n = 3), and miscellaneous (n = 5). None of these patients had received streptokinase.

In the early phase of AMI, cardiac antigens are released. The immunological response to this release often results clinically in the so-called Dressler syndrome, which can be observed in 1 to 7% of patients with a recent AMI (2). Ten days after AMI, antibodies against myosin, actin, and myoglobin could be shown in a large proportion of patients (3).

Also, enzyme molecules may act as antigens in this process; 10 days after AMI, we observed macro-CK complexes, which were absent at the early stages of the patient's AMI (4). In patients with atherosclerosis, which is the pathological condition underlying AMI, the incidence of macro-CK was increased (4). According to Ross and Glomset (5, 6), autoimmunity plays an important role in the pathogenesis of atherosclerosis. In the post-pericardiotomy syndrome, antigenic determinants are apparently modified and give rise to an immune response (7).

When we examined atherosclerotic aortic walls for their LDH-isoenzyme pattern according to the method of Wieme (8), we observed a relative predominance of LDH-3 and LDH-4. Table 1 summarizes the results of the LDH-electrophoresis of inner (intima + media) and outer (adventitia) regions of three aortic walls.

In macro-CK formation, the antibody always shows an affinity for CK-BB, which is abundant in the vascular wall (9). In macro-LDH forms observed after AMI, the antibody was found to be complexed to LDH-3, which is also predominant in atherosclerotic vascular wall. As in the case of CK-BB, LDH-3 can be regarded as a typical example of a vascular wall protein, which may undergo structural changes in the post-AMI period.

We believe the occurrence of macro-LDH after AMI can be explained as an autoimmune reaction against proteins of the cardiovascular system. The presence of macro-LDH in the absence of therapy with streptokinase and the evidence of autoimmune features in atherosclerosis support this view.

References

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Correction of Precision Equations in NCCLS EP5-T

To the Editor:

The NCCLS document EP5-T (1) is a valuable resource used often in method evaluations. However, the equations for calculating total precision in EPS-T are incorrect. The purpose of this Letter is to explain the error and to provide correct equations. Equations 1 and 2 are recommended by EP5-T for calculating total precision for the cases when there are one or two runs per day, respectively. \( S_P = \text{total precision, } B = \text{standard deviation of daily means, } A = \text{standard deviation of run means, } S_w = \text{within-run precision, and } N = \text{number of observations within a run.} \)

\[
S_P = \left[ B^2 + \left( N - 1 \right) S_w^2 \right]^{1/2} \\
S_T = \left[ \left( 2B^2 + A^2 + S_w^2 \right) N \right]^{1/2} \\
S_M = \left[ B^2 - \frac{S_w^2}{N} \right]^{1/2} \\
S_I = \left[ S_M^2 + S_T^2 \right]^{1/2}
\]

This is valid if and only if the estimate of \( S_M \) is greater than or equal to zero. For example, if \( B = 0 \) [i.e., the mean on all days are the same] and \( N = 2 \), then according to equation 1, \( S_P = S_w / \sqrt{2} \), which is theoretically impossible (2), because total precision cannot be less than within-run precision. If the true \( S_M \) were zero, 50% of the time \( S_M \) would be estimated to be less than zero due to sampling error.

The correct method is to calculate the day-to-day component of precision by using equation 3. If the result for equation 3 is negative, set it to 0. Then use equation 4. (One may wish to test for statistical significance for positive estimates of \( S_M \) using an \( F \) test. Non-statistically significant results would also be set to zero before using equation 4.)

References

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Plasma Acidification Increases Atrial Natriuretic Peptide as Measured by Radioimmunoassay

To the Editor:

Considerable evidence now suggests that the atrial natriuretic peptides
(ANP) may be a new hormone of potential importance in the control of sodium and water balance (1–2). Many radioimmunoassay (RIA) procedures have now been described for measuring this peptide in plasma (3–11), and different laboratories have reported various ranges for ANP concentrations in normal subjects, with means ranging from 8 to greater than 100 ng/L. Several factors could account for these variations: differences in subjects' characteristics, such as sodium intake (5, 6) and age (12), and important methodological differences. In some assays ANP is measured directly (3, 4), in others indirectly after extraction of plasma on Sep-pak cartridges (4–8) or other material (9–11). Differences in analytical recovery after extraction and the use of different antibodies and radiolabeled ANP, which could differ in their binding characteristics, could presumably influence the concentrations measured. In addition, however, acidification of plasma before extraction has been widely used in RIA procedures for ANP (4, 5, 7, 8), although the reason for this acidification has not been made explicit. To investigate whether this step might influence the values obtained, we measured the concentrations of immunoreactive ANP (IrANP) in plasma with and without prior acidification.

Blood was sampled from either normal subjects (peripheral blood) or by cardiac catheterization from patients with known coronary artery disease but without overt clinical cardiac failure. The samples, collected in tubes containing EDTA and aprotinin (2000 kallikrein units per 10 mL of blood), were centrifuged and the plasma was stored in two 5-mL aliquots at −20 °C. Before the assay, one aliquot of each sample was acified with 0.5 mL of 1 mol/L hydrochloric acid (final pH 3.3–3.9); the other aliquot (control) received an equal volume of distilled water (final pH 7.7–8.4). All aliquots were then extracted by using Sep-pak C18 cartridges (Waters Associates) activated with 5 mL of methanol followed by 5 mL of water. The adsorbed IrANP was eluted from each cartridge with 4.5 mL of ethanol/acetic acid/water (90/4/16 by vol) into glass tubes; the solvent was evaporated at 50 °C, under nitrogen, and the residues were redissolved in 250 μL of phosphate/Triton X-100 buffer (pH 7.5), as previously described (6). The pH was checked in every extract before RIA; in each case it was between 6.9 and 7.1. As Figure 1 shows, acidification of the plasma before extraction increased the measured values of immunoreactive ANP in all plasma samples, irrespective of the concentrations in the non-acified samples, by a mean of 70% (range 20 to 190%). Recovery of ANP from the Sep-pak cartridge, tested by adding 50 μL (50 nCi) of iodinated rat ANP (Amer sham International, Amerham, U.K.) to 5-mL plasma samples, was 86.8 (SD 10.1)% in four non-acified plasma samples, 63.7 (SD 11.9)% in four acified plasma samples. Thus the higher concentration of IrANP measured in acified samples cannot be accounted for by a supposed increased recovery from the Sep-pak cartridge.

Moreover, because the pH of the final plasma extracts immediately before RIA were about the same (6.9–7.1) for both the non-acified and acidified plasma extracts, differences in measured IrANP concentrations cannot be attributed to pH-induced alterations in ANP–antibody binding during the assay. Perhaps acidification of plasma releases free peptide from a protein-bound form, dissociates the peptide from a polymerized form, or cleaves larger forms of atrial peptides into smaller immunoreactive fragments. Whatever the mechanism, this preanalytical step is an important contributor to the observed variations in the concentrations of IrANP in plasma as reported by different workers.

References


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The Abbott ER-EIA Monoclonal Kit

To the Editor:

In the report by Ream and Vrabel (1) evaluating the Abbott ER-EIA monoclonal kit, they conclude that (a) the antibody-coated beads (plastic, not glass) in the kit fail to bind unoccupied (hormone-free) estrogen receptor (ER) and bind the receptor in amounts unrelated to the total amount of ER in the cytosol, and (b) the antibody used in the kit may not be specific for ER. These conclusions are not consistent with a large body of data compiled on the antibodies and, in our opinion, are not supported by the data presented in the paper.

The antibodies in question, D547Spy and H222Spy, have been well characterized by Drs. G. L. Greene and E. V. Jensen and colleagues at the University of Chicago, who have shown that