

Original Article

Modified Microsample ACT Test for Heparin Monitoring

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ABSTRACT

The activated clotting time (ACT) is a standard method of heparin monitoring during cardiac angioplasty and cardiac surgery. A modified ACT (ACT+) which employs a microsample blood volume (0.05 ml) and yields faster clotting time results than a conventional Hemochron ACT was evaluated and compared to the standard ACT.

The ACT+, determined using a single channel general purpose analyzer (Hemochron Jr.), employs a plastic cuvette containing dried ACT reagents (kaolin-silica-phospholipid). In vitro evaluation of heparinized normal donor blood demonstrated the test insensitivity to aprotinin. Using split blood specimens, duplicate ACT and ACT+ were performed (n=92) and a correlation between the two was established.

In evaluation of 79 cardiac surgery and 53 angioplasty patients, good correlation was observed between ACT+ and Hemochron ACT ($r=0.93$, $n=574$). Mean versus difference plots demonstrated an improved reproducibility of the ACT+ (Mean SD=35 seconds) compared to the ACT (Mean SD=53 seconds), presumably due to automation of the blood sample sizing and blood/reagent mixing. These studies indicate that the microsample ACT+ is an alternative to the conventional ACT with an advantage of small sample requirement and rapid reproducible results.

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INTRODUCTION

Anticoagulation with heparin, necessary for invasive cardiac procedures, requires close monitoring to reduce the deleterious side effects associated with inadequate or excessive drug dosing. Over the course of the last two decades the activated clotting time (ACT) test has proven the standard method for heparin monitoring during cardiac surgery requiring cardiopulmonary bypass (CPB) (1,2) and percutaneous transluminal coronary angioplasty (PTCA) (3). The Hemochron ACT^a which is performed using either a celite or kaolin activator, measures the integrity of the intrinsic blood coagulation pathway and the degree to which heparin inhibits clotting. Target Hemochron ACT values have been established for safe anticoagulation during PTCA (3) and CPB (4).

The Hemochron Jr.^a is a new microsample blood coagulation analyzer which performs a modified ACT, the ACT+. The Hemochron Jr. ACT+ offers the advantage of small sample volume requirement, automated blood sampling, and standardized blood/reagent mixing. A novel blood coagulation activator consisting of a combination of kaolin and silica is employed to enhance the heparin sensitivity of the test and reduce the interference of aprotinin on the test result. Aprotinin is a protease inhibitor which has recently been approved for use in cardiac surgery to reduce post-operative blood loss (5,6). Aprotinin artificially prolongs the celite ACT (7), rendering the test of little use in these clinical situations.

This study demonstrates the precision of the Hemochron Jr. ACT+ and its utility as an alternative test to monitor heparin anticoagulation during CPB and PTCA.

MATERIALS AND METHODS

ACT tests

Correlation studies were conducted using a Hemochron and a Hemochron Jr. blood coagulation analyzer. The Hemochron employs a mechanical clot detection methodology to measure clotting time. Blood (2 ml) is added to a test tube, containing a celite (Hemochron CA510)^a or kaolin activator (Hemochron K-ACT)^a, agitated, and placed in the rotating incubated test well. The blood fluidity is monitored with an electromagnetic field. As a fibrin clot forms, the displacement of a magnetic rod in the test tube causes a change of the electromagnetic field strength which signals an endpoint.

The Hemochron Jr. employs a combination of mechanical-optical clot detection mechanism. Blood (less than 0.05 ml) which is applied to the test cuvette well is automatically drawn into the instrument by a pump. The blood is mixed with lyophilized ACT+ reagent (a mixture of kaolin, silica and phospholipid) and moved back and forth, across a restriction area in the test channel. As a fibrin clot forms the decreased rate of movement of the blood in the test channel is monitored by the optical system and signals an endpoint.

In vitro studies

Blood was collected from normal donors, free from medication, by venipuncture. Baseline ACT+ and ACT were performed in samples from 20 donors. In another four donors' samples, porcine heparin^b was added to aliquots of the blood samples in amounts corresponding to 0, 2, 3, 4, 5 and 6 units/ml. Both Hemochron Jr. ACT+ and Hemochron celite ACT tests were performed immediately from these aliquots. In some samples, aprotinin^c was added at concentration of 500 KIU/ml and ACT+ and kaolin ACT were performed to determine the effect upon the ACT+ test.

Clinical studies

Patients were randomly selected from those requiring PTCA or CPB. No patients were excluded. None of the patients received aprotinin. Pre-heparin bolus and post-heparin bolus blood samples were collected from patients prior to initiation of PTCA (n=53) and CPB (n=79). ACT+ and celite ACT tests were performed concurrently at the bedside with the same sample draw and at regular intervals during the procedure.

Quality controls

The Hemochron and Hemochron Jr. analyzers employed two levels of electronic quality control for daily instrumental verification as well as two levels of coagulation control reagent for test precision validation.

Instrument Quality Control: The electronic quality control cartridges perform a pre-programmed simulation of a clot formation. In the Hemochron a measured decrease of the magnetic field strength is used to simulate a clot and the analyzer response is determined. In the Hemochron Jr. the blockage of the optical detector simulates a clot and assesses analyzer response.

Wet Test Quality Control: Each lot of ACT+ or ACT tests is quality controlled using lyophilized control plasma (Hemochron) or whole blood (Hemochron Jr.). Each is performed by rehydration of the citrated control mixture with diluent, addition of calcium chloride and application to the ACT test tube or ACT+ cuvette.

Statistical analyses

Correlation between ACT+ and ACT were established using linear regression models. Descriptive statistics are used to identify measures of mean, standard deviation (SD) and coefficient of variation (CV).

RESULTS

Generation of conversion correlations

In a pilot evaluation employing a split sample design, concurrent ACT+ and celite ACT tests were performed in dupli-

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cate in CPB (n=20) and PTCA (n=12) patients. Regression analysis of the averaged ACT+ results and the averaged ACT results were conducted (n=92 paired data points) (Figure 1). A bimodal result was observed with two distinct linear regressions corresponding to an ACT+ cut off value of 132 seconds (regression 1: ACT+≤132 sec, regression 2: ACT+>132 sec).

Based on these correlation equations, a conversion table was generated and programmed into the Hemochron Jr. Analyzer such that Hemochron Jr. ACT+ results are reported in conven-

tional Hemochron celite ACT values, values familiar to the clinician managing the cardiac patients. All subsequent results reported below are expressed as the converted celite ACT values.

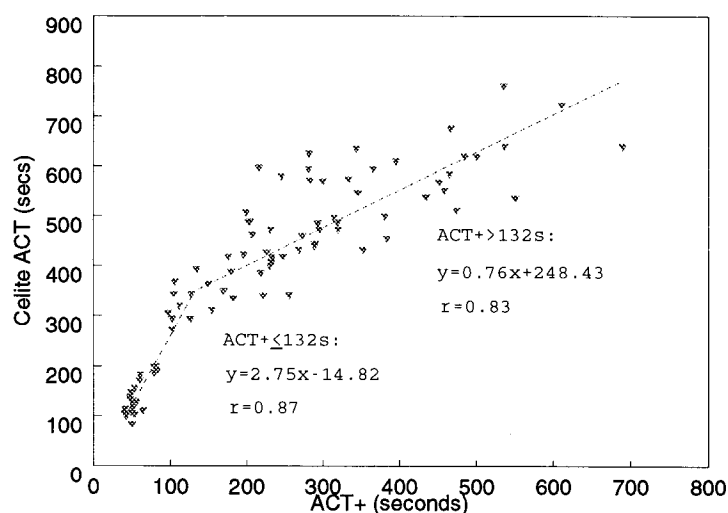
In vitro studies

Comparative Heparin Sensitivity of the Hemochron Jr. ACT+ and Hemochron ACT: In a normal population (n=20), the mean baseline ACT+ was 103 ± 11 seconds (expressed as converted celite ACT values). The in vitro heparin sensitivity curve shown in Figure 2 was generated by adding increasing amounts of porcine heparin to aliquots of normal donor blood (n=4 donors). The ACT+ and celite ACT tests were performed in duplicate. Both tests show an increase of clotting times proportional to the increase in the blood heparin concentration. The sensitivity curves for both assays are linear. The heparin sensitivity curve is variable for each donor, attributing to the large standard deviation observed in the graph.

Correlation of the Hemochron Jr. ACT+ to Hemochron ACT: The results of this normal donor study were graphed to illustrate the correlation of the two assays. Good correlation was observed between the Hemochron Jr. ACT+ and Hemochron ACT in this normal donor population (Figure 3).

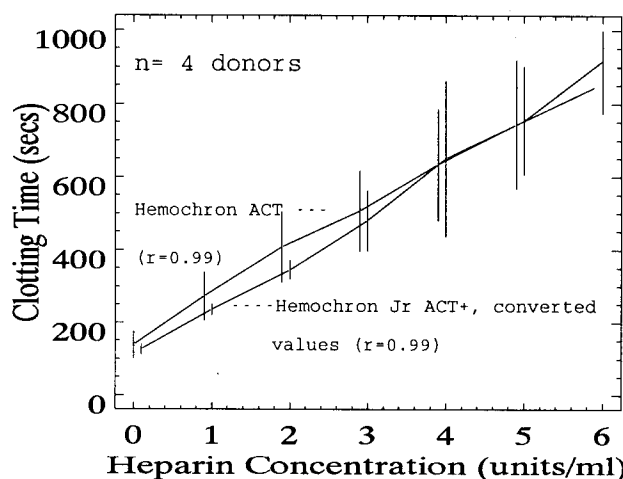
Test Sensitivity to Aprotinin: Aprotinin was added to normal donor blood samples to attain a concentration of 500 KIU/ml blood. Each sample was aliquoted and tested using the ACT+ and a control kaolin-ACT (Hemochron K-ACT)(Figure 4). The celite ACT was not employed due to the known interference of aprotinin with this test (4). Each sample was also tested with the ACT+ prior to the addition of aprotinin. The heparin

Figure 1: Correlation of the Hemochron celite ACT and the Hemochron Jr. ACT+



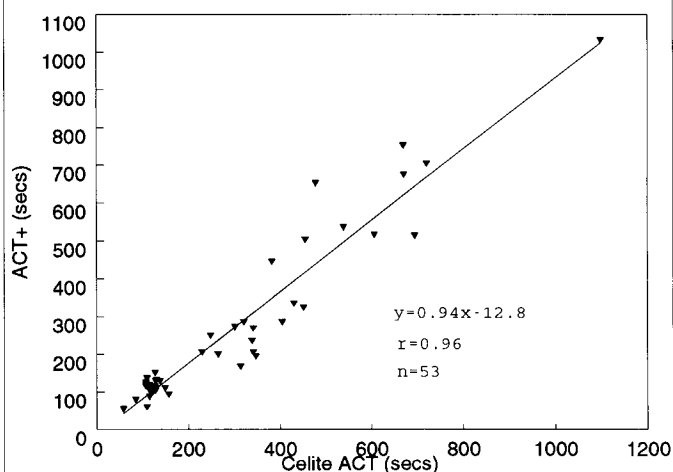
Each value represents the mean of duplicate determinations. The individual regression equation is shown for each phase of the bimodal correlation.

Figure 2: Heparin sensitivity expressed as heparin concentration versus ACT

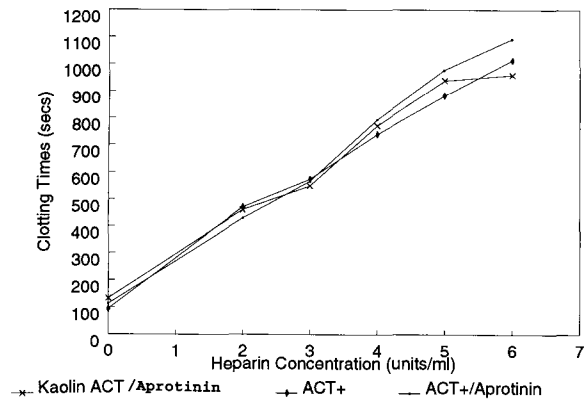


The values shown represent the mean $\pm$ 1SD of four different normal donor blood samples to which porcine heparin had been added. Both tests demonstrate a similar heparin sensitivity (paired t-test: $p > 0.1$, no significant difference).

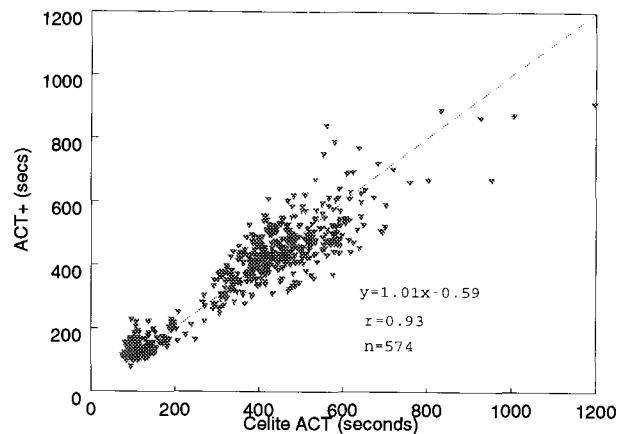
Figure 3: Correlation of the celite ACT and the converted ACT+ values



Clotting time results obtained from the in vitro heparin experiments shown in Figure 2 are graphed to illustrate the correlation between the two tests in 4 normal donors. Three test results were over 1000 seconds (Hemochron) and non-recordable on the Hemochron Jr.

Figure 4: Influence of aprotinin on the ACT+ test

ACT+ results were not affected by aprotinin (500 KIU/ml) and were significantly correlated to the kaolin-ACT.

Figure 5: Celite ACT and ACT+ correlation in PTCA and CPB patients

sensitivity of the ACT+ was not altered in the presence of aprotinin (paired t-test: $p > 0.1$, no significant difference). The converted ACT+ clotting time was significantly correlated to the kaolin-ACT ($r = 0.994$) in the samples with aprotinin.

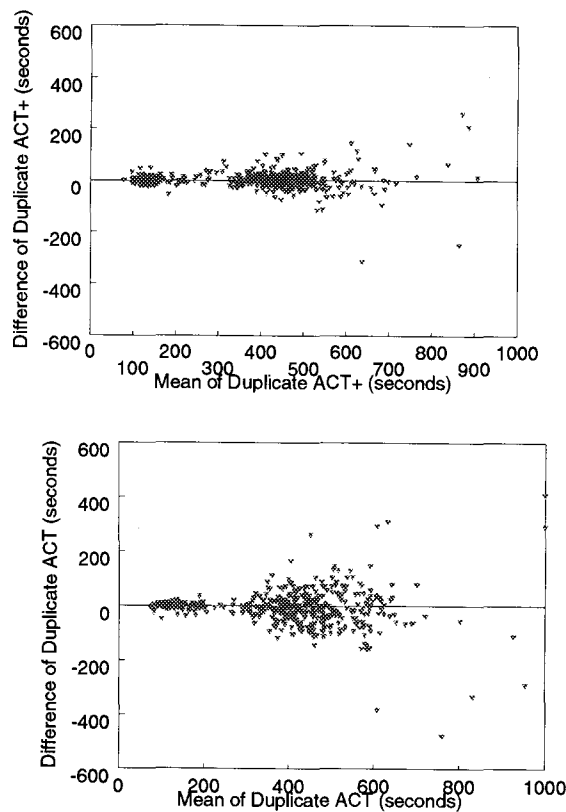
Clinical studies

Correlation of the ACT+ to celite ACT: Following incorporation of the programmed celite ACT conversion in the Hemochron Jr., a subsequent clinical trial was conducted to verify the correlation of the ACT+ and celite ACT. Blood samples were obtained from 79 CABG patients at baseline, following heparinization, and during CPB, and from 53 PTCA patients before and following heparinization for angioplasty. A total of 574 samples were compared for concurrent ACT tests. The mean baseline ACT+ was 124 ± 14 seconds in patients not receiving heparin treatment ($n = 100$). The ACT+ and ACT results were highly correlated ($r = 0.93$) and not significantly different (paired t-test: $p = 0.36$) (Figure 5).

Reproducibility of the tests: Reproducibility of the ACT+ test was determined using the analysis of duplicate testing represented as the clinical data in Figure 5. Mean versus difference plots (Figure 6) demonstrated a significantly improved reproducibility of the ACT+ (Mean SD=35 seconds, $n = 509$ paired data) compared to the ACT (Mean SD=53 seconds, $n = 516$ paired data), $p < 0.001$.

DISCUSSION

The Hemochron ACT has been a standard method to monitor anticoagulation and hemostasis during invasive cardiac procedures for more than twenty years. This

Figure 6: Mean versus difference plots of celite ACT and ACT+ tests

The mean versus difference plot illustrates the reproducibility of duplicate clotting time measure. The ACT+ (top) shows less variability of the duplicate measure compared to the ACT (bottom).

test has been shown to be valuable as an indicator of heparin resistance and sensitivity and as an appropriate guide to maintain proper heparin and protamine dosing during CPB and PTCA (2,8). Hemochron ACT clotting times have been identified as target anticoagulation ranges in these patients (3,4).

The problematic aspects of the ACT test are its dependence upon operator technique to yield precise results. In a properly controlled clinical setting precision results are routinely available provided the volume of blood added to the test tube and the degree of agitation of the test tube are standardized.

The modified Hemochron Jr. ACT+ described in this report is an alternative means to monitor heparin anticoagulation during invasive cardiac procedures. The test displays a linear sensitivity to heparin over the range of concentrations employed in these settings and demonstrates good correlation to the Hemochron celite ACT and kaolin ACT. Display of converted celite ACT values on the Hemochron Jr. provides the clinician familiar reference ranges such that hemostasis may be assessed, and drug dosing adjusted, easily and quickly. Furthermore the ACT+ displays an improved reproducibility compared to the traditional ACT. This may be attributable to automation and standardization of the blood sample volume measurement and the blood/reagent mixing.

A complete quality assurance program can be established for the Hemochron Jr. ACT+ by utilizing electronic quality control to confirm daily operation of the instrument and a lyophilized whole blood control to confirm performance of the test cuvette.

In laboratory evaluation the ACT+ which employs a kaolin-silica activator was not influenced by the presence of aprotinin. The kaolin ACT is known to be unaffected at moderate aprotinin concentration while the celite ACT is artificially prolonged (7,9). As the use of aprotinin becomes more common in cardiac surgery the ACT+ may prove to be an alternate method for anticoagulation monitoring of patients receiving this drug. The test is directly applicable in all adult cardiac patients irrespective of the concurrent use of aprotinin. Initial studies which demonstrate the utility of the ACT+ in pediatric cardiac patients suggest its utility in this patient population where blood volume conservation may be important but further confirmatory studies are required.

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