Serum Glutamic-Oxaloacetic Transaminase Activity: Diagnostic Accuracy of the Revised Spectrophotometric and the Dinitrophenylhydrazine Methods

Elias Amador, Robert J. Franey, and Mary F. Massod

The diagnostic accuracy of two methods for measuring serum glutamic-oxaloacetic transaminase activity was examined in 50 cases of acute myocardial infarction proven by autopsy and in 12 nonfatal cases documented by rigid clinical criteria. All activities measured with the revised spectrophotometric method were elevated during the second day after infarction, and all patients had an elevated activity. The spectrophotometric method therefore had a diagnostic accuracy of 100%.

Activities measured with the dinitrophenylhydrazine method of Reitman and Frankel were elevated in only 68% of cases during the second day after infarction, and the activity was elevated in only 76% of all the patients during the first 4 days. Dinitrophenylhydrazine-measured activities were therefore 20-30% inaccurate. The diagnostic inaccuracy of the dinitrophenylhydrazine method could result in 300,000 false-negative diagnoses of acute myocardial infarction each year in the United States alone.

Serum glutamic-oxaloacetic transaminase (SGOT) activity, measured spectrophotometrically, has been shown to be highly accurate in the diagnosis of acute myocardial infarction (1).

The dinitrophenylhydrazine method of Reitman and Frankel has also been proposed for diagnostic use (13, 19). Recently we have become aware of falsely normal dinitrophenylhydrazine SGOT activities during acute myocardial infarction. In reviewing the literature, we found that the diagnostic accuracy of the dinitrophenylhydrazine method has not been examined critically. Therefore, we have scrutinized the diagnostic accuracy of the spectrophotometric and dinitrophenylhydrazine methods in groups of consecutive patients with acute myocardial infarction proven by autopsy, or by rigid clinical criteria.

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Materials and Methods

Group 1 Fatal Acute Myocardial Infarction

Diagnostic accuracy is defined as the correlation of elevated SGOT activity with acute myocardial infarction proved to be present at autopsy (21). The protocols of the 1000 autopsies performed at these hospitals from January 1963 to June 1965 were reviewed for cases of acute myocardial infarction. A total of 50 cases was found in which the SGOT activity had been measured during the first 4 days following the onset of infarction. These 50 autopsy-proved cases accordingly constitute the standard of reference for examining the diagnostic accuracy of the SGOT determination methods. The clinical, laboratory, and electrocardiographic criteria of Snodgrass and co-workers (21) were employed to date the onset of infarction, and the histologic criteria of Mallory and co-workers to confirm the age of the infarct (25).

The SGOT activities were measured in the chemistry laboratories of each of the two participating hospitals, one of which used the revised spectrophotometric method and the other the dinitrophenylhydrazine method.

In the first series of 25 consecutive autopsy cases of acute myocardial infarction the SGOT activity was measured spectrophotometrically. The assay used the optimum conditions defined by Henry et al., and by Amador and Wacker (3, 14, 17). Normal spectrophotometric activities, measured at 25° ± 0.1° in 110 healthy adults, ranged from 9 to 25.8 units. The upper limit of the normal range (mean ± 2 standard deviations) was 25 units. Consequently, an activity above 25 units had a 97.8% or greater chance of being abnormal, and was considered elevated (28).

In a second series of 25 consecutive autopsy cases of acute myocardial infarction, the dinitrophenylhydrazine activity was measured with commercial SGOT reagent kits, as recommended by Frankel and Reitman (13). Activities above 50 units were considered elevated (13, 19).

Group 2 Nonfatal Acute Myocardial Infarction

The SGOT activity was measured on the same serums by both methods in another 12 consecutive patients, during the first 4 days following nonfatal infarctions. The rigid clinical criteria of Snodgrass et al. served to document the diagnosis and to estimate the date of infarction (21).

Results

Group 1 Fatal Acute Myocardial Infarction

Elevated SGOT activity is known to appear usually 1 day after the onset of infarction, reaching a peak in 2 days, and subsiding within
3–5 days. With both methods, SGOT activities were measured from the first through the fourth day after the onset of myocardial infarction.

The number of elevated SGOT activities during the first 4 days following the onset of infarction was examined. The spectrophotometric activities were elevated in 100% of the cases during the second day. On the other hand, the Reitman-Frankel activities were elevated in only 68% of the cases during the second or peak day (Table 1).

Spectrophotometric activities remained elevated during an average of 3.3 days, whereas the dinitrophenylhydrazine activities remained elevated for only 2.5 days (Table 2).

The over-all incidence of elevated SGOT activities during the first 4 days after the onset of infarction was also examined. The spectrophotometric activities were elevated for all patients during this period, a diagnostic accuracy of 100% (Table 2). In marked contrast, the dinitrophenylhydrazine activities were persistently normal in 5 of 25 patients, a diagnostic accuracy of 80%. Most important is the clinical consequence of the persistently negative dinitrophenylhydrazine activities, as the diagnosis of an acute myocardial infarction was missed in 3 of these patients during life.

**Group 2  Nonfatal Acute Myocardial Infarction**

The diagnostic accuracy of both methods was compared by simultaneous measurements of SGOT activity on the same sera of 12 consecutive patients with nonfatal acute myocardial infarction. Here again, the revised spectrophotometric activities were elevated in 100% of the patients during the second day, while the dinitrophenylhydrazine activities were elevated in only 67% of them (Table 1). The high incidence of false negative dinitrophenylhydrazine activities and the

**Table 1. Elevated SGOT Activities During Acute Myocardial Infarction Proved on Autopsy (%)**

<table>
<thead>
<tr>
<th>Measurement method</th>
<th>1</th>
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<td>68</td>
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<td></td>
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<td>67</td>
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*The SGOT activities were measured separately in each method in 2 series of 25 autopsy cases each.
†The SGOT activities were measured simultaneously by both methods in one group of 12 patients.
marked discrepancy in the duration of elevated activities measured by both methods were also evident in these nonfatal cases (Table 2).

Discussion

SGOT activity is known to increase consistently after an acute myocardial infarction. Unlike leukocytosis, increased erythrocyte sedimentation rate, increased levels of C-reactive protein, fever, and other nonspecific abnormalities, elevated SGOT activity is relatively specific for myocardial infarction, if injury or inflammation of the liver and skeletal muscle can be excluded. Furthermore, experimental studies indicate that even small areas of infarction induce a significant elevation of activity (1).

The method employed plays a crucial role in the diagnostic accuracy of the SGOT test. As shown by the results of this study, the spectrophotometric method is the method of choice, as it is 100% accurate in myocardial infarction. This excellence could not be maintained with the dinitrophenylhydrazine method of Reitman and Frankel: In fact, fully 20% of the fatal cases of myocardial infarction were missed with it. A comparatively high proportion of false negative Reitman-Frankel activities has also been reported by others (8, 12, 20, 22, 23).

This high incidence of false negative dinitrophenylhydrazine activities abolishes the highly useful function of SGOT assays, differentiating angina pectoris from acute myocardial infarction, or detecting a silent myocardial infarct when the electrocardiographic findings are masked or nonspecific. The danger of false negative activities is further underlined by the fact that an estimated 1,500,000 persons suffer an acute myocardial infarction, and 546,000 persons die from it each year in the United States (18, 24). Widespread use of the dinitro-

<table>
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<tr>
<th>Measurement method</th>
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<th>Patients with elevated SGOT</th>
<th>Mean duration (days)</th>
<th>False negative SGOT (%)</th>
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*The SGOT activities were measured separately in 2 series of 25 autopsy cases each.
†The SGOT activities were measured simultaneously in one group of 12 patients.
The dinitrophenylhydrazine method therefore could potentially result in false negative SGOT activities for 300,000 persons each year in the United States alone. The diagnostic pitfalls of the dinitrophenylhydrazine method are not limited to myocardial infarction. In viral hepatitis, an endemic and highly contagious disease, the SGOT activity is consistently elevated when measured spectrophotometrically (26). In contrast the dinitrophenylhydrazine method is falsely negative in 25% of cases (27).

The diagnostic inaccuracy of the Reitman-Frankel dinitrophenylhydrazine method results from its marked lack of correlation with the standard spectrophotometric method (2-7, 9, 10, 15-17). The analytical factors responsible for this are:

1. Dinitrophenylhydrazine is a nonspecific reagent that reacts with both substrates and products, and with ketoacids in serum. The absorbance of the reagent-serum blank is many times larger than that of the colored product, and therefore small variations in the blank produce large errors in the measured activity.

2. The concentration of substrate is rate-limiting.

3. The reagent “calibration curve” relates a baseline of pyruvic dinitrophenylhydrazine with glutamic-oxaloacetic transaminase activities, thereby violating the basic rule of analytical chemistry that the standard should have the same composition as the test substance. Moreover, this “calibration curve” was established against the rate-limiting spectrophotometric assay of Karmen that employs suboptimal concentrations of substrate and coenzyme, and that does not control the temperature of catalysis.*

4. For a method to be diagnostically accurate a statistically defined normal range must be included. This has not been done for the Reitman-Frankel method. The use of a “borderline” range is diagnostically hazardous, abolishes the sensitivity of the method, and lacks a statistical basis.†

These factors deprive the Reitman-Frankel method of its main diagnostic function: the clarification of mild, albeit highly significant, elevations of activity (Table 3).

The readiness with which the claims made for “simplified” SGOT

*The spectrophotometric method of Karmen uses rate-limiting concentrations of L-aspartic acid (33 mM) and NADH (0.1 mM) and neglects to control the temperature of catalysis (5, 14, 15). As shown recently, the optimum concentrations of substrate and coenzyme are 125 and 0.5 mM, respectively, and the use of a constant temperature cuvet compartment is essential for accuracy (5, 14, 15).

†Chinsky et al. initially defined the “borderline” range thus: “An arbitrary definition of range of activity was made as follows: Normal up to 40, Borderline 41-50, High above 50 units.” (11). The marked inaccuracy of this definition has been shown to result from the technical defects of the Karmen and Reitman-Frankel assays (5, 14, 15, 17).
kits have been accepted is remarkable because there is a striking lack of analytical chemical data and autopsy documentation to support their accuracy and diagnostic validity. The widespread adoption of accurate methods for the assay of SGOT activity, based on optimum assay conditions, specific reagents, and statistically defined normal activities, should eliminate the highly dangerous findings of false-negative activities and should permit the clinical chemist to fulfill his responsibility to the patient.

References