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Preliminary Pre-Clinical Studies on the Side Effects of Breast Cancer Treatment

Purpose: Technological advancement in the treatment of cancer together with early detection and diagnosis have considerably improved the survival of breast cancer patients. On the other hand, the potential of patients developing side effects from cancer treatment are not negligible. Despite the progress that has been made in terms of early diagnosis, therapy, and survival, including improvements in the chemotherapeutic agents, radiation and molecular targeted therapies, cardiotoxicity of cancer therapy is still cause for concern. Radiation therapy for breast cancer is associated with increased risk of heart disease and myocardial infarction. Furthermore, the association of radiation therapy to chemotherapy is an important aspect to be considered in the development of cardiac disease, as this could play an additional role as a risk factor. Besides the heart effect, other side effects can be observed in the bone, ovary, uteri, and other organs. This paper aims to review the recent literature to present the current understanding of side effects associated with breast cancer treatment. The focus is on recent preclinical studies that have assessed potential changes in different organs that may be injured after breast cancer treatment, both due to both radiation and chemotherapy agents. Conclusion: Radiation-induced heart disease is one important side effect that must be considered during the treatment planning and patient follow-up. The cardiac damage can be potentialized when chemotherapy is associated to radiotherapy, and the literature findings indicate that heart fibrosis plays an important role at the radio-chemotherapy induced cardiac damage. Literature findings also showed important side effects at the bone, that can lead to ospeoporosis, due to the decrease of calcium, after radio or chemotherapy treatments. This decrease could be explained by the ovarian failure observed at rats after chemotherapy treatment. It is of great importance to acknowledge the complications originating from the treatment, so that new

strategies can be developed. In this way, it will be possible to minimize side effects and improve the patients' quality of life.

Introduction

Breast cancer (BC) is the most common cancer (excluding skin cancers) and the second leading cause of cancer death (after lung cancer) in women. One in eight to ten women will get BC during their lifetime (DeSantis et al. 2019). In 2018, there were 2,088,849 people newly diagnosed with BC and 626,679 BC deaths worldwide, the vast majority occurring in women (Lovelace et al. 2019). Mortality from BC has decreased both in North America and the European Union, which is attributed to early detection and effective local and systemic therapies (Malvezzi et al. 2016; Harbeck & Gnant 2017). In South America, Africa, and Asia, however, the number of BC cases continues to grow, most likely due to changes in lifestyle and screening programs, in addition access to state-of-the-art diagnosis and therapy is often limited (Harbeck & Gnant 2017; Bray et al. 2018). It is clear that BC survival varies considerably by the stage at diagnosis. For patients diagnosed during 2009 through 2015 in the United States, the overall 5-year BC survival rate was 98% for stage I, 92% for stage II, 75% for stage III, and 27% for stage IV (DeSantis et al. 2019).

Early BC without detectable distant metastases is potentially curable through the application of a multidisciplinary approach (Harbeck & Gnant 2017). Most patients with early-stage disease receive treatment that includes radiotherapy (RT) and/or chemotherapy, and about 45% of patients with stage III will receive these treatments as

well as surgery (DeSantis et al. 2019). The risk of local recurrence can also be reduced by adjuvant RT, which is recommended for all BC patients undergoing breast-conserving surgery (EBCTCG et al. 2011; Duma et al. 2019).

In the past, the life span of a patient with metastatic disease was often too short for cardiovascular complications induced by BC treatment to become evident, but this is now a matter of concern. Despite the progress that has been made regarding early diagnosis, therapy, and survival, including improvements in RT, molecular targeted therapies, and chemotherapeutic agents, the cardiotoxicity of cancer therapy is still a point of concern (Albini et al. 2010). To accurately evaluate the cardiac exposure, so that a prediction on the occurrence of cardiac adverse events can be made, is a complex problem yet to be solved, and currently is a subject of intense examination (Loap et al. 2020). The use of agents to protect against the adverse cardiovascular events due to chemotherapy and RT in early-diagnosed BC survivors have, however, already been tested (Lage et al. 2019).

The Radiological Sciences Laboratory (LCR/UERJ) has been investigating the side effects of BC treatment for more than ten years. In this review we present a consolidation of the studies developed and published by the laboratory during recent years, and their connection with the current papers in the literature.

Cardiotoxicity induced by radiotherapy

In most developed countries, RT is received by around half of women with BC (Taylor & Kirby 2015). Radiation therapy in BC may be delivered to the whole or a portion of the breast (after lumpectomy), the chest wall (after mastectomy), and the regional lymph nodes. Radiation therapy in BC can be delivered to the breast as a whole or just a

portion (after lumpectomy), the chest wall (after mastectomy), and the regional lymph nodes. Post-lumpectomy whole-breast radiation is a regular component of breast-conserving therapy (Waks & Winer 2019). In the majority of early-stage BCs, RT following breast-conserving surgery is a widely accepted and selected approach for organ preservation (van Dongen 2000; Fisher et al. 2002; Veronesi et al. 2002; Castaneda & Strasser 2017).

Breast-conserving therapy with breast-conserving surgery and adjuvant RT for early-stage BC and ductal carcinoma *in situ* (DCIS) is one of the greatest achievements of evidenced-based modern cancer care. For women with early-stage invasive BC and DCIS who opt to undergo a breast-conserving approach, whole-breast radiotherapy (WBRT) has become the accepted standard of care. According to numerous studies, the risk of local recurrence in invasive cancer and noninvasive disease is reduced by 60%-70% and 50%-60%, respectively, following RT (van Dongen 2000; Fisher et al. 2002; Veronesi et al. 2002; Houghton 2003; Bijker et al. 2006; Castaneda & Strasser 2017). Furthermore, a 5.3% reduction in overall mortality after adjuvant RT has been reported in a 15-year follow-up (EBCTCG 2005).

Recently, the American Society for Radiation Oncology (ASTRO) published guidelines for the radiation therapy of BC, based on several prospective randomized studies, recommending 15 daily fractions as a standard protocol for whole-breast radiation (Smith et al. 2018). This is a reduction from the classical recommendation of 25–35 fractions (Smith et al. 2018); however, there is still no consensus as to whether this reduction will lessen the radiation-induced cardiac damage (Caron & Nohria 2018).

Radiation-induced heart disease (RIHD) can occur in a few days after RT but mostly occurs several years after exposure. In general, the radiation-induced cardiotoxicity risk depends on multiple factors, which include a combination of total dose, the dose per

fraction, the age of the exposed patient, the proximity of the heart to the tumor, the irradiated heart volume, in which the substructures of the heart are subject to radiation, and the total proportion of the heart within the radiation field (Caron & Nohria 2018). As the 10-year survival rates continue to rise, long-term toxicities potentially induced by RT become more evident with regards to late cardiac events (EBCTCG et al. 2011; Janssen, Käsmann, et al. 2018; Janssen, Rades, et al. 2018; Duma et al. 2019). Darby et al. (Darby et al. 2013) and Sardaro et al. (Sardaro et al. 2012) reported a 7.4% and 4% risk of cardiac disease for each gray (Gy) mean heart dose, respectively. Recently, van den Bogaard et al. (van den Bogaard et al. 2017) reported a relative increase in the cumulative incidence of acute coronary events dependent on the mean heart dose within 9 years of RT. Some strategies can be used to avoid heart irradiation: respiratory gating, treating the patient in prone position, reducing the target volume, and using techniques like intensity-modulated radiation therapy (IMRT) to improve tumor conformation, though there is not a consensus regarding the best technique to implement in order to minimize the heart irradiation (Duma et al. 2019). de Almeida et al. (DeAlmeida et al. 2012) demonstrated that, by contouring each slice of the left descending coronary artery using gated computed tomography images and drawing the whole coronary artery, it was possible to substantially reduce the dose to the coronary artery by a factor of 4. It is fair to say that most of the data available have been gathered from retrospective studies using patients treated with 2D technology or simple 3D planning without a major effort to contour the substructures of the heart. Data from literature about the use of modern techniques, like IMRT, volumetric modulated arc radiotherapy (VMAT) and image-guided radiation therapy (IGRT), to reduce heart doses are still in need of confirmation via several years of patient follow-up. While some studies have demonstrated a significant reduction in the mean heart dose when using IMRT compared to three-

dimensional conformal radiotherapy (3D-CRT) (Mast et al. 2013; LIN & WANG 2015), only small differences were found in others (Taunk & Prosnitz 2012; Ravichandran et al. 2013; Ma et al. 2015). Two systematic reviews of studies published between 2003 and 2013 and between 2014 and 2017 demonstrated a higher mean heart dose for IMRT when compared 3D-CRT (Taylor et al. 2015; Drost et al. 2018). Taylor et al. (Taylor et al. 2015) reported an overall mean heart dose of 5.4 Gy among all left-sided regimens, while Drost et al. (Drost et al. 2018) reported mean heart doses of 2.6 Gy to 5.0 Gy. All of these are beyond the recommended constraint for mean heart doses, which is 2.5 Gy to the whole heart (Piroth et al. 2019). Until now, a threshold dose below which the cardiac damage would be abolished has not been identified. Even though modern RT techniques are capable of significantly reducing the heart doses, studies are still required for the evaluation of the cardiac risks as a function of the delivered dose using modern techniques (Zhu et al. 2018).

It is now known that RIHD can be associated with changes in collagen concentration, the main component of the extracellular matrix (ECM). Upon injury of myocardial tissue, transforming growth factor-beta 1 (TGF-β1) augments ECM through (myo)fibroblast proliferation and activation (Liu et al. 2009). TGF-β1 expression can also be enhanced by angiotensin II (AngII) via the angiotensin II type 1 receptor (AT1); both cardiomyocyte hypertrophy and the development of cardiac fibrosis can develop through the activation of this pathway (Gao et al. 2009; Yokono et al. 2020; AlQudah et al. 2020).

The first study of the mechanisms involved in RIHD was to investigate alterations in cardiac functional parameters and the cardiac expression of angiotensin-converting enzyme (ACE), AT1, procollagen type I (proc-I) and TGF-β1 in Wister rats irradiated at the heart directly (Ferreira-Machado et al. 2010). A single dose of radiation (0, 5, 10

and 15 Gy) was delivered and at specific times post-irradiation (two days, fifteen days, and four months), these evaluations were conducted. At the four-month period, 15 Gy-irradiated rats exhibited a slight, but statistically significant, reduction in the ejection fraction, from 91–85%, as well as a significative increase in TGF-β1, proc-I, and AT1 mRNA. This study was important to confirm that the heart renin-angiotensin-system (RAS) plays an important role in RIHD, and it is associated to the radio induced heart fibrosis through the expression of TGF-β1, and all those enhanced expressions were only observed with the 15 Gy dose, four months after irradiation suggesting late fibrosis. Since a possible drawback of this study was the use of male rats neglecting to consider the role of the hormones in the tissue response to irradiation, in the following studies female rats were used.

In the previous study it was identified that a single 15 Gy dose and at least four months were necessary to induce measurable heart damage, simulating RT treatment, which uses 45-60 Gy for the BC treatments. It should be mentioned that that clinical breast radiation is fractionated at much lower doses. Additionally, although a large number of centers still uses 2D tangential breast radiation fields, the present doses to the coronary may be considerably less when modern techniques such as IMRT and VMAT are used in association with CT images for better target delineation. In order to study the possible involvement of fibrosis and apoptosis in this process, the presence of proc-I, TGF-β1, and caspase-3-cleaved were evaluated (Ferreira-Machado et al. 2013). Female Wistar rats were given a single radiation dose of 15 Gy. At thirteen months post-irradiation, histological and physiological evaluations were carried out to understand the late effects of RIHD. In the irradiated group, the levels of proc-I, TGF-β1 and caspase-3-cleaved were amplified. Physiological changes (degeneration of heart tissue, microvascular lesions, and collagen deposition) and functional (reduced ejection fraction) were

observed. These results indicated a decline in cardiac function following exposure to ionizing radiation that is somewhat related to an increase in collagen and in the apoptosis-related caspase-3-cleaved.

It is important to highlight most of the research about the mechanisms of RIHD has been performed mostly with a high-dose rate single high dose of radiation to the heart, or a limited number of fractions. While these radiation protocols seem to cause similar late cardiac remodeling, there is few data available from animal models to determine whether fractionated sections as used in the clinic cause cardiovascular effects similar to single high-doses (Boerma et al. 2016). Boria A.J. and Perez-Torres Carlos J. compared fractionated and single doses mice models of radiation necrosis. They found that all fractionation schemes produced radiation necrosis comparable to what would be achieved with single fraction doses (Boria & Perez-Torres 2019). The single fraction animal model is frequently used as it causes less animal stress due to manipulation and anesthesia (as all the animals are anesthetized during irradiation), and avoids possible confounds due to the potentially limited reproducibility of positioning for focal treatments.

Cardiotoxicity induced by chemotherapy

The association of chemotherapy and RT is an important aspect to be considered in the potential development of cardiac disease in cancer patients and plays a key role as a risk factor. Cardiovascular diseases are gaining more attention as undesirable effects of anticancer drugs (Alexandre et al. 2020). Up to 30% of patients receiving chemotherapy may develop a cardiac side effect during their lifetime (Mihalcea et al. 2017).

Cardiotoxicity can vary from clinically asymptomatic forms to cardiomyopathy with severe heart failure and even death (Pizzino et al. 2015; Mihalcea et al. 2017).

Systemic therapy may be preoperative (neoadjuvant), postoperative (adjuvant), or both (DeSantis et al. 2019). Neoadjuvant systemic treatment has clearly appeared as a standard of care for treatment situations in which primary breast conservation is not possible because of tumor size or the association of the tumor and breast size (Schmidt 2014; Harbeck & Gnant 2017).

Despite the short- and long-term risks, chemotherapy continues to be crucial in the prevention of many patients with stage I-III BC. Various neoadjuvant and adjuvant chemotherapy regimens may be considered for the treatment of early BC. At large, the regimens using docetaxel/cyclophosphamide, adriamycin/cyclophosphamide, and cyclophosphamide/methotrexate/5-fluorouracil are all reasonable choices in lower risk patients where the benefits of chemotherapy outweigh the associated toxicities (Waks & Winer 2019). The current chemotherapy standards for early BC are anthracyclines and taxanes, which can be administered as either a combination or in sequence over a period of 18–24 weeks and usually do not differ between neoadjuvant and adjuvant settings. The Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis indicated that anthracycline-containing and taxane-containing chemotherapy reduced 10-year BC mortality by approximately a third (EBCTCG 2012; Harbeck & Gnant 2017).

Though cardiotoxicity is frequently associated with anthracycline use, recently, this toxicity has also been attributed to BC cytostatic drugs (Curigliano et al. 2016). Mackey et al. reported that heart failure occurred in 26 (3%) patients in a TAC (docetaxel, doxorubicin, and cyclophosphamide) group and 17 (2%) patients in a FAC (5-fluorouracil, doxorubicin, and cyclophosphamide) group, whilst death occurred in 2 and

4 patients in the TAC and FAC groups, respectively. A considerable reduction in the left ventricular ejection fraction (LVEF) was also observed in 58 (17%) TAC-receiving patients and 41 (15%) FAC-receiving patients (Mackey et al. 2013; Schmidt 2014).

Chemotherapy-associated cardiovascular side effects can be classified as either a direct toxic effect on the heart, involving cardiomyopathy and cardiac dysfunction, cardiac ischemia, arrhythmias, and hypertension; and the effect on the coagulation system. These can lead to the development of cardiac dysfunction or heart failure (Mihalcea et al. 2017).

The polymerization of tubulin, leading to microtubule dysfunction and altered cell division can be considered a feature of taxane-induced cardiotoxicity, but in addition to this, the release of larger quantities of histamine resulting in arrhythmias, conduction disturbances or myocardial ischemia have been identified (Albini et al. 2010; Florescu et al. 2013; Mihalcea et al. 2017). Between 2.3-8% of taxane-treated patients are reported to develop left ventricular dysfunction (Schlitt et al. 2014).

Cyclophosphamide is a nitrogen mustard alkylating agent with potent antineoplastic, immunosuppressive, and immunomodulatory properties. Cardiac toxicity associated to this chemotherapeutic agent may often be a lethal complication. A total cyclophosphamide dose greater than 150 mg/kg is predictive for an increased risk of acute heart failure, with an estimated incidence between 7% and 33%. The exact mechanism of cyclophosphamide-induced cardiac toxicity is yet to be established (Dhesi et al. 2013).

After the promising results in the previous studies about the RIHD (Ferreira-Machado et al. 2010; Ferreira-Machado et al. 2013), the RAS-related genes that could be altered by BC treatments were investigated using female Wistar rats and also chemotherapy agents

associated to irradiation (Salata et al. 2013). Three animal groups were used: non-treated control, chemotherapy regimen and irradiation (TC+IR), and irradiation only (IR). The chemotherapy agents given were a combination of docetaxel and cyclophosphamide. Five months after the treatment procedures, the left ventricle (LV) was processed for molecular analysis. Renin was only detected in the treated groups, both TC+IR and IR, in comparison to the control group. ACE and vascular endothelial growth factor (VEGF) levels were decreased, while AT1 mRNA was enhanced in the TC+IR and IR groups as compared to the control group. The presence of mast cells in the LV tissue of all groups was evaluated through the use of a toluidine blue solution. In both treated groups (TC+IR and IR), mast cells were identified, but these were not observed in the control (Figure 1). It was concluded that chemotherapy and irradiation may have a synergistic effect provoking significant changes in some RAS-related genes. These alterations are crucial in deciphering the pathways and consequences beyond cardiotoxicity induced by BC treatments.

FIGURE 1

The last study (Salata et al. 2014), considering the RAS genes, revealed that the association of chemotherapy agents and irradiation could cause greater damage to the heart. In this direction, knowing that the induced damage caused by the RAS alterations could be associated to fibrosis, after 5 months the same Wistar rat groups were submitted to echocardiography, the animals were then euthanized, and the LV was analyzed visually using light microscopy and the gene expression was determined by PCR (Salata et al. 2014). Echocardiography indicated decreases in the ejection fraction

and cardiac output of the TC+IR group. Both the TC+IR and IR groups had reduced intramyocardial vessel-to-cardiomyocyte ratio, increased connective tissue, cardiomyocyte hypertrophy, increased numbers of apoptotic nuclei and increased Bax/Bcl2 expression. An increase of TGF-β1 mRNA expression in both groups was also observed but proc-I expression was augmented only in the TC+IR group. Figure 2 shows the difference among the LV of rats from the three groups, and it was possible to see that chemotherapy and irradiation induced the disorganization of the tissue and vacuolization of the cytoplasm. The study indicated that the induced cardiac remodeling commences with the reduction of intramyocardial vessels in the LV tissue. The main consequence is the loss of cardiomyocytes through apoptosis, leading to the replacement of healthy tissue with fibrous tissue. The damage caused by the combination of therapies induced functional alterations that did not occur when the mice were only submitted to irradiation

Endothelial injury appears to be the major event triggering late damage in the RIHD. Microvascular lesions (endothelial dysfunction) may be responsible for sustaining the chronic nature of the fibrosis induced by irradiation. Due to low blood perfusion, late myocardial degeneration has been observed and associated with the progressive increase of fibrosis and cardiomyocyte apoptosis (Ferreira-Machado et al. 2013).

FIGURE 2

Considering this last observation of cardiac remodeling, changes in the low-Z element distribution in heart tissue after irradiation and chemotherapy using the low-energy X-ray fluorescence (LEXRF) technique were investigated (Mantuano et al. 2016).

quantitative analysis of the tissues under different conditions can be achieved by obtaining elemental maps of low Z elements through this technique. This could help to better understand the pathways participating in the induced heart damage. Low atomic weight elements were of interest in this work, this included: sodium (Na), as it is strongly related to cardiomyocyte contraction; magnesium (Mg) for its importance in the cardiac metabolism; and iron (Fe), as cardiotoxicity induced by BC treatment can be linked to oxidative stress. The LV of the same IR, TC+IR and control female Wistar rat groups were analyzed 5 months after the treatment. Results showed more damage upon chemotherapy and radiotherapy compared to the healthy myocardium of the control group. LEXRF maps displayed a reduction in the intensities of Na and Fe, and an increase in the relative intensity of Mg in the TC+IR and IR groups when compared to the control group. These elemental changes were corroborated by the observation of nuclei and fiber disorganization, as well as some other structures. The association of chemotherapy and irradiation was shown to be more aggressive since there was reduced Na intensity and increased Mg intensity, when compared to the IR and control groups. The LEXRF images taken together with light microscopy, X-ray absorption and phase contrast images, was satisfactory in characterizing the cardiac tissue, for the first time in the literature, from the structural and morphological perspectives (Mantuano et al. 2016).

The same technique, LEXRF, was used to investigate the aorta of these animals, as increased intimal and medial calcifications of the aorta contribute, in part, to the incidence of cardiovascular mortality in BC patients (Mantuano et al. 2018). The aorta is an active participant in the cardiovascular system, and arterial stiffness has been highlighted as a predictor of cardiovascular morbidity and mortality. The work of Mantuano et al. (2018) looked into the aorta distribution of low atomic number

elements such as Mg, Fe, and Na, which are linked to endothelial cell damage. The analyses demonstrated that when the tissue was exposed to the chemotherapy agents such as (docetaxel + cyclophosphamide) and/or irradiation, some normal structures became disorganized, and consequently the intensity of some elemental compounds was altered. The results of the Mg and Fe fluorescent intensities of this study indicate potential aorta calcification. Na and Mg were significantly reduced in the TC+IR group in comparison to the control, as shown in Figure. 3. Na and Mg are essential for regular aortic function such as contraction and inducing its relaxation. Through LEXRF and attenuation coefficient maps acquired simultaneously, it was possible to see that the combined chemotherapy and radiotherapy caused more damage to the aortic tissue compared to the radiation therapy alone. Thus, these findings provide a deeper understanding of elemental distribution in the tissue.

FIGURE 3

Pathophysiology of cardiotoxicity according to the present knowledge

When considering organs at risk during treatment planning, the heart is typically viewed as homogeneous: its substructures are not often considered nor delineated. However, the functional complexity and the histological diversity of the cardiac substructure can account for the extensive range of radiation-induced cardiac adverse events (Taunk et al. 2015; Loap et al. 2020). Dosimetric studies have demonstrated the importance of dose evaluation on the left anterior descending coronary artery independently, as the mean heart dose only weakly correlates with the mean doses to cardiac substructures. Thus, adjustments to the RT strategy, such as the tangential angle, should be considered in order to reduce the high doses at some hot spots (Kirova et al. 2011; DeAlmeida et al.

2012; Vennarini et al. 2013; Zhu et al. 2018). As the principle cardiotoxic effect is on the coronary artery, it is fair to say that the mean dose to the coronary artery is indeed more important than the mean dose to the whole heart. The left anterior descending artery is a long thin structure, thus the dose along its entire length was assessed and the segments that experienced the greatest exposure were identified (Kirova et al. 2011; DeAlmeida et al. 2012).

Literature findings show radiogenic effects on the micro and macrovascular cardiac systems. These effects include inflammation, oxidative effects, cytokine activity, and endothelial damage, and lead to an accelerated atherosclerotic process (Mehta et al. 2018). Radiation-induced cardiovascular damage can be directed towards the coronary arteries, leading to fibrosis of the pericardium and myocardium, microvascular damage, and valve stenosis (Stewart et al. 2013; Tapio 2016; Piroth et al. 2019). To precisely evaluate cardiac radiation exposure, all cardiac substructures require delineation. Multiple manual cardiac segmentation and auto-segmentation algorithms have been proposed to this end, based either on delineation atlases or on deep-learning. For daily routine, however, the performance of these are not yet satisfactory, and thus coronary auto-segmentation continues to be a challenge, even for deep-learning algorithms (Zhuang et al. 2019; Loap et al. 2020).

Possible mechanisms by which cardiotoxicity triggers heart failure have been suggested, depending on the antineoplastic drug, focusing the origin to oxidative stress-induced cardiomyocyte apoptosis (Spallarossa et al. 2016). The use of chemotherapy exacerbates preexisting hypertension, a common comorbidity in patients with cancer, with long-term cardiac effects, including left ventricular hypertrophy, augmented arterial stiffness, and heart failure (Albini et al. 2010; Mihalcea et al. 2017). Currently, the measurement

of the LVEF is the standard parameter used to assess the manifestation of cardiotoxicity (Plana et al. 2014).

Atherosclerosis plays an important role in RIHD. Endothelial cells are radiosensitive, and doses superior to 2 Gy can induce inflammatory effects that may result in arteriosclerosis (Lusis 2000; Piroth et al. 2019). This is in corroboration with previous results associating the RAS to the cardiotoxicity induced by chemotherapy agents and irradiation. In order to prevent the heart side effects due to BC treatment, new studies are in progress with the aim of determining the possible role of angiotensin receptor blockers (ARB) as a cardioprotective agent (Pickler, A. Mantuano, et al. 2019; Pickler, A Mantuano, et al. 2019). The effects of two ARBs (Losartan and Olmesartan) were evaluated, at the elemental level, in the aortic arch and in the coronary artery of healthy and hypertensive rats. To assess elemental and morphological differences in aortic and coronary samples, the LEXRF technique was employed. The results revealed that the amounts of some key elements in the aorta were reduced upon Olmesartan treatment, while the original amounts of most of the elements were maintained after Losartan treatment. Since the amounts of O, Na, Mg, and zinc (Zn) in the aorta and in the coronary artery of the rats treated with Losartan were comparable to those of healthy rats, it was concluded that Losartan is more efficient in protecting, at an elemental level, the aortic arch and coronary artery of hypertensive rats. The next step will be to use Losartan during irradiation and chemotherapy administration on Wistar rats and evaluate the LV, aorta, and coronary artery, to verify if Losartan can prevent, or minimize, cardiotoxicity.

Other side effects of breast cancer treatment

Besides the heart effect, other side effects can be observed in women submitted to BC treatment. It is known that apoptosis of osteoblasts, osteocytes, osteoprogenitor cells, and endothelial cells occurs after RT, which leads to progressive hyalinization and fibrosis of medullary spaces and, subsequently, reduction of osseous vascularization (Blanco & Chao 2008). Radiation-induced rib fracture has been recognized as a normal tissue complication after conventional RT when the radiation field is in the thoracic region, such as for breast or lung cancer (Pettersson et al. 2009; Kim et al. 2020). Rib fractures occur in approximately 1.8% of patients with BC (Pierce et al. 1992; Harris 2016). A study was published comparing the potential RT-induced alterations in the rib microstructure of Wistar rats, using synchrotron radiation computed microtomography (SR-µCT) (Nogueira, de Almeida, et al. 2012). Significant differences between irradiated and non-irradiated groups were identified in ventral and dorsal rib sites. The most apparent change was in the dorsal ribs, where a significant increase in the bone volume fraction (BV/TV) was observed, most likely as consequence of disruption to the balance of bone resorption and bone formation. Since the number of testing samples was low, it is acknowledged that further studies are necessary to confirm these preliminary results. It is important to clarify that due to the rat's anatomy, the dorsal and ventral ribs are remarkably close to each other, differently from humans. It is not possible to make a parallel of rat's ventral or dorsal ribs to human's ventral or dorsal ribs. Although it is possible to compare the ribs effects that were found in the rats after irradiation, to the possible effects on the ribs structure of treated patients, as the bone tissues are very similar.

In a sequential study, the variation of calcium (Ca) distribution in the ribs of female Wistar rats given a single dose of 20 Gy was investigated (Nogueira, Barroso, et al.

2012). Ca distribution was determined using synchrotron radiation microfluorescence (SR-μXRF) and the rats were designated into two groups: control and irradiated (IR). In this study, Ca content was found to be decreased within the dorsal ribs of the RT group compared to the control group, indicating the presence of osteoporosis. As it was previously discussed, it is not possible to affirm that 20 Gy single doses would have the effect if they were delivered in lower-doses fractions. But single doses animal models are widely used at literature (Boerma et al. 2016).

Additional studies reported possible effects of chemotherapeutic drugs in the induction of amenorrhea in premenopausal women, with a consequent decrease in estrogen production. It is known that premenopausal women undergoing adjuvant chemotherapy in the treatment of BC experience significant bone loss from the first year after treatment commencement, particularly at the femur (Shapeero et al. 2009; Kim et al. 2019; Stumpf et al. 2019). Evaluation of the chemical elemental changes of the bone may be highly important in understanding bone alterations such as those due to osteoporosis. Taking this into account, a study was done to evaluate the effect of the multidrug combination of docetaxel and cyclophosphamide in the elemental distribution in femurs compared to a non-treated control group (Andrade et al. 2014; Pickler et al. 2015; Nogueira et al. 2017), using female Wistar rats euthanized 5 months after the end of the TC treatment. The uteri mass was determined. Maps of calcium (Ca), iron (Fe), zinc (Zn), potassium (K) and strontium (Sr) concentrations were obtained by SR-μXRF, and bone morphological parameters were obtained by 3D-μCT images. The TC+IR group displayed a significant reduction in uterine mass compared to the control. Qualitative analysis performed by μXRF showed that rats of the TC+IR group had iron in femur composition, as shown in Figure 4. This same result was not observed in animals from the other groups. A significant decrease in the Ca and Zn concentrations

was observed in the rats treated with docetaxel and cyclophosphamide as compared to the control group, characterizing the bone fragility caused by the chemotherapy treatments. Increase of canal/pore thickness could be noticed in the treated groups, as well as the increase in the number of canal/pores (Figure 5). Together, this alludes to the occurrence of early menopause and osteoporosis, likely due to the absence, or reduced, production of estrogen. The presence of iron in the TC+IR samples indicates the process of osteoporosis, as according to literature, this ion competes with calcium ions.

FIGURE 4

To better understand the pathway that leads to osteoporosis the structural and ultrastructural alterations in the ovarian stroma induced by TC treatment was investigated (Moraes et al. 2016). Wistar rats were divided into a control group and the TC group, which were euthanized 5 months after the end of treatment, and their plasma and ovaries were collected. The serum estradiol level was significantly reduced in the TC group in comparison to the control group, and there was a greater number of apoptotic nuclei in the TC group. Furthermore, the role of the inflammatory response in the development of ovarian damage was investigated, and a greater number of mast cells and increased expression of tumor necrosis factor-alpha (TNF-α) was found in the TC group. In addition, the involvement of fibrosis was assessed, showing that the TC group had increased expression levels of TGF-β1, collagen type I (col-I) and collagen type III (col-III) compared to the control group. Ultrastructural analysis revealed the presence of collagen fibrils in the treated group and illustrated that the ovarian tissue architecture was more disorganized in this group than in the control group. The results

from this study were fundamental in providing further insight into the mechanisms involved in the development of this disease and the chemotherapy-induced ovarian failure.

Conclusion

BC can be described as a disease of good prognosis, if diagnosed early. The options of treatment include surgery, radiotherapy and/or chemotherapy, and in this review, we have discussed the main side effects of BC treatment in the heart, bone and ovary, as described in the literature.

The LCR/UERJ has been investigating the side effects of BC treatment for more than ten years. Table 1 summarizes the main findings observed in studies with rats after exposure to radiation and/or chemotherapy agents (docetaxel and cyclophosphamide), developed by the LCR/UERJ.

TABLE 1

Combined irradiation and chemotherapy for BC can lead to serious heart complications that begin with a reduction of intramyocardial vessel density. The latter observations indicate that therapeutic strategies for BC must consider the RAS component alterations in order to avoid the known cardiac complications. The maps obtained by LEXRF enabled the evaluation of whether an excess or a lack of some crucial elements in the tissues occurred and thus helps to localize critical changes. This has generated new prospects for the control of these elements in order to maintain good functioning of the

heart. This control can aid in avoiding cardiac diseases, infarction and other problems that can be fatal immediately or in the long term. Optimizing treatment to balance anti-cancer efficacy and cardiovascular safety is vital in achieving the best outcomes for breast cancer patients.

With respect to the complications of BC treatment in bone tissue, it can be concluded that treatment with cyclophosphamide and docetaxel leads to ovarian alterations, resulting in decreased estrogen concentration and subsequent change in bone matrix, which promote bone fragility.

DECLARATION OF INTERESTS

The authors report no conflicts of interest.

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TABLE TITLE

Table 1. Main findings observed in rats after exposure to radiation and/or chemotherapy
agents (docetaxel and cyclophosphamide)

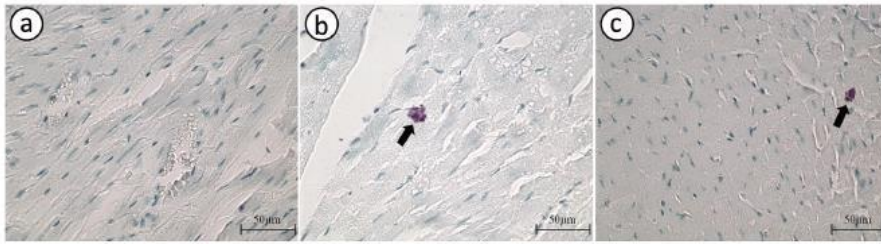| Left Ventricle			
Functional	**Molecular**	**Histological**	**Elemental**
↓ Ejection Fraction [41]	↑ TGF-β1, ↑ Proc-I, ↓ VEGF [41,53]	↓ intramyocardial vessel-to-cardiomyocyte ratio [53]	↓ Na [55]
↓ Cardiac Output [53]	↑ AT1, ↑ Renin, ↓ ACE [41,53]	cardiomyocyte hypertrophy [53]	↓ Fe [55]
	↑ caspase 3 [42]	↑ apoptotic nuclei [54]	↑ Mg [55]
		↑ Bax/Bcl2 expression [54]	
Bone Matrix		**Ovary**	
SR-μCT	**SR-μXRF**	**Histological**	**Hormonal**
↑ bone volume fraction [74]	↓ Ca [74]	↑ mast cells, ↑ TNF-α [83]	↓ serum estradiol level [83]
↑ Porosity [80]		↑ TGF-β1, ↑ col-I, ↑ col-III [83]	

Figure 1. Toluidine blue-stained photomicrographs showing the mast cells in the left ventricle of Wister rats. (a) Control (received no treatment); (b) TC+IR (received chemotherapy and were irradiated); (c) IR (were only irradiated). Figure from (Salata et al. 2013).

Figure 2. Light microscopy image of the left ventricle of Wister rats stained with picrosirius red: (a) healthy cardiac tissue, where nuclei, fibers and structures are intact; (b) chemotherapy + irradiation group, disorganization of the tissue and vacuolization; (c) irradiation only group, disorganization of the fibers and nuclei. Figure from (Mantuano et al. 2016).
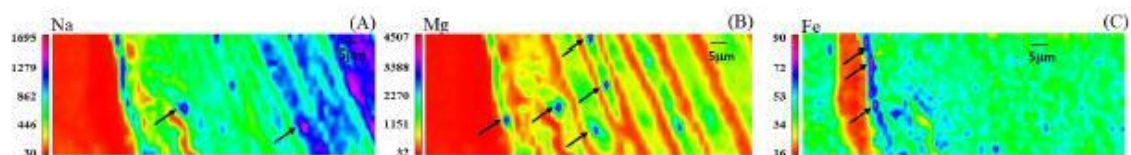
Figure 3. LEXRF intensity maps of aorta samples of Wistar rats submitted to chemotherapy and irradiation. (a) Na; (b) Mg; and (c) Fe. The arrows indicate the endothelial cell nuclei. Figure modified from (Pickler, A. Mantuano, et al. 2019).
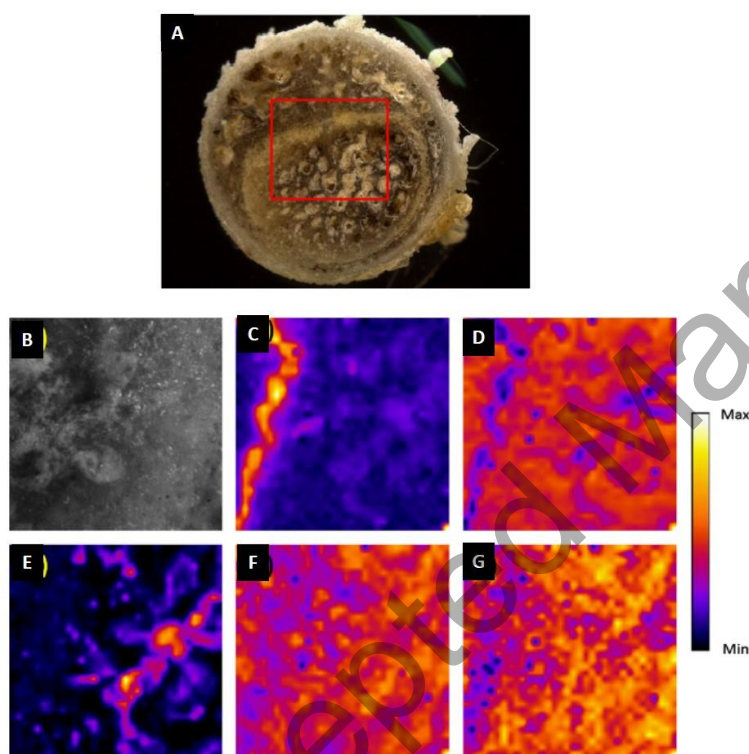

Figure 4. (a) Optical micrograph of the femur head. The red square represents the selected region for SR-μXRF. (b) Photograph of the analyzed region; (c-g) μXRF distribution for (c) K, (d) Ca, (e) Fe, (f) Zn, and (g) Sr elements, of a Wister rat submitted to chemotherapy with docetaxel and cyclophosphamide. Figure modified from (Andrade et al. 2014).
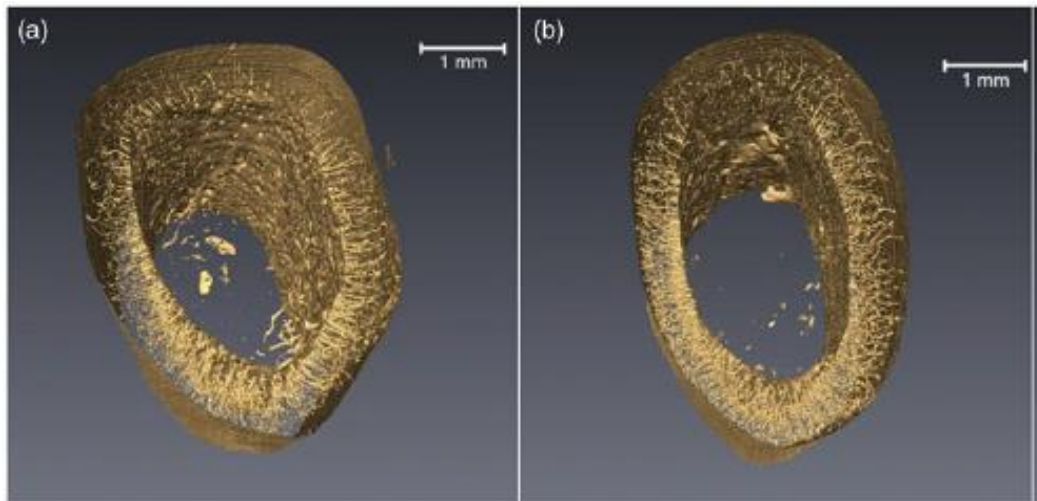
Figure 5. 3D-μCT of femoral diaphysis samples of (a) the non-treated control group and (b) the group treated with docetaxel and cyclophosphamide TC. Increase of canal/pore thickness can be noticed in the treated group, as well as an increase in the number of canal/pores. Figure modified from (Nogueira et al. 2017).