Studying the association between some genetic polymorphisms and Doxorubicin-Induced Cardiotoxicity

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Abstract
Anthracyclines are a crucial component of chemotherapy regimens used to treat a range of malignancies in children and adults. However, cardiac dysfunction and heart failure frequently limit the therapeutic efficacy of anthracyclines. The risk of heart dysfunction increases with increasing doses of anthracycline. Cardiotoxicity is a challenging side effect of DOX, which is cumulative and irreversible; this necessitates assessing the cardiac functions in cancer patients before and after the use of DOX to avoid permanent cardiac damage. Serial measurements of left ventricle ejection fraction (LVEF) are commonly used for cardiac monitoring during anthracycline treatment. In some studies, cardiotoxicity was defined as LVEF decrease by an absolute 10% and/or below 55%; in others, cardiotoxicity was defined as a decrease below 45%. A serious disadvantage of this test is radioactivity exposure and the low predictability of pre-symptomatic cardiac damage. Blood cardiac biomarkers, such as cardiac troponins and B-type natriuretic peptide (BNP), have been used in heart failure diagnostics. Susceptibility to DOX cardiotoxicity is largely individual, with some patients developing cardiomyopathy at low doses and others tolerating much higher cumulative doses, and this may suggest the presence of genetic predisposition factors. Genetic variations in CBR3 and ABCC1 genes were suggested to contribute to DOX adverse effects. This review highlights the genetic basis for anthracycline-related cardiac dysfunction, focuses on particular genes that have been implicated in innate predisposition to ACT, and assessment of cardiotoxicity.

Keywords: Breast cancer, Polymorphisms, Doxorubicin, Cardiotoxicity.

Introduction:
Anthracyclines are a class of chemotherapeutics that have been indispensable to improvements in cancer survival, particularly for common childhood and young adult cancers such as leukemia, lymphoma, sarcoma, as well as breast cancer⁴. Their anticancer effect is mediated via DNA intercalation, generation of hydroxyl free radicals, and inhibition of topoisomerase II, a key enzyme in DNA replication and transcription⁵. In reality, the World Health Organization classifies doxorubicin as a necessary cytotoxic drug⁶. The therapeutic efficacy of anthracyclines is diminished by cardiac dysfunction, which is frequently detected by imaging investigations and may proceed to heart failure. The mechanisms underlying anthracycline-induced cardiotoxicity (ACT) are not fully understood, but studies indicate that processes related to those underlying their antitumor activity, specifically the production of reactive oxygen species (ROS) and direct DNA binding that results in the inhibition of topoisomerase-II-mediated DNA repair and ultimately cell death, contribute to cardiotoxicity [⁴]. Increasing anthracycline dosage raises the risk of cardiac dysfunction (Figure 1)⁵, ⁶-⁹. Other risk variables include extreme ages at anthracycline exposure (4 years; >65 years), female sex, chest radiation, the existence of cardiovascular risk factors (diabetes, hypertension), and concomitant treatment of cyclophosphamide, paclitaxel, and trastuzumab⁷,⁹. Despite the proven dose-response relationship, there is interpatient variation in the likelihood of cardiac dysfunction for any given anthracycline dosage; clinical factors alone have limited predictive potential for diagnosing cardiac dysfunction¹⁰,¹¹.
However, there are currently no reliable tools available to evaluate a patient's individual risk of ACT prior to starting therapy. This information could be used to tailor anthracycline regimen, cardioprotective medications, and follow-up to reduce risk of cardiac morbidity and mortality. Pharmacologic and surveillance strategies to slow the progression of cardiotoxicity as well as predictive tools that account for treatment exposure are also available. To improve initial risk assessment and ultimately create tailored pharmacogenetic therapy for patients at high risk, it is essential to identify genetic ACT susceptibilities. Clinical practice recommendations call for genetic testing of certain variations prior to the two most widely prescribed anthracyclines, doxorubicin or daunorubicin.

Recently, single nucleotide polymorphisms (SNPs) linked with cardiac dysfunction have received more attention\(^\text{10, 12–20}\). Due to the poor prognosis and interindividual diversity in risk, there is a growing interest in creating prediction techniques to identify individuals at the highest risk of having this condition\(^\text{16, 21–23}\). Such a risk prediction instrument might guide individualized decisions for anthracycline-based therapy and post-treatment monitoring.

A total of 170 survivors with cardiomyopathy (cases) were compared with 317 survivors with no cardiomyopathy (control subjects; matched on cancer diagnosis, year of diagnosis, length of follow-up, and race/ethnicity) using conditional logistic regression techniques. A dose-dependent association was observed between cumulative anthracycline exposure and cardiomyopathy risk\(^\text{20}\).

### 2. Pathogenesis of Anthracycline-Related Cardiomyopathy

Single cell myocytolysis is the initial stage of myocardial damage. Patchy myocardial necrosis, interstitial fibrosis, and multifocal myocardial fibrosis are the next stages\(^\text{24}\). If left untreated, this causes cardiac structure to be disrupted and finally manifests clinically as overt heart failure. There is ongoing research on the aetiology of anthracycline-related cardiomyopathy. The quinone and hydroquinone moieties on the neighbouring rings of the tetracyclic ring structure of anthracyclines allow for electron gains and losses. Anthracyclines bind to DNA by intercalating between certain bases and preventing the production of DNA and ribonucleic acid (RNA), leading to DNA strand scission and impeding cell replication. An iron-dependent, enzyme-mediated reductive mechanism is used by anthracyclines to produce semiquinone and free radicals while inhibiting topoisomerase II. Anthracyclines bind to cellular membranes to change ion transport and fluidity\(^\text{25}\). Anthracyclines containing quinone groups create free radicals that combine with oxygen to form superoxide anion radicals, which can induce cardiotoxicity\(^\text{26}\). Anthracyclines also cause cardiotoxicity by reduced adenosine triphosphate synthesis, direct mitochondrial damage, mitochondria-dependent cardiomyocyte apoptosis, and lipid peroxidation of the cardiac myocyte membrane\(^\text{26,27}\). Also contributing to the anthracycline-induced cardiotoxicity is DNA damage caused by TOP2B\(^\text{28}\). Targeting topoisomerase-II (Top2b), doxorubicin creates a compound with iron (III)\(^\text{29}\). Reactive oxygen species production and faulty mitochondrial biogenesis can both be reduced by cardiomyocyte-specific ablation of Top2b\(^\text{29}\). Due to the high
density of mitochondria in cardiomyocytes, which make about 35% of the total cell volume, the heart is particularly sensitive to anthracyclines. Other cellular alterations in anthracycline-exposed cardiomyocytes include a decrease in cardiac stem cells, a decrease in DNA synthesis, a decrease in cell death signaling, changes in gene expression, an inhibition of calcium release from the sarcoplasmic reticulum, a decrease in the formation of the protein titin in sarcomeres, and a decrease in mitochondrial creatine kinase activity and function.

3. Cardiotoxicity assessment

Anthracycline-induced cardiotoxicity is evaluated in a variety of methods, with the majority of studies classifying it as a considerable decline in either the left ventricular ejection fraction (LVEF) or the left ventricular shortening fraction (SF), or as the presence of clinical signs of cardiac illness. Acute, early-onset chronic, and late-onset chronic cardiotoxicity are the three categories for temporal onset. The bulk of research on genetic vulnerability concentrates on early-onset chronic toxicity, the most prevalent form of ACT, which first manifests as an asymptomatic reduction in LVEF/SF and eventually develops into symptomatic HF. Higher cumulative doses (standardised to doxorubicin equivalents), shorter infusion times, pre-existing cardiovascular disease, hypertension, diabetes, use of additional cardiotoxic medications, mediastinal radiotherapy, female sex, and age 4 years or > 65 years have all been linked to an increased risk of developing ACT. However, there is a lot of variation in ACT incidence even across groups at low and high risk, indicating that inherent susceptibility probably plays a role. During anthracycline therapy, the left ventricular ejection fraction (LVEF) is often employed for cardiac monitoring. In some studies, cardiotoxicity was defined as a reduction in LVEF of at least 10 percent requiring medical intervention and/or below 55 percent, but in others, it was defined as a reduction below 45 percent. Radioactivity exposure and the limited prediction of pre-symptomatic heart injury are major disadvantages of this test. Diagnostics for heart failure have utilized blood cardiac biomarkers such as cardiac troponins and NTproBNP (N-terminal pro-B-type natriuretic peptide).

4. Genetic polymorphism and anthracycline Cardiomyopathy

Given the notion that genetic risk factors contribute to ACT, a growing body of research has investigated this link. However, the heterogeneity in ACT definition, ACT measurement, and ACT assessment scheduling makes it difficult to draw broad findings. Candidate gene analysis has been the most popular form of study examining genetic risk for ACT development to date. On the basis of previously known biological connections, candidate genes are investigated, with an emphasis on genes involved in anthracycline binding and metabolism, as well as genes implicated in the cardiotoxicity pathway, notably in the production of reactive oxygen species (ROS). Less commonly, unbiased genome-wide association studies (GWAS) have also been conducted. Despite the fact that the effect size of variations linked with cardiotoxicity is often greater than that of variants associated with cancer development, the influence on echocardiography results might be very tiny and difficult to interpret clinically. Figure 2 depicts a continuum of rigour spanning from biased candidate gene techniques through unbiased genome studies to independent replication in model systems, along which these investigations fall.
4.1 Genes Involved in Regulation and Metabolism: CBR3. Carbonyl Reductases (CBRS)

Catalyze anthracycline reduction to cardiotoxic alcohol metabolites. CBR3 polymorphisms affect the production of these metabolites. A missense mutation in CBR3 (G>A, rs1056892) leading to Val244Met was related with decreased LVEF and an increased incidence of cardiomyopathy in breast cancer survivors. An independent investigation of breast cancer patients treated with anthracyclines with or without trastuzumab confirmed the correlation between CBR3 and left ventricular ejection fraction reduction. The effect of CBR3 SNPs (CBR3 V244M) on the dose-dependent risk of anthracycline-associated cardiomyopathy was examined by Blanco et al. 317 survivors without cardiomyopathy and 170 survivors with cardiomyopathy were compared. When compared to those with CBR3:GA/AA genotypes who were not exposed to anthracyclines, people with CBR3 V244M homozygous G genotypes (CBR3:GG) had an elevated risk of cardiomyopathy (OR: 5.48; p = 0.003), as well as those exposed to low- to moderate-dose anthracyclines (OR: 3.30; p = 0.006). Regardless of the CBR3 genotype, high doses of anthracyclines (>250 mg/m2) were linked to an elevated risk of cardiomyopathy. This study shows that anthracyclines enhance the incidence of cardiomyopathy at dosages as low as 101 to 150 mg/m2. This study shows an enhanced risk of anthracycline-related cardiomyopathy at doses as low as 101 to 150 mg/m2, as seen in Figure 3. With approval of Blanco and associates, it is conceivable that variations in the CBR family might increase the risk of ACT given the function that these genes play; however, this has not yet been thoroughly explored. The strength of the association between the Val244Met missense mutation in CBR3 and other cancer types to be assessed by examining larger cohorts. Despite having modest baseline levels in the liver, CBR3 is robustly inducible by the transcription factor Nrf2. Compared to wild-type mice, the livers of Gclm/ mice (a mouse model of glutathione deficiency) contain significant levels of the CBR3 messenger RNA and CBR3 protein. Schaupp et al. looked at CBR3's capacity to break down doxorubicin. Using high-performance liquid chromatography, the incubation of doxorubicin and purified recombinant murine CBR3 was examined for the production of doxorubicinol, demonstrating that doxorubicin is a substrate of recombinant murine CBR3. Furthermore, doxorubicinol production was higher in Gclm+/+ hepatocytes compared to Gclm/ hepatocytes. Furthermore, doxorubicin-induced cytostasis and/or cytotoxicity were more readily produced in cocultures of differentiated rat myoblasts (C2C12 cells) and primary Gclm/ murine hepatocytes than incubations with Gclm+/+ hepatocytes. These findings suggest that CBR3 may play a significant role in the cardiotoxicity brought on by doxorubicin.

4.2 Genes Involved in Transport ABC-Encoded ATP Binding Cassette

The ABC family consists of 49 genes that code for a family of transmembrane proteins found in numerous cell types, including the myocardium, that utilize adenosine triphosphate (ATP) as active transporters. ABC transporters help to export numerous chemotherapeutics, including anthracyclines, from cardiac cells. Doxorubicin is transported through the transporters that ABCC1 and ABCB1 encode for, and they contribute to tumor multidrug resistance. Although doxorubicin has also been demonstrated to be transported via
the ABCB4 and ABCC2 genes, these genes typically encode transporters for phospholipid and bile acid transport\textsuperscript{47,48}. Although doxorubicin transfer has also been proven, the ABCB5 protein mostly transports cyclic nucleotides. [49] Anthracyclines will build up in cardiomyocytes as a result of variations in these ABC genes that reduce or interfere with expression, which increases the risk of ACT. Particularly, it has been discovered that a synonymous mutation in ABCC1 (T>C, rs246221) is linked to decreased SF in children with acute lymphoblastic leukaemia (ALL) and decreased LVEF in breast cancer survivors\textsuperscript{50,51}. Other ABCC1 mutations linked to ACT include a C>T variant in the 3' UTR (rs3743527) linked to lower SF in childhood ALL survivors, a G>T missense mutation resulting in Gly671Val (rs45511401) linked to a higher risk of acute ACT in non-Hodgkin lymphoma (NHL) patients, and a G>T intron variant (rs4148350) linked to higher odds of clinically (CCSs)\textsuperscript{52,53}. Other ABC family polymorphisms have been linked to an increased risk of ACT in the CCS population, including an A>C intron variant (rs2235047) in ABCB1 and a non-coding transcript variant (A>G, rs4148808) in ABCB4 that were both linked to decreased SF; an A>C missense variant (rs3740066) leading to Ile1324Met in ABCC2 that was linked to decreased SF; an A>T\textsuperscript{52,56}. A C>T synonymous variation (rs1045642) in ABCB1 decreased the likelihood of low LVEF in breast cancer survivors\textsuperscript{58}. While candidate gene analyses rather than GWAS have largely been used to evaluate variants in the ABC gene family, researchers have consistently discovered a relationship with elevated risks of ACT. In reality, the bulk of research has involved sizable populations of survivors with sufficient follow-up time, and the ABC gene family contains some of the most compelling data to date supporting its participation in ACT risk. 40 percent of a cohort of 15 CCSs with early onset ACT shared the ABCC2 polymorphism (rs8187710), resulting in Cys1515Tyr, which supports the idea that SNPs in the ABC gene family increase the risk of ACT\textsuperscript{59}. These very small cohort-based studies used a wide variety of anthracycline dosage parameters, which might prevent independent validation. Future research should focus on bigger studies with carefully defined patient groups as well as functional investigations to give supporting evidence for the validity of these variations.

5. CLINICAL ACTIONABILITY AND THE PROMISE OF GENOMIC MEDICINE

ACT secondary prevention and early diagnosis are now targeted by a number of clinical practice techniques, including pharmacological treatments and screening to identify subclinical ACT. Modifying anthracycline delivery and co-administering cardioprotective medicines are the mainstays of primary prevention. Although it hasn’t been well-researched in children, liposomal-encapsulated doxorubicin has the potential to be less cardiotoxic than conventional doxorubicin in adults\textsuperscript{60}. The cardioprotectant dexrazoxane is utilized in high-risk patients and treatment regimens since it has been shown to reduce ACT in both adults and children [61]. There are risk prediction models for the CCS population to evaluate the risk of treatment-related cardiotoxicity. These, however, solely consider cardiotoxic exposures and are not frequently carried out before therapy. As a result, there is no recognized method for using patient genotype to assess risk prior to anthracycline exposure, and there is no agreed-upon advice on how surveillance for cardiotoxic sequelae should be altered in light of genetic risk. Furthermore, there are no widely accepted recommendations on how to put the results of such risk-assessment tests into practice through adjustments to dosage, frequency, or the addition of cardioprotective measures. Pre-treatment ACT risk assessment in the adult cancer survivor population now emphasizes age, sex, medical history, cardiac history, cardiac biomarkers, and health habits\textsuperscript{62}. Recent research in the juvenile cancer population has demonstrated that adding genetic information to an ACT risk prediction model improved prediction accuracy compared to a model that just took into account clinical parameters\textsuperscript{63}. Additionally, a clinical experiment that aimed to integrate genetic screening, innovative biomarkers, and imaging techniques to create a risk-prediction model to identify pediatric patients who are most vulnerable to ACT before starting therapy has just been completed\textsuperscript{64}. Increased use of predictive genomic medicine in this field is encouraging, especially as clinical practice guideline groups are starting to advise genetic screening in children for certain variations prior to doxorubicin or daunorubicin treatment.

6. Future Perspective

Several attempts have been made to construct risk prediction algorithms to identify people at the highest risk for anthracycline-associated cardiomyopathy. Clinical risk prediction models have yielded modest predictive power for identifying patients at risk for anthracycline-related cardiomyopathy\textsuperscript{65,66}, resulting in studies that combined genetic variants with clinical and demographic variables to improve the ability to classify anthracycline-exposed survivors according to their risk of developing cardiomyopathy.
7. CONCLUSIONS

An increasing amount of evidence indicates a correlation between inherent genetic variation and ACT risk. The bulk of research has centered on the targeted genotyping of genes-producing products known to be involved in anthracycline metabolism or believed to be involved in the cardiotoxicity pathway. It is possible to improve the capacity to predict the risk for doxorubicin-associated cardiomyopathy by integrating genetics with clinical features. While this strategy has produced initial relevant results, more functional investigations are required to corroborate findings and describe the clinical impact of variations.

References
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