Current perspectives

Chemotherapy-related cardiotoxicity: new diagnostic and preventive strategies

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Chemotherapy is an established approach for several malignancies, but its utility may be hampered by induced cardiac toxicity possibly leading to heart failure, with a negative impact on the patient's quality of life and ultimately survival. Prospective left ventricular systolic function monitoring has demonstrated that cardiotoxicity could be subclinically present for many months or years before its overt manifestation. Although considered irreversible, some reports suggested recovery or delayed progression of cardiac dysfunction by preventive cardioactive therapies. Thus, the identification of earlier instrumental or biochemical markers of cardiac injury able to predict heart failure remains a major task. Diastolic indexes as a primary expression of hemodynamic dysfunction after cardiac damage, analyzed by means of conventional or newer Doppler technologies (tissue Doppler, color M-mode, etc.) are discussed. Moreover, brain natriuretic peptides, troponins and endothelin-1, as possible sensitive/specific markers/predictors of subclinical cardiotoxicity are reviewed in order to update and possibly improve the strategy for the detection and clinical management of chemotherapy-related cardiotoxic effects.

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Introduction

Single antineoplastic agents or more frequently combination regimens at standard doses are "state of the art" therapy for several types of solid and hematological malignancies, both post-surgery (i.e. adjuvant chemotherapy) and for advanced disease. Moreover, high-dose chemotherapy (HD-C) followed by hemopoietic stem cell transplantation has improved the clinical outcome of patients with selected chemosensitive diseases, such as Hodgkin and non-Hodgkin lymphomas, multiple myeloma, acute myelogenous and lymphatic leukemia and possibly breast cancer and germ cell tumors[1]. The therapeutic potential of both standard and HD-C may be limited by acute and chronic cardiotoxicity (CTX), a clinical problem not yet completely addressed in its pathogenic, diagnostic and therapeutic aspects. Unfortunately, clinical CTX may have a dramatic impact on the patient's quality of life and survival, particularly in childhood cancer survivors and young adults cured of hematological malignancies and women receiving adjuvant chemotherapy for breast cancer.

Besides the long known cardiotoxic potential of anthracyclines and anthraquinones (ANT), several antineoplastic agents with different mechanisms of action have been associated with cardiac damage, possibly leading to congestive heart failure (CHF): 5-fluorouracil, cyclophosphamide (CY), paclitaxel (PCT) alone or in combination with ANT, but also monoclonal antibodies, e.g. trastuzumab (TZB)[2-4].

The incidence and severity of CTX varies depending upon the drug used, whether as a single agent or in combination, the peak and cumulative dose administered and on possible, occasionally unrecognized, pharmacokinetic interactions between antineoplastic or supportive care drugs. This is well exemplified by CY, widely used in the treatment of lymphomas and breast cancer, both at standard and high doses: while not cardiotoxic at standard doses, when administered at high doses (i.e. > 5 g/m^2) it has resulted in CTX, with a wide variability in mortality and morbidity, ranging from 1 to 9% and from 5 to 30% respectively. Several reasons account for this wide variability, particularly the co-administration of other chemotherapeutic agents

as part of conditioning regimens in high-dose settings, patient selection and pre-treatment with ANT[5-7]. Another example is provided by TZB, used as a single drug or in association with ANT or drugs such as vinorelbine, not known to be cardiotoxic *per se*, in the treatment of advanced breast cancer.

Variability in grading the entity of clinical or subclinical (i.e. defined as any alteration of the functional and/or biochemical values from baseline following chemotherapy) CTX may hamper the comparability in different series, while even a true differentiation between direct cardiotoxic effects and indirect cardiac lesions (vascular, renal, pulmonary damage, etc.) by chemotherapy is sometimes difficult. Finally, some cardiac events occurring in cancer patients may be related to other clinical conditions or to radiation therapy and therefore they are not to be straightaway considered as drug-related adverse effects.

Clinical scenario

Etiopathogenesis. As suggested by the models of ANT-related cardiomyopathies two forms of CTX may be distinguished: 1) acute CTX, occurring during or soon after drug administration; 2) chronic or late CTX, characterized by the progressive development of cardiomyopathy and occurring several months or years after the last drug administration. Chronic CTX may also be differentiated in "early onset" and "late onset", depending on the relative interval of its clinical presentation. The "early onset" form is usually more severe.

Typically, early CTX is unusual, more likely to be seen in elderly patients after large single doses, is frequently reversible, and it does not translate into late CTX even though some fatalities may occur. Several signs or symptoms may be observed: cardiopalm, chest pain, tachycardia, ECG abnormalities, atrial and ventricular arrhythmias, and hypotension[2-7]. An ominous pericarditis-myocarditis is very rare, more frequent after high-dose CY (HD-CY) in the autoptic cases, hemorrhagic myocardial necrosis and pericardial effusion have been described in relation to direct endothelial damage[3].

Chronic CTX is clinically more relevant and needs to be increasingly studied and recognized, particularly in its subclinical phase when it is still amenable to treatment with preventive cardioactive therapy. ANT used as single agent or in combination (with PCT, TZB, etc.) is the drug most commonly implied in causing a direct myocardial injury, potentially evolving to a decreased myocardial function and CHF. The exact mechanism underlying ANT-induced CTX has not been fully elucidated, but the formation of free radicals and calcium overload into myocytes play a crucial role taking into account the lack of catalase and superoxide dismutase within the cardiac muscle[8]. Other mechanisms suggesting apoptotic cell death of endothelial cells and cardiomyocytes secondary to ANT exposure even at submicromolar concentrations have been proposed[9]. The ANT-induced generation of H_2O_2 free radicals has been shown to be responsible for this mechanism through enhanced endothelial nitric oxide synthase transcription in endothelial cells and myocytes[9]. The alcohol metabolite of doxorubicin, doxorubicinol, because of its inhibiting effect on several ion pumps and owing to its ability to inactivate the mitochondrial aconitase-iron regulatory protein-1, a post-transcriptional regulator of iron uptake and storage proteins, is probably the key effector in myocardial damage[8].

PCT has unique pharmacological features, relevant to its use in combination with ANT: it follows non-linear pharmacokinetics and it is formulated in Cremophor EL, a vehicle involved in the non-linear disposition of the drug. It can enhance the ANT activity by a metabolic interaction (increased conversion of doxorubicin to cardiotoxic doxorubicinol), and by pharmacokinetic interference with its vehicle Cremophor EL and the consequent reduced elimination of ANT resulting in increased doxorubicinol plasma levels and tissue exposure[10]. Therefore, in association with PCT, a reduced ANT cumulative dose of 340 mg/m^2 has been established[11].

Among the new agents TZB, a monoclonal antibody against human epidermal receptor-2 (HER-2), has been recently approved for the treatment of metastatic breast cancer. HER-2 is a member of the epidermal growth factor receptor family, considered important mediators of cell differentiation and survival. Over-expression of HER-2 is present in a variety of human tumors (e.g. it is detectable in about 30% of breast cancers) and this biological hallmark identifies a subset of patients with more aggressive disease. TZB exhibits synergistic antitumoral effects when associated with ANT or PCT; however, its use in combination is limited by the high incidence of CTX, ranging from 7% in monotherapy up to 28% when used in combination with ANT or PCT in patients previously treated with ANT[12]. Because of the retrospective nature of these observations, combination regimens of TZB and ANT or PCT are now being prospectively evaluated worldwide to assess their exact cardiotoxic potential. The mechanisms underlying TZB-related CTX are largely unknown and besides, the pharmacokinetic interactions have not been explored as a potential explanation of the clinical findings. Nevertheless, both HER receptors and HER ligands are physiologically expressed in the normal human heart, and their activation regulates factors involved in the cellular response to oxidative stress (nuclear factor-kB) and the expression of cardiac proteins involved in the regulation of cardiac hypertrophy[12]. In contrast, the deletion of HER proteins abrogates normal cardiac development and provokes the transition from compensated hypertrophy to heart failure. Moreover, the ability of the heart to withstand stress is due to a program of cell survival against apoptosis, properly activated by HER ligands. Thus, the HER protein and ligand interaction plays a heretofore unrecognized role in cardiac func-

tion and in cardiac protection from different stressors. These results strongly suggest that HER blockade and the inhibition of downstream myocardial signaling by HER-2 antagonists such as TZB may have deleterious effects on the heart, especially when subjected to further stress, such as myocardial ischemia overload or other cardiotoxic agents such as ANT[13]. With regard to this, further investigation of the role of normal and aberrant HER-2 signaling in the development of cardiac dysfunction could also lead to an improved understanding of the pathophysiology of cardiomyopathies and – possibly – to novel therapies for the management of all forms of CHF, regardless of etiology[14].

Epidemiology and risk factors. In ANT-treated adult patients cardiac dysfunction is consistent with mild dilated cardiomyopathy, while in young cancer survivors a reduced left ventricular mass and thinning of the left ventricular walls resulting in a restrictive pattern of cardiomyopathy were observed[15]. The commonly reported incidence of 7% for ANT-related CHF[2,3,8,16] is felt to be much higher by other investigators[17]: in large retrospective studies an actuarial estimation of 15% of patients presented with a 25% reduction in the left ventricular ejection fraction (LVEF) within 1 month of the end of chemotherapy and 11% of them deteriorated to severe CHF, increasing to 20% after 5 years of follow-up[2,3,17]. Many patients (about 60%) may recover from clinical CHF[18], especially if treated with recent cardioactive drugs such as angiotensin-converting enzyme (ACE)-inhibitors, carvedilol and antialdosterone drugs[19]. Nevertheless, the mortality of patients in NYHA class III-IV exceeds 50% within 2 years, so that the overall prognosis of this form of cardiomyopathy is similar to that of CHF in idiopathic cardiomyopathy[10]. Finally, a relevant percentage (approximating 5%) of these patients may require heart transplantation, as a last resort therapy[20]. Although worrisome, these data are likely to represent an underestimation of the problem: the numbers may be even higher with longer observation times nowadays possible owing to the improved survival of cancer patients. In fact, the increasing survival rate among children and young adults with neoplasms such as leukemia, lymphoma or breast cancer treated with ANT or HD-C has led to the observation of cardiac damage and dysfunction manifesting even several years after treatment. In their review, Kremer et al.[21] report that up to 57% of children may present with such problems. In another recent study, more than 65% of such patients were reported to have suffered from CHF 6 years following chemotherapy[22]. This clinical scenario has caused many investigators to raise doubts about the utility of monitoring the cardiac function only by LVEF evaluation during the course of treatment, since the value of such monitoring is not sensitive and specific enough for the early prediction of a future cardiomyopathy[6,17,23]. More specific and sensitive markers of subclinical CTX would be needed to allow the distinction between patients who need careful cardiologic follow-up after the completion of therapy and those who do not. More importantly, the interval before the detection of subclinical CTX and clinical deterioration provides the cardiologist with a unique opportunity for preventive interventional strategies. Hence, there is growing expectation among cardiologists and oncologists for newer non-invasive and cost-effective diagnostic tools for the early identification of patients prone to develop left ventricular dysfunction.

Nevertheless, two available Consensus guidelines[24,25] for the monitoring of chemotherapy-induced CTX have proposed radionuclide or echo LVEF, during and shortly after chemotherapy, as a prognostically valuable parameter. The reported severe prognosis of chronic CTX has led to the inclusion in these guidelines of the recommendation for extensive and expensive functional monitoring programs during treatment, in order to reduce the estimated patients' risk for the development of cardiomyopathy to less than 5%[26]. However, probably not all patients need to be submitted to such frequent and multiple diagnostic evaluation[27]. Of course, the relevance of such a strategy lies in the ethical objective of administering the highest dose of active drug against cancer while minimizing the risk of potentially severe side effects, such as cardiomyopathy. Monitoring cardiac function would allow clinicians to adjust drug dosages and the rate of administration, or to shift high-risk patients toward less cardiotoxic therapies.

Several risk factors that may increase the incidence of CTX have been identified. The strongest one is either the total dose during a course (i.e. CY) or the administration rate of cumulative doses (i.e. ANT). Previous studies have demonstrated a 30% incidence of CHF in patients receiving a cumulative dose of ANT > 550 mg/m^2, and an incidence < 10% when the cumulative dose was below this limit; for epirubicin, an ANT analogue with less CTX, a threshold of 900 mg/m^2 has been defined[28,29]. However, these thresholds are relevant only from an epidemiological point of view, as wide variations in the individual sensitivity to CTX linked to one's genetic background both for drug activity and related toxicity exist. Even for CY, on the basis of retrospective studies, a threshold of 100 mg/kg i.v. bolus has been proposed, beyond which the risk of severe and occasionally fatal myocarditis is considered too high and ethically not acceptable[5]. However, recently both we and other authors[5,6,30] have shown that multiple fractionated doses allowed the administration of nearly double dosages of CY (200 mg/kg, equal to about 7 g/m^2), so increasing the antitumor activity without any clinical CTX. Besides the dose, a patient age > 60 years and female gender represent other independent risk factors for CTX[6]. An increasing age-dependent susceptibility occurs even for other cardiac diseases, such as myocardial infarction and cardiomyopathy (the normal aging heart is potentially a diseased heart!). Even the susceptibility of the female gender to

CTX is about 2-fold that of men, again in the same manner as in other cardiac conditions (i.e. morbidity for thrombolytic therapy, diabetes, cigarette smoking, etc.). Finally, preexisting coronary valvular or myocardial disease, hypertension, electrolyte imbalances (hypokalemia, hypomagnesemia), mediastinal radiotherapy or previous chemotherapy – especially ANT – are all considered risk factors for CTX[6]. As a means of prevention, these conditions should be well assessed before the onset of therapy and attempts to modify them should be undertaken when feasible.

Conventional and newer diagnostic strategies

The mentioned Consensus guidelines proposed radionuclide LVEF as prognostically useful for monitoring the chemotherapy-induced CTX, with echocardiographic LVEF as an alternative, depending on the local facilities and relative clinician's experience[25]. Undoubtedly, endomyocardial biopsy obtained from the right ventricle by catheterization is considered the most sensitive and specific tool for any anatomic type of cardiac damage, but in clinical practice the use of this technique is obviously limited by its invasiveness, lack of universal expertise in obtaining and interpreting biopsy specimens and also by sampling errors due to scattered cardiac damage, especially in the preclinical phase[16]. Therefore, this test is mainly useful in situations where the specific cause of cardiac dysfunction cannot be established with certainty. Other standard diagnostic tools, always performed in clinical practice, include ECG and chest radiography: the latter, however, is strictly limited only to exclude other causes of cardiac-like patient symptoms, because of its very low sensitivity for cardiac function monitoring.

Electrocardiography. Twelve-lead ECG is readily available in all oncology units: during or immediately after the administration of cardiotoxic drugs ECG abnormalities are seen in about 10% (range 0-40%) of patients receiving ANT[2-4,8] and in about 25% of patients treated with HD-CY[5-7]. They include non-specific ST-T alterations and benign atrial or ventricular arrhythmias: these findings usually are transient and do not reflect nor predict the development of late CTX[2,3,8,31]. Also, comparisons of the QRS voltages, measured as summations of the R and S waves in the six-limb leads, have been used to diagnose or predict CTX[32]; however, a decrease in the QRS voltages is generally a late phenomenon, is not always present, and may even coincide with the onset of CHF. In addition, a decrease in the QRS voltages can also be observed in patients who develop pleural or pericardial effusion, as a result of tumor progression, without any chemotherapy-related cardiomyopathy[5,31]. In this respect, in about 40% of patients treated with HD-CY we observed a decrease in the QRS voltages exceeding 10% of the basal values: it appears related to interstitial edema secondary to endothelial injury, the so-called "electrical insulation"[5]. In our experience, it seemed reversible, disappearing within 4 days of chemotherapy and not predicting clinical CTX at a 2-year follow-up[30,31]. Finally, some authors suggested a prolonged QTc interval as an early sign of CHF which is independent of the actual echo LVEF and which may predispose patients to the development of malignant arrhythmias[33-35]. However, other authors showed that a prolongation of the QTc interval > 0.45 s was transient in most patients and moreover no correlation was found between it and echo LVEF or clinical outcome[23]. Besides, a novel ECG-derived diagnostic tool, signal-averaging ECG, has been recently evaluated in this setting. Signal-averaging ECG was performed in 29 children with cancer, treated with ANT, in addition to echo LVEF calculation: at signal-averaging ECG, CTX was detected at lower cumulative doses compared to echo LVEF and in a subclinical phase, when the LVEF was still within normal values[36]. Finally, the QT dispersion (difference between the maximum and minimum QTc interval on the 12-lead ECG) has been evaluated as a predictor of CHF after HD-CY: in this study 5 out of 19 patients presented with CHF within 2 weeks of HD-CY and the only difference between patients who presented with CHF and those who did not (> 60 vs < 30 ms, respectively) was QT dispersion[37]. However, in this context all ECG parameters globally have quite a low sensitivity and specificity.

Left ventricular systolic function. Nowadays, the techniques routinely employed to assess LVEF are the most used tools for monitoring chemotherapy-induced CTX: by the two mentioned modalities, radionuclide or echo, serial LVEF determination is universally applied, and its value has been thoroughly investigated[26,27,38-44]. In a large cohort of patients a decline of LVEF by at least 15% to a final value < 45% usually preceded CHF[43]; however, this and other studies have shown that the future development of CHF does not always follow the same functional pathway. For this reason, serial LVEF determination is considered as having a good specificity but a poor sensitivity. In another study, in which ANT was suspended when basal LVEF declined by more than 15% or to an absolute value < 50%, it was reported that this approach resulted in a 4-fold reduction in the risk of CHF during follow-up, compared to the control group[38]. A confirmation that rest LVEF is not a very sensitive tool for early risk detection (it is a late manifestation of the process from myocardial damage to CHF) derives from a study in which LVEF determination was compared to biopsy: its sensitivity was only 53% and its specificity 75%[44]. Moreover, it should be kept in mind that LVEF may be influenced by preload and afterload changes, often occurring in the diverse clinical settings of these patients (i.e. fever, septicemia, anemia, exaggerated sympathetic tone due to anxiety, uroprotective fluid overload, etc.).

To increase its sensitivity, one *ad hoc* study also measured radionuclide LVEF by exercise testing[41]. During exercise, a normal heart increases the cardiac output leading to an increase in LVEF well beyond 5% of the resting value; failure to increase LVEF by this percentage or a downright decrease indicates a reduced myocardial systolic reserve, which is considered as a more sensitive index of CTX. By this method, the sensitivity increased from 53 to 89%, but the specificity decreased to 41 from 75%; as a consequence, some patients with a resting LVEF < 50% but with a stress LVEF increasing by more than 5% could continue treatment with ANT without any adjunctive risk[41]. Other authors using exercise echo[45,46] or dobutamine echo[47,48] obtained analogous variations of the systolic indexes such as LVEF or left ventricular end-systolic wall stress (radius/thickness ratio × systolic pressure), considered as an index of the contractility reserve. However, during chemotherapy the feasibility of stress tests may be hampered by the poor physical conditions of many cancer patients; to overcome this obstacle, some investigators studied the contractility reserve more directly and proposed the stress-velocity index to monitor CTX[22]. This is a complex technique that requires the measurement of a carotid pulse tracing, a phonocardiogram, and of blood pressure during an echocardiographic recording: the index incorporates the measurement of the contractility afterload and preload, allowing for their independent contribution to the overall left ventricular performance. Lipshultz et al.[22] applied this index to study a large cohort of survivors of childhood cancer and showed a strong correlation between the variations of this parameter and the cumulative dose of ANT and the risk of late CHF; however, due to its technical complexity, the wider application of this technique is limited.

Overall, at present comprehensive echo study remains the most widely used tool by cardiologists for non-invasive monitoring in patients receiving chemotherapy: the chamber dimensions, wall thickness and several indexes of ventricular function and afterload or ventricular remodeling can be assessed. For this purpose, an outcome study about childhood malignancies after sequential ANT therapy reported a new-onset (mild or moderate) or worsening (from mild to moderate-severe) mitral regurgitation at color Doppler imaging as a sensitive predictor of late CHF manifesting at a median interval of 18 months[49].

However, we would like to give a special warning to physicians taking decisions regarding patients treated with chemotherapy: decision making solely on the basis of the echo LVEF index, without any comprehensive echo-Doppler exam evaluation, could be harmful in terms of the patient's response to treatment and of the prognosis. This warning is particularly significant when LVEF is calculated by non-echo-skilled operators, where the variability may even reach 15% (just what we can consider as a timely sign of CTX!), not to mention the poor quality of the echo windows in adults, particularly in cancer patients. To overcome the difficulties in calculating the left ventricular volumes, the utilization of the automatic border detection technique or, better, the application of second harmonic imaging, available in newer echo machines (possibly in association with the use of transpulmonary contrast agents that may better outline the entire endocardial surface of the left ventricle) could result in a decreased variability when determining LVEF.

Diastolic function. Since in other forms of heart failure diastolic dysfunction generally precedes systolic dysfunction, several studies have focused on diastolic function parameters as earlier markers of CTX[50]. Several indexes of the diastolic phase can be assessed either at radionuclide angiography[51] or echocardiography[31,52,53]. The peak filling rate and time to peak filling may be evaluated at radionuclide angiography; the early (E) and atrial (A) diastolic velocities and their ratio (E/A), the deceleration time and the isovolumic relaxation time have been investigated at echocardiography. Besides, the same automatic border detection technique accurately permits to on-line measure the peak filling rate and time to peak filling indexes[54]. All these studies showed an earlier increase in the diastolic indexes abnormalities regarding LVEF or cavity dilation, and a strict correlation with risk factors, total doses and the patient outcome. The exercise diastolic function has been specifically tested in one study using the radionuclide technique: in 4 out of 25 long-term survivors treated with HD-C only a prolongation of the time to peak filling during exercise correlated with the development of left ventricular dysfunction[55]. Even dobutamine stress echo has been evaluated as a tool for revealing latent diastolic dysfunction secondary to subclinical CTX: Cottin et al.[56] reported normal values of the peak E and E/A ratio at rest but a significant decrease during dobutamine infusion, and it was related to the ANT cumulative dose.

A novel index, combining the systolic and diastolic time intervals (Tei index), has been prospectively evaluated for the assessment of subclinical CTX in patients undergoing chemotherapy[57,58]. Ishii et al.[57] observed a significant difference in the Tei index between patients who received a low dose and those who received a moderate to high dose of ANT. Thus, they considered the Tei index as a sensitive and easy approach for the detection of subclinical CTX due to ANT.

However, we should keep in mind that even the diastolic indexes are influenced by changes in hemodynamics, particularly heart rate and blood volume and pressure[59,60].

New echo-Doppler indexes. Recent ultrasound techniques by tissue Doppler imaging and color M-mode mitral flow propagation have been proposed as methods which allow for the study of the intrinsic myofiber

properties, such as relaxation and elastic recoil, almost independently of the hemodynamic conditions, in several clinical settings and cardiac diseases[61-64]. The tissue Doppler imaging technique (color and pulsed wave mode) measures the longitudinal velocities of all segments of the left ventricular wall during the entire diastolic period, as an estimation of myocardial relaxation: it is generally analyzed on the basis of the early (Ea) and atrial (Aa) velocities and their ratio[61]. The color M-mode mitral flow propagation technique measures the propagation of the mitral flow velocity (Vp) into the left ventricular cavity, as an expression of left ventricular elastic recoil after systolic emptying[64,65]. These indexes are accurately and linearly (absence of the "U-phenomenon" such as occurs for the conventional E/A ratio) correlated with the progression of diseases, such as ischemic[66,67] and hypertrophic heart diseases[68]. In a cohort of 60 patients treated with various antineoplastic regimens (ANT, TZB and PCT or HD-CY) we showed wider and more significant changes from basal values (usually more than -35%) of the newer Doppler indexes Ea-Aa-Ea/Aa ratio-Vp than those observed for the conventional diastolic indexes deceleration time-E/A ratio-isovolumic relaxation time. Besides, these changes occurred earlier during chemotherapy when LVEF was still > 50%[69]. Interestingly, in the 5 patients who developed CHF during a mean 2-year follow-up, at the end of chemotherapy the modifications of these indexes were even more marked (approximately -50% from the basal value) when the systolic and conventional Doppler indexes were respectively still normal or inconclusive. Similar studies and analogue results regarding both pulsed tissue Doppler imaging and strain rate imaging[70-73] have been recently published. These promising techniques should be applied in larger cohorts of patients in order to assess their clinical utility for monitoring chemotherapy-induced CTX. Their advantages include the applicability to virtually all patients, including those with poor quality echogenic windows, and a very low intra/interobserver variability[74]. However, specific skill and experience are needed to accurately perform and interpret these new echo technologies[74,75].

In this context, another promising ultrasound technique, ultrasonic tissue characterization by integrated backscatter, has to be considered as a sensitive tool for the detection of myocardial damage related to specific diseases, i.e. coronary ischemic necrosis and idiopathic cardiomyopathy[76]. In fact, some of their clinical applications have been successfully exploited even in children receiving ANT: in particular, the integrated backscatter of the myocardium of these children, obtained from the left ventricular posterior wall, was significantly more abnormal than that of a normal age-matched control group and, moreover, it was correlated with the ANT cumulative dose and the late development of CHF[77].

Newer candidate markers of subclinical cardiotoxicity

In order to predict morbidity and future events, just as for other cardiomyopathies, there is increasing interest in other physiological changes that may precede or coincide with chemotherapy-related left ventricular dysfunction. Hence, sensitive indexes of autonomic nervous activity, such as heart rate variability (HRV) or the mentioned QT dispersion, and biochemical markers of specific myocardial damage (troponins) or neurohormonal markers of cardiac dysfunction [natriuretic peptides and endothelin-1 (ET-1)] have recently been investigated in this setting.

Heart rate variability. HRV is the circadian fluctuation of heartbeats, which is controlled by the parasympathetic-sympathetic balance of the autonomic nervous system: normal subjects are able to modulate the heart rate in a wide range of intervals during different physiological or physical activities, with rapid modifications. HRV may be assessed both by spectral analysis and time-domain analysis and may be calculated from 24-hour ECG recordings using specific software. To date, few studies have evaluated HRV as a prognostic parameter in chemotherapy-induced CTX. In other chronic diseases such as post-infarction[78] and idiopathic cardiomyopathy[79,80] this parameter is impaired in patients with left ventricular dysfunction and it represents an independent index of cardiac morbidity and mortality. Two studies[23,81] assessed this marker: the first one demonstrated a better correlation between the amount of ANT received and the degree of HRV indexes abnormalities than with radionuclide and echo LVEF[23]. With regard to the other study, high differences were found between patients treated with HD-C and normal age-matched subjects[81]. However, the same authors failed to reproduce these results in patients treated with various cycles of HD-C by serial measurements of HRV over 1 year of follow-up after chemotherapy: in particular, HRV was reduced only during the first days after chemotherapy and then returned to basal values, with the mental stress of patients as a possible confounding factor[82]. Hence, HRV analysis seems to be a sensitive test for the detection of CTX but further studies are needed to clarify its specificity; until then, HRV is still of limited clinical utility in the evaluation of individual patients[83].

Natriuretic peptides. Atrial natriuretic peptides (ANP) and brain natriuretic peptides (BNP and pro-BNP) belong to a family of structurally related peptides that are synthesized, stored and secreted from the heart in response to atrial or ventricular overload. ANP is mainly synthesized in the atria, while BNP is synthesized both in the atria and ventricles as the prohormone proBNP, but is mainly released from the ventricles[84]. Plasma concentrations of N-terminal proBNP (NT-

proBNP) are likely to reflect *de novo* synthesis rather than the release of stored BNP, possibly more precisely reflecting natriuretic pathway activation[85]. Moreover, its practical usefulness lies in the possibility of accurate assay with easier handling of serum samples and applicability to routine immunoanalyzers than ANP and BNP sampling that necessitate more complex determinations, not suitable for routine use[86]. Plasma levels of natriuretic peptides are elevated in patients with severe CHF of various etiologies: ischemic[87-89], hypertensive[90], idiopathic[91,92], but also in patients with asymptomatic left ventricular systolic[93] or diastolic dysfunction[94]. Besides, it is important to bear in mind that increased levels are strictly correlated with outcome and future major cardiac events[95-97]. Also, elevated BNP and NT-proBNP are strictly related to patient prognosis[84-86]. These results have proved to be an interesting field for the investigation of the pathological mechanisms of cardiac diseases and also an accurate marker for predicting disease outcome, so allowing physician orientation and monitoring of different therapeutic strategies[98,99]. However, at the moment the results are not conclusive and these biochemical markers cannot be used as a unique diagnostic tool for primary care in the community[100-102]: they seem to have a strong negative predictive value (about 90%) but a weak positive predictive value (even lower than 41%)[103]. Their significance mainly seems to be in terms of prediction of events: death and hospitalizations for symptomatic patients[84-86], and the use of ACE-inhibitors for asymptomatic patients with left ventricular dysfunction[98,99].

However, there are only a few small studies[104-108] investigating these peptides in chemotherapy-treated patients, especially in conjunction with the indexes of systolic and diastolic function. One cross-sectional study compared various parameters (cardiac enzymes, echo LVEF, Doppler E/A ratio, catecholamines, angiotensin II) before and after HD-C and showed that only ANP and BNP levels were significantly changed[104]. Recently, another small prospective follow-up study[105] demonstrated that during the evolution of ANT-induced left ventricular dysfunction the secretion of natriuretic peptide was more closely associated with the impairment of left ventricular diastolic dysfunction (decreased Doppler E wave peak velocity and percent first one third left ventricular filling period, increased left ventricular end-systolic diameter) than with the deterioration of LVEF. Finally, another study performed serial determinations of the basal BNP and then weekly for 5 weeks in 15 patients receiving HD-CY: a value > 43 pmol/l was present in 7 patients already 1 week after and 3 of them, with the highest BNP peaks, developed CHF 1-3 weeks later[107]. Finally, in 36 patients treated with ANT we detected a significant increase from the basal plasma NT-proBNP concentrations (baseline values: mean 77 ± 122 pg/ml; end-therapy values: mean 167 ± 235 pg/ml) and, importantly, we observed a direct correlation between increasing plasma NT-proBNP concentrations and tissue Doppler diastolic indexes (Ea, Ea/Aa ratio). On the other hand, the correlation with echo LVEF was not significant. The correlation was even stricter when only the 4 patients who developed CHF within 18 months of follow-up were considered[109].

Hence, natriuretic peptides could become candidate markers of early chemotherapy-induced CTX, but at present they still need to be adequately evaluated both during treatment and follow-up.

Troponins. Cardiac troponins (cTnT and cTnI) too are emerging as specific biochemical markers of myocardial damage and have been evaluated also in such a context. These thin-filament contractile proteins are present in high concentrations in the myocardium and released into the circulation after cardiac injury such as myocardial infarction[110,111], myocarditis[112], non-ischemic cardiomyopathy[113,114], and even in minimal myocardial cell damage as occurs in unstable angina[115] or after percutaneous coronary angioplasty[116]. In all these clinical settings, cTnT or cTnI – as their diagnostic and prognostic values seem clinically identical[115] – constitute precise hallmarks for future events and cardiac mortality[110,114-117].

An interesting experimental study has paved the way to utilize the serum levels of troponins for the detection of chemotherapy-induced CTX[118]: the investigators studied spontaneously hypertensive rats treated with increasing doses of ANT and measured both the serial serum levels of cTnT and its cardiac tissue localization by immunohistochemical staining and confocal microscopy. Then, they correlated these results with the corresponding grade of pathological lesions in biopsy specimens, evaluated by the classical 6-grade Billingham score. A close linear relationship between the cumulative doses of doxorubicin, serum levels of cTnT, decreased staining for tissue cTnT and pathological score of cardiomyopathy was observed. The authors conclude that cTnT is released from ANT-damaged myocytes and measurements of cTnT serum levels provide a means for assessing early ANT-related CTX[118]. Moving to the clinical field, Lipshultz et al.[119] found that cTnT levels were increased in about 30% of children treated with ANT for lymphoblastic leukemia, that they were positively correlated with dose, and that increased concentrations of cTnT sometimes persisted for months suggesting long-term myocardial injury. Finally, Cardinale et al.[120,121] serially measured serum cTnI levels during different HD-C regimens (18 samples for each patient) and found increased cTnI levels in about one third of 211 adults, particularly those receiving ANT: during the subsequent 12-month follow-up, the cTnI-positive group showed a progressive decrease of echo LVEF with a close relationship between the cTnI increase as well as the number of cTnI-positive assays and the maximal LVEF decline (by an absolute 16% compared with only 5% in the cTnI-nega-

tive group). On the contrary, two other studies did not find similar data: in 22 children treated with ANT-containing cycles, Fink et al.[122] did not observe any significant increase in cTnT measured within 3 days of chemotherapy. Raderer et al.[123] reported 10 patients treated with ANT as a part of HD-C with no changes in cTnT levels during a 48-hour observation period. Finally, for 16 patients receiving HD-CY (7 g/m^2) we did not detect any increase in the cTnI serum levels over a 72-hour interval from treatment[31]. Analogous results were observed in another recent study including about 30 patients treated with HD-CY: although cTnT was serially determined for a median of 14 days after the completion of therapy, no elevated levels were observed[124].

Overall, these preliminary studies are not yet conclusive for the routine monitoring of troponins in patients receiving chemotherapy, especially within 24 hours of the administration of the drugs[125], and more *ad hoc* studies are necessary to establish the true diagnostic and prognostic significance of such biochemical markers in this setting[126].

Endothelin-1. ET-1 is a potent vasoconstrictor peptide, counteracting the vasodilator neurohormonal factors such as ANP and BNP; it is synthesized by endothelial cells and ventricular myocytes and is involved in the human pathophysiology of left ventricular dysfunction as a participating factor to the progression of heart failure; however, its precise role is not yet fully understood[127]. The same 5-fluorouracil-induced CTX seems related to coronary artery vasospasm and mediated by increased levels of ET-1, as recently reported by Porta et al.[128]. Plasma levels of ET-1 have been reported in humans with advanced CHF[96,129]: in these two studies ET-1 levels have been considered as a useful prognostic indicator of survival. But, a more recent study by Tsutamoto et al.[96] including about 290 patients demonstrated, by stepwise multivariate analysis, that ET-1, in contrast to BNP and left ventricular end-diastolic volume or pressure, was not a useful predictor of mortality and morbidity: probably, this is due to the fact that ET-1 is a local factor[96].

With regard to chemotherapy-related CTX, Suzuki and Miyauchi[130] reported elevated plasma levels in ANT-treated patients with breast cancer and they hypothesized that ET-1 plays a role in the pathophysiology of ANT-related CTX. Then, they experimentally investigated this issue using ANT-treated cultured neonatal rat cardiomyocytes. The conclusion of this study was that ET-1 acutely increased the expression of an endogenous antioxidant, manganese superoxide dismutase, as a cytoprotective effect on ANT-toxic cardiomyocytes (and pre-treatment with an antisense of manganese superoxide dismutase attenuated such a cytoprotective effect); but, in conditions of prolonged exposure of cultured myocytes to ANT, this cytoprotective effect of ET-1 against ANT-toxicity was found not to persist[130]. In the clinical scenario, Yamashita et al.[131] reported the observation of 2 patients in whom the levels of ET-1 progressively increased during ANT treatment for breast cancer and who subsequently developed CHF. Then, they conducted a prospective study of 30 consecutive patients treated with ANT and serially monitored the changes in the echo LVEF, ANP and ET-1. The plasma levels of ET-1 increased progressively in 5 patients; later on, 2 of these 5 patients developed clinically overt CHF. On the other hand, LVEF and ANP values showed no abnormalities until the manifestation of CHF. This suggests a potential role of ET-1 sampling for the prediction of ANT-induced CTX[131].

Old and new preventive interventional strategies: lights and shades ("all that glitters is not gold")

We have already mentioned some traditional preventive strategies applied to reduce the risk of chemotherapy-induced CTX: 1) modifying, where possible, the pre-therapy risk factors (diabetes, hypertension, cigarette smoking, electrolyte abnormalities, etc.); 2) modulating the dose rate and schedule of drug administration, in order to reduce the cardiotoxic risk by lowering the load doses of tissue exposure.

The use of several agents that have proved to reduce the incidence of CTX could be employed. However, to date only the Fe-chelator dexrazoxane (Cardioxane®), a cardioprotective agent specific for ANT, has been approved by the Food and Drug Administration[4]. Its administration, in accordance with the American Society of Clinical Oncology guidelines, is indicated only for delayed use in patients with metastatic breast cancer who have already received 300 mg/m^2 of ANT, in pediatric malignancies, in patients using high-dose ANT therapy and finally in patients at high risk for CTX. Otherwise, agents such as trimetazidine[132], carnitine[133,134] and coenzyme Q_{10}[135,136] all are being experimentally (in cell culture or animal studies) investigated, particularly in Italy, but without relevant human beneficial effects, as reported by Horenstein et al.[137] in a recent review.

One biological device used for reducing the CTX risk is the liposomes, "lipid shells" incorporating ANT. Liposomal encapsulation of doxorubicin (Doxil®) may reduce both the non-specific drug delivery to normal tissue as well as the high peak plasma levels of free drug responsible for its toxicity.

Stealth liposomal doxorubicin is a formulation in which the drug is encapsulated in liposomes that escape instant recognition and uptake by the mononuclear phagocyte system. As a result, the formulation has a long half-life, and the liposomes may eventually be extravasated through the abnormally permeable vessels characteristic of many tumors. Once concentrated in tumors, liposomes can deliver high levels of doxorubicin to malignant cells, without affecting normal tissue. Phase III studies in patients have indicated that Doxil®

produces less nausea, vomiting, alopecia and stomatitis than conventional ANT, and is also less cardiotoxic[138].

Nevertheless, all these preventive strategies have still not been able to significantly reduce the incidence of chemotherapy-related CTX in the clinical real world. Nowadays, many investigators believe that better prevention of CTX still remains a prospective monitoring of timely accurate markers of cardiac damage, either of a functional or of an anatomic type, that may lead to develop an ominous CHF[17,22,23].

We also believe that a precocious diagnosis of left ventricular dysfunction could provide cardiologists with a unique opportunity to initiate preventive cardioactive therapy, such as ACE-inhibitors and carvedilol, recognized as being able to modify the natural history of all forms of cardiomyopathies. In fact, several *ad hoc* studies such as SOLVD and SAVE have demonstrated that treatment with ACE-inhibitors decreases morbidity and mortality in patients with mild asymptomatic left ventricular dysfunction from other causes. For this purpose, an outcome study[17] evaluated a preliminary ACE-inhibitor therapy for more than 3 months in 60 cancer patients treated with low or high-dose ANT and belatedly developing mild or moderate asymptomatic left ventricular dysfunction. The ACE-inhibitor caused a remarkable and long-lasting recovery while in control patients no spontaneous regain was observed; moreover, some asymptomatic patients experienced a new deterioration of LVEF after interruption of ACE-inhibitor therapy. In this direction, the ongoing prospective placebo-controlled blinded study by the same authors, in 120 patients treated with cardiotoxic chemotherapy and subsequently given ACE-inhibitor therapy, will hopefully confirm the positive results from the already cited pilot study[17]. Even Keefe[19] reported a significant improvement in LVEF and also symptomatic benefits, following the use of carvedilol and spironolactone, which also proved to be well tolerated; finally Fazio et al.[139] described in 1998 the first case of ANT-induced cardiomyopathy, unresponsive to standard medical treatment (digoxin, diuretics and ACE-inhibitors), successfully treated with carvedilol.

Conclusions

Chemotherapy-related CTX is a well-recognized side effect of some antineoplastic agents that may have a dramatic impact on patient survival and quality of life. To date, no universally accepted and valuable tools are available for its early recognition and treatment. Hence, CTX remains a clinical challenge, particularly in view of the increasing number of long-term cancer survivors. Because of the limited success of different preventive strategies, the clinical evaluation of patients and regular monitoring of cardiac function are and will be essential to prevent severe and progressive cardiac dysfunction. The monitoring of CTX is mandatory both during chemotherapy and follow-up, especially in young persons and high-risk patients and in those in whom LVEF decreases to below 50% or other systolic-diastolic indexes are changing in a pathological direction. Although current guidelines indicate LVEF alone as the "gold standard" tool, it should be taken into account that, apart from the risk of inaccurate measurements, a decrease in this parameter may also be due to changes in patient hemodynamics or other extracardiac conditions. However, it should be safer to perform a comprehensive multiparametric echo-Doppler exam and to monitor some indexes of both the systolic and diastolic left ventricular functions. For patients at risk with a non-conclusive LVEF or diastolic indexes evaluation, an adjunctive echo-Doppler exercise or dobutamine stress evaluation may be useful to assess their cardiac reserve. Hence, prospective monitoring could facilitate the early utilization of cardioactive intervention that may prevent further cardiac deterioration.

For the future, the results of ongoing studies including large cohorts of patients and comparing newer functional or biochemical markers of early cardiac damage (such as tissue Doppler, BNP or others) with the conventional indexes of cardiac function are awaited.

These studies should establish which is the best tool for the accurate identification of patients at risk of developing CHF, who could benefit from more precocious therapeutic intervention in order to prevent or, at least, delay the development of cardiomyopathy. This is especially true for those patients who have already been endowed with a good life expectancy following cancer.

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