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Serum Biomarkers for the Detection of Cardiac Dysfunction in Childhood Cancers Receiving Anthracycline-Based Treatment

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Anthracyclines are routinely used in cancer chemotherapy in many childhood cancers. A serious adverse effect of doxorubicin chemotherapy is cardiotoxicity which may lead to congestive heart failure for long-term survivors years after treatment. Currently, echocardiography is used to control the heart function during anthracyclines therapy. B-type natriuretic peptide (BNP) and NT-proBNP as well as cardiac troponins have been proposed as clinical markers for subclinical anthracycline-induced cardiotoxicity. The BNP and pro-BNP can be easily measured in plasma and initial data indicate that the NT-proBNP could be sensitive predictor for the development of congestive heart failure.

Keywords: Anthracyclines; Children; pro-BNP; Troponin.

Anthracyclines are widely used to treat solid and hematologic malignancies in children. Up to 60% of children diagnosed with cancer receive anthracycline-containing chemotherapy as part of their treatment. The most used anthracyclines are doxorubicin, daunorubicin, idarubicin, and epirubicin. However, the use of anthracyclines is burdened by the risk of severe cardiotoxicity[1,2].

However, anthracyclines can induce dose-dependent and cumulative cardiotoxicity, resulting in heart failure. Several factors contribute to increased cardiotoxicity from anthracyclines (Table 1).

Scientific data so far obtained show that the molecular mechanisms of antitumor cytotoxicity and cardiotoxicity are different. The mechanisms underlying the cardiotoxic effects of doxorubicin are not fully understood, but lipid peroxidation and free radical generation appear to play important roles.

Endomyocardial biopsy is the most sensitive and specific method for diagnosing and monitoring anthracycline cardiotoxicity, but the invasive nature of this procedure limits its use. Evaluation of left ventricular systolic function by echocardiography represents the most frequently used non-invasive method in clinical practice[3-5]. Measurements of fractional shortening and left ventricular (LV) ejection fraction are generally used during treatment with anthracyclines. However, these measurements have the disadvantage of detecting heart defects late, ie, when the decline in left ventricular ejection fraction (LVEF) has already occurred. Once symptoms of heart failure develop, discontinuation of anthracycline treatment is unable to reverse this condition (Table 2).

Therefore, there is a need for early detection of cardiotoxic effects. Therefore, there is growing interest in the use of biomarkers, such as BNP (B-type natriuretic peptide) and NT-proBNP (N-terminal pro B-type natriuretic peptide), cardiac troponins T (TnT) and I (TnI), and ST2 (Suppression of Tumorigenicity 2) for the detection of myocardial damage[6,7].

Troponin

Troponin is a high molecular weight protein present in muscle tissue and cardiac cells, composed of three domains: the I domain, which binds F-actin, the C domain, which binds Ca2+ ions, and the T domain, which binds tropomyosin. It plays a key role in the excitation-contraction phase of striated muscle fibers, which begins with the binding of Ca2+ to the C-site of troponin.

This process causes the I domain to move away from the T domain, with subsequent sliding of tropomyosin on the actin filament, which gives way to the myosin heads. The sliding of the myosin heads on the actin ones, at an angle of 45°, causes muscle contraction. Because they have high specificity and sensitivity for myocardial tissue, troponins (cTnT) are the most suitable biomarkers in clinical practice to detect myocardial infarction or unstable angina.

The release of troponins T and I after acute myocardial infarction occurs between 12-48 h and 10-24 h, respectively, reflecting early cardiac injury [8]. Troponins may, therefore, be considered subclinical markers of ischemia, but they also play a role in long-term cardiac damage.

Studies in children undergoing cardiovascular interventions have shown a higher diagnostic value of troponin I than T, because it is not affected by any alterations in renal function[9-11]. In contrast, the increase in serum troponin as a prognostic marker of other cardiovascular events in patients without acute coronary syndromes (such as chemotherapy cardiotoxicity) is much discussed. Several studies have shown that troponin I positivity during anthracycline cycles identifies patients at increased risk of developing systolic dysfunction[12].

Although it appears to be a promising biomarker of early anthracycline injury, the troponin I assay has not yet been validated in clinical practice in this setting. The limitations of studies in this regard are represented by the timing of sampling, the duration of follow-up, the different definition of cardiac endpoints, the heterogeneity of patients, and the limited sample size[13,14].

The first study reported in the literature on this topic was performed by assaying TnT levels in doxorubicin-treated mice, finding an increase in serum TnT and myocardial injury as the cumulative dose of the drug increased[15]. The prognostic role of troponin T in the development of anthracycline myocardial dysfunction in ALL children is, also, somewhat controversial. Only one study reported a significant correlation between cardiac dysfunction and TnT: in particular, the increased levels of TnT (cut off: 0.03 ng/ml) found correlated significantly with increased left ventricular telediastolic volume and reduced ventricular wall thickness, during follow-up[16,17]. The increase in troponin T in patients exposed to minimal doses of anthracyclines would be evidence that cardiomyocytic damage begins early during therapy and may establish as early as the first dose of drug administration[18-20]. No dose-based study of troponin I in children treated with anthracyclines, however, has shown a significant increase in troponin I[21]. Whereas in anthracycline-treated adults a close relationship has been shown to exist between increased TnI and reduced LV ejection fraction, the same could not be affirmed in children, because troponin levels were found to be always below threshold values[21,22].

Since a reduction in FEVS is a late event indicative of at least a sub-clinical picture of heart disease, research has developed in in recent years the search for biochemical markers has developed, which at intervals subsequent to the administration of anthracyclines are able to identify the subjects in whom there has been a cardiac insult predictive of evolutionary tendency.

Elevation of troponin I above 0.08 ng/ML within the first 72 hours and even troponin I elevation beyond 0.08 ng/ML within the first 72 hours and even more afterwards seems to be one of the most effective markers. possible usefulness of treatment with ACE inhibitors for the prevention of late cardiotoxicity in late cardiotoxicity in patients at high clinical risk identified by post-chemotherapy troponin I elevation. However, the major problem hindering the widespread use of troponins as a certain marker of myocardial damage is that it is not yet clear whether at each cycle of chemotherapy, a single measurement has sufficient

predictive value or whether multiple evaluations are necessary.

NT Pro-BNP

NT pro-BNP (N-terminal fraction of Brain Natriuretic Peptide precursor) is the N-terminal fragment of the BNP protein precursor (pro-BNP), a pro-hormone produced by ventricular cardiac myocytes (first isolated from porcine brain) in response to cardiac stretch and overload, whose function is to reduce afterload and increase natriuresis. Following an increase in pressure or volume stress of the ventricles, the heart responds, in fact, by increasing the increment of this hormone to normalize hemodynamic parameters. N-terminal pro-BNP has a longer half-life than BNP and is less affected by withdrawal[7].

There is no evidence about this hormone as an early marker of cardiotoxicity. From studies that assessed NT-pro-BNP levels in children after or during anthracycline therapy, no striking results emerged. In a group of children exposed to a first dose of doxorubicin greater than 25 mg/m^2, NT-pro-BNP levels were increased, despite the complete absence of echocardiographic abnormalities[23]. One should keep in mind a possible bias in the increase of NT-pro-BNP levels represented by the hyper-hydration therapy performed in all cancer patients, for the prevention of tumor lysis syndrome. A study performed in another pediatric population undergoing treatment with anthracyclines showed, at the end of therapy, a positive correlation between increased levels of the hormone and reductions in LVEF and left ventricular mass, respectively[24].

Another aspect that should not be overlooked is the variability of NT-pro-BNP values in relation to sex, renal function, ventricular stiffness, and above all age[24-27]. In the pediatric population, there is a wide variability in NT-pro-BNP levels among the various age groups with very high values for children under six years of age, which are far from the adult reference values[28].

Elevated BNP levels represent a recognized biomarker for heart failure, so much so that their high sensitivity makes BNP a particularly useful marker for ruling out heart failure (high negative predictive value). However, specificity is low and the variability of normal levels is very wide, reducing their practical usefulness as markers of heart failure.

Nevertheless, especially in children, promising results compared with troponins have been obtained with the determination of natriuretic peptide during anthracycline therapy. High levels of BNP and its precursor pro-BNP correlate well with echocardiographic indices of myocardial dysfunction in most studies. However, the variability and overlap of serum levels in patients with and without myocardial dysfunction makes it difficult to establish accurate cut-off values for the identification of subclinical heart failure.

ST2

ST2 is a protein encoded by the ST2 gene belonging to the interleukin 1 (IL-1) cluster(29). Of the four isoforms encoded by the gene, the major products are two receptors of the IL-1 receptor family: sST2 and ST2L. ST2L is a membrane receptor whereas sST2 is a soluble receptor that can be assayed in serum. The functional ligand of the ST2L receptor is interleukin 33 (IL-33)[30].

IL-33 - ST2L binding occurs on the membranes of inflammatory cells and activates mitogen-activated protein kinases (MAPKs) and other biochemical pathways. The final reaction of this activation cascade is the inhibition of the NF-êB complex (nuclear factor with pro-inflammatory role)[29,31].

sST2 acts as a truncated receptor for IL-33 and, as such, functions as a molecular trap for it, removing it from interaction with ST2L (it possesses the sequence that recognizes IL-33, but lacks the intracellular domain, which is important for signal transduction). IL-33 regulates the transcription of sST2 and ST2[32]. In research performed in high-grade pancreatic adenocarcinoma cell lines, the role of the cytokine in the expression of ST2 gene isoforms was observed: it stimulates the expression of ST2L and inhibits that of sST2, respectively. sST2 interacts directly with macrophages in the presence of circulating bacterial lipopolysaccharide, inhibiting the production of cytokines modulating the inflammatory response (IL-6, IL-12 TNFá).

ST2L is, on the other hand, able to activate T helper type 2 (Th2) lymphocytes and induce the production of IL-4, IL-5, and IL-13, which stimulate the Th2-mediated inflammatory response itself[33-36]. ST2 receptors are also involved in cardiac dynamics. Under stressful conditions,

cardiomyocytes and cardiac fibroblasts show increased release of both.

Notably, it has been observed that, under conditions of cardiovascular disease, endothelial cells are the major source of expression of mRNAs encoding for the two isoforms of ST2, compared with myocardial cells[37]. The rapid increase in sST2 levels found after myocardial infarction is considered a negative prognostic factor in terms of survival[38-40].

IL-33, by interacting with ST2L, exerts an antihypertrophic function on the cardiac organ. Myocardial hypertrophy is promoted, in fact, by the induction of factor NF- Kb by angiotensin II and phenylephrine[41]. sST2, by sequestering IL-33, reduces its cardioprotective action.

Administration of IL-33 in patients with cardiovascular disease activates the intracellular pathway of ST2L, reducing degeneration of myocardial function[41]. In addition, IL-33 would have a stabilizing role on atherosclerotic plaques, preventing their rupture. This action is related to the fact that interleukin reduces serum levels of INF-ã, which activates the production of metallo-proteases by macrophages, which in turn damage the fibrous cap of atherosclerotic plaques, rendering them unstable[33,41,42]. An antioxidant action of the cytokine has also been proven, as it is able to reduce the levels of intracellular oxygen free radicals.

The possibility of considering plasma ST2 levels as early bio-humoral markers of cardiovascular events related to heart failure and ischemic disease has been hypothesized, such as to allow an early diagnosis of these, interrupt their evolution, and prevent their negative outcomes[43-45].

Elevation of sST2 levels in patients with dyspnea has been found but was not useful in identifying the possible cardiac cause of this[46-48]. ST2 would also present an anti-apoptotic role within the cell, exerting an inhibitory effect on the action of caspases and an excitatory effect on the expression of anti-apoptotic proteins[49]. sST2 is significantly increased in patients with chest pain, systemic inflammatory states, and alcohol abuse, in conjunction with pulmonary diseases such as asthma, COPD, pulmonary embolism, and pulmonary hypertension[49-52].

sST2 increases in very different clinical conditions, not only in pathological conditions of the cardiovascular system; this does not make it a specific marker of disease. Despite its limited use as a diagnostic marker, its role as a prognostic marker in several clinical conditions is more credited.

Table 1. Risk factors for anthracycline-related toxicity.

Risk Factors
Cumulative dose (> 250 mg/sqm)
Concomitant use of other drugs and chemotherapeutics (alkylating, antimicrotubule agents, targeted therapies)
Radiotherapy to the mediastinum
Female sex
Time since end of treatment
Age less than 5 years or more than 65 years
Infusion duration
Cardiovascular risk factors (obesity, hypertension, dyslipidemia, pre-existing heart disease)

Table 2. Mechanisms of chemotherapy-induced cardiac toxicity

Mechanisms of Toxicity	
Type 1	Typical toxicity induced by all drugs of the anthracycline family. It is dose-dependent. The damage to myocardial tissue begins immediately after the first administration and is irreversible at the cellular level, since it leads to the death of the biological unit
Type 2	Represents the toxicity that typically occurs following treatment with trastuzumab (Herceptin). It is not dose-dependent and creates cellular dysfunction that does not lead to cell death with a reversible damage

DISCUSSION

Anthracyclines are cardiotoxic and chronic damage is more frequent than acute damage.

The susceptibility to damage is individual; in fact, cases of myocardial damage have been reported in the literature in patients receiving even very low doses of doxorubicin[53]. The mechanism underlying the damage is still not completely clear. To date, it is not easy to define which are the most sensitive and specific markers able to detect subclinical anthracycline-induced cardiomyopathy. Ejection fraction and shortening fraction are often used to assess systolic heart function but are not characterized by high sensitivity[54-56].

Both parameters are influenced by ventricular geometry, so they cannot be considered accurate indicators under conditions of altered ventricular conformation. The myocardial performance index (MPI), on the other hand, is not affected by ventricular geometry and does not require any form of normalization, is applicable for the study of the function of both ventricles, and correlates well with other forms of ventricular function measurement used in the pediatric patient[57].

These data suggest that MPI may represent a more sensitive and accurate parameter for investigating subclinical anthracycline injury in children than current methods of assessment. The study also showed that alterations in echocardiographic parameters, such as ejection fraction and shortening fraction, occur even for moderate doses of anthracycline treatment, but later than the timing of MPI increase. The latter would therefore be a better quality marker than the other echocardiographic parameters, although its prognostic value is still uncertain.

The significant increase in MPI is mainly related to the prolongation of the iso-volumetric contraction time (ICT). The significant elevation of MPI is mainly related to the prolongation of ICT, which leads to the hypothesis that, at the beginning, anthracyclines damage starts with a picture of systolic dysfunction. Confirmation of this hypothesis is provided by the finding of a decreasing trend in FS and LVEF and the absence of significant alterations in E/A ratio and iso-volumetric release time (IRT) (both, the latter, parameters assessing diastolic function)[58,59].

To date, the measures to be implemented in the treatment of asymptomatic patients with preserved ventricular function and alterations in the MPI alone are unclear, precisely because of the dubious prognostic value of the latter. About bio-humoral markers, no connection was found between these and the above echocardiographic parameters. NT-pro-BNP, cTnT, and ST2 were chosen as markers of cardiotoxicity in the population examined, being widely used to investigate cardiac damage (whether or not related to ischemic phenomena) in adults and children[60,61].

In particular, NT-pro-BNP is a diagnostic and prognostic marker of heart failure not only in the adult but also in the pediatric patient[62,63] Several studies performed in adult patients treated with anthracyclines have shown the presence of a correlation between the levels of some bio-humoral markers and some echocardiographic parameters[22,64-67]. NT-pro-BNP correlates very well with signs and symptoms of heart failure in both adults and children, allowing differentiation between respiratory and cardiac causes of respiratory distress[68-72]. Several studies have shown the importance of NT-pro-BNP as a marker of systolic dysfunction in children[73].

Therefore, it would be useful in pediatrics to combine the measurement of NT-pro-BNP levels with the assessment of signs and symptoms at rest and in response to physical exertion for the purpose of risk stratification of pediatric patients with heart failure.

It has been proposed that measurement of this bio-humoral marker be included in the new risk stratification classification of children who develop heart failure, compared with the old Ross classification[74]. In the pediatric patients this stratification, should be performed by age group, since the clinical presentations of decompensation vary widely between infants and older children.

Normal NT-pro-BNP values are highly variable in the pediatric population and differ greatly from those in adults. Children have highly variable measures of the marker, depending on the age groups considered (with higher values in children younger than 3 years of age, compared with older children)[75]. However, there are few studies in the literature evaluating the normal ranges of the NT-pro-BNP marker in the

pediatric patient[76,77]. Equally few are the studies in which increased levels of the marker in children undergoing anthracycline treatment have been found to be accompanied by abnormalities on echocardiographic examination, and only in one of these was it related to dose accumulation[78-81]. This relationship is still somewhat controversial, and more data could confirm the prospective sensitivity of this marker in the early detection of anthracycline cardiotoxicity in the pediatric patient.

Transient increase in NT-pro-BNP during anthracycline treatment is not predictive of cardiotoxicity and development of CHF; it is only predictive if persistently elevated[82].

The NT-pro-BNP levels that emerged were not shown to be persistently elevated in any of the 19 patients analyzed. Furthermore, because they are influenced by a variety of confounding factors such as hyperhydration during chemotherapy, NT-pro-BNP levels do not appear to be useful in the choice of modulation of ongoing therapy. Levels of TnT did not change, confirming the lack of development of cardiotoxicity by the children under investigation.

To date, the predictive role of troponin levels in anthracycline cardiotoxicity is controversial: a study reported by Fink et. al denies elevation in levels of the marker when assayed in children exposed to doxorubicin; in contrast, Lipshultz et al. observed slight increases in serum TnT values after the first administrations of doxorubicin, hypothesizing its predictive role about the risk of developing cardiomyopathy[83,84].

At present, it has been found that there is no clinical cut-off of cTn that takes into account some important variables: sex of patients, age, time of measurement and dosing method. Models capable of combining serum biomarkers, cardiac risk factors, type of antineoplastic treatment, and imaging parameters are needed to increase accuracy in identifying subjects at higher risk of antineoplastic therapy-related cardiotoxicity. Thus, further studies are needed for the troponin assay to identify standards regarding cut-off values, sampling times, and then evaluate the real evidence that intervention strategies based on the use of troponin are effective and have an impact in reducing cardiotoxicity-induced morbidity and mortality in patients with cancer.

ST2 was selected for its release under conditions of myocardial stress. Cardiomyocytes and cardiac fibroblasts show an increased release of both ST2L and sST2 receptors in the setting of heart failure and myocardial fibrosis. sST2, in addition to being a marker of cardiac stress, increases in different clinical conditions, especially inflammatory ones.

In particular, in pediatric patients significant increases of the marker have been found during asthma, Kawasaki disease, and pulmonary hypertension. It is possible that the decreasing trend of ST2 found in children treated for acute lymphoblastic leukemia can be partly related to the prolonged steroid therapy (immunosuppressive) to which they are exposed during the induction and re-induction phases[85]. Ultimately, ST2 does not appear to be a specific marker of cardiac damage, as it increases in different clinical conditions, such as inflammatory and/or autoimmune diseases.

Over the past decade, new biomarkers have been studied although not yet incorporated into everyday clinical use[86-88]. Promising biomarkers such as PlGF, GDF-15, sFlt1, hs-CRP, glycogen phosphorylase BB and H-FABP, galectin-3, , myeloperoxidase, and fibrocytes that could provide new data on tissue inflammation in heart failure are being evaluated[89-92].

CONCLUSIONS

Anthracyclines can increase the risk of cardiotoxicity to the point of congestive heart failure years after recovery. Currently, cardiac function during doxorubicin therapy is monitored using echocardiography. However, this method is unable to detect subclinical cardiac deterioration. Therefore, more sensitive markers are needed. Evaluation of biochemical parameters such as troponin and the natriuretic peptides ANP and BNP in the blood can be an aid in the diagnosis of subclinical cardiac damage. Troponin is released following myocardial cell damage, whereas natriuretic peptides are secreted depending on the pressure in the atria or ventricles. Detection of high concentrations of these parameters in the blood could help predict patients at higher risk for congestive heart failure.

However, the data provided by measurements of serum biomarkers in the assessment of cardiotoxicity is still difficult to

interpret: the diversity of data in the literature, the insufficient sample size, the heterogeneity of the population studied and the different cardiological evaluation make the comparison of the results of different studies rather complex. Further prospective studies on larger populations will allow to better define whether the dosage of BNP can be an effective preventive strategy, but at present there is no clear evidence for this hormone as an early marker of cardiotoxicity.

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Authors' contributions

All authors participated in the research design, data analysis, and the writing of the manuscript. All authors approved the final version of the manuscript.

Conflict of interest statement

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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