

Low prevalence of detectable serum cardiac troponin I among healthy Tanzanian adults: observational study

MOHAMED Y. JANABI*, PATRICIA MUNSERI¹, SAID ABOUD¹, ERIC SANDSTROM² and MUHAMMAD BAKARI¹

¹Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

²Karolinska Institutet, Sodersjukhuset Stockholm Sweden

Abstract

Background: Cardiac troponin test is used in detecting various heart disorders. The objective of this study was to establish normal reference levels for serum cardiac Troponin I which could be utilized for selection of vaccines and determine any electrocardiogram (EKG) changes among healthy volunteers.

Methods: A total of 263 healthy blood donors from Dar es Salaam, Tanzania were included in this sub-study. A thorough medical history and physical examination to rule out any major chronic disease like heart failure, chronic kidney diseases, diabetes mellitus and HIV was undertaken. Ten mL of blood sample for the purpose of establishing normal reference values for Troponin I assessment and parallel EKG was performed to all participants.

Results: Of the 263 subjects, males were 156 (59.3%) and females were 107 (40.7%). Median (range) age was 34 years old. The manufacturer's reference level for serum Cardiac Troponin I was 0.00-0.39 µg/L. Serum Cardiac Troponin I was detected in two blood donors (0.76%). However, their Troponin I levels were within the manufacturer's normal range (0.01-0.36 µg/L). Clinically both subjects were healthy and their EKG tracing were unremarkable.

Conclusions: Our study has shown that among healthy subjects, detectable serum cardiac Troponin I is a rare finding. The manufacturer's range is applicable in our setting and can be used in the ongoing vaccine trial. The significance of minimally elevated serum cardiac Troponin I may represent a subclinical cardiac injury and have important clinical implications, a hypothesis that should be tested in future longitudinal outcome studies.

Keywords: cardiac Troponin I, electrocardiogram, blood donors, vaccine trial, Tanzania

Introduction

Cardiac troponins are well established as specific biomarkers for myocyte injury in the setting of acute coronary syndromes (ACS) (Safford *et al.*, 2012). Troponin measurement aids in the diagnosis of acute myocardial infarction (MI), facilitates risk stratification, and helps to direct treatment modalities (Montagnana *et al.*, 2008). Although troponin elevation in disorders such as congestive heart failure (CHF) and chronic kidney disease (CKD) portends worsened prognoses, physicians struggle to interpret troponin values in non-ACS settings (Freda *et al.*, 2002; Apple *et al.*, 2004). Compounding these clinical issues is controversy with regard to the appropriate lower limits to define troponin elevation and the importance of imprecision with current troponin assays around these lower limits of detection. Expert committees have recommended that troponin elevation be defined, with the use of a normal reference population, as the lowest detectable troponin concentration above the 99th percentile that has 10% coefficient of variation (CV) (Venge *et al.*, 2002; Uettwiller-Geiger *et al.*, 2002). For cardiac troponin I (cTnI) upper level using the study assay, is 0.39 µg/L, whereas the lower detection limit is 0.00 µg/L. The choice of the threshold has important clinical implications because recent studies suggest that in patients with ACS, cTnI levels between 0.01 and 0.03 µg/L may have important prognostic and therapeutic implications (Meier *et al.*, 2002). In addition, minimally increased cardiac troponin I levels in patients without definite ACS are also associated with poor prognosis. To date, few data are available with regard to the prevalence of cTnI elevation in healthy general population.

* Correspondence: E-mail m_janabi@yahoo.com

Troponin levels are increasingly being measured in subjects without typical ischemic chest pain and the resultant uncertainty as to the interpretation of values in these settings may promote clinical confusion. The Tanzania-Mozambique Vaccine (TaMoVaC) 01 trial is a phase II HIV vaccine trial that is intended to ascertain the safety and immunogenicity of a DNA prime and an MVA boost vaccine. Since it is still unclear on whether MVA causes myocarditis, pericarditis and/or myopericarditis, cardiac troponin *I* levels will be measured in enrolled volunteers receiving MVA so as to monitor the adverse events if any on the study participants and EKG will be performed in all participants. However, reference (normal) levels for cardiac troponin levels in the Tanzanian adult population is still unknown leading to difficulty in interpretation of results. Therefore there is a need to establish normal reference levels for cardiac troponin in our own population. Establishment of normal reference levels will not only assist in trials that use MVA but will also be of clinical value during clinical interpretation of cardiac troponin levels that will be of much use, due to the rise in cardiac conditions such as acute coronary syndromes.

Our research group has just completed a phase I/II HIV vaccine trial (HIVIS 03) among healthy volunteers using a DNA priming and MVA (received from Walter Reeds-USA) boosting vaccination approach in Dar es Salaam, Tanzania. The HIVIS03 trial has shown excellent safety and immunogenicity results. This led to new grant from the European and Developing Countries Clinical Trials Partnership Programme (EDCTP) to conduct another HIV vaccine trial (TaMoVac 01 and 02) on improved DNA priming and MVA boosting strategies in healthy volunteers. Since MVA is closely related to small pox, there have been some concerns of possible perimyocarditis associated with MVA administration. Though there is no data available to prove this concept. However, the US Food and Drug Authority (FDA) requires that all vaccines of MVA be under close monitoring for cardiac injury including serum troponin assessment and EKG monitoring.

In the general healthy population the prevalence of detectable serum cardiac Troponin *I* (cTn *I*) is unknown and the significance of minimally increased Troponin *I* remains controversial. Since our group is using MVA as a boost vaccine we are required by FDA regulations to measure serum Troponin *I* levels and perform EKG to all volunteers. To better understand the prevalence of cTnI detection that occurs in the general population without cardiovascular disease, kidney or metabolic complaints we measured cardiac cTnI levels among healthy blood donors using manufacturers range as standard reference to be able to establish our own normal reference values.

Material and Methods

Study area and design

This cross-sectional community-based study was carried out in Dar es Salaam City, Tanzania. The City has three municipal hospitals (Ilala, Temeke and Kinondoni) with approximate population of 5 million residents. The study included 263 participants consisting of demographic and medical history data, as well as objective measurements (weight, heart rate, and blood pressure). Participants aged between 25-65 years old were invited to participate in the study. Clinically all those who had history of heart failure, chronic kidney diseases, diabetes or HIV were excluded from the study.

ECG measurement

Minnesota Code for ECG analysis was used (Minnesota Code is used mainly for population research and clinical trials and not for hospital practice). The coding for Q-QS Waves start with 1 (1-Codes). Frontal Plane QRS Axis codes start with 2 (2-Codes), tall R Waves with 3 (3-Codes), ST Segment Depression (4-Codes) and Negative T-Waves (5-Codes), Atrioventricular (A-V) Conduction Defects (6-Codes), Intraventricular Conduction Defects (7-Codes), Arrhythmias (8-Codes) and Miscellaneous Codes (9-Codes). ST elevations are coded 9-2. And 6-2-3 codes for Wenckebach's Phenomenon while the WPW code is 6-4-1. 7-1-1 codes for complete left bundle

branch block (LBBB), 7-2-1 for complete right bundle branch block (RBBB), 8-2-1 for Ventricular fibrillation or ventricular asystole and 9-8-1 Technical problems which interfere with coding.

Determination of cardiac Troponin I (Biomarker Assays)

Blood samples were collected in red top tubes and processed within 8 hours (Abbott Laboratories the AxSYM Troponin-I ADV assay).

Data analysis

All statistical analysis was performed using SAS (version 9.1; Cary, NC) statistical software. Because of the skewed distribution, cTnI levels were analyzed as categorical variables as undetectable (<0.00 µg/L), detected (≥0.01 µg/L). To estimate the population prevalence of detectable cTnI, we used the SAS approach. Statistical comparisons of variables between cTnI categories were made with the use of the χ^2 or Fisher exact test.

Ethical considerations

The study received ethical approval from the Muhimbili University of Health and Allied Review Board. All study subjects provided written informed consent.

Results

A total of 263 subjects were recruited in the study. The proportion of males was 49.3% and 40.7% were females (Figure 1). The overall median age was 34 (range= 25-56) years. No significant differences in demographics, medical history, blood pressure, or body mass index (BMI) occurred between males and females.

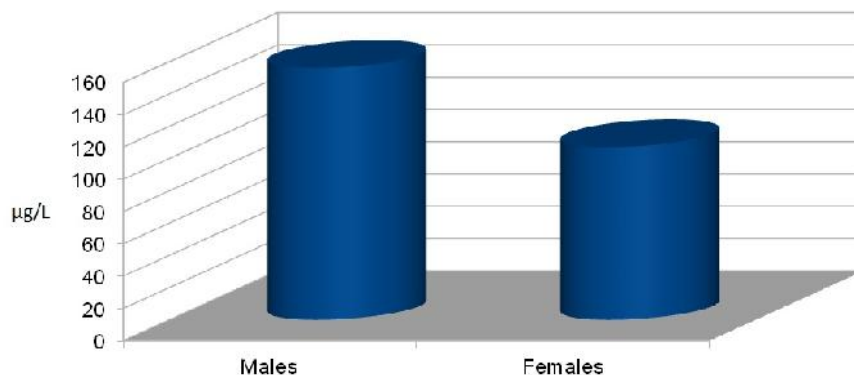


Figure 1. Distribution of serum cardiac Troponin I among blood donors by sex

Among the 263 subjects who provided blood samples for cTnI testing, 261 subjects (99.3%) had undetectable values (0.00 µg/L) and only two had detectable levels (0.01 µg/L and 0.36 µg/L) which were within normal reference range of the assay used (0.00-0.39 µg/L) (Figure 2). Clinically both were healthy and their ECGs were unremarkable (Figures 3).

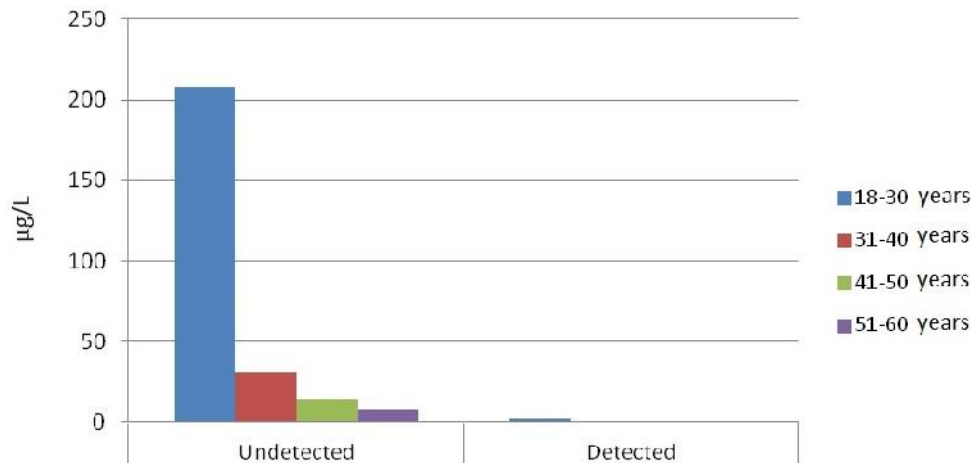


Figure 2. Cardiac Troponin I levels among blood donors and their age group

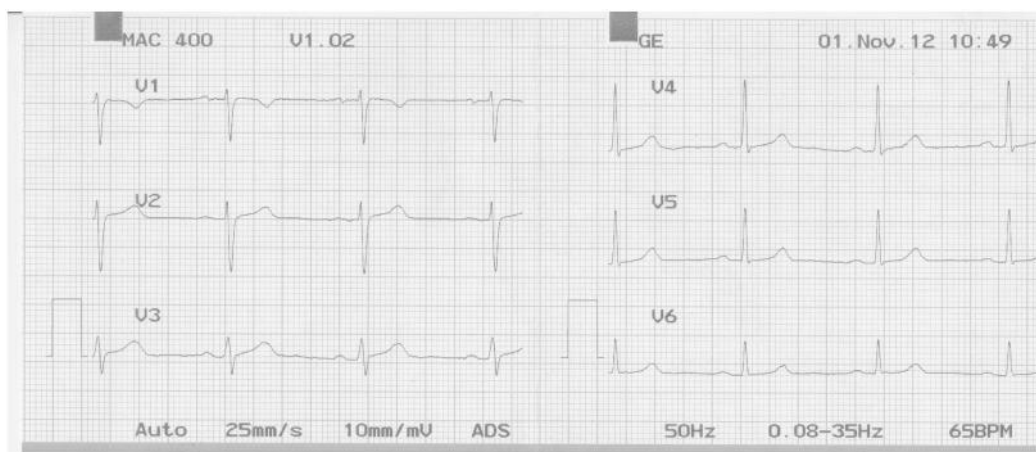


Figure 3. Sample of normal ECG tracing of volunteers

Discussion

Cardiac troponins are increasingly being measured in patients without typical symptoms of ischemic chest pain, and physicians struggle to interpret troponin elevation in these settings, especially when their levels are only minimally elevated. In part, this difficulty arises because little is known about the frequency and clinical correlates of troponin elevation in the ambulatory setting. We measured cardiac troponin I (cTnI) in healthy blood donors in Dar es Salaam Tanzania and identified that majority 99.3 % of the subjects had undetectable cTnI (0.00 µg/L) levels. We identified 2 (0.7%) participants with detectable cTnI, however both were within the normal manufactures reference values, and it was not associated with any ECG changes. Echocardiography was performed in the two individuals that revealed a normal systolic and diastolic function. Few data are available evaluating the prevalence of cTnI elevation in the general healthy population.

Previous studies have reported the prevalence of cardiac troponin elevation among dialysis recipients, patients visiting emergency departments with angina and patients presenting to outpatient clinics with unspecific chest pain. In addition, LVH, CKD, and CHF have previously

been associated with elevated cardiac troponin levels in small populations or subgroups of patients (Rosamond *et al.*, 2014); this study was not designed to investigate such an association. It was designed to detect cardiac troponin I among normal healthy individuals using manufactures' assay range as standard reference to enable us to establish our own normal range. Apple and coworkers found in their study higher troponin I values in blacks our study with 100% blacks did not find such an elevation. The contrast between the two studies Apples group used patients with cardiac manifestation while here all volunteers were healthy blood donors.

The European Society of Cardiology and American College of Cardiology recommended that the lower detection limit for cardiac troponin be the 99th percentile of the assay in a standard reference population provided that the CV at that level was 10% (Scharnhorst V *et al.*, 2000). If an assay did not meet that criterion, the recommendation was to set the lower detection limit for troponin T or I at the lowest level at which the CV was 10% (Gerhardt *et al.*, 2000). The cTnI immunoassay used in the present study had a 99th percentile value of 0.00 µg/L-0.03 µg/L at which the CV was 10%. Previous studies have shown strong associations between troponin levels of 0.01 to 0.029 µg/L and cardiovascular abnormalities in a population-based sample arguing that the detection limit for cTnI should be lowered to 0.01 µg/L, so that all detectable cTnI values are characterized as abnormal if the goal of testing is optimal sensitivity (Apple *et al.*, 2003). In the present study we observed no apparent clinical differences between those subjects with undetected vs. detected cTnI values. According to the assay used in this study all subjects fell within normal range (0.00 µg/L-0.39 µg/L). The findings suggest that the mandate for assay development should focus on greater sensitivity to detect lower levels of cTnI, and such efforts will require increased assay precision. Previous clinical trials also demonstrated that patients with minimally increased cTnI levels below the 10% CV level (regardless of etiology or the magnitude of elevation) have worse prognoses (Jaffe *et al.*, 2000). Furthermore, a recent simulation study using assay imprecision values representative of current cTnI assays demonstrated a very low false-positive rate for diagnosing MI when the 99th percentile was used instead of the 10% CV level, thus supporting the use of 99th percentile to define troponin elevation (Sasse *et al.*, 2002).

Multiple mechanisms exist of troponin elevation indicating cardiac injury and concomitant troponin elevations in population-based sample (Myerson *et al.*, 2009). For instance microvascular coronary disease, which occurs in CHF, Diabetes Mellitus (DM), and CKD, may contribute to troponin elevation in these disease processes. LV strain leads to decreased subendocardial perfusion, endothelial dysfunction, and apoptosis are potential etiologies of troponin elevation in CHF subjects (Jauch *et al.*, 2013). Troponin elevation seen with LVH may be the result of a supply/demand mismatch whereby hypertrophied myositis physically impair adequate endocardia tissue perfusion. Both micro vascular disease and lipotoxic mechanisms may lead to myocardial damage in diabetic patients (Guest *et al.*, 1995). Microvascular disease has been demonstrated to be present in CKD patients, and uremia may lead to silent ischemia similar to that seen in diabetic subjects.

Elevation of cardiac Troponins has been reported to have clinical implications and associated with a large number of cardiac risk factors and evidence of cardiac structural and functional abnormalities. In multivariable analyses, cTnI elevation has shown strong and independent association with 4 independent risk determinants: CHF, LVH, DM, and CKD. Importantly, in the absence of these 4 risk determinants, cTnI elevation reported was extremely rare (0.18%) (Balamuthusamy *et al.*, 2007; Sato *et al.*, 2003). Collectively, this result agrees with our study where detectable cTnI was only found in two volunteers. Thus, in any population-based cohort, cTnI elevation might represent either prevalent cardiovascular disease or a high risk for cardiovascular disease. These findings suggest that primary and secondary prevention efforts should be intensified in subjects with otherwise unexplained troponin elevation and that additional cardiovascular evaluation might be warranted. Given the associations reported elsewhere between troponin elevation and LV structural and functional abnormalities, DM, and

renal failure, echocardiography, measurement of fasting glucose, and assessment of renal function would be reasonable.

Finally, our findings suggest that cTnI may merit consideration for further studies as a screening test for myocardial injury in high-risk population subsets. Although sensitivity of the test to detect cardiovascular disease will be low in apparently healthy subjects, given the low detectable levels of troponin observed (0.7%) specificity appears to be quite high. Sensitivity may be improved in high-risk subjects such as those with cardiovascular diseases. These results are significant to us as stated earlier our group is conducting HIV vaccine trial phase I/II using MVA boost that has been associated with myocardial injury.

Findings from our study can be used as normal reference range as no such study has been conducted among healthy indigenous Tanzanians to establish local normal reference values. If future studies demonstrate increased risk for cardiac events in high-risk subjects with troponin elevation, and appropriate therapies for these subjects are identified, we speculate that troponin may prove to be a useful component of multiple-biomarker panels for screening of at-risk subjects in the general population and vaccine trial cohorts. Development of more sensitive troponin assays may improve the performance of troponin as a screening test.

However, it's worth noting this study has several limitations, which should be noted. The number of subjects with troponin elevation was small, limiting the depth of multivariable analyses that could be performed. Being a cross-sectional analysis of a small cohort thus may not be applicable to other populations. Finally, cardiovascular outcome data from this cohort are not available, and the prognostic implications of troponin elevation in the general population require further outcome studies, which this study was not designed to investigate.

Our study demonstrated a low prevalence of Cardiac cTnI (0.7%) among healthy subjects. The clinical and therapeutic implications of these findings require further prospective study with outcome data. Furthermore, findings of cTnI levels from our study subjects agrees with manufactures levels hence can be used in our local settings as a standard reference values.

Acknowledgments

The authors wish to thank Tanzania Red Cross for organizing the blood donation camps, all nurses from vaccine trial group (TaMoVaC) for assisting in sample collection and the blood donors for their generous cooperation, and Mr. Mfinanga from Central pathology Laboratory of Muhimbili National Hospital for performing all the assays. And the cardiac team for the echocardiography examination.

References

- Apple, F.S., Murakami, M.M., Pearce, L.A., Herzog, C.A. (2004) Multi-biomarker risk stratification of N-terminal pro-B-type natriuretic peptide, high-sensitivity C-reactive protein, and cardiac troponin T and I in end-stage renal disease for all-cause death. *Clinical Chemistry* 50, 2279–2285.
- Apple, F.S., Quist, H.E., Doyle, P.J., Otto, A.P. & Murakami, M.M. (2003) Plasma 99th percentile reference limits for cardiactroponin and creatine kinase MB mass for use with European Society of Cardiology/American College of Cardiology consensus recommendations. *Clinical Chemistry* 49, 1331-1336.
- Balamuthusamy, S., Khosla, S., Meka, S., Saha, S., Srinivasan, L., Ahmed, A., Benatar, D., Trivedi, A., Siddique, M. & Arora, R. (2007) Clinical utility of cardiac troponin I in the diagnosis of acute coronary syndrome in patients with renal failure. *American Journal of Therapeutics* 14, 356-360.
- Freda, B.J., Tang, W.H., Van Lente, F. et al. (2002) Cardiac troponins in renal insufficiency: review and clinical implications. *Journal of the American College of Cardiology* 40, 2065–2071.

- Gerhardt, W., Nordin, G., Herbert, A.K., Gustavsson, E., Muller-Bardorff, M. & Katus, H.A. (2000) Troponin T and I assays show decreased concentrations in heparin plasma compared with serum: lower recoveries in early than in late phase of myocardial injury. *Clinical Chemistry* 46, 817-821.
- Guest, T.M., Ramanathan, A.V., Tuteur, P.G., Schechtman, K.B., Ladenson, J.H., Jaffe, A.S. (1995) Myocardial injury in critically ill patients. A frequently unrecognized complication. *JAMA* 273, 1945-1949.
- Jaffe, A.S., Ravkilde, J., Roberts, R., Naslund, U., Apple, F.S., Galvani, M., et al. (2000) It's time for a change to a troponin standard. *Circulation* 102, 1216-1220.
- Jauch, E.C., Saver, J.L., Adams, H.P.Jr, Bruno, A., Connors, J.J., Demaerschalk, B.M., Khatri, P., McMullan, P.W. Jr., Qureshi, A.I., Rosenfield, K., Scott, P.A., Summers, D.R. et al. (2013) Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 44, 870-947.
- Meier, M.A., Al-Badr, W.H., Cooper, J.V., Klin-Rogers, E.M., Smith, D.E., Eagle, K.A. & Mehta, R.H. (2002) The new definition of myocardial infarction: diagnostic and prognostic implications in patients with acute coronary syndromes. *Archives of Internal Medicine* 162, 1585-1589.
- Montagnana, M., Lippi, G., Salvagno, G.L., Guidi, G.C. (2008) Reference ranges and diagnostic thresholds of laboratory markers of cardiac damage and dysfunction in a population of apparently healthy black Africans. *Clinical Chemistry and Laboratory Medicine* 46, 714-716.
- Myerson, M., Coady, S., Taylor, H., Rosamond, W.D., Goff, D.C. Jr, ARIC Investigators. (2009) Declining severity of myocardial infarction from 1987 to 2002: the Atherosclerosis Risk in Communities (ARIC) Study. *Circulation* 119, 503-514.
- Rosamond, W.D., Chambless, L.E., Heiss, G., Mosley, T.H., Coresh, J., Whitsel, E., Wagenknecht, L., Ni, H., Folsom, A.R. (2012) Twenty-two-year trends in incidence of myocardial infarction, coronary heart disease mortality, and case fatality in 4 US communities, 1987-2008. *Circulation* 125, 1848-1857.
- Safford, M.M., Brown, T.M., Muntner, P.M., Durant, R.W., Glasser, S., Halanych, J.H., Shikany, J.M., Prineas, R.J., Samdarshi, T., Bittner, V.A., Lewis, C.E., Gamboa, C., Cushman, M., Howard, V., Howard, G., REGARDS Investigators (2012) Association of race and sex with risk of incident acute coronary heart disease events. *JAMA* 308, 1768-1774.
- Sasse, E.A. (2002) *How to Define and Determine Reference Intervals in The Clinical Laboratory*. NCCLS document C28-A, 2nd ed. NCCLS Wayne, PA.
- Sato, Y., Yamada, T., Taniguchi, R., Nagai, K., Makiyama, T., Okada, H., Kataoka, K., Ito, H., Matsumori, A., Sasayama, S. & Takatsu, Y. (2003) Persistently increased serum concentrations of cardiac troponin t in patients with idiopathic dilated cardiomyopathy are predictive of adverse outcomes. *Heart* 89, 659-660.
- Scharnhorst, V., Vader, H.L. & van der Graaf, F. (2002) Characteristics of the cardiac troponin I assay on the Immulite 2000 analyzer. *Clinical Chemistry* 48, 1626-1627.
- Uettwiller-Geiger, D., Wu, A.H.B., Apple, F.S., Jevans, A.W., Venge, P., Olson, M.D., et al. (2002) Multicenter evaluation of an automated assay for troponin I. *Clinical Chemistry* 48, 869-876.
- Venge, P., Lagerquist, B., Diderholm, E., Lindahl, B. & Wallentin, L. (2002) Clinical performance of three cardiac troponin assays in patient with unstable coronary artery disease. FRISC II Study Group. *American Journal of Cardiology* 89, 1035-1041.