Bol Med Hosp Infant Mex 2013;70(2):72-76

RESEARCH ARTICLE

Timely diagnosis of myocardial damage in patients treated with anthracyclines: a challenge for the 21st century

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ABSTRACT

Background. Currently used methods for assessment of myocardial damage in patients treated with anthracyclines are deficient in detecting mild myocardial damage. Troponin I is part of the protein contractile machinery in the myofibril and is used as a specific biomarker of myocardial damage. The aim of the study was to compare troponin I levels in patients with prior anthracycline use after a new cycle of chemotherapy.
Methods. We included patients aged from 9 to 18 years who were diagnosed with cancer and were being treated with anthracyclines at the Hospital Infantil de México Federico Gómez. We analyzed serum troponin I prior to and after the new cycle of chemotherapy and compared the results, always in a blinded manner.
Results. The mean cumulative dose of anthracyclines in the study population was 234 mg/m^2 SC for daunorubicin and 269 mg/m^2 SC for doxorubicin. There was no significant systolic dysfunction according to echocardiography. Impaired mobility of left ventricular walls was observed using SPECT-CT. There was no evidence of increased levels of troponin I in serum after application of a new dose of anthracyclines.
Conclusions. Extensive research with mixed results has been carried out in regard to biomarkers that aid in the early diagnosis of cardiomyopathy secondary to anthracycline. Taking into account the kinetics of troponin I in myocardial damage is a critical step for evaluation. Using this premise, we did not find an increase of this biomarker in blood after myocardial damage secondary to administration of anthracyclines.

Key words: anthracyclines, cardiac function test, troponin I, cardiotoxicity.

INTRODUCTION

Anthracyclines and their derivatives are among the most important antitumor agents and belong to a class of pigmented antibiotics produced by the fungus *Streptococcus peucetius* var. *caesius*. The major agents are daunorubicin, doxorubicin, epirubicin and idarubicin, which is a synthetic derivative.[1,2]

Currently, anthracyclines have been incorporated into >50% of the cancer treatment protocols in children and each year >750 patients receive anthracyclines in the U.S.[3] The clinical usefulness of these agents is limited by the onset of cardiomyopathies, whose presence depends on the total dose of the pharmaceutical and is often irreversible.[1,2] The reported prevalence of myocardial damage for the Hospital Infantil de México Federico Gómez (HIMFG) corresponds to 3%.[4]

The studied mechanism of anthracycline-induced myocardial injury is the generation of free radicals, which induce peroxidation of myocyte membranes. A source of free radicals are the anthracycline iron complexes that increase the permeability of the mitochondrial membrane (due to an increased sensitivity to its calcium-dependent channels), which conditions ATP depletion.[2,5]

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Received for publication: 2-5-13
Accepted for publication: 3-5-13

The heart is more vulnerable to free radical damage because the protective antioxidant enzymes are present at lower levels than in other tissues such as the kidney or liver.[3] Risk factors for developing secondary damage to the use of anthracyclines include hypertension, preexisting heart disease, advanced age and mediastinal radiation.[2,6] It has been shown that female gender is an independent risk factor.[4,7]

Finally, many antineoplastic agents such as trastuzumab, cyclophosphamide, dactinomycin, mithramycin, mitomycin, etoposide, melphalan, vincristine, bleomycin, paclitaxel, docetaxel, and dacarbazine may have an additive effect on anthracycline-induced cardiotoxicity.[2]

Approximately 25% of the patients have myocardial failure when the dose of anthracyclines is >500 mg/m^2; 50% have cardiac events with cumulative doses >600 mg/m^2, and almost all patients have cardiotoxicity with doses >800 mg/m^2. The reported frequency of subclinical myocardial alterations after treatment with anthracyclines reaches 57%[5] and for the symptomatic cardiac abnormalities up to 16%.[8,9]

Myocardial biopsy results show strong evidence that the damage begins at the time of the initial exposure despite the fact that cardiac reserves impede clinical recognition until the damage has been sufficient to offset the heart.[10]

The gold standard for detection of this toxicity in patients treated with anthracyclines is an endomyocardial biopsy. Mortality associated with this procedure reaches 0.05% and complications range from cardiac perforation, thromboembolism, cardiac tamponade, arrhythmia, bundle branch block, valvular dysfunction, vascular lesions, as well as only local complications.

Currently used methods for assessment of myocardial damage in patients using anthracyclines include EKG, echocardiogram, radionuclide ventriculography and myocardial biopsy. Despite the great progress made in this area, most of these studies have shortcomings in detecting mild myocardial damage.

Myocardial damage in children may remain clinically occult for a long period before the onset of signs and symptoms consistent with heart failure. Damage has been observed in patients with previously reported imaging studies within the normal range for their age. Enzymatic measurements have been used for many years to aid in the detection of damaged tissue and monitoring disease processes.

The first biomarker used to detect myocardial damage was aspartate aminotransferase, reported for the first time by Karmen et al. in 1954. Soon after, lactate dehydrogenase was described as an additional marker. Later, creatinine kinase was added, with its MB fraction mainly expressed in cardiac tissue.[11,12]

Immunoassay confirms the nonspecificity of these biomarkers for myocardial tissue. This was resolved with the study of troponin T and troponin I.[13,14] Troponins are the regulatory proteins of contraction machinery in myofibrils and have been used as biomarkers of myocardial damage. They form a complex of three units (troponin I, T and C) together with tropomyosin and are found in the actin filament, resulting in being essential to skeletal muscle contraction and cardiac calcium regulation.[15,16]

Recently, troponin I has been proposed as a marker of early damage in patients treated with anthracyclines. The normal value of troponin I is <0.03 ng/ml[14,16-18] and usually increases within 4 to 6 h after the onset of myocardial damage, reaching its maximum value at 24 h. This increase has a 10- to 14-day duration.

Based on the above, the objective of this study was to compare troponin I levels in patients treated with anthracyclines prior to and after a new cycle of chemotherapy.

PATIENTS AND METHODS

We included patients with a diagnosis of cancer with ages ranging from 9 to 18 years and who were treated with anthracyclines in the Oncology Department of HIMFG during the period from September-November of 2012. We excluded patients with congenital or acquired cardiac disease and those with diseases that altered troponin I measurement during the previous month (chronic renal failure, vascular disease, stroke, endocarditis, myocarditis, sepsis, hypertension, chest trauma).

Sampling was performed in hospitalized patients for chemotherapy administration from August to November, 2012 as well as those patients receiving outpatient chemotherapy. In all cases, clinical records were reviewed and information was obtained regarding demographic, clinical, treatment background and monitoring, including calculation of creatinine clearance. For further standardization and comparison of cumulative anthracycline dose, the conversion was done according to their relative toxicity (Table 1).[5]

Serum levels of troponin I were determined prior to the new chemotherapy cycle; anthracycline-based chemotherapy was administered in the conventional manner ac-

cording to the hospital-established protocols. Later, serum level measurements of troponin I were taken during the first 24 h after chemotherapy.

The serum was stored for subsequent analysis using electrochemiluminescence (Cobas e601, Roche), at a temperature of -20°C in the central laboratory. Results are reported in ng/ml, and those with levels >0.03 ng/ml were interpreted as positive.

For assessment of myocardial function we used two-dimensional, M-mode, tissue spectral Doppler transthoracic echocardiography (Phillips IE33 four-chamber echocardiography) in the parasternal long and apical axis. Left ventricular systolic function was assessed using ejection fraction, fractional shortening, and myocardial performance index (MPI) as well as right ventricular systolic pressure (Table 2).

In addition, all patients had myocardial function assessed using single photon emission SPECT-CT ventriculography (in a double gamma SPET-CT detector, model T16 Symbia, Siemens). Marking of erythrocytes *in vitro* was performed using the application of case ultratag, and processing of the study, once obtained, with the program-specific CEDARS SINAI radionuclide ventriculography.

At all times, both the chemist responsible for the processing of troponin T samples as well as the physician responsible for the single-photon emission radionuclide ventriculography and the cardiologist responsible for conducting the echocardiogram were blinded to the results obtained by their counterparts.

For data analysis we used the statistical program SPSS for MAC v.20.0 using descriptive statistics. Changes in troponin I levels were compared using Student t test; $p < 0.05$ was considered significant.

This current study complied with the provisions of Title II of the General Health Law for Health Research corresponding to category II (Research with Minimal Risk). Furthermore, this study was evaluated by the Ethics Committee of the HIMFG. Patients and relatives were informed of the features and objectives of the study and all patients/relatives provided informed consent.

RESULTS

We included 16 patients treated with anthracyclines: nine were diagnosed with lymphoblastic leukemia, two with osteosarcoma and five with other types of cancer. All patients were undergoing treatment. The mean age was 146 months (range: 103-183 months). Of these, two patients had recently initiated treatment with anthracyclines and four patients (25%) were being treated due to relapse.

Cumulative values of anthracycline doses are shown at baseline and after the new treatment cycle (Table 3). The average for daunorubicin was 204 mg/m^2 SC for before and 234 mg/m^2 SC after the new cycle of chemotherapy. The average for doxorubicin was 200 mg/m^2 SC and 269 mg/m^2 SC, respectively.

The values of estimated creatinine clearance were 86-170 ml/min/1.73 m^2 SC, with an average of 136 ml/min/1.73 m^2 SC (normal values for age). Results of the assessment of cardiac function obtained by echocardiography and SPECT-CT are shown in Tables 4 and 5, respectively.

Serum troponin I measurements were performed in all study patients. The average was 0.005 ng/ml. The second measurement of troponin I obtained during the first 24 h postchemotherapy showed almost no change. The average of these results was also ~0.005 ng/ml.

DISCUSSION

There has been much discussion about the benefit that cardiac troponins would provide as markers of cardiac injury and as a potential monitoring method. However, most studies conducted so far in this field have methodological

Table 1. Relative toxicity of specific anthracyclines

Anthracycline	*Toxicity*
Doxorubicin	1.0
Daunorubicin	0.75
Mitroxantrone	0.5

Table 2. Normal values of echocardiograph measurements for evaluation of left ventricle

Measurement	*Normal values*
EF	50-70%
FS	28-41%
RVSP	15-30 mmHg
MPI	0.34-0.42

EF, ejection fraction; FS, fractional shortening; RVSP, right ventricular systolic pressure; MPI, myocardial performance index

shortcomings, mainly in the time troponin level measurements are taken after the last dose of chemotherapy.

Although there is a variety of information about the possible benefit of the determination of troponin to detect myocardial damage, multiple studies show conflicting data. There are two important aspects to consider when planning a study on the use of troponin I to measure myocardial damage. The first arises from the fact that this is a biomarker reflecting acute cellular injury, which would imply that the main increases would occur immediately after the application of the anthracycline dose. However, studies were found in which measurements were made up to 168 months after use of these chemotherapeutic agents. In the literature, it has not been well-defined whether there is a persistent increase in this biomarker detectable by conventional tests. It is believed that there is an intracellular pool that can be released constantly, which could maintain an elevated blood level with prolonged cell damage. However, it is also believed that these levels would not be high enough to be detected using currently marketed kits. Reference has even been made to the measurement of troponin I as a test providing these elevations are not being detected by it. The second aspect is related to the time of the completion of the echocardiogram. Theoretically, it should be done at the time of the sampling to measure the troponin and to compare the results with respect to each patient's baseline echocardiogram, which has not been performed in the previously described studies.

Another important point to consider is that these results report cardiotoxicity based on the changes of myocardial function reflecting this and not the measurable cellular damage that could be evaluated only by a myocardial biopsy. In other words, it referred to the functional definition of cardiotoxicity.

In this study there was no increase in troponin I levels after chemotherapy administration. Although the global cardiac function was measured by ejection fraction and demonstrated no significant alterations or dose-related cumulative anthracyclines, it was evident that there was an early dysfunction in motility—mainly in the left ventricle—after the administration of chemotherapy.

Heart failure secondary to cardiotoxicity as a result of the use of anthracyclines is well documented in the literature. It is dose related, and the risk remains throughout the patient's life, despite no longer being under treatment with anthracyclines. Mortality rate exceeds 50%.

Biopsy results showed strong evidence that damage begins at the time of the initial exposure, although cardiac reserves prevent clinical recognition until sufficient damage has occurred for cardiac compensation. The prognosis of children with cancer has improved substantially over time. Unfortunately, the rate of secondary complications due to oncological management is increasing. The use of biomarkers to help detect incipient myocardial damage has been the subject of study in recent years with contradictory results depending on the methods used.

In this study we evaluated the concentrations of troponin I with a method consistent with its appearance in the blood after myocardial damage. No correlation was observed between the administration of chemotherapy and increased levels of this substance. Although the number of patients included in the study is low, there are no data

Table 3. Cumulative dose of doxorubicin and daunorubicin prior to and after the new cycle of chemotherapy

Cumulative dose		*Minimum*	*Maximum*	*Average*
Daunorubicin	Pre	0	570	204
	Post	30	600	234
Doxorubicin	Pre	0	375	200
	Post	40	450	269

Pre, prior to the new cycle of chemotherapy; Post, after the new cycle of chemotherapy

Table 4. Myocardial function evaluated using echocardiogram

Parameter	*Minimum*	*Maximum*	*Average*
EF	56%	72%	66.9%
FS	31%	41%	37.5%
RVSP	19 mmHg	47 mmHg	29.78 mmHg
MPI	0.32	0.67	0.4786

EF, ejection fraction; FS, fractional shortening; RVSP, right ventricular systolic pressure; MPI, myocardial performance index

Table 5. Myocardial function evaluated using SPECT-CT

Parameter	*Minimum*	*Maximum*	*Average*
EF	46.5%	60.4%	53.4%
PFR	469.3	1232.2	802.3
TMFR	185.0	310.0	248.5
PFR	661.0	1489.2	930.5

EF, ejection fraction; PFR, peak filling rate (normal: 429-556 cm^3/sec); TMFR, time to the maximum filling rate (normal: <180 msec)

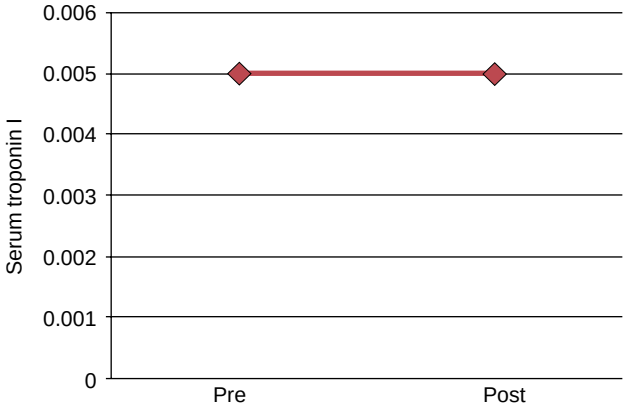

Figure 1. Serum troponin I values prior to and after the new cycle of chemotherapy.

to offer ideas that increasing the number of patients would obtain different results.

ACKNOWLEDGMENTS

The authors acknowledge the "Programa de Becas Carlos Slim" for providing suppor to EMN-R.

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