MONASH University

Anthracycline-Induced Cardiotoxicity: Understanding the Incidence, Risk Factors and Development of a Prediction Model

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Doctor of Philosophy

A thesis submitted for the degree of Doctor of Philosophy at

Monash University in 2018

School of Pharmacy

Acknowledgement

First and foremost, I would like to express my gratitude to my supervisor Dr. Shaun Lee Wen Huey for taking me as his apprentice. His useful comments, remarks and engagement through the learning process of this doctorate thesis. Without his guidance and persistent help this thesis would not have been possible. My thanks also go to Prof. Nathorn Chaiyakunapruk, my co-supervisor. He was at great help in times of need.

My thanks and appreciations also go to member of Hospital Ampang, University Kebangsaan Malaysia Medical Centre and University Malaya Medical Centre for their kind co-operation and valuable information which help me in completion of the project.

A very special gratitude goes out to all down at Monash University Malaysia Merit Scholarship for supporting me financially throughout the three and a half year journey.

I am grateful to my siblings and husband, who have provided me through moral and emotional support in my life and especially during this life-changing journey. I am also grateful to my parents and friends who have supported me along the way.

Thanks for all your encouragement.

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Thesis by Publication – General Declaration

This thesis includes one original papers published in peer reviewed journals and two unpublished publications. The core theme of the thesis is anthracycline-induced cardiotoxicity. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the School of Pharmacy under the supervision of Dr. Shaun Lee Wen Huey and Professor Nathorn Chaiyakunapruk.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of chapter 2.1.2, 4.1.2, 4.2.3, and 4.2.4 my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status (published, in press, accepted or returned for revision, submitted)	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution*	Co-author(s), Monash student Y/N*
2.1.2	Burden of Antineoplastic-related Cardiovascular Toxicity in Asia: A systematic review and meta-analysis.	Submitted	80%. Concept and collecting data and writing first draft	Shaun Lee Wen Huey, input into manuscript 15% Nathorn Chaiyakunapruk, input into manuscript 5%	N N
4.1.2	Roles of pharmacogenomics in antineoplastic-induced cardiovascular toxicities: A Systematic Review and Meta-analysis.	Submitted	70%. Concept and collecting data and writing first draft	Shaun Lee Wen Huey, input into manuscript 10% Nathorn Chaiyakunapruk, input into manuscript 5% Wichittra Tassaneeyakul, input into manuscript 5% Poukwan Arunmanakul, input into manuscript 5% Surakit Nathisuwan, input into manuscript 5%	N N N N N

Thesis Chapter	Publication Title	Status (published, in press, accepted or returned for revision, submitted)	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution*	Co-author(s), Monash student Y/N*
4.2.3	Candidate Gene Association Studies of Anthracycline-induced Cardiotoxicity: A Systematic Review and Meta-analysis	Published	80%. Concept and collecting data and writing first draft	Shaun Lee Wen Huey, input into manuscript 15% Nathorn Chaiyakunapruk, input into manuscript 5%	N N
4.2.4	Potential of Oncocardiology	Published	80%. Concept and collecting data and writing first draft	Shaun Lee Wen Huey, input into manuscript 15% Nathorn Chaiyakunapruk, input into manuscript 5%	N N

1 General Introduction

Anthracycline antibiotics are among the most potent chemotherapeutic agents since their introduction 50 years ago. Agents in this pharmacological group of antineoplastic drugs include doxorubicin, daunorubicin, epirubicin, idarubicin and mitoxantrone. They are the back bone for many chemotherapy regimens in the treatment of breast cancer, lymphoma, and leukaemia. This may be due to the wide range of mechanisms which anthracycline is thought to act on. These include: (i) initiation of apoptosis via inhibition of topoisomerase II, (ii) DNA synthesis inhibition, (iii) DNA binding and alkylation, (iv) DNA cross-linking, (v) interference with DNA strand separation and helicase activity, and (vi) free radical formation and lipid peroxidation[1]. Among these, topoisomerase II inhibition is widely accepted as the core mechanisms for the anti-tumour activity of anthracyclines[2], while its free radical formation and lipid peroxidation activity have been widely discussed as the mechanism underlying its predominant cardiac toxicity[3].

Anthracycline-induced cardiac toxicity (ACT) was first described since 1970s[4, 5]. ACT are classified into acute, chronic or early-onset and late-onset cardiac toxicity. Arrhythmias, abnormal ST and T wave and acute heart failure which present immediately to weeks after treatment are examples of acute cardiotoxicity. Although the damage may be reversed after withdrawal of therapy, most patients may suffer permanent cardiac damage. Chronic or early-onset cardiotoxicity occurs within the first year after completion of treatment. It accounts for 89.5% of all ACT cases[6]. Left ventricular dysfunction, chronic heart failure and QT dispersion are common presentation. Late-onset

cardiotoxicity which is always presented as heart failure that develops after the first year of therapy was found to increase with the length of follow up[7].

Anthracyclines increases clinical, subclinical and both cardiotoxicity risk by 5.43, 6.25 and 2.27 fold respectively with risk of cardiac death by 4.94 fold compared to non-anthracycline regimens[8]. Due to its significant consequence to cancer survivors, huge attempts had been carried out to identify the risk factors with the aim to identify patients at risk for ACT[4, 6, 8, 9]. Several significant risk factors for developing cardiac toxicity had been identified, including extreme age that is more than 65 years old or less than 4 years old, female gender, hypertension, pre-existing cardiac disease, mediastinal radiation, treatment with cyclophosphamide, paclitaxel or trastuzumab and higher individual anthracycline dose. Cumulative anthracycline dose is one of the strongest predictors for developing cardiac toxicity, with a clear dose-response relationship. However, these demographic and clinical factors incompletely explain inter-individual variability of sensitivity to anthracycline suggestive of the potential role of genetic[10, 11].

1.1 Research Questions and Hypotheses

1.1.1 Research Questions

As discussed above, there were several key questions which we would like to address in the following theses. These can be categorised as below:

1. Findings on the incidence of ACT were mainly North America- or Europe-based population.
    a. What is the incidence of antineoplastic-related cardiotoxicity and ACT in a multi-ethnic -based population?
2. The effort to identify potential genetic biomarkers for antineoplastic-related cardiovascular toxicity had increased over the years especially after the introduction of targeted therapy in cancer treatment.
    a. Is there any genetic biomarker that is potentially used as predictor for antineoplastic-related cardiovascular have been identified?
    b. Is there any genetic biomarker that is potentially used as predictor for anthracycline has been identified?
3. A few risk factors for ACT have been identified.
    a. How these factors stratified ACT risk in patients receiving anthracycline-based chemotherapy?

1.1.2 Hypotheses

We hypothesize that

1. There was a difference in the incidence of antineoplastic-related cardiotoxicity and ACT in an Asia-based population compared to North American or European population
2. There are genetic biomarkers that can be potentially used as predictor for antineoplastic-related cardiovascular
3. A risk prediction model to stratify ACT risk in patients receiving anthracycline-based chemotherapy can be developed and be useful in clinical setting.

1.2 Research aims and objectives

1.2.1 Aims

The aim of this study is to identify the burden of ACT in a multi-ethnic population, to develop a risk prediction model to identify individual at risk and to determine the usefulness of the developed model in clinical setting.

1.2.2 Objectives

1. To identify the incidence of antineoplastic-related cardiotoxicity and ACT in a multi-ethnic-based population.
2. To develop and validate a multivariable risk prediction model for ACT.
3. To determine the usefulness of the developed model in clinical setting.
4. To explore the acceptance of the end user of in term of the content and usability of the developed prediction model.
5. To identify genetic biomarker that is potentially used as predictor for antineoplastic-related cardiovascular with focus in anthracycline-induced cardiotoxicity.

1.2.3 Conceptual Framework

Studies were planned and executed to achieve above objectives (Table 1-1).

Table 1-1: Conceptual framework of the research.

Sections	Titles	Objectives
2	Incidence of cancer chemotherapy-related cardiovascular complications in Asia	To identify the incidence of antineoplastic-related cardiotoxicity and ACT in a multi-ethnic-based population.
3.1	To develop and validate a multivariable risk prediction model for ACT.	Development and Validation of ACT Prediction Model
3.2	To determine the usefulness of the developed model in clinical setting.	Utility Evaluation of Prediction Model in Clinical Settings: A Pilot Study
3.3	To explore the acceptance of the end user of in term of the content and usability of the developed prediction model.	A qualitative exploration on the content and usability of a 4-factors anthracycline-induced cardiotoxicity (ACT) prediction model
4	To identify genetic biomarker that is potentially used as predictor for antineoplastic-related cardiovascular with focus in anthracycline-induced cardiotoxicity.	Pharmacogenomics in antineoplastic-related cardiovascular toxicity

1.3 Literature Review

1.3.1 History and types of anthracycline

The first anthracycline isolated is daunorubicin. It was isolated from *Streptomyces peuceticus* in the 1963 by an Italian research company, Farmitalia Research Laboratories[12]. Daunorubicin showed a promising therapeutic effect in acute childhood leukaemia since its introduction[12]. Soon after that doxorubicin was isolated from *Streptomyces peuceticus* var. *caesius*, a mutant strain derived from *Streptomyces peuceticus*[13]. Doxorubicin differs from daunorubicin just by a single hydroxyl group, however doxorubicin has broader therapeutic activity which includes both solid tumour and haematological malignancies[2, 14, 15].

Epirubicin and idarubicin are the two newer members of the family after many attempts to identify better anthracyclines. Similarly, chemical structures of both newer anthracyclines are only slightly changed from doxorubicin and daunorubicin, but their spectrum of activity and/or side effects are significantly different[2]. Epirubicin, a semisynthetic derivative of doxorubicin, is preferred in breast cancer treatment compared to doxorubicin because its maximum tolerated dose is almost double to that of doxorubicin[16]. Better safety profile of epirubicin is attributed to the positional change of the hydroxyl group which increased its volume of distribution and clearance[2]. Contrary, idarubicin, a derivative of daunorubicin after removing of the 4-methoxy group, possess an extended therapeutic activity in multiple myeloma, non-Hodgkin's lymphoma and breast cancer[2].

Mitoxantrone, a synthetic anthracenedione, was synthesised with the aim to reduce anthracycline side effects by the American Cyanamid Company and the Midwest Research Institute independently[17]. It is structurally similar to doxorubicin and daunorubicin.

1.3.2 Mechanism of Actions and Usages

The most accepted mechanism for its therapeutic effects is topoisomerase II inhibition[2]. The inhibition of topoisomerase II enzyme causes cell death by preventing the cut of both strands of the DNA double helix, an essential step in DNA replication. Others suggested mechanisms include DNA synthesis inhibition, DNA binding and alkylation, DNA cross-linking, interference with DNA strand separation and helicase activity, and free radical formation and lipid peroxidation[1]. The mode of doxorubicin anti-tumour activity is illustrated in Figure 1-1. Anthracycline usage in solid tumours (

Table 1-2) and haematological malignancies (Table 1-3) may be related to the wide range of anti-tumour mechanism of anthracyclines.

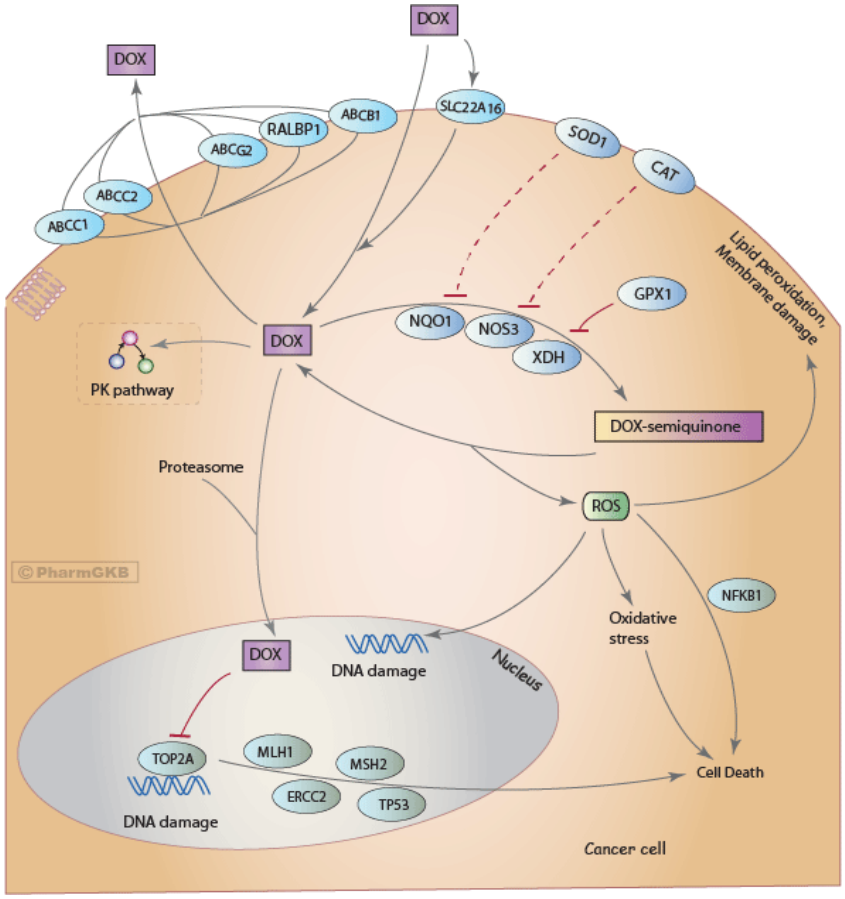

Figure 1-1: Mode of doxorubicin anti-tumour activity and related genes.
ABCB1, ATP binding cassette subfamily B member 1; ABCC1, ATP binding cassette subfamily C member 1; ABCC2, ATP binding cassette subfamily C member 2; ABCG2, ATP binding cassette subfamily G member 2; CAT, catalase; DOX, doxorubicin; ERCC2, ERCC excision repair 2; GPX1, glutathione peroxidase 1; MLH1, MutL homolog 1; MSH2, MutS homolog 2; NKFB1, nuclear factor kappa B subunit 1; NOS3, nitric oxide synthase 3; NQO1, NAD(P)H quinone dehydrogenase 1; RALBP1, RalA binding protein 1; ROS, reactive oxygen species; SLC22A16, solute carrier family 22 member 16; SOD1, superoxide dismutase 1; TOP2A, topoisomerase II alpha; TP53, tumour protein P53; XDH, xanthine dehydrogenase. Reproduce with permission from PharmGKB and Stanford University[18].

Table 1-2: Anthracycline used in solid tumours based on guidelines used in Malaysia and National Comprehensive Cancer Network (NCCN) guidelines.

Cancer	**Regimens**	**Guidelines**
Breast cancer	5-fluorouracil, doxorucibin, cyclophosphamide (FAC)	Malaysia
	5-fluorouracil, epirubicin, cyclophosphamide (FEC)	Malaysia, NCCN
	Doxorubicin, cyclophosphamide (AC)	Malaysia, NCCN
	Docetaxel, doxorubicin, cyclophosphamide (TAC)	Malaysia, NCCN
	Epirubicin, cyclophosphamide (EC)	NCCN
Small cell lung cancer	Cyclophosphamide, doxorubicin, vincristine (CAV)	Malaysia, NCCN
	Cyclophosphamide, epirubicin, vincristine (CEV)	Malaysia
Oesophageal cancer	Epirubicin, cisplatinum, 5-fluorouracil (ECF)	Malaysia, NCCN
	Epirubicin, oxaliplatin, fluorouracil	NCCN
	Epirubicin, cisplatin/oxaliplatin, capecitabine	NCCN
Gastric cancer	ECF	Malaysia,NCCN
	Epirubicin, oxaliplatin, capecitabine (EOX)	Malaysia
	Epirubicin, oxaliplatin, fluorouracil	NCCN
	Epirubicin, cisplatin/oxaliplatin, capecitabine	NCCN
Liver cancer	Doxorubicin monotherapy	Malaysia
Epithelial uterine cancer	Doxorubicin, cisplatinum	Malaysia, NCCN
Uterine leiomyosarcoma	Doxorubicin	Malaysia, NCCN
Sarcomatoid renal cell carcinoma	Doxorubicin, gemcitabine	Malaysia
Soft tissue sarcoma	Doxorubicin	Malaysia, NCCN
	Epirubicin, ifosfamide (EI)	Malaysia
	Doxorubicin, dacarbazine, ifosfamide (MAID)	Malaysia, NCCN
	Doxorubicin, ifosfamide (AI)	Malaysia, NCCN
	Doxorubicin, dacarbazine (AD)	NCCN
	Doxorubicin, ifosfamide, mesna (AIM)	NCCN
	Doxorubicin, olaratumab	NCCN
	Epirubicin	NCCN
	Vincristine, doxorubicin, cyclophosphamide (VAC)	NCCN
	Vincristine, doxorubicin, ifosfamide (VAI)	NCCN
Ewing sarcoma	VAC	NCCN
	VAI	
	Vincristine, ifosfamide, doxorubicin, etoposide (VIDE)	NCCN
Osteosarcoma	Cisplatin, doxorubicin	NCCN
	Methotrexate, cisplatin, doxorubicin (MAP)	NCCN
	Doxorubicin, cisplatin, ifosfamide, methotrexate	NCCN
	Ifosfamide, cisplatin, epirubicin	NCCN
Bladder cancer	Ifosfamide, doxorubicin	NCCN
Ovarian cancer	Doxorubicin	NCCN

Table 1-3: Anthracycline used in haematological malignancies based on guidelines used in Malaysia and National Comprehensive Cancer Network (NCCN) guidelines.

Cancer	Regimens	Guidelines
Acute lymphoblastic leukaemia	Vincristine, daunorubicin, dexamethasone, L-asparaginase, methotrexate (Induction Phase I)	Malaysia
	Fludarabine, cytarabine, idarubicin, GCSF (FLAG-Ida consolidation Week 16)	Malaysia
	Vindesine, doxorubicin, prednisolone (Reinduction Phase I Week 22)	Malaysia
	Cytarabine, L-asparaginase, daunorubicin, thioguanine (CART)	Malaysia
	Vincristine, dexamethasone, pegaspargase ± daunorubicin (COG AALL-0031)	NCCN
	TKIs, cyclophosphamide, vincristine, doxorubicin, dexamethasone	NCCN
	TKIs, daunorubicin, vincristine, prednisolone, cyclophosphamide	NCCN
	Daunorubicin, vincristine, prednisone, pegaspargase	NCCN
	Doxorubicin, vincristine, prednisone, methotrexate, pegaspargase	NCCN
	Daunorubicin, vincristine, prednisone, pegaspargase, cyclophosphamide	NCCN
	Cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate/cytarabine (hyper CVAD)	NCCN
	Cyclophosphamide, vincristine, doxorubicin, dexamethasone, pegaspargase, methotrexate/cytarabine	NCCN
	Idarubicin, dexamethasone, vincristine, cyclophosphamide, cytarabine ± rituximab	NCCN
Acute myeloid leukaemia	Daunorubicin, cytarabine (DA 3+7)	Malaysia, NCCN
	Idarubicin, cytarabine (IA 3+7)	Malaysia, NCCN
	Mitoxantrone, cytarabine (MA 3+7)	Malaysia, NCCN
	Mitoxantrone, cytarabine (MIDAC)	Malaysia
	GCSF, fludarabine, cytarabine, idarubicin (FLAG-Ida)	Malaysia, NCCN
	Cytarabine, idarubicin (Ida-HIDAC)	Malaysia, NCCN
	Thioguanine, daunorubicin, cytarabine, prednisolone (TRAP)	Malaysia
	Cytarabine, daunorubicin, midostaurin	NCCN
Acute promyelocytic leukaemia	ATRA, idarubicin (APML induction)	Malaysia
	Idarubicin (APML consolidation)	Malaysia
	ATRA, daunorubicin, cytarabine	NCCN
	ATRA, idarubicin	
	Arsenic trioxide, ATRA, daunorubicin	
	Daunorubicin, cytarabine	
	ATRA, idarubicin, mitoxantrone	
	ATRA, idarubicin, arsenic trioxide	
Burkitt's lymphoma/leukaemia	Methotrexate, vincristine, cyclophoaphamide, doxorubicinm dexamethasone (Block B)	Malaysia

Table 1-3: Anthracycline used in haematological malignancies based on guidelines used in Malaysia and National Comprehensive Cancer Network (NCCN) guidelines. *(cont.)*

Cancer	Regimens	Guidelines
Hodgkin's lymphoma	Doxorubicin, bleomycin, vinblastine, dacarbazine (ABVD)	Malaysia, NCCN
	Cyclophosphamide, vincristine, procarbazine, prednisolone, doxorubicin, bleomycin, vinblastine (COP-ABV)	Malaysia
	Doxorubicin, vinblastine, mechlorethamine, etoposide, vincristine, bleomycin, prednisone (Stanford V)	NCCN
	Bleomycin, etoposide, doxorubicin, vinvristine, cyclophosphamide, procarbazine, prednisone (BEACOPP)	NCCN
	Cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP)	NCCN
	Prednisone, vinblastine, doxorubicin, gemcitabine (PVAG)	NCCN
Non-Hodgkin's lymphoma	Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone (RCHOP-21)	Malaysia
	Methotrexate, vincristine, daunorubicin, ifosfamide, mesna, dexamethasone, thioguanine (R2)	Malaysia
	Mesna, Ifosfamine, mitoxantrone, etoposide (MINE)	Malaysia
	Fludarabine, mitoxantrone, dexamethasone (FMD)	Malaysia
	Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone (RCHOP)	Malaysia, NCCN
	Hyper CVAD	NCCN
	Rituximab, ifosfamide, carboplatin, etoposide (RICE)	NCCN
	Bortezomib, rituximab, cyclophosphamide, doxorubicin, prednisone (VR CAP)	NCCN
	Etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, rituximab (EPOCH-R)	NCCN
	Rituximab, cyclophosphamide, vincristine, doxorubicin (R-CODOX)	NCCN
	Cyclophosphamide, doxorubicin, vincristine, etoposide, prednisone (CHOEP)	NCCN
Multiple myeloma	Vincristine, doxorubicin, dexamethasone (VAD)	Malaysia
	Bortezomib, doxorubicin, dexamethasone	NCCN
	Dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, etoposide, bortezomib (VTD-PACE)	NCCN
	Dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, etoposide (DT-PACE)	NCCN

1.3.3 Type of anthracycline-induced cardiotoxicity

ACT is becoming clinical significance as the use of anthracycline is increasing and the cancer survivor rate is improving overtimes. Doxorubicin was first suspected to cause cardiomyopathy during its Phase I and Preliminary Phase II study in 1969[18]. In the same year, two types of cardiac abnormalities were suggested, electrocardiographic (ECG) changes and congestive heart failure[19]. Since then, numerous researches investigating various areas related to ACT had been carried out.

Irreversibility of ACT is another factor that caused concern among cardiologists and oncologists. As a type I cardiotoxicity, ACT is caused by cardiomyocytes death and thus it is irreversible. On the contrary, type II cardiotoxicity is likely reversible because it is caused by cardiomyocytes dysfunction.

ACT is also broadly classified into three types according to the time of onset: acute ACT occurs immediately to weeks after a single dose or end of treatment; early-onset chronic ACT occurs within the first year from the end of treatment and late-onset chronic ACT develops after a year from the end of treatment. Vasodilation with hypotension and transient cardiac rhythm changes maybe observed although acute ACT is always subclinical[14]. Early-onset ACT is the most common type of ACT and often presented as left ventricular dysfunction, chronic heart failure and QT dispersion.

1.3.4 Frequency of anthracycline-induced cardiotoxicity

ECG changes were first reported in almost half of patients receiving doxorubicin (48 of 97 cases)[18]. Thirty years later, the incidence of doxorubicin induced congestive heart failure in cohorts treated for breast cancer or small cell lung cancer was found to be 5.1%[20]. The incidence of early and late onset cardiotoxicity in children treated with daunorubicin for acute myeloid leukaemia was 13.7% and 17.4%[21]. A recent review of eighteen studies reported the incidence of subclinical and clinical cardiotoxicity were 17.9% and 6.3%, leading to an overall cardiovascular event of 10.6%[22]. In a recent prospective study involving 2625 patients receiving doxorubicin or epirubicin with a median follow-up of 5.2 years reported cardiotoxicity incidence was 9% with 9.7% in patients with breast cancer and 6.2% in patients with non-Hodgkin's lymphoma[23]. Frequency of ACT could be differs according to types and the cumulative dose of anthracycline[24] (Table 1-4).

Table 1-4: Reported incidence of left ventricular dysfunction for doxorubicin, epirubicin and idarubicin.

Anthracycline	Incidence of Left ventricular dysfunction (%)
Doxorubicin	3 – 26*
Epirubicin	0.9 – 3.3
Idarubicin	5 – 18

*At a cumulative dose of 550mg/m^2.

1.3.5 Mechanism of anthracycline-induced cardiotoxicity

Similar to the mode of anti-tumour effect, the pathophysiology of ACT is also uncertain. The most accepted mechanism for ACT is via reactive oxygen species (ROS). Other possible mechanisms include impairment of calcium homeostasis, dysregulation of protein degradation, induction of mitochondrial DNA lesions or interference with topoisomerase II[14] (Figure 1-2). Cardiomyocytes have lower tolerance to oxidative stress than other tissues due to lower concentration of enzymatic defences in the heart. Free radicals released in the reduction of DOX by NADH dehydrogenase and in the formation of DOX-iron complexes subsequently cause cell death through apoptotic pathways[25]. This theory is further supported by the cardio-protective activity of dexrazoxane, an iron chelator, when used together with DOX but contradicted by finding that deferasirox do not possess similar protective effects.

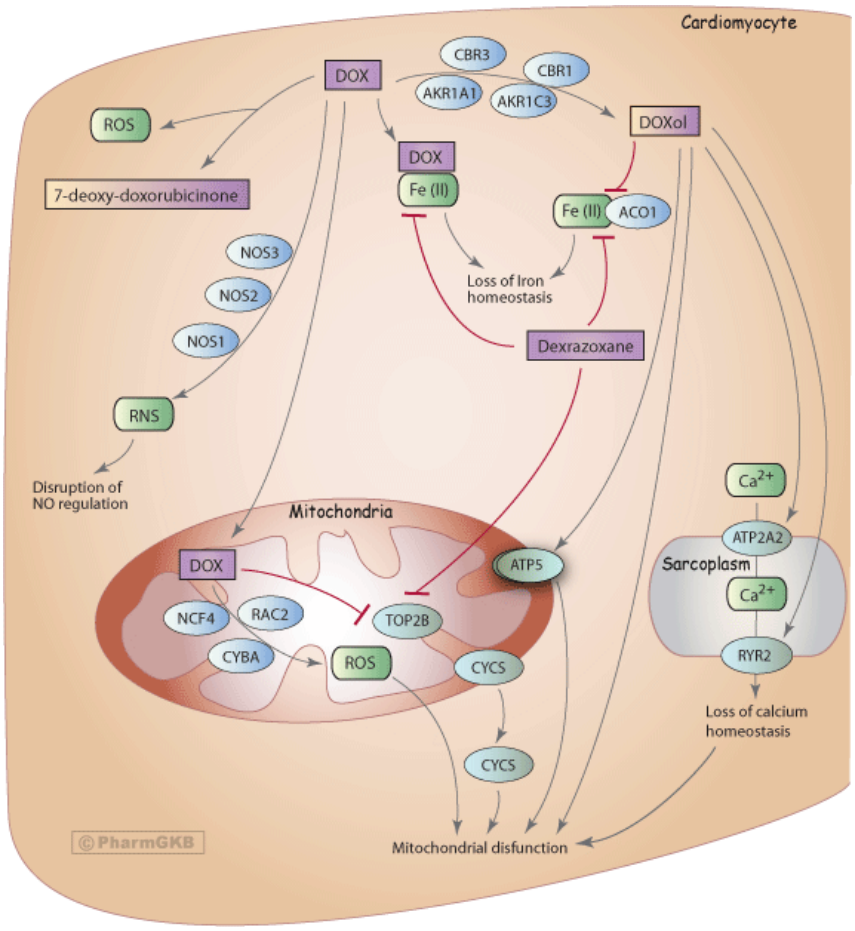

Figure 1-2: Mechanism of cardiotoxicity and related genes.
ACO1, aconitase-iron regulatory protein-1; AKR1A1, aldo-keto reductase family 1 member A1; AKR1C3, , aldo-keto reductase family 1 member C3; ATP2A2, ATPase sarcoplasmic; ATP5, ATP synthase; CBR, carbonyl reductase; CYBA, cytochrome B-215 alpha chain; CYCS, cytochrome c; DOX, doxorubicin; DOXol, doxorubicinol; NCF4, neutrophil cytosolic factor 4; NOS, nitric oxide synthases; RAC2, Ras-related C3 botulinum toxin substrate 2; RNS, reactive nitrogen species; ROS, reactive oxygen species; RYR2, ryanodine receptor 2; TOP2B, topoisomerase II beta. Reproduce with permission from Thorn et al.[25]

1.3.6 Risk factors for anthracycline-induced cardiotoxicity

Thirteen factors have been suggested to increase the risk of ACT, namely age more than 65 years or less than 4 years, female gender, African-American ethnicity, very high or very low body weight, hypertension, diabetes, pre-existing cardiac disease, mediastinal radiation, treatment with cyclophosphamide, paclitaxel or trastuzumab, cumulative anthracycline dose, higher individual anthracycline doses and follow-up duration (Table 1-5).

Among well recognised factors associated with increased risk of ACT, cumulative anthracycline dose is the most prominent. Total cumulative dose of doxorubicin was found to be related to ACT since 1969[19]. This is further supported by a retrospective analysis of three trials by Swain *et al.* and maximum dose of 550 mg/m^2 was suggested[20]. Besides, the study also found that patient older than 65 years have a greater risk for congestive heart failure especially with a cumulative dose of more than 400 mg/m^2. Conversion to doxorubicin isotoxic equivalents was suggested in the calculation of total cumulative anthracycline dose in view of each anthracycline possesses different risk for ACT. Although supportive literatures are limited, widely accepted conversion formulas is as below[26-28] (Table 1-6).

Table 1-5: Risk factors associated with increase anthracycline-induced cardiac toxicity and reporting literatures.

Risk factors	Reporting literature
Older age	Swain *et al.*, 2003[20], Lotrionte *et al.*, 2013[22]
Younger age	Von Hoff *et al.*, 1977[29], Lotrionte *et al.*, 2013[22], Lipshultz *et al.*, 1995[30], Silber *et al.*, 1993[31]
Female	Lipshultz *et al.*, 1995[30], Silber *et al.*, 1993[31]
African-American ethnicity	Lotrionte *et al.*, 2013[22]
Very high or very low body weight	Lotrionte *et al.*, 2013[22]
Higher cumulative dose	Lefrak *et al.*, 1973[19] Lotrionte *et al.*, 2013[22], Lipshultz *et al.*, 1995[30], Silber *et al.*, 1993[31], Steinherz *et al.*, 1991[32]
Cumulative dose >350mg/m^2	Alexander *et al.*, 1979[33], Buzdar *et al.,* 1985[34]
Cumulative dose >300mg/m^2	Hayakawa *et al.*, 2001[35]
Uncontrolled hypertension	Minow et al., 1977[36], Lotrionte *et al.*, 2013[22]
Diabetes mellitus	Lotrionte *et al.*, 2013[22]
Severe co-morbidities	Lotrionte *et al.*, 2013[22]
Concurrent cyclophosphamide use	Minow *et al.*, 1975[37]
Concurrent trastuzumab use	Cobleigh *et al.*, 1999[38]
Concurrent paclitaxel use	Nabholtz *et al.*, 2001[39]
Chest radiation	Minow *et al.*, 1975[37] Lotrionte *et al.*, 2013[22], Steinherz *et al.*, 1991[32]
Follow-up duration	Lipshultz *et al.*, 1995[30], Steinherz *et al.*, 1991[32]

Table 1-6: Commonly used conversion formula in calculating doxorubicin isotoxic equivalent for different type of anthracyclines.

Anthracyclines	Conversion Formula
Daunorubicin	Total dose x 0.833
Doxorubicin	Total dose x 1
Epirubicin	Total dose x 0.67
Idarubicin	Total dose x 5
Mitoxantrone	Total dose x 4

1.3.7 Pharmacogenetics in ADRs risk prediction

Development in molecular biology has increased our understanding of the role of genetic variation underlying adverse drug reactions (ADRs). To dates, a few prominent genetic testing are recommended in identifying patient at risk for ADRs. Examples are thiopurine methyltansferase (TMPT) gene variation and human leukocyte antigen (HLA)-B*1502. Polymorphisms of TMPT gene have been widely studied and are recommended to be use in dose adjustment of thiopurines in some institutions. *TPMT*2*, *TPMT*3A* and *TPMT*3C* are known to cause lowered TPMT activity, thus a reduced dose is recommended for heterozygous and homozygous patients to prevent hematopoietic toxicity[40].

It is widely acknowledge that due to difference in allele frequency, genetic association can also be ethnicity specific[41]. HLA-B*1502 screening is recommended for Han Chinese, Malay, and Thai population to identify patients at risk for carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis[42]. The genetic association between hypersensitivity induced by abacavir and HLA-B*5701 is also an ethnic difference in which it is prevalent in Caucasians, but not in Hispanics or Africans[43].

Recent studies found that SLC22A17 and SLC22A7 variants are significantly associated with ACT and improved patient risk stratification[44]. Other therapeutics products used in oncology with pharmacogenomics screening recommendation due to adverse drug reactions include abemaciclib, afatinib, anastrazole, lenelidomide, lapatinib, nilotinib and more. A list of valid genomic biomarkers for clinical guidance can be found on the FDA website 'Table of Pharmacogenomic Biomarkers in Drug labels' (http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm).

2 Incidence of cancer chemotherapy-related cardiovascular complications in Asia

We recognised the need to estimate the incidence of cancer chemotherapy-related cardiovascular complications in Asia because currently available incidence of cancer chemotherapy-related cardiovascular complications was based studies conducted in North America or Europe. Besides, evidences suggested that ethnicity such as African-American are at higher risk for cancer-chemotherapy-related cardiovascular complications[22] and ethnicities in Asia is diverse and is greatly distinct from other continents. Thus, we conducted a systematic review to estimate the incidence of cancer chemotherapy-related cardiovascular complications in Asia (Section 2.1, page 22). Besides, we also reported the incidence and characteristics of anthracycline-induced cardiotoxicity in a multi-ethnic population in Asia (Section 2.1.2, page 23).

2.1 Burden of Antineoplastic-related Cardiovascular Toxicity in Asia: A systematic review and meta-analysis.

2.1.1 Introduction

This chapter has been submitted to Heart Failure Reviews awaiting editorial decision. The candidate, Leong Siew Lian was primarily responsible for searching, analysis and writing of the manuscript. The paper's co-authors, Shaun Lee Wen Huey and Nathorn Chaiyakunapruk contributed in various aspects of this article.

2.1.2 Submitted manuscript and supplementary materials

Title: Antineoplastic-related Cardiovascular Toxicity: A systematic review and meta-analysis in Asia.

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Abstract

Introduction: Cancer and heart diseases are the leading causes of morbidity and mortality in many countries worldwide. Recent advancement in chemotherapy has led to an improvement in cancer survival rates, but at a cost of higher cardiac side effects. However, report on chemotherapy-related cardiotoxicities incidence in Asia is lacking.

Methods: We systematically searched multiple databases to identify studies reporting incidence of antineoplastic-related cardiovascular toxicity in Asia published from inception to September 2017. Pre-specified subgroups were performed to explore heterogeneity and study quality assessed and reported according to PRISMA guidelines.

Results: A total of 54 studies across 11 countries in Asia reported 8 types of cardiovascular toxicities were included. These studies mostly reported on adult populations, and usually examined cardiotoxicities related to anthracycline use. The most frequently reported cardiotoxicities were heart failure, electrocardiogram abnormalities and left ventricular dysfunction. The incidence of cardiotoxicity was between 0.5 to 69%. Subgroup analysis showed higher incidence in middle income countries compared to high income countries.

Conclusions: Although robust incidence studies are sparse, cardiovascular complications affects approximately one in twenty cancer patients in Asia. This highlights a unique opportunity of cancer patients caring that need cardiologists and oncologist to become familiar with this emerging sub-specialty.

Keywords: chemotherapy; cancer therapy; heart adverse effect; cardiac toxicity; incidence; Asian

Introduction

Cardiovascular (CV) toxicities such as heart failure, systemic hypertension and thromboembolic events are commonly experienced by patients who have received chemotherapeutic agents[1, 2]. While CV toxicities are commonly associated with older antineoplastic agents such as anthracycline, there have been an increasing number of reports associated with newer chemotherapeutics such as trastuzumab, bevacizumab and tyrosine kinase inhibitors[1, 2]. These adverse effects are mainly due to the direct cytotoxic cardiac injury associated with chemotherapy and can be classified into either: cardiac systolic dysfunction, cardiac ischaemia, arrhythmias, pericarditis and repolarisation abnormalities[3]. While the exact mechanisms are unknown, these adverse effects are thought to be related to the interaction of chemotherapy with concurrent drugs or changes in physiology of the patient such as hepatic metabolism. It has been reported that 5.3% five years survivors of childhood cancer experienced cardiac conditions such as congestive heart failure, valvular abnormalities, pericardial disease and myocardial infarction[4]. These rates are expected to increase with the advancement in cancer management[5].

While understanding the pathophysiology of these adverse effects is important in the development of preventive measures, recognising the risk and burden is the first crucial step towards developing new strategies to promote cardiac risk prevention, detection and management. Antineoplastic-related CV toxicities have been widely studies and reported in other continents especially North America and Europe[6-8]. Reviews based on data from western countries reported the incidence rate of cancer treatment-induced cardiotoxicity with several chemotherapeutic agents, including anthracycline (0.9% - 57%)[1, 9-11], cyclophosphamide (2% - 28%)[9-11], trastuzumab (0% - 28%)[9, 11, 12] and bevacizumab (1.7% - 10.9%)[1, 9]. However, to date there has been no studies that have quantified the incidence of cardiotoxicities in Asia, which may differ due to the presence of interethnic difference[13]. This has been evidence in abacavir- and carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis[14]. Thus, we performed a systematic review to provide collective evidence on the incidence and characteristics of antineoplastic-related CV toxicities which in turn to guide future research in this region.

Method

Search strategy and selection criteria

The following databases were searched: Ovid Medline, EMBASE and Cochrane Central Register of Controlled Studies, without language restriction, for studies reporting antineoplastic related cardiovascular toxicity in Asia (Online Resource 1) from database inception until September 30, 2017. This was supplemented with a manual search of cited references from retrieved articles. Any article which reported the incidence of CV toxicity in cancer patients treated with an antineoplastic agent in Asian countries was included. Studies were excluded if they were case report, conference abstracts, reviews and non-patient or lab studies.

Data extraction and quality assessment

Information about geographic location, study design, participant demographics, types of cancer, frequency of CV toxicity and definition of CV toxicity were extracted independently by two reviewers (SLL and SWHL) using a piloted data extraction table. All data were reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement[15].

Study quality was independently assessed using Risk of Bias In Non-randomised Studies – of Interventions (ROBINS-I)[16] for non-randomised studies while the Cochrane risk of bias(RoB 2.0) tool[17] was used for randomised studies.

Data Analysis

We performed a meta-analysis of proportions to estimate incidence of pre-specified subgroups, using the Freeman-Tukey Double Arcsine Transformation[18] to establish variance of raw proportions. DerSimonian-Laird random effects models[19] was used to combine the transformed proportions and to incorporate heterogeneity anticipated among included studies. Heterogeneity of the studies was assessed using Cochran's Q and I^2 statistics. Pre-specified subgroup analyses were carried out to assess the difference in incidence according to country, country's income as reported in World Economic Situation Prospects[20] and regions[21], study design, study period, age at diagnosis (<18 years, ≥ 18 years, or both), chemotherapy (anthracycline- vs non anthracycline-based), CV toxicity definition used and type of CV toxicity. All analyses were performed using Stata 15.0 (StataCorp, College Station, TX).

Result

Study characteristics

The initial search yielded 1,285 articles, of which 104 articles were selected, and 54 articles met the inclusion criteria (Online Resource 1). These studies were reported across eleven Asian countries, mainly from East Asia (45 studies, 13 223 patients), Middle East (7 studies, 27 271 patients) and South Asia (2 studies, 456 patients). Thirty-eight (70%) studies were conducted in high-income countries, including Japan (n=26), Taiwan (n=8), Hong Kong, Israel and South Korea (n=1 each). One was a multicentre study expanding across 5 countries (Hong Kong, Japan, South Korea, Singapore and Taiwan). Fourteen studies were from upper-middle income countries (China, Iran and Turkey), with only two from lower-middle countries (India and Pakistan). The reported age at diagnosis of the patient populations ranged from birth to 89 years, but most studies reported an adult (18 – 65 years) and elderly (> 65 years) population (32 studies, 34 559 patients; Online Resource 1).

Thirty-nine (72%) studies reported CV toxicity among patients who received antineoplastic treatment. Twelve of these studies studied more than one type of CV toxicities. The National Cancer Institute criteria was the most common criteria used to define CV toxicity. Heart failure (n=14, 26%) was the most frequently reported toxicity followed by electrocardiogram (ECG) abnormalities (n=10, 19%) and left ventricular dysfunction (n=9, 17%). In terms of chemotherapy agents, twenty-eight (52%) studies reported the effect of anthracycline-based chemotherapy. Ten (19%) studies reported both the combination effect of anthracycline- and non-anthracycline-based chemotherapy. Among studies that reported the effect of non-anthracycline-based chemotherapy, three studies included bevacizumab (6%), one each on 5-fluorouracil, carboplatin, cyclophosphamide-based, gemcitabine, nintedanib and paclitaxel (2%). Type of antineoplastic agents was not specified in two studies.

Quality assessment

Twenty-six studies were judged to have low risk of bias, three have moderate risk of bias and sixteen had serious risk of bias when assessed using the ROBINS-I assessment tool, (Online Resource 1). These were mainly due to presence of confounding factor as well as poor reporting and measurement of outcomes (Online Resource 1). All nine randomized controlled trials were reported to have a low risk of bias (Online Resource 1).

Pooled incidence according to type of cardiotoxicity

Fifty-two studies were selected for meta-analysis, as two studies did not report the number of CV cases[22, 23] . The overall estimated incidence of antineoplastic-related CV toxicity in Asia was 4.7% (95% CI, 3.36 – 6.11), but there was considerable heterogeneity (I^2 = 94%) suggesting differences in effect sizes which exists within this set of studies. Stratification by CV toxicity showed that the most common reported toxicity was hypertension, with a pooled incidence of 22.7% (95% CI, 8.83 – 40.44). Other reported toxicities include ECG abnormality (7.4%, 95% CI, 3.44 – 12.59), heart failure (6.9%,

95% CI, 4.04 – 10.26) and left ventricular dysfunction (5.3, 95% CI,2.76 – 8.50; Fig. 1). When stratified by chemotherapy agent, the highest incidence was observed in patients receiving non-anthracycline based chemotherapy with a incidence of 11.7% (95% CI, 2.57 – 15.89). A relatively lower incidence was reported in patients receiving anthracycline-based chemotherapy, 3.2% (95% CI, 1.68 – 4.96).

Incidence according to country

We stratified CV toxicities by country's income and found that the incidence was higher in upper-middle income countries, with a pooled incidence of 13.1% (95% CI: 5.45 – 23.37; Fig. 2). Incidence of CV toxicities was the highest in Pakistan (19.9%, 95% CI, 15.57 – 24.90) and lowest in India (1.3%, 95% CI, 0.16 – 4.58). Other countries with incidence that exceeded 10% were China (18.2%, 95% CI, 3.50 – 40.21), Iran (15.5%, 95% CI, 7.35 – 27.42) and South Korea (12.1%, 95% CI, 6.93 – 19.17). All these findings were based on single study in the country except for China which was a pooled incidence of seven studies with a total study population of 735 patients. The estimated incidence for Japan which had the highest number of studies (n=25) with 3,698 patients was 1.8% (95% CI, 0.58 – 3.38). Estimated incidence in Israel from one study with the largest study population (n = 26,310) was 3.6% (95% CI, 3.34 – 3.79).

Incidence of cardiotoxicity by chemotherapy

We further stratified by types of chemotherapy used. In the twenty-nine studies which reported incidence of cardiac event with anthracycline use, presence of cardiac event was reported in eighteen (62%) studies. Analysis of type of CV toxicities among this sub-population showed different distribution compare to the overall population (Fig. 3). In anthracycline recipients, ECG abnormality had the highest incidence of 9.2% (95% CI, 2.12 – 19.89) followed by unspecified cardiotoxicity (8.2%, 95% CI, 3.07 – 15.24), LV dysfunction (5.5%, 95% CI, 1.57 – 11.30), other cardiac disorders (0.7%, 95% CI, 0.05 – 1.79) and heart failure (2.4%, 95% CI, 1.06 – 4.02). Other cardiac disorders include CV dysfunction[24] and acute cardiac complications[25].

Discussion

To our knowledge this is the first and only systematic review which assessed the incidence and characteristics of CV toxicity in Asia. We found a total of fifty-four studies, reporting frequency of various types of CV toxicity related to various types of antineoplastic agent. Results from our meta-analysis revealed that nearly one in every twenty recipients of antineoplastic agents will develop CV related toxicity with higher rate in middle-income countries. Given the approximate incidence of cancers in Asia was 13.2 million, this translates to 620,000 recipients of antineoplastic agents who will develop CV[26] in their lifetime. Commonly reported CV toxicities were targeted therapies related hypertension and anthracycline-related ECG abnormalities and left ventricular dysfunction.

Over the past few years, there has been increasing reports of adverse events associated with antineoplastic use, which had led to a new branch of interest of onco-cardiology. As noted in our review, we found that the number of studies reported has increased from an average of one study per year during 1996 to 2000 to four studies per year over the past 2 years (Fig. 4). This increase is mainly fuelled by an in increasing in number of studies for newer antineoplastic agents such as bevacizumab[27] and trastuzumab[28]. This increasing prevalence has potential ramification to the healthcare systems, as studies have shown that the economic burden of cardiotoxicities are very high, ranging from international dollar (Int$) 908 to Int$40 971 per patient[29] for treatment of heart failure to USD 485.06 to USD 817.73 per 100 patients per month[30].

Hypertension had the highest-incidence among all types of CV toxicity found in this study, which are related to the antineoplastic nintedanib[31] and bevacizumab[32-34]. Although the underlying pathophysiological mechanism for antineoplastic related hypertension remains unknown, increase in vascular tone due to inhibition of VEGF-mediated vasodilation is the most accepted hypothesis for the mechanism of hypertension by these agents[35]. Given that tyrosine kinase inhibitors and VEGF-A inhibitors acts on this pathway, it is expected to cause some degree of increase in blood pressure. As such recipients of these agents should be considered at higher risk for CV toxicity if they have systolic blood pressure of more than 160 mmHg or diastolic blood pressure of more than 100 mmHg; diabetes mellitus or established CV disease[9]. Strategies such as serial monitoring of blood pressure and aggressive management of blood pressure elevations are necessary to avoid cardiac dysfunction and early termination of cancer therapy. Besides, an improved collaboration between oncology and cardiology is needed to address the clinical gaps experienced by this at risk patient population[36].

Among antineoplastic agents, ACT related CV toxicity is commonly reported and well defined, with incidences ranging from 0.9% to 26%, depending to type and cumulative dose of anthracycline[37]. Systematic review and meta-analysis by Lotrionte et al. which found that the ACT incidence was from 3.5% to 17.6%[38]. The pooled estimated of the ACT incidence in our study was within the range reported by previous studies. Our study further confirms finding from other reviews which have reported risk factors for cardiotoxicity, including the use of doxorubicin at doses of 550mg/m^2 [39]. In addition, our study found that the use of synthetic anthracycline amrubicin[40-43] and pirarubicin[25] were relatively safe. Among the 367 patients received amrubicin or pirarubicin, only two (0.5%) patients reported to have decreased left ventricular ejection fraction of more than 15% from baseline.

There are several strengths of this systematic review and meta-analysis. Our study is the first of its kind to quantify incidence estimates derived using a comprehensive search strategy and included

additional studies that are not found in academic sources. We also quantify the degree of heterogeneity using I^2 index and noted that wide confidence intervals, suggesting the importance of further research in this area to identify further sources of this large variance.

Despite its strengths, some aspects in this study need to be considered when interpreting our findings. Due to the diversity of language in Asia, our search may have missed studies which were not published in English. Most of the studies had not adequately controlled for baseline CV functions at the start of follow-up with missing crucial data on definitions and measurements, except for several characteristics such as gender and age. This information is important for further methodological analyses to identify for sources of heterogeneity and how different cardiac outcomes definitions, measurements and study period affect incidence estimates. As such, future studies might benefit from examining in different sub-populations such as elderly and children as this would provide a basis for developing effective strategies to prevent and respond to CV related toxicities due to antineoplastic use. Inclusion of studies with serious risk of bias may affect the accuracy of our findings, thus cautions is advised during interpretation.

The findings on the type of cardiotoxicities and antineoplastic agent may guide clinicians in monitoring CV functions in patient receiving antineoplastic agent in general and anthracycline in specific.

Conclusion

Cardiovascular toxicities due to antineoplastic use affects almost one in every twenty (approximately 620 thousand) cancer patients in Asia. These findings strengthen the case to expand for efforts to identify and prevent CV related toxicities due to antineoplastic use, and the need for early CV screening in this population. Considering the serious health consequences, more efforts are needed to raise awareness of, and provide guidance especially to both oncologists and cardiologist on the best way to respond to this and become familiar with this emerging subspecialty.

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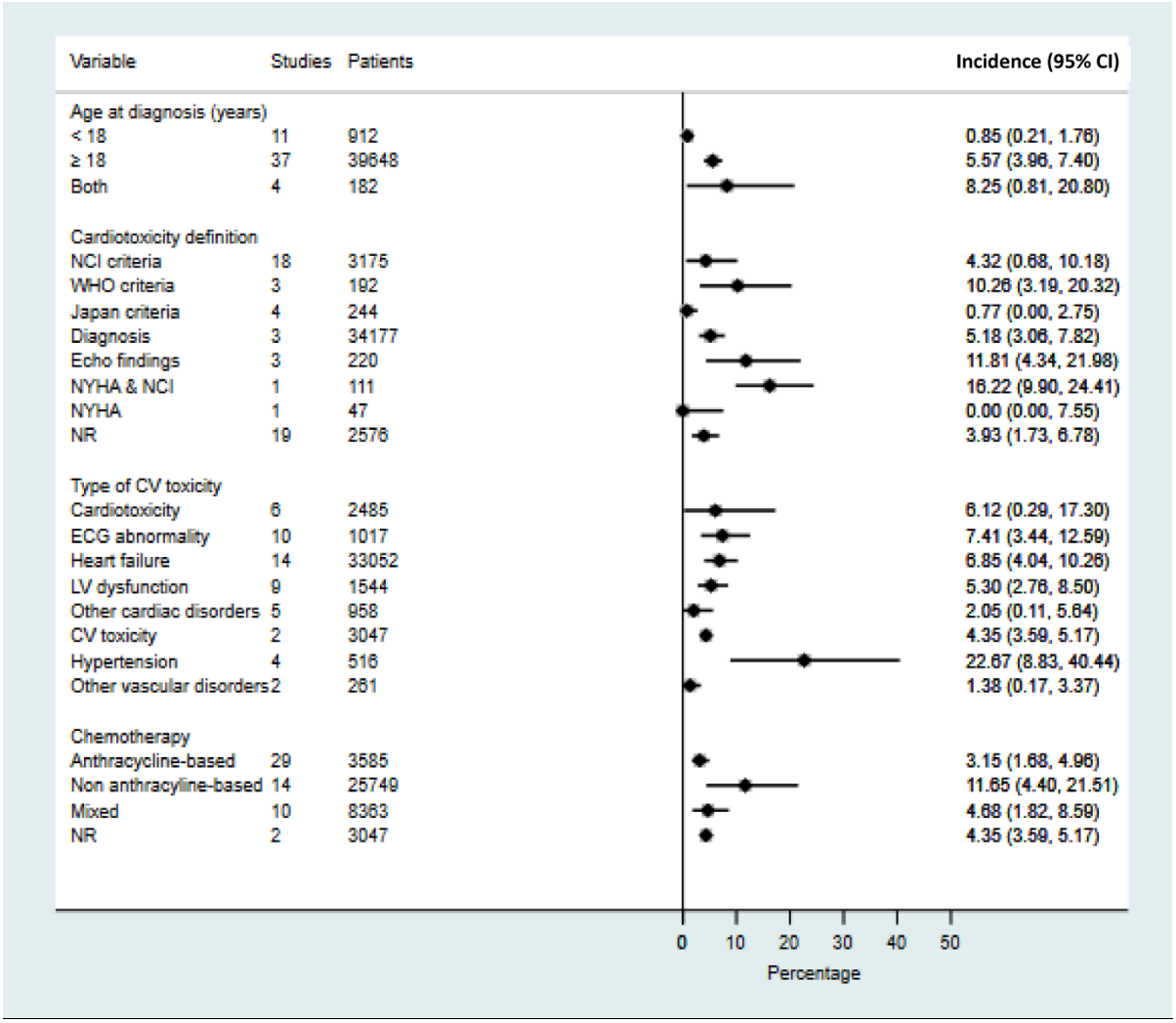

Fig. 1 Pooled estimated incidence of cardiovascular toxicity according to characteristics of participants. Overall pooled incidence is 4.65 (95% CI: 3.36 - 6.11) with high heterogeneity, (I^2 = 93.91%).

CI, confidence interval; Echo, echocardiogram; NCI, National Cancer Institute; NR: not reported; RCT, randomised controlled trial; WHO, World Health Organisation.

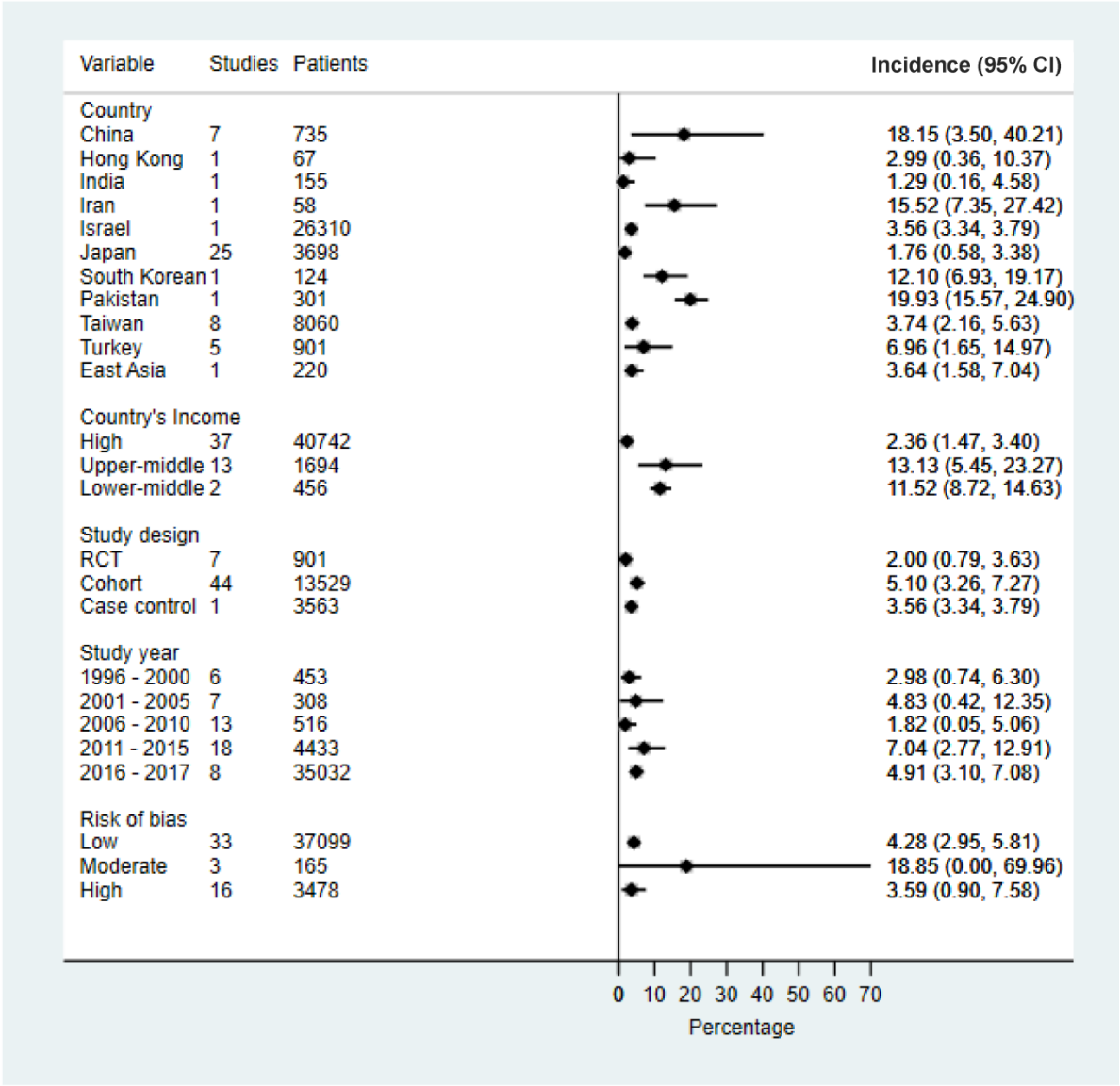

Fig. 2 Pooled estimated incidence of cardiovascular toxicity according to characteristics of included studies. Overall pooled incidence is 4.65 (95% CI: 3.36 - 6.11) with high heterogeneity, (I^2 = 93.91%). CI, confidence interval; Echo, echocardiogram; NCI, National Cancer Institute; NR: not reported; RCT, randomised controlled trial; WHO, World Health Organisation.

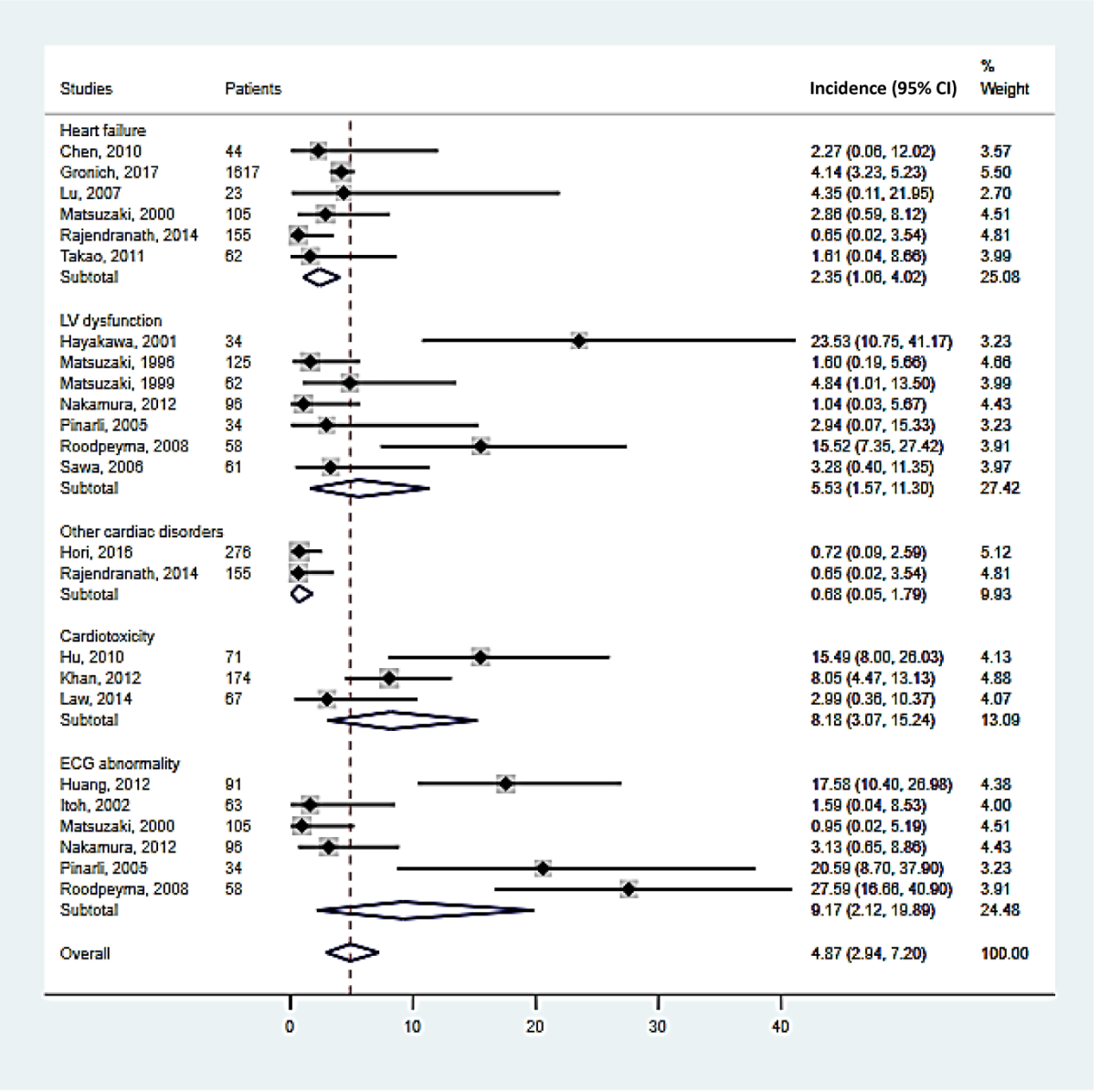

Fig. 3 Type of CV toxicities among anthracycline recipients. Overall pooled incidence is 6.57 (95% CI: 5.06 – 8.24) with high heterogeneity, (I^2 = 95.35%).

CI, confidence interval; ECG, electrocardiogram; LV, left ventricular.

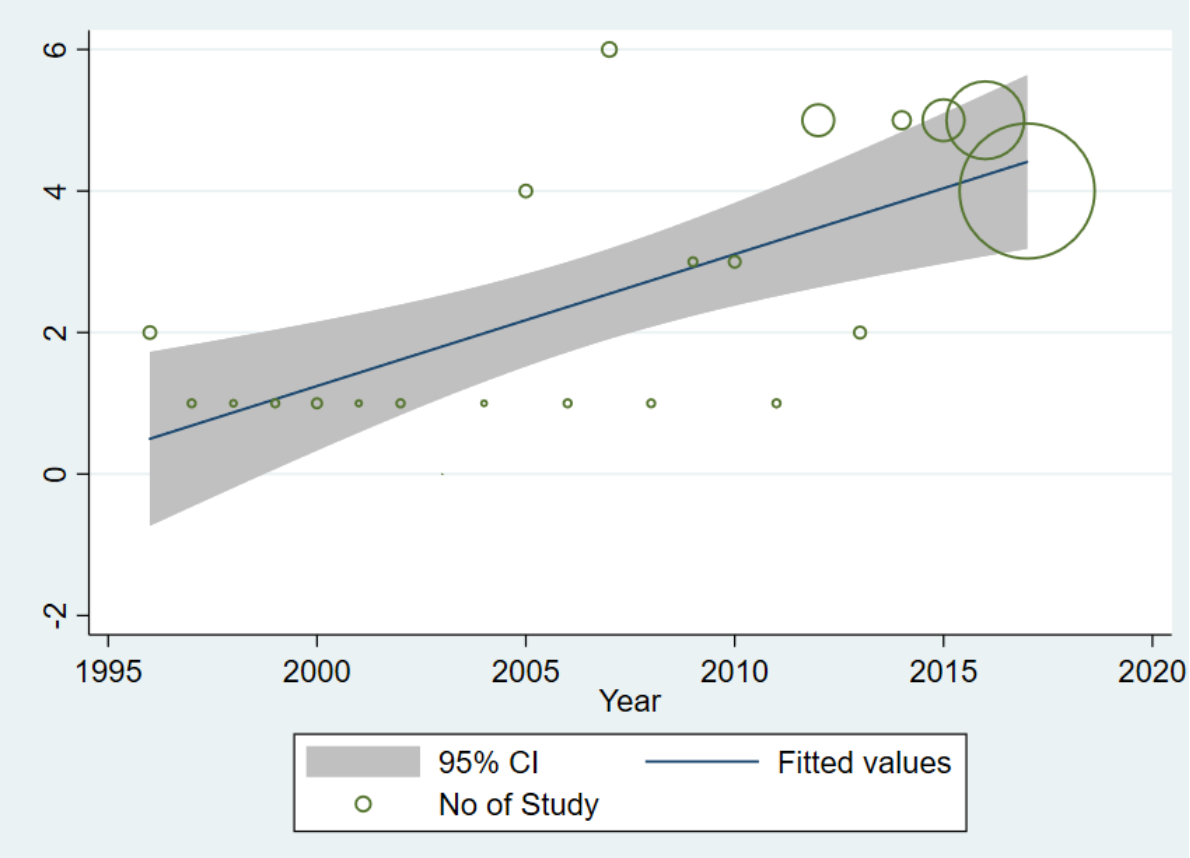

Fig. 4 Scatter plot of study distribution according to years weighted by number of participants.

Title: Antineoplastic-related Cardiovascular Toxicity: A systematic review and meta-analysis in Asia.

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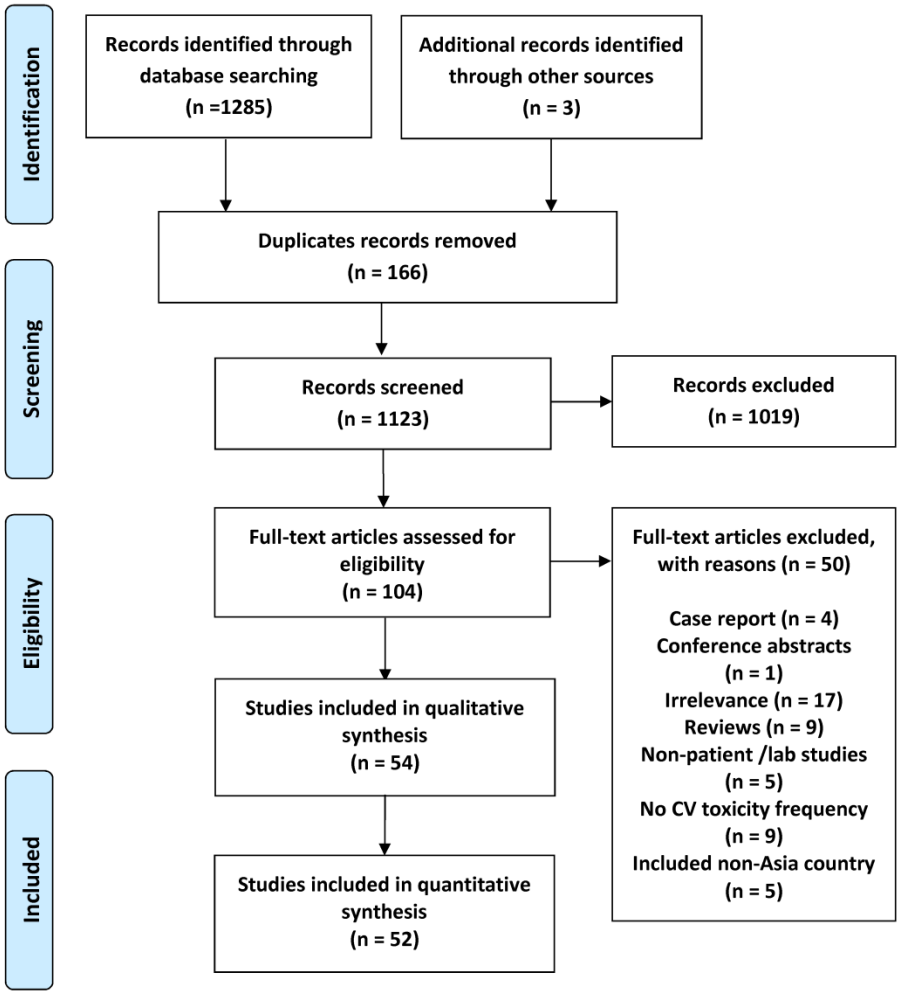
Supplementary material

eTable 1: Databases and search terms used.

Ovid Medline, Cochrane Central Register of Controlled Studies and EMBASE Keyword search (1) antineoplast*.mp.; (2) anticancer*.mp.; (3) antitumo?r*.mp.; (4) "cancer drug*".mp.; (5) "cancer chemotherapy*".mp; (6) cardiotoxic*.mp.; (7) "cardiac toxic*".mp.; (8) arrhythmia*.mp.; (9) "heart failure*".mp.; (10) cardiomyopathy*.mp.; (11) myocardiopath*.mp.; (12) "cardiovascular disease*".mp.; (13) cardiovascular.mp; (14) Asia.mp.; (15) (Japan or Brunei or China or Hong Kong or Indonesia or Malaysia or Myanmar or Papua or Philippines or Korea or Singapore or Taiwan or Thailand or Vietnam).mp.; (16) (Bangladesh or India or Iran or Nepal or Pakistan or Sri Lanka).mp.; (17) (Bahrain or Iraq or Israel or Jordan or Kuwait or Lebanon or Oman or Qatar or Saudi Arabia or Syrai or Turkey or Emirates or Yemen).mp.; (18) (Armenia or Azerbaijan or Georgia or Kazakhstan or Kyrgyzstan or Russia or Tajikistan or Turkmenistan or Uzbekistan).mp.; (19) 1 or 2 or 3 or 4 or 5; (20) 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13; (21) 14 or 15 or 16 or 17 or 18; (22) 19 and 20 and 21.



eFigure 1: Process of study selection.

eTable 2: Studies characteristics.

Author (Year)	Country	Study design; number of patients	Age (years)	Male / Female	Cancer	Chemotherapy (Intervention vs Control)	Definition of cardiotoxicity	Study aim
Hu, 2010	China	Prospective cohort; 71	Mean CAP/CEF = 64(4.13)/ 61(2.57)	0/71	Stage IIa breast cancer	Oral capecitabine (CAP) vs cyclophosphamide/ epirubicin/5-fluorouracil (CEF)	NCI-CTCAE v3.0	Oral capecitabine as adjuvant monotherpay in women 55 years of age or older with stage IIa breast cancer
Huang, 2012	China	Prospective cohort; 254	Mean= 66.6 (60 - 77)	122 / 132	acute myeloid leukaemia (AML)	Daunorubicin- or homoharrungtonine-based regimens	WHO	Compare the antitumour efficacy and safety profile of high dose homoharringtonine
Xu, 2012	China	Prospective cohort, 202	Med = 48 (22 - 74)	NR	HER2-negative locally recurrent/metastatic breast cancer	Bevacizumab	NR	Compare safety and efficacey of fisrt line bevacizumab between Chinese and Western patients with HER2-negative
Cai, 2014	China	Prospective cohort; 57	Med = 37 (6 - 54)	34/23	Myeloid hematological malignancies (CML, AML)	Fludarabine, cytarabine, busulfan, cyclophosphamide	NCI-CTC, v2.0	Analyse the efficacy of a Flu & Ara-c containing regimen as a myeloablative conditioning regimen
Dai, 2015	China	Prospective cohort; 62	Med = 64.2 (33 - 83)	31/31	NSCLC	Nintedanib	NCI-CTCAE v3.0	Phase II clinical study
Wang, 2015	China	Prospective cohort; 114	Med = 63.4 (29 - 81)	64/50	metastatic colorectal cancer (mCRC)	Bevacizumab	NCI-CTCAE v3.0	Efficacy and safety of continuous usage of bevacizumab

eTable 2: Studies characteristics. *(cont.)*

Author (Year)	Country	Study design; number of patients	Age (years)	Male / Female	Cancer	Chemotherapy (Intervention vs Control)	Definition of cardiotoxicity	Study aim
Zhou, 2015	China	RCT; Control/Intervention: 138/138	B: Med = 57 (30 - 75); Placebo: Med = 56 (23 - 74)	Control: 77/61 ,Intervention: 75/63	NSCLC	Bevacizumab (B) + carboplatin/paclitaxel vs placebo + carboplatin/paclitaxel	NCI-CTCAE v4	Efficacy of first-line bevacizumab plus platinum in Chinese
Luan, 2017	China	RCT; 111	EC: 50.2 (10.2), TC: 51.5 (9.7)	0 / Control: 55; Intervention: 56	Breast cancer	Epirubicin/ cyclophosphamide (EC) vs docetaxel/ cyclophosphamide (TC)	NA	Differenced in myocardial toxicity induced by different chemotherapy regimens
Law, 2014	Hong Kong	Retrospective cohort; 67	Med = (16 - 80)	38/29	HL	ABVD	NR	Clinical and histopathological characteristics, treatment types, clinical course and treatment outcomes
Rajendranath, 2014	India	Prospective cohort, 155	NR	114/41	ALL, HL, NHL, sarcoma, germ cell tumour, Wilms tumour, retinoblastoma, AML, others	Anthracyclines, prednisolone, vincristine, cyclophosphamide, etoposide, cisplatin	NR	Long-term sequela associated with therapy in childhood cancer survivors
Roodpeyma, 2008	Iran	Prospective cohort, 58	Mean = 17.5 (4.6), med = 17.5 (9 - 29)	36/22	ALL, HL, Wilms tumour, lymphoma, others	Anthracycline	NA	Frequency of changes in left ventricular function 5 years after completion of anthracycline-therapy

eTable 2: Studies characteristics. *(cont.)*

Author (Year)	Country	Study design; number of patients	Age (years)	Male / Female	Cancer	Chemotherapy (Intervention vs Control)	Definition of cardiotoxicity	Study aim
Gronich, 2017	Israel	Nested case-control; Control/Cases: 25 382/930	Mean = 68.74 (10.57)/69.35 (11.40)	NR	Malignant disease	Tyrosine-kinase inhibitors (TKIs)	HF, cardiomyopathy	Which TKIs are associated with greater risk for new onset HF
Matsuzaki, 1996	Japan	Prospective cohort; 125	Med = AL851 / ALHR88: 7.6/7.2	71/54	High-risk ALL	Vincristine, daunorubicin, L-asparaginase, prednisolone, methotrexate, cytarabine, enocitabine, 6-mercaptopurine,, cyclophosphamide, doxorubicin, dexamethasone	NR	Treatment results of AL851 and ALHR88 protocols for high-risk ALL in children
Sekine, 1996	Japan	Prospective cohort, 60	Med- 63 (27 - 74)	45/15	NSCLC	Paclitaxel	Toxicity criteria of the Japan Society for Cancer Therapy	Efficacy and toxicity of paclitaxel given over 3h
Furuse, 1997	Japan	Prospective cohort, 60	Med = 65 (45-74)	49/11	Stage III/IV NSCLC	Paclitaxel	WHO criteria & SWOG	Dose-limiting toxic effects of paclitaxel
Matsuzaki, 1999	Japan	RCT; Control/Intervention: 31/31	Med = 4.7 (2-9.3)	Control: 14/15, Intervention:23/10	ALL	vincristine, prednisolone, L-asparaginase, daunorubicin, cytarabine, methotrexate, 6-mercaptopurine, cyclophosphamide	FS <29%, abnormal regional wall motion	Long-term myocardial function of the patients treated with AL841

eTable 2: Studies characteristics. *(cont.)*

Author (Year)	Country	Study design; number of patients	Age (years)	Male / Female	Cancer	Chemotherapy (Intervention vs Control)	Definition of cardiotoxicity	Study aim
Matsuzaki, 2000	Japan	Retrospective cohort; 105	NR	NR	ALL	vincristine, prednisolone, L-asparaginase, daunorubicin, cytarabine, methtrexate, 6-mercaptopurine, enocitabine, doxorubicin, dexamethasone, cyclophosphamide	NR	Long-term outcome and late effects of childhood ALL treatment.
Hayakawa, 2001	Japan	Prospective cohort, 34	Mean = 11.5 (0.7 - 21.7)	18/16	NR	Doxorubicin-based regimens	EF < 60% or FS < 30	ANP and BNP as specific markers for doxorubicin-induced cardio toxic effects in children
Itoh, 2002	Japan	RCT; 63: control, 32, intervention, 31	Med = Cont/Interv: 62 (37 - 69)/62 (35 - 69)	Control: 18/14, Intervention: 20/111	aggressive NHL	dose-escalated CHOP	JCOG toxicity criteria	Explore a suitable therapeutic-intensified regimen for the treatment of aggressive NHL
Sawaki, 2004	Japan	Prospective cohort: 27	Med = 54.2 (32 - 72)	0/27	HER2-overexpressing metastatic breast cancer	Trastuzumab	NCI-CTC v2	Efficacy and safety of trastuzumab as a single agent in second-third line treatment of HER2-overexpressing metastatic breast cancer

eTable 2: Studies characteristics. *(cont.)*

Author (Year)	Country	Study design; number of patients	Age (years)	Male / Female	Cancer	Chemotherapy (Intervention vs Control)	Definition of cardiotoxicity	Study aim
Atagi, 2005	Japan	RCT; Control/Intervention: 23/23	Med = Contr/Interv: 77 (72-84)/77 (71 - 83)	Control: 19/4; Intervention: 16/7	Locally advanced NSCLC	Radiotherapy vs Carboplatin + radiotherapy	NCI-CTC v2	Compare the impact of chemo-radiotherapy and radiotherapy alone on survival in unresectable locally advanced NSCLC elderly
Matsui, 2005	Japan	Prospective cohort; 59	Med = 62 (38 - 74)	43/16	advanced NSCLC, chemonaive	Gemcitabine - docetaxel	NCI-CTC v2	Phase I To compare 2 schedules of gemcitabine-docetaxel
Sawa, 2006	Japan	Prospective cohort, 61	Med = 65 (33-75)	46/15	NSCLC	Amrubicin	Japan Society for Cancer Therapy criteria	To evaluate the efficacy and safety of amrubicin in patients with NSCLC
Senju, 2007	Japan	Prospective cohort, 23	Mean 47.2 (18.1, 17-72)	12/11	AML, T-cell leukaemia, lymphoma	Doxorubicin-based regimens	NR	Tei-index in early myocardial damage induced by anthracycline
Takeda, 2007	Japan	Prospective cohort, 60	Med = 65.5 (41 - 75)	37/23	Stage III/IV NSCLC	Amrubicin	Japan Society for Cancer Therapy criteria	Efficacy and safety of amrubicin in patients with NSCLC
Tokuda, 2007	Japan	RCT; 97, control 48, intervention 49	Med = 46 (27 - 55)	0/97	Stage I to IIIB breast cancer involving 10 or more axillary lymph nodes	Cyclophosphamide, thiotepa	JCOG toxicity criteria	Efficacy of high-dose chemotherapy as consolidation of the treatment of high-risk postoperative breast cancer

eTable 2: Studies characteristics. *(cont.)*

Author (Year)	Country	Study design; number of patients	Age (years)	Male / Female	Cancer	Chemotherapy (Intervention vs Control)	Definition of cardiotoxicity	Study aim
Saito, 2009	Japan	Retrospective cohort; NR	Med = 53 (27 - 68)	NR	Metastatic breast cancer	trastuzumab, 5'-deoxy-5-fluorouridine, cyclophosphamide	NCI-CTC v2	Evaluate combination therapy with trastuzumab, 5'-deoxy-5-fluorouridine, cyclophosphamide as third- to six-line treatment.
Shimokawa, 2009	Japan	Retrospective cohort, 32	66 (50 - 83)	26/6	Refractory and relapsed SCLC	Amrubicin	NCI-CTCAE v3.0	Efficacy and safety of amrubicin in SCLC
Hongo, 2010	Japan	Retrospective cohort; 39	Med = 56 (33 - 69)	0/39	Endometrial carcinoma	Paclitaxel, pirarubicin, carboplatin	NCI-CTCAE v3.0	Evaluation of the efficacy and feasibility of TPC regimen
Takao, 2011	Japan	Prospective cohort, 62	Med = 53 (25 - 74)	0/62	Metastatic breast cancer	Gemcitabine	NCI-CTCAE v3.0	Safety of long-term gemcitabine monotherapy
Nakamura, 2012	Japan	RCT, 96 : control 49, Intervention 47	PH/DH: med = 51(34-65)/53 (28 - 63)	NR	Stage II or IIIA HER2-positive breast cancer	FEC + trastuzumab + paclitaxel vs FEC + trastuzumab + docetaxel	NCI-CTCAE v3.0	Compare pathologic complete response rates with FEC + trastuzumab + paclitaxel vs FEC + trastuzumab + docetaxel
Kurokawa, 2014	Japan	Prospective cohort, 54	Med = 66 (34 - 75)	42/12	HER2-positive gastric cancer	Trastuzumab, cisplatin	NCI-CTCAE v4.0	Efficacy and safety of trastuzumab and cisplatin in HER2-positive advanced gastric cancer

eTable 2: Studies characteristics. *(cont.)*

Author (Year)	Country	Study design; number of patients	Age (years)	Male / Female	Cancer	Chemotherapy (Intervention vs Control)	Definition of cardiotoxicity	Study aim
Murakami, 2014	Japan	Nonrandomised single-arm; 82	Med = 66 (44 - 74	65/17	Refractory small-cell lung cancer	Amirubin	NCI-CTCAE v3.0	Evaluate the effectiveness of amirubin therapy
Yamshiro, 2015	Japan	Retrospective and prospective cohort; 1890	Mean = 54.4 (18-88)	0/1890	HER2-positive incasive breast cancer	Trastuzumab	NCI-CTCAE v4.0	Efficacy and safety of trastuzumab in Japanese patients.
Hori, 2016	Japan	RCT; Control/Intervention: 144/132	DNR: mean = 7.04 (0.38), THP: 6.31 (0.39)	Control: 73/71, Intervention: 70/62	B-ALL	Pirarubicin (THP), Daunorubicin (DNR)	NCI-CTC	Comparing the toxicities of pirarubicin and daunorubicin
Shirakawa, 2016	Japan	Retrospective cohort, 394	Med = 62 (18 - 87)	223/171	Advanced or recurrent solid tumour	Mixed	NR	Examine CVDs in association with chemotherapy in solid tumours to establish prediction model
Koizumi, 2017	Japan	Retrospective cohort; 27	NR	18/9	Cancer	Antitumour	NR	Investigate the differences in the incidence and severity of adverse drug events in paediatric with and without cancer.

eTable 2: Studies characteristics. *(cont.)*

Author (Year)	Country	Study design; number of patients	Age (years)	Male / Female	Cancer	Chemotherapy (Intervention vs Control)	Definition of cardiotoxicity	Study aim
Nishikawa, 2017	Japan	Prospective cohort, 47	Med = 69 (32 - 89)	37/9	HER2-positive gastric cancer	Trastuzumab, paclitaxel	New York Heart Association classification system	Efficacy and safety of trastuzumab and paclitaxel in gastric cancer
Cha, 2013	South Korean	Retrospective cohort; 124	Med = 50 (27 - 730	0/124	Breast cancer	Trastuzumab	Grade I, asymptomatic decline in LVEF>10% from baseline; grade II, asymptomatic decline in LVEF below 50% or ≥ 20% from baseline; grade III, treatment-responsive heart failure; grade IV, severe or refractory heart failure, and grade V, death due to cardiac toxicity	Clinical presentation of trastuzumab-associated cardiac toxicity
Khan, 2012	Pakistan	Retrospective cohort; 301	Mean = 47 (18 - 81)	75/226	Mixed	5 -Fluorouracil	NR	To study cardiotoxicities, especially bradycardia in cancer patients treated with 5-Fluouracil

eTable 2: Studies characteristics. *(cont.)*

Author (Year)	Country	Study design; number of patients	Age (years)	Male / Female	Cancer	Chemotherapy (Intervention vs Control)	Definition of cardiotoxicity	Study aim
Wang, 1998	Taiwan	Prospective cohort; 41	Med = 62 (35-68)	33/8	Advanced colorectal cancer	5-fluorouracil (5-FU)	WHO	Efficacy and toxicity of weekly 24-hour infusion of high-dose 5-FU and leucovorin in Chinese patients with metastatic colorectal carcinoma
Chen, 2005	Taiwan	Prospective cohort; 45	Med = 47 (30 - 64)	NR	Breast cancer	Epirubicin + paclitaxel	NCI-CTC v2	Evaluate toxicity of a weekly administration of epirubicin and paclitaxel.
Juan, 2007	Taiwan	Prospective cohort; 19	Mean = 12.5 (4.6)	11/8	Osteogenic sarcoma, ALL, AML, neoblastoma, NHL	Standard chemotherapy using Taiwan Paediatric Oncology Group protocols (Anthracycline-based)	NR	Determine the changed in left ventricular function in children with malignancies undergoing long-term chemotherapy
Lu, 2007	Taiwan	Prospective cohort; 23	Med = 55 (33-73)	0/23	Metastatic breast cancer	Paclitaxel	NR	Efficacy of weekly paclitaxel in women with metastatic breast cancer
Hou, 2009	Taiwan	Retrospective cohort; 23	Med = 1 (0 - 5.9	15/8	Hepatoblastoma	Cisplatin + doxorubicin/ epirubicin	NR	Treatment experience of hepatoblastoma

eTable 2: Studies characteristics. *(cont.)*

Author (Year)	Country	Study design; number of patients	Age (years)	Male / Female	Cancer	Chemotherapy (Intervention vs Control)	Definition of cardiotoxicity	Study aim
Chen, 2010	Taiwan	Prospective cohort; 44	ALL: Med = 6.3 (0.4 - 11.5), AML: Med = 6.7 (0 - 11.1)	ALL: 11/12, AML: 11/10	ALL, AML	TPOG-911, TPOG-ALL-97 VHR, TPOG-ALL-2002 VHR (Anthracycline-based)	NR	Describe clinical features, molecular diagnoses and outcomes of infant leukaemia
Chien, 2016	Taiwan	Retrospective cohort; Control/Intervention: 3794/1051	Mean = 50.99	0/4845	Breast cancer	Chemotherapy +/- trastuzumab	HF (ICD-9 code 402.x1), hypertensive heart and chronic kidney disease with HF (ICD-9 codes 404.x1 and 404.x3), cardiomyopathies (ICD-9 code 425), HF (ICD-9 code 428) and cardiogenic shock (ICD-9 code 785.51)	The rate and risk of HF and/or CM in Asian women undergoing trastuzumab treatment
Tan, 2016	Taiwan	Retrospective cohort; CT: 1708, CRT: 1312	Mean = 51 (12)	NR	Breast cancer	Chemotherapy	Ischemic heart disease (ICD-9-CM 410-414) & congestive heart failure (ICD-(-CM 428)	Estimate the risk of CV complications with a follow-up period of 5 years.

eTable 2: Studies characteristics. *(cont.)*

Author (Year)	Country	Study design; number of patients	Age (years)	Male / Female	Cancer	Chemotherapy (Intervention vs Control)	Definition of cardiotoxicity	Study aim
Pinarli, 2005	Turkey	Prospective cohort; 34	Mean = 12.2 (3.44); Med = 12.5 (5 - 20)	23/11	NHL, ganglioneuroblastoma, HL, sarcoma, Wilms tumour, hepatoblastoma, neuroblastoma	Anthracycline	NA	Assess cardiac functions by electrocardiography, exercise electrocardiography testing, echocardiography and plasma BNP levels
Uysal, 2007	Turkey	Retrospective cohort;38	Med = 10	24/14	HL	COPP - ABVD based regimens, GPOH 90 treatment protocol, CHOP	NR	Epidemiologic and clinicopathological characteristics and treatment outcome of paediatric HL
İçli, 2012	Turkey	Retrospective cohort; 680	Med 9 wk/ 1yr: 47 (18 - 80)/ 48 (18 - 82)	NR	HER2-positive early breast cancer	Trastuzumab	NR	Compare the outcome of 9 weeks and 1 year adjuvant trastuzumab in early breast cancer patients
Arslan, 2013	Turkey	Retrospective cohort; 38	Mean = 7.86 (1 - 16)	21/17	Lymphoma & solid tumour	Anthracyclines	NA	Assess the value of the plasma levels of GDF-15 and TDI in detecting late myocardial dysfunction in childhood cancer survivors.

eTable 2: Studies characteristics. *(cont.)*

Author (Year)	Country	Study design; number of patients	Age (years)	Male / Female	Cancer	Chemotherapy (Intervention vs Control)	Definition of cardiotoxicity	Study aim
Gunaldi, 2016	Turkey	Retrospective cohort, 111	Med = 49 (33 - 72)	0/111	HER2-positive breast cancer	Trastuzumab	NYHA & NCI-CTCAE v4	The rate of cardiotoxicity related to trastuzumab and its potential risk factors

ABVD, doxorubicin/bleomycin/vinblastine/dacarbazine; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CHOP, cyclophosphamide/doxorubicin/ vincristine/ prednisolone; CM, cardiomyopathy; CML, chronic myeloid leukaemia; COPP, cyclophosphamide/vincristine/prednisone/procarbazine; CT, chemotherapy; CRT, combination of chemotherapy and radiotherapy; CV, cardiovascular; EF, ejection fraction; FEC, 5-fluorouracil/epirubicin/cyclophosphamide; FS, fractional shortening; GDF-15, growth-differentiation factor-15; HER2, human epidermal growth factor receptor 2; HF, heart failure; HL, Hodgkin's lymphoma; ICD, International Classification of Diseases; JCOG, Japan Clinical Oncology Group; LVEF, left ventricular ejection fraction; med, median; NA, not applicable; NCI-CTC, National Cancer institute-Common Toxicity Criteria; NCI-CTCAE v, National Cancer Institute-Common Terminology Criteria for Adverse Events; NR, not reported; NHL, non-Hodgkin lymphoma; NSCLC, non-small cell lung cancer; NYHA, New York Heart Association; RCT, randomised control trial; SCLC, small cell lung cancer; SWOG, Southwest Oncology Group; TDI, Tissue Doppler imaging; TKIs, tyrosine-kinase inhibitors; TPOG, Taiwan Pediatric Oncology Group ; WHO, World Health Organisation;

eTable 3: Quality of the included cohort studies.

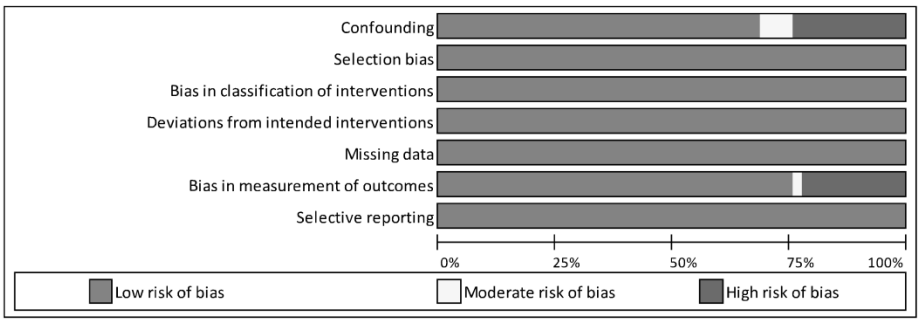
Study ID	Domain of bias in ROBINS-I assessment tool							Overall risk of bias
	A	B	C	D	E	F	G	
Arslan, 2013	Low	Low	Low	Low	Low	Low	Low	Low
Cai, 2014	Serious	Low	Low	Low	Low	Low	Low	Serious
Cha, 2013	Low	Low	Low	Low	Low	Low	Low	Low
Chen, 2005	Low	Low	Low	Low	Low	Low	Low	Low
Chen, 2010	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Chien, 2016	Low	Low	Low	Low	Low	Low	Low	Low
Dai, 2015	Moderate	Low	Low	Low	Low	Low	Low	Moderate
Furuse, 1997	Low	Low	Low	Low	Low	Low	Low	Low
Gronich, 2017	Low	Low	Low	Low	Low	Low	Low	Low
Gunaldi, 2016	Low	Low	Low	Low	Low	Low	Low	Low
Hayakawa, 2001	Low	Low	Low	Low	Low	Low	Low	Low
Hongo, 2010	Low	Low	Low	Low	Low	Low	Low	Low
Hou, 2009	Low	Low	Low	Low	Low	Serious	Low	Serious
Hu, 2010	Low	Low	Low	Low	Low	Low	Low	Low
Huang, 2012	Serious	Low	Low	Low	Low	Low	Low	Serious
Içli, 2012	Low	Low	Low	Low	Low	Low	Low	Low
Juan, 2007	Low	Low	Low	Low	Low	Low	Low	Low
Khan, 2012	Serious	Low	Low	Low	Low	Serious	Low	Serious
Koizumi, 2017	Low	Low	Low	Low	Low	Serious	Low	Serious
Kurokawa, 2014	Low	Low	Low	Low	Low	Low	Low	Low
Law, 2014	Serious	Low	Low	Low	Low	Serious	Low	Serious

eTable 3: Quality of the included cohort studies *(cont.)*

Study ID	Domain of bias in ROBINS-I assessment tool							Overall risk of bias
	A	B	C	D	E	F	G	
Lu, 2007	Serious	Low	Low	Low	Low	Serious	Low	Serious
Matsui, 2005	Moderate	Low	Low	Low	Low	Low	Low	Moderate
Matsuzaki, 1996	Serious	Low	Low	Low	Low	Serious	Low	Serious
Matsuzaki, 2000	Low	Low	Low	Low	Low	Serious	Low	Serious
Murakami, 2014	Low	Low	Low	Low	Low	Low	Low	Low
Nishikawa, 2017	Low	Low	Low	Low	Low	Low	Low	Low
Pinarli, 2005	Low	Low	Low	Low	Low	Low	Low	Low
Rajendranath, 2014	Low	Low	Low	Low	Low	Low	Low	Low
Roodpeyma, 2008	Low	Low	Low	Low	Low	Low	Low	Low
Saito, 2009	Low	Low	Low	Low	Low	Low	Low	Low
Sawa, 2006	Low	Low	Low	Low	Low	Low	Low	Low
Sawaki, 2004	Low	Low	Low	Low	Low	Low	Low	Low
Sekine, 1996	Low	Low	Low	Low	Low	Low	Low	Low
Senju, 2007	Low	Low	Low	Low	Low	Low	Low	Low
Shimokawa, 2009	Serious	Low	Low	Low	Low	Low	Low	Serious
Shirakawa, 2016	Moderate	Low	Low	Low	Low	Serious	Low	Serious
Takao, 2011	Serious	Low	Low	Low	Low	Low	Low	Serious
Takeda, 2007	Low	Low	Low	Low	Low	Low	Low	Low

Study ID	Domain of bias in ROBINS-I assessment tool							Overall risk of bias
	A	B	C	D	E	F	G	
Tan, 2016	Low	Low	Low	Low	Low	Low	Low	Low
Uysal, 2007	Low	Low	Low	Low	Low	Serious	Low	Serious
Wang, 1998	Serious	Low	Low	Low	Low	Low	Low	Serious
Wang, 2015	Low	Low	Low	Low	Low	Low	Low	Low
Xu, 2012	Serious	Low	Low	Low	Low	Serious	Low	Serious
Yamshiro, 2015	Serious	Low	Low	Low	Low	Low	Low	Serious

A, Confounding bias; B, Selection bias; C, Bias in classification of interventions; D, Bias due to deviations from intended intervention; E, Bias due to missing data; F, Bias in measurement of outcomes; G, Bias in selection of the reporter result.



eFigure 2: Risk of bias according to 7 domains in ROBINS-I of included cohort studies.

eTable 4: Risk of bias of included randomised trials.

Study ID	Outcome	Randomization process	Deviations from intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	Overall Bias
Hori, 2017	Cardiovascular toxicity	Low	Low	Low	Low	Low	Low
Atagi, 2005	Cardiovascular toxicity	Low	Low	Low	Low	Low	Low
Itoh, 2002	Cardiovascular toxicity	Low	Low	Low	Low	Low	Low
Luan, 2017	Cardiovascular toxicity	Low	Low	Low	Low	Low	Low
Matsuzaki, 1999	Cardiovascular toxicity	Low	Low	Low	Low	Low	Low
Muro, 2016	Cardiovascular toxicity	Low	Low	Low	Low	Low	Low
Nakamura, 2012	Cardiovascular toxicity	Low	Low	Low	Low	Low	Low
Tokuda,	Cardiovascular toxicity	Low	Low	Low	Low	Low	Low
Zhou, 2015	Cardiovascular toxicity	Low	Low	Low	Low	Low	Low

2.2 Incidence and characteristics of cardiotoxicities induced by anthracycline and anthracycline based chemotherapy regimens in Malaysian cancer patients.

2.2.1 Introduction

Since the initial investigations of the family of anthracycline drugs, they have been employed in the treatment of a wide variety of hematologic malignancies and solid tumours. Among others are lymphoma, leukaemia, breast cancer, and sarcoma. The use of anthracyclines in cancer treatments may be different from country to country. When compared to National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology, Malaysia have some chemotherapy protocols that are different for the treatment of solid cancer and haematological malignancies as outlined in Table 1-2 and Table 1-3.

Studies reporting incidence of ACT in adult population in Malaysia or other Asian countries are lacking. To address these gaps of knowledge, we conducted a retrospective study of ACT in three large tertiary hospitals in Malaysia. The key objectives of this study were to determine the incidence and types of ACTs that occur among cancer patients.

2.2.2 Methods

Study sample, Inclusion, and Exclusion Criteria
This retrospective-observational study was done in three tertiary level hospitals in Klang Valley. This study had been approved by Malaysia Medical Research and Ethics Committee (NMRR-15-612-24156)(Appendix 1), Monash University Human Research Ethics Committee (CF15/3029 – 2015001271) (Appendix 2), UKM Medical Centre Secretariat for Medical Research and Innovation (FF-2015-402) (Appendix 3), and UMMC Medical Research Ethics Committee (2016930-4304) (Appendix 4).

Data from medical records of all cancer patients receiving anthracycline-based chemotherapy at the hospitals between August 2016 and June 2017 were analysed retrospectively. Patients of all age range who received anthracycline for cancer therapy were eligible for inclusion. However, we exclude patients where chemotherapy was not administered, chemotherapy records were not available, anthracycline was not administered or they had incomplete anthracycline administered record.

The following data were recorded from medical records: demographics, past medical history, type of cancer, cancer treatment (chemotherapy: regimens, including type and cumulative dose of anthracycline, radiation therapy: field, dose and fraction) blood pressure, serum creatinine, ejection fraction, and cardiac event using a pretested data collection form (Appendix 5).

Anthracycline-induced Cardiotoxicity

Cardiotoxicity was defined as a diagnosis of left ventricular dysfunction, heart failure, cardiomyopathy, coronary artery disease, QT dispersion or arrhythmia; or a decrease in ejection fraction (EF) to less than 50%; or an absolute decrease in EF of 10% or more with or without heart failure signs or symptoms (subclinical)[45] after administration of any type of anthracyclines.

Statistical analysis

The relationship between demographic and clinical variables and ACT was assessed using univariate logistic regression with normative category or largest category as reference category. Odds ratio with confidence intervals and p-value were reported. For categorical variables, logistic regression analysis was used to assess each variable. Each continuous variable was tested in logistic regression as both a continuous and categorical variable. Variables with p-value <0.05 were considered significant risk factors. All analyses were performed using Stata 15.0 (StataCorp, College Station, TX).

2.2.3 Results

A total of 2034 patients who received anthracycline-based chemotherapy were eligible for inclusion in this study: 1191 were Malay, 540 Chinese, 252 Indian and 51 others. There were 781 males and 1253 females with a median age of 49 years (range 1 – 89 years). The median follow-up was 19.2 months (range: 0 – 219.8 months). Among these patients, 94 (4.6%) patients experienced ACT during the duration of follow-up. The demographic and clinical characteristics of study population and patients with ACT are summarised in Table 1.

Characteristic of patients with ACT

Patients experienced ACT received anthracycline between the ages of 12 – 82 years with a median age of 49 years. Of the patients who experienced ACT 56 (60%) were female and 38 (40%) were male. The results of the ethnicity categorisation revealed that the Malay patients experienced maximum ACT which was about 54%, followed by 32% in Chinese, 13% in Indian and 1% in other ethnicity. The incidence of ACT within a particular ethnic was between 2.0% to 5.6% with highest for Chinese and lowest for other ethnicity while 4.7% for Indian and 4.3% for Malay. Diffused large B-cell lymphoma (42, 45%), breast cancer (20, 21%) and acute myeloid leukaemia (12, 13%) were the three most common indications for anthracyclines treatment in these patients. Of these patients 20 (21%) had hypertension (Table 1).

Regarding the type of anthracycline received by these patients, 49 (52%) received doxorubicin, followed by 23% who received epirubicin and 18% who received a combination of anthracyclines. Others received daunorubicin, idarubicin or mitoxantrone. The incidence of ACT for specific types of anthracycline was between 1.5% and 9.1% with highest for idarubicin and lowest for daunorubicin, ACT incidence for doxorubicin, epirubicin and mitaxantrone were similar which is approximately 4.3%. ACT incidence for combination of anthracycline was 7.1%. The cumulative isotoxic equivalent doses received were between 17 – 536mg/m^2 with a median dose of 249.5mg/m^2.The results of the cumulative isotoxic equivalent doses categorisation revealed that maximum ACT were experienced by patients received doses between 201 – 300 mg/m^2. Of the patients who experienced ACT 66 (70%) received cyclophosphamide and 20 (21%) received chest radiation as concomitant treatment (Table 1).

Coronary artery disease (CAD), 30% were the most common type of ACT documented followed by subclinical, 23 and then arrhythmia which was 16% (Table 1, Figure 1). The associations between potential risk factors for ACT are presented in Table 2. Old age (≥ 50 years), a high cumulative dose (≥250mg/m^2), cardiovascular comorbid (hypertension, diabetes, and hypertension, dyslipidaemia and/or diabetes), diagnosis of haematological malignancy (acute lymphoblastic leukaemia and Hodgkin's lymphoma), diagnosis of breast cancer, concomitant use of cyclophosphamide and trastuzumab and past medication history of cardio-protective drugs (beta-blocker, angiotensin converting enzyme inhibitor or angiotensin receptor blocker) were the six significant risk factors for ACT in this study.

Table 2-1: Demographic and clinical characteristics of study population (N = 2034) and patients with anthracycline-induced cardiotoxicity (N = 94).

Characteristic	Full Population (N = 2034)		Patient with Cardiac Event (N = 94)	
	No	%	No	%
Age at primary cancer diagnosis				
Mean	45.6		55.1	
SD	17.2		15.2	
Median	49.0		49.0	
Range	1 - 89		12 - 82	
Gender				
Male	781	38.4	38	40.4
Female	1253	61.6	56	59.6
Race/ethnicity				
Malay	1191	58.6	51	54.3
Chinese	540	26.5	30	31.9
Indian	252	12.4	12	12.8
Others	51	2.5	1	1.1
Average BSA kg/m2				
Mean	1.61		1.63	
SD	0.23		0.22	
Median	1.61		1.61	
Range	0.31 – 2.52		1.11 – 2.26	
Primary diagnosis				
Acute lymphoblastic leukaemia	133	6.5	3	3.2
Acute myeloid leukaemia	222	10.9	12	12.8
Hodgkin's lymphoma	209	10.3	6	6.4
Diffuse large B-cell lymphoma	519	25.5	42	44.7
Other type of lymphoma	149	7.3	5	5.3
Others haematological cancer	5	0.2	0	0.0
Breast cancer	613	30.1	20	21.3
Sarcoma	127	6.2	4	4.3
Others solid tumour	57	2.8	2	2.1
Length of follow-up, months				
Mean	27.6		22.2	
SD	26.9		20.1	
Median	19.2		19.2	
Range	0 – 219.8		0 – 85.1	

Table 2-1: Demographic and clinical characteristics of study population (N = 2034) and patients with anthracycline-induced cardiotoxicity (N = 94). *(cont.)*

Characteristic	Full Population (N = 2034)		Patient with Cardiac Event (N = 94)	
	No	%	No	%
Type of anthracycline				
Daunorubicin	67	3.3	1	1.1
Doxorubicin	1128	55.4	49	52.1
Epirubicin	518	25.5	22	23.4
Idarubicin	33	1.6	3	3.2
Mitoxantrone	48	2.4	2	2.1
Combination of anthracyclines	240	11.8	17	18.1
Cumulative anthracycline exposure, mg/m^2				
Mean	239.4		213.7	
SD	102.0		108.6	
Median	249.5		249.5	
Range	17.0 – 639.2		17.0 – 536.5	
Categories of anthracycline exposure, mg/m^2				
1-100	242	11.9	17	18.1
101-150	203	10.0	11	11.7
151-200	241	11.8	13	13.8
201-250	362	17.8	19	20.2
251-300	461	22.7	16	17.0
301-350	322	15.8	10	10.6
351-400	103	5.1	6	6.4
401-450	57	2.8	0	0.0
≥ 451	43	2.1	2	2.1
Concomitant cytotoxic drugs				
Cyclophosphamide	1348	68.0	66	70.2
Paclitaxel	19	0.9	0	0.0
Trastuzumab	1	0.0	0	0.0
Cyclophosphamide and Paclitaxel	60	2.9	3	3.2
Cyclophosphamide and trastuzumab	29	1.4	5	5.3
Paclitaxel and trastuzumab	0	0.0	0	0.0
Cyclophosphamide, paclitaxel and trastuzumab	3	0.1	0	0.0
Chest radiation	526	25.9	20	21.3
Pre-anthracycline CV risk factors				
Hypertension only	212	10.4	20	21.3
Diabetes only	84	4.1	7	7.4
Dyslipidaemia only	28	1.4	2	2.1
Hypertension and diabetes	163	8.0	8	8.5
Hypertension and dyslipidaemia	62	3.0	8	8.5
Diabetes and dyslipidaemia	16	0.8	0	0.0
Hypertension, diabetes and dyslipidaemia	60	2.9	5	5.3

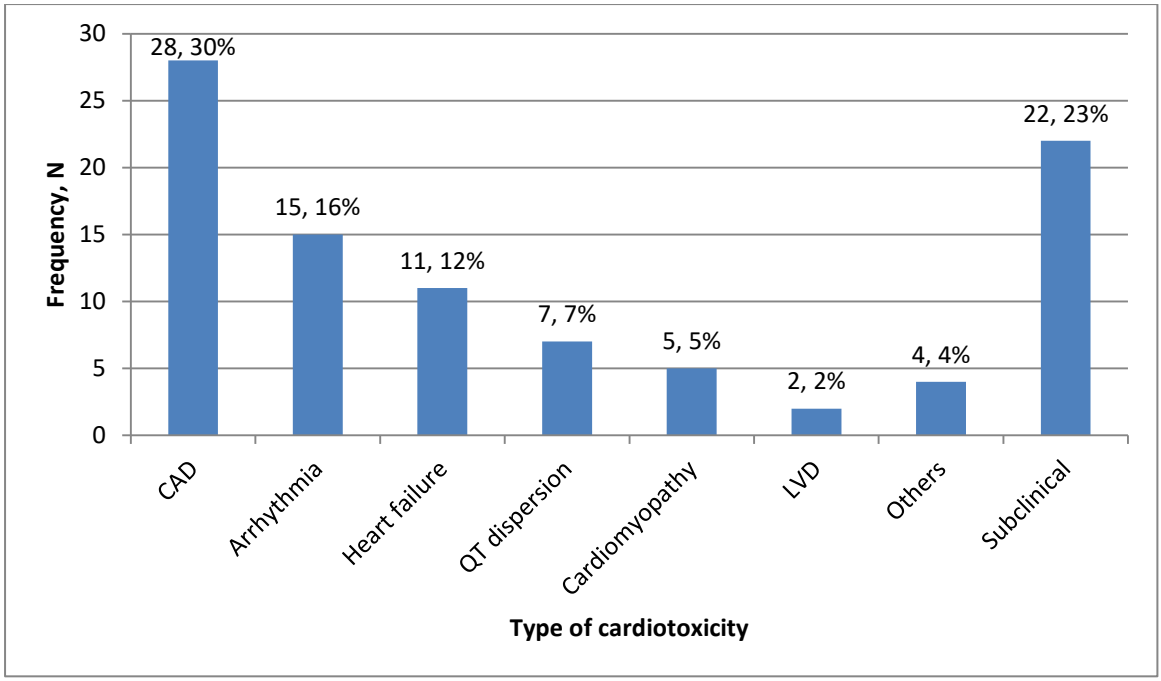


Figure 2-1: Frequency and percentage of different type of cardiotoxicity found among patients with anthracycline-induced cardiotoxicity (N = 94).
Others include supraventricular tachycardia, hypertension, bradycardia and first degree heart block.
CAD, coronary artery disease; LVD, left ventricular dysfunction.

Table 2-2: Univariate analysis of risk factor for anthracycline-induced cardiotoxicity.

Covariates	p-Value	Odds Ratio (95% CI)
Age (years)*	<0.001	1.04 (1.03 – 1.06)
Age ≥ 50 years	<0.001	3.81 (2.33 – 6.25)
Male	0.679	1.09 (0.72 – 1.67)
Race		
Malay	Ref	
Chinese	0.246	1.31 (0.83 – 2.09)
Indian	0.735	1.12 (0.58 – 2.13)
Others	0.430	0.45 (0.06 – 3.30)
Follow-up duration (month)	0.049	1.00 (1.00 – 1.00)
Body surface area (m^2)*	0.469	1.41 (0.55 – 3.56)
Cumulative dose (mg/m^2)*	0.013	1.00 (1.00 – 1.00)
Cumulative dose ≥ 250mg/ m^2	0.026	0.61 (0.40 – 0.94)
Cardiovascular comorbid	<0.001	2.70 (1.77 – 4.12)
No of cardiovascular comorbid*	<0.001	1.51 (1.23 – 1.87)
Type of cardiovascular comorbid		
Hypertension only	<0.001	3.48 (2.00 – 6.03)
Diabetes only	0.014	2.85 (1.24 – 6.52)
Dyslipidaemia only	0.241	2.41 (0.55 – 10.46)
Hypertension and diabetes	0.223	1.62 (0.75 – 3.49)
Hypertension and dyslipidaemia	<0.001	4.64 (2.08 – 10.33)
Hypertension, diabetes and dyslipidaemia	0.033	2.85 (1.09 – 7.46)
Primary diagnosis		
Acute lymphoblastic leukaemia	0.03	0.26 (0.08 – 0.86)
Acute myeloid leukaemia	0.20	0.65 (0.33 – 1.26)
Hodgkin's lymphoma	0.01	0.34 (0.14 – 0.80)
Diffuse large B cell lymphoma	Ref	
Other type of lymphoma	0.05	0.38 (0.15 – 0.98)
Breast cancer	0.001	0.38 (0.22 – 0.66)
Sarcoma	0.06	0.37 (0.13 – 1.05)
Others solid tumour	0.23	0.41 (0.10 – 1.75)
Haematological malignancy	0.02	1.72 (1.08 – 2.74)
Type of anthracycline		
Daunorubicin	0.28	0.33 (0.05 – 2.45)
Doxorubicin	Ref	
Epirubicin	0.93	0.98 (0.58 – 1.63)
Idarubicin	0.21	2.20 (0.65 – 7.47)
Mitoxantrone	0.95	0.96 (0.23 – 4.06)
Combination of anthracyclines	0.08	1.68 (0.95 – 2.97)
Concomittant cytotoxic agents	0.128	1.48 (0.89 – 2.44)
Cyclophosphamide	0.174	1.42 (0.86 – 2.37)
Cyclophosphamide and Paclitaxel	0.534	1.48 (0.43 – 5.15)
Cyclophosphamide and Trastuzumab	0.001	5.77 (2.00 – 16.69)
Chest radiation	0.300	0.77 (0.46 – 1.27)
Use of cardio-protective drugs	<0.001	3.97 (2.50 – 6.30)
No of cardio-protective drugs used*	<0.001	2.93 (2.14 – 4.02)

2.2.4 Discussion

To our knowledge, this is the first report on the incidence of ACT in a multi-ethnic population. The key findings from this study are that 4.6% of anthracycline recipients experienced ACT, and 35% received cumulative isotoxic equivalent doses between 201 – 300mg/m^2.

Reported incidence of ACT was varied across the globe. Pooled incidence as reported by a meta-analysis of eighteen studies conducted mainly in North America and Europe reported the incidence of overall cardiovascular events, subclinical and clinical, was 10.6%[22]. Two studies conducted in Japan reported very different ACT rate of 1.4%[46] and 23.5%[35]. This could be due to the relatively small number of patients, 114 and 34 relatively. With 2034 participants in our study, our incidence is in between the reported ACT incidence.

Congestive heart failure with clinical signs and symptoms is one of the earliest reported manifestations of ACT[4, 47]. Other reported manifestations include conduction abnormalities and dysrhythmias[47]. Cardiac arrhythmia was reported in sixteen (21%) elderly Chinese patients who received standard-dose daunorubicin as induction and post induction therapy for newly diagnosed acute myeloid leukaemia[48]. Recently, a case of sudden cardiogenic shock characterised by a severe left ventricular systolic dysfunction in a 60-year-old main treated with anthracycline-based chemotherapy for Hodgkin's lymphoma was reported[49]. In our studies, a wide range of cardiovascular diseases were reported after anthracycline therapy (Figure 2). Cardiac dysfunction were manifested as heart failure (n = 11), cardiomyopathy (n =5) or left ventricular dysfunction (n = 2). Twenty-four (26%) of our cases were conduction abnormality and dysrhythmias which were reported as arrhythmia (n = 15), QT dispersion (n = 7), supraventricular tachycardia (n = 1) and first degree heart block (n = 1).

Reviewers of eighteen North American- and Europe-based studies conclude that cumulative dose of anthracycline was the most consistently reported risk factor[22]. Other admissible predictors were chest radiotherapy, African-American ethnicity, very young or very old age, diabetes, hypertension, very high or very low body weight, or severe co-morbidities[22]. Studies had suggested cumulative dose of more than 350mg/m^2 as risk factor[33, 34]. A more recent study in Japan reported abnormal echocardiography incidences tripled in patients received anthracycline more than 300mg/m^2 compare to patients received total anthracycline dose of 300mg/m^2 or less[35]. Our findings corresponded to most of the reported risk factors including cumulative dose, old age, diabetes and hypertension. However, our study population was at risk for ACT at a lower cumulative dose (250mg/m^2) and a younger age (50 years). These may implicate that our population is more susceptible to ACT. We did not find ethnicity and very young age as a risk factor. Very young age was not associated with ACT in our study most probably due to the small number of included paediatric participants (n = 37). We did not assess the role of body weight and non-cardiovascular-related comorbidities.

Data were collected in three out of five cancer referral centres in central region of Malaysia, thus relinquishing selection bias. However, our study has some inherent limitations associated with its retrospective design. Despite limited availability of LVEF results, LVEF measured by echocardiography has inherent risk of 14% inter-observer variation[50]. Besides, our patients received uncontrolled regimen of chemotherapy which may be an unidentified confounder.

2.2.5 Conclusion

This population-based study is the first study to evaluate cardiotoxicity risk of anthracycline in multi-ethnic Asian population. The ACT incidence was 4.6%. Our findings will provide essential information for clinicians and Malaysian patients to make informed decisions.

3 Prediction Model for Anthracycline-induced Cardiotoxicity

We recognised the need to predict the individual risk for anthracycline-induced cardiotoxicity (ACT) based on the significant incidence worldwide which is expected to increase with the improved cancer survival rate[51]. Besides, various demographic and clinical characteristics had been identified as risk factors for ACT, however prediction model to stratify the ACT risk of patients with various type of cancer is unavailable. Thus, we developed and validate a demographic and clinical characteristics-based prediction model for ACT (Section 3.1, page 65). In addition, we pilot its usability in a prospective population (Section 3.2, page 88) and explore the opinion of healthcare professionals on the content and usability of the developed prediction model (Section 3.3, page 94).

3.1 Development and Validation of ACT Prediction Model

3.1.1 Introduction

A risk prediction model is a clinical prediction model that provides risk estimates for the presence of a diagnosis. It has become increasingly popular to support clinical decision-making[52]. Development of prediction models involves seven steps: deliberation of research question and initial data examination, coding of predictors, model specification, model estimation, assessment of model performance, internal validation and model presentation[53]. While proposed key measures for assessment of model performance are the model intercept, calibration slope, discrimination and clinical usefulness[53].

Cardiotoxicity is a broad term which encompassed cardiomyocytes damage, diastolic dysfunction, heart failure, conduction abnormalities, arrhythmias, and ischaemic heart disease[54-56]. The classification of anthracycline cardiotoxicities into acute, subacute or chronic is generally accepted although it could be merely a phenomenon being clinically identified at various stages[23]. Changes in left ventricular ejection fraction are the gold standard for reporting anthracycline-induced cardiotoxicities (ACT).

Risk factors for ACT had been identified soon after the introduction of doxorubicin in medical use. The effect of total cumulative dose on doxorubicin cardiotoxicity was first discovered in 1973[19] and supported by later studies[4, 29, 36, 37]. In addition, Minow *et al.* also found that radiation to the heart and concurrent use of cyclophosphamide were factors which increase the risk of doxorubicin-related cardiac toxicity[37]. Two years later, uncontrolled hypertension was found to be a risk factor in potentiating the development of doxorubicin cardiomyopathy[36]. von Hoff *et al.* found that dosing schedule of doxorubicin and advancing patient age were associated with congestive heart failure[4]. In another study, von Hoff *et al.* found that children were more susceptible to daunorubicin-induced cardiomyopathy[29]. Cobleigh *et al.* reported association of concomitant therapy with trastuzumab and anthracycline-induced cardiotoxicity slightly after approval of trastuzumab for medical use in the United States[38].

In an era of patient-centred care, it is important that clinicians provide tailored assessments of risk and benefit. Besides, identifying patients at risk may also play a role for identifying individuals who most benefit from prophylaxis measures. A clinical risk model can be a valuable tool to achieve these goals. Thus, the purpose of this study was to develop and internally validate a simple model that would be predictive of anthracycline-induced cardiotoxicity using demographic and clinical information.

3.1.2 Method

Ethical consideration

This study had been approved by Malaysia Medical Research and Ethics Committee (NMRR-15-612-24156)(Appendix 1), Monash University Human Research Ethics Committee (CF15/3029 – 2015001271) (Appendix 2), UKM Medical Centre Secretariat for Medical Research and Innovation (FF-2015-402) (Appendix 3), and UMMC Medical Research Ethics Committee (2016930-4304) (Appendix 4).

Study sample, Inclusion, and Exclusion Criteria

We conducted a retrospective observation study at three tertiary level hospitals in Klang Valley between August 2016 and June 2017. We reviewed the medical records of patients with diagnosis of cancer or prescribed with anthracycline therapy. Inclusion criteria included patients of all age range who received anthracycline for cancer therapy. Exclusion criteria included chemotherapy not administered, chemotherapy record not available, anthracycline not administered and incomplete anthracycline administered record. The sample size for model development was calculated using N = (n*10)/I where N is sample size, n is number of covariates and I is the estimated event rate in the population[57]. A minimum of 1300 patient were estimated to be required for a model with thirteen effective covariates and an estimated event rate of 10%[58, 59]. Number of covariates was determined based on literatures (refer Statistical analyses)

Data collection

The following data were recorded from medical records: demographics, past medical history, type of cancer, cancer treatment (chemotherapy: regimens, including type and cumulative dose of anthracycline, radiation therapy: field, dose and fraction) blood pressure, serum creatinine, ejection fraction, and cardiac event using a pretested data collection form (Appendix 5). The primary outcome, cardiac event after anthracycline administration, was defined as a diagnosis of left ventricular dysfunction, heart failure, cardiomyopathy, coronary artery disease, QT dispersion or arrhythmia; or a decrease in ejection fraction (EF) to less than 50%; or an absolute decrease in EF of 10% or more without heart failure signs or symptoms. According to Common Terminology Criteria for Adverse Events (CTCAE) version 4, such endpoint would be classified as grade II cardiac toxicity

and above[60]. Duration of follow up was calculated as the duration between the first dose of anthracycline and the last medical record entry.

Randomisation

These patients were randomised into two groups for model development stage as development set (⅔) and model validation stage as test set (⅓). Randomisation was done by ordering the patients according to age and then stratified to gender (female and male), four race groups (Chinese, Indian, Malay and others) and nine diagnosis (acute lymphoblastic leukaemia, acute myeloid leukaemia, breast cancer, diffuse large B-cell lymphoma, Hodgkin's lymphoma, others non-Hodgkin's lymphoma, others haematological malignancies, sarcoma and others solid tumour) resulting in 72 strata. Simple random samplings were then performed based on ration of 2:1 in each stratum and each patient was attributed to either development or test sample. Number 1, 2 or 3 was assigned to each patient continuously and repetitively until the patient list was exhausted. Patient with number 1 and 2 were attributed to development sample while patients assigned with number 3 were attributed to test sample. Description and number of patients in each stratum is shown in Table 3-1.

Model development stage

In development stage, the relationship between covariate and the outcome in each sample consisting of development set was assessed using univariate logistic regression. Odds ratio with confidence intervals and p-value were reported. For categorical covariate, logistic regression analysis was used to assess each variable. Each continuous covariate was tested in logistic regression as both a continuous and categorical variable. Covariate with univariate p-value <0.20 were considered in a series of multivariable logistic regression analyses. A backward stepwise selection process which eliminating the variables with the largest p-value first was used. Multicollinearity among significant predictors in multivariable logistic regression ($p < 0.05$) was tested using Spearman rank correlation and Variance Inflation Factor (VIF). The cut-off point for Spearman rank correlation and VIF were 0.8 and 10 respectively. The performance of the final model was assessed using Brier score, R squared, AUROC and the Hosmer-Lemeshow goodness-of-fit test. The final model was decided based on model with the highest area under the receiver operating characteristic curve (AUROC) and best calibration slope.

Internal validation stage

Both split-sample method and bootstrap procedure were performed for internal validation[61]. For split-sample method, we applied the split ⅓ method where ⅓ of the patients were kept for independent evaluation part and ⅔ of the patients for the logistic regression model estimation. Bootstrap procedure was done by bootstrap resampling where the logistic model was fitted in a bootstrap sample of 678 patients which was drawn with replacement from the test set. Averages of performance measures were taken over 100 repetitions. Model performance as determine by the AUROC, R-squared, Hosmer-Lemeshow goodness-of-fit test, brier score and calibration slope of the final model in development set, testing set, and bootstrap samples was compared. Predicted risk for individual

Table 3-1: Description and number of patients in each stratum in randomisation.

No.	Description			No of patients
	Gender	Race	Diagnosis	
1	Female	Chinese	Acute lymphoblastic leukaemia	16
2			Acute myeloid leukaemia	24
3			Breast cancer	208
4			Diffuse large B-cell lymphoma	47
5			Hodgkin's lymphoma	8
6			Others non-Hodgkin's lymphoma	8
7			Others haematological malignancies	0
8			Sarcoma	19
9			Others solid tumour	18
10		Indian	Acute lymphoblastic leukaemia	3
11			Acute myeloid leukaemia	13
12			Breast cancer	113
13			Diffuse large B-cell lymphoma	15
14			Hodgkin's lymphoma	9
15			Others non-Hodgkin's lymphoma	4
16			Others haematological malignancies	0
17			Sarcoma	8
18			Others solid tumour	3
19		Malay	Acute lymphoblastic leukaemia	39
20			Acute myeloid leukaemia	71
21			Breast cancer	273
22			Diffuse large B-cell lymphoma	159
23			Hodgkin's lymphoma	76
24			Others non-Hodgkin's lymphoma	53
25			Others haematological malignancies	1
26			Sarcoma	23
27			Others solid tumour	10
28		Others	Acute lymphoblastic leukaemia	3
29			Acute myeloid leukaemia	1
30			Breast cancer	15
31			Diffuse large B-cell lymphoma	7
32			Hodgkin's lymphoma	1
33			Others non-Hodgkin's lymphoma	1
34			Others haematological malignancies	0
35			Sarcoma	3
36			Others solid tumour	0

Table 3-1: Description and number of patients in each stratum in randomisation. (*cont.*)

No.	Description			No of patients
	Gender	**Race**	**Diagnosis**	
37	Male	Chinese	Acute lymphoblastic leukaemia	9
38			Acute myeloid leukaemia	34
39			Breast cancer	0
40			Diffuse large B-cell lymphoma	84
41			Hodgkin's lymphoma	10
42			Others non-Hodgkin's lymphoma	15
43			Others haematological malignancies	2
44			Sarcoma	28
45			Others solid tumour	10
46		Indian	Acute lymphoblastic leukaemia	11
47			Acute myeloid leukaemia	10
48			Breast cancer	1
49			Diffuse large B-cell lymphoma	12
50			Hodgkin's lymphoma	20
51			Others non-Hodgkin's lymphoma	11
52			Others haematological malignancies	0
53			Sarcoma	15
54			Others solid tumour	4
55		Malay	Acute lymphoblastic leukaemia	50
56			Acute myeloid leukaemia	66
57			Breast cancer	2
58			Diffuse large B-cell lymphoma	191
59			Hodgkin's lymphoma	85
60			Others non-Hodgkin's lymphoma	53
61			Others haematological malignancies	2
62			Sarcoma	27
63			Others solid tumour	0
64		Others	Acute lymphoblastic leukaemia	2
65			Acute myeloid leukaemia	3
66			Breast cancer	0
67			Diffuse large B-cell lymphoma	4
68			Hodgkin's lymphoma	0
69			Others non-Hodgkin's lymphoma	4
70			Others haematological malignancies	0
71			Sarcoma	4
72			Others solid tumour	2

Risk score and Predicted risk

Risk score for each covariate was calculated as regression coefficient of the covariates divided by smallest coefficient in the model and then rounded to the nearest integer[62]. Individual risk score was the total sum of points. Natural breakpoints of risk scores were evaluated to identify high- (> 10%), medium- (6 - 10%), low-risk (1 – 5%) and no-risk (< 1%) groups[63].

Statistical analyses

Candidate covariates were selected based on literature review and priori agreement of clinical importance. Previously reported ACT risk factors include age, gender, ethnicity, cumulative dose, hypertension, diabetes mellitus, concurrent cyclophosphamide, trastuzumab and paclitaxel use, chest radiation and follow-up duration[22, 30, 37-39]. Besides, we also included dyslipidaemia because it is a well-known risk factor for cardiovascular diseases[64]. Concurrent used of cardio-protective agents: beta-blocker, angiotensin converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB) were assessed because literatures suggested that they may decreased risk of ACT[65, 66].

Age and cumulative anthracycline exposure were categorised to identify their thresholds. Age was categorised according to The Framingham Heart Study[64]. Cumulative anthracycline exposure was categorised with an interval of 50mg/m^2 based on the different in literature findings[33-35]. The threshold was the lower limit of the lowest category with significant odds ratio in univariate analysis.

Considering all continuous variables were skewed in Shapiro-Wilk normality test, development set and test set were compared using Mann-Whitney test for continuous variables. Pearson's chi-squared test or Fisher's Exact test were used to compare categorical variables. All analyses were done using STATA 15.0 software (STATA Corp., College Station., Texas, USA).

3.1.3 Results

Descriptive analysis

We managed to review 4181 out of 7084 medical records of patients with diagnosis of cancer or prescribed with anthracycline therapy between August 2016 and June 2017. Only 2034 patients met the inclusion and exclusion criteria (Figure 3-1). These patients were randomised into two groups for model development stage as development set (⅔, n = 1356) and model validation stage as test set (⅓, n = 678).

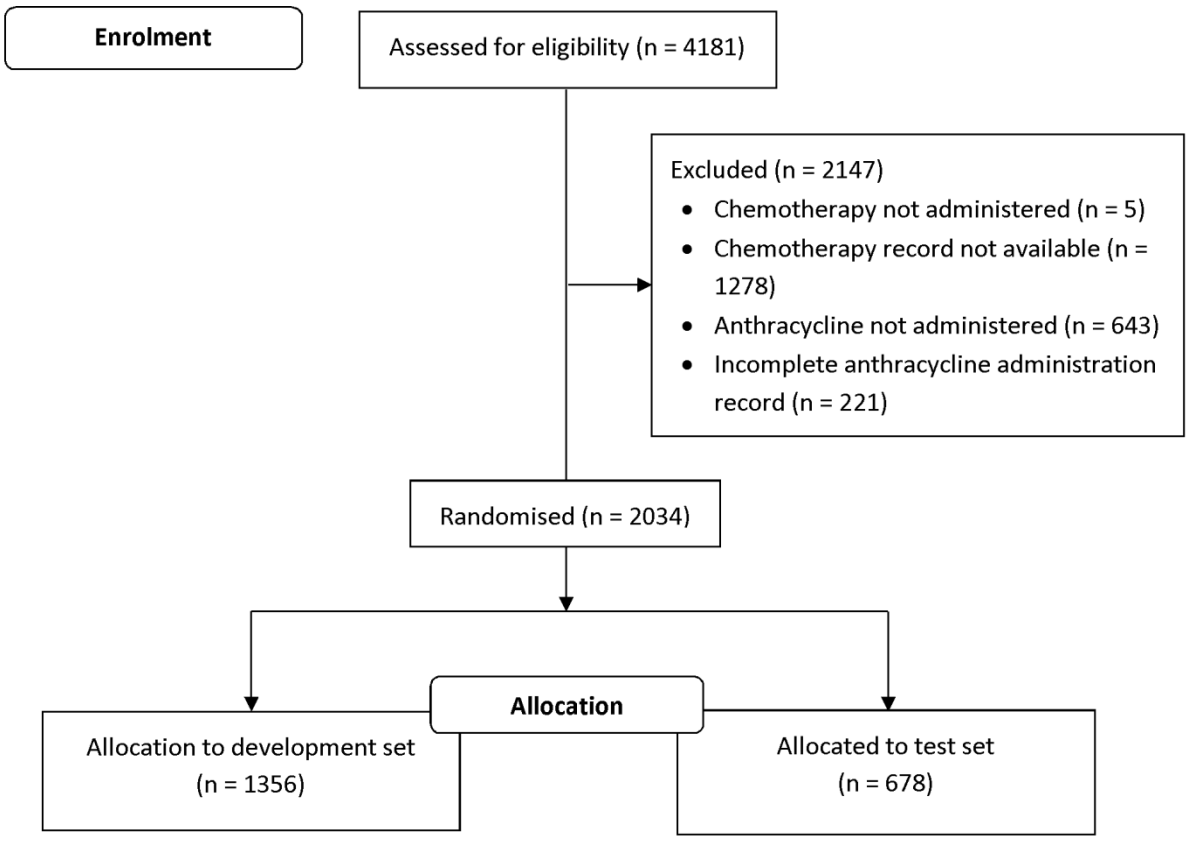


Figure 3-1: Flow diagram of patients' enrolment into the study.

Demographic and epidemiologic distributions

Characteristics of the study populations are summarised in Table 3-2. Of the 2034 patients who met the inclusion criteria and enrolled, 1237 were haematological malignancies and 797 were solid tumour. Median age of the 2034 patients was 49 years (range 1 – 89 years). There were 1253 (61.6%) women and 781 (38.4%) men. One thousand one hundred ninety one (58.6%) were Malay, 540 (26.5%) were Chinese, and 252 (12.4%) were Indian. The remaining 50 patients were Sabah and Sarawak indigenous, Indonesians, Myanmars, Filipinos, Syrians, and Yemeni. The median of average body surface area was 1.61m^2 (range 0.31 – 2.52). The most common cardiac comorbid was hypertension (24.4%) with more than half of them also presented with diabetes mellitus (32.8%) or dyslipidaemia (12.5%) or both (12.1%).

Cancer distribution

Cancers were classified to nine types: acute lymphoblastic leukaemia, acute myeloid leukaemia, breast cancer, diffuse large B-cell lymphoma, Hodgkin's lymphoma, others non-Hodgkin's lymphoma, others haematological malignancies, sarcoma and others solid tumour (Figure 3-2). Others haematological malignancies included chronic lymphoblastic leukaemia, chronic myeloid leukaemia and multiple myeloma. Others solid tumours were mainly lung cancer, uterine cancer, gastric cancer and oesophageal cancer. In our study populations, cancers in female peak at age 50 – 59 years old with breast cancer as the most common type of cancer (Figure 3-2). Cancer incidence was the highest in male patients aged 20 – 29 years with Hodgkin's lymphoma as the most common type cancer. Incidence of diffused large B-cell lymphoma was the highest in patients between 60 -69 years old. Most breast cancer cases (44.9%) and diffused large B-cell lymphoma cases (67.4%) were in Malay patients followed by Chinese and Indian (Figure 3-3).

Anthracycline usage

Among the five types of anthracycline included in this study, the most commonly used anthracycline was doxorubicin (Figure 3-4). It was used across all types of cancers with highest usage in the treatment of diffused large B-cell lymphoma and lowest usage in acute myeloid leukaemia. The second most used anthracycline, epirubicin, was mainly used in breast cancer treatment. The least used anthracycline in our population was idarubicin which was mainly used in acute myeloid leukaemia treatment. Daunorubicin, and mitoxantrone were also mainly use in the treatment of acute myeloid leukaemia.

Table 3-2: Demographic and clinical characteristics of patients in the development set (N = 1356) and test set (N = 678).

	Development Set (N = 1356)		Test Set (N = 678)		
Demographic or clinical Characteristic	**No**	**%**	**No**	**%**	**p-value[a]**
Age at primary cancer diagnosis					
Mean	45.7		45.4		
SD	17.19		17.31		
Median	49		49		0.8651
Range	1 - 89		3 - 88		
Gender					0.974
Male	521	38.4	260	38.3	
Female	835	61.6	418	61.7	
Race/ethnicity					1.000 [b]
Malay	794	58.6	397	58.6	
Chinese	359	26.5	181	26.7	
Indian	168	12.4	84	12.4	
Others	35	2.6	16	2.4	
Average BSA kg/m^2					
Mean	1.61		1.62		
SD	0.22		0.23		
Median	1.61		1.61		0.2629
Range	0.31 - 2.52		0.58 - 2.32		
Primary diagnosis					0.999 [b]
Acute lymphoblastic leukaemia	91	6.7	42	6.2	
Acute myeloid leukaemia	148	10.9	74	10.9	
Hodgkin's lymphoma	140	10.3	69	10.2	
Diffuse large B cell lymphoma	345	25.4	174	25.7	
Other type of lymphoma	100	7.4	49	7.2	
Others haematological cancer	3	0.2	2	0.3	
Breast cancer	409	30.2	204	30.1	
Sarcoma	84	6.2	43	6.3	
Others solid tumour	36	2.7	21	3.1	
Length of follow-up, months					
Mean	27.6		27.5		
SD	27.09		26.7		
Median	19.2		19.2		0.7306
Range	0 - 219.8		0 - 216.2		
Cumulative anthracycline exposure, mg/m2					
Mean	239.2		239.7		
SD	102.58		100.76		
Median	249.3		249.5		0.6933
Range	17.0 - 577.3		24.7 - 639.2		

Table 3-2: Demographic and clinical characteristics of patients in the development set (N = 1356) and test set (N = 678). (*cont.*)

	Development Set (N = 1356)		Test Set (N = 678)		
Demographic or clinical Characteristic	**No**	**%**	**No**	**%**	**p-value**[a]
Categories of anthracycline exposure					0.692[b]
1-100	171	12.6	71	10.5	
101-150	128	9.4	75	11.1	
151-200	161	11.9	80	11.8	
201-250	230	17.0	132	19.5	
251-300	308	22.7	153	22.6	
301-350	222	16.4	100	14.7	
351-400	69	5.1	34	5.0	
401-450	38	2.8	19	2.8	
≥ 451	29	2.1	14	2.1	
Concomitant cytotoxic drugs					0.284[b]
Cyclophosphamide	905	66.7	443	65.3	
Paclitaxel	10	0.7	9	1.3	
Trastuzumab	0	0.0	1	0.1	
Cyclophosphamide and Paclitaxel	42	3.1	18	2.7	
Cyclophosphamide and trastuzumab	18	1.3	11	1.6	
Paclitaxel and trastuzumab	0	0.0	0	0.0	
Cyclophosphamide, paclitaxel and trastuzumab	1	0.1	2	0.3	
Chest radiation	344	25.4	182	26.8	0.474
Pre-anthracycline CV risk factors					0.819[b]
Hypertension only	137	10.1	75	11.1	
Diabetes only	58	4.3	26	3.8	
Dyslipidaemia only	18	1.3	10	1.5	
Hypertension and diabetes	109	8.0	54	8.0	
Hypertension and dyslipidaemia	39	2.9	23	3.4	
Diabetes and dyslipidaemia	8	0.6	8	1.2	
Hypertension, diabetes and dyslipidaemia	37	2.7	23	3.4	
Cardiac events	62	4.6	32	4.7	0.881

[a] Mann-Whitney test for continuous variables and Pearson's chi-squared test for categorical variables unless otherwise specified.
[b] Fisher's Exact test

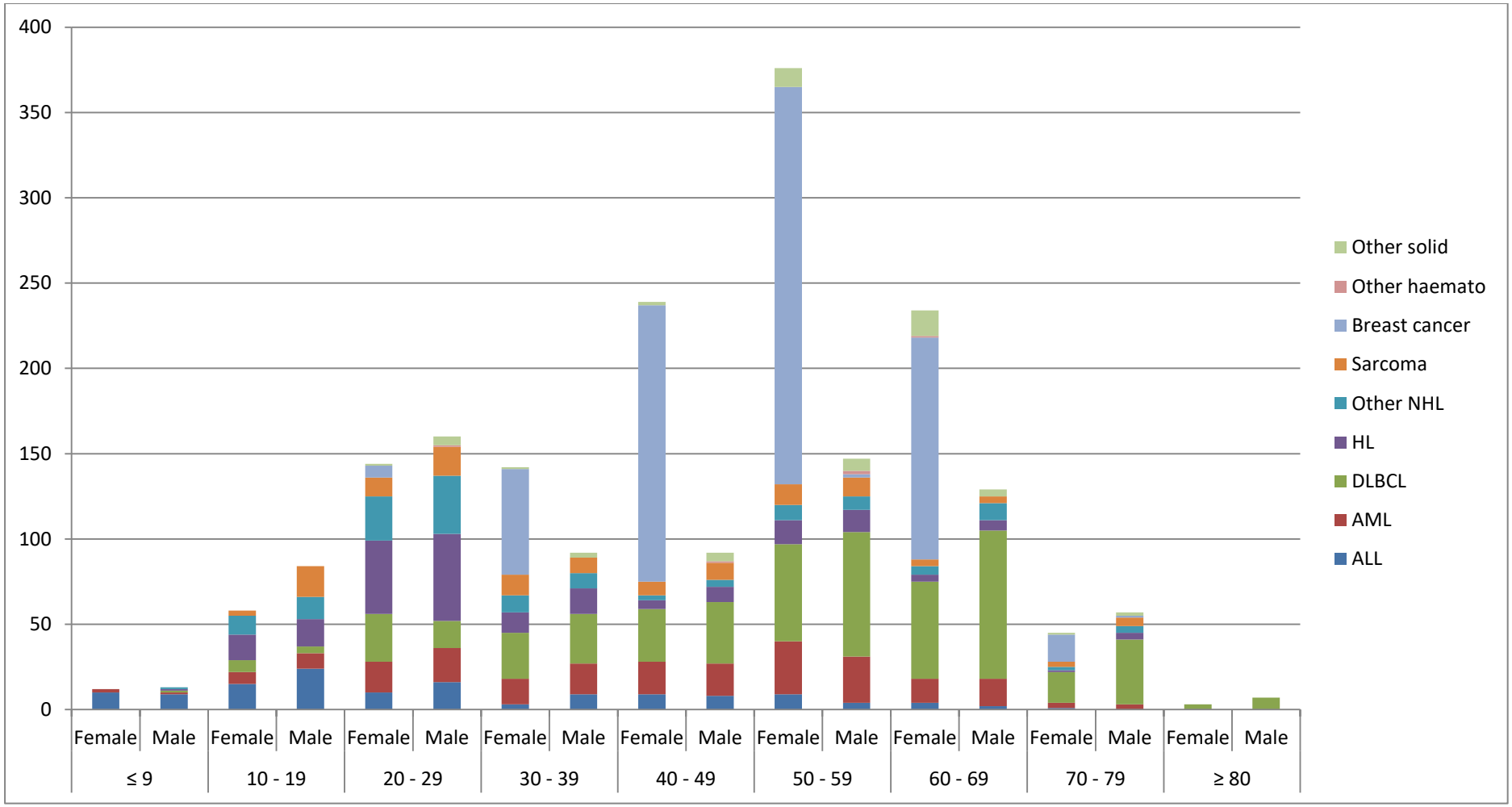


Figure 3-2: Cancer distribution according to age and gender (N = 2034).

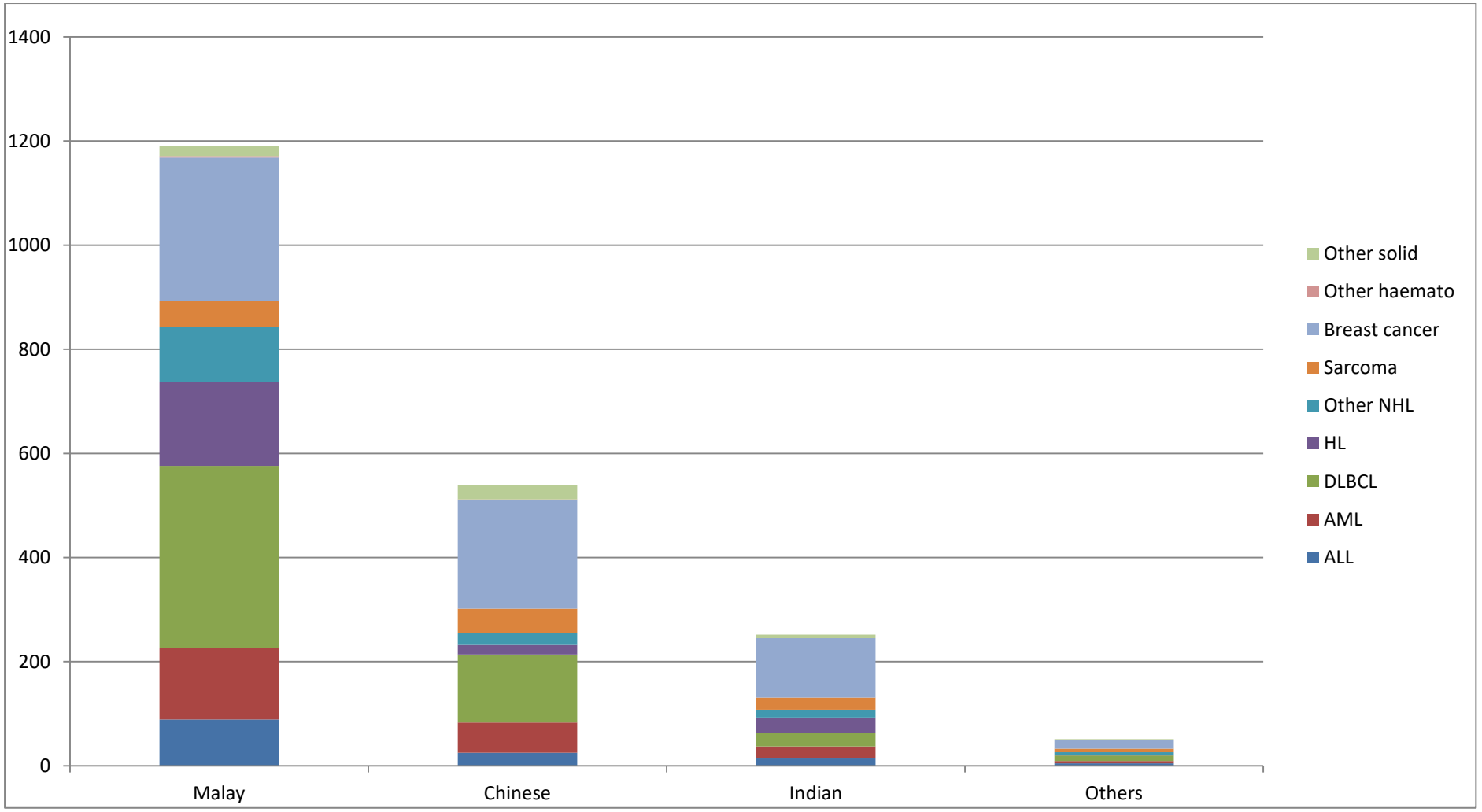


Figure 3-3: Cancers distribution according to races (N = 2034).

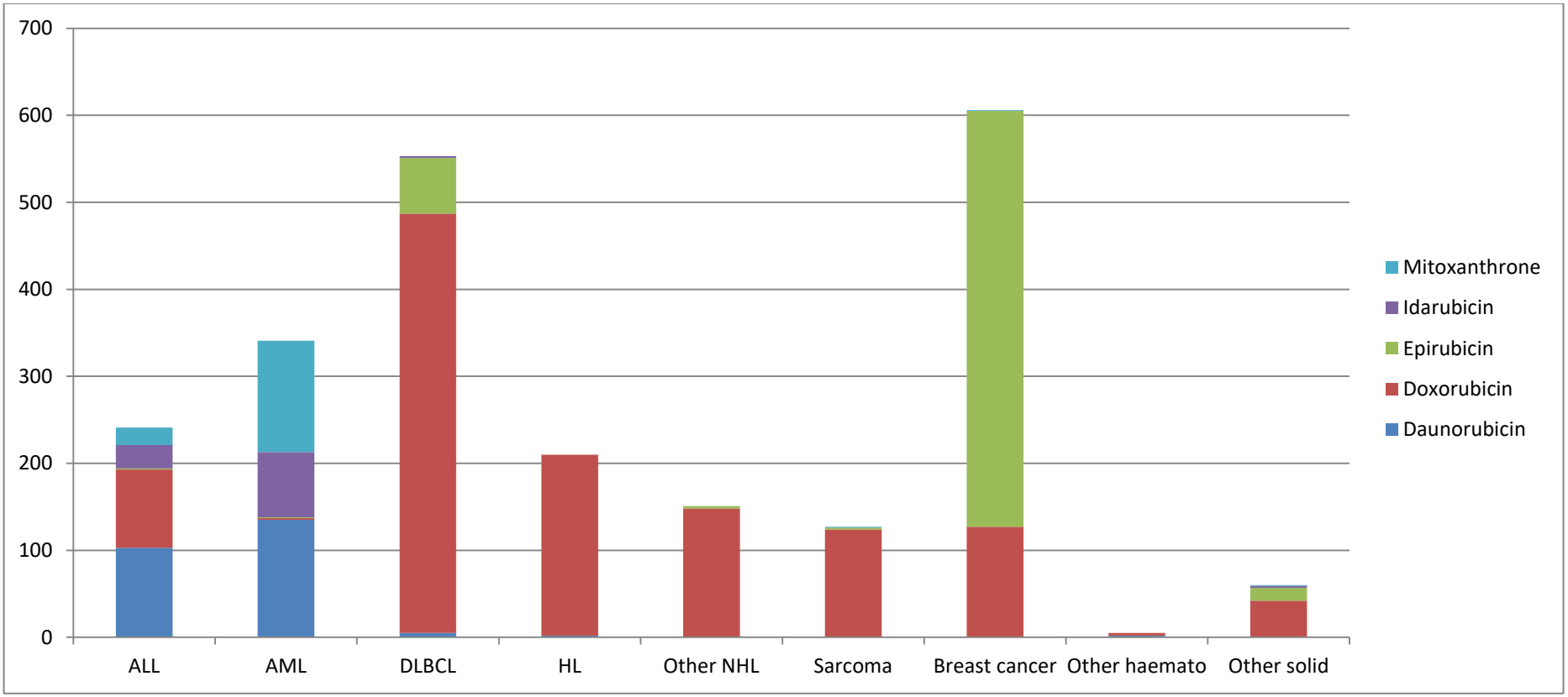


Figure 3-4: Anthracyclines used in the treatment of different types of cancers with 240 (11.8%) cases used more than one type of anthracycline.

Univariate analysis

The only demographic factors that increased the odds for cardiac event after anthracycline exposure was if the patient was aged 50 year-old and above. Clinical factor which were significantly increase the odds for cardiac event were presence of cardiovascular comorbid especially hypertension and diabetes mellitus, increase number of cardiovascular comorbid, concomitant cyclophosphamide and trastuzumab, concomitant use of cardio-protective agent and increase number of cardio-protective agent used (Table 3-3).

Table 3-3: Odds ratio from univariate analysis of covariates for the development set (N = 1356).

			95% CI	
Covariates	**p-Value**	**Odds Ratio**	**Lower**	**Upper**
Age (years)[a]	<0.001	1.052	1.032	1.072
Age ≥ 50 years	<0.001	5.813	2.929	11.537
Male	0.753	1.087	0.647	1.828
Race				
Malay		1.00 (Ref)		
Chinese	0.179	1.459	0.841	2.533
Indian	0.675	0.828	0.342	2.005
Others	1			
Follow-up duration (month)	0.062	1.000	0.999	1.000
Body surface area (m^2)[a]	0.997	0.998	0.320	3.111
Cumulative dose (kg/m^2)[a]	0.053	0.998	0.995	1.000
Cumulative dose ≥ 250 kg/m^2	0.155	0.686	0.408	1.153
Cardiovascular comorbid	<0.001	3.010	1.799	5.034
No of cardiovascular comorbid[a]	0.002	1.524	1.173	1.980
Type of cardiovascular comorbid				
Hypertension only	<0.001	4.328	2.245	8.343
Diabetes only	0.024	3.130	.162	8.432
Dyslipidaemia only	0.066	4.147	0.910	18..911
Hypertension and diabetes	0.154	1.933	0.782	4.777
Hypertension and dyslipidaemia	0.107	2.765	0.803	9.519
Hypertension, diabetes and dyslipidaemia	0.089	2.928	0.848	10.105
Primary diagnosis				
Acute myeloid leukaemia	0.471	0.761	0.362	1.60
Hodgkin's lymphoma	0.011	0.152	0.035	0.646
Diffuse large B cell lymphoma		1.00 (Ref)		
Other type of lymphoma	0.037	0.214	0.050	0.913
Breast cancer	0.002	0.345	0.177	0.672
Sarcoma	0.127	0.389	0.116	1.306
Other solid tumours	0.522	0.617	0.141	2.698
Haematological malignancies	0.102	1.595	0.912	2.792
Concomitant cytotoxic drugs	0.069	1.850	0.953	3.588
Cyclophosphamide	0.087	1.794	0.919	3.503
Cyclophosphamide and Paclitaxel	0.491	1.710	0.368	8.043
Cyclophosphamide and trastuzumab	0.007	6.709	1.693	26.589

Table 3-3: Odds ratio from univariate analysis of covariates for the development set (N = 1356). (cont.)

			95%	CI
Covariates	**p-Value**	**Odds Ratio**	**Lower**	**Upper**
Chest radiation	0.416	0.772	0.414	1.441
Use of cardio-protective drugs	<0.001	3.353	1.866	6.029
No of cardio-protective drugs used[a]	<0.001	2.602	1.728	3.917

Ref, reference group

*Age, body surface area, cumulative dose, no of cardiovascular comorbid and no of cardio-protective drugs used were treated as continuous variable in the analysis.

Multivariable analyses and the final model

The final model was selected from 51 possible models based on AUROC and calibration slope (Appendix 6).The final model was reduced to four covariates (Table 3-4). The four covariates, age more than 50 year-old, haematology malignancies, concomitant use of cardio-protective agent, and concomitant administration of cyclophosphamide and trastuzumab, can reliably (Prob > F = 0.0000) predict 3.8% of the variance in cardiac event. A low Brier score or mean squared error of 0.042 showed that the overall performance of the final model was good. Its discrimination power was acceptable as evidence by its AUROC of 0.75. The Hosmer-Lemeshow goodness-of-fit test (p = 0.82) revealed that the predicted likelihood was highly concordance to the observed likelihood. At the ACT incidence of study population of 4.6%, estimated overall rate of correct classification was 70%, with 70% of no cardiac event group correctly classified (specificity) and 66% of the cardiac event group correctly classified (sensitivity). At this threshold, this model has positive predictive value (PPV) of 9.6% and negative predictive value (NPV) of 97.0% (Figure 3-5).

Table 3-4: Regression coefficient and odds ratios from multivariable analysis and risk score of covariates in the final model.

Covariate	Regression Coefficient (Intercept = -0.011)	Odds Ratio (95% confidence interval)	p-Value	Risk score
Age more than 50 year-old	0.059	5.50 (2.71 - 11.19)	<0.001	2
Haematology malignancies	0.034	2.07 (1.26 - 4.12)	0.007	1
Concomitant use of cardio-protective agent	0.055	2.07 (1.12 - 3.82)	0.021	2
Concomitant administration of cyclophosphamide and trastuzumab	0.108	5.07 (1.31 - 19.62)	0.019	3

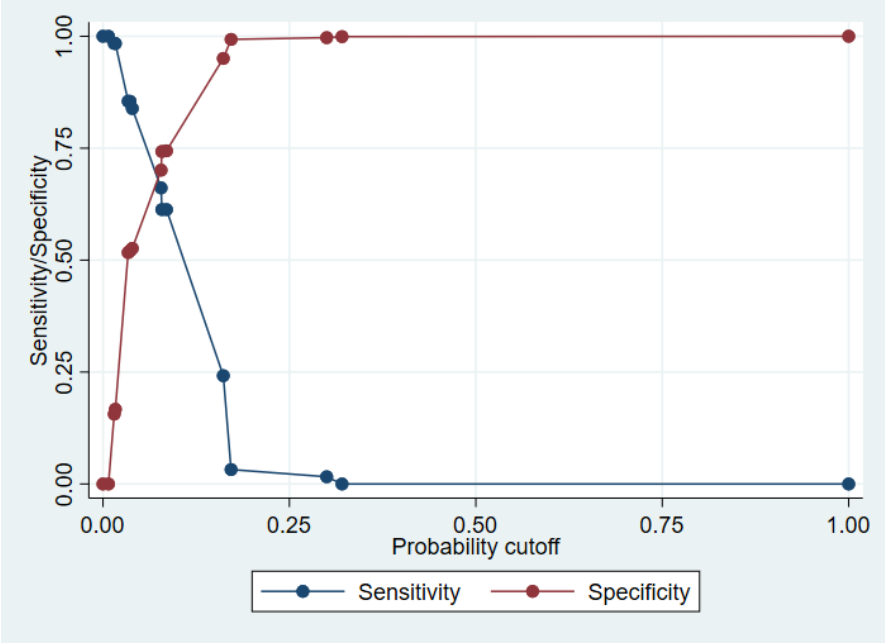
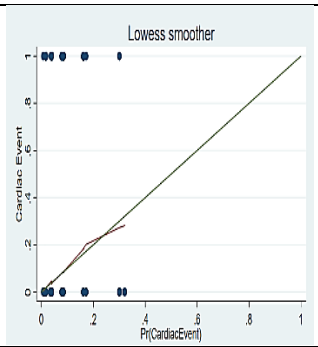
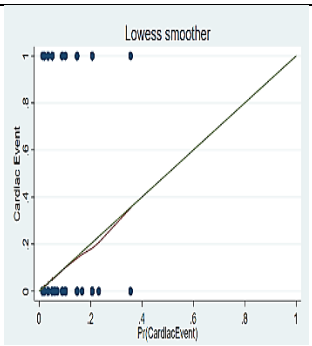
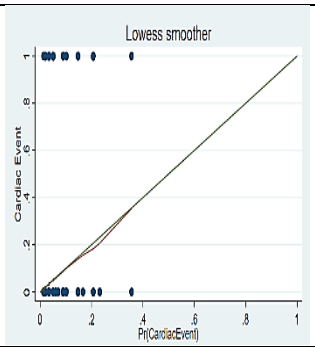


Figure 3-5: Sensitivity and specificity of the final model at different risk thresholds.

Model Validation

The stability of predictive capability of the final model was ascertained by applying the model in the test set and bootstrap samples. Table 3-5 showed the details of the internal validations.

Table 3-5: Estimated apparent and test performance in test set and bootstrap samples of the final model.

Performance measures	Apparent	Test set	Bootstrap
R-squared	0.0384	0.0442	0.0455
Brier score	0.0418	0.0427	0.0427
AUROC	0.7479	0.7217	0.7217
Hosmer-Lemeshow goodness-of-fit test	0.8178	0.9643	0.9643
Calibration slope			

Predicted risk

In the development set, the risk score for patients ranged from 0 to 7. The median risk score was 2, with cardiac event rate of 4.4%. The risk of cardiotoxicity increased with higher risk score, ranging from 0.5% for a risk score of 0 to 20% for a risk score of 6 to 7. Natural breakpoints of 0, 1 – 2, 3 – 4 and ≥5 points were selected to stratify the patients into cardiotoxicity event rates of <1% (no risk), 1 – 5 % (low risk), 6 – 10% (medium risk) and >10% (high risk). Similar pattern was observed in cardiotoxicity outcomes in the test set (Table 3-6, Figure 3-6).

Table 3-6: Risk of anthracycline-induced cardiotoxicity (ACT) by risk score in development and test set.

ACT risk	Risk score	Development Set			Test Set		
		Total, N	ACT, N	%	Total, N	ACT, N	%
No risk	0	203	1	<1	106	1	<1
Low	1 – 2	712	19	2.4	346	10	2.9
Medium	3 – 4	362	27	7.5	180	13	7.2
High	≥ 5	79	15	19.0	46	8	17.4

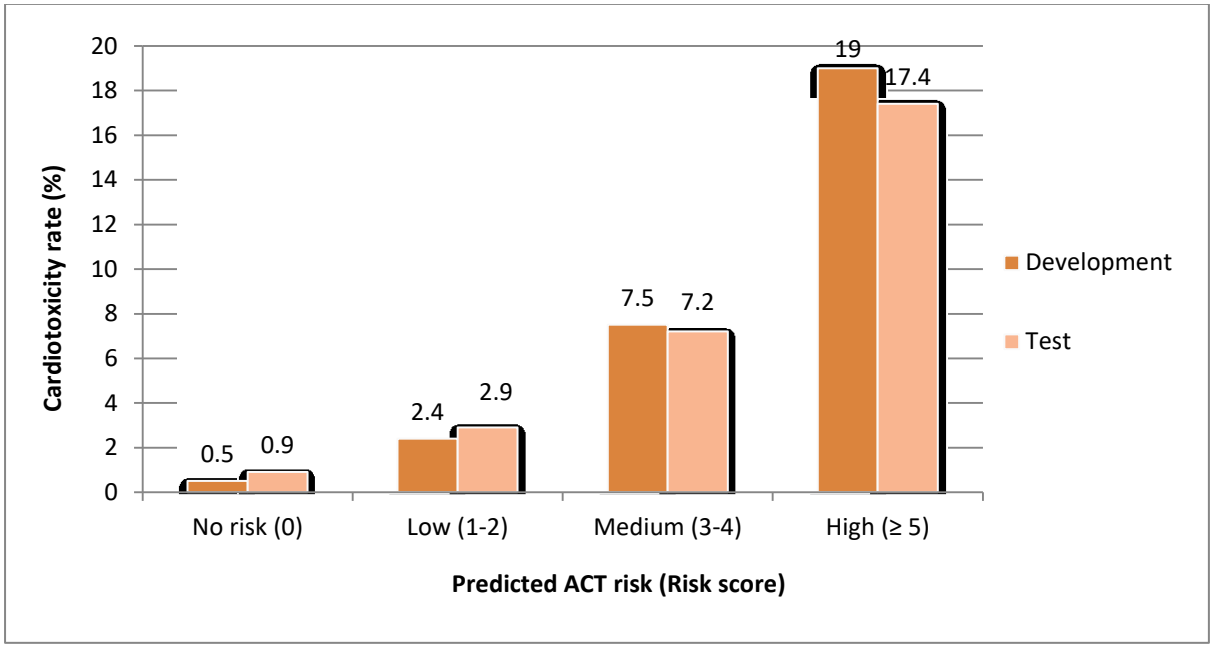


Figure 3-6: Cardiotoxicity rate by predicted risk and risk score in development and test set.

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3.1.4 Discussion

We developed and internally validated a prediction model to predict the cardiac event in patient receiving anthracycline for cancer treatment. Age more than 50 year-old, haematology malignancies, concomitant use of cardio-protective agent, and concomitant administration of cyclophosphamide and trastuzumab were the strongest independent predictors of cardiac event. This model has four predictors provided an AUROC of 0.75 and R^2 of 0.38%. At population with similar ACT incidence, at least nine of 100 anthracycline recipients (number need to screen, NNS = 19) with any of the predictors had most likely acquired ACT (PPV = 9.6%). One advantage of our prediction model is its practicality because it focused on covariates that are readily measured. Among the four covariates in the model, age, concomitant use of cardio-protective agent and concomitant administration of cyclophosphamide and trastuzumab had been identified in other studies.

In previous studies in United State of America, age less than 4-year[67] or more than 65-year[68] were found as risk factor for anthracycline-related cardiotoxicity. However, a study in Turkey reported similar incidence of decreased left ventricular ejection fraction between patients age less than 50-year and 55-year or more[69]. In our study, one (2.8%) of thirty-six included paediatric patients (age $\leq$ 12-year) developed cardiac event after fifteen months of follow-up. He was diagnosed sarcoma and treated with total cumulative dose of 148.6 mg/m^2 of doxorubicin at the age of 12-year. In our study population, patients received anthracycline were at cardiotoxicity risk at an age of more than 50-year, which is younger than previous studies. Age more than 50-year was chosen over age as continuous covariate in the final model because it gave a better calibration slope (Appendix 6).

To our knowledge, haematology malignancies were not reported before as a risk factor for anthracycline-related cardiotoxicity (ACT). However, previous study found that cardiovascular mortality in lymphoma patients was relatively high (14 - 30%)[70-72]. In our study, approximately 60% of the patients were diagnosed with haematology malignancies with diffused large B-cell lymphoma as the most common diagnosis (n = 519). Sixty-eight (72%) of cardiac events were in this group. Similarly, Khan et al. who conducted their study in Australia reported a higher cardiotoxicity incidence in patients with lymphoma (9.3%) than patients with breast cancer (6.7%). In contrary, Cardinale *et al.* who conducted their study in Italy reported that more cardiotoxicity occurred in patients with breast cancer (9.7%) than those with non-Hodgkin's lymphoma (6.2%)[59].

Previous studies found that prior cardiac pathology such as hypertension, coronary artery disease were additional risk factors for ACT[72]. In our population, hypertension and diabetes mellitus were significantly associated with ACT, but not in multivariable regression. However, we found that the use of cardio-protective agents, beta-blocker, angiotensin converting enzyme inhibitor (ACEi) and/or angiotensin II receptor blocker (ARB), in the treatment of underlying comorbid was an independent risk factor for ACT in multivariable analysis. This supported the finding by Reinbolt *et al.* who also reported a significantly greater use of beta blockers, ACE inhibitors, diuretics, and ARBs was demonstrated in the cardiotoxicity group in their study[73]. Their underlying comorbid may have increased their susceptibility to ACT; however, this hypothesis could only be confirmed with further analysis.

Another major finding of this study is confirmation of earlier work that has shown the ACT is augmented by additional of trastuzumab to adjuvant or neoadjuvant breast cancer treatment regimen with doxorubicin or epirubicin with cyclophosphamide and/or 5-fluorouracil had led to increase cardiac events[73, 74]. Our findings support these findings where cyclophosphamide and trastuzumab increase the risk for ACT approximately 5 times (95% CI, 1.31 - 19.62, p = 0.019). The worsening of ACT by trastuzumab could be related to its effect on cell repair mechanisms of the heart, which expresses HER2[75]. The similar effect was not observed for paclitaxel most likely because paclitaxel which is related to increase formation of cardiotoxic doxorubicinol [76] is not routinely administrated concurrently with anthracycline.

Our data does contrast with the literature regarding cumulative dose as risk factor. We did not find increase in cumulative dose of anthracycline to be significantly associated with cardiac event in both univariate and multivariable analysis. Gender and radiation to the chest was also not found to be a significant risk factor for cardiotoxicity. Taken together, these data suggest that risk factors for anthracycline-related cardiotoxicity in our population may be different from study population in previous studies.

The strengths of our multicentre study are its large sample size of Asia population and complete treatment data were available. The multicentre study design improves the representativeness of the prediction model. Cardio-toxic treatment was well characterised, including cumulative anthracycline doses and type of anthracycline derivate. We were able to analyse the influence of different anthracycline derivatives. Furthermore, the cohort represents a heterogeneous group of diagnosis, treatment across a broad spectrum of different doses of anthracycline, and a variety of ages at diagnosis and ethnicity.

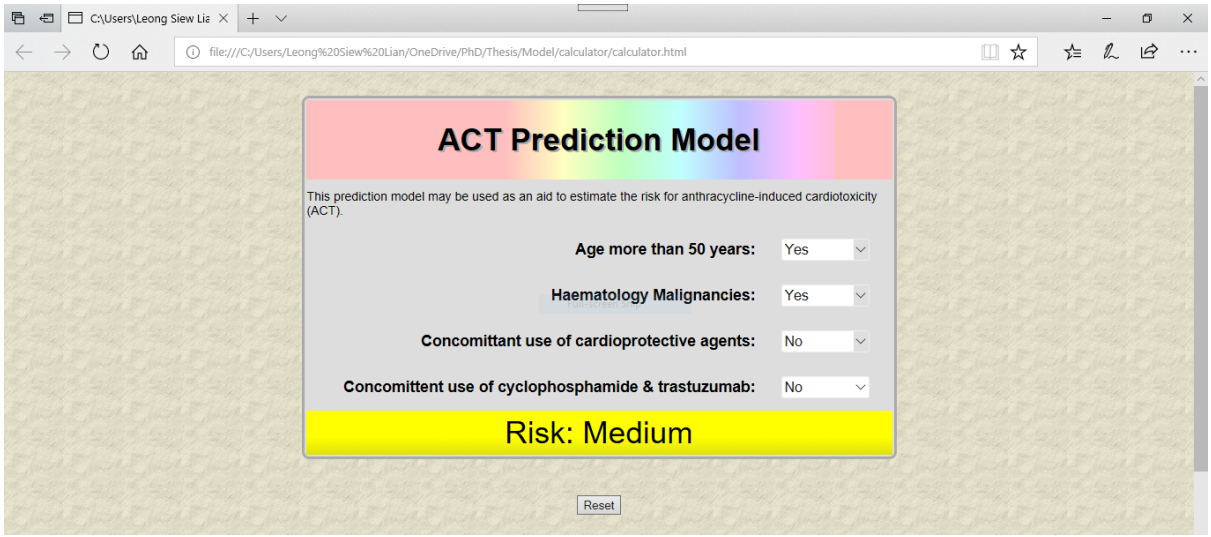
Compares to other adverse events prediction models which has PPV between 12.8% and 18.9%[77], the predictive ability of this model which is lower (9%). However, its ability to identify cancer patients at high risk for ACT is undeniable. Furthermore, it serves as the cornerstone of future ACT prediction model development.

There are several potential limitations of this study. The data was collected retrospectively rather than prospective. Thus, the credibility of our result is very much depending on accurate recordkeeping. Echocardiography monitoring record was limited in the study population. Approximately 19% were monitored prior to anthracycline administration and 20% had echocardiography monitored after anthracycline monitoring. Among them, only 4.5% were monitored during both point of time. Other cardiovascular risk factors such as smoking, high density lipoprotein cholesterol (HDL-C) level and triglyceride level were not able to be included due to lack of availability in medical record. Future prospective studies should include these four covariates and significant covariates in univariate analysis to further develop and validate prediction model for ACT.

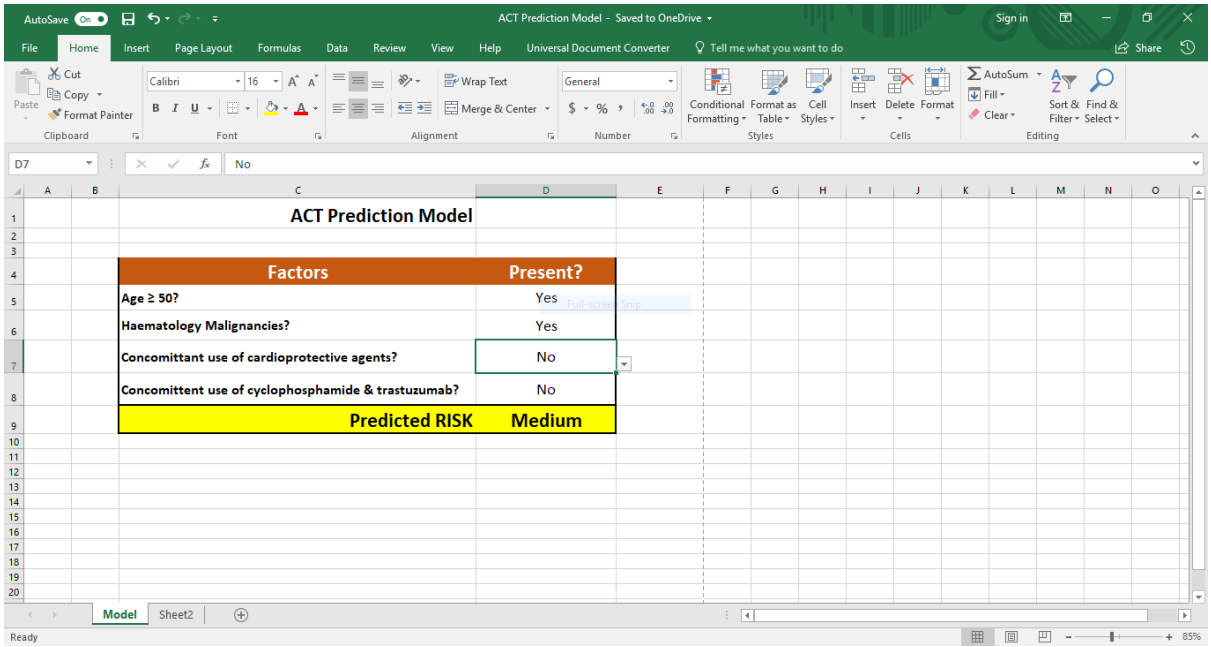
3.1.5 Conclusion

With future supportive prospective data, this four covariates prediction model with good overall performance, acceptable discrimination and stable prediction can potentially contribute in better prediction of ACT in cancer patients receiving anthracycline.

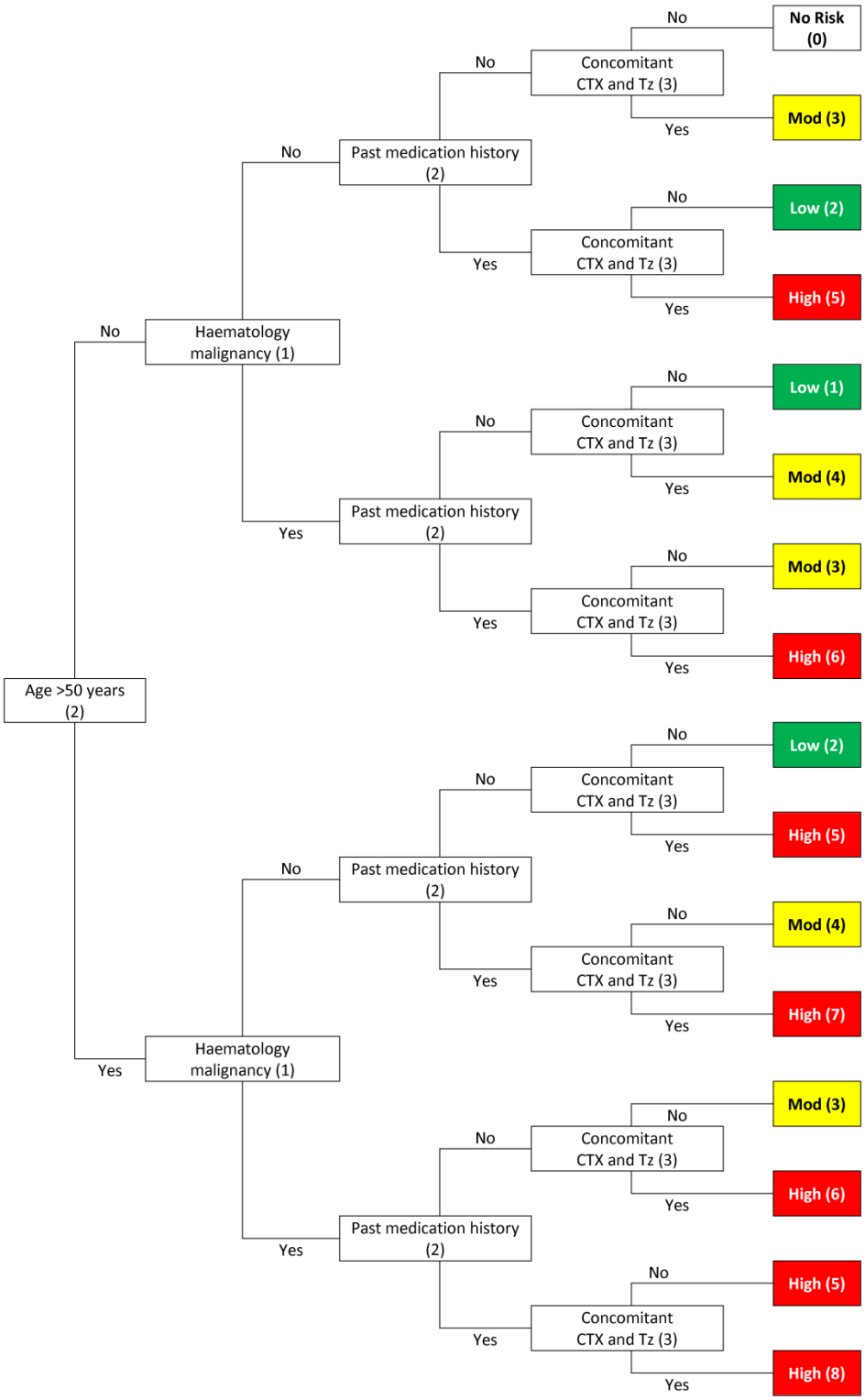
3.1.6 Web page version of the model



3.1.7 Excel spreadsheet of the model



3.1.8 Regression Tree of the model



Past medication history refers to angiotensin converting enzyme inhibitor, angiotensin receptor blocker or beta-blocker. CTZ, cyclophosphamide; Tz, trastuzumab

3.1.9 Poster Presentation

Part of this chapter has been presented as poster presentation during 12th MOH-AMM Scientific Meeting on 30 October-1 November 2017, Malaysia. The candidate, Leong Siew Lian was primarily responsible for data collection, analysis and preparation of the poster. The poster's co-authors, Chang Kian Meng, Oteh Maskon, Kong Zhen Ying, Habiba Nazeera Begum Kamarul Jaman, Kong Su Shan, Loong Ly Sia, S. Fadilah Abdul Wahid, Wan Fariza Wan Jamaludin, Shawal Faizal Mohamad, Samir Kumar Paul, Nathorn Chaiyakunapruk, Shaun Lee Wen Huey, contributed in various aspects of this research.

Predicting Anthracycline-Induced Cardiotoxicity: Development and Validation of Prediction Model Based on Demographic and Clinical Characteristics

12th MOH-AMM Scientific Meeting 2017
Incorporating the 20th NIH Scientific Seminar and the 5th International Clinical Conference in Emergency Medicine (ICCEM) 2017

SL Leong, EM Chang, O Maslina, ZY Kong, HNB Kamarul Imran, SS Kong, LS Loong, SF Abdul Wahid, WF Wan Jamaluddin, SF Mohamad, SK Paul, N Chaiyakunapruk, SWH Lee

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Introduction

- Anthracycline-based chemotherapy is the mainstay treatment for breast cancer therapy and many types of haematological malignancies even after introduction of newer antineoplastic agents such as monoclonal antibodies and angiogenesis inhibitors.
- Advancement in cancer management increased overall cancer survival, but also exposed cancer survivors to the risk of developing treatment-related complications such as cardiac complications of anthracycline-based treatment[1].
- The purpose of this study was to develop and validate a prediction model that would stratify patients at risk of anthracycline-induced cardiotoxicity.

Method

- A total of 2,034 patients treated with anthracycline from three hospitals in Klang Valley, Malaysia were included into this study.
- The clinical data were collected, and cardiotoxicity outcomes were defined as a diagnosis of left ventricular dysfunction, heart failure, cardiomyopathy, coronary artery disease, QT dispersion or arrhythmia; or a decrease in ejection fraction to less than 50%; or an absolute decrease in ejection fraction of 10% or more without heart failure signs or symptoms.
- Multivariable logistic regression analysis was used to determine covariates that contribute to cardiac event after anthracycline treatment.
- Multicollinearity among significant predictors in multivariable logistic regression ($p < 0.05$) was tested using Spearman rank correlation (rho <0.8) and Variance Inflation Factor (VIF <10).
- The final model was decided based on model with the highest area under the receiver operating characteristic curve (AUROC) and best calibration slope.
- It was then validated internally using split-sample and bootstrapping technique by comparing its performance (Brier score, R squared, AUROC and the Hosmer-Lemeshow goodness-of-fit test).
- A risk score was constructed by assigning the number of points to each covariate[2].

Table 1: Demographic and clinical characteristics of study population.

	Development Set (N = 1356)		Test Set (N = 678)		p-value*
Demographic or clinical Characteristic	No	%	No	%	
Age at primary cancer diagnosis					
Mean	45.7		45.4		
SD	17.19		17.31		
Median	49		49		0.865
Range	1 - 89		3 - 88		
Gender					0.974
Male	521	38.4	260	38.3	
Female	835	61.6	418	61.7	
Race/ethnicity					1.000**
Malay	794	58.6	397	58.6	
Chinese	359	26.5	181	26.7	
Indian	168	12.4	84	12.4	
Others	35	2.6	16	2.4	
Average BSA kg/m^2					
Mean	1.61		1.62		
SD	0.22		0.23		
Median	1.61		1.61		0.263
Range	0.31 - 2.52		0.58 - 2.32		
Primary diagnosis					0.999**
Acute lymphoblastic leukaemia	91	6.7	42	6.2	
Acute myeloid leukaemia	148	10.9	74	10.9	
Hodgkin's lymphoma	140	10.3	69	10.2	
Diffuse large B cell lymphoma	345	25.4	174	25.7	
Other type of lymphoma	100	7.4	49	7.2	
Others haematological cancer	3	0.2	2	0.3	
Breast cancer	409	30.2	204	30.1	
Sarcoma	84	6.2	43	6.3	
Others solid tumour	36	2.7	21	3.1	
Length of follow-up, months					
Mean	27.6		27.5		
SD	27.09		26.7		
Median	19.2		19.2		0.731
Range	0 - 219.8		0 - 216.2		
Cumulative anthracycline exposure, mg/m^2					
Mean	239.2		239.7		
SD	102.58		100.76		
Median	249.3		249.5		0.693
Range	17.0 - 577.3		24.7 - 639.2		

*Mann-Whitney test for continuous variables and Pearson's chi-squared test for categorical variables unless otherwise specified.
**Fisher's Exact test

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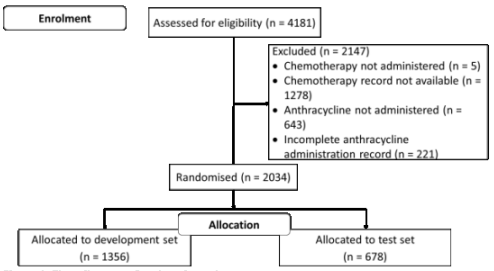

Figure 1: Flow diagram of patients' enrolment.

Table 2: Odds ratios and score of covariates in the final model.

Covariate	Regression Coefficient (Intercept = -0.011)	Odds Ratio (95% CI)	p-Value	Points assigned
Age more than 50 year-old	0.059	5.50 (2.71 - 11.19)	<0.001	2
Haematology malignancies	0.034	2.07 (1.26 - 4.12)	0.007	1
Concomitant use of cardio-protective agent	0.055	2.07 (1.12 - 3.82)	0.021	2
Concomitant administration of cyclophosphamide and trastuzumab	0.108	5.07 (1.31 - 19.62)	0.019	3

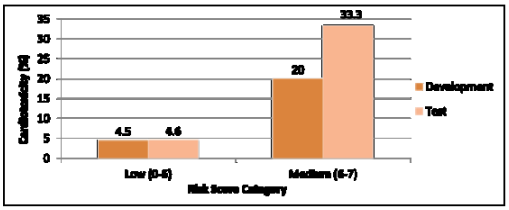

Figure 2: Cardiotoxicity rate by risk score category.

Discussion & Conclusion

- We developed and internally validated a prediction model to predict anthracycline-induced cardiotoxicity (ACT) risk in patient received anthracycline for cancer treatment.
- This multivariable prediction model provided an AUROC of 0.75 and R^2 of 0.38%. At optimum risk threshold of 6%, the model is more capable of eliminating patients at risk for ACT than identifying patient at risk for cardiotoxicity (PPV= 10.3%, NPV= 97.6%).
- Our patients were at cardiotoxicity risk at an age of more than 50-year, which is younger than previous study[3].
- Our findings supported findings on the association of use of beta blockers, ACE inhibitors, diuretics, and ARBs with increased ACT[4] and ACT is augmented by additional of trastuzumab to adjuvant or neoadjuvant breast cancer treatment[4,5].
- Although haematology malignancies were not reported before as a risk factor for ACT, cardiovascular mortality in lymphoma patients was relatively high (14 - 30%)[6-8].
- The strengths of our study are its large sample size of Asia population and complete treatment data were available. Cardio-toxic treatment was well characterised, including cumulative anthracycline doses and type of anthracycline derivate.
- The study limitation is the data was collected retrospectively, thus, the credibility of our result is very much depending on accurate recordkeeping.
- The model developed is the first and only model developed based on Asia population to predict anthracycline induced cardiotoxicity, using covariates that are readily measured. Further validation with prospective cohort is required for credibility.

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3.2 Utility Evaluation of Prediction Model in Clinical Settings: A Pilot Study

3.2.1 Introduction

A clinical prediction models improve understanding of the determinants of the course of outcome of patients with a particular condition[78]. It can be applied to several challenging clinical scenarios and assist medical-decision-making. However, models developed from retrospective study design have a few limitations. This mainly arise from the accuracy in patient's selection[79] as missing data or inaccuracy in recording may lead to selection bias. To overcome these limitations, most models developed warrant external validation using a prospective population to ensure the data are valid and reproducible[80].

In this pilot study, we wish to evaluate the usability of the prediction model which we have developed previously using a prospective cohort of patients who were treated for haematology malignancies.

3.2.2 Method

Ethical consideration
This study had been approved by Malaysia Medical Research and Ethics Committee (NMRR-15-612-24156) (Appendix 1), Monash University Human Research Ethics Committee (CF15/3029 – 2015001271) (Appendix 2) and UKM Medical Centre Secretariat for Medical Research and Innovation (FF-2015-402) (Appendix 3).

Study sample, Inclusion, and Exclusion Criteria
The cohort was prospectively identified from Hospital Ampang, Selangor and UKM Medical Centre, Kuala Lumpur. Patients who were planned for anthracycline-based chemotherapy were screened for eligibility before recruitment. We recruited patients from August 2015 until June 2016 and followed-up consented patient from inclusion until June 2017. Inclusion criteria were patient aged above 18 years old who were diagnosed with lymphoma. We excluded patient who has history of prior anthracycline use and/or cardiac irradiation because their heart function may be caused by previous treatment.

Characteristic of Prediction model
The prediction model was internally validated using both split-sample method and bootstrap procedure. Key factor used in the model are age more than 50 year-old, haematology malignancies, concomitant use of cardio-protective agent, and concomitant administration of cyclophosphamide and trastuzumab. The AUROC is 0.75 and R^2 is 0.38%. At population with ACT incidence of 4.6%, the NNS and PPV of the model is 19 and 9.6%.

Data collection

Demographics, past medical history, type of cancer and cancer treatment (chemotherapy: regimens, including type and cumulative dose of anthracycline, radiation therapy: field, dose and fraction) were recorded. Blood pressure, liver function, renal function (serum creatinine) and cardiac function were followed-up periodically using a pretested data collection form (Appendix 5).

Cardiac function monitoring was done using measures as listed below.

1. Echocardiogram: Echocardiogram (echo) was done by certified sonographer using a calibrated transthoracic echocardiography machine. Echo was done after participant consented and within 1 week before the first dose of anthracycline and repeated after at least one month after the last dose of anthracycline. Cardiotoxicity is defined as decline in LVEF to less than 50%; or an absolute decrease in EF of 10% or more with or without heart failure signs or symptoms[45].

2. 12- Lead electrocardiography: 12-Lead electrocardiography (ECG) was done by nurse or doctor using a calibrated electrocardiography machine. QT interval was determined by averaging five QT intervals of the same results. Any QT interval greater than or equal to 0.45 seconds is considered prolonged[45]. ECG was done after participant consented and within 1 week before the first dose of anthracycline and repeated at least one month after the last dose of anthracycline.

Statistical analyses

Continuous data are reported as median (range) and mean (standard deviation. Categorical variables are reported as n (%). Individual anthracycline-induced cardiotoxicity (ACT) risk score was the sum of score for each factor (Table 3-7) the patient had and classified into no-, low-, moderate- and high-risk groups (Table 3-8).

Table 3-7: Risk score for each risk factor used to estimate individual risk for anthracycline-induced cardiotoxicity.

Risk factor	**Risk score**
Age more than 50 year-old	2
Haematology malignancies	1
Concomitant use of cardio-protective agent	2
Concomitant administration of cyclophosphamide and trastuzumab	3

Table 3-8: Four anthracycline-induced cardiotoxicity (ACT) risk groups according to the total risk score of each patient.

ACT risk group	**Total risk score**
No risk	< 1
Low	1 – 2
Medium	3 – 4
High	≥ 5

3.2.3 Results

We recruited 48 out of 102 patients screened for eligibility. Thirty-four patients remained active at the end of data collection period (Figure 3-7).

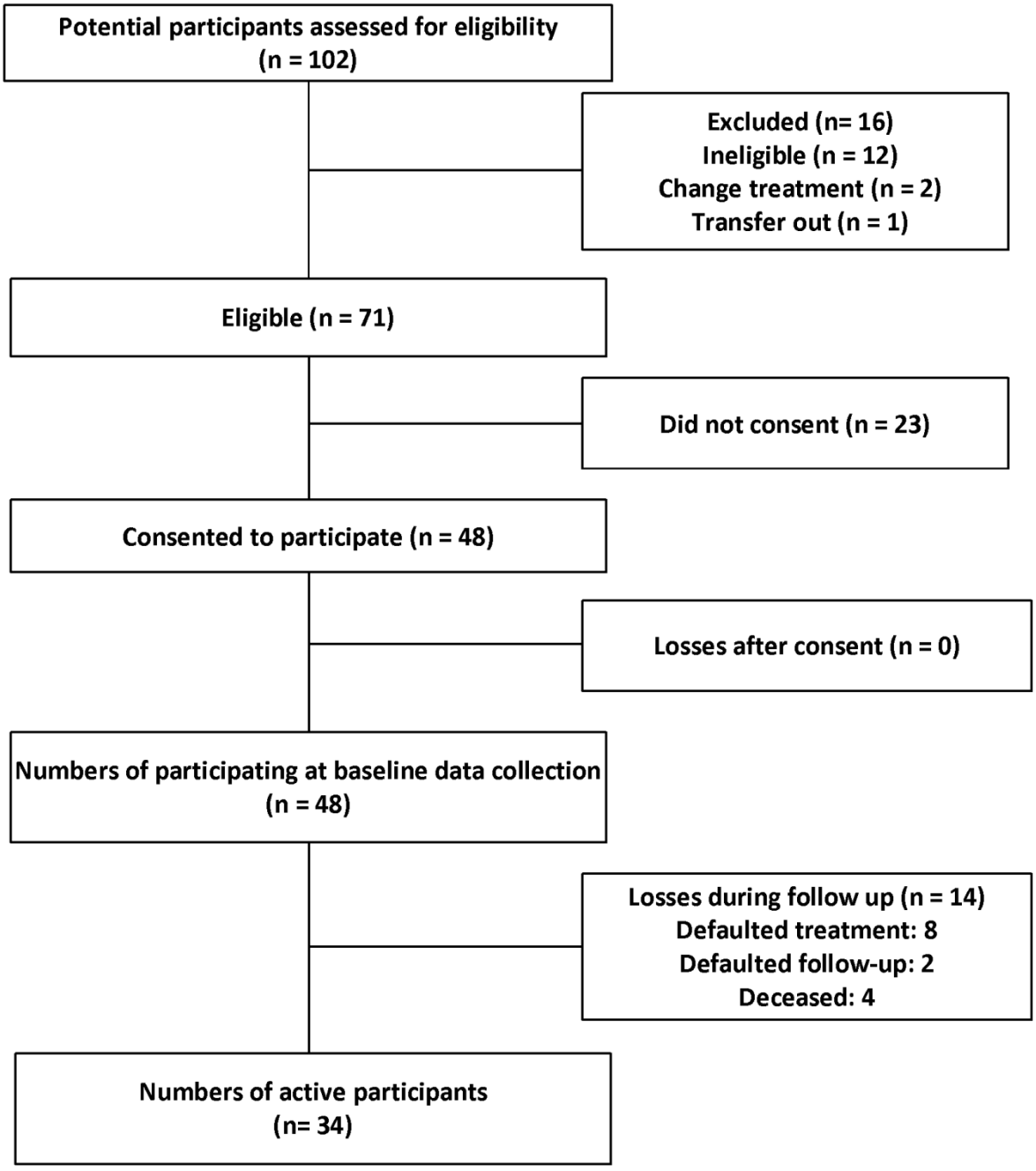


Figure 3-7: Diagram showing participant flow through study.
Defaulted treatment refers to patients who didn't show up for subsequent treatment appointment, defaulted follow-up refers to patients didn't show up for subsequent follow-up appointment.

Table 3-9: Demographic and clinical characteristics of the included patients (N = 34).

	Study population (N = 34)	
Demographic or clinical Characteristic	**No**	**%**
Age at primary cancer diagnosis		
Mean	47.7	
SD	16.61	
Median	51	
Range	22 - 74	
Gender		
Male	14	41.2
Female	20	58.8
Race/ethnicity		
Malay	22	64.7
Chinese	7	20.6
Indian	4	11.8
Others (Sarawak indigenous)	1	2.9
Average BSA kg/m^2		
Mean	1.60	
SD	0.21	
Median	1.59	
Range	1.20 – 2.09	
Primary diagnosis		
Hodgkin's lymphoma	10	29.4
Diffuse large B cell lymphoma	19	55.9
Other type of lymphoma	5	14.7
Length of follow-up, months		
Mean	11.7	
SD	3.38	
Median	11.7	
Range	3.7 – 18.9	
Cumulative anthracycline exposure, mg/m^2		
Mean	276.9	
SD	51.3	
Median	298.4	
Range	80 – 324.2	
Categories of anthracycline exposure, mg/m^2		
1-100	1	2.9
101-150	1	2.9
151-200	1	2.9
201-250	2	5.9
251-300	23	67.6
301-350	6	17.6
Concomitant cytotoxic drugs		
Cyclophosphamide	26	76.4
Chest radiation	4	11.8

Table 3-9: Demographic and clinical characteristics of the included patients (N = 34). *(cont.)*

	Study population (N = 34)	
Demographic or clinical Characteristic	**No**	**%**
Pre-anthracycline CV risk factors		
Hypertension only	2	5.9
Diabetes only	2	5.9
Dyslipidaemia only	1	2.9
Hypertension and diabetes	2	5.9
Hypertension and dyslipidaemia	1	2.9
Diabetes and dyslipidaemia	1	2.9
Use of cardio-protective drugs	3	8.8
Individual risk		
Low	15	44.1
Medium	16	47.1
High	3	8.8
Cardiac events	3	8.8

Characteristics of the study populations are summarised in Table 3-9. Of the thirty-four patients who completed follow-up, nineteen (55.9%) were diffused large B-cell lymphoma, ten (29.4%) were Hodgkin's lymphoma and five (14.7%) were other type of non-Hodgkin's lymphoma. Median age of the thirty-four patients was 51 years (range 22 – 74 years). There were twenty (58.8%) women and fourteen (41.2%) men. Twenty-two (64.7%) were Malay, seven (20.6%) were Chinese, four (11.8%) were Indian and one Sarawak indigenous. The median of average body surface area of these patients was 1.59m^2 (range 1.20 – 2.09).

The types of anthracycline administered were doxorubicin (n = 33) and epirubicin (n = 1). The median of cumulative anthracycline exposure, the sum of cardio-toxic potential adjusted cumulative dose (mg/m^2) of each anthracycline, was 298.4 (range 80 – 324 mg/m^2). Patients were mainly in medium and low risk category according to the classification of ACT predictive model. At the median follow-up duration of 11.7 months (range 3.7 – 18.9 months), three (8.8%) patients were diagnosed with cardiac event with different risk score (Table 3-10). The number of ACT cases was as predicted by the positive predictive value of the model developed previously.

Table 3-10: Risk of anthracycline-induced cardiotoxicity (ACT) by risk score.

ACT risk	**Risk score**	**Total, N**	**ACT, N**	**%**
No risk	0	0	0	0
Low	1 – 2	15	1	6.7
Medium	3 – 4	16	1	6.3
High	≥ 5	3	1	33.3

3.2.4 Discussion

Three ACT cases were identify as expected by the ACT prediction model (PPV = 9.6%). However, the number need to screen (NNS) of eleven in this study is less than required in the model development study (NNS = 19).

The baseline characteristics of this prospective population were fairly similar to the study population in the model development studies. Median age, gender and ethnic distribution and median body surface average area were comparable. The median length of follow-up was lesser while cumulative anthracycline exposure was higher in this population. Due to the nature of the study sites of the study as haematology referral centre, the distribution of primary diagnosis of the study population were double or more for Hodgkin's lymphoma, diffuse B-cell lymphoma and other type of lymphoma while there were none solid tumour patients.

The main advantage of the ACT prediction model is the nature of the risk factors in the model which are readily available before the initiation of anthracycline therapy. Thus, baseline cardiac function for patient at risk can be plan prior to anthracycline administration. Besides, the risk factors in the model can be identified from interviewing the patient and their past medical records. Therefore, the assessment of ACT risk using the model can be done by all healthcare professionals.

There are a few limitations warrant discussion. First is the lack of heterogeneity in diagnosis in our study population. The diagnosis of haematology malignancy put all the patients at risk for ACT with risk score of one. Our patient selection criteria that exclude patient who has history of prior anthracycline use might be the cause that none of the patients received cyclophosphamide and trastuzumab concurrently, a treatment choice mainly for breast cancer, which carries a risk score of three.

3.2.5 Conclusion

The use of ACT prediction model in clinical practice is promising. However, supportive findings from diverse and large prospective data from different centre(s) are needed.

3.3 A qualitative exploration on the content and usability of a 4-factors anthracycline-induced cardiotoxicity (ACT) prediction model

3.3.1 Introduction

Besides utility evaluation, we also explore the necessity and practicality aspect of clinical usefulness of the developed model. Exploration of the necessity and practicality is required to support future work in developing a more extensive prediction model. Thus, this qualitative survey aims to explore the opinion of healthcare professionals on the content and usability of the developed prediction model.

3.3.2 Method

Participants

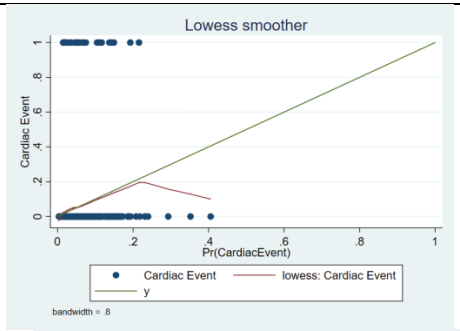
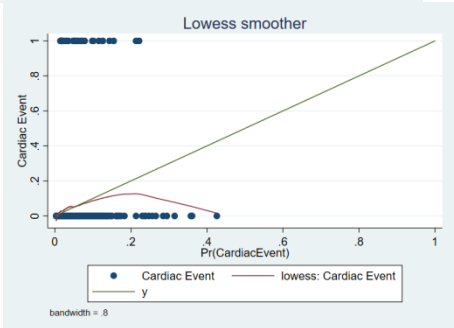
The study was conducted in Klang valley, where most oncology referral centers in Malaysia are located. Participants were conveniently selected from consultants, specialists and pharmacists practicing in the field of haematology oncology, oncology and cardiology in any of the five cancers referral centers in Klang Valley, namely Hospital Kuala Lumpur, Hospital Ampang, National Cancer Institute, UKM Medical Center and UM Medical Center. This group of healthcare professionals was targeted because they will be the main user of the 4-factors ACT prediction model.

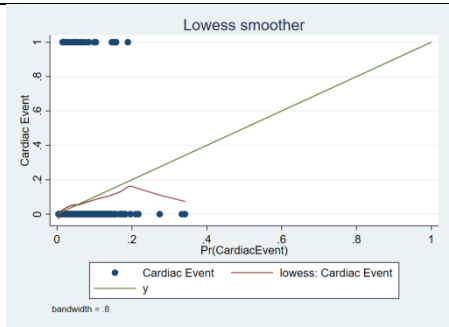
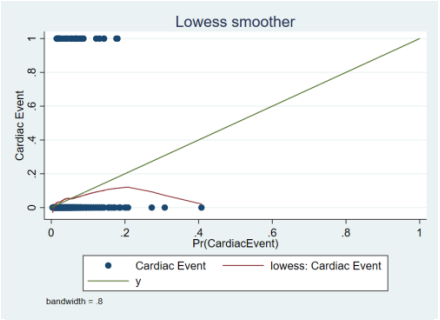
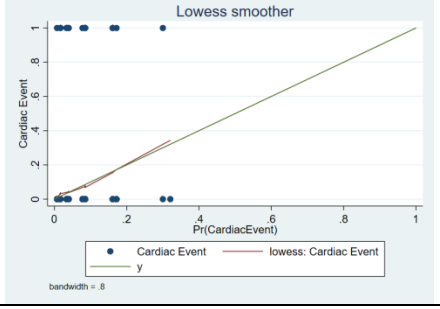
Procedure and survey process

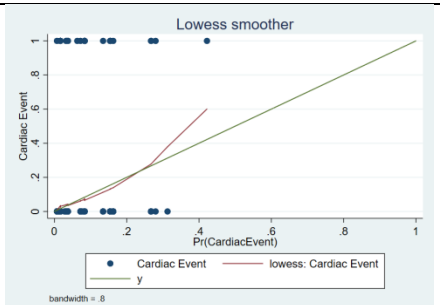
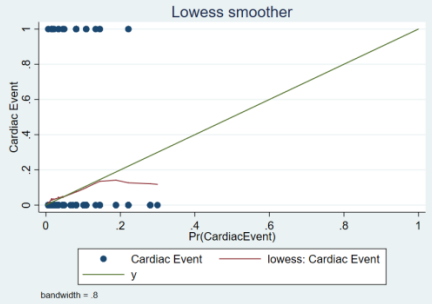
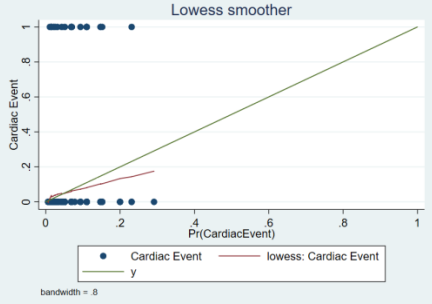
A semi-structured survey form which consists of seven open-ended questions (

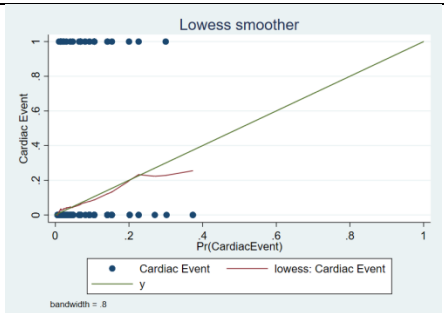
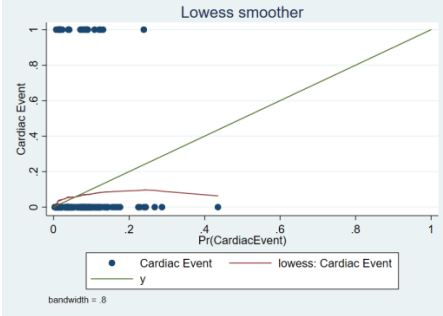
Appendix 6: Performance of potential models

Ten of fifty-one models have AUROC of 0.75 or more (rounded to two decimal points). Model 5 was selected as final model based on the AUROC and calibration slope.

Model	Description	R-squared	AUROC	Hosmer-Lemeshow test	Calibration slope
1	Age + Cardio-protective agent + Haematology + Cyclophosphamide & Trastuzumab	0.0412	0.7508	0.0151	
2	Age + ACEi + Haematology + Cyclophosphamide & Trastuzumab	0.0446	0.7534	0.0002	

Model	Description	R-squared	AUROC	Hosmer-Lemeshow test	Calibration slope
3	Age + Cardiac comorbid + Haematology + Cyclophosphamide & Trastuzumab	0.0356	0.751	0.0020	
4	Age + Haematology + Cyclophosphamide & Trastuzumab	0.0333	0.7472	0.0002	
5	**Age50 + Cardio-protective agent + Haematology + Cyclophosphamide & Trastuzumab**	**0.0384**	**0.7479**	**0.8178**	

Model	Description	R-squared	AUROC	Hosmer-Lemeshow test	Calibration slope
6	Age50 + Number of cardiac comorbid + Haematology + Cyclophosphamide & Trastuzumab	0.0454	0.7457	0.5998	
7	Age50 + Chinese + ACEi + Haematology + Cyclophosphamide & Trastuzumab	0.0462	0.7487	0.2456	
8	Age50 + Chinese + Cardiac Comorbid + Haematology + Cyclophosphamide & Trastuzumab	0.038	0.75	0.5049	

Model	Description	R-squared	AUROC	Hosmer-Lemeshow test	Calibration slope
9	Age50 + Chinese + Cardiac Comorbid + Haematology + Cyclophosphamide & Trastuzumab + breast cancer	0.0402	0.7529	0.3541	
10	Age50 + Haematology + Creatinine clearance + Cyclophosphamide & Trastuzumab	0.0354	0.754	0.9932	

Age, age as continuous covariate; Age50, age as 50 or more years

Appendix 7: Qualitative survey form

) was sent to potential participants together with the 4-factors ACT prediction model in excel format (Figure 3-8). Face-to-face or video conference interview were conducted upon request which was audio recorded. All interviews were transcribed in verbatim. The resulting transcribes were then sent to the participant for approval. A minimum of one week was given to the participants to approve the transcript by signing the validation form. The survey continued until saturation point was reached, when no new information was obtained from subsequent interviews.

Answers were subjected to thematic content analysis, and the transcripts are analysed for relevant content to identify the emerging categories[81] with every additional survey until saturation. Consensus of themes' definition and naming was achieved through discussions between two researchers.

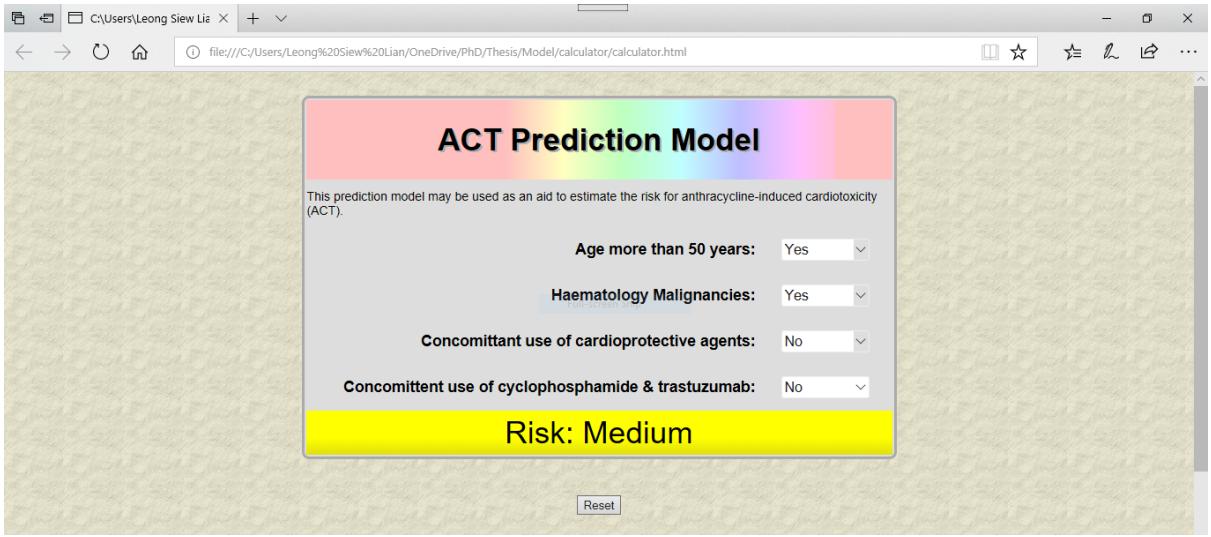


Figure 3-8: Excel format of the 4-factor prediction model.

3.3.3 Results

A total of six healthcare professionals, three consultants and three pharmacists practising in the area of hematology-oncology, oncology and cardiology responded to the survey. During analysis, two themes were identified: lack of scoring system as well as content and usability of the developed model.

Theme 1: Lack of scoring system

In this preliminary investigation, all respondents lamented the lack of scoring system to predict the occurrence of cardiotoxicity among their patients receiving anthracycline therapy. In general, most respondents would usually base their judgement on their experience as well as other medical history such as cardiac assessments.

> No specific scoring systems if no history of heart disease. If history of cardiovascular, would use TIMI score, CHAD score and NYHA classification. [C1]

> Not a formal scoring system but will be based on co morbidities but baseline cardiac function test is required. [C2]

Theme 2: Content and usability of the developed model

Subtheme 1: Simple validated tool

In general, most respondents commented that they would prefer to use a simple tool which requires very minimal input and was validated. Indeed, all respondents expressed the need to have a validated tool which they felt can be universally applied to all their patients.

> Validated, universal. [C2]

> Validation of the system. [C3]

> Simple to use. [P1]

> Ease of use. [P2]

Respondents suggested that any tool which will be developed needs to be validated using some form of data or cohort to ensure that the results are applicable and widely accepted.

> Supported with validation study. [P3]

> Validation with supporting data. [C3]

Subtheme 2: Characteristics of the developed model.

When shown the developed prediction model, respondents replied that they particularly liked the model as they found it was very simple and easy to use. They were particularly impressed that the model only relied on existing demographic data which was easy to obtain. There has even been suggestion that the tool could be further developed into an app to improve its audience base.

> Straightforward and easy to use. [P3]
>
> Fairly easily available. [C2]
>
> Readily available. [P2 and P3]
>
> Possible if easy access, if has phone app. [C1]

There was however some reservation on how applicable the tool could be as a prediction model

> Great and simple, but hard to believe how it works for my patients. [C2]
>
> Would need to be convinced that model has validated use over a reasonably large population of patients and has accurately predicted the risk of cardiotoxicity. [P2]

3.3.4 Discussion

The ACT prediction model has a good overall performance (Brier score = 0.042), acceptable discrimination (AUROC = 0.75) and stable prediction with a positive predictive value of 9.6%. It also can potentially use in clinical practice. Thus, the objective of this study was to explore healthcare professionals' perspectives regarding the content and usability of the ACT prediction model, as well as their suggestion to improve the model. The current study portrays the respondents from hematology-oncology, oncology and cardiology and represents doctors and pharmacists who will be the main potential user of the model.

The study results suggest that the formal scoring system to predict ACT risk for anthracycline recipients is lacking. At the same time, healthcare professionals in the related fields are anticipating a straightforward, validated and readily accessible tool for the purpose.

The most dominant reasons why healthcare professionals are ready to adopt the developed 4-factors ACT prediction model are readily available factors and its straightforwardness. However, the model needs to be validated with supporting data.

We managed to include healthcare professionals from all related clinical areas. The major limitation of the survey is the low respond rate of 15.7%. Nevertheless, we manage to reach saturation point at 4^{th} respondents and no new information emerged with the subsequent two responds. Future study to validate the prediction model is required.

3.3.5 Conclusion

This qualitative exploratory study investigated the perspectives of doctors and pharmacists from hematology-oncology, oncology and cardiology practice area. Currently there is no formal scoring system to predict the risk for ACT among anthracycline recipients and the developed 4-factors ACT prediction model is easy to use. Respondents were ready to adopt a validated ACT prediction model in their clinical practice.

4 Pharmacogenomics in antineoplastic-related cardiovascular toxicity

We also recognised that in the effort to pave way for personalised medicine, beside stratify patients' individual risk using prediction model, it will only do it justice by considering the possibility of genetic role in predicting cancer chemotherapy-related cardiovascular (CV) complications in the era of genetic. Therefore, we conducted systematic reviews to identify single nucleotide polymorphisms related to antineoplastic-related CV complications in general (Section 4.1, page 103) and anthracycline-induced cardiotoxicity in specific (Section 4.2, page 167).

4.1 Pharmacogenetics in non-anthracycline based antineoplastic-induced cardiovascular toxicities: A Systematic Review and Meta-analysis

4.1.1 Introduction

This chapter has been submitted to JAMA Cardiology awaiting editorial decision. The candidate, Leong Siew Lian was primarily responsible for searching, analysis and writing of the manuscript. The paper's co-authors, Shaun Lee Wen Huey, Nathorn Chaiyakunapruk, Wichittra Tassaneeyakul, Poukwan Arunmanakul and Surakit Nathisuwan contributed in various aspects of this article.

4.1.2 Submitted manuscript and supplementary materials

Title Page

Title: Pharmacogenetics in non-anthracycline based antineoplastic-induced cardiovascular toxicities: A Systematic Review and Meta-analysis of genotypes effect.

Authors: Siew Lian Leong, MClinPharm[1,2], Nathorn Chaiyakunapruk, PhD[1,3,4,5], Wichittra Tassaneeyakul, PhD[6], Poukwan Arunmanakul, Pharm.D. BCPS[7], Surakit Nathisuwan, PhD[8], Shaun Wen Huey Lee, PhD[1,5*]

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Word count of the manuscript text: 2413 words

Title: Pharmacogenomics in antineoplastic-induced cardiovascular toxicities: A Systematic Review and Meta-analysis of genotypes effect.

Key Points

Question: What is the role of genetic biomarker in chemotherapy-related cardiovascular toxicities?

Findings: In this systematic review and meta-analysis of 35 gene association studies, 219 SNPs were identified. Human epidermal growth factor receptor 2 (HER2) rs1136201 was found to increase the risk for trastuzumab-related heart failure by 2.4 times (2.13 – 8.68). Besides, number of variant allele also may be a contributor factor.

Meaning: While the role of many other SNPs warrants further evidence, HER2 rs1136201 is a potential predictors for trastuzumab-related cardiotoxicity.

Abstract

Importance: Exploration on genetic role in antineoplastic-related cardiovascular toxicity has increased with the advancement of genotyping technology. However, knowledge on the extent of genetic determinants in affecting the susceptibility to the cardiovascular toxicities of antineoplastic is limited.

Objective: This study aims to identify potential single nucleotide polymorphism (SNP) in predicting antineoplastic-related cardiovascular toxicity.

Data sources: We systematically searched for original research in PubMed, Cochrane Central Register of Controlled Studies, CINAHL Plus, EMBASE and HuGE Navigator from database inception until January 2018, reporting.

Study selection: Studies on association between polymorphism and antineoplastic-induced cardiovascular toxicity in patients treated for cancer of all antineoplastic agents were included except for anthracycline. Case report, conference abstracts, reviews and non-patient studies were excluded.

Data extraction and Synthesis: Data extracted by two independent reviewers were combined with random-effects model and reported according to PRISMA and MOOSE guidelines.

Main Outcome and measure: The primary outcome was association between SNP and the odds for cardiovascular toxicity.

Results: The 35 studies included examined a total of 219 SNPs in 80 genes, 11 antineoplastic and 5 types of cardiovascular toxicities. Meta-analyses showed that human epidermal growth factor receptor 2 (HER2) rs1136201, a risk variants (pooled OR: 2.92; 1.66 – 5.11, $p < 0{\cdot}001$) is a potential predictors for trastuzumab-related cardiotoxicity. Gene dose effect analysis number of variant allele may contribute to the risk too.

Conclusions and relevance: This review found HER2 rs1136201 is potential in predicting trastuzumab-related heart failure. Studies on clinical use and economic aspect of the SNP are required to support its implementation as a clinical practice.

Keywords: Chemotherapy, Cardiac toxicity, Genetics, Trastuzumab, Left ventricular dysfunction, Single nucleotide polymorphism

Introduction

The discipline of cardio-oncology is growing rapidly with the growing of number of cancer survivors [1] and awareness of the cardiovascular (CV) toxicity as one of the most significant complications of cancer therapy [2]. Recognised CV adverse effects of cancer chemotherapies are diverse and include chemotherapy related cardiac dysfunction (CRCD), hypertension, ischemia vascular effects, coronary disease, thromboembolism and arrhythmias. Anthracyclines, alkylating agents, monoclonal antibobies included HER2-targeted agents and VEGF-targeted agents, small molecule tyrosine kinase inhibitors (TKIs), antimicrotubule, antimetabolites and proteasome inhibitors groups have been associated with CV adverse effects. Some antineoplastic causes a specific CV adverse effect, while others causes various CV adverse effects. For example, the most common CV adverse event associated with bevacizumab is hypertension [3] while trastuzumab therapy is associated with congestive heart failure and decreased left ventricular ejection fraction (DLVEF) [4]. These adverse effects can impede or disrupt cancer treatment and subsequently worsen the cancer outcomes and quality of life, increase cost of care and utilization of healthcare resources.

Risk factors for CV toxicity during cancer therapy vary among antineoplastic. History of heart failure, coronary artery disease and lower body mass index were reported to be risk factors for sunitinib-related CV adverse effects [5]. In addition, trastuzumab-induced CV toxicity is associated with prior anthracycline use, pre-existing DLVEF, hypertension, elevated body mass index and age [6]. Awareness of these risks for CV toxicity is important in early prevention, identification, and treatment of the adverse effects. Because of the incompleteness of demographic and clinical risk factors to stratify individual at risk and the growth of targeted therapeutics discovery and development, attempts to understand genetic contribution have increasingly been explored over the past few years. However, the extent of knowledge of the genetic determinants which increases susceptibility to the CV toxicities of antineoplastic is limited. Our goal was to perform a systematic review and meta-analysis

of studies of antineoplastic agents to understand the contribution of genetic polymorphism to the risk of antineoplastic-induced CV adverse events.

Methods

Search strategy

We searched EMBASE, Cochrane Central Register of Controlled Studies, PubMed, CINAHL Plus and HuGE Navigator from inception until January 2018. Search terms used include CV toxicity and genetic. This was supplemented with a manual search of cited references from retrieved articles. Primary studies reporting the results of studies examining the association between polymorphism and antineoplastic-induced CV toxicity in patients treated for cancer were included. All antineoplastic were included except for anthracycline, which has been reported before separately [7]. Case report, conference abstracts, reviews and non-patient or lab studies were excluded.

Data extraction

Information about geographic location, study design, participant demographics and clinical characteristics, genotyping technique and definition of cardiotoxicity were extracted by reviewers (SLL and SWHL). Effects of genotypes and number of CV adverse event for each genotype were also collected. CV adverse events were categorised as follow: decreased left ventricular ejection fraction (DLVEF), hypertension, arrhythmia, venous thromboembolism (VTE) and cardiovascular disease (CVD). We reported these data in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement [8] and Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines [9].

Quality assessment

Quality of the included studies was assessed independently by reviewers using quality of genetic association studies (Q-Genie) tool developed by Sohani *et al.*[10] The validation tool was developed based on the Strengthening the Reporting of Genetic Association Studies (STREGA)[11] and Strengthening the Reporting of Genetic Risk Prediction Studies (GRIPS)[12]. It consists of nine domains;

rationale for study, selection and definition of outcome of interest, selection and comparability of comparison groups, technical classification of the exposure, non-technical classification of the exposure, other sources of bias, sample size and power, a priori planning of analyses, statistical methods and control for confounding, testing of assumptions and inferences for genetic analyses and appropriateness of inferences drawn from results.

Statistical analysis

We presented all data narratively. We used the odds ratio (OR) for CV adverse event, estimating 95% confidence intervals (95% CI). In studies with similar outcomes (minimum 2 studies), we performed pairwise random effects meta-analysis [13]. Heterogeneity of the studies was assessed using Cochran's Q and I^2 statistics. Gene dose effect which compares the effect of number of allele was conducted for polymorphism with sufficient data available. All analyses were performed using Stata 15.0 (StataCorp, College Station, TX).

Results

Study Selection and characteristics

Our search retrieved 7,883 potentially relevant articles. After screening, 187 articles were identified for review, with 152 articles excluded. A total of thirty-five articles describing CV adverse events of eleven antineoplastic from five drug classes were included in the current review (eFigure 1). The antineoplastic include tyrosine kinase inhibitor (axitinib, sorafenib and sunitinib), monoclonal antibody (bevacizumab, cetuximab and trastuzumab), antimetabolite (fluorouracil), alkylating agent (cisplatin and temozolamide) and immunomodulatory agent (lenalidomide and thalidomide). These studies mostly described CV events which include hypertension induced by four antineoplastic agents namely bevacizumab [14-23], sunitinib [24-31], axitinib [32,33] and sorafenib [22,34]; DLVEF induced by trastuzumab [35-42] and VTE induced by bevacizumab [21,43,44], cisplatin [45], lenalidomide [46], temozolamide [43] and thalidomide [44,47]. Majority of these studies used either the National Cancer Institute Common Toxicity Criteria version 2, 3, or 4 to assess severity of CV adverse events. The characteristics of the included studies are presented in eTable 1.

Most of the studies were cohort studies [15-38,40-43,45,46,48] (n=31) while the remaining were case-control (n=3)[39,44,47] and randomised controlled trial (n = 1)[14]. These studies were done in Europe (n = 16)[16,17,20,21,24,25,29,31,34,35,37,38,40,45-47], North America (n = 5)[22,23,36,39,41], Asia (n = 5)[18,30,33,42,48], and another 4 studies were multi-centred studies conducted in several countries. Five studies did not report the study location [14,15,26,43,44]. Thirty studies included adults in their report with eight studies did not report the age of included population. Twenty-two studies described the ethnicity of their participants [14,16-19,22,24,25,27-29,31-33,36,39,41,42,44,46-48].

The most common type of diseases examined were breast cancer (n = 10), renal cancer (n = 9), colorectal cancer (n = 4), multiple myeloma (n = 3), testicular cancer (n = 1), and glioma (n = 1). The

remaining seven studies mixed type of cancer were examined. All the studies reported single type of cardiovascular adverse event except for three studies [17,21,45] which reported two cardiovascular toxicities. Twenty studies reported the genetic association with hypertension [14-34], eight studies each on DLVEF [35-42] and VTE [17,21,43-47], one study on coronary artery disease [45] and one on arrhythmia [48].

The quality of the reporting in the studies

All the thirty-two included studies were rated to be of good quality, with mean scores of >3 on all domains assessed using the Q-Genie tool (Supplementary eTable 2).

Polymorphism

The thirty-five included studies identified a total of 219 single-nucleotide polymorphisms (SNPs) in eighty genes (Table 1, eTable 3-7). Seventy-four (34%) of SNPs in forty genes were found to be significantly associated with antineoplastic-induced cardiovascular toxicities in at least one study. These SNPs were mainly associated with hypertension, decreased LVEF and VTE. However, only SNPs from vascular endothelial growth factor (VEGF) and human epidermal growth factor receptor 2 (HER2) in association with bevacizumab-related hypertension and trastuzumab-related decreased LVEF respectively have sufficient data for quantitative analysis.

SNPs in bevacizumab-related hypertension

Three retrospective cohort studies which included a total of 366 patients examining the role of five SNPs associated with vascular endothelial growth factor (VEGF) were included in quantitative analysis[15,16,18]. All the SNPs associated with increased risk of bevacizumab-related hypertension were: heterozygous and homozygous variant of VEGF -2574C>A (rs699947)[18], heterozygous and homozygous variant of VEGF -1498T>C (rs833061)[15], heterozygous and homozygous variant of VEGF -1154G>A (rs1570360)[16] and heterozygous and homozygous variant of VEGF 936C>T (rs3025039)[18]. Meta-analysis of these SNPs showed that patients with heterozygous and homozygous variant in the

VEGF (rs699947, rs833061, rs1570360, rs2101963, rs3025039) were 1.56 times higher risk of developing bevacizumab-induced hypertension (Pooled odds ratio (OR): 1.56, 95% CI, 1.07 – 2.88, p = 0.006; Figure 1).

SNPs in trastuzumab-related decreased LVEF

Six cohort studies examined the role of human epidermal growth factor receptor 2 (HER2) variant 655A>G rs1136201 in developing cardiotoxicity[35-38,40,41] (eTable 4). In the 1322 patients examined, cardiotoxicity was defined as either a decline of 10-20% of LVEF from baseline [35-38,40]; or an absolute LVEF value of less than 45 – 50% [35-38,40]. The dose of trastuzumab used was a loading dose of 8mg/kg followed by 6mg/kg [35-38]. In the study by Beauclair *et al.* which included 63 HER2-positive breast cancer patients, the authors noted significant association for heterozygotes of rs1136201 with increased risk of developing cardiotoxicity [35]. Similarly, Roca *et al.* studied with a doubled number of HER2-positive breast cancers patients (n = 132) and found similar association for heterozygous and homozygous variant genotypes of rs1136201 (OR = 3.83, 95% CI: 1.11 – 13.18, p = 0.025)[37]. Pooled analyses showed that the presence of HER2 heterozygous and homozygous variant genotypes of rs1136201 increased the risk of developing heart failure by 2.4 times (95% CI: 1.17 – 5.06, p = 0.018; Figure 2).

Four studies examined the role of rs1058808 SNP in heart failure among patients with HER2 polymorphism[36,39-41] (eTable 4). These studies had a very similar definition of cardiotoxicity and dosage of transtuzumab in their cohort examined. In the study by Stanton *et al.* which included 140 HER2-positive breast cancer patients, the authors noted significant association for heterozygous and homozygous variants for reduced risk of developing cardiotoxicity [39]. Similarly, Boekhout *et al.* studied 206 early-stage HER2-positive breast cancer patients and found similar association for homozygous variant (OR = 0.09; 95% CI, 0.02 – 0.45; p = 0.003)[40]. However, the study by Lemieux *et al*. did not find significant association between heterozygous variant of rs1058808 SNP (OR = 0.19,

95% CI, 0.19 – 4.71, p = 0.95) or homozygous variant (OR = 1.62, 95% CI, 0.32 – 8.29, p = 0.57)[36]. Pooled analysis showed that the presence of rs1058808 SNP was potentially cardio-protective, and reduced the risk of developing heart failure by 31% (OR: 0.69; 95% CI: 0.47 – 1.02, p = 0.061, Figure 2).

Analysis of gene dose effect showed odds ratios changed with the number of variant allele although the differences were not statistically significant (Table 2). Only FCGR2A rs1801274 has enhanced risk effect with increased number variant allele. The risk or protective effect in SNPs HER2 rs1136201, HER2 rs1058808 and FCGR3A rs396991 is stronger for heterozygous genotypes compared to homozygous genotypes. For example, heterozygous genotype of FCGR3A rs396991 has greatest protective effect (OR: 0.60; 95% CI: 0.25 – 1.48, p = 0.27) compared to homozygous (OR: 0.99; 95% CI: 0.41 – 2.41, p = 0.98).

Discussion

We found a total of thirty-five studies, exploring the effect of 219 SNPs on eleven antineoplastic and five types CV toxicities. All the studies utilized candidate-gene approach, and only two used genome-wide approaches [14,41]. Our findings indicate that seventy-four (34%) SNPs are significantly associated with risk of particular antineoplastic related CV toxicities. Among these findings, association of SNPs rs1136201 and rs1058808 of HER2 with trastuzumab-related cardiotoxicity was most prominent. This effect has potential clinical ramification since trastuzumab is specifically used for HER2 receptor positive breast cancer. It has been reported that the incidence of trastuzumab-induced CV toxicities are likely between 20-33% [49-51] which are frequently manifested as decreased LVEF (7.5%) and congestive heart failure (1.9%)[52]. While most of these side effects are often mild and reversible, the long-term implication on CV morbidity and mortality are uncertain [53]. Indeed, in patients with the SNP rs1136201 close monitoring and attention should be given to this particular cohort of breast cancer patients as they have 2.4 times increased risk of developing heart failure. This could include compulsory screening for the variant, which currently cost approximately USD1 per SNP.

Heterogeneity for pooled estimates of SNP rs113601 was most likely attributed by population stratification and difference in classification of cardiotoxicity events. The ethnicity of study population various between studies from French, White, Black, to Asian with more than half of the studies (67%) did not specify ethnicity. Although age was similar among studies, half of them did not report the average age of the included participants. Besides, the definition of cardiotoxicity also varied which contributed to difference in classification of clinical outcome.

Although the role of these SNPs in the pathophysiology of cardiotoxicity is still unknown, it has been proposed that trastuzumab-related cardiotoxicity could be related to the disruption in signalling between the HER2 receptor and ligand growth factor. It had been shown that HER2 is critical for

normal myocyte growth, survival and homeostasis in mouse studies [54-56]. Studies by Crone *et al.* and Özcelik *et al* using HER2-deficient conditional mutant mice and found evidence of dilated cardiomyopathy [54,55]. Meanwhile, in-vitro study using culture of neonatal rat ventricular myocytes found that anti-HER2 related impairment of mitochondrial integrity and disruption of cellular energetics is caused by the activation the mitochondrial apoptosis pathway [56].

Before these SNPs could ultimately be used to mitigate risk of developing this highly morbid adverse effect, these results should be confirmed and validated with larger sample sizes and well-designed genetic association studies. Given the large volume of patients who receive these agents and the relative frequency of which CV toxicity occurs, it seems logical that more advanced approaches such as human genome-wide association studies or whole exome or whole genome sequencing should be undertaken with proper population stratification. Besides, preclinical molecular and novel non-human genetic research will also enhance opportunities for broader genomic analysis. Further investigation on the mechanism of SNPs rs1136201 and rs1058808 on trastuzumab-related cardiotoxicity is also recommended since it is still unknown.

This study has several strengths. Firstly, included studies examining the role of HER2 SNPs had included a relatively large sample of participants. All studies had used an objective outcome of ejection fraction and had a relatively homogenous definition of decreased LVEF. Although the participants of these studies were recruited from Europe and North America, generalization of these findings in other populations is probable with large multi-ethnic genetic studies in the future.

There are some limitations of this study which warrants discussion. Current evidence suggests that several SNPs are associated with CV toxicities. However, a number of methodological concerns may limit the interpretation and comparability of the results. Although 219 SNPs were identified, meta-analysis could only be performed for nine of the SNPs, as most of the SNPs found in this study have

been studied only once. In addition, there were inconsistencies in reporting result and lacking in required data for meta-analysis, which further curbed the ability to combine the data. Another study limitation was the heterogeneity of the included studies, which limited the precision of overall estimates. For example, although meta-analysis can be performed for five SNPs in relation to bevacizumab-related HTN, the moderate to high values of I^2 values suggest that there exists heterogeneity across studies. Collinearity among the genotypes also hinders further analysis such as network meta-analysis.

Conclusion

This review found that SNPs rs1136201 human epidermal growth factor receptor 2 (HER2) is a potential predictor for trastuzumab-related cardiotoxicity. There might be a potential role of the SNP testing as part of pre-treatment screening prior to the use of trastuzumab. However, more clinical and economic evidence are needed before a concrete recommendation can be made.

Acknowledgment
Monash University Malaysia had fully supported supply of articles.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Table 1: Summary of SNPs investigated in studies.

Hypertension

Bevacizumab

EGF (rs4444903)[a]	AGTR1 (rs12695902)	KLKB1 (rs4253296)
EGF (rs9992755)[a]	AGTR1 (rs12721331)	KLKB1 (rs4253315)
FIP200 (rs1129660)[a]	AGTR1 (rs1492099)	KLKB1 (rs4253327)
GRK4 (rs1419044)[a]	AGTR1 (rs2675511)	KLKB1 (rs4253331)
HT (rs1937506)[a]	AGTR1 (rs275649)	KLKB1 (rs925453)
KLKB1 (rs1912826)[a]	AGTR1 (rs2933249)	SCNN1A (rs2041375)
SV2C (rs6453204)[a]	AGTR1 (rs3772616)	SCNN1A (rs2228576)
ULK1 (rs9481)[a]	AGTR1 (rs385338)	SCNN1A (rs2286600)
VEGF (rs699947)[a]	AGTR1 (rs389566)	SCNN1A (rs3764874)
VEGF (rs833061)[a]	AGTR1 (rs4681440)	SCNN1A (rs3764875)
VEGF (rs2010963)[a]	AGTR1 (rs5182)	SCNN1A (rs3782723)
VEGF (rs3025039)[a]	ATG13 (rs13448)	SCNN1A (rs4764585)
VEGF (rs3097)[a]	ATG3 (rs9831088)	SCNN1A (rs7973914)
VEGF (rs13207351)[a]	ATG5 (rs633724)	ULK1 (rs11616018)
VEGF (rs25569394)[a]	ATG8 (rs11149841)	ULK1 (rs12303764)
VEGF (rs1005230)[a]	ATG8 (rs8060972)	UVRAG (rs1458836)
VEGF (rs35864111)[a]	BDKRB1 (rs10147171)	VEGF (rs10434)
VEGFR2 (rs1870377)[a]	BDKRB1 (rs11622768)	VEGF (rs1570360)
WNK1 (rs11064560)[a]	BDKRB1 (rs2071083)	VEGF (rs2146323)
WNK1 (rs2286028)[a]	BDKRB1 (rs2071084)	VEGF (rs25648)
WNK1 (rs2158501)[a]	BDKRB1 (rs885845)	VEGF (rs3024994)
WNK1 (rs11064519)[a]	BECN1 (rs11552192)	VEGF (rs3025030)
WNK1 (rs7953912)[a]	CYP11B2 (rs12050217)	VEGF (rs3025035)
ACE (rs4295)	CYP11B2 (rs1799998)	VEGF (rs833069)
ACE (rs4305)	CYP11B2 (rs4543)	VEGFR2 (rs2305948)
ACE (rs4309)	CYP11B2 (rs6433)	WNK1 (rs10774461)
ACE (rs4311)	FIP200 (rs17337252)	WNK1 (rs10849582)
ACE (rs4343)	GNB3 (rs5446)	WNK1 (rs10935724)
ACE (rs4357)	GRK4 (rs1010290)	WNK1 (rs11064524)
AGT (rs11568054)	GRK4 (rs1419043)	WNK1 (rs11064547)
AGT (rs2004776)	GRK4 (rs1557213)	WNK1 (rs11068756)
AGT (rs2478523)	GRK4 (rs17835422)	WNK1 (rs11611231)
AGT (rs2478543)	GRK4 (rs1801058)	WNK1 (rs12314329)
AGT (rs2478544)	GRK4 (rs2067003)	WNK1 (rs12816718)
AGT (rs2478545)	GRK4 (rs2105380)	WNK1 (rs1468326)
AGT (rs2493131)	GRK4 (rs2515936)	WNK1 (rs17223420)
AGT (rs2493132)	GRK4 (rs2857845)	WNK1 (rs2286007)
AGT (rs3789678)	KLKB1 (rs1511802)	WNK1 (rs4980968)
AGT (rs3889728)	KLKB1 (rs3087505)	WNK1 (rs4980973)
AGT (rs4762)	KLKB1 (rs3775302)	WNK1 (rs6489755)
AGT (rs5050)	KLKB1 (rs4253251)	WNK1 (rs7967755)
AGT (rs6687360)	KLKB1 (rs4253260)	WNK1 (rs953361)
AGT (rs7079)	KLKB1 (rs4253292)	WNK1 (rs2269937)
AGT (rs1926722)		

Sorafenib

ABCG2 (rs2231137)[a]	UGT1A9 (rs72551330)[a]	ABCG2 (rs2622604)
VEGFR1 (rs9513070)[a]	ABCB1 (rs1045642)	VEGFR2 (rs2305948)
VEGFR2 (rs1870377)[a]	ABCB1 (rs2032582)	VEGFR2 (rs2305948)
UGT1A9 (rs178868320)[a]	ABCG2 (rs2231142)	CYP3A5 (rs776746)
UGT1A9 (rs6714486)[a]		

Table 1: Summary of SNPs investigated in studies (cont).

Hypertension		
Sunitinib		
VEGF (rs833061)[a]	IL8 A>T (rs1128847)[a]	VEGFR3 (rs448012)
VEGF (rs2010963)[a]	eNOS (rs2070744)[a]	VEGFR3 (rs307821)
VEGF (rs699947)[a]	CYP3A4 (rs4646437)[a]	VEGFR3 (rs307826)
VEGF (rs1570360)[a]	ABCG2 (rs2622604)	VEGFR1 (rs9582036)
VEGFR2 (rs1870377)[a]	ABCB1 (rs1045642)	VEGF (rs3025039)
ABCB1 (rs1128503)[a]	ABCG2 (rs55930652)	CYP3A5 (rs776746)
ABCB1 (rs2032582)[a]	VEGFR1 (rs9554320)	CYP3A4 (rs2740574)
ABCG2 (rs2231142)[a]	VEGFR2 (rs2305948)	PDGFR-α (rs35597368)
Cetuximab		
FIP200 (rs1129660)		
Axitinib		
VEGRF2 (rs2305948) [a]	ABCG2 (rs2231142)	VEGFR1 (rs9513070)
Decreased left ventricular ejection fraction		
Trastuzumab		
BRINP1 (rs10117876)[a]	BRINP1 (rs7027658)[a]	CREBRF (rs201763080)
BRINP1 (rs7038923)[a]	BRINP1 (rs75912020)[a]	EYS (rs139944387)
BRINP1 (rs7041012)[a]	BRINP1 (rs76890184)[a]	FCGR2A (rs1801274)
BRINP1 (rs1160584)[a]	BRINP1 (rs58944852)[a]	FCGR3A (rs396991)
BRINP1 (rs230145)[a]	BRINP1 (rs6256837)[a]	FIG4 (rs56378532)
BRINP1 (rs230144)[a]	HER2 (rs1058808)[a]	GTF3C3 (rs146213213)
BRINP1 (rs230142)[a]	HER2 (rs1136201)[a]	KRT15 (rs78272919)
BRINP1 (rs62573809)[a]	Intergenic (rs4305714) [a]	MYADM (rs140387622)
BRINP1 (rs16908078)[a]	LDB2 (rs55756123)[a]	PHF3 (rs139503277)
BRINP1 (rs7851490)[a]	LINC01060 (rs7698718)[a]	PLEKHA6 (rs149581993)
BRINP1 (rs7854066)[a]	RAB22A (rs707557)[a]	SFTPA2 (rs150273659)
BRINP1 (rs62573837)[a]	TRPC6 (rs77679196)[a]	ZNRF3 (rs5762940)
BRINP1 (rs76586195)[a]		
Venous thromboembolism		
Thalidomide		
PPARD (rs2267669)[a]	DCLRE1B (rs12022378)[a]	CINP (rs7011)*
CASP3 (rs1049216)[a]	XRCC5 (rs2440)[a]	ABCB4 (rs2302387)
SERPINE (rs2070682)[a]	IL12A (rs582537)[a]	ALDH-1A1 (rs168351)
NAT2 (rs2410558)[a]	HMMR (rs299295)[a]	ALDH-1A1 (rs610529)
TNFRSF17 (rs12922317)[a]	LEP (rs10249476)[a]	PARP1 (rs1805414)
LIG1 (rs20579)[a]	ALDH1A1 (rs2161811)[a]	VEGF (rs699947)
COMT (rs4633)[a]	ERCC6 (rs4253211)[a]	CETP (rs289747)
MT (rs13815)[a]	CHEK1 (rs506504)[a]	GAN (rs2608555)
CDKN1A (rs3829963)[a]		
Cisplatin		
PAI-1 (rs1799889)	PAI-1 (rs1799889)	Factor V (rs6025)
Factor II (rs1799963)		
Lenalidomide		
CINP (rs7011)	CDKN1A (rs3829963)	CHEK1 (rs506504) CC
ALDH 1A1 (rs610529)	XRCC5 (rs2440)	TNFRSF17 (rs12922317)
NFKB1 (rs3774968)		

Table 1: Summary of SNPs investigated in studies (cont).

Venous thromboembolism		
Bevacizumab		
VEGF (rs2010963)[a]	VEGF (rs833061)[a]	FIP200 (rs1129660)
VEGF (rs13207351)[a]	ATG3 (rs9831088)	FIP200 (rs17337252)
VEGF (rs1570360)[a]	ATG5 (rs633724)	ULK1 (rs11616018)
VEGF (rs699947)[a]	ATG8 (rs8060972)	UKL1 (rs12303764)
VEGF (rs35569394)[a]	ATG8 (rs11149841)	ULK1 (rs9481)
VEGF (rs1005230)[a]	ATG13 (rs13448)	UVRAG (rs1458836)
VEGF (rs35864111)[a]	BECN1 (rs11552191)	
Temozolomide		
VEGF (rs2010963)		
Coronary heart disease		
Cisplatin		
Factor V (rs1799963)[a]	Factor II (rs6025)	PAI-1 (rs1799889)
Arrhythmia		
DYPD (rs1801159)[a]		

[a]significant association found in at least one study; ABCB, ATP binding cassette subfamily B member; ABCG2, ATP binding cassette subfamily G member 2; ACE, angiotensin I converting enzyme; AGT, angiotensinogen; AGTR1, angiotensin II receptor type 1; ALDH 1A1, aldehyde dehydrogenase 1 family member A1; ATG, autophagy related; BDKRB1, bradykinin receptor B1; BECN1, beclin 1; BRINP1, BMP/Retinoic acid inducible neural specific 1; CASP3, caspase 3; CDKN1A, cyclin dependent kinase inhibitor 1A; CETP, cholesteryl ester transfer protein; CHEK1, checkpoint kinase 1; CINP, cyclin dependent kinase 2 interacting protein; CREBRF, CREB3 regulatory factor; CYP11B2, cytochrome P450 family 11 subfamily B member 2; CYP3A5, cytochrome P450 family 3 subfamily A member 5; DCLRE1B, DNA cross-link repair 1B; DPYD, dihydropyrimidine dehydrogenase; EGF, epidermal growth factor; eNOS, endothelial nitric oxide synthase; ERCC6, ERCC excision repair 6, chromatin remodelling factor; EYS, eyes shut homolog (drosophila); FIG4, FIG4 phosphoinositide 5-phosphatase; FCGR2A, Fc fragment of IgG receptor IIa; FCGR3A, Fc fragment of IgG receptor IIIa; FIP200, focal adhesion kinase family interacting protein of 200kDa; GAN, gigaxonin; GNB3, G protein subunit beta 3; GRK4, G protein-coupled receptor kinase 4; GTF3C3, general transcription factor IIIC subunit 3; HER2, human epidermal growth factor receptor 2; HMMR, hyaluronan mediated motility receptor; IL, interleukin; KLKB1, kallikrein B1; KRT15, keratin 15; LDB2, LIM domain binding 2; LEP, leptin; LIG1, DNA ligase 1; LINC01060, long intergenic non-protein coding RNA 1060; MT, mitochondrially; MYADM, myeloid associated differentiation marker; NAT2, N-acetyltransferase 2; NFKB1, nuclear factor kappa B subunit 1; PAI-1, plasminogen activator inhibitor-1; PARP1, Poly(ADP-Ribose) polymerase 1; PHF3, PHD finger protein 3; PLEKHA6, pleckstrin homology domain containing A6; PPARD, peroxisome proliferator activated receptor delta; RAB22A, RAB22A member RAS oncogene family; SCNN1A, sodium channel epithelial 1 alpha subunit; SERPINE1, serpin family E member 1; SFTPA2, surfactant protein A2; SV2C, synaptic vesicle glycoprotein 2C; TNFRSF17, tumour necrosis factor receptor superfamily member 17; TRPC6, transient receptor potential cation channel subfamily C member 6; UGT1A9, UDP glucuronosyltransferase family member A9; ULK1, unc-51 like autophagy activating kinase 1; UVRAG, UV radiation resistance associated; VEGF, vascular

endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; WNK1, WNK lysine deficient protein kinase 1; XRCC5, X-ray repair cross complementing 5; ZNRF3, zinc and ring finger 3.

Table 2: Gene dose effect of trastuzumab-induced decreased LVEF.

SNPs	Pooled OR (95% CI)			p-value[a]
	Combination (Aa/aa)	Heterozygous (Aa)	Homozygous (aa)	
HER2 655A>G (rs1136201)	**2.43**[b] **(1.17 – 5.06)**	1.71 (0.91 – 3.23)	1.24 (0.70 – 2.18)	0.78
HER2 1170C>G (rs1058808)	0.69 (0.47 – 1.02)	0.76[b] (0.48 – 1.19)	0.44 (0.13 – 1.50)	0.36
FCGR2A 131C>T (rs1801274)	1.10 (0.42 – 2.87)	1.06 (0.36 – 3.12)	1.70[b] (0.83 – 3.49)	0.48
FCGR3A 158T>G (rs396991)	0.83 (0.37 – 1.89)	0.60[b] (0.25 – 1.48)	0.99 (0.41 – 2.41)	0.62

Bold indicate significant odds ratios. [a]Statistical test of difference between odds ratio for Aa and aa.
[b]Greatest effect among genotypes.

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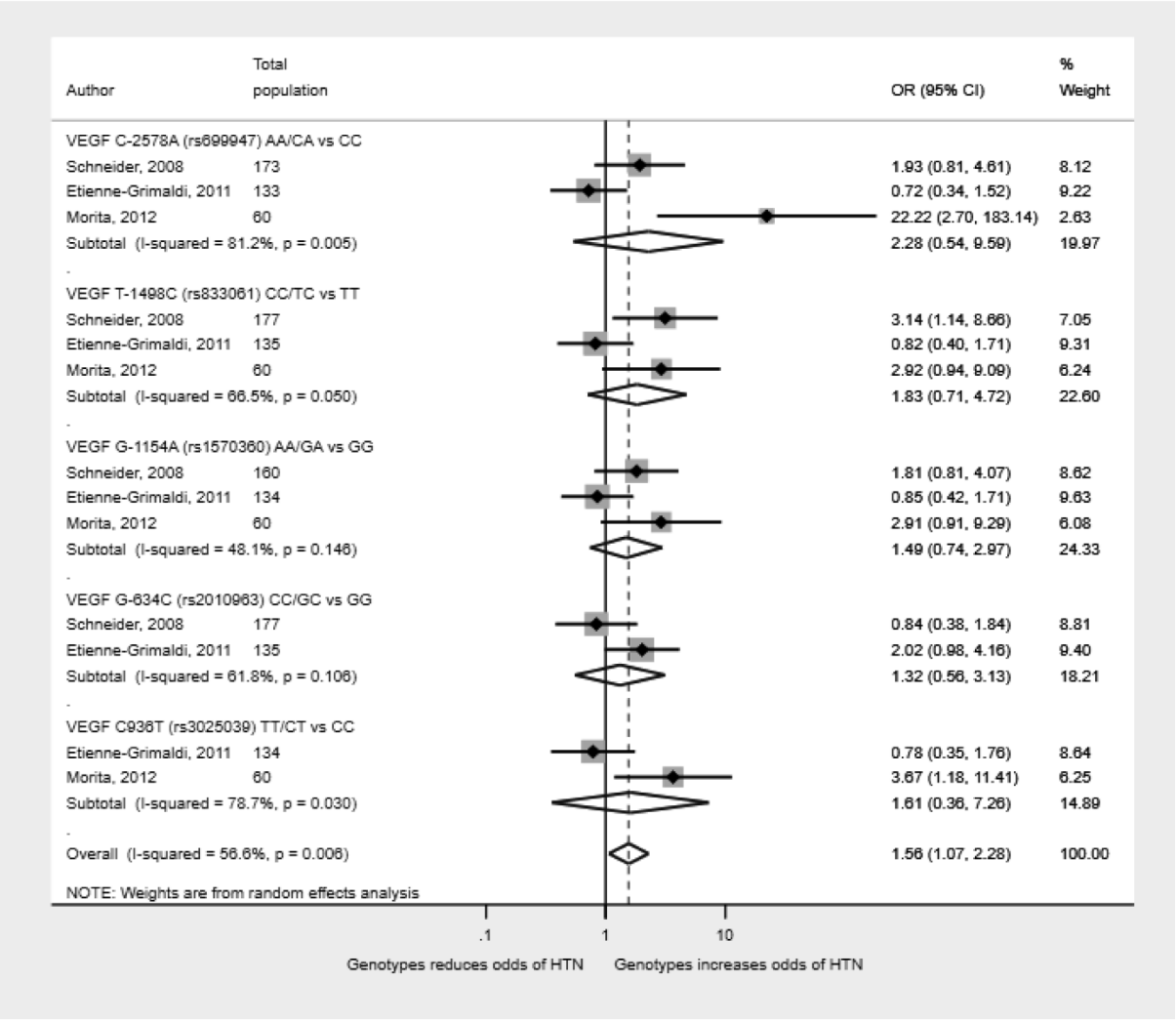


Figure 1: Meta-analysis of genotypes associated with bevacizumab-induced hypertension (HTN). VEGF, vascular endothelial growth factor

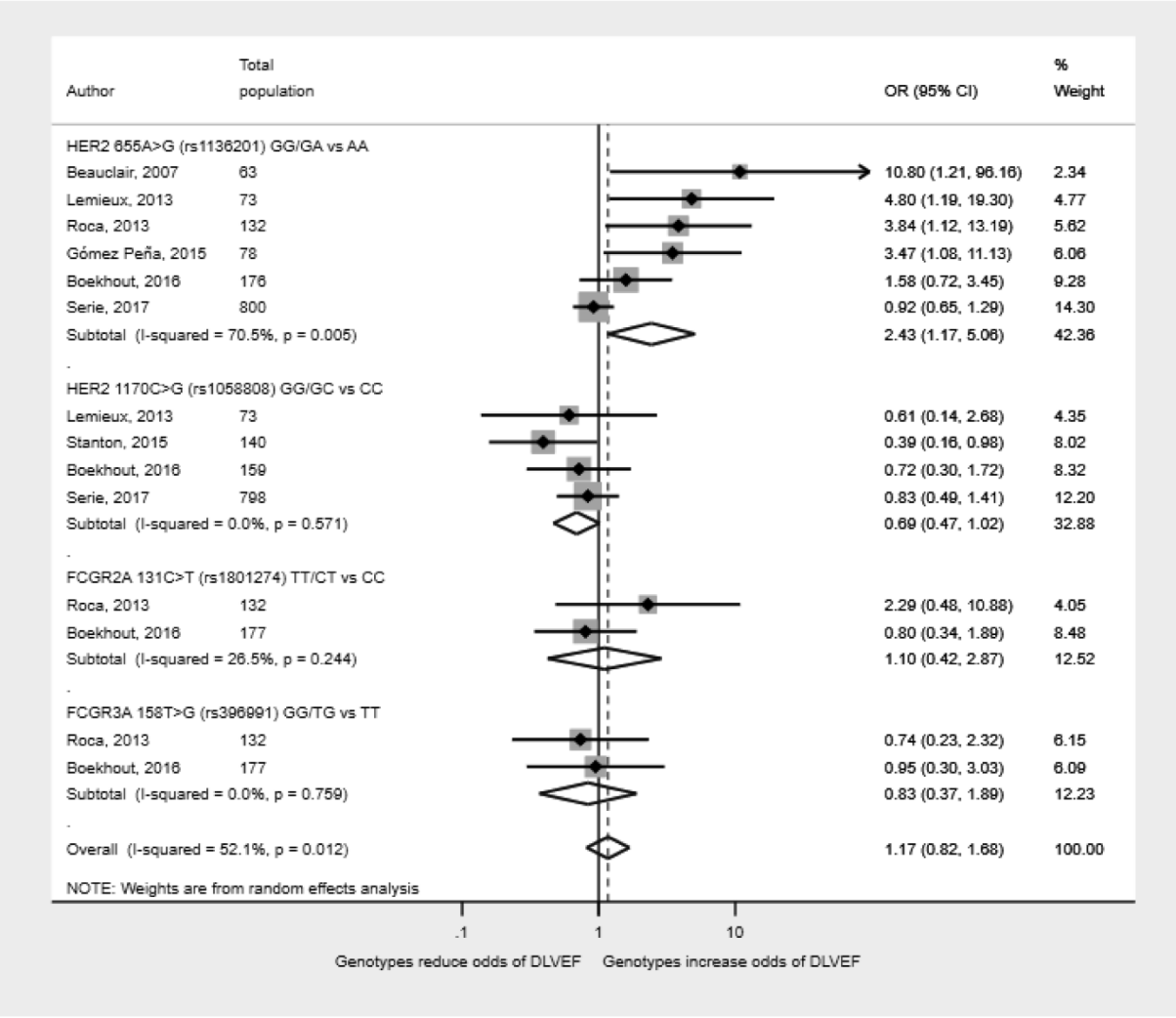


Figure 2: Meta-analysis of genotypes associated with trastuzumab-induced decreased left ventricular ejection fraction (DLVEF).
HER2, human epidermal growth factor receptor 2

Title: Roles of pharmacogenomics in antineoplastic-induced cardiovascular toxicities: A Systematic Review and Meta-analysis of genotypes effect.

Authors: Siew Lian Leong, Nathorn Chaiyakunapruk, Wichittra Tassaneeyakul, Poukwan Arunmanakul, Surakit Nathisuwan, Shaun Wen Huey Lee

Online-Only Supplements

Search Term

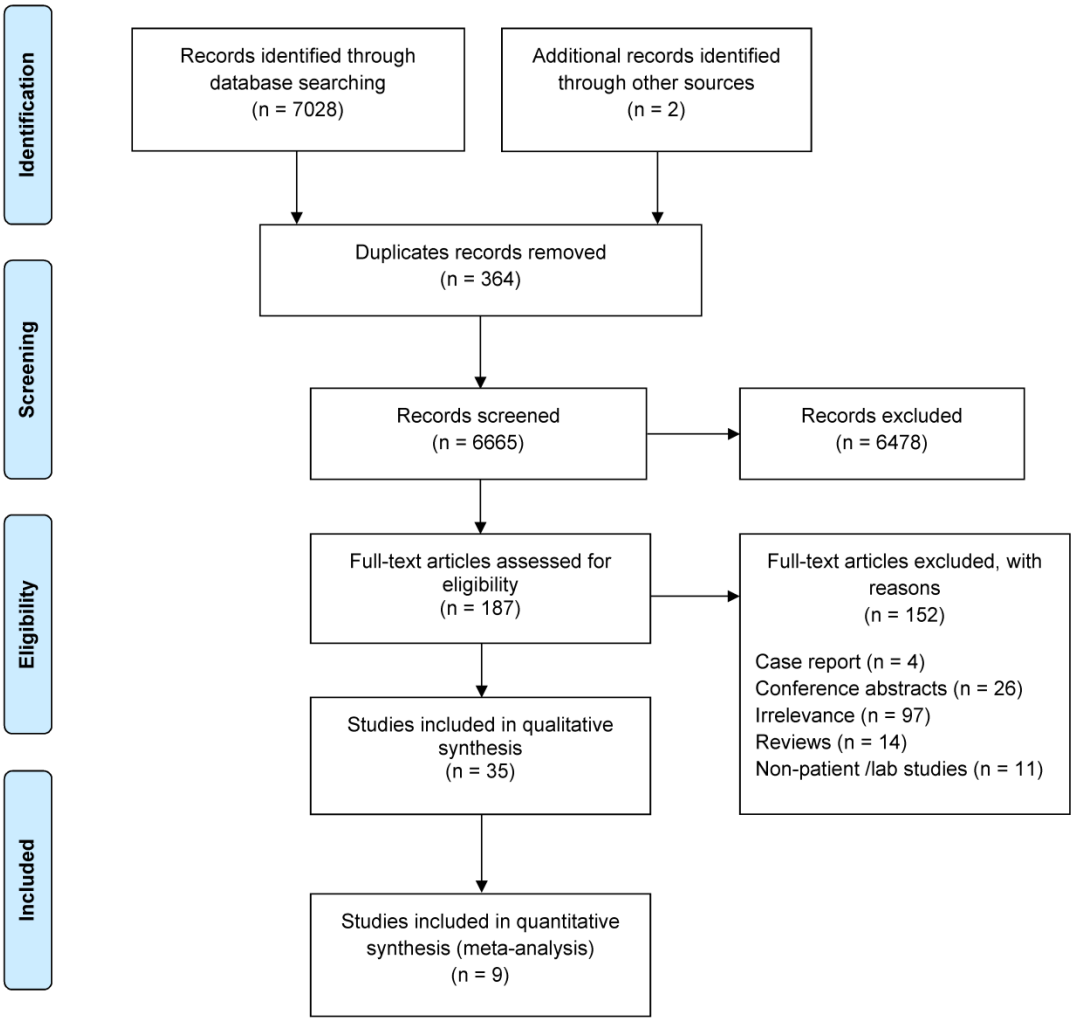
Search terms

1. Cochrane Central Register of Controlled Studies and EMBASE Keyword search
(1) antimetabolite*.mp.; (2) fluorouracil*.mp.; (3) mercaptopurine*.mp.; (4) capecitabine*.mp.; (5) cytarabine*.mp.; (6) fludarabine*mp.; (7) methotrexate*mp.; (8) bleomycin*.mp.; (9) gemcitabine*mp.; (10) "angiogenesis inhibitor".mp.; (11) bevacizumab*.mp.; (12) thalidomide*.mp.; (13) sorafenib*mp.; (14) sunitinib*.mp.; (15) pazopanib*.mp.; (16) evarolimus*.mp.; (17) "histone deacetylase inhibitor".mp.; (18) vorinostat*.mp.; (19) romidepsin*.mp.; (20) chidamide*.mp.; (21) panobinostat*.mp.; (22) belinostat*.mp.; (23) "tyrosine kinase inhibitor".mp.; (24) imatinib*.mp.; (25) gefitinib*.mp.; (26) erlotinib*.mp.; (27) cabozantinib*.mp; (28) "proteasome inhibitor".mp.; (29) bortezomib*.mp.; (30) carfilzomib*.mp.; (31) ixazomib*.mp.; (32) "antimicrotubule agent".mp.; (33) vinblastine*.mp.; (34) vincristine*.mp.; (35) "vinca alkaloid".mp.; (36) taxane*.mp.; (37) paclitaxel*.mp.; (38) docetaxel*.mp. (39) "monoclonal antibody".mp.; (40) trastuzumab*.mp.; (41) rituximab*.mp.; (42) 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41; (43) genetic*.mp.; (44) polymorphism*.mp.; (45) pharmacogenomics*.mp.; (46) variant*.mp.; (47) 43 or 44 or 45 or 46; (48) "left ventricular dysfunction".mp.; (49) "heart failure".mp.; (50) cardiomyopathy*.mp.; (51) stroke*.mp.; (52) "QTc prolongation".mp.; (53) arrhythmia*.mp.; (54) "myocardial ischemia".mp.; (55) hypertension*.mp.; (56) "ejection fraction".mp.; (57) embolism*.mp.; (58) "cardiovascular toxicity".mp. (59) cardiotoxicity*.mp.; (60) 48 or 49 or 50 or 51 or 52 or 53 or 54 or 55 or 56 or 57 or 58 or 59; (60) 42 and 47 and 60

2. PUBMED Text Word Search
(1) antimetabolite* or fluorouracil* or mercaptopurine* or capecitabine* or cytarabine* or fludarabine* or methotrexate* or bleomycin* or gemcitabine* or "angiogenesis inhibitor" or bevacizumab* or thalidomide* or sorafenib* or sunitinib* or pazopanib* or evarolimus* or "histone deacetylase inhibitor" or vorinostat* or romidepsin* or chidamide* or panobinostat* or belinostat* or "tyrosine kinase inhibitor" or imatinib* or gefitinib* or erlotinib* or cabozantinib* or "proteasome inhibitor" or bortezomib* or carfilzomib* or ixazomib* or "antimicrotubule agent" or vinblastine* or vincristine* or "vinca alkaloid" or taxane* or paclitaxel* or docetaxel* or "monoclonal antibody" or trastuzumab* or rituximab*; (2) genetic* or polymorphism* or pharmacogenomics* or variant*; (3) "left ventricular dysfunction" or "heart failure" or cardiomyopathy* or stroke* or "QTc prolongation" or arrhythmia* or "myocardial ischemia" or hypertension* or "ejection fraction" or embolism* or "cardiovascular toxicity" or cardiotoxicity*; (4) 1 and 2 and 3

3. CINAHL Plus All Text search
(1) antimetabolite* or fluorouracil* or mercaptopurine* or capecitabine* or cytarabine* or fludarabine* or methotrexate* or bleomycin* or gemcitabine* (2) "angiogenesis inhibitor" or bevacizumab* or thalidomide* or sorafenib* or sunitinib* or pazopanib* or evarolimus* (3) "histone deacetylase inhibitor" or vorinostat* or romidepsin* or chidamide* or panobinostat*or belinostat* (4) "tyrosine kinase inhibitor" or imatinib* or gefitinib* or erlotinib* or cabozantinib* (5) "proteasome inhibitor" or bortezomib* or carfilzomib* or ixazomib* (6) "antimicrotubule agent" or vinblastine* or vincristine* or "vinca alkaloid" or taxane* or paclitaxel* or docetaxel* (6) "monoclonal antibody" or trastuzumab* or rituximab*; (8) 1 or 2 or 3 or 4 or 5 or 6 or 7; (9) genetic* or polymorphism* or pharmacogenomics* or variant*; (10) "left ventricular dysfunction" or "heart failure" or cardiomyopathy* or stroke* or "QTc prolongation" or arrhythmia* or "myocardial ischemia" or hypertension* or "ejection fraction" or embolism* or "cardiovascular toxicity" or cardiotoxicity*; (11) 8 and 9 and 10

4. HuGE Navigator search
(1) antimetabolite and cardiovascular toxicity and genetic; (2) angiogenesis inhibitor and cardiovascular toxicity and genetic; (3) histone deacetylase inhibitor and cardiovascular toxicity and genetic; (4) tyrosine kinase inhibitor and cardiovascular toxicity and genetic (5) proteasome inhibitor and cardiovascular toxicity and genetic (6) antimicrotubule agent and cardiovascular toxicity and genetic (7) vinca alkaloid and cardiovascular toxicity and genetic (8) taxane and cardiovascular toxicity and genetic (9) monoclonal antibody and cardiovascular toxicity and genetic



eFigure 1: Selection process of included studies.

eTable 1: Descriptions of included studies (N = 35).

Author (Year)	Geographic location; ethnic group	Study design; number of patients	Age (years)	Gender: male/ female	Cancer	Chemotherapy	Source of DNA sample	Genotyping	Definition of cardiotoxicity
Yamaguchi (2001)[1]	Japan; Japanese	Prospective cohort; 107	NR	NR	Mixed type	5-fluorouracil	Whole blood	PCR-SSCP	Arrhythmia
Kato (2016)[2]	Japan, Japanese	Retrospective cohort; 20	Med = 67 (35 – 78)	14/6	RCC	Axitinib	Whole blood	TaqMan® SNP genotyping assay	NCI CTCAE grade ≥2 hypertension
Escudier (2015)[3]	Worldwide*; White (288), Black (1), Asian (15), Other (2)	Retrospective cohort; 306	NR	221/85	mRCC	Axitinib, Sorafenib	Whole blood	TaqMan® SNP Genotyping Assay	Grade ≥hypertension, ≥ 1 diastolic BP >90mmHg, diastolic BP increase ≥15mmHg
Schneider (2008)[4]	NR	Retrospective cohort; 363	NR	NR	Breast cancer	Bevacizumab	Paraffin-embedded tissue	TaqMan-based real-time PCR	NCI CTCAE v2.0 grade 3-4 hypertension
Etienne-Grimaldi (2011)[5]	France; Caucasian (136), other (1)	Retrospective cohort; 137	Med = 56 (24 – 79)	0/137	Breast cancer	Bevacizumab	Whole blood	PCR-RFLP	NCI CTCAE v3.0 grade 1- 4 hypertension
Formica (2011)[6]	Italy; Caucasian (38), African/American (1), Hispanic (1)	Prospective cohort; 40	Med = 61 (30 – 78)	22/18	Metastatic colorectal cancer	Bevacizumab and FOLFIRI	Whole blood	PCR	NCI CTCAE v3.0 grade 3 – 4 hypertension or DVT
Morita (2012)[7]	Japan; Japanese	Retrospective cohort; 60	Med = 62 (36 – 80)	38 / 22	Metastatic colorectal cancer	Bevacizumab	Whole blood	TaqMan® SNP Genotyping Assay	Hypertension

eTable 1: Descriptions of included studies (N = 35) (cont).

Author (Year)	Geographic location; ethnic group	Study design; number of patients	Age (years)	Gender: male/ female	Cancer	Chemotherapy	Source of DNA sample	Genotyping	Definition of cardiotoxicity
Lambrechts (2014)[8]	Worldwide[2]; White	Retrospective cohort; 1631	Mean = 58.2 ± 11.2	816/815	NSCLC, renal cancer, pancreatic cancer, breast cancer, colorectal cancer, gastric cancer	Bevacizumab	Whole blood	MassARRAY iPLEX Gold	NCI CTCAE v3.0 grade 1 – 4 hypertension
Schneider (2014)[9]	NR; ECOG-5103: European Ameican ECOG-2100: Caucasion	RCT; ECOG-5103: 824, ECOG-2100: 149	NR	NR	Breast cancer	Bevacizumab	ECOG-2100: Paraffin-embedded tissue	ECOG-5103: HumanOmnil-Quad array, Human OmniExpress array ECOG-2100: TaqMan SNP Genotyping Assay	ECOG-5103: Hypertension (SBP >160mmHg) ECOG-2100: CTC v2.0 grade 3-5 hypertension
Di Stefano (2015)[10]	NR	Retrospective cohort; 225	Med = 61.6 (15 – 80)	132/93	Grade II – IV glioma	Bevacizumab, temozolamide	Whole blood	TaqMan® SNP Genotyping Assay	NCI CTCAE v4.0 grade 1 – 4 thrombo-haemorrhagic
Sibertin-Blanc (2015)[11]	France; NR	Retrospective cohort; 89	63.6	63/26	Metastatic colorectal cancer	Bevacizumab	Whole blood, paraffin-embedded tissue	TaqMan® endpoint PCR	NCI-CTCAE v3.0 grade 2-3 hypertension

eTable 1: Descriptions of included studies (N = 35) (cont).

Author (Year)	Geographic location; ethnic group	Study design; number of patients	Age (years)	Gender: male/ female	Cancer	Chemotherapy	Source of DNA sample	Genotyping	Definition of cardiotoxicity
Berger (2017)[12]	Italy; NR	Retrospective cohort; 657	Med (range) = Discovery: 60 (19 – 75), Validation 65 (31 – 76), Control 64 (38 – 76)	Discovery: 134/85, Validation: 158/76, Control 143/61	Metastatic colorectal cancer	Bevacizumab and FOLFIRI; cetuxumab and FOLFIRI	Whole blood, formalin-fixed paraffin embedded tissue	PCR-based direct DNA sequence analysis	NCI CTCAE v3.0 grade ≥2 hypertension, VTE
Frey (2017)[13]	North America; NR	Prospective; 114	Mean = 57 ± 11.6 (range = 29 - 86)	19/95	Breast cancer, NSCLC, serous ovarian cancer, other advanced solid tumours	Bevacizumab	Blood	Sequenom iPLEX matrix-assisted laser desorption/ionization (MALDI)-time of flight (TOF) mass spectrometry	NCI CTCAE v3.0 grade 3 – 4 hypertension
De Haas (2010)[14]	Netherlands; NR	Retrospective cohort; 324	Med = 28 (16 – 64)	324/0	Non-seminomatous testicular cancer	Cisplatin	Whole blood	Allelic discrimination assay	VTE (DVT and/or PE), CHD (Myocardial infarction /CAD)
Bagratuni (2013)[15]	Greece; Greek (105), others (3)	Retrospective cohort; 108	Med = 71 (39 – 87)	58/50	Multiple myeloma	Lenalidomide	Whole blood		VTE
Jain (2010)[16]	United States; Hispanic, Caucasians, African-Americans, Asians	Retrospective cohort; 178	NR	143/35	mCRPC, NSCLC, CRC, KS, ST	Sorafenib and/or Bevacizumab	Whole blood	Single / nested PCR	NCI CTCAE v3.0 hypertension ≥ grade 2

eTable 1: Descriptions of included studies (N = 35) (cont).

Author (Year)	Geographic location; ethnic group	Study design; number of patients	Age (years)	Gender: male/ female	Cancer	Chemotherapy	Source of DNA sample	Genotyping	Definition of cardiotoxicity
Boudou-Rouquette (2012)[17]	France; NR	Prospective cohort; 54	Med = 64 (58 – 76)	38/16	Advanced or metastatic solid tumours	Sorafenib	Whole blood	TaqMan® drug metabolism genotyping assay	NCI CTCAE v4.0 hypertension
Garcia-Donas (2011)[18]	Spain; white Spanish (94), African (1), Asian (1)	Prospective cohort; 95	Med = 65 (42 -87, 56 – 73)	65/30	Clear-cell RCC	Sunitinib	Whole blood	KASPar SNP genotyping system	NCI CTCAE v3.0 grade 3 – 4 hypertension
Eechoute (2012)[19]	Netherlands; Caucasian (232), Other (15), Unknown (8)	Retrospective cohort; 255	Med = 60 (20 – 89)	161/94	Renal cell carcinoma, GIST, others	Sunitinib	Whole blood	TaqMan® SNP Genotyping Assay	NCI CTCAE v3.0 grade 3 hypertension
Kim (2012)[20]	NR	Retrospective cohort; 63	Med = 60 (35 – 80)	49/14	mRCC	Sunitinib	Whole blood, frozen tissue	PCR	Hypertension (SBP ≥150mmHg and/or DBP ≥90mmHg)
Diekstra (2015)[21]	Netherlands, Spain, United States; White (321), Black (5), Asian (3), Latin American (1), Arabian (3)	Retrospective cohort; 333	Med = 61 (Percentiles: 55, 69)	228/105	mRCC	Sunitinib	Whole blood mononuclear cell samples	TaqMan®, KASPar SNP genotyping system	NCI CTCAE v3.0 or 4.0 grade >2 hypertension
Diekstra (2015)[22]	Netherlands, Spain, United States; White (358), Asian (3), Black (8), Arabian (4), Latin American (1)	Retrospective cohort; 374	Med = 61 (Percentiles: 54, 68)	258/116	mRCC	Sunitinib	Whole blood mononuclear cell samples	TaqMan®	NCI CTCAE v3.0 or 4.0 grade >2 hypertension

eTable 1: Descriptions of included studies (N = 35) (cont).

Author (Year)	Geographic location; ethnic group	Study design; number of patients	Age (years)	Gender: male/ female	Cancer	Chemotherapy	Source of DNA sample	Genotyping	Definition of cardiotoxicity
Diekstra (2017)[23]	Netherlands; Caucasian (277), Black (4), Asian (2), Arab (3), Latin American (1)	Retrospective cohort; 287	Med = 61 (34 – 87)	195/92	mRCC	Sunitinib	Whole blood, serum, plasma	TaqMan® SNP Genotyping Assay	NCI CTCAE v 3.0 or 4.0 grade 3 – 4 hypertension
Dornbusch (2016)[24]	German; Caucasian	Retrospective cohort; 121	Med = 59 (IQR = 53.5 – 67.0)	95/26	mRCC	Sunitinib	Cryo-preserved non-malignant kidney tissue specimen, whole blood	TaqMan® SNP Genotyping Assays	Hypertension
Low (2016)[25]	Japan, NR	Prospective cohort; 219	Med = 63 (32 – 83)	161/58	RCC	Sunitinib	NR	PCR	NCI CTCAE v3.0 grade 3 – 4 hypertension
Johnson (2008)[26]	NR; European descent	Nested case control; 702	NR	NR	Multiple myeloma	Thalidomide	Whole blood	Affymetrix targeted genotyping	VTE
Almasi (2011)[27]	Czech Republic; Caucasian	Case control; 111	NR	53/58	Multiple myeloma	Thalidomide	Whole blood	TaqMan® SNP Genotyping Assay	NCI CTCAE grade 3 -4 VTE
Beauclair (2007)[28]	France; NR	Prospective cohort; 61	Med = 50.7 (30.5 - 83.1)	NR	Advanced breast cancer	Trastuzumab	Whole blood	PCR-RFLP-based assay	A decline in LVEF of ≥ 20%

eTable 1: Descriptions of included studies (N = 35) (cont).

Author (Year)	Geographic location; ethnic group	Study design; number of patients	Age (years)	Gender: male/ female	Cancer	Chemotherapy	Source of DNA sample	Genotyping	Definition of cardiotoxicity
Lemieux (2013)[29]	Canada; French	Retrospective cohort, 73	Med = 55	0/73	non-metastatic HER2-positive invasive breast cancer	Trastuzumab	Normal breast tissue / Whole blood	TaqMan® SNP Genotyping Assay	A decline in LVEF of ≥ 10% from baseline with a resulting LVEF <50% or absolute LVEF value of <45%
Roca (2013)[30]	France; NR	Prospective cohort, 132	Med = 48.0 (24.0 - 65.0)	0/132	HER2-positive breast cancer	Trastuzumab	Whole blood	NR	A relative decline in LVEF of >15% or left ventricular toxicity or LVEF absolute value of <50%
Gómez Peña (2015)[31]	Europe; NR	Prospective cohort; 78	Mean = 51.72 ± 12.26	0/78	HER2-positive breast cancer	Trastuzumab	Saliva	TaqMan® SNP Genotyping Assay	A decline in LVEF ≥ 10% from baseline with a resulting LVEF <50% or a decline in LVEF of >15% or absolute LVEF value of <45% at least once
Stanton (2015)[32]	New York; White (112), Asian (18), Black (10)	Case control; 140	Med = 56 (32 - 85)	0/140	Breast cancer	Trastuzumab	Whole blood, buccal	real-time PCR	A decline in LVEF of 15% or a decline in LVEF of 10% if LVEF <55%, or symptomatic CHF
Boekhout (2016)[33]	Europe; NR	Prospective cohort; 206	Mean = 49	NR	Early-stage HER2-positive breast cancer	Trastuzumab	Whole blood	TaqMan® SNP Genotyping Assays	Decline in LVEF of >15% or LVEF absolute value of <45%

eTable 1: Descriptions of included studies (N = 35) (cont).

Author (Year)	Geographic location; ethnic group	Study design; number of patients	Age (years)	Gender: male/ female	Cancer	Chemotherapy	Source of DNA sample	Genotyping	Definition of cardiotoxicity
Serie (2017)[34]	North America; White/non-Hispanic	Prospective; 800	NR	NR	Early HER2+ breast cancer	Trastuzumab	Blood	Affymetrix Axiom array	Decline in LVEF of >20% or LVEF absolute value of <50%
Udagawa (2018)[35]	Japan; Japanese	Retrospective cohort; Screening: 1460 Replication: 243	Med (range) = Replication: Case: 52 (32 – 72), Control 56 (29 – 86)	Replication: Case: 1/18, Control: 56/168	Breast cancer, gastric, others and unknown	Trastuzumab	Formalin-fixed paraffin embedded lymph nodes or blood	Sanger sequencing or TaqMan®	Decline in LVEF of >10%

*Australia, Austria, Brazil, Canada, China, France, Germany, Greece, India, Ireland, Italy, Japan, Korea, Poland, Russian, Singapore, Slovakia, Spain, Sweden, Taiwan, United Kingdom, United States; [2]Argentina, Australia, Austria, Belgium, Brazil, Bulgaria, Canada, China, Czech Republic, Denmark, Finland, France, Germany, Greece, Guatemala, Hong Kong, Hungary, Ireland, Israel, Italy, Korea, Mexico, Netherlands, New Zealand, Norway, Panama, Peru, Poland, Portugal, Puerto Rico, Russian, Singapore, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, Turkey, United Kingdom, United States. CAD, coronary artery disease; CHD, coronary heart disease; CHF, congestive heart failure; CRC, colorectal cancer; DVT, deep vein thrombosis; EF, ejection fraction; FOLFIRI, irinotecan + 5-fluorouracil+folinic acid regimen; GIST, gastrointestinal stromal tumour, IQR, interquartile range; LVEF, left ventricular ejection fraction; mCRPC, metastatic castrate resistant prostate cancer; Med, median; mRCC, metastatic renal cell carcinoma; NCI-CTCAE , National Cancer Institute Common Terminology Criteria for Adverse Events; NR, not reported; NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PCR-SSCP, polymerase chain reaction-single strand conformation polymorphism; PE, pulmonary embolism; RCC, renal cell carcinoma; RCT, randomised control trial; ST, solid tumours; VTE, venous thromboembolism;

eTable 2: Quality of studies (N = 35).

Item Study	A	B	C	D	E	F	G	H	I	J	K	Total	Score
Yamaguchi, 2001[1]	4.5	4.0	2.0	4.0	3.0	4.5	5.5	4.0	4.0	4.5	4.0	44.0	Good
Beauclair, 2007[28]	6.0	5.0	0.0	5.0	3.0	3.5	4.0	3.0	4.0	5.0	5.0	43.5	Good
Schneider, 2008[4]	6.0	5.0	3.0	4.5	4.0	4.0	6.0	5.0	4.5	4.5	5.0	51.5	Good
Johnson, 2008[26]	7.0	5.0	5.5	5.5	3.0	4.5	5.5	6.5	5.0	5.0	6.0	58.5	Good
de Haas, 2010[14]	6.0	5.0	0.0	5.5	4.5	4.5	6.0	5.0	4.5	5.0	5.0	51.0	Good
Jain, 2010[16]	4.5	4.5	0.0	4.0	3.0	4.0	5.5	5.0	5.0	5.5	5.5	46.5	Good
Almasi, 2011[27]	5.5	5.0	0.0	5.0	4.0	4.0	5.0	5.5	5.5	5.0	5.0	49.5	Good
Etienne-Grimaldi, 2011[5]	6.5	6.5	0.0	4.0	3.0	4.0	5.0	5.0	5.0	5.0	5.0	49.0	Good
Formica, 2011[6]	5.5	5.0	0.0	5.5	3.5	4.0	4.5	5.5	5.0	5.5	5.5	49.5	Good
Garcia-Donas, 2011[18]	5.0	4.0	0.0	6.0	5.0	4.0	5.0	6.5	4.5	5.0	5.0	50.0	Good
Boudou-Rouquette, 2012[17]	5.0	5.5	0.0	5.5	3.0	4.5	4.5	5.5	4.0	5.0	5.0	47.5	Good
Eechoute, 2012[19]	5.0	7.0	0.0	6.0	4.5	4.0	6.0	6.0	5.5	6.0	5.5	55.5	Good
Kim, 2012[20]	5.0	5.5	4.0	5.0	3.0	4.5	4.5	5.5	5.5	5.0	5.0	52.5	Good
Morita, 2013[7]	6.5	6.5	0.0	5.5	3.0	3.5	4.5	6.0	5.5	5.0	5.5	51.5	Good
Bagratuni, 2013[15]	5.0	6.0	5.0	5.5	4.5	4.5	5.5	5.0	5.5	4.5	5.0	56.0	Good
Lemieux, 2013[29]	6.0	6.5	4.5	5.0	3.0	4.5	6.0	5.5	5.0	5.5	6.0	57.5	Good
Roca, 2013[30]	6.0	4.5	5.5	4.0	4.0	4.5	6.0	4.5	5.5	5.0	5.5	55.0	Good
Lambrechts, 2014[8]	6.0	7.0	6.0	6.0	4.0	5.0	6.0	5.0	6.0	5.5	6.0	62.5	Good
Schneider, 2014[9]	6.5	6.5	6.0	6.5	4.5	4.5	6.5	6.0	6.5	5.5	6.0	65.0	Good
Di Stefano, 2015[10]	5.0	5.0	0.0	6.0	3.0	4.0	6.0	5.5	5.0	4.5	5.0	49.0	Good
Diekstra, 2015 [22]	6.5	6.0	0.0	6.0	4.0	5.0	6.5	6.0	5.5	6.5	6.5	58.5	Good

eTable 2: Quality of studies (N = 32) (cont).

Item Study	A	B	C	D	E	F	G	H	I	J	K	Total	Score
Diekstra, 2015[21]	5.0	5.5	5.0	5.0	4.0	5.5	5.5	6.0	5.0	6.0	5.0	57.5	Good
Escudier, 2015[3]	4.5	5.5	0.0	4.0	3.0	5.0	6.0	5.0	5.0	6.0	5.5	49.5	Good
Sibertin-Blanc, 2015[11]	7.0	7.0	0.0	5.0	3.0	4.0	4.0	5.0	5.0	6.0	5.0	51.0	Good
Gomez Pena, 2015[31]	7.0	6.5	0.0	6.0	4.5	4.0	5.0	5.5	5.5	5.5	6.5	56.0	Good
Stanton, 2015[32]	5.0	6.0	0.0	4.0	4.0	4.0	4.5	5.5	4.5	5.5	5.5	48.5	Good
Boekhout, 2016[33]	6.0	7.0	6.0	5.5	6.0	5.5	6.5	6.0	6.0	5.5	6.5	66.5	Good
Dornbusch, 2016[24]	6.5	4.5	0.0	5.5	4.0	4.5	5.0	5.5	5.0	4.5	5.0	50.0	Good
Kato, 2016[2]	6.0	4.0	0.0	5.0	3.0	3.5	5.0	5.5	4.0	5.0	5.0	46.0	Good
Low, 2016[25]	5.0	5.5	0.0	4.5	3.0	4.5	5.5	6.0	5.0	5.0	5.0	49.0	Good
Berger, 2017[12]	5.0	5.0	5.0	6.0	4.0	4.5	6.0	5.0	5.0	4.5	5.0	55.0	Good
Diekstra, 2017[23]	5.0	5.0	0.0	5.5	3.0	4.5	6.0	6.0	4.5	6.0	5.5	51.0	Good
Frey, 2017[13]	6.5	7.0	7.0	6.0	4.0	5.0	5.0	5.5	5.5	5.0	5.5	62.0	Good
Serie, 2017[34]	7.0	7.0	7.0	6.0	4.0	5.0	6.0	6.0	6.5	6.0	6.5	67.0	Good
Udagawa, 2018[35]	7.0	6.5	6.5	6.0	4.0	5.0	5.5	6.0	6.5	5.5	6.0	64.5	Good

Reported rate is an average rate given by two investigators. Investigators agrees on 56.1% of the items with moderate agreement (kappa = 0.41).
A, Rationale for study; B, Selection and definition of outcome of interest; C, Selection and comparability of comparison groups (if applicable); D, Technical classification of the exposure; E, Non-technical classification of the exposure; F, Other sources of bias; G, Sample size and power; H, A priori planning of analyses; I, Statistical methods and control for confounding; J, Testing of assumptions and inferences for genetic analyses; K, Appropriateness of inferences drawn from results.

eTable 3: Single nucleotide polymorphism in antineoplastic-induced hypertension.

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
ABCB1 (rs1128503)	Garcia-Donas, 2011	Sunitinib	**Significant association. HR = 0.41 (0.20 – 0.81), p = 0.011**	Yes (> 0.05)
ABCB1 (rs2032582)	Garcia-Donas, 2011	Sunitinib	**Significant association. HR = 0.42 (0.21 – 0.84), p = 0.014**	Yes (> 0.05)
	Boudou-Rouquette, 2012	Sorafenib	No significant association.	Yes (NR)
ABCG2 (rs2231142)	Garcia-Donas, 2011	Sunitinib	No significant association. HR = 1.29 (0.34 – 4.93), p = 0.71	Yes (> 0.05)
	Diekstra, 2015	Sunitinib	**Significant association. OR = 0.03 (0.001 – 0.85), p = 0.04[f]**	Yes (NR)
	Boudou-Rouquette, 2012	Sorafenib	No significant association.	Yes (NR)
	Low, 2016	Sunitinib	No significant association. AA vs AC/CC: OR = 1.08 (0.29 – 4.05), p = 0.91[b] CC vs AC/AA: OR = 0.74 (0.37 – 1.47), p = 0.39[b]	NR
	Kato, 2016	Axitinib	No significant association. With A allele vs without A allele: p = 1.00	NR
CYP3A4 (rs4646437)	Diekstra, 2017	Sunitinib	**Significant association. GG vs AG/AA: OR = 2.51 (1.21 – 5.22), p = 0.013**	Yes (NR)
EGF (rs4444903)	Lambrechts, 2014	Bevacizumab	**Significant association. OR = 1.57 (1.17 – 2.11), p = 0.0025**	Yes (0.46)
EGF (rs9992755)	Lambrechts, 2014	Bevacizumab	**Significant association. OR = 1.45 (1.08 – 1.96), p = 0.014**	Yes (0.29)
eNOS (rs2070744)	Eechoute, 2012	Sunitinib	**Significant association. CC/CT vs TT: OR = 2.62 (1.08 – 6.35), p = 0.045[f]**	NR
FIP200 (rs1129660)	Berger, 2017	FOLFIRI + bevacizumab	**Significant association. AG/GG vs AA: OR = 0.30 (0.12 – 0.67), p = 0.002**	Yes (NR)
	Berger, 2017	FOLFIRI + cetuximab	No significant association. AG/GG vs AA: OR = 1.40 (0.45 – 4.04), p = 0.60	Yes (NR)
GRK4 (rs1419044)	Frey, 2017	Bevacizumab	**Significant association. Allelic: OR = 0.09 (0.01 – 0.46), p = 0.001**	Yes (>0.1)
HT (rs1937506)	Lambrechts, 2014	Bevacizumab	**Significant association. OR = 1.53 (1.09 – 2.15), p = 0.015**	Yes (0.52)
IL8 A>T (rs1126647)	Garcia-Donas, 2011	Sunitinib	No significant association. HR = 0.94 (0.49 – 1.80), p = 0.85	Yes (> 0.05)
	Diekstra, 2015	Sunitinib	**Significant association. AA vs AT/TT: OR = 1.70 (1.08 – 2.68), p = 0.022**	Yes (>0.05)

eTable 3: Single nucleotide polymorphism in antineoplastic-induced hypertension (cont).

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
KLKB1 (rs1912826)	Frey, 2017	Bevacizumab	**Significant association. Allelic: OR = 2.19 (1.17 – 4.13), p = 0.01**	Yes (>0.1)
SV2C (rs6453204)	Schneider, 2014 (ECOG-5103)	Bevacizumab	No significant association. OR = 2.0, p = 6.4 x 10^{-5}[c]	Yes (p ≥ 0.0001)
	Schneider, 2014 (ECOG-2100)	Bevacizumab	**Significant association. OR = 2.4, p = 0.037**	Yes (p ≥ 0.0001)
ULK1 (rs9481)	Berger, 2017	FOLFIRI + bevacizumab	**Significant association. GA/AA vs GG: OR = 0.25 (0.03 – 1.05), p = 0.047**	Yes (NR)
VEGF (rs1570360)	Schneider, 2008	Bevacizumab	No significant association. AA vs GA/GG: p = 0.29 GG vs GA/AA: p = 0.15	NR
	Etienne-Grimaldi, 2011	Bevacizumab	No significant association. AA vs GA/GG: OR = 0.60 (0.20 – 1.81), p = 0.36[f] GG vs GA/AA: OR = 1,17 (0.59 – 2.35), p = 0.65[f]	Yes (NR)
	Morita, 2012	Bevacizumab	No significant association. GG vs GA/AA: OR = 0.34 (0.11 – 1.10), P = 0.07	Yes (NR)
	Kim, 2012	Sunitinib	No significant association. GG vs GA/AA: p = 0.14[e] AA vs GA/GG: p = 0.45[e]	NR
	Dornbusch, 2016	Sunitinib	No significant association. Variants vs wild type: OR = 0.60 (0.26 – 1.38), p = 0.29	Yes (0.830)
	Garcia-Donas, 2011	Sunitinib	**Significant association. HR = 2.04 (1.05 – 3.96), p = 0.035**	Yes (> 0.05)
	Diekstra, 2015	Sunitinib	No significant association. GG/GA vs AA: OR = 1.86 (0.76 – 4.52), p = 0.17[b]	Yes (NR)
	Formica, 2011	FOLFIRI + bevacizumab	No significant different	Yes (NR)

eTable 3: Single nucleotide polymorphism in antineoplastic-induced hypertension (cont).

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
VEGF (rs2010963)	Schneider, 2008	Bevacizumab	**Significant association. CC vs GC/GG: p = 0.005**	NR
	Etienne-Grimaldi, 2011	Bevacizumab	No significant association. GG vs GC/CC: OR = 0.49 (0.24 – 1..02), p = 0.06[b] CC vs GC/GG: OR = 2.11 (0.74 – 6.07), p = 0.16[b]	Yes (NR)
	Morita, 2012	Bevacizumab	No significant association. CC vs GC/GG: OR = 0.17 (0.02 – 1.12), p = 0.10[b]	Yes (NR)
	Kim, 2012	Sunitinib	**Significant association. CC vs GC/GG: p = 0.03**[d]	NR
	Dornbusch, 2016	Sunitinib	No significant association. Variant vs wild type: OR = 1.53 (0.66 – 3.55), p = 0.40	Yes (0.846)
	Garcia-Donas, 2011	Sunitinib	No significant association. HR = 0.63 (0.32 – 1.20), p = 0.16	Yes (> 0.05)
	Formica, 2011	FOLFIRI + bevacizumab	No significant different	Yes (NR)
VEGF (rs3025039)	Sibertin-Blanc, 2015	Bevacizumab	**Significant association. CC vs CT/TT: p = 0.019**	Yes (NR)
	Diekstra, 2015	Sunitinib	No significant association. OR = 1.58 (0.62 – 4.03), p = 0.3[f]	Yes (NR)
	Dornbusch, 2016	Sunitinib	No significant association. OR = 0.56 (0.21 – 1.53), p = 0.34	Yes (1.000)
	Etienne-Grimaldi, 2011	Bevacizumab	No significant association. TT vs CT/CC: OR = 0.76 (0.08 – 8.60), p = 0.82[b] CC vs CT/TT: OR = 1.27 (0.57 – 2.86), p = 0.56[b]	Yes (NR)
	Morita, 2012	Bevacizumab	**Significant association. CC vs CT/TT: OR = 0.27 (0.09 – 0.85), p = 0.02**[b]	Yes (NR)
	Kim, 2012	Sunitinib	No significant association. CC vs CT/TT: p = 0.52[b] TT vs CT/CC: p = 1.0[b]	NR
VEGF (rs3097)	Frey, 2017	Bevacizumab	**Significant association. Allelic: OR = 2.04 (1.05 – 3.85), p = 0.03**	Yes (>0.1)

eTable 3: Single nucleotide polymorphism in antineoplastic-induced hypertension (cont).

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
VEGF (rs699947)	Schneider, 2008	Bevacizumab	No significant association. AA vs CA/CC: p = 0.32 CC vs CA + AA: p = 0.16	NR
	Etienne-Grimaldi, 2011	Bevacizumab	No significant association. AA vs CA/CC: OR = 0.54 (0.23 – 1.29), p = 0.16[f] CC vs CA + AA: 1.38 (1.66 – 2.91), p = 0.16[f]	Yes (NR)
	Morita, 2012	Bevacizumab	**Significant association.** **CC vs CA/AA: OR = 0.05 (0.01 – 0.37), p = 0.004**[f]	Yes (NR)
	Kim, 2012	Sunitinib	**Significant association.** **CC vs CA/AA: p = 0.03**§	NR
	Dornbusch, 2016	Sunitinib	No significant association. Variants vs wild type: OR = 2.38 (0.71 – 7.97), p = 0.18	Yes (0.196)
	Garcia-Donas, 2011	Sunitinib	**Significant association.** **HR = 2.43 (1.27 – 4.66), p = 0.0074**	Yes (> 0.05)
	Formica, 2011	FOLFIRI + bevacizumab	No significant different	No
VEGF (rs833061)	Schneider, 2008	Bevacizumab	**Significant association.** **TT vs CC/CT: p = 0.02**	NR
	Etienne-Grimaldi, 2011	Bevacizumab	No significant association. CC vs CT/TT: OR = 0.47 (0.19 – 1.14), p = 0.10[b] TT vs CC/CT: OR = 1.22 (0.59 – 2.53), p = 0.60[b]	Yes (NR)
	Morita, 2012	Bevacizumab	No significant association. TT vs CC/CT: OR = 0.34 (0.11 – 1.07), p = 0.07[b]	Yes (NR)
	Kim, 2012	Sunitinib	**Significant association.** **TT vs CC/CT: p = 0.03**[d]	NR
	Formica, 2011	FOLFIRI + bevacizumab	No significant different.	No
VEGFR1 (rs9513070)	Escudier, 2015	Sorafenib	**Significant association.** **GG vs AA: OR = 3.89 (1.2 – 1.31), p = 0.035**	Yes (0.504)
	Escudier, 2015	Axitinib	No significant association. AG vs AA: OR = 0.98 (0.5 – 1.9), p = 1.00 GG vs AA: OR = 1.18 (0.5 – 3.1), p = 0.81	Yes (0.504)

eTable 3: Single nucleotide polymorphism in antineoplastic-induced hypertension (cont).

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
VEGFR2 (rs1870377)	Jain, 2010	Sorafenib & Bevacizumab	**Significant association. Variant vs wild type: OR = 2.3 (1.2 – 4.6), p = 0.0154**	Yes (p ≥ 0.77)
	Kim, 2012	Sunitinib	No significant association. AA vs AT/TT: p = 0.98[e] TT vs AT/AA: p = 0.29[e]	NR
	Dornbusch, 2016	Sunitinib	No significant association. Variant vs wild type: OR = 1.01 (0.43 – 2.34), p = 1.00	Yes (0.628)
	Garcia-Donas, 2011	Sunitinib	**Significant association. HR = 2.62 (1.32 – 5.20), p = 0.0058**	Yes (> 0.05)
VEGFR2 (rs2305948)	Garcia-Donas, 2011	Sunitinib	No significant association. HR = 1.09 (0.43 – 2.77), p = 0.85	Yes (> 0.05)
	Escudier, 2015	Sorafenib	No significant association. CT vs CC: OR = 0.70 (0.3 – 1.8), p = 0.47	Yes (0.738)
	Escudier, 2015	Axitinib	**Significant association. CT vs CC: OR = 0.36 (0.2 – 0.8), p = 0.016**	Yes (0.738)
WNK1 (rs11064560)	Lambrechts, 2014	Bevacizumab	**Significant association. OR = 1.41 (1.04 – 1.92), p = 0.028**	No (0.04)
	Frey, 2017	Bevacizumab	**Significant association. Allelic OR = 2.27 (1.25 – 4.17), p = 0.01**	Yes (>0.1)
WNK1 (rs11064519)	Frey, 2017	Bevacizumab	**Significant association. Allelic OR = 2.10 (1.15 – 3.84), p = 0.01**	Yes (>0.1)
WNK1 (rs2158501)	Frey, 2017	Bevacizumab	**Significant association. Allelic OR = 0.42 (0.22 – 0.80), p = 0.01**	Yes (>0.1)
WNK1 (rs2286028)	Frey, 2017	Bevacizumab	**Significant association. Allelic OR = 0.35 (0.11 – 0.91), p = 0.03**	Yes (>0.1)
WNK1 (rs7953912)	Frey, 2017	Bevacizumab	**Significant association. Allelic OR = 1.91 (0.99 – 3.65), p = 0.05**	Yes (>0.1)
ABCB1 (rs1045642)	Garcia-Donas, 2011	Sunitinib	No significant association. HR = 0.56 (0.29 – 1.09), p = 0.09	Yes (> 0.05)
	Boudou-Rouquette, 2012	Sorafenib	No significant association.	Yes (NR)
ABCG2 (rs2231137)	Boudou-Rouquette, 2012	Sorafenib	No significant association.	Yes (NR)
ABCG2 (rs2622604)	Diekstra, 2015	Sunitinib	No significant association. OR = 0.51 (0.20 – 1.34), p = 0.17[f]	Yes (NR)
	Boudou-Rouquette, 2012	Sorafenib	No significant association.	Yes (NR)

eTable 3: Single nucleotide polymorphism in antineoplastic-induced hypertension (cont).

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
ABCG2 (rs55930652)	Diekstra, 2015	Sunitinib	No significant association. OR = 0.45 (0.18 – 1.13), p = 0.45[f]	Yes (NR)
ACE (rs4295)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.20 (0.65 – 2.22), p = 0.62	Yes (<0.1)
ACE (rs4305)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.97 (0.54 – 1.76), p = 1.00	Yes (<0.1)
ACE (rs4309)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.91 (0.49 – 1.68), p = 0.87	Yes (<0.1)
ACE (rs4311)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.83 (0.46 – 1.49), p = 0.60	Yes (<0.1)
ACE (rs4343)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.83 (0.45 – 1.52), p = 0.61	Yes (<0.1)
ACE (rs4357)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = Inf (0.05 – Inf), p = 0.66	Yes (<0.1)
AGT (rs11568054)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 2.04 (0.27 – 15.56), p = 0.64	Yes (<0.1)
AGT (rs2004776)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.47 (0.74 – 2.86), p = 0.28	Yes (<0.1)
AGT (rs2478523)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.32 (0.71 – 2.38), p = 0.42	Yes (<0.1)
AGT (rs2478543)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.97 (0.54 – 1.75), p = 1.00	Yes (<0.1)
AGT (rs2478544)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.69 (0.31 – 1.45), p = 0.39	Yes (<0.1)
AGT (rs2478545)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.02 (0.51 – 2.08), p = 1.00	Yes (<0.1)
AGT (rs2493131)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.87 (0.19 – 3.25), p = 1.00	Yes (<0.1)
AGT (rs2493132)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.95 (0.50 – 1.82), p = 0.98	Yes (<0.1)
AGT (rs3789678)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.53 (0.60 – 3.78), p = 0.43	Yes (<0.1)
AGT (rs3889728)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.94 (0.46 – 1.85), p = 0.97	Yes (<0.1)
AGT (rs4762)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.75 (0.27 – 1.90), p = 0.68	Yes (<0.1)

eTable 3: Single nucleotide polymorphism in antineoplastic-induced hypertension (cont).

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
AGT (rs5050)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.87 (0.36 – 2.00), p = 0.89	Yes (<0.1)
AGT (rs6687360)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.22 (0.67 – 2.22), p = 0.58	Yes (<0.1)
AGT (rs7079)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.15 (0.60 – 2.25), p = 0.77	Yes (<0.1)
AGT (rs1926722)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.67 (0.18 – 2.09), p = 0.65	Yes (<0.1)
AGTR1 (rs12695902)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.81 (0.22 – 2.59), p = 0.93	Yes (<0.1)
AGTR1 (rs12721331)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.99 (0.23 – 3.70), p = 1.00	Yes (<0.1)
AGTR1 (rs1492099)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.89 (0.41 – 1.98), p = 0.88	Yes (<0.1)
AGTR1 (rs2675511)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.01 (0.52 – 1.94), p = 1.00	Yes (<0.1)
AGTR1 (rs275649)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.00 (0.53 – 1.92), p = 1.00	Yes (<0.1)
AGTR1 (rs2933249)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.23 (0.60 – 2.50), p = 0.65	Yes (<0.1)
AGTR1 (rs3772616)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.33 (0.64 – 2.70), p = 0.51	Yes (<0.1)
AGTR1 (rs385338)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.24 (0.62 – 2.44), p = 0.62	Yes (<0.1)
AGTR1 (rs389566)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.06 (0.56 – 2.02), p = 0.99	Yes (<0.1)
AGTR1 (rs4681440)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.63 (0.17 – 1.87), p = 0.53	Yes (<0.1)
AGTR1 (rs5182)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.68 (0.37 – 1.25), p = 0.24	Yes (<0.1)
ATG13 (rs13448)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. TC/CC vs TT: OR = 0.81 (0.45 – 1.43), p = 0.49	Yes (NR)
ATG3 (rs9831088)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. AG/GG vs AA: OR = 0.61 (0.22 – 1.57), p = 0.29	Yes (NR)
ATG5 (rs633724)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. CT/TT vs CC: OR = 1.01 (0.40 – 2.63), p = 1.00	Yes (NR)

eTable 3: Single nucleotide polymorphism in antineoplastic-induced hypertension (cont).

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
ATG8 (rs11149841)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. GT/TT vs GG: OR = 1.53 (0.15 – 1.56), p = 0.25	Yes (NR)
ATG8 (rs8060972)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. AT/TT vs AA: OR = 1.41 (0.46 – 3.89), p = 0.61	Yes (NR)
BDKRB1 (rs10147171)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.92 (0.80 – 2.70), p = 1.00	Yes (<0.1)
BDKRB1 (rs11622768)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.99 (0.29 – 2.98), p = 1.00	Yes (<0.1)
BDKRB1 (rs2071083)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 2.94 (0.77 – 12.50), p = 0.13	Yes (<0.1)
BDKRB1 (rs2071084)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.18 (0.49 – 2.72), p = 0.82	Yes (<0.1)
BDKRB1 (rs885845)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.25 (0.68 – 2.28), p = 0.53	Yes (<0.1)
BECN1 (rs11552192)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. AT/TT vs AA: OR = 0.68 (0.12 – 2.47), p = 0.59	Yes (NR)
CYP3A4 (rs2740574)	Garcia-Donas, 2011	Sunitinib	No significant association. HR = 1.31 (0.21 – 8.28), p = 0.77	Yes (> 0.05)
CYP3A5 (rs776746)	Garcia-Donas, 2011	Sunitinib	No significant association. HR = 0.96 (0.27 – 3.48), p = 0.96	Yes (> 0.05)
	Diekstra, 2015	Sunitinib	Significant association. OR = 4.70 (1.47 – 15.0), p = 0.009[f]	No (0.014)
	Boudou-Rouquette, 2012	Sorafenib	No significant association.	No
CYP11B2 (rs12050217)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.97 (0.45 – 2.17), p = 1.00	Yes (<0.1)
CYP11B2 (rs1799998)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.62 (0.89 – 2.94), p = 0.12	Yes (<0.1)
CYP11B2 (rs4543)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 2.38 (0.72 – 8.33), p = 0.17	Yes (<0.1)
CYP11B2 (rs6433)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.42 (0.77 – 2.59), p = 0.28	Yes (<0.1)
FIP200 (rs17337252)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. AG/GG vs AA: OR = 1.03 (0.37 – 3.37), p = 1.00	Yes (NR)
GNB3 (rs5446)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.72 (0.37 – 1.37), p = 0.36	Yes (<0.1)

eTable 3: Single nucleotide polymorphism in antineoplastic-induced hypertension (cont).

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
GRK4 (rs1010290)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.05 (0.55 – 2.03), p = 1.00	Yes (<0.1)
GRK4 (rs1419043)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.59 (0.40 – 900024), p = 0.72	Yes (<0.1)
GRK4 (rs1557213)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.12 (0.85 – 2.17), p = 0.79	Yes (<0.1)
GRK4 (rs17835422)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.46 (0.08 – 1.78), p = 0.36	Yes (<0.1)
GRK4 (rs1801058)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.17 (0.64 – 2.13), p = 0.69	Yes (<0.1)
GRK4 (rs2067003)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.14 (0.63 – 2.07), p = 0.74	Yes (<0.1)
GRK4 (rs2105380)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.00 (0.00 – 2.94), p = 0.38	Yes (<0.1)
GRK4 (rs2515936)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.78 (0.38 – 1.54), p = 0.56	Yes (<0.1)
GRK4 (rs2857845)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.79 (0.88 – 3.57), p = 0.11	Yes (<0.1)
KLKB1 (rs1511802)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.61 (0.32 – 1.15), p = 0.14	Yes (<0.1)
KLKB1 (rs3087505)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.86 (0.29 – 2.33), p = 0.96	Yes (<0.1)
KLKB1 (rs3775302)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.70 (0.28 – 1.61), p = 0.49	Yes (<0.1)
KLKB1 (rs4253251)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.71 (0.34 – 1.51), p = 0.42	Yes (<0.1)
KLKB1 (rs4253260)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.56 (0.74 – 3.22), p = 0.27	Yes (<0.1)
KLKB1 (rs4253292)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.64 (0.76 – 2.47), p = 0.23	Yes (<0.1)
KLKB1 (rs4253296)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.67 (0.62 – 4.35), p = 0.36	Yes (<0.1)
KLKB1 (rs4253315)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.77 (0.28 – 1.93), p = 0.71	Yes (<0.1)
KLKB1 (rs4253327)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.85 (0.44 – 1.64), p = 0.70	Yes (<0.1)

eTable 3: Single nucleotide polymorphism in antineoplastic-induced hypertension (cont).

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
KLKB1 (rs4253331)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 2.20 (0.73 – 6.56), p = 0.18	Yes (<0.1)
KLKB1 (rs925453)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.37 (0.71 – 2.61), p = 0.39	Yes (<0.1)
PDGFR-α (rs35597368)	Garcia-Donas, 2011	Sunitinib	No significant association. HR = 1.26 (0.51 – 3.10), p = 0.62	Yes (> 0.05)
SCNN1A (rs2041375)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.91 (0.45 – 1.77), p = 0.89	Yes (<0.1)
SCNN1A (rs2228576)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.72 (0.37 – 1.37), p = 0.36	Yes (<0.1)
SCNN1A (rs2286600)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.99 (0.55 – 1.83), p = 1.00	Yes (<0.1)
SCNN1A (rs3764874)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.70 (0.83 – 3.47), p = 0.16	Yes (<0.1)
SCNN1A (rs3764875)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.34 (0.71 – 2.51), p = 0.41	Yes (<0.1)
SCNN1A (rs3782723)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.50 (0.78 – 2.96), p = 0.26	Yes (<0.1)
SCNN1A (rs4764585)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.92 (0.51 – 2.67), p = 0.88	Yes (<0.1)
SCNN1A (rs7973914)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.77 (0.40 – 1.48), p = 0.50	Yes (<0.1)
UGT1A9 (rs178868320)	Boudou-Rouquette, 2012	Sorafenib	No significant association.	Yes (NR)
UGT1A9 (rs6714486)	Boudou-Rouquette, 2012	Sorafenib	No significant association.	Yes (NR)
UGT1A9 (rs72551330)	Boudou-Rouquette, 2012	Sorafenib	No significant association.	No
ULK1 (rs11616018)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. TC/CC vs TT: OR = 0.91 (0.30 – 2.45), p = 1.00	Yes (NR)
ULK1 (rs12303764)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. TG/GG vs TT: OR = 0.64 (0.25 – 1.67), p = 0.37	Yes (NR)
UVRAG (rs1458836)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. CT/TT vs CC: OR = 0.37 (0.04 – 1.59), p = 0.27	Yes (NR)
VEGF (rs1005230)	Formica, 2011	FOLFIRI + bevacizumab	No significant different.	No
VEGF (rs10434)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.54 (0.83 – 2.78), p = 0.18	Yes (<0.1)
VEGF (rs13207351)	Formica, 2011	FOLFIRI + bevacizumab	No significant different.	Yes (NR)

eTable 3: Single nucleotide polymorphism in antineoplastic-induced hypertension (cont).

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
VEGF (rs2146323)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.90 (0.45 – 1.75), p = 0.88	Yes (<0.1)
VEGF (rs25648)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.17 (0.45 – 2.85), p = 0.87	Yes (<0.1)
VEGF (rs3024994)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 2.04 (0.27 – 15.56), p = 0.64	Yes (<0.1)
VEGF (rs3025030)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.54 (0.83 – 2.78), p = 0.18	Yes (<0.1)
VEGF (rs3025035)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 0.92 (0.20 – 3.45), p = 1.00	Yes (<0.1)
VEGF (rs35569394)	Formica, 2011	FOLFIRI + bevacizumab	No significant different.	No
VEGF (rs35864111)	Formica, 2011	FOLFIRI + bevacizumab	No significant different.	No
VEGF (rs833069)	Frey, 2017	Bevacizumab	No significant association. Allelic: OR = 1.30 (0.71 – 2.36), p = 0.44	Yes (<0.1)
VEGFR1 (rs9582036)	Dornbusch, 2016	Sunitinib	No significant association. Variant vs wild type: OR = 1.09 (0.24 – 4.86), p = 1.00	Yes (0.480)
VEGFR1 (rs9554320)	Dornbusch, 2016	Sunitinib	No significant association. Variant vs wild type: OR = 1.12 (0.40 – 3.16), p = 1.00	Yes (0.713)
VEGFR2 (rs2305948)	Jain, 2010	Sorafenib & Bevacizumab	No significant association. Variant vs wild type: OR = 1.09 (0.50 – 2.37), p = 0.83	Yes (p ≥ 0.77)
	Kim, 2012	Sunitinib	No significant association. GA vs GG: p = 0.14[e]	NR
VEGFR3 (rs307826)	Dornbusch, 2016	Sunitinib	No significant association. Variant vs wild type: OR = 1.72 (0.59 – 5.09), p = 0.40	Yes (0.298)
	Garcia-Donas, 2011	Sunitinib	No significant association. HR = 1.31 (0.42 – 4.06), p = 0.64	Yes (> 0.05)
VEGFR3 (rs307821)	Dornbusch, 2016	Sunitinib	No significant association. Variant vs wild type: OR = 2.32 (0.82 – 6.56), p = 0.12	Yes (0.616)
	Garcia-Donas, 2011	Sunitinib	No significant association. HR = 2.01 (0.75 – 5.37), p = 0.17	Yes (> 0.05)
VEGFR3 (rs448012)	Garcia-Donas, 2011	Sunitinib	No significant association. HR = 1.13 (0.60 – 2.12), p = 0.72	Yes (> 0.05)

eTable 3: Single nucleotide polymorphism in antineoplastic-induced hypertension (cont).

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
WNK1 (rs10774461)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 0.57 (0.30 – 1.04), p = 0.07	Yes (>0.1)
WNK1 (rs10849582)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 1.35 (0.73 – 2.50), p = 0.37	Yes (>0.1)
WNK1 (rs10935724)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 0.85 (0.44 – 1.63), p = 0.72	Yes (>0.1)
WNK1 (rs11064524)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 0.67 (0.29 – 1.45), p = 0.37	Yes (>0.1)
WNK1 (rs11064547)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 0.95 (0.43 – 2.17), p = 1.00	Yes (>0.1)
WNK1 (rs11068756)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 1.41 (0.78 – 2.56), p = 0.28	Yes (>0.1)
WNK1 (rs11611231)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 0.38 (0.09 – 1.22), p = 0.12	Yes (>0.1)
WNK1 (rs12314329)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 1.81 (0.69 – 4.63), p = 0.25	Yes (>0.1)
WNK1 (rs12816718)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 0.78 (0.34 – 1.71), p = 0.65	Yes (>0.1)
WNK1 (rs1468326)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 2.17 (0.90 – 5.26), p = 0.09	Yes (>0.1)
WNK1 (rs17223420)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 0.50 (0.19 – 1.20), p = 0.14	Yes (>0.1)
WNK1 (rs2286007)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 1.67 (0.62 – 4.37), p = 0.35	Yes (>0.1)
WNK1 (rs4980968)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 1.67 (0.88 – 3.13), p = 0.13	Yes (>0.1)
WNK1 (rs4980973)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 1.23 (0.45 – 3.23), p = 0.80	Yes (>0.1)
WNK1 (rs6489755)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 1.79 (0.87 – 3.70), p = 0.12	Yes (>0.1)
WNK1 (rs7967755)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 0.81 (0.33 – 1.87), p = 0.75	Yes (>0.1)
WNK1 (rs953361)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 0.54 (0.27 – 1.06), p = 0.08	Yes (>0.1)

eTable 3: Single nucleotide polymorphism in antineoplastic-induced hypertension (cont).

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
WNK1 (rs2269937)	Frey, 2017	Bevacizumab	No significant association. Allelic OR = 0.76 (0.41 – 1.41), p = 0.44	Yes (>0.1)

[a]Significant associations are in bold (p<0.05) unless otherwise specified; [b]calculated; [c]statistical significant is defined as p-value<5.0 x10^{-8}; [d]Cochran-Armitage trend test; [e]Fischer's exact test; [f]multivariate analyses; HR, hazard ratio; HWE, Hardy-Weinberg equilibrium; NR, not reported

eTable 4: Single nucleotide polymorphism in antineoplastic-induced decreased left ventricular ejection fraction.

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
BRINP1 (rs10117876)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 5.86 x 10^{-7}**	Yes (>1.0x10^{-4})
BRINP1 (rs7038923)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 5.86 x 10^{-7}**	Yes (>1.0x10^{-4})
BRINP1 (rs7041012)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 1.34 x 10^{-6}**	Yes (>1.0x10^{-4})
BRINP1 (rs1160584)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 1.62 x 10^{-6}**	Yes (>1.0x10^{-4})
BRINP1 (rs230145)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 1.62 x 10^{-6}**	Yes (>1.0x10^{-4})
BRINP1 (rs230144)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 1.70 x 10^{-6}**	Yes (>1.0x10^{-4})
BRINP1 (rs230142)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 1.62 x 10^{-6}**	Yes (>1.0x10^{-4})
BRINP1 (rs62573809)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 5.70 x 10^{-6}**	Yes (>1.0x10^{-4})
BRINP1 (rs16908078)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 2.95 x 10^{-6}**	Yes (>1.0x10^{-4})
BRINP1 (rs7851490)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 3.12 x 10^{-6}**	Yes (>1.0x10^{-4})
BRINP1 (rs7854066)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 3.04 x 10^{-6}**	Yes (>1.0x10^{-4})
BRINP1 (rs62573837)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 2.88 x 10^{-6}**	Yes (>1.0x10^{-4})
BRINP1 (rs76586195)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 2.90 x 10^{-6}**	Yes (>1.0x10^{-4})
BRINP1 (rs7027658)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 2.95 x 10^{-6}**	Yes (>1.0x10^{-4})
BRINP1 (rs75912020)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 2.95 x 10^{-6}**	Yes (>1.0x10^{-4})
BRINP1 (rs76890184)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 2.95 x 10^{-6}**	Yes (>1.0x10^{-4})
BRINP1 (rs58944852)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 2.87 x 10^{-6}**	Yes (>1.0x10^{-4})
BRINP1 (rs6256837)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 6.01 x 10^{-7}**	Yes (>1.0x10^{-4})

eTable 4: Single nucleotide polymorphism in antineoplastic-induced decreased left ventricular ejection fraction.

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
HER2 (rs1136201)	Beauclair, 2007	Trastuzumab	**Significant association. AG/GG vs AA: OR = 19.59 (1.03 – 372.41), p = 0.047**[b]	Yes (0.72)
	Lemieux, 2013	Trastuzumab	**Significant association. AG vs AA: OR = 6.00(1.46 - 24.69), p = 0.01 AG/GG vs AA: OR = 4.80 (1.19 - 19.30), p = 0.03**	Yes (0.39)
	Roca, 2013	Trastuzumab	**Significant association. AG vs AA: OR = 4.32 (1.25 - 14.94), p = 0.015 AG/GG vs AA: OR = 3.83 (1.11 - 13.18), p = 0.025**	Yes (0.62)
	Gómez Peña, 2015	Trastuzumab	**Significant association. AG vs AA: OR = 3.80 (1.12 - 13.63), p = 0.031 AG/GG vs AA: OR = 3.41 (1.02 - 11.96), p = 0.039**	Yes (0.52)
	Serie, 2017	Trastuzumab	No significant association. AG/GG vs AA: OR = 0.94 (0.71 – 1.25), p = 0.67	Yes ($>1.0x10^{-4}$)
HER2 (rs1058808)	Lemieux, 2013	Trastuzumab	No significant association. GC vs GG: OR = 0.96 (0.19 - 4.71), p = 0.95 CC vs GG: OR = 1.62 (0.32 - 8.29), p = 0.57	Yes (0.03)
	Stanton, 2015	Trastuzumab	**Significant association. CG/GG vs CC: p = 0.04**	Yes (NR)
	Boekhout, 2016	Trastuzumab	**Significant association. GG vs CG/CC: OR = 0.09 (0.05 – 0.36), p = 0.003**	NR
	Serie, 2017	Trastuzumab	No significant association. CG/GG vs CC: OR = 1.03 (0.80 – 1.34), p = 0.80	Yes ($>1.0x10^{-4}$)
Intergenic (rs4305714)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 1.39×10^{-6}**	Yes ($>1.0x10^{-4}$)
LDB2 (rs55756123)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 8.93×10^{-8}**	Yes ($>1.0x10^{-4}$)
LINC01060 (rs7698718)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 7.73×10^{-6}**	Yes ($>1.0x10^{-4}$)
RAB22A (rs707557)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 5.62×10^{-6}**	Yes ($>1.0x10^{-4}$)
TRPC6 (rs77679196)	Serie, 2017	Trastuzumab	**Significant association. Variant vs wild type: p = 7.725×10^{-6}**	Yes ($>1.0x10^{-4}$)

eTable 4: Single nucleotide polymorphism in antineoplastic-induced decreased left ventricular ejection fraction.

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
CREBRF (rs201763080)	Udagawa, 2018	Trastuzumab	No significant association[b]. Allelic: OR = 29.4 (5.5 – 156.6), p = 0.0039 Dominant: OR = 31.1 (5.6 – 171.5), p = 0.0038	NR
EYS (rs139944387)	Udagawa, 2018	Trastuzumab	No significant association[b]. Allelic: OR = 12.2 (4.0 – 37.0), p = 0.00064 Dominant: OR = 13.7 (4.3 – 44.2), p = 0.00056	NR
FCGR2A (rs1801274)	Roca, 2013	Trastuzumab	No significant association. HR vs HH: OR = 1.72 (0.32 - 8.99) RR vs HH: OR = 3.80 (0.67 - 21.29)	Yes (0.86)
FCGR3A (rs396991)	Roca, 2013	Trastuzumab	No significant association. FV vs FF: OR = 0.59 (0.17 – 2.08) VV vs FF: OR = 1.70 (0.29 – 9.82)	Yes (0.08)
FIG4 (rs56378532)	Udagawa, 2018	Trastuzumab	No significant association[b]. Allelic: OR = 4.4 (1.5 – 12.8), p = 0.018 Dominant: OR = 5.0 (1.6 – 15.5), p = 0.015	NR
GTF3C3 (rs146213213)	Udagawa, 2018	Trastuzumab	No significant association[b]. Allelic: OR = 11.6 (3.3 – 40.9), p = 0.0035 Dominant: OR = 12.6 (3.4 – 46.7), p = 0.0033	NR
KRT15 (rs78272919)	Udagawa, 2018	Trastuzumab	No significant association[b]. Allelic: OR = 6.9 (2.6 – 18.3), p = 0.0015 Dominant: OR = 7.9 (2.8 – 22.8), p = 0.0012	NR
MYADM (rs140387622)	Udagawa, 2018	Trastuzumab	No significant association[b]. Allelic: OR = 3.9 (1.5 – 10.2), p = 0.014 Dominant: OR = 3.5 (1.1 – 10.7), p = 0.045 Recessive: OR = 15.2 (1.7 – 137.0), p = 079	NR
PHF3 (rs139503277)	Udagawa, 2018	Trastuzumab	No significant association[b]. Allelic: OR = 16.6 (4.6 – 60.3), p = 0.0014 Dominant: OR = 18.1 (4.7 – 69.0), p = 0.0013	NR
PLEKHA6 (rs149581993)	Udagawa, 2018	Trastuzumab	No significant association[b]. Allelic: OR = 37.8 (6.1 – 233.3), p = 0.0031 Dominant: OR = 40.0 (6.3 – 255.0), p = 0.0031	NR

eTable 4: Single nucleotide polymorphism in antineoplastic-induced decreased left ventricular ejection fraction.

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
SFTPA2 (rs150273659)	Udagawa, 2018	Trastuzumab	No significant association[b]. Allelic: OR = 9.4 (2.7 – 32.7), p = 0.0060 Dominant: OR = 10.7 (2.9 – 39.1), p = 0.0050	NR
ZNRF3 (rs5762940)	Udagawa, 2018	Trastuzumab	No significant association[b]. Allelic: OR = 6.9 (2.6 – 18.2), p = 0.0016 Dominant: OR = 6.0 (1.9 – 18.7), p = 0.0085 Recessive: OR = 75.8 (4.6 – 1259.3), p = 0.027	NR

[a]Significant associations are in bold (p<0.05) unless otherwise specified; [b]statistical significant is defined as p-value<0.0019; HWE, Hardy-Weinberg equilibrium; NR, not reported

eTable 5: Single nucleotide polymorphism in antineoplastic-induced venous thromboembolism.

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationshipa	HWE (p-Value)
ALDH1A1 (rs2161811)	Johnson, 2008	Thalidomide	**Significant association. Minor allele vs major allele: OR = 1.64 (1.19 – 2.26)**	Yes (p≥0.001)
CASP3 (rs1049216)	Johnson, 2008	Thalidomide	**Significant association. CC vs TT: OR = 0.52 (0.36 – 0.77)**	Yes (p≥0.001)
CDKN1A (rs3829963)	Almasi, 2011	Thalidomide	**Significant association. AC vs CC: 3.64 (1.28 – 10.36), p = 0.015**	Yes (NR)
CHEK1 (rs506504)	Johnson, 2008	Thalidomide	**Significant association. TT vs CC: OR = 2.97 (1.45 – 6.10)**	Yes (p≥0.001)
CINP (rs7011)	Johnson, 2008	Thalidomide	**Significant association. TT vs CC: OR = 1.63 (1.15 – 2.32)**	Yes (p≥0.001)
	Almasi, 2011	Thalidomide	No significant association. CT vs CC: OR = 1.05 (0.94 – 1.03), p = 0.92 TT vs CC: 1.10 (0.38 – 2.89), p = 0.92 CT/TT vs CC: 1.06 (0.40 – 2.79), p = 0.91 TT vs CT/CC: OR = 1.07 (0.20 – 5.85), p = 0.94	Yes (NR)
	Bagratuni, 2013	Lenalidomide	No significant association. CC vs CT/TT: OR = 0.90 (0.23 – 3.57), p = 0.89[b] TT vs CT/CC: OR = 3.78 (0.68 – 20.92), p = 0.13[b]	NR
COMT (rs4633)	Johnson, 2008	Thalidomide	**Significant association. Minor allele vs major allele: OR = 0.68 (0.49 – 0.94)**	Yes (p≥0.001)
DCLRE1B (rs12022378)	Johnson, 2008	Thalidomide	**Significant association. TT vs CC: OR = 1.50 (1.09 – 2.07)**	Yes (p≥0.001)
ERCC6 (rs4253211)	Johnson, 2008	Thalidomide	**Significant association. CC vs GG: OR = 1.80 (1.13 – 2.88)**	Yes (p≥0.001)
Factor II (rs6025)	De Haas, 2010	Cisplatin	**Significant association. Wild type vs heterozygous variant: 0.06 (0.01 – 0.27), p = 0.003[b]**	Yes (p>0.05)
HMMR (rs299295)	Johnson, 2008	Thalidomide	**Significant association. TT vs CC: OR = 1.50 (1.04 – 2.17)**	Yes (p≥0.001)
IL12A (rs582537)	Johnson, 2008	Thalidomide	**Significant association. AA vs CC: OR = 1.42 (1.03 – 1.95)**	Yes (p≥0.001)
LEP (rs10249476)	Johnson, 2008	Thalidomide	**Significant association. TT vs CC: OR = 1.55 (1.11 – 2.15)**	Yes (p≥0.001)

eTable 5: Single nucleotide polymorphism in antineoplastic-induced venous thromboembolism (cont).

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
LIG1 (rs20579)	Johnson, 2008	Thalidomide	**Significant association. TT vs CC: OR = 0.44 (0.24 – 0.80)**	Yes (p≥0.001)
MT (rs13815)	Johnson, 2008	Thalidomide	**Significant association. CC vs GG: OR = 0.58 (0.41 – 0.83)**	Yes (p≥0.001)
NAT2 (rs2410558)	Johnson, 2008	Thalidomide	**Significant association. TT vs CC: OR = 0.65 (0.45 – 0.94)**	Yes (p≥0.001)
PPARD (rs2267669)	Johnson, 2008	Thalidomide	**Significant association. GG vs AA: OR = 0.49 (0.31 – 0.78)**	Yes (p≥0.001)
PPARD (rs1805414)	Johnson, 2008	Thalidomide	**Significant association. CC vs TT: OR = 0.73 (0.57 – 0.95)**	Yes (p≥0.001)
SERPINE1 (rs2070682)	Johnson, 2008	Thalidomide	**Significant association. CC vs TT: OR = 0.71 (0.51 – 0.99)**	Yes (p≥0.001)
TNFRSF17 (rs12922317)	Johnson, 2008	Thalidomide	**Significant association. GG vs AA: OR = 0.60 (0.43 – 0.85)**	Yes (p≥0.001)
VEGF (rs2010963)	Formica, 2011	FOLFIRI + bevacizumab	No significant different	Yes (NR)
	Di Stefano, 2015	Bevacizumab	**Significant association. CC vs CG/GG: HR = 2.44, p = 0.006**	Yes (0.68)
XRCC5 (rs2440)	Johnson, 2008	Thalidomide	**Significant association. TT vs CC: OR = 1.48 (1.07 – 2.05), p = 0.02**[b]	Yes (p≥0.001)
ABCB4 (rs2302387)	Johnson, 2008	Thalidomide	No significant association. AA vs GG: OR = 0.67 (0.40 – 1.10)	Yes (p≥0.001)
ALDH-1A1 (rs1683511)	Almasi, 2011	Thalidomide	No significant association. AG vs AA: 0.78 (0.26 – 2.35), p = 0.66	Yes (NR)
ALDH1A1 (rs610529)	Almasi, 2011	Thalidomide	No signification association. CT vs CC: OR = 0.64 (0.21 – 1.95), p = 0.43 TT vs CC: OR = 1.00 (0.29 – 3.51), p = 1.00 CT/TT vs CC: OR = 0.75 (0.28 – 2.01), p = 0.58 TT vs CT/CC: OR = 1.25 (1.40 – 3.92), p = 0.71	Yes (NR)
	Bagratuni, 2013	Lenalidomide	No significant association. TT vs CT/CC: OR = 0.39 (0.05 – 3.28), p = 0.39	NR
ATG13 (rs13448)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. TC/CC vs TT: OR = 1.33 (0.36 – 4.55), p = 0.78	Yes (NR)
ATG3 (rs9831088)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. AG/GG vs AA: OR = 0.41 (0.09 – 1.46), p = 0.17	Yes (NR)

eTable 5: Single nucleotide polymorphism in antineoplastic-induced venous thromboembolism (cont).

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
ATG5 (rs633724)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. CT/TT vs CC: OR = 0.45 (0.12 – 1.57), p = 0.18	Yes (NR)
ATG8 (rs11149841)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. GT/TT vs GG: OR = 1.66 (0.46 – 5.73), p = 0.38	Yes (NR)
ATG8 (rs8060972)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. AT/TT vs AA: OR = 0.84 (0.14 – 3.34), p = 1.00	Yes (NR)
BECN1 (rs11552192)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. AT/TT vs AA: OR = 2.98 (0.74 – 10.71), p = 0.07	Yes (NR)
CDKN1A (rs3829963)	Bagratuni, 2013	Lenalidomide	No significant association. CC vs CA/AA: OR = 2.56 (0.30 – 21.52), p = 0.37[b]	NR
CHEK1 (rs506504)	Bagratuni, 2013	Lenalidomide	No significant association. CC vs CT/TT: OR = 1.10 (0.13 – 9.61), p = 0.93	NR
Factor V (rs1799963)	De Haas, 2010	Cisplatin	No significant association. Wild type vs heterozygous variant: OR = 0.69 (0.15 – 3.18), p = 0.64[b]	Yes (p>0.05)
FIP200 (rs1129660)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. AG/GG vs AA: OR = 0.56 (0.10 – 2.23), p = 0.56	Yes (NR)
FIP200 (rs17337252)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. AG/GG vs AA: OR = 0.60 (0.17 – 2.38), p = 0.53	Yes (NR)
GAN (rs2608555)	Almasi, 2011	Thalidomide	No significant association. CT vs CC: OR = 0.79 (0.29 – 2.19), p = 0.65 TT vs CC: OR = 1.26 (0.12 – 13.26), p = 0.85 CT/TT vs CC: OR = 0.83 (0.31 – 2.21), p = 0.71 TT vs CT/CC: OR = 1.37 (0.13 – 14.08), 0.79	Yes (NR)
NFKB1 (rs3774968)	Bagratuni, 2013	Lenalidomide	No significant association. CC vs CT/TT: OR = 0.49 (0.06 – 4.18), p = 0.52[b] TT vs CT/CC: OR = 3.16 (0.78 – 12.86), p = 0.11[b]	NR
PAI-1 (rs1799889)	De Haas, 2010	Cisplatin	No significant association. 4G/4G vs 4G/5G + 5G/5G: OR = 1.06 (0.43 – 2.61), p = 0.90[b] 5G/5G vs 4G/5G + 4G/4G: OR = 0.76 (0.28 – 2.09), p = 0.60[b]	Yes (p>0.05)
TNFRSF17 (rs19222317)	Bagratuni, 2013	Lenalidomide	No significant association. AA vs AG/GG: OR = 0.39 (0.09 – 1.58), p = 0.19[b] GG vs AG/AA: OR = 0.99 (0.24 – 4.07), p = 0.98[b]	NR

eTable 5: Single nucleotide polymorphism in antineoplastic-induced venous thromboembolism (cont).

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
ULK1 (rs11616018)	Berger, 2017	FOLFIRI + bevacizumab	TC/CC vs TT: OR = 1.73 (0.47 – 5.94), p = 0.37	Yes (NR)
ULK1 (rs12303764)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. TG/GG vs TT: OR = 0.55 (0.16 – 1.91), p = 0.39	Yes (NR)
ULK1 (rs9481)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. GA/AA vs GG: OR = 0.80 (0.14 – 3.20), p = 0.77	Yes (NR)
UVRAG (rs1458836)	Berger, 2017	FOLFIRI + bevacizumab	No significant association. CT/TT vs CC: OR = 0.71 (0.07 – 3.39), p = 0.75	Yes (NR)
VEGF (rs1005230)	Formica, 2011	FOLFIRI + bevacizumab	No significant different	No
VEGF (rs13207351)	Formica, 2011	FOLFIRI + bevacizumab	No significant different	Yes (NR)
VEGF (rs1570360)	Formica, 2011	FOLFIRI + bevacizumab	No significant different	Yes (NR)
VEGF (rs35569394)	Formica, 2011	FOLFIRI + bevacizumab	No significant different	No
VEGF (rs35864111)	Formica, 2011	FOLFIRI + bevacizumab	No significant different	No
VEGF (rs699947)	Formica, 2011	FOLFIRI + bevacizumab	No significant different	No
	Almasi, 2011	Thalidomide	No significant association. AC vs AA: OR = 0.46 (0.16 – 1.38), p = 0.17 CC vs AA: OR = 0.48 (0.13 – 1.77), p = 0.27 AC/CC vs AA: OR = 0.47 (0.17 – 1.25), p = 0.13 CC vs AC/AA: OR = 0.71 (0.21 – 2.38), p = 0.58	Yes (NR)
VEGF (rs833061)	Formica, 2011	FOLFIRI + bevacizumab	No significant different	No
VEGF (rs2010963)	Di Stefano, 2015	Temozolomide	No significant association. CC vs CG/GG: OR = 0.85 (0.24 – 3.07), p = 0.81[b] GG vs CG/CC: OR = 3.43 (0.74 – 15.98), p = 0.12[b]	Yes (0.68)
XRCC5 (rs2440)	Bagratuni, 2013	Lenalidomide	No significant association. CC vs CT/TT: 1.10 (0.26 – 4.67), p = 0.90[b] TT vs CT/CC: 1.60 (0.30 – 8.46), p = 0.58[b]	NR

[a]Significant associations are in bold (p<0.05); [b]calculated; HWE, Hardy-Weinberg equilibrium; NR, not reported

eTable 6: Single nucleotide polymorphism in antineoplastic-induced coronary heart disease.

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
Factor II (rs6025)	De Haas, 2010	Cisplatin	No significant association. Wild type vs heterozygous variant: OR = 0.28 (0.03 – 2.49), p = 0.25[b]	Yes (p>0.05)
Factor V (rs1799963)	De Haas, 2010	Cisplatin	**Significant association. Wild type vs heterozygous variant: OR = 0.19 (0.05 – 0.75), p = 0.018**[b]	Yes (p>0.05)
PAI-1 (rs1799889)	De Haas, 2010	Cisplatin	No significant association. 4G/4G vs 4G/5G + 5G/5G: OR = 1.46 (0.48 – 4.39), p = 0.50[b]	Yes (p>0.05)

[a]Significant associations are in bold (p<0.05); [b]calculated; HWE, Hardy-Weinberg equilibrium

eTable 7: Single nucleotide polymorphism in antineoplastic-induced arrhythmia.

Polymorphism (SNP-ID)	Study	Antineoplastic	Relationship[a]	HWE (p-Value)
DYPD (rs1801159)	Yamaguchi, 2001	5-fluorouracil	NR	Yes

[a]Significant associations are in bold (p<0.05); HWE, Hardy-Weinberg equilibrium; NR, not reported

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4.2 Pharmacogenetics role in anthracycline-induced cardiotoxicity prediction

A systematic review and meta-analysis was done with the aim to identify single nucleotide polymorphism that can be considered as a risk factor in the risk prediction model. Although a significant number of SNPs were studies however the evidences are limited to support any of them to be use as predictor. Thus, genetic biomarker was excluded in the development of ACT risk prediction model.

4.2.1 Introduction

Part of this chapter has been published in the Scientific Reports (2017), Volume 7, Issue 1, page 39. The candidate, Leong Siew Lian was primarily responsible for searching, analysis and writing of the manuscript. The paper's co-authors, Shaun Lee Wen Huey and Nathorn Chaiyakunapruk, contributed in various aspects of this article.

Based on the finding of this chapter, a letter to editor has been published in JAMA Cardiology (2017), Volume 2, Issue 7, page 817. The candidate, Leong Siew Lian was primarily responsible for writing of the letter. The paper's co-authors, Shaun Lee Wen Huey and Nathorn Chaiyakunapruk, contributed in various aspects of this letter.

4.2.2 Unpublished findings

The use of anthracycline has also been associated with atrial and ventricular fibrillation, resulting in supraventricular extrasystoles, premature ventricular contractions as well as torsades de pointes[29]. Genetic studies have suggested that several genes plays a role in the ECG abnormalities including sodium channel, voltage gated, type V alpha subunit (*SCN5A*), potassium channel, voltage gated KQT-like subfamily Q, member 1 (*KCNQ1*)[82], and matrix metallopeptidase (*MMP-1* and *MMP-3*)[83]. In the current study, forty genes contained within the family and mutation of KCNQ1 and KCNH2 has been reported to cause long QT syndrome[84]. The effect of these genes in arrhythmia is outlined in Figure 4-1. Although Kitagawa *et al.* did not find significant association between polymorphisms in these genes and electrocardiogram changes; they provide the basis for future research in this area as they found that there is significant increase in QTc interval after anthracycline therapy[85]. Thus, more studies are needed to assess the association of polymorphs and anthracycline induced electrocardiogram changes.

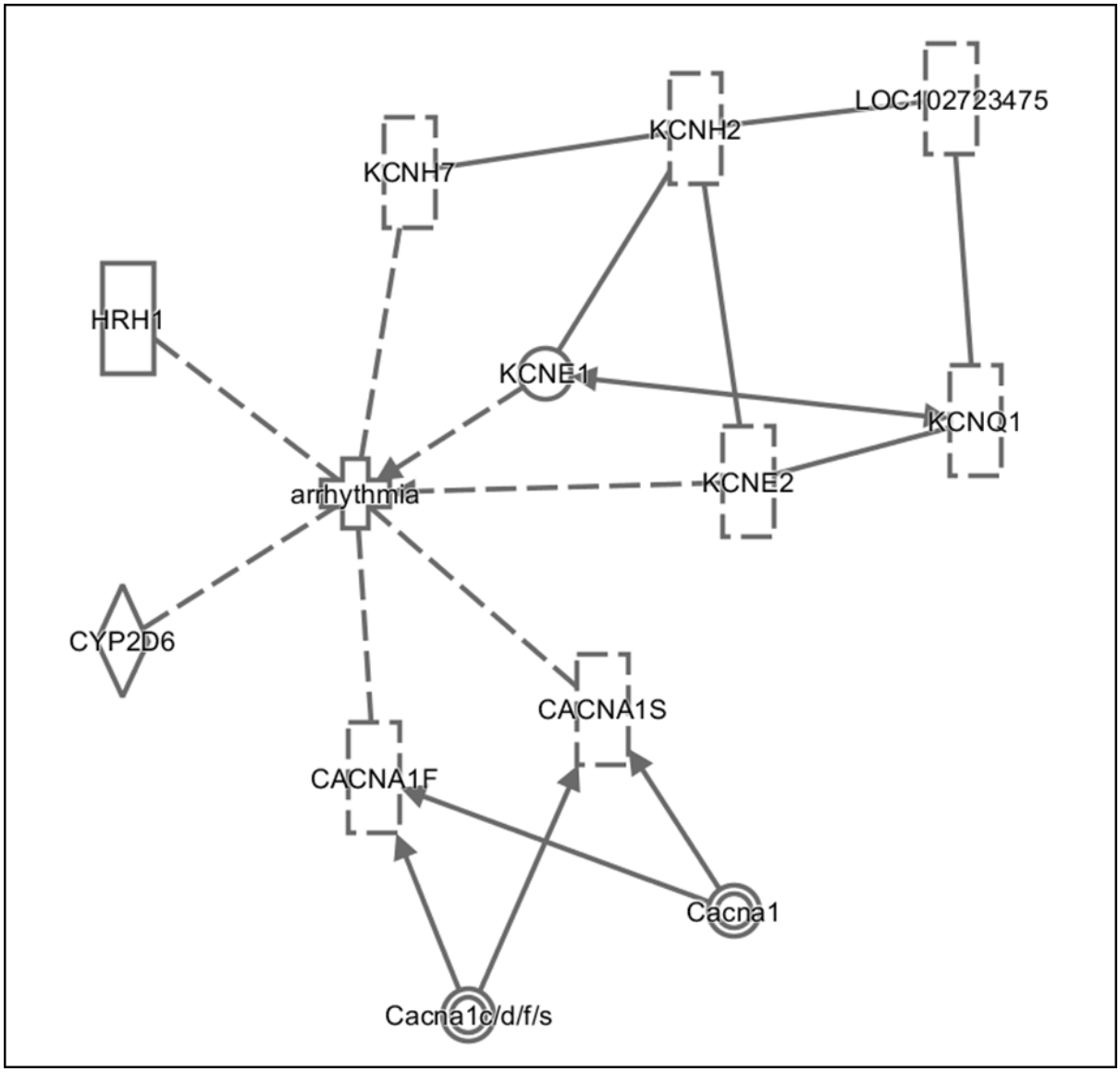

Figure 4-1: The effects of potassium voltage-gated channel subfamily H member 2 (KCNH2) and potassium voltage-gated channel subfamily Q member 1 (KCNQ1) in arrhythmia were generated using QIAGEN's Ingenuity Pathway Analysis.
IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity.

4.2.3 Full article and supplementary materials

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Candidate Gene Association Studies of Anthracycline-induced Cardiotoxicity: A Systematic Review and Meta-analysis

Received: 16 August 2016
Accepted: 27 January 2017
Published online: 27 February 2017

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Anthracyclines play an important role in the management of patients with cancer but the development of anthracycline-induced cardiotoxicity (ACT) remains a significant concern for most clinicians. Recently, genetic approach has been used to identify patients at increased risk of ACT. This systematic review assessed the association between genomic markers and ACT. A systematic literature search was performed in Medline, PubMed, Cochrane Central Register of Controlled Studies, CINAHL Plus, AMED, EMBASE and HuGE Navigator from inception until May 2016. Twenty-eight studies examining the association of genetic variants and ACT were identified. These studies examined 84 different genes and 147 single nucleotide polymorphisms. Meta-analyses showed 3 risk variants significantly increased the risk for ACT; namely ABCC2 rs8187710 (pooled odds ratio: 2.20; 95% CI: 1.36–3.54), CYBA rs4673 (1.55; 1.05–2.30) and RAC2 rs13058338 (1.79; 1.27–2.52). The current evidence remains unclear on the potential role of pharmacogenomic screening prior to anthracycline therapy. Further research is needed to improve the diagnostic and prognostic role in predicting ACT.

Anthracycline antibiotics are among the most potent chemotherapeutic agents since their introduction 50 years ago. Agents in this pharmacological group of antineoplastic drugs include doxorubicin, daunorubicin, epirubicin, and idarubicin. They are the backbone for many chemotherapy regimens in the treatment of breast cancer[1,2], lymphoma[3–7], leukaemia[8,9] and sarcomas[10,11]. This may be due to the wide range of mechanisms which anthracyclines are thought to act on including: (i) initiation of apoptosis via inhibition of topoisomerase II, (ii) DNA synthesis inhibition, (iii) DNA binding and alkylation, (iv) DNA cross-linking, (v) interference with DNA strand separation and helicase activity, and (vi) free radical formation and lipid peroxidation[12]. While anthracyclines have revolutionised the management of both early and advance-stage diseases, the clinical usefulness of anthracyclines is compromised by the adverse effects of cardiac toxicity. Regimens using anthracyclines were reported to increase the risk of clinical and subclinical cardiac toxicity as well as death by more than 5-fold[13–15].

Thus, the early identification of patients at risk of cardiotoxicity is a primary goal for many cardiologist and oncologist. Research over the past few decades have identified several risk factors associated with ACT including: aged $\geq$ 65 years old or less than 4 years old, female gender, pre-existing hypertension and/or cardiac disease, mediastinal radiation, high doses of anthracycline as well as concurrent treatment with cyclophosphamide, paclitaxel and trastuzumab[16,17]. Nevertheless, most of these approaches have low diagnostic sensitivity and predictive power to detect subclinical myocardial injury[18,19]. Several studies have recently reported the use of genetic variants as prognostic biomarkers for early detection of ACT[20–23]. The aim of the current study was to provide an overview on studies using genetic markers for identification of patients at risk of ACT and summarise these associations.

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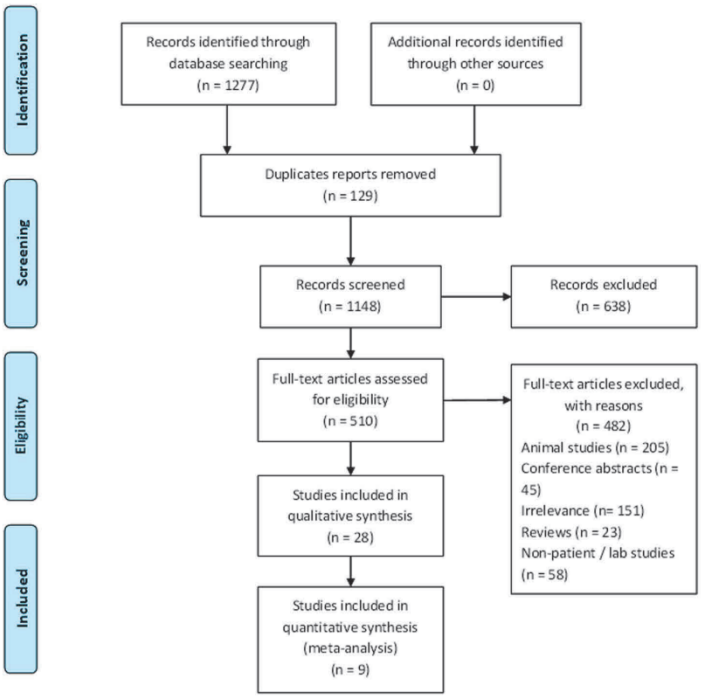

Figure 1. PRISMA flow diagram showing the selection process and criteria of the included studies.

Methods

Search strategy. We searched OVID Medline, PubMed, Cochrane Central Register of Controlled Studies, CINAHL Plus, AMED, EMBASE and HuGE Navigator from inception until May 2016. The search terms include anthracycline, cardiotoxicity and genetic (The full search term can be found in Supplementary Information: Search Strategies). This was supplemented with a manual search of cited references from retrieved articles.

Study selection. Studies that met the following criteria were included: (i) primary studies that determined an association between genetic polymorphism (including single nucleotide polymorphism (SNPs), deletions, duplication and copy-number variants) and cardiotoxicity; (ii) anthracycline was used and (iii) conducted in human population. Articles titles and abstracts were screened for relevancy by two independent reviewers (SWHL and SLL) and full text retrieved in accordance to the inclusion criteria. Any disagreement was resolved through adjudication with input by a third reviewer.

Data extraction. Two reviewers (SWHL and SLL) independently extracted data from identified studies using standardised data extraction form. Reviewers compared the results and resolved any differences by discussion. Information extracted include: geographic location, ethnic group, study design, participant demographics and clinical characteristics, genotyping technique, and definition of cardiotoxicity. The study was conducted following the process specified in the PRISMA statement.

Quality assessment. The reviewers independently assessed the quality of the included studies using quality of genetic association studies (Q-Genie) tool developed by Sohani *et al.*[24]. This validated tool consisting of nine categories was developed based on the Strengthening the Reporting of Genetic Association Studies (STREGA)[25] and Strengthening the Reporting of Genetic Risk Prediction Studies (GRIPS)[26] guidelines.

Statistical analysis. In studies which had assessed for polymorphisms of the same genotype (minimum 2 studies), we conducted a meta-analysis using a random effects model[27]. Study heterogeneity was assessed using the Cochran Q and the I^2 statistics. We also calculated the departure from Hardy-Weinberg equilibrium (HWE), which if violated, may bias the estimates and replication of postulated gene-disease associations across different

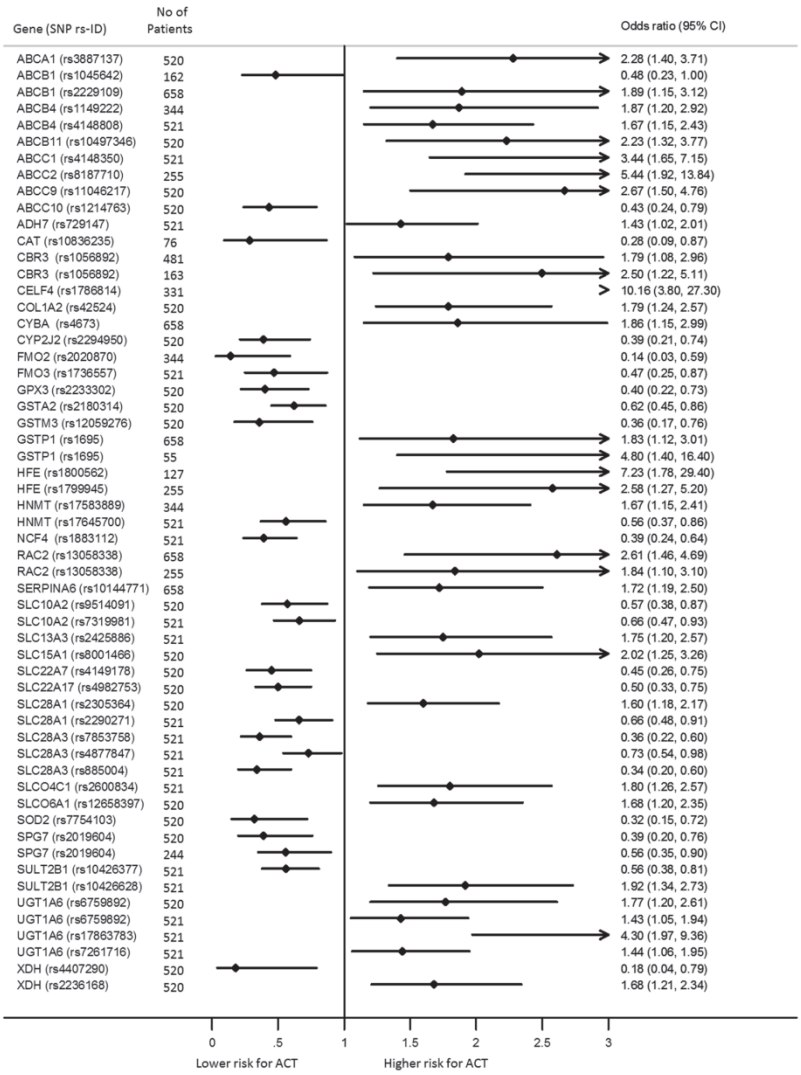

Figure 2. Forest plot of SNPs which examined the association of developing anthracycline-induced cardiotoxicity. SNPs significantly associated with ACT with no odds ratio or confidence interval reported are ABCC1 (rs3743527, rs246221, rs45511401), ABCC5 (rs7627754), AKR1C4 (rs7083869, rs2151896), CBR3 (rs10483032), CYP1A2 (rs2069522, rs2069526, rs4646427), CYP2B6 (rs7255904, rs1709115), CYP4B1 (rs837400, rs4646495), CYP4F11 (rs8112732, rs12610962, rs2072270), HSD17B2 (rs16956248, rs13333826, rs7196087, rs2955159, rs2966245), HSD17B4 (rs257970, rs2636968), KCNH2 (rs3807375), POR (rs2868177, rs13240755, rs4732513), SLC22A17 (rs11625724, rs12882406, rs12896494). The diamond in each line represents the effect estimate and weight of each study. The width of the line across the diamond shows the 95% confidence interval of the effect estimate of individual studies. ACT, Anthracycline-induced cardiotoxicity; CI, confidence interval.

studies[28]. All analyses were performed using Stata 13.0 (StataCorp, College Station, TX) and Review Manager 5.3 packages (http://comunity.cochrane.org/tools/review-production-tools/revman-5)[29].

Study: Author (year)	Geographic location, ethnic group	Study design; number of participants	Age (years)		Gender: male/female		Type of Cancer examined	Anthracycline used/ Cumulative dose (mg/m^2)	Source of DNA sample	Genotyping	Definition of cardiotoxicity
			Cases	Controls	Cases	Controls					
Wojnowski (2005)[32]	Germany; 98% Germans	NCC; 550	Mean = 62.0 ± 10.9	Mean = 61.3 ± 11.0	50/37	212/151	NHL	Doxorubicin/*Cases:* Med = 504 mg IQR = 160.5 mg *Controls:* Med = 540 mg IQR = 90 mg	Peripheral blood	i) Pyrosequencing ii) RFLP	i) arrhythmia in the absence of arrhythmia before treatment ii) myocarditis-pericarditis iii) acute heart failure iv) LVEF <50% or SF <25%
Weiss (2006)[33]	USA; 85% Caucasian	CC; 197	Med = 68 (56–88)		Approx. 98/99		AML	Daunorubicin/NR	BM/ peripheral blood	i) Multiplex PCR ii) Sequenom's high-throughput matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS)	i) SWOG toxicity criteria for SWOG 9031 ii) CTCAEv2.0 for SWOG-9333.
Blanco (2008)[36]	USA; Whites, Blacks & Others	NCC; 145	Mean = 10.3 ± 6.5	Mean = 9.1 ± 5.8	10/20	57/58	Leukaemia, brain tumour, HL, NHL, Wilms tumour, bone tumour neuroblastoma, soft tissue sarcoma,	Doxorubicin/<100 = 1 (2)* 100–350 = 13 (46) 350–500 = 7 (31) >500 = 9 (36)	Buccal cells/ saliva	i) PCR-RFLP ii) Allelic discrimination with specific fluorescent probes	Self-reporting of signs and symptoms of CHF and use of medication for CHF management.
Rajic (2009)[40]	Slovenia; Caucasian	CC; 76	Mean = 25.8 ± 5.3		32/44		ALL	Not specified/ Mean = 199 ± 108 Range = 24–540	Bone marrow smears	i) qPCR ii) Custom TaqMan® genotyping assay	i) Clear conduction disturbances, depolarization and repolarization changes in ECG ii) SF < 30%, LVEF <54% iii) Derangement of (reference range) E (0.75 ± 0.13), A (0.51 ± 0.11), E/A (1.53 ± 0.4), IVRT (67 ± 8), PV-A (0.21 ± 0.08), PV-D (0.47 ± 0.11) PV-S (0.44 ± 0.1)
Rossi (2009)[41]	Italy; NR	CC; 106	Med = 66 (56–75)		55/51	55/51	DLBCL	Doxorubicin/15 mg/m^2/week	Peripheral blood	SNP minisequencing	Grade 2–4 cardiotoxicity according to CTCAEv 0.3
Blanco (2012)[35]	USA; Non-Hispanic whites, Hispanics, Blacks & Others	NCC; 487	Mean = 8.3 ± 6	Mean = 8.2 ± 6	76/94	162/155	HL, NHL, bone tumours, soft tissue sarcoma, ALL, AML, other.	Not specified/*Cases:* Med = 300 (0–575) *Controls:* Med = 140 (0–1050)	Peripheral blood/ buccal cells/ saliva	Allelic discrimination with specific fluorescent probes	i) signs and symptoms of cardiac compromise based on American Heart Association criteria 2005 ii) Absence of symptoms/ signs with echo evidence of left ventricular dysfunction (EF ≤ 40% and/ or SF ≤ 28%).
Kitagawa (2012)[59]	Japan; Japanese	PC; 34	Med = 49 (21–71)		0/34		Breast cancer	Epirubicin/NR	Whole blood	TaqMan® genotyping assay	i) QTc interval prolongation ii) other toxic effects based on CTCAEv3
Lubieniecka (2012)[44]	Canada; Caucasian	PC; 185	Med = 46 (14–74)		86/99		AML	Daunorubicin/NR	Blood	Sequenom genotyping assay	Percentage drops in LVEF.
Sachidanandam (2012)[53]	NR	CS; 2	Adult		−/2		Breast cancer	Doxorubicin	Blood	PCR	NR
Continued											

Study: Author (year)	Geographic location, ethnic group	Study design; number of participants	Age (years)		Gender: male/female		Type of Cancer examined	Anthracycline used/ Cumulative dose (mg/m^2)	Source of DNA sample	Genotyping	Definition of cardiotoxicity
			Cases	Controls	Cases	Controls					
Semsei (2012)[51]	Hungary; Hungarian	RC; 235	Mean = 5.7 ± 3.8		126/109		ALL	Daunorubicin, doxorubicin/NR	Peripheral blood	i) Mini-sequencing ii) GenomeLab SNPstream genotyping assay	Changes in LVFS
Visscher (2012)[30]	Canada; 78% Canadian, 22% Dutch	CC; 440	Discovery Med = 5.5 (0.04–17.0) Replication Med = 6.2 (0.4–17.6) Dutch-EKZ Med = 9.0 (0.5–16.8)	Discovery Med = 3.9 (0.5–16.5) Replication Med = 3.7 (0.05–16.9) Dutch-EKZ Med = 10.6 (2.1–17.1)	Discovery = 17/21 Replication = 22/18 Dutch-EKZ = 22/21	Discovery = 66/52 Replication = 82/66 Dutch-EKZ = 27/26	ALL, AML, other leukemia, HL, NHL Osteosarcoma, Rhabdomyosarcoma, Ewing's sarcoma, Other sarcoma, Nephroblastoma, Hepatoblastoma, Neuroblastoma, Carcinoma	Doxorubicin, Daunorubicin/ Discovery *Cases:* Med = 300 (36–540) *Controls:* Med = 175 (60–600) Replication *Cases:* Med = 270 (45–840) *Controls:* Med = 250 (25–600) Dutch-EKZ *Cases:* Med = 360 (100–720) *Controls:* Med = 300 (50–720)	NR	Custom Illumina GoldenGate SNP genotyping assay	i) SF ≤ 26% ii) sign and symptoms requiring for cardiac compromise intervention based on CTCAEv3
Volkan-Salanci (2012)[45]	Turkey; Turkish	PC; 70	Mean = 49.1 ± 13.6		7/63		Breast cancer, lymphoma, mesenchymal tumour, nasopharyngeal cancer, duodenal cancer, sarcoma	Doxorubicin, epirubicin/ Mean = 317.1 ± 94.9	NR	TaqMan® genotyping assay	i) LVEF decrease > 10% ii) LVEF ≤ 50%
Windsor (2012)[39]	UK, Caucasian, Afro-Caribbean, Indian/ Asian	CC, 58	Med = 18 (10–51)		34/24		Osteosarcoma	Doxorubicin/NR	Peripheral blood	i) Standard PCR, ii) PRC- RFLP, iii) Multiplex PCR, iv) Illumina microarray	Decrease in LVEF by ≥ 1 CTCAEv3 grade.
Armenian (2013)[36]	USA; Non-Hispanic whites, Hispanics, Blacks & Others	NCC; 255	Med = 49.2 (16–68.8)	Med = 51.0 (6.4–72.6)	34/43	119/59	Haematology malignancy + haematopoietic cell transplant	Not specified/*Cases:* Med = 300 (60–650) *Controls:* Med = 300 (40–600)	Peripheral blood stem cells, FFPE BM core biopsies, unstained slides of BM smears	Sequenom MassARRAY	Sign and symptoms of cardiac compromise requiring intervention based American Heart Association criteria 2005
Lipshultz (2013)[46]	USA; NR	PC; 184	Med = 15.2 (3.1–31.4)		101/83		ALL	Doxorubicin/Med = 300 (204–420)	Peripheral blood	i) Pyrosequencing ii) Sequenom genotyping assay iii) TaqMan® genotyping assay	i) cTnT > 0.01 ng/mL ii) NT-proBNP > 150 pg/mL (< 1 year old) iii) NT-proBNP > 100 pg/mL (≥ 1 year old)
Lubieniecka (2013)[52]	Canada; NR	RC; 91	Mean = 48.4 Range = 19–74		48/43		AML	Daunorubicin	Blood	Sequenom genotyping assay	Percentage drop in LVEF
Visscher (2013)[31]	Canada; 41% Canadian, 69% Dutch	CC; 218	Canadian-CPNDS Med = 12.6 (0.9–17.0) Dutch-EKZ Med = 9.1 (0.5–16.8)	Canadian-CPNDS Med = 4.9 (0.5–16.0) Dutch-EKZ Med = 11.2 (1.8–17.7)	Canadian-CPNDS = 8/4 Dutch-EKZ = 23/21	Canadian-CPNDS = 31/47 Dutch-EKZ = 44/40	ALL, AML, other leukemia, HL, NHL Osteosarcoma, Rhabdomyosarcoma, Ewing's sarcoma, Other sarcoma, Nephroblastoma, Hepatoblastoma, Neuroblastoma, Carcinoma, Germ cell tumour	Doxorubicin, daunorubicin/Canadian CPDNS *Cases:* Med = 300 (175–550) *Controls:* Med = 150 (50–540) Dutch-EKZ *Cases:* Med = 360 (100–720) *Controls:* Med = 280 (50–720)	Blood/ saliva/ buccal swab	Custom Illumina GoldenGate SNP genotyping assay	i) SF ≤ 26% ii) sign and symptoms of cardiac compromise requiring intervention based on CTCAEv3
Vivenza (2013)[49]	NR	PC; 48	57.5 (28–73)		1/47		Breast cancer	Epirubcin/540	Blood	i) Allelic discrimination using Applera SNP assay ii) TaqMan® genotyping assay	i) overt CHF (grade III) based on CTCAEv2 ii) LVEF < 50% (grade II) based on CTCAEv2

Continued

Study: Author (year)	Geographic location, ethnic group	Study design; number of participants	Age (years)		Gender: male/female		Type of Cancer examined	Anthracycline used/ Cumulative dose (mg/m^2)	Source of DNA sample	Genotyping	Definition of cardiotoxicity
			Cases	Controls	Cases	Controls					
Wang (2014)[37]	USA; Non-Hispanic whites	NCC; 363	Discovery cohort Med = 19.4 (0.4–41.7)	Discovery cohort Med = 18.5 (3.5–49.2)	40/53	94/100	HL, NHL bone tumours, soft tissue sarcoma, ALL, AML, other.	Not specified/ Discovery *Cases:* Med = 300 (0–630) *Controls:* Med = 152 (0–825) Replication Med = 300 (60–649)	Peripheral blood, buccal cells/ saliva	Illumina IBC cardiovascular SNP array	American Heart Association criteria for cardiac compromise: i) symptoms and/or signs of cardiac compromise and echo evidence of LV dysfunction. ii) absence of symptoms/ signs with echo evidence of LV dysfunction (LVEF $\leq$ 40% and/or SF $\leq$ 28%).
Wasielewski (2014)[38]	The Netherlands; Dutch	CC; 21 (Cohort I = 5; Cohort II = 13, Cohort III = 3)	Cohort I Med = 49 (2–57) Cohort II Med = 46 (34–61) Cohort III Med = 4 (4–9)		NR		Breast cancer, ALL, neuroblastoma, Wilm's tumour, primary neuroectodermal tumour	Epirubicin, Doxorubicin, Daunorubicin/ Range = 175–600	NR	Targeted next-generation DNA sequencing	i) signs and symptoms of cardiac compromise based on American Heart Association criteria (ii) echo evidence of LV dysfunction. iii) absence of symptoms/ signs with echo evidence of LV dysfunction (LVEF $\leq$ 40% and/or SF $\leq$ 28%).
Aminkeng (2015)[47]	Canada; European, African, East Asia, Aboriginal Canadian	PC; Discovery = 280 Replication = 96	Discovery Med = 9.0 (2.5–14) Replication Med = 7.5 (5–12)	Discovery Med = 4.0 (2–7.5) Replication Med = 11 (6–14)	Discovery 15/17 Replication 12/10	Discovery 136/112 Replication 38/36	ALL, AML, other leukaemia, HL, NHL, osteosarcoma, rhabdomyosarcoma, Ewing's sarcoma, other sarcoma, hepatoblastoma, neuroblastoma, Wilms tumour	Doxorubicin, Daunorubicin, Epirubicin/Discovery *Cases:* Med = 260 (177.5–365) *Controls:* Med = 175 (140–295) Replication *Cases:* Med = 407.5 (270–480) *Controls:* Med = 277.5 (180–364)	NR	Illumina HumanOmniExpress assay	i) LVEF < 45% ii) Dilation of LV-end-diastolic dimension >117%.
Krajinovic (2015)[43]	Canada, French-Canadian	CC; 295	QcALL cohort Mean = 6.16 DFCI cohort Mean = 5.27		QcALL cohort = 134/117 DFCI cohort = 21/23		ALL	Doxorubicin/300–360	Blood, buccal swabs	PCR allele-specific-oligonucleotide hybridization assays.	Reduction in SF and EF
Reichwagen (2015)[22]	Germany, Czech Republic & Switzerland; NR	NCC; 520	Med = 68 (61–80)	Med = 67 (62–79)	25/31	46/48	NHL	Doxorubicin/*Cases:* Med = 309 *Controls:* Med = 318	Blood	i) Pyrosequencing ii) TaqMan® genotyping assays	Grade >0 based on CTCAEv2
Visscher (2015)[21]	Canada & The Netherlands; NR	CC; 536	Med = 7.4 (0.04–17.6)	Med = 4.9 (0.1–17.7)	64/58	211/187	Leukaemia, lymphoma, sarcoma, blastoma and others	Doxorubicin, Daunorubicin/*Cases:* Med = 300 (36–840) *Controls:* Med = 200 (25–740)	Blood, saliva, buccal swabs	Custom Illumina GoldenGate SNP genotyping assay	i) Shortening fractions <26% ii) Echo and/ or symptoms of cardiac compromise requiring intervention based on CTCAEv3
Vulsteke (2015)[48]	Belgium; NR	PC; 877	Mean = 50.3		NR		Breast cancer	Epirubicin/NR	Blood	Sequenom MassARRAY	(ii) asymptomatic decrease of LVEF>10%
Hertz (2016)[39]	USA; White, Black, Other	CC, 166	Med = 50 (35–64)	Med = 50 (24–80)	0/19	0/147	Breast cancer	Doxorubicin/*Cases:* Med = 240 (240–350) *Controls:* Med = 240 (120–366)	Blood	i) Sequenom MassARRAY ii) TaqMan® allelic discrimination assay	EF<55%

Continued

Study: Author (year)	Geographic location, ethnic group	Study design; number of participants	Age (years)		Gender: male/female		Type of Cancer examined	Anthracycline used/ Cumulative dose (mg/m^2)	Source of DNA sample	Genotyping	Definition of cardiotoxicity
			Cases	Controls	Cases	Controls					
Reinbolt (2016)[42]	USA; NR	NCC, 162	Mean = 51.9 ± 11.9	Mean = 50.1 ± 9.3	0/52	0/110	Breast cancer	NR	NR	i) TaqMan® allelic discrimination assay ii)	i) EF <50% ii) decrease of LVEF>15% iii) new arrhythmia iv) new myocardial infarction
Wang (2016)[22]	USA; Non-Hispanic white, Hispanic, others	NCC; 385 (Discovery = 331, Replication = 54)	Discovery Set Mean = 8.4 ± 5.7 Med = 7.5 (0–20) Replication Set Mean = 7.7 ± 5.0 Med = 7.7 (0.02–20.6)	Discovery Set Mean = 8.3 ± 5.8 Med = 7.9 (0–21)	Discovery Set: 46/66 Replication Set: 30/24	Discovery Set:106/113	HL, NHL, Sarcoma, AML, ALLand others	NR/Discovery *Cases:* Med = 319 (0–760) *Controls:* Med = 180 (0–825) Replication *Cases:* Med = 350 (0–668) *Controls:* Med = 301 (0–668)	Blood, buccal cells, saliva	i) Illumina HumanOmniExpress assay ii) Sequenom MassARRAY	i) signs and symptoms of cardiac compromise based on American Heart Association criteria 2009 ii) absence of symptoms/ signs with echo evidence of LV dysfunction (LVEF ≤ 40% and/or SF < 28%).

Table 1. Descriptions of included studies. ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia, BM, bone marrow; CC, case-control; EF, ejection fraction; FFPE, Formalin-fixed, paraffin-embedded; HL, Hodgkin's lymphoma; LVEF, left ventricular ejection fraction; LVFS, left ventricular shortening fraction; Med, median; CTCAE, National Cancer Institute Common Toxicity Criteria; NCC, nested case control; NHL, non-Hodgkin's lymphoma, NR, not reported, PC, prospective cohort; RC, retrospective cohort; RFLP, restriction fragment length polymorphism; SF, shortening fraction.

Result

Study and patient characteristics. Our search identified 1,277 studies and 510 underwent assessment. A total of twenty-eight studies involving 7,082 patients were included in the current review (Fig. 1). The characteristics of the included studies are presented in Table 1. Eighteen of the studies were case control studies[20–23,30–43], of which eight were nested case-control studies[22,23,32,34–37,42]. Another seven were prospective cohort studies[44–50] while two were retrospective cohort study[51,52]. The remaining one was a case report[53]. These studies were conducted in the North America (n = 16)[20,21,23,30,31,33–37,42–44,46,47,52], Europe (n = 9)[22,32,38–41,45,48,51], and Asia (n = 1)[50] while two did not report the study location[49,53]. Almost equal number of studies were conducted in children (n = 10) and adults (n = 13) population. Five studies included both children and adults in their report[36–39,44]. Nineteen studies described the ethnicity of their participants[20,23,30–40,43–45,47,50,51] but, there were inconsistencies in reporting of race/ethnicity. For example, Weiss *et al.*[33] described their participants either as Caucasian or not while Blanco *et al.*[34] described their participants as White, Black and others.

The most common type of cancer examined were leukaemia (n = 7), breast cancer (n = 6), lymphoma (n = 3) and osteosarcoma (n = 1). In the other eleven studies, the authors examined a mix types of cancer. Doxorubicin (n = 8), daunorubicin (n = 4) and epirubicin (n = 3) were the common anthracyclines examined. Only eight studies reported the cumulative anthracycline dose in doxorubicin isotoxic equivalent doses[21,23,30,31,35–37,47]. The median cumulative doses in doxorubicin isotoxic equivalent dose ranged from 240 to 504 mg/m^2 for cases and 175 to 540 mg/m^2 for controls. These conversions were mainly derived based upon the guidelines of the Children's Oncology Group[54,55].

The definition of cardiotoxicity varied across studies, with most studies using either a subjective outcome (n = 5), objective outcome (n = 8) or both (n = 14) while one study did not define cardiotoxicity[53] (Supplementary Table 1). Most studies using subjective outcomes defined cardiotoxicity as the presence of signs and symptoms requiring intervention[21–23,30,31,33,35–37,41,47–49]. In addition, some studies have used the left ventricular ejection fraction (LVEF) or shortening fraction (SF) as an objective measure, but the cut-off points varies. For example, the cut-off values of less than 40% to 55% of LVEF[20] or decrease of more than 10–15% have been used. Three studies also included electrocardiogram changes in the definition of cardiotoxicity i.e. arrhythmia[22,32,42] and abnormalities in ECG[40] while one study solely examined the effect of anthracycline on QT interval and arrhythmia[50].

Blood and buccal cells were the most common bio-specimen used for genotyping. Fifteen studies used single bio-specimen of either, blood[20,22,32,39,41,44,46,48–53], buccal swab[34] or bone marrow smear[40] while seven studies used more than one type of bio-specimens[21,23,31,33,35–37]. Six studies did not report the bio-specimen used for genotyping[30,38,42,43,45,47]. Seventeen studies used single genotyping assay[21,30,31,35–38,41–45,47,48,50,52,53] while the remaining eleven studies use multiple genotyping assays[20,22,23,32–34,39,40,46,49,51]. The most commonly used assay technique were TaqMan® genotyping assay (n = 7), Sequenom MassARRAY (n = 4), Sequenom genotyping assay (n = 3), custom Illumina GoldenGate SNP genotyping assay (n = 3) and pyrosequencing (n = 3). Twenty-one studies assessed their cohort or control group for compliance with the HWE[20–23,30–32,34–39,41,44,45,47–49,51,52].

The quality of the reporting in the studies. Among the reviewed studies, twenty-six studies were rated to have high quality (mean score of 45 for studies with control group and 40 for studies without control group)

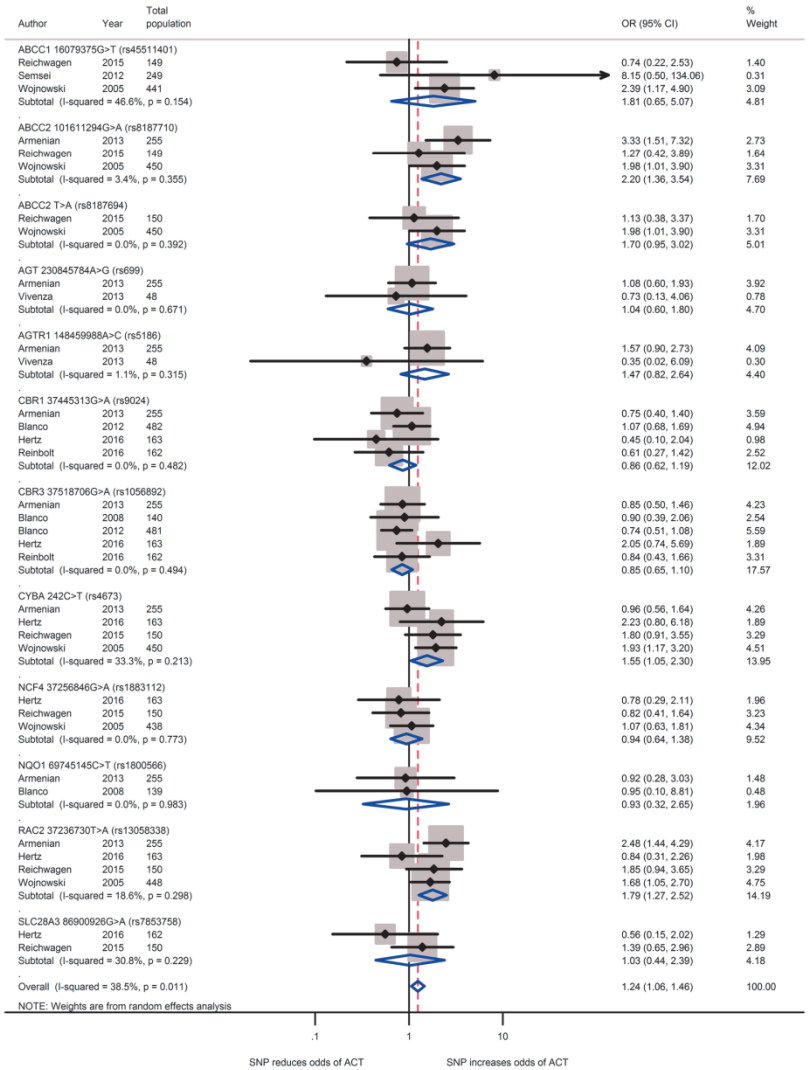

Figure 3. Forest plot of meta-analysis for 12 SNPs. Three variants, ABCC2 rs8187710, CYBA rs4673 and RAC2 rs13058338, are significantly increased the odds for ACT.

except for one study[44], which was rated to be of moderate quality (Supplementary Table 2). On average, included studies were rated as good for most of the items on the Q-Genie tool except for the domain: sample size and power as studies had not described or determined the sample size required for their studies. In most cases, these were either retrospectively analyses of a research datasets/cohort assembled for different purposes.

Anthracycline-induced cardiotoxicity and genotype. A total of 147 SNPs involving eighty-four genes were reported by the studies (Supplementary Table 3). Three genome-wide association studies[23,39,47] were identified, and the remaining studies involved using a candidate gene approach. Most of the studies focused on variation in genes implicated in biosynthesis of anthracyclines or cardiac function. Eighty-seven of the SNPs were reported to be significantly associated with ACT by at least one study (Fig. 2). Quantitative analysis was possible for twelve polymorphs in eleven genes (Fig. 3). Most of the SNPs were from genes which encode transporter

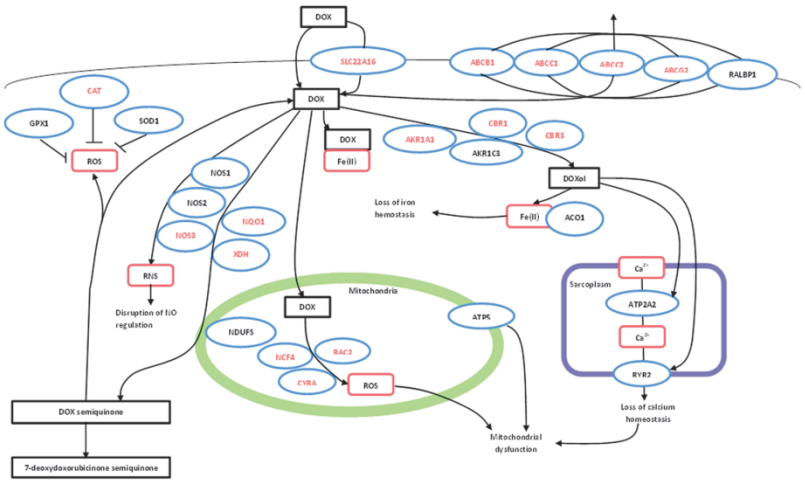

Figure 4. Diagrammatic representative of the candidate genes involved in transport and metabolism of doxorubicin and doxorubicin induced cardiotoxicity. ABCB1, ATP-Binding Cassette Subfamily B Member 1; ABCC1, ATP-Binding Cassette Subfamily C Member 1; ABCC2, ATP-Binding Cassette Subfamily C Member 2; ABCG2, ATP-Binding Cassette Subfamily G Member 2, ACO1, Aconitase 1; AKR1A1, Aldo-Keto Reductase Family 1 Member A1, AKR1C3, Aldo-Keto Reductase Family 1 Member C3; ATP2A2, ATPase Sarcoplasmic/Endoplasmic Reticulum Ca^{2+} Transporting 2; ATP5E, ATP synthase H^+ Transporting, mitochondrial F1 Complex, Epsilum Subunit; CAT, Catalase gene; CBR1, Carbonyl Reductase 1; CBR3, Carbonyl Reductase 3; CYBA, Cytochrome B-245 Alpha Chain; GPX1, Glutathione Peroxidase 1; NCF4, Neutrophil Cytosolic Factor 4; NDUFS, NADH: Ubiquinone Oxidoreductase Subunit; NOS1, Nitric Oxide Synthase 1; NOS2, Nitric Oxide Synthase 2; NOS3, Nitric Oxide Synthase 3; NQO1, NAD(P)H Quinone Dehydrogenase 1; RAC2, Ras-related C3 Botulinum Toxin Substrate 2; RALBP1, RalA Binding Protein 1; RYR2, Ryanodine Receptor 2; SLC22A16, Solute Carrier Family 22 Member 16; SOD1, Superoxide Dismutase 2, mitochondrial; XDH, Xanthine Dehydrogenase.

proteins; of which twenty-eight SNPs were from eleven ATP-binding cassette (ABC) transporters gene while nineteen SNPs were eleven genes encode solute carriers (SLC). The most studied genes encoding metabolising proteins were genes encode aldo/keto reductase (AKR) superfamily and carbonyl reductase (CBR). A discussion on the genes included in meta-analysis follows below.

ATP Binding Cassette (ABC) gene. ABC transporters genes encode a superfamily of transmembrane proteins that actively transport substrates including doxorubicin across membranes using adenosine triphosphate[56]. Fourteen of the twenty-eight variants in ABC transporters were found to significantly increase the risk for ACT[20,21,30–32,36,41,43,48,51] (Supplementary Table 3). ABCC1 is the most studied gene with nine SNPs followed by ABCB1 (5 SNPs) and ABCC2 (3 SNPs). The rs246221 polymorphism of ABCC1 gene was found to significantly deteriorate cardiac function in both studies[48,51]. Seven SNPs, rs1045642[20,41], rs1149222[20,30,31,53], rs4148808[20,31], rs45511401[22,32,51], rs4148350[20,30], rs8187710[22,32,36] and rs8187694[22,30,32] were found to increase the risk in only one of the studies assessing their association with ACT.

Armenian *et al.* recruited 77 cases and 178 controls from a population of haematological patients that underwent haematopoietic cell transplantation reported that rs8187710 increased ACT risk (OR: 5.22; 95% CI: 1.92–13.84; false discovery rate-adjusted p = 0.02)[36]. Using similar study design and a larger sample size (87 cases and 363 controls) of only non-Hodgkin lymphoma survivors, Wojnowski *et al.* reported the heterozygous or homozygous genotypes risk of acute ACT was statistically significant (OR: 2.3; 95% CI: 1.0–5.4; Fisher exact test p = 0.06)[32]. In contrast, Reichewagen *et al.* did not find significant association between the mutation and risk for ACT (OR: 1.3; 95% CI: 0.4–3.9; p = 0.67)[22]. When combined, the missense mutation was associated with a large increase in risk (pooled OR: 2.20; 95% CI: 1.36–3.54; p = 0.001).

Meta-analysis of three studies in European[22,32,51] populations revealed that the missense mutation of rs45511401 increased the risk for ACT (pooled OR: 1.81; 95% CI: 0.65–5.07; p = 0.26) with moderate heterogeneity (I^2 = 47%). Similarly the combined effect of ABCC2 rs8187694 from two studies in European[22,32] populations showed no significant association (pooled OR: 1.70; 95% CI: 0.95–3.02; p = 0.07).

Carbonyl reductases (CBR) gene. Carbonyl reductases (CBR) genes encode enzymes that catalyse the reduction of endogenous aliphatic aldehydes and ketones and various xenobiotic, thus offering cardio-protective role

against ACT. Four SNPs on carbonyl reductases (CBR) were studied, one on carbonyl reductase 1 gene (CBR1) and three on carbonyl reductase 3 gene (CBR3). However, two SNPs, rs9024 of CBR1 and rs1056892 of CBR3 were associated with cardio-protection, but this did not reach statistical significance (pooled OR: 0.86; 95% CI: 0.62–1.19 and 0.85; 0.65–1.10 respectively, Fig. 3).

Cytochrome b-245, alpha polypeptide (CYBA) gene. Cytochrome B-245, alpha polypeptide gene (CYBA, NC_000016.10) encodes the primary component of the microbicidal oxidase system of phagocytes. We identified six studies which assessed associations of the rs4673 missense SNP of CYBA with ACT, three studies[20,22,32] are included in qualitative analysis due to unavailability of required information in the other two studies[30,41]. Among the samples, the SNP was found to increase the odds of developing ACT (pooled OR: 1.55; 95% CI: 1.05–2.30; $p = 0.03$) with moderate heterogeneity ($I^2 = 33\%$).

Neutrophil cytosolic factor 4 (NCF4) gene. Neutrophil cytosolic factor 4 gene (NCF4, NC_00002.10) encodes the p40phox subunit of the NAD(P)H oxidase[57]. The rs1883112 polymorphism at the putative promoter of NCF4 blocks oxidase activation of the enzyme thus reduces the formation of reactive oxidant intermediates[58]. Two of the six studies examined the effect of SNP rs1883112 found that SNP was significantly associated with cardiac toxicity[32,36]. The combined effect of this synonymous substitution from two studies in North America[20,36] and European[22,32] populations showed no significant association (pooled OR: 0.94; 95% CI: 0.64–1.38; $p = 0.75$).

Ras-Related C3 Botulinum Toxin Substrate 2 (RAC2) gene. Ras-Related C3 Botulinum Toxin Substrate 2 gene (RAC2, NC_000022.11) encodes the protein regulating diverse processes including secretion, phagocytosis, cell polarisation and generation of reactive oxygen species. Three of six studies reported SNP rs13058338 on RAC2 significantly increase risk for ACT[32,36,41]. Analysis of this intron variant in four studies showed that RAC mutation increased the risk of cardiotoxicity by nearly two times (pooled OR: 1.79; 95% CI: 1.27–2.52; $p < 0.001$).

Discussion

To our knowledge, this is the first and only systematic review which examined the role of genetic polymorphisms with ACT induced cardiotoxicity. We found a total of twenty-eight studies, examining eighty-four different genes. Most of the genes studied were linked to the biochemical pathway of anthracycline, oxidative stress or cardiac function (Fig. 4). As such, it is not surprising that all but one[47] genetic studies described in this article have included these candidate genes in their study. Results from our meta-analyses revealed that polymorphism in three (3.6%) of the eight-four genes were significantly associated with an increased odds of cardiotoxicity in individuals treated with anthracyclines. However, the individual risk provided by any of these candidate genes were moderate only (OR: 1.55–2.20), in agreement with previous studies which have examined other complex diseases, such as stroke[59] and ischaemic heart diseases[60,61].

For the genes that were found to have a positive association, animal and mechanistic studies have shown that these alleles alter the expression or activity of the encoded protein and thus contribute to disease pathogenesis. ABCC2 gene encodes for proteins that are involved the efflux of substances from cells, and mutation of ABCC2 significantly reduces ATPase activity, resulting in a decrease in efflux activity leading to intracellular accumulation of anthracycline[62]. Similarly, the Rac2 (Ras-related C3 botulinum toxin substrate 2) encoded by RAC2 gene is a mitochondrial protein that is required in electron transfer reaction of NADPH oxidase[63] during the formation of reactive oxygen species (ROS)[64]. Alteration of the gene results in mitochondrial dysfunction and thus an increase ROS production, which ultimately leads to myocytes damages. Taken together, mutations in these genes are thought to result in cardiomyopathy due to accumulation of anthracycline and excessive ROS in myocytes.

We also observed that some of these genes were not only related to cardiotoxicity, but also other adverse drug reactions (ADRs) of chemotherapy such as myelosuppression and infection as well as overall survival. The SNPs ABCG2 rs2231142[41], NCF4 rs1883112[41], GSTP1 rs1695[39,41], CYBA rs4673[39] and GSTM1 null allele[39] significantly increased odds for grade 3–4 hematologic toxicity in patients treated with anthracycline-based chemotherapy regimen. Similarly, ABCB1 rs1045642, ABCG2 rs2231137 and NCF4 rs1883112 significantly increased odds for grade 2–4 infection[41]. In addition, rs1695 of GSTP1[39], rs17222723 of ABCC2[41] and rs4673 of CYBA[41] were significantly related to progression-free survival or event-free survival.

This study has some limitations which warrant discussion. Firstly, we found a total of 147 SNPs which were examined for the possible association with ACT. Most of the SNPs have only been examined once; which limited our ability to perform a meta-analysis. In addition, there were inconsistencies in reporting of results between studies. As such, our meta-analyses only included between two to five studies, which restricted subgroup analyses. The included studies were also heterogeneous and had not adjusted for confounders, which further limits the precision of overall estimates. We also selectively discussed the roles of genes included in the meta-analysis. It should be noted that the SNPs discussed in this review does not imply that they are superior in any aspect to other SNPs identified. Many of the studies were not prospectively designed but had used a convenience sampling, which is reinforced by the fact that none of the studies had adequately reported the sample size calculations. Similarly, nearly all of the studies (96%) of the studies were carried out in Western populations, thus limiting the generalisability to other populations. Furthermore, most of the studies had not reported the demographics of their population. Finally, only a handful studies had adjusted for some confounding factors in their analysis, although these have been shown to increase the risk factor for AIC.

Over the past few decades, the development in molecular biology has increased our understanding on the role of genetic variation underlying adverse drug reactions (ADRs). Currently, genetic testing is recommended for identifying patients at risk for ADRs. Examples include testing of thiopurine methyltansferase (TMPT) gene variation prior to thiopurine therapy in inflammatory bowel disease and human leukocyte antigen (HLA)-B*1502

for treatment of seizures with carbamazepine. Polymorphisms of TMPT gene have been known to cause lowered TPMT activity, and thus a reduced dose is recommended for heterozygous patients to prevent hematopoietic toxicity[65]. Meanwhile, HLA-B*15:02 screening is recommended for Asian populations to identify patients at risk for carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis[66].

However, results from this study suggest that unlike examples listed above, several polymorphs may be involved in ACT. As such, a genome-wide association studies which could examine SNPs across the whole genome should be conducted. In order to ensure that study findings can be more effective to influence the development of personalised medicine for addressing drug toxicities in general and ACT in specific, future studies should ideally be conducted in a prospective large cohort. Multicentre studies including patients from other continents especially Africa, Asia, South America, Australia and Oceania, are encouraged. In addition, the use of an objective definition of cardiotoxicity and reporting the frequency of events for each genotype should be considered.

Conclusions

Results of this study indicate that several polymorphisms of pharmacogenetics candidates across the anthracyclines biochemistry and cardiomyopathy pathways are potentially a predictor for ACT. However, the evidences are limited and too heterogeneous for a significant quantitative analysis. Further studies are needed to generate robust genetic predictor(s) for ACT to achieve the goal of individualising anthracycline therapy.

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Acknowledgements

Siew Lian Leong is funded by the Research Degrees Scholarships from Monash University Malaysia.

Author Contributions

S.L.L. and S.W.H.L. are joint first authors who take responsibility for the planning of the methods and preparation of the manuscript. Leong had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. N.C. is senior author who takes responsibility for final editing of the manuscript.

Additional Information

Supplementary information accompanies this paper at doi:10.1038/s41598-017-00075-1

Competing Interests: The authors declare no competing financial interests.

Supplementary Information

Search Strategies
Complete list of search terms used.

Supplementary Table 1: Definition of cardiotoxicity used by included studies. *This table summarised the various definition for cardiotoxicity used in the included studies.*
(Words)

Supplementary Table 2: The quality assessment of reporting in each study (N = 27).
Each study was scored independently by 2 reviewers according the 11 items in Q-Genie tool. The average score of each item and total average score for each study were summarised in this table. (Words)

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status.
This table summarised the findings on the association between each of the 147 SNPs in 84 genes and anthracycline-induced cardiotoxicity.
(Words)

Search Strategies

1. OVID Medline, Cochrane Central Register of Controlled Studies, EMBASE and AMED Text word search
(1) anthracycline*.mp.; (2) doxorubixin*.mp.; (3) daunorubicin*.mp. (4) epirubicin*.mp. (5) idarubicin*.mp. (6) 1 or 2 or 3 or 4 or 5; (7) cardiotoxicity*.mp.; (8) cardiomyopathy*mp.; (9) heart*.mp.; (10) failure*.mp.; (11) 9 and 10; (12) arrhythmia*.mp.; (13) 7 or 8 or 11 or 12; (14) genetic*.mp.; (15) polymorphism*.mp.; (16) pharmacogenomics*.mp.; (17) variant*.mp. (18) 14 or 15 or 16 or 17; (19) 6 and 13 and 18

2. PUBMED Text word Search
(1) anthracycline or doxorubicin or daunorubicin or epirubicin or idarubicin; (2) heart and failure; (3) 2 or cardiotoxicity or cardiomyopathy or arrhythmia; (4) genetic or pharmacogenomics or variant or polymorphism (5) 1 and 3 and 4

3. CINAHL Plus Text word search
(1) anthracycline or doxorubicin or daunorubicin or epirubicin or idarubicin; (2) cardiotoxicity or cardiomyopathy or arrhythmia or heart failure; (3) genetic or pharmacogenomics or variant or polymorphism; (4) 1 and 2 and 3

4. HuGE Navigator Text word search
anthracycline and cardiotoxicity and genetic

Supplementary Table 1: Definition of cardiotoxicity used by included studies.

Studies	Definition
Wojnowski, 2005	arrhythmia in the absence of arrhythmia before treatment *or* myocarditis-pericarditis *or* acute heart failure *or* LVEF <50% or SF <25%
Weiss, 2006	SWOG toxicity criteria for SWOG 9031 *or* CTCAEv2.0 for SWOG-9333.
Blanco, 2008	Self-reporting of signs and symptoms of CHF and use of medication for CHF management.
Rajic, 2009	Clear conduction disturbances, depolarization and repolarization changes in ECG *or* SF < 30%, *or* LVEF <54% *or* derangement of (reference range) E (0.75±0.13), A (0.51±0.11), E/A (1.53±0.4), IVRT (67±8), PV-A (0.21±0.08), PV-D (0.47±0.11) PV-S (0.44±0.1)
Rossi, 2009	Grade 2-4 cardiotoxicity according to CTCAEv 0.3
Blanco, 2012	Signs and symptoms of cardiac compromise based on AHA criteria 2005 *or* absence of symptoms/signs with echo evidence of left ventricular dysfunction (EF ≤ 40% and/or SF ≤ 28%).
Kitagawa, 2012	QTc interval prolongation *or* other toxic effects based on CTCAEv3
Lubieniecka, 2012	Percentage drops in LVEF.
Semsei, 2012	Changes in LVFS
Visscher, 2012	SF ≤ 26% *or* sign and symptoms requiring for cardiac compromise intervention based on CTCAEv3
Volkan-Salanci, 2012	LVEF decrease > 10% *or* LVEF ≤ 50%
Windsor, 2012	Decrease in LVEF by ≥ 1 CTCAEv3 grade.
Armenian, 2013	Sign and symptoms of cardiac compromise requiring intervention based AHA criteria 2005
Lipshultz, 2013	cTnT > 0.01 ng/mL or NT-proBNP > 150 pg/mL (< 1 year old) or NT-proBNP > 100 pg/mL (≥ 1 year old)
Lubieniecka, 2013	Percentage drop in LVEF
Visscher, 2013	SF ≤ 26% *or* sign and symptoms of cardiac compromise requiring intervention based on CTCAEv3
Vivenza, 2013	Overt CHF (grade III) based on CTCAEv2 *or* LVEF < 50% (grade II) based on CTCAEv2
Wang, 2014	AHA criteria for cardiac compromise i.e. symptoms and/or signs of cardiac compromise and echo evidence of LV dysfunction *or* absence of symptoms/signs with echo evidence of LV dysfunction (LVEF ≤ 40% and/or SF ≤ 28%).
Wasielewski, 2014	Signs and symptoms of cardiac compromise based AHA criteria *or* echo evidence of LV dysfunction *or* absence of symptoms/signs with echo evidence of LV dysfunction (LVEF ≤ 40% and/or SF ≤ 28%).
Aminkeng, 2015	LVEF < 45% *or* dilation of LV-end-diastolic dimension >117%.
Krajinovic, 2015	Reduction in SF and EF
Reichwagen, 2015	Grade >0 based on CTCAEv2
Visscher, 2015	SF <26% *or* echo and/or symptoms of cardiac compromise requiring intervention based on CTCAEv3
Vulsteke, 2015	Asymptomatic decrease of LVEF>10%
Hertz, 2016	EF<55%
Reinbolt, 2016	EF <50% *or* decrease of LVEF>15% *or* new arrhythmia *or* new myocardial infarction
Wang, 2016	Signs and symptoms of cardiac compromise based on AHA criteria 2009 *or* absence of symptoms/signs with echo evidence of LV dysfunction (LVEF ≤ 40% and/or SF ≤ 28%).

Sachidanandam, 2012 did not report the definition of cardiotoxicity used.
Abbreviation: AHA, American Heart Association; CHF, congestive heart failure; ECG, electrocardiogram; EF, ejection fraction; LV, left ventricular; LVEF, left ventricular ejection fraction; LVFS, left ventricular shortening fraction; CTCAE, National Cancer Institute Common Toxicity Criteria; SF, shortening fraction; SWOG, Southwest Oncology Group

Supplementary Table 2: The quality assessment of reporting in each study (N = 27[1]).

Study / Item	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	6	5.5	5	7	7	6	6	6	6.5	4.5	6	6	6	6
B	5.5	5	5	7	6	7	6	5	5.5	5	5	6	6	5
C	5.5	4	5.5	5	3	6.5	0	0	0	5	0	4	7	5
D	5	5	5	6	6	5	6	5	5.5	4.5	4.5	6	5	5.5
E	5	4.5	4.5	2	4	4.5	4	3	5	3.5	3.5	7	4.5	3.5
F	4	3	4	2	5	4	3.5	3.5	4	3.5	3.5	6	5	3.5
G	3	3.5	2.5	1	7	3	2.5	3	3	3	2.5	1	2.5	6
H	5	4.5	4.5	4	7	5	4	3	5.5	5	5	6	5	6
I	6	4.5	5.5	4	7	6	3	2.5	6	4.5	5	6	5	5
J	3.5	4	4	5	4	4	3.5	3	3.5	4	3.5	6	4	4
K	6	5	4	5	6	6	4	4.5	5.5	5	5	6	5	4
Total scoring	54.5	48.5	49.5	48	62	57	42.5	38.5	50	47.5	43.5	60	55	53.5
Quality[1]	G	G	G	G	G	G	G	M	G	G	G	G	G	G

Reported rate is an average rate given by two investigators. [1]Study by Sachinandam, 2012 is not rated because it is a case study

1, Wojnowski, 2005; 2, Weiss, 2006; 3, Blanco, 2008; 4, Rajic, 2009; 5, Rossi, 2009; 6, Blanco, 2012; 7, Kitagawa, 2012; 8, Lubieniecka, 2012; 9, Semsei, 2012; 10, Visscher, 2012; 11, Volkan-Salanci, 2012; 12, Windsor, 2012; 13, Armenian, 2013; 14, Lipshultz, 2013; 15, Lubieniecka, 2013; 16, Visscher, 2013; 17, Vivenza, 2013; 18, Wang, 2014; 19, Wasielewski, 2014; 20, Aminkeng,2015; 21, Krajinovic, 2015; 22, Reichwagen, 2015; 23, Visscher, 2015; 24, Vulsteke, 2015; 25, Hertz, 2016; 26, Reinbolt, 2016; 27, Wang, 2016.

A, Rationale of Study; B, Selection and definition of outcome; C, Selection and comparability of comparison groups (0 if not applicable); D, Technical classification of the exposure; E, Non-technical classification of the exposure; F, Other source of bias; G, Sample size and power; H, A priopri planning of analyses; I, Statistical methods and control for confounding; J, Testing of assumption & inferences for genetic analyses; K, Appropriateness of inferences drawn from results. [1]G = Study of good quality, M =Study of moderate quality.

Supplementary Table 2: The quality assessment of reporting in each study (N = 27[1]). *(continued)*

Study / Item	15	16	17	18	19	20	21	22	23	24	25	26	27
A	6	5.5	6	5	6.5	5.5	6	6	6	5.5	6	6	6
B	6	6	6.5	5	7	5	5.5	6.5	6.5	5	6	6	6
C	0	5.5	6	0	7	5.5	0	6	6	5.5	5	7	7
D	6	5	6	3.5	6	4.5	6	6	6	4.5	6	6	6
E	5	4	4	4	7	4.5	3	2	2	4.5	4	6	6
F	5	3.5	5	4.5	6.5	4.5	5	5.5	5.5	4.5	5	4	5
G	3	2	1	2.5	1	1	3	1	1	1	4	1	7
H	5	5	5	4.5	7	5	5	6.5	6.5	5	6	5	6
I	6	6	5.5	5	6.5	5.5	5.5	6	6	5.5	4	6	7
J	5	4.5	3	3.5	6	4.5	5	5.5	5.5	4.5	6	5	6
K	5	5	6.5	4	6.5	6.5	6	6	6	6.5	6	6	6
Total scoring	52	52	54.5	41.5	67	52	50	57	57	52	58	58	68
Quality*	G	G	G	G	G	G	G	G	G	G	G	G	G

Reported rate is an average rate given by two investigators. [1]Study by Sachinandam, 2012 is not rated because it is a case study

1, Wojnowski, 2005; 2, Weiss, 2006; 3, Blanco, 2008; 4, Rajic, 2009; 5, Rossi, 2009; 6, Blanco, 2012; 7, Kitagawa, 2012; 8, Lubieniecka, 2012; 9, Semsei, 2012; 10, Visscher, 2012; 11, Volkan-Salanci, 2012; 12, Windsor, 2012; 13, Armenian, 2013; 14, Lipshultz, 2013; 15, Lubieniecka, 2013; 16, Visscher, 2013; 17, Vivenza, 2013; 18, Wang, 2014; 19, Wasielewski, 2014; 20, Aminkeng,2015; 21, Krajinovic, 2015; 22, Reichwagen, 2015; 23, Visscher, 2015; 24, Vulsteke, 2015; 25, Hertz, 2016; 26, Reinbolt, 2016; 27, Wang, 2016.

A, Rationale of Study; B, Selection and definition of outcome; C, Selection and comparability of comparison groups (0 if not applicable); D, Technical classification of the exposure; E, Non-technical classification of the exposure; F, Other source of bias; G, Sample size and power; H, A priopri planning of analyses; I, Statistical methods and control for confounding; J, Testing of assumption & inferences for genetic analyses; K, Appropriateness of inferences drawn from results. [1]G = Study of good quality, M =Study of moderate quality.

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
ABCA1 (NC_000009.11)	106738433G>A	None	rs3887137	Visscher,2015	**SNP is significantly associated with ACT in combined cohort OR = 2.28 (1.40 – 3.71), p = 9.5x10^{-4}**	No
ABCB1 (NC_000007.13)	g.87138532A>C	None	rs2235047	Visscher, 2013	No significant association between SNP and ACT OR = 1.79 (1.05 – 3.04), p = 0.036	No
	g.87138645A>G	Ile1145	rs1045642	Rossi, 2009	No significant association between genotypes and cardiac toxicity TT vs CT/CC OR = 1.16 (0.73 – 1.84), p = 0.515 CT/TT vs CC OR = 1.09 (0.69 – 1.73), p = 0.680	No
				Hertz, 2016	**CC vs CT/TT OR = 0.48 (0.23 – 1.00), p = 0.049**	No
	g.87179809C>T	Ser400Asn	rs2229109	Rossi, 2009	**AG vs GG OR = 1.89 (1.15 – 3.12), p = 0.010**	No
	g.87179601A>G	Gly412	rs1128503	Hertz, 2016	No significant association between genotype and cardiotoxicity GG vs GA/AA OR = 0.57 (0.27 – 1.21), p = 0.14	No
	g.87160618G>T	Ser893Thr	rs2032582		No significant association between genotype and cardiotoxicity GG vs GT/TT OR = 0.72 (0.36 – 1.43), p = 0.35	

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
ABCB4 (NC_000007.13)	g.87073775T>G	None	rs1149222	Visscher, 2012	**Risk variant OR = 1.87 (1.20 – 2.92), p = 0.0054**	No
				Visscher, 2013	No significant association between SNP and ACT OR = 1.36 (0.97 – 1.90), p = 0.075	No
				Hertz, 2016	No significant association between genotype and cardiotoxicity TT vs GT/GG OR = 1.06 (0.48 – 2.34), p = 0.14	No
				Sachidanandam, 2012	No significant association	NR
	g.87105795A>G	None	rs4148808	Visscher, 2013	**SNP is significantly associated with ACT in combined cohort OR = 1.67 (1.15 – 2.43), p = 0.0073**	No
				Hertz, 2016	No significant association between genotype and cardiotoxicity GG vs GA/AA OR = 3.92 (0.34 – 45.39), p = 0.27	No
ABCB11 (NC_000002.12)	169479422A>G	None	rs10497346	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 2.23 (1.32 – 3.77), p = 0.0033**	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
ABCC1 (NC_000016.10)	g.15983430G>A	None	rs215060	Semsei, 2012	No significant reduction in LVFS after chemotherapy, p = 0.2	No
	g.15991778T>C	None	rs246219		No significant reduction in LVFS after chemotherapy, p = 0.6	No
	g.16108028G>A	None	rs11864374		No significant reduction in LVFS after chemotherapy, p = 0.2	No
	g.16120105G>A	None	rs6416666		No significant reduction in LVFS after chemotherapy, p = 0.3	No
	g.16141824C>T	None	rs3743527		**TT genotype is associated with lower mean LVFS, p = 0.001**	No
	g.16044465T>C	Val275=	rs246221	Semsei, 2012	**TT/TC genotypes are associated with lower mean LVFS, p = 0.027**	No
				Vulsteke, 2015	**SNP is associated with an asymptomatic LVEF decline > 10%, p = 0.038**	No
	g16079375G>T	Gly671Val	rs45511401	Semsei, 2012	No significant reduction in LVFS after chemotherapy, p = 0.3	No
				Reichwagen, 2015	No significant association between genotypes and cardiotoxicity GG vs GT/TT OR = 0.7 (0.2 – 2.5), p = 0.632	No
				Wojnowski, 2005	**GT/TT genotypes are associated with acute cardiotoxicity (p = 0.005) and chronic or acute cardiotoxicity (p = 0.029)**	No (0.14)
	g.6093318C>T	None	rs4148358	Semsei, 2012	No significant reduction in LVFS after chemotherapy, p = 0.3	No
	g.16076620G>T	None	rs4148350	Visscher, 2012	**Risk variant** **OR = 2.40 (1.33 – 4.33), p = 0.0040**	No
				Hertz, 2016	No significant association between genotype and cardiotoxicity GG vs GT/TT OR = 0.93 (0.21 – 4.04), p = 0.92	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
ABCC2 (NC_000010.10)	g.101611294G>A	Cys1515Tyr	rs8187710	Armenian, 2013	**At risk genotype GA/AA OR = 5.44 (1.92 – 13.84) FDR-adjusted p = 0.02**	No
				Wojnowski, 2005	No significant association between GA/AA genotypes and cardiotoxicity, p = 0.05	No
				Reichwagen, 2015	No significant association between genotypes and cardiotoxicity GG vs GA/AA OR = 1.3 (0.4 – 3.95), p = 0.669	No
	T>A	Val1188Glu	rs8187694	Visscher, 2012	No significant association p = 0.90	No
				Reichwagen, 2015	No significant association between genotypes and cardiotoxicity TT vs TA/AA OR = 1.1 (0.4 – 3.4), p = 0.822	No
				Wojnowski, 2005	**TA/AA genotypes are associated with chronic or acute cardiotoxicity, p = 0.05**	No
	g.101595996T>A	Val1085Glu	rs17222723	Rossi, 2009	No significant association between genotypes and cardiac toxicity AT/AA vs TT OR = 0.85 (0.50 – 1.43), p = 0.546	Yes
ABCC5 (NC_000003.11)	g.183737356A>T	None	rs7627754	Krajinovic, 2015	**Homozygote variant vs heterozygote + homozygote wt, EF reduction of 12%: p <0.0005 and SF reduction of 8%: p = 0.001 using t-test**	NR
ABCC6 (NC_000016.10)	g.16150272T>C	None	rs212097	Semsei, 2012	No significant reduction in LVFS after chemotherapy, p = 0.2	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
ABCC9 (NG_000012.11)	g.22017395G>C	Pro739Ala	rs201223488	Wasielweski, 2014	No causal relation between mutation and development of anthracycline-associated cardiomyopathy	No
	21908424G>C	None	rs11046217	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 2.67 (1.50 – 4.76), p = 9.9x10^{-4}**	No
ABCC10 (NC_00006.11)	43468329A>G	None	rs1214763	Visscher, 2015	Yes **SNP is significantly associated with ACT in combined cohort OR = 0.43 (0.24 – 0.79), p = 0.0035**	No
ABCG2 (NC_000004.11)	g.89052323G>T	Gln141Lyn	rs2231142	Rossi, 2009	No significant association between genotypes and cardiac toxicity	No
	g.89061114C>T	Val12Met	rs2231137		AC/AA vs CC OR = 1.05 (0.70 – 1.57), p = 0.784 AG/AA vs GG OR = 1.01 (0.56 – 1.80), p = 0.974	No
ACE (NC_000017.10)	Ins/Del	-	rs4340	Vivenza, 2013	Del/Del + Del/Ins vs Ins/Ins, p = 0.37 using Fischer's exact test	No
	g.61566031G>A	Thr202	rs4343	Armenian, 2013	At risk genotype AG/GG OR = 1.28 (0.67 – 2.45) FDR-adjusted p = 0.72	No
ADH7 (NC_000004.11)	g.100333267G>A	None	rs729147	Visscher, 2013	**SNP is significantly associated with ACT in combined cohort OR = 1.43 (1.02 – 2.01), p = 0.041**	No
ADRB2 (NC_000005.10)	g.148206440G>A	Gly16Arg	rs1042713	Armenian, 2013	At risk genotype GG OR = 0.60 (0.31 – 1.20) FDR-adjusted p = 0.35	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
AGT (NC_000001.10)	g.230845977G>A	Thr174Met	rs4672	Vivenza, 2013	Homozygote variant vs heterozygote + homozygote wt, p = 0.70 using Pearson's chi-square test	No
	g.230845794A>G	Met235Thr	rs699	Vivenza, 2013	Homozygote variant + heterozygote vs homozygote wt, p = 0.31 using Fischer's exact test	No
				Armenian, 2013	At risk genotype GA/AA OR = 1.15 (0.57 – 2.30) FDR-adjusted p = 0.88	No
AGTR1 (NC_000003.11)	g.148459988A>C	None	rs5186	Vivenza, 2013	Homozygote variant + heterozygote vs homozygote wt, p = 0.31 using Pearson's chi-square test	No
				Armenian, 2013	At risk genotype CA/AA OR = 0.68 (0.37 – 1.25) FDR-adjusted p = 0.88	No
AKR1A1 (NC_000001.10)	g.46032311A>G	Asn52Ser	rs2229540	Lubieniecka, 2012	No significant association, p = 0.1667	No
AKR1C4 (NC_000010.10)	g.5260682C>G	Leu311Val	rs17134592	Lubieniecka, 2012	No significant association, p = 0.9556	No
	g.5244295G>A	None	rs7083869	Lubieniecka, 2013	**SNP is associated with LVEF drop p = 0.0045**	No
	g.5244441A>G	None	rs2151896		**SNP is associated with LVEF drop p = 0.045**	No
AKR7A2 (NC_000001.10)	g.19635011C>T	Ala142Thr	rs1043657	Lubieniecka, 2012	No significant association, p = 0.4379	No
CAT (NC_000011.10)	g.34438684C>T	None	rs1001179	Rajic, 2009	No significant correlation between SNP and late cardiac damage OR = 3.222 (0.341 – 30.426) p = 0.307	NR
	g.34439157C>T	None	rs10836235		**Heterozygote is correlated with late cardiac damage OR = 0.284 (0.093 – 0.867), p = 0.020**	NR

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
CBR1 (NC_000021.9)	g.37445313G>A	None	rs9024	Blanco, 2012	AA/GA vs GG OR = (0.45 – 1.47), p = 0.49	No
				Hertz, 2016	No significant association between genotype and cardiotoxicity GG vs GA/AA OR = 0.45 (0.10 – 2.04), p = 0.30	No
				Reinbolt, 2016	No significant different between major allele (G) and minor allele (A), p = 0.480 No significant different between genotypes, p = 0.261	NR
				Sachidonandam, 2012	No significant association	NR
				Armenian, 2013	At risk genotype GG OR = 1.51 (0.76 – 3.03) FDR-adjusted p = 0.46	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
CBR3 (NC_000021.8)	g.37518706G>A	Val244Met	rs1056892	Blanco, 2008	GG vs AA OR = 5.63 (0.80 – 39.57), p = 0.083 GA vs AA OR = 3.66 (0.64 – 21.07), p = 0.15	No
				Lubieniecka, 2012	No significant association, p = 0.6988	No
				Armenian, 2013	At risk genotype GG OR = 1.08 (0.59 – 1.87) FDR-adjusted p = 0.88	No
				Visscher, 2015	No significant association between SNP and ACT OR = 0.93, p = 0.67	No
				Blanco, 2012	**AA/GA vs GG** **OR = 1.79 (1.08 – 2.96), p = 0.02**	No
				Volkan-Salanci, 2012	Presence of A allele is associated with deterioration in cardiac function.	No
				Hertz, 2016	**AA vs GA/GG** **OR = 6.19 (0.08 – 19.76), p = 0.002** **GG vs GA/AA** **OR = 2.50 (1.22 – 5.11), p = 0.012**	No
				Reinbolt, 2016	No significant different between major allele (G) and minor allele (A), p = 0.395 No significant different between genotypes, p = 0.556	NR
	g.37507501G>A	Cys4Tyr	rs8133052	Lubieniecka, 2012	No significant association, p = 0.7788	No
	g.37512565A>G	None	rs10483032	Lubieniecka, 2013	**SNP is associated with LVEF drop p = 0.0045**	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
CELF4 (NC_000018.10)	g.37497065G>A	None	rs1786814	Wang, 2016	**GG genotype is associated with anthracycline-related cardiomyopathy OR = 10.16 (3.8 – 27.3), p < 0.001 at cumulative anthracycline exposure of > 300mg/m^2**	No
COL1A2 (NC_000007.13)	g.93881175C>G	Pro549Ala	rs42524	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 1.79 (1.24 – 2.57), p = 0.0020**	No
CYBA (NC_000016.10)	242C>T	His72Tyr	rs4673	Visscher, 2012	No significant association p = 0.63	No
				Reichwagen, 2015	No significant association between genotypes and cardiotoxicity CC vs CT/TT OR = 1.8 (0.9 – 3.6), p = 0.090	No
				Hertz, 2016	No significant association between genotype and cardiotoxicity GG vs GA/AA OR = 1.31 (0.66 – 2.59), p = 0.45	No
				Wojnowski, 2005	**CT/TT genotypes are associated with chronic or acute cardiotoxicity, p = 0.01**	No
				Rossi, 2009	**TT vs CT/CC OR = 1.86 (1.15 – 2.99), p = 0.010**	No
				Armenian, 2013	At risk genotype GA/AA OR = 1.29 (0.72 – 2.44) FDR-adjusted p = 0.65	No
CYP1A2 (NC_000015.10)	g.74746892T>G	None	rs2069522	Lubieniecka, 2013	**SNP is associated with LVEF drop p = 0.004**	No
	g.74749000T>G	None	rs2069526		**SNP is associated with LVEF drop p = 0.0045**	No
	g.74753351T>C	None	rs4646427		**SNP is associated with LVEF drop p = 0.0045**	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
CYP2B6 (NC_000019.10)	g.41023115G>A	None	rs7255904	Lubieniecka, 2013	**SNP is associated with LVEF drop p = 0.0475**	No
CYP2F1 (NC_000019.10)	g.41113670T>C	None	rs1709115	Lubieniecka, 2013	**SNP is associated with LVEF drop p = 0.042**	No
CYP2J2 (NC_000001.10)	60087084A>C	None	rs2294950	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 0.39 (0.21 – 0.74), p = 0.0014**	No
CYP3A4 (NC_000007.13)	g.99366316G>A	None	rs35599367	Hertz, 2016	No significant association between genotype and cardiotoxicity GG vs GA/AA OR = 0.65 (0.09 – 4.73), p = 0.67	No
CYP3A5 (NC_000007.13)	g.99270539C>T	None	rs776746	Hertz, 2016	No significant association between genotype and cardiotoxicity GG vs GA/AA OR = 2.17 (0.80 – 5.94), p = 0.13	No
CYP4B1 (NC_000001.10)	g.47265776A>G	None	rs837400	Lubieniecka, 2013	**SNP is associated with LVEF drop p = 0.021**	No
	g.47283505G>T	None	rs4646495		**SNP is associated with LVEF drop p = 0.041**	No
CYP4F11 (NC_000019.10)	g.15906807G>A	None	rs8112732	Lubieniecka, 2013	**SNP is associated with LVEF drop p = 0.0235**	No
	g.15912567A>G	None	rs12610962		**SNP is associated with LVEF drop p = 0.028**	No
	g.15913964A>G	None	rs2072270		**SNP is associated with LVEF drop p = 0.0285**	No
	g.15921833A>C	None	rs11086012		No significant associated between SNP and LVEF drop, p = 0.051	No
	g.15906196G>A	None	rs2108623	Visscher, 2013	No significant association between SNP and ACT OR = 0.77 (0.57 – 1.04), p = 0.084	No
CYP11B2 (NC_000008.10)	g.143999600A>G	None	rs1799998	Vivenza, 2013	Homozygote variant + heterozygote vs homozygote wt, p = 0.31 using Fischer's exact test	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
FMO2 (NC_000001.10)	37236730T>A	Asp36Gly	rs2020870	Visscher, 2012	**Protective variant OR = 0.14 (0.03 – 0.59), p = 4.2x10^{-4}**	No
FMO3 (NC_000001.10)	g.171080080G>A	Val257Met	rs1736557	Visscher, 2013	**SNP is significantly associated with ACT in combined cohort OR = 0.47 9 0.25 – 0.87), p = 0.011**	No
GPX3 (NC_000005.10)	150395291G>C	None	rs2233302	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 0.40 (0.22 – 0.73), p = 0.0011**	No
GSTA1 (NC_000006.11)	NR	NR	NR	Weiss, 2006	No significant associations between genotype and cardiac events	No (0.34)
GSTA2 (NC_000006.11)	52725690C>G	Ser112Thr	rs2180314	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 0.62 (0.45 – 0.86), p = 0.0036**	No
GSTM1 (NC_000001.10)	NA	NA	NA	Weiss, 2006	No significant associations between genotype and cardiac events	NR
	NA	NA	NA	Rajic, 2009	No significant correlation between SNP and late cardiac damage OR = 1.089 (0.312 – 3.798) p = 0.895	NR
	NA	NA	NA	Rossi, 2009	No significant association between genotypes and cardiac toxicity Null vs wt OR = 0.92 (0.62 – 1.36), p = 0.681	No
	NA	NA	NA	Vivenza, 2013	Present (+/- and +/+) vs Null (-/-), p = 0.147 using Fisher's exact test	Yes
GSTT1 (NC_000022.10)	NA	NA	NA	Weiss, 2006	No significant associations between genotype and cardiac events	NR
	NA	NA	NA	Rajic, 2009	No significant correlation between SNP and late cardiac damage OR = 6.222 (0.726 – 53.348) p = 0.063	NR
	NA	NA	NA	Vivenza, 2013	Present (+/- and +/+) vs Null (-/-), p = 0.687 using Fisher's exact test	Yes

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
GSTM3 (NC_000001.11)	110075064 A>G	None	rs12059276	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 0.36 (0.17 – 0.76), p = 0.0031**	No
GSTP1 (NC_000011.10)	g.67585218A>G	Ile105Val	rs1695	Volkan-Salanci, 2012	Presence of G allele is associated with deterioration in cardiac function.	No
				Rossi, 2009	**GG vs AG/AA OR = 1.83 (1.12 – 3.01), p = 0.015**	No
				Windsor, 2012	**Variants are associated with increased risk of cardiotoxicity OR = 4.8 (1.4 – 16.4), p = 0.011**	No
				Vivenza, 2013	Homozygote variant + heterozygote vs homozygote wt, p = 0.20 using Pearson's chi-square test	Yes
	g.67586108C>T	Ala114Val	rs1138272	Rossi, 2009	No significant association between genotypes and cardiac toxicity CT vs CC OR = 0.99 (0.52 – 1.87), p = 0.978	No
HAS3 (NC_000016.10)	g.69109674A>C	Ala93=	rs2232228	Wang, 2014	**Presence of allele A increase the risk of cardiomyopathy with the increase of cumulative anthracycline exposure.**	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
HFE (NC_000006.11)	g.26093141G>A	Cys282Tyr	rs1800562	Armenian, 2013	At risk genotype GA/AA OR = 0.30 (0.05 – 1.23) FDR-adjusted p = 0.28	No
				Lipshultz, 2013	**Heterozygote is associated with multiple elevations in cTnT concentration** **OR = 7.23 (1.78 – 29.4), p = 0.006**	NR
	g.26091179C>G	His63Asp	rs1799945	Armenian, 2013	**At risk genotype CG/GG** **OR = 2.58 (1.27 – 5.20)** **FDR-adjusted p = 0.03**	No
				Lipshultz, 2013	No association between heterozygote or homozygote with cardiac markers	NR
HNMT (NC_000002.11)	g.13876039C>A	None	rs17583889	Visscher, 2012	**Risk variant** **OR = 1.91 (1.21 – 3.02), p = 0.0057**	No
				Visscher, 2013	**SNP is significantly associated with ACT in combined cohort** **OR = 1.67 (1.15 – 2.41), p = 0.0073**	No
				Sachidanandam, 2012	Presence of homozygote variant (high risk) and heterozygote (intermediate risk)	NR
	g.138780932T>C	None	rs17645700	Visscher, 2013	**SNP is significantly associated with ACT in combined cohort** **OR = 0.56 (0.37 – 0.86), p = 0.0054**	No
HSD17B2 (NC_000016.10)	g.82028304T>G	None	rs16956248	Lubieniecka, 2013	**SNP is associated with LVEF drop** **p = 0.023**	No
	g.820829177T>A	None	rs13333826		**SNP is associated with LVEF drop** **p = 0.024**	No
	g.82054058C>T	None	rs7196087		**SNP is associated with LVEF drop** **p = 0.028**	No
	g.82079586C>T	None	rs2955159		**SNP is associated with LVEF drop** **p = 0.028**	No
	g.82072694G>A	None	rs2966245		**SNP is associated with LVEF drop** **p = 0.0305**	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
HSD17B4 (NC_000005.9)	g.119502865G>T	None	rs257970	Lubieniecka, 2013	**SNP is associated with LVEF drop p = 0.003**	No
	g.119502373G>A	None	rs2636968		**SNP is associated with LVEF drop p = 0.007**	No
NCF4 (NC_000022.10)	g.37256846GA>G	None	rs1883112	Visscher, 2012	No significant association, p = 0.76	No
				Hertz, 2016	No significant association between genotype and cardiotoxicity AA vs GA/GG OR = 1.44 (0.51 – 4.07), p = 0.49	No
				Reichwagen, 2015	No significant association between genotypes and cardiotoxicity GG/GA vs AA OR = 0.6 (0.3 – 1.4), p = 0.280	No
				Armenian, 2013	At risk genotype AA OR = 0.06 (0.54 – 2.13) FDR-adjusted p = 0.88	No
				Rossi, 2009	**AG/GG vs AA** **OR = 0.39 (0.24 – 0.64), p = 1.4×10^{-4}**	No
				Wojnowski, 2005	**AA genotype is associated with chronic cardiotoxicity (p = 0.013) and chronic or acute cardiotoxicity (p = 0.031)**	No
NOS3 (NC_000007.13)	g.150696111T>G	p.Asp298Glu	rs1799983	Krajinovic, 2015	Protective effect of the TT genotype on ejection fraction (p = 0.02	NR
NQO1 (NC_000016.9)	g.69745145C>T	Pro187Ser	rs1800566	Armenian, 2013	At risk genotype CT/CC OR = 0.88 (0.23 – 3.42) FDR-adjusted p = 0.88	No
				Blanco, 2008	CC vs TT OR = 1.26 (0.14 – 11.39), p = 0.84 CT vs TT OR = 0.65 (0.06 – 6.77), p = 0.72	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
NR1I2 (NC_000003.11)	g.119501039C>A	None	rs1523127	Hertz, 2016	No significant association between genotype and cardiotoxicity TT vs TG/GG OR = 0.76 (0.37 – 1.54), p = 0.44	No
	g.119530858G>A	None	rs3732357		No significant association between genotype and cardiotoxicity AA vs GA/GG OR = 1.44 (0.76 – 2.70), p = 0.26	No
	g.119499507T>C	None	rs1523130		No significant association between genotype and cardiotoxicity GG vs GA/AA OR = 1.11 (0.57 – 2.17), p = 0.76	No
POR (NC_000007.13)	g.75589903A>G	None	rs2868177	Lubieniecka, 2013	**SNP is associated with LVEF drop p = 0.0025**	No
	g.75606109G>A	None	rs13240755		**SNP is associated with LVEF drop p = 0.0035**	No
	g.75607608C>T	None	rs4732513		**SNP is associated with LVEF drop p = 0.004**	No
	g.75601169G>A	None	rs6953065		SNP is associated with LVEF drop p = 0.0445	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
RAC2 (NC_000022.11)	g.37236730T>A	None	rs13058338	Armenian, 2013	**At risk genotype TA/AA OR = 2.61 (1.46 – 4.69) FDR-adjusted p = 0.02**	No
				Wojnowski, 2005	**TA/AA genotypes are associated with acute cardiotoxicity (p = 0.005) and chronic or acute cardiotoxicity (p = 0.04)**	No (0.95)
				Rossi, 2009	**AA vs AT/TT OR = 1.84 (1.10 – 3.10), p = 0.019**	No
				Reichwagen, 2015	No significant association between genotypes and cardiotoxicity GG vs GT/TT OR = 0.7 (0.2 – 2.5), p = 0.632	No
				Hertz, 2016	No significant association between genotype and cardiotoxicity TT vs TA/AA OR = 1.8 (0.9 – 3.7), p = 0.077	No
				Visscher, 2012	No significant association p = 0.13	
RARG (NC_000012.11)	g.53605545G>A	Ser4271Leu	rs2229774	Aminkeng, 2015	All population OR 4.7 (2.7-8.3), p = 4.3 x 10^{-11}	No
SERPINA6 (NC_000014.8)	93848406G>A	None	rs10144771	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 1.72 (1.19 – 2.50), p = 0.0042**	No
SLC10A2 (NC_000013.10)	g.103714254G>A	None	rs9514091	Visscher, 2012	**Protective variant OR = 0.43 (0.23 – 0.78), p = 0.0033**	No
				Visscher, 2013	**SNP is significantly associated with ACT in combined cohort OR = 0.57 (0.38 – 0.87)**	No
	g.103723722G>A	None	rs7319981	Visscher, 2013	**SNP is significantly associated with ACT in combined cohort OR = 0.66 (0.47 – 0.93), p = 0.016**	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
SLC13A3 (NC_000020.10)	44693250A>G	None	rs2425886	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 1.75 (1.20 – 2.57), p = 0.0037**	No
SLC15A1 (NC_000013.10)	98153602G>C	None	rs8001466	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 2.02 (1.25 – 3.26), p = 0.0042**	No
SLC22A2 (NC_000006.11)	g.160670282A>C	Ser270Ala	rs316019	Visscher, 2013	No significant association between SNP and ACT OR = 0.75 9 0.46 – 1.21), p = 0.23	No
SLC22A7 (NC_000006.12)	43389166A>G	None	rs4149178	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 0.45 (0.26 – 0.75) p = 0.0013**	No
SLC22A16 (NC_000006.11)	g.110778128T>C	His49Arg	rs714368	Hertz, 2016	No significant association between genotype and cardiotoxicity AA vs GA/GG OR = 0.78 (0.31 – 1.96), p = 0.60	No
	g.110777962A>G	Asn104	rs6907567		No significant association between genotype and cardiotoxicity AA vs GA/GG OR = 0.74 (0.30 – 1.83), p = 0.51	No
	g.110763875A>G	Val252Ala	rs723685		No significant association between genotype and cardiotoxicity TT vs TC/CC OR = 0.44 (0.10 – 2.03), p = 0.29	No
	g.110760008A>G	Met377Thr	rs12210538		No significant association between genotype and cardiotoxicity AA vs GA/GG OR = 1.30 (0.63 – 2.71), p = 0.48	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
SLC22A17 (NC_000014.8)	22884409G>A	None	rs4982753	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 0.50 (0.33 – 0.75), p = 4.4x10^{-4}**	No
	23814995A>T	None	rs11625724		**SNP is significantly associated with ACT in combined cohort OR = 1.63 (0.0020)**	No
	23812237G>C	None	rs12882406		**SNP is significantly associated with ACT after adjusting the effect of rs4982753 OR = 1.52, p = 0.042**	No
	23816998T>C	None	rs12896494		**SNP is significantly associated with ACT after adjusting the effect of rs4982753 OR = 0.65, p = 0.031**	No
SLC28A1 (NC_000015.10)	g.84909044T>C	None	rs2305364	Visscher, 2013	**SNP is significantly associated with ACT in combined cohort OR = 1.60 (1.18 – 2.17), p = 0.0020**	No
	g.84904404A>C	None	rs2290271	Visscher, 2013	**SNP is significantly associated with ACT in combined cohort OR = 0.66 (0.48 – 0.91)**	No
SLC28A3 (NC_000009.11)	g.86900926G>A	Leu461=	rs7853758	Visscher, 2012	**Protective variant OR = 0.31 (0.16 – 0.60), p = 1.0x10^{-4}**	No
				Visscher, 2013	**SNP is significantly associated with ACT in combined cohort OR = 0.36 (0.22 – 0.60), p = 1.6 x 10^{-5}**	No
				Reichwagen, 2015	No significant association between genotypes and cardiotoxicity GG vs GA/AA OR = 1.4 (0.7 – 3.0), p = 0.393	No
				Hertz, 2016	No significant association between genotype and cardiotoxicity GG vs GA/AA OR = 0.55 (0.16 – 1.91), p = 0.35	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
SLC28A3 (NC_000009.11)	g.86946417A>C	None	rs4877847	Visscher, 2012	**Protective variant OR = 0.60 (0.41 – 0.89), p = 0.0092**	No
				Visscher, 2013	**SNP is significantly associated with ACT in combined cohort OR = 0.73 (0.54 – 0.98), p = 0.037**	
	g.86909550G>A	None	rs885004	Visscher, 2013	**SNP is significantly associated with ACT in combined cohort OR = 0.34 (0.20 – 0.60), p = $3.0x10^{-5}$**	No
SLCO4C1 (NC_000005.10)	g.101633540G>A	None	rs2600834	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 1.80 (1.26 – 2.57), p = 0.0011**	No
SLCO6A1 (NC_000005.10)	g.101779552A>G	None	rs12658397	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 1.68 (1.20 – 2.35), p = 0.0025**	No
SOD2 (NC_000006.11)	g.159981080G>A	None	rs7754103	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 0.32 (0.15 – 0.72), p = 0.0024**	No
	g.160113872A>G	Val16Ala	rs4880	Rajic, 2009	No significant correlation between SNP and late cardiac damage OR = 0.917 (0.328 – 2.562) p = 0.907	NR
				Armenian, 2013	At risk genotype GA/AA OR = 1.79 (0.90 – 3.38) FDR-adjusted p = 0.28	No
SPG7 (NC_000016.10)	g.89549357T>G	None	rs2019604	Visscher, 2012	**Protective variant OR = 0.39 (0.20 – 0.76), p = 0.0021**	No
				Visscher, 2013	**SNP is significantly associated with ACT in combined cohort OR = 0.56 (0.35 – 0.90), p = 0.012**	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
SULT2B1 (NC_000019.10)	g.48588977C>A	None	rs10426377	Visscher, 2013	**SNP is significantly associated with ACT in combined cohort OR =0.56 (0.38 – 0.81), p = 0.0015**	No
	48589173A>G		rs10426628	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 1.92 (1.34 – 2.73), p = 3.2x10^{-4}**	No
TOP2B (NC_000003.11	g.24929802C>A	None	rs10865801	Hertz, 2016	No significant association between genotype and cardiotoxicity CC vs CT/TT OR = 1.32 (0.67 – 2.61), p = 0.43	No
TP53 (NC_000017.10)	g.757972G>C	Pro72Arg	rs1042522	Rossi, 2009	No significant association between genotypes and cardiac toxicity CG/CC vs GG OR = 1.20 (0.81 – 1.78), p = 0.357	No
				Vivenza, 2013	Homozygote variant + heterozygote vs homozygote wt, p = 0.33 using Pearson's chi-square test	Yes
UGT1A6 (NC_000002.11)	g.234601669T>G	Ser7Ala	rs6759892	Visscher, 2012	**Risk variant OR = 1.77 (1.20 – 2.61), p = 0.0038**	No
				Visscher, 2013	**SNP is significantly associated with ACT in combined cohort OR = 1.43 (1.05 – 1.94), p = 0.022**	No
	g.234602277G>T	Val209=	rs17863783	Visscher, 2013	**SNP is significantly associated with ACT in combined cohort OR = 4.30 (1.97 – 9.36), p = 2.4x10^{-4}**	No
				Hertz, 2016	Only homozygote wt were presence	No
	g.234593117G>T	None	rs4261716	Visscher, 2013	**SNP is significantly associated with ACT in combined cohort OR = 1.44 (1.06 – 1.95), p = 0.018**	
DSG2 (NC_000018.10)	g.31521193T>G	Val158Gly	rs191143292	Wasielewski, 2014	No causal relation between mutation and cardiomyopathy	No

Supplementary Table 3: Genetic polymorphisms, association with ACT and HWE status *(continued)*

Gene	Nucleotide change	Amino acid change	SNP rsID	Study (Name, Year)	Association[2]	Deviation from HWE (exact test mid p-value)[1]
JUP (NC_000017.10)	g.39913771C>T	Val648Ile	rs143043662	Wasielewski, 2014	No causal relation between mutation and cardiomyopathy	No
VCL (NC_000010.10)	g.75873961C>T	Ala990Val	rs150595117	Wasielewski, 2014	No causal relation between mutation and cardiomyopathy	No
MYH7 (NC_000014.8)	NR	Asp(545, 955)Asn	NR	Wasielewski, 2014	No causal relation between mutation and cardiomyopathy	No
	NR	Tyr1375	NR		No causal relation between mutation and cardiomyopathy	No
TTN (NC_000002.11)	NR	Ser31346Leu	NR	Wasielewski, 2014	No causal relation between mutation and cardiomyopathy	No
	g.179602871T>C	Tyr4453Cys	rs371552518		No causal relation between mutation and cardiomyopathy	No
	NR	Glu10855dup	NR		No causal relation between mutation and cardiomyopathy	No
DSP (NC_000006.11)	NR	Arg1425Lys	NR	Wasielewski, 2014	No causal relation between mutation and cardiomyopathy	No
PKP2 (NC_000012.11)	g.32994058A>C	Ile531Ser	rs147240502	Wasielewski, 2014	No causal relation between mutation and cardiomyopathy	No
XDH (NC_000002.11)	g.31460174G>A	Val279	rs4407290	Visscher, 2015	**SNP is significantly associated with ACT in combined cohort OR = 0.18 (0.04 – 0.79), p = 0.0039**	No
	g.31460632A>G	None	rs2236168		**SNP is significantly associated with ACT in combined cohort OR = 1.68 (1.21 – 2.34), p = 0.0017**	No

FDR, false discovery rate; HWE, Hardy-Weinberg Equilibrium; NR, not reported
[1]In studies that had not reported for HWE testing we used the BGI Cognitive Genomics (available at https://www.cog-genomics.org/software/stats to test for the deviation. A mid p-value of less than 0.05 is considered statistical deviation from HWE.
[2]Significant associations are in bold (p<0.05)

4.2.4 Letter to editor

COMMENT & RESPONSE

Potential of Oncocardiology

To the Editor Yeh and Chang[1] described the new discipline of oncocardiology as well as the opportunities for research and practice of cardiology and oncology. However, the authors failed to highlight the potential of personalized medicine. The significant strides made since the emergence of human genome sequencing has led to a fundamental shift in cancer biology and treatment.

Since the early work by Wojnowski et al[2] in 2005, research on genetic association with anthracycline-induced cardiotoxicity (ACT) has grown swiftly, with wide coverage of genes and single-nucleotide polymorphisms. Some of these single-nucleotide polymorphisms have since been found to be associated with ACT, including the genes *ABCC2* (rs8187710), *CYBA* (rs4673), and *RAC2* (rs13058338).[3] These studies demonstrate that pharmacogenetic testing has the potential to improve the prediction of anthracycline cardiotoxicity and distinguish between individuals at higher risk for ACT from those with lower risk for ACT. Thus, high-risk individuals can start preventive pharmacological management and closer cardiac function monitoring. We believe that pharmacogenetic testing will be a part of the future for oncocardiology.

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Published Online: March 8, 2017. doi:10.1001/jamacardio.2017.0116

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

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To the Editor We read the recent review by Yeh and Chang[1] with interest and congratulate them for reinforcing the role of cardio-oncology (oncocardiology), an emergent discipline in the cardiology field. This article is timely in the context of the proliferation of new cancer therapies and the increase in the number of cancer survivors. Cardiovascular care for cancer patients has become challenging because they live longer and are at greater risk of cardiovascular events. Yeh and Chang[1] pointed out the well-known toxic effects of anthracyclines, but they also underlined the cardiovascular toxicity resulting from the anti-HER2 and anti-VEGF antibodies, inhibitors of tyrosine kinases and of other intracellular signals. This description is very important because it aims at educating the cardiology community about the growing issue of cardiotoxicity in cancer patients.[1]

However, the authors did not address the potential cardiovascular effects of the emergent immune checkpoint-modulating immunotherapy. To our knowledge, this therapy, including anti-PD1 and anti-CTLA-4 antibodies, represents the most promising therapeutic approach against cancer. These treatments have revolutionized cancer therapy, but their application is also associated with a spectrum of immune-related adverse events, including heart failure due to dysimmune acute myocarditis or dilated cardiomyopathy with fibrosis. Although the rate of left ventricular dysfunction was low in clinical trials, safety signals were issued, and selected cases of cardiotoxicity were recently published.[2] A 2016 article[3] reported on patients with cardiotoxcity observed during immune checkpoint-blocking therapy in 6 large clinical American and European cancer centers. These adverse events were sometimes fatal and occurred mostly in patients who had previous cardiovascular diseases or risk factors. Most of these therapies act by blocking PD-1 or CTLA-4 receptors on T cells and then stimulating their antitumor effect. The mechanisms of their cardiotoxicity have not been fully elucidated, but many years before their use, PD-1 deficiency was described to predispose for spontaneous myocarditis and cardiomyopathy in mice. Severe myocarditis also occurred in a model of CTLA-4-deficient mice.[4] Interestingly, the epidemiologic data presented in a white population showed evidence for an association of the *CTLA-4* +49A>G polymorphism with dilated cardiomyopathy.[5] The authors of this work suggested that upregulated immune reactions in the myocardium induced by CTLA-4 modulation might contribute to inflammatory responses and favor reparative fibrosis.[5] This example of cardiotoxicity induced by the new immunotherapies strengthens the importance of cardio-oncology for patient care and development of cancer and cardiovascular basic research.

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5 Integrated Discussion

5.1 Main findings of the research

Studies reporting incidence of anthracycline-induced cardiotoxicity (ACT) are increasing despite 5 decades of experience ACT use. It seems that ACT affects cancer survivors worldwide at comparable incidence rate. Reported risk factors for ACT appeared to be similar but differ in sensitivity across the globe. Findings suggest that Malaysians were at risk for ACT at younger age and lower cumulative dose of anthracycline. Previous studies reported patients' age 65-year or more were at risk for ACT while our study found that the age threshold was 50-year. Studies in United States suggested cumulative dose of more than 350mg/m^2 as risk factor[33, 34] and 300mg/m^2 by study in Japan[35] while our study population were at risk at a cumulative dose of 250mg/m^2.

A demographic and clinical characteristics-based multivariable model was developed with 1356 patients and internally validated with 678 patients. The developed and validated four-factor model showed acceptable performance and stability. The four covariates are age more than 50 year-old, haematology malignancies, concomitant use of cardio-protective agent, and concomitant administration of cyclophosphamide and trastuzumab. It discrimination power was evidence by its AUROC of 0.75. At the ACT incidence of study population of 4.6%, estimated overall rate of correct classification was 70%, with 70% of no cardiac event group correctly classified (specificity) and 66% of the cardiac event group correctly classified (sensitivity). Pilot study on its utility in clinical setting and qualitative analysis on its content and usability showed it is likely to be used in ACT prediction risk following anthracycline use.

Parallel to the advancement in genotyping techniques, researchers had embarked on genetic association studies in search for more sensitive predictors for antineoplastic-related cardiotoxicity and ACT. Single nucleotide polymorphism (SNP) may affect cardiotoxicity risk positively or negatively. ABCC2 rs8187710, CYBA rs4673 and RAC2 rs13058338 were the three SNPs contributes to significantly increase of risk for ACT. The findings seem promising although is premature to be conclusive.

5.2 Implications of findings

Although ACT has been recognised for almost half a century, the incidence and risk remain a significant concern among clinicians. Studies on risk factors for ACT in different populations are warranted as sensitivity towards risk factors may differ.

The use of model in clinical practice which personalised risk assessment for ACT following anthracycline therapy could offer several benefits to cancer patients. Identifying cancer patients at high risk for ACT may inform decisions on the intensity of the treatment. More precise risk stratification may also identify patients who may benefits from more frequent cardiac function screening for earlier detection of ACT. Screening strategies such as biomarkers or novel technologies aimed at identifying cancer patients at high risk may be employed more effectively too.

Our systematic reviews showed the results from published genetic association studies on ACT are promising and should be developed. It also provides directions for future studies in exploring the role of genetics biomarker for ACT prediction.

Based on our findings, patients undergoing chemotherapy especially anthracycline-based regimens should have proper clinical evaluation and assessment of cardiovascular risk factors. Regular cardiac function monitoring is recommended for anthracycline recipients with ACT risk factors especially the four risk factors in the model at baseline and up to at least four years after end of anthracycline therapy.

With these preventive measures in place for patients at risk for ACT, the vitally important expected outcome is reduction in both short and long term cardiac toxicity event. The latter is particularly unfavourable in childhood cancer survivors. Besides, optimal dose of anthracycline can be administered with confident which will improve success rate of chemotherapy in cancer treatment. These include survival, disease control and/or quality of life, depending on the indication of the therapy.

The readily available covariates of the model will enhance the usability of the model and diminish the screening cost. Given the number to screen of nineteen for this model and the annual cost of heart failure management of Int$ 908 to Int$ 40, 971 per patient[86], this model offers potential to reduce healthcare cost. In cases where anthracycline use is inevitable, frequent cardiac monitoring based on the risk stratified by the model enable early detection of declined heart function.

5.3 Limitations of the research

Other than the limitations discussed in each section of the thesis, we also encountered other challenges while conducting the project.

The main challenge we encountered was the lack of prospective data. While the proposal targeted to include 240 patients from two cancer referral centres, only forty-two patients consented among 102 patients available after one year of data collection. The total number of patients was less than estimation from consensus of previous years. This unexpected recruitment necessitated us to use retrospective data instead. As such, we used the limited prospective data to test the utility of our prediction model.

Besides, we also faced roadblocks in the attempts to validate the prediction model using external data that we could not overcome at this point of time. We identified two possible databases that potentially used for external validation, The Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute database and Clalit Health Services database. However, to access to both databases require a significant monetary contribution which we could not provide since this project was not funded. Thus, we conducted a qualitative survey to gather expert opinions on the content and usability of the prediction model.

Another shortcoming we dealt with was the lack of funding to incorporate genetic markers in prediction model. While multiple attempts to obtain funding from various organisations, these were all rejected. Due to time constraint we dropped the plan and kept the blood samples for future studies. Lack of consented patients was the other reason for the decision.

We also experienced issue in getting additional data from authors for systematic reviews. Among eight authors contacted, only one author provided us with additional data for meta-analysis. This may have led to less comprehensive meta-analyses.

5.4 Recommendation for future research

Studies to determine incidence and risk factors for ACT in population yet to be reported should be encourage as susceptibility may differ. This will impart in the effort to pave way for personalised medicine.

Based on the potential of the prediction model, it warrants external validation and utility evaluation in clinical settings. Besides, its development attempts are encouraged, especially studies designed to improve its sensitivity using prospective studies with more complete cardiac function results and risk factors cardiac disease. With improved evidence on genetic role in ACT, genetic biomarkers such as SNPs and gene-dose effect should be incorporated in the prediction model.

Future studies to determine genetic role in ACT should employ more advanced approaches such as human genome-wide association studies or whole exome or whole genome sequencing should be undertaken with proper population stratification. Future genetic studies should strategize to determine the gene-dose effect in ACT.

Research on preventive strategies for patients at risk for ACT should be encouraged such as developing anthracycline with similar efficacy but less cardiac toxic and preventive agents.

6 Conclusion

Systematic review and meta-analysis of the incidence of antineoplastic-related cardiovascular toxicities in Asia showed a pooled incidence for ACT of 3.2%. However, a retrospective study in Malaysia which included a multi-ethnic population in Asia which found that incidence of ACT was higher at 4.6%.

To predict the risk for ACT among anthracycline recipients, a prediction model which was developed based on demographic and clinical data of the 2034 anthracycline recipients resulting in a 4-factors prediction model. The factors are age more than 50 years, presence of haematology malignancy, concomitant use of cardioprotective agents and concomitant use of cyclophosphamide and trastuzumab. In a small prospective study, the developed and validated prediction model showed a potential to stratify individual at risk for ACT in clinical settings. Qualitative study on the perspective of healthcare professionals in the related-clinical area on the developed prediction model showed that the scoring system to predict ACT risk is lacking and the model was welcomed once it is validated with supporting data.

Systematic reviews and meta-analyses on genetic biomarkers associated to antineoplastic-related cardiovascular toxicities showed that HER2 rs1136201 is potentially predictor for trastuzumab-related decreased left ventricular ejection fraction while ABCC2 rs8187710, CYBA rs4673 and RAC2 rs13058338 were risk variants for ACT.

These imply that clinical practice is in need for a tool to predict and thus prevent ACT because number of ACT cases is expected to increase with the improvement in cancer survival rates. An ideal tool would require a combination of demographic data, clinical data and genotypes effects.

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Appendices

Appendix 1: Malaysia Medical Research and Ethics Committee (NMRR-15-612-24156)

(Medical Research & Ethics Committee)
KEMENTERIAN KESIHATAN MALAYSIA
d/a Institut Pengurusan Kesihatan
Jalan Rumah Sakit, Bangsar
59000 Kuala Lumpur

[redacted]

Ruj. Kami : (12)KKM/NIHSEC/ P15-706
Tarikh : 31hb Julai 2015

Leong Siew Lian
Jeffrey Cheah School of Medicine and Health Sciences,Monash University - Sunway Campus

Dr.Shaun Lee Wen Huey
MONASH UNIVERSITY - SUNWAY CAMPUS

Tuan/ Puan,

NMRR-15-612-24156 (IIR)
Anthracycline-induced Cardiotoxicity: Polymorphisms Role and Cost

Dengan hormatnya perkara di atas adalah dirujuk.

2. Bersama dengan surat ini dilampirkan surat kelulusan saintifik dan etika bagi projek ini. Segala rekod dan data subjek adalah SULIT dan hanya digunakan untuk tujuan kajian dan semua isu serta prosedur mengenai *data confidentiality* mesti dipatuhi. Kebenaran daripada Pegawai Kesihatan Daerah/Pengarah Hospital dan Ketua-Ketua Jabatan atau pegawai yang bertanggung jawab disetiap lokasi kajian di mana kajian akan dijalankan mesti diperolehi sebelum kajian dijalankan. Dato'/ Tuan/ Puan perlu akur dan mematuhi keputusan tersebut.

3. Penyelidik bersama yang terlibat dalam kajian ini ialah :
- Dato' Dr Chang Kian Meng
- Kong Su Shan

4. Adalah dimaklumkan bahawa kelulusan ini adalah sah sehingga **31 Julai 2016**. Tuan/Puan perlu menghantar dokumen-dokumen seperti berikut selepas mendapat kelulusan etika. Borang-borang berkaitan boleh dimuat turun daripada laman web Jawatakuasa Etika & Penyelidikan Perubatan (JEPP) (http://www.nih.gov.my/mrec).

i. ***Continuing Review Form*** selewat-lewatnya 2 bulan sebelum tamat tempoh kelulusan ini bagi memperbaharui kelulusan etika.
ii. ***Study Final Report*** pada penghujung kajian.
iii. Mendapat kelulusan etika sekiranya terdapat pindaan ke atas sebarang dokumen kajian/ lokasi kajian/ penyelidik.
iv. Kajian berkenaan intervensi klinikal sahaja: Laporan mengenai ***all Serious Adverse Events (SAEs), Suspected Unexpected Serious Adverse Reaction (SUSARs)*** dan ***Protocol Deviation/Violation*** di lokasi kajian yang diluluskan oleh JEPP jika berkenaan. *SAE* perlu dilaporkan dalam tempoh 15 hari kalender dari *awareness of event* oleh penyelidik. Laporan awal *SUSAR*

perlu dikemukakan seawal mungkin tapi tidak melewati 7 hari calendar dari *awareness of event* oleh penyelidik, disusuli dengan laporan lengkap dalam tempoh tambahan 8 hari kalender.

4. Bilangan subjek/ pesakit/ responden yang disasarkan untuk menyertai kajian ini di Malaysia adalah **480 orang**.

5. Sila ambil maklum bahawa sebarang urusan surat-menyurat berkaitan dengan penyelidikan ini haruslah dinyatakan nombor rujukan surat ini untuk melicinkan urusan yang berkaitan.

Sekian terima kasih.

BERKHIDMAT UNTUK NEGARA

Saya yang menurut perintah,

[redacted]

Timbalan Pengerusi
Jawatankuasa Etika & Penyelidikan Perubatan
Kementerian Kesihatan Malaysia

Appendix 2: Monash University Human Research Ethics Committee (CF15/3029 – 2015001271)

MONASH University

Monash University Human Research Ethics Committee (MUHREC)
Research Office

Human Ethics Certificate of Approval

This is to certify that the project below was considered by the Chair of the Monash University Human Research Ethics Committee. The Chair was satisfied that the proposal meets the requirements of the *National Statement on Ethical Conduct in Human Research* and has granted approval.

Project Number:	**CF15/3029 - 2015001271**
Project Title:	**Anthracycline-induced Cardiotoxicity: Polymorphisms Role and Cost (AIC-RC)**
Chief Investigator:	**Dr Wen Huey Shaun Lee**
Approved:	**From: 17 August 2015 To: 17 August 2020**

Terms of approval - ***Failure to comply with the terms below is in breach of your approval and the Australian Code for the Responsible Conduct of Research.***

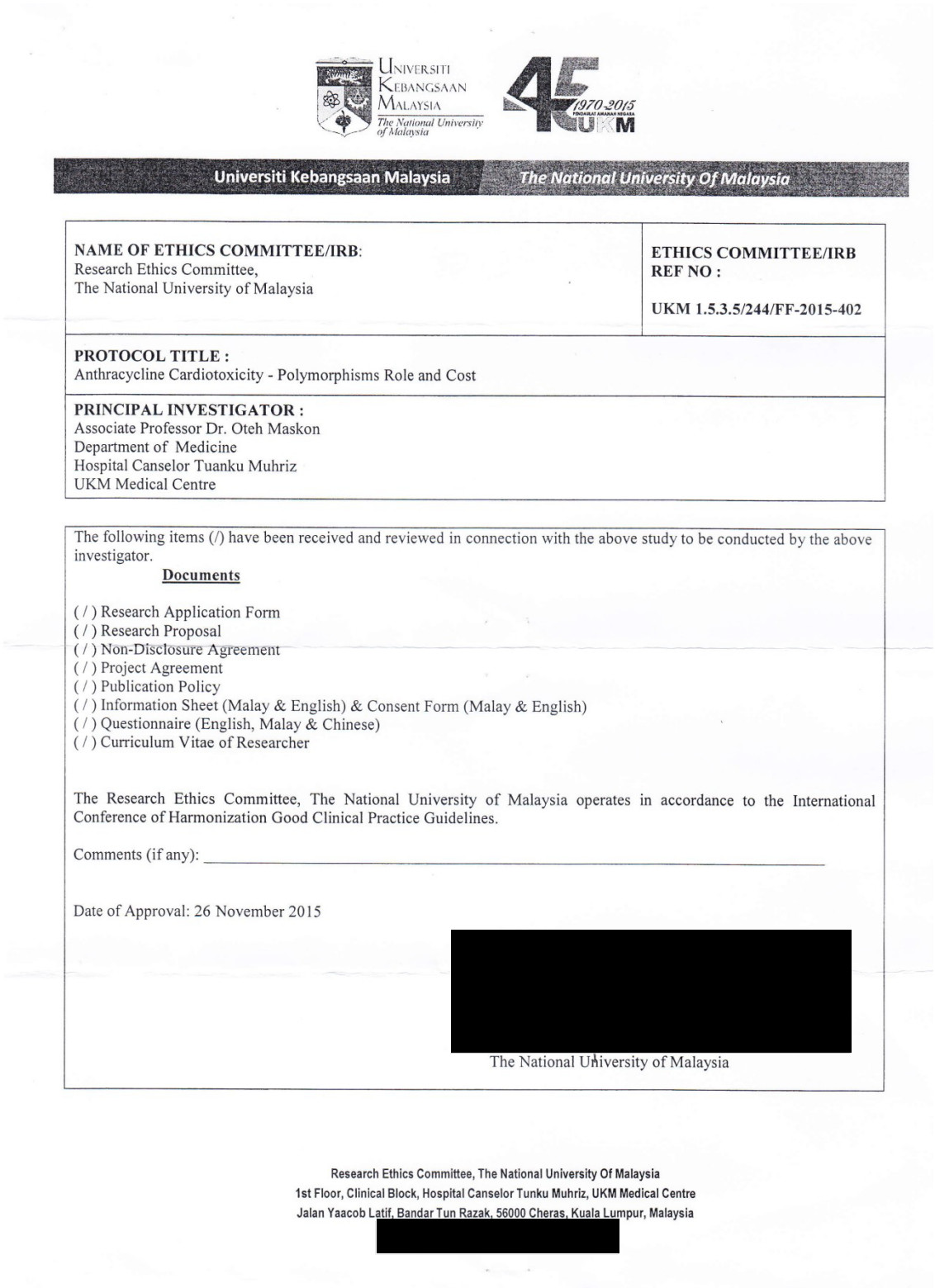
1. Approval is only valid whilst you hold a position at Monash University and approval at the primary HREC is current.
2. **Future correspondence:** Please quote the project number and project title above in any further correspondence.
3. **Final report:** A Final Report should be provided at the conclusion of the project. MUHREC should be notified if the project is discontinued before the expected date of completion.
4. **Retention and storage of data:** The Chief Investigator is responsible for the storage and retention of original data pertaining to a project for a minimum period of five years.

[redacted]
Professor Nip Thomson
Chair, MUHREC

cc: Mrs Siew Lian Leong, Dr Chang Kian Meng, Mrs Su Shan Kong

Human Ethics Office
Monash University
Room 111, Chancellery Building E
24 Sports Walk, Clayton Campus, Wellington Rd, Clayton VIC 3800, Australia
[redacted]

Appendix 3: UKM Medical Centre Secretariat for Medical Research and Innovation (FF-2015-402)



UNIVERSITI KEBANGSAAN MALAYSIA
The National University of Malaysia

45 (1970-2015) UKM

Universiti Kebangsaan Malaysia | The National University Of Malaysia

NAME OF ETHICS COMMITTEE/IRB: Research Ethics Committee, The National University of Malaysia	ETHICS COMMITTEE/IRB REF NO : UKM 1.5.3.5/244/FF-2015-402
PROTOCOL TITLE : Anthracycline Cardiotoxicity - Polymorphisms Role and Cost	
PRINCIPAL INVESTIGATOR : Associate Professor Dr. Oteh Maskon Department of Medicine Hospital Canselor Tuanku Muhriz UKM Medical Centre	

The following items (/) have been received and reviewed in connection with the above study to be conducted by the above investigator.

Documents

(/) Research Application Form
(/) Research Proposal
(/) Non-Disclosure Agreement
(/) Project Agreement
(/) Publication Policy
(/) Information Sheet (Malay & English) & Consent Form (Malay & English)
(/) Questionnaire (English, Malay & Chinese)
(/) Curriculum Vitae of Researcher

The Research Ethics Committee, The National University of Malaysia operates in accordance to the International Conference of Harmonization Good Clinical Practice Guidelines.

Comments (if any): _____

Date of Approval: 26 November 2015

[redacted]

The National University of Malaysia

Research Ethics Committee, The National University Of Malaysia
1st Floor, Clinical Block, Hospital Canselor Tunku Muhriz, UKM Medical Centre
Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia

[redacted]

Appendix 4: UMMC Medical Research Ethics Committee (2016930-4304)

UNIVERSITY OF MALAYA MEDICAL CENTRE
MEDICAL ETHICS COMMITTEE
UNIVERSITY MALAYA MEDICAL CENTER
ADDRESS : LEMBAH PANTAI, 59100 KUALA LUMPUR, MALAYSIA
TELEPHONE : 03-79493209/2251 FAXIMILE : 03-79492030

NAME OF ETHICS COMMITTEE/IRB Medical Ethics Committee, University Malaya Medical Center	**MECID.NO**: 2016930-4304
ADDRESS : LEMBAH PANTAI, 59100 KUALA LUMPUR	
PROTOCOL.NO(if applicable) :	
TITLE: Anthracycline Cardiotoxicity - Risk Stratification Model	
PRINCIPAL INVESTIGATOR : MS KONG ZHEN YING	**SPONSOR** -

The following item [✓] have been received and reviewed in connection with the above study to conducted by the above investigator.

[✓]	Application to Conduct Research Project(form)	Ver.No :	Ver.Date : 06-10-2016
[✓]	Study Protocol	Ver.No : 1	Ver.Date : 06-10-2016
[ ]	Patient Information Sheet	Ver.No :	Ver.Date :
[ ]	Consent Form	Ver.No :	Ver.Date :
[ ]	Questionnaire	Ver.No :	Ver.Date :
[✓]	Investigator's CV / GCP (MS KONG ZHEN YING,Leong Siew Lian, Shaun Lee Wen Huey,)	Ver.No :	Ver.Date :
[ ]	Insurance certificate	Ver.No :	Ver.Date :
[✓]	Other Attachments		
	1) data collection form	Ver.No : -	Ver.Date :

and the decision is [✓]

[✓] Approved

[ ] Rejected(reasons specified below or in accompanying letter)

Comments:

A retrospective study involving medical records review of patients who have received anthracyclines.

Investigator are required to:

1) *follow instructions, guidelines and requirements of the Medical Ethics Committee.*
2) *report any protocol deviations/violations to Medical Ethics Committee.*
3) *provide annual and closure report to the Medical Ethics Committee.*
4) *comply with International Conference on Harmonization – Guidelines for Good Clinical Practice (ICH-GCP) and Declaration of Helsinki.*
5) *obtain a permission from the Director of UMMC to start research that involves recruitment of UMMC patient.*
6) *ensure that if the research is sponsored, the usage of consumable items and laboratory tests from UMMC services are not charged in the patient's hospital bills but are borne by research grant.*
7) *note that he/she can appeal to the Chairman of MEC for studies that are rejected.*
8) *note that Medical Ethics Committee may audit the approved study.*
9) *ensure that the study does not take precedence over the safety of subjects.*

Date of approval : 16-10-2016

This is a computer generated letter. No signature required.

Appendix 5: Data collection form

Anthracycline-Induced Cardiotoxicity Study		
Patient ID : __ __ __ __	Patient Initials __ __ __	Study Site: __

Demographics

1. Date of birth	__ __/__ __/__ __ __ __ (DD/MM/YYYY)	
2. Gender	☐1 Male	☐2 Female
3. Race		
a. Malay	☐1 Yes	☐0 No
b. Chinese	☐1 Yes	☐0 No
c. Indian	☐1 Yes	☐0 No
d. Sabah indigenous (Please specify):_____	☐1 Yes	☐0 No
e. Sarawak indigenous (Please specify):_____	☐1 Yes	☐0 No
f. Peninsular indigenous (Please specify):_____	☐1 Yes	☐0 No
g. Others (Please specify):_____	☐1 Yes	☐0 No
4. Birth (≤ 12 years old only)	☐88 NA	
a. Preterm (< 37 weeks)	☐1 Yes	☐0 No
b. Term (≥ 37 weeks)	☐1 Yes	☐0 No
5. Last follow-up date	__ __/__ __/__ __ __ __ (DD/MM/YYYY)	
6. Duration of follow-up from 1st dose of anthracycline	_____ days	
7. Duration of follow-up from last dose of anthracycline	_____ days	

Radiotherapy	☐1 Yes	☐0 No

Medical History

1. Cardiovascular Disease	☐1 Yes	☐0 No	☐88 U/S
If **Yes**, which of the following:			
a. Hypertension	☐1 Yes	☐0 No	☐88 U/S
b. High cholesterol	☐1 Yes	☐0 No	☐88 U/S
c. Coronary artery disease (heart attack, chest pain)	☐1 Yes	☐0 No	☐88 U/S
d. Stroke	☐1 Yes	☐0 No	☐88 U/S
e. Arrhythmia	☐1 Yes	☐0 No	☐88 U/S
2. Endocrine or metabolic disease	☐1 Yes	☐0 No	☐88 U/S
If **Yes**, which of the following:			
a. Diabetes	☐1 Yes	☐0 No	☐88 U/S
b. Hypothyroid disease	☐1 Yes	☐0 No	☐88 U/S
c. Hyperthyroid disease	☐1 Yes	☐0 No	☐88 U/S
d. Other endocrine or metabolic disease	☐1 Yes	☐0 No	☐88 U/S
3. Gastrointestinal Disease	☐1 Yes	☐0 No	☐88 U/S
If **Yes**, which of the following:			
a. Irritable bowel syndrome	☐1 Yes	☐0 No	☐88 U/S
b. Dierticulitis	☐1 Yes	☐0 No	☐88 U/S
c. Ulcerative Colitis / Crohn's Disease	☐1 Yes	☐0 No	☐88 U/S
d. Other gastrointestinal disease	☐1 Yes	☐0 No	☐88 U/S
4. Genitourinary Disorders	☐1 Yes	☐0 No	☐88 U/S
If **Yes**, which of the following:			
a. Bladder cancer	☐1 Yes	☐0 No	☐88 U/S
b. Urinary tract infection	☐1 Yes	☐0 No	☐88 U/S
c. Pelvic inflammatory disease	☐1 Yes	☐0 No	☐88 U/S
d. Endometriosis	☐1 Yes	☐0 No	☐88 U/S
e. Vulvodynia	☐1 Yes	☐0 No	☐88 U/S
f. Gynaecologic cancer	☐1 Yes	☐0 No	☐88 U/S
g. Vulvovestibulitis	☐1 Yes	☐0 No	☐88 U/S
h. Acute / chronic bacterial prostatitis	☐1 Yes	☐0 No	☐88 U/S
i. Epididymitis	☐1 Yes	☐0 No	☐88 U/S
j. Benign prostate hyperplasia	☐1 Yes	☐0 No	☐88 U/S
5. Hematopoietic, lymphatic, or infectious disease	☐1 Yes	☐0 No	☐88 U/S
If **Yes**, which of the following:			
a. Epstein-Barr virus / Chronic Fatigue Syndrome	☐1 Yes	☐0 No	☐88 U/S
b. Tuberculosis	☐1 Yes	☐0 No	☐88 U/S
c. HIV / AIDS	☐1 Yes	☐0 No	☐88 U/S
d. Viral Hepatitis (A, B, C, D, E)	☐1 Yes	☐0 No	☐88 U/S

Anthracycline-Induced Cardiotoxicity Study		
Patient ID : __ __ __ __	Patient Initials __ __ __	Study Site: __

6. Neurologic Disease	☐1 Yes	☐0 No	☐88 U/S
If **Yes**, which of the following:			
a. Lumbosacral / vertebral disc disease	☐1 Yes	☐0 No	☐88 U/S
b. Numbness or tingling in limbs	☐1 Yes	☐0 No	☐88 U/S
c. History of seizures	☐1 Yes	☐0 No	☐88 U/S
d. Migraine headaches	☐1 Yes	☐0 No	☐88 U/S
e. Peripheral neuropathy	☐1 Yes	☐0 No	☐88 U/S
f. Other neurological disease	☐1 Yes	☐0 No	☐88 U/S
7. Psychiatric Disease	☐1 Yes	☐0 No	☐88 U/S
If **Yes**, which of the following:			
a. Depression	☐1 Yes	☐0 No	☐88 U/S
b. Eating disorder	☐1 Yes	☐0 No	☐88 U/S
c. Anxiety / panic attacks	☐1 Yes	☐0 No	☐88 U/S
d. Suicide attempt	☐1 Yes	☐0 No	☐88 U/S
e. Other psychiatric disease	☐1 Yes	☐0 No	☐88 U/S
8. Respiratory Tract Disorders / Allergies	☐1 Yes	☐0 No	☐88 U/S
If **Yes**, which of the following:			
a. Asthma	☐1 Yes	☐0 No	☐88 U/S
b. Drug allergies	☐1 Yes	☐0 No	☐88 U/S
c. Food allergies	☐1 Yes	☐0 No	☐88 U/S
d. Skin allergies (contact dermatitis)	☐1 Yes	☐0 No	☐88 U/S
e. Sinusitis	☐1 Yes	☐0 No	☐88 U/S
f. Hayfever, allergic rhinitis	☐1 Yes	☐0 No	☐88 U/S
g. Latex allergies	☐1 Yes	☐0 No	☐88 U/S
h. Other allergies	☐1 Yes	☐0 No	☐88 U/S
9. Sexually Transmitted Disease	☐1 Yes	☐0 No	☐88 U/S
If **Yes**, which of the following			
a. Gonorrhoea	☐1 Yes	☐0 No	☐88 U/S
b. Syphilis	☐1 Yes	☐0 No	☐88 U/S
c. Chlamydia	☐1 Yes	☐0 No	☐88 U/S
d. HIV / AIDS	☐1 Yes	☐0 No	☐88 U/S
e. Genital herpes	☐1 Yes	☐0 No	☐88 U/S
f. Genital warts	☐1 Yes	☐0 No	☐88 U/S
g. Trichomonas	☐1 Yes	☐0 No	☐88 U/S
h. Other sexually transmitted disease	☐1 Yes	☐0 No	☐88 U/S
i. Nongonococcal urethritis	☐1 Yes	☐0 No	☐88 U/S
10. Autoimmune / Other Disorders	☐1 Yes	☐0 No	☐88 U/S
If **Yes**, which of the following:			
a. Fibromyalgia or Fibromyositis	☐1 Yes	☐0 No	☐88 U/S
b. Autoimmune Disorders (eg Lupus, Rheumatoid Arthritis, Sjogren's Scleroderma)	☐1 Yes	☐0 No	☐88 U/S
c. Other musculoskeletal, rheumatologic, or connective tissue disease	☐1 Yes	☐0 No	☐88 U/S

Anthracycline Treatment

1. Diagnosis requiring anthracycline treatment

a.	AML (stage: _____)	☐1 Yes	☐0 No
b.	CML (stage: _____)	☐1 Yes	☐0 No
c.	ALL (stage: _____)	☐1 Yes	☐0 No
d.	CLL (stage: _____)	☐1 Yes	☐0 No
e.	HL (stage: _____)	☐1 Yes	☐0 No
f.	DLBCL (stage: _____)	☐1 Yes	☐0 No
g.	Other type of lymphoma (please specify):_____	☐1 Yes	☐0 No
h.	MM	☐1 Yes	☐0 No
i.	Breast cancer (stage: _____)	☐1 Yes	☐0 No
j.	Sarcoma (please specify):_____	☐1 Yes	☐0 No
k.	Others (please specify):_____		

2. Type of anthracycline received

a.	Daunorubicin	☐1 Yes	☐0 No
b.	Doxorubicin	☐1 Yes	☐0 No
c.	Epirubicin	☐1 Yes	☐0 No
d.	Idarubicin	☐1 Yes	☐0 No
e.	Mitoxantrone	☐1 Yes	☐0 No

3. Administration method

a.	IV bolus	☐1 Yes	☐0 No
b.	IV infusion	☐1 Yes	☐0 No

4. Date for the first dose of anthracycline received __ __/__ __/__ __ __ __ (DD/MM/YYYY)

5. Date for the last dose of anthracycline received __ __/__ __/__ __ __ __ (DD/MM/YYYY)

6. Average body surface area _____ m^2
(calculated using formula $((Wt*Ht)/3600)^{½}$)

7. Total dose received

a.	Daunorubicin	_____ mg /m^2
b.	Doxorubicin	_____ mg /m^2
c.	Epirubicin	_____ mg /m^2
d.	Idarubicin	_____ mg /m^2
e.	Mitoxantrone	_____ mg /m^2

Doxorubicin isotoxic equivalent _____ mg /m^2
(conversion factor: Daunorubicin, total dose x 0.833; Doxorubicin, total dose x 1, Epirubicin, total dose x 0.67; Idarubicin, total dose x 5; Mitoxantrone, total dose x 4)

Anthracycline-Induced Cardiotoxicity Study		
Patient ID : __ __ __ __	Patient Initials __ __ __	Study Site: __

Anthracycline Treatment (cont)

8. Anthracycline treatment regimen

Date (DD/MM/YYYY)	Anthracycline*	Prescribed dose (mg/m^2)	BSA (m^2)	Administered dose (mg)	Administration method	Chemo regimen

Anthracycline: Dauno, Daunorubicin; Doxo, Doxorubicin; Epi, Epirubicin; Ida, Idarubicin; Mito, Mitoxanthrone

9. Concomitant cytotoxic drugs

	Drug	Total dose received (mg)		Drug	Total dose received (mg)
1.			9.		
2.			10.		
3.			11.		
4.			12.		
5.			13.		
6.			14.		
7.			15.		
8.			16.		

10. Concomitant non-cytotoxic drugs

No	Drugs	Total dose received (mg)	Pharmacology group
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
11.			
12.			
13.			

Vital Sign & Laboratory Data[@]

Date (DD/MM/YYYY) / Parameters						
Blood pressure (mm/Hg)						
Hear rate (pulse/min)						
Respiratory rate (breath/min)						
Temperature (°C)						
Performance status (Score (System))						
ALT (U/L)						
Total bilirubin (µmol/L) / Direct bilirubin						
Serum Creatinine (µmol/L)						
Creatinine clearance[#] (mL/min/1.73 m^2)						
Date (DD/MM/YYYY) / Parameters						
Blood pressure (mm/Hg)						
Hear rate (pulse/min)						
Respiratory rate (breath/min)						
Temperature (°C)						
Performance status (Score (System))						
ALT (U/L)						
Total bilirubin (µmol/L) / Direct bilirubin						
Serum Creatinine (µmol/L)						
Creatinine clearance[#] (mL/min/1.73 m^2)						

[@] Last monitoring prior to administration of every anthracycline dose.

[#] - For adult (≥ 18 years old) : estimate using *CKD-EPI equation*, GFR = $141 \times \min(S_{cr}/\kappa, 1)^{\alpha} \times \max(S_{cr}/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] × 1.159 [if black] **,** S_{cr} is serum creatinine in µmol/L, κ is 61.9 for females and 79.6 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of S_{cr}/κ or 1, and max indicates the maximum of S_{cr}/κ or 1

- For children (1 - 18 years old): estimate using *Bedside Schwartz equation*, GFR = (36.2 × Height in cm) / Creatinine in µmol/L

Anthracycline-Induced Cardiotoxicity ☐1 **Yes** ☐0 **No**

1. Date of diagnosis __ __/__ __/__ __ __ __ (DD/MM/YYYY)

2. Type of AIC
   a. Left ventricular dysfunction ☐1 Yes ☐0 No
   b. Chronic heart failure ☐1 Yes ☐0 No
   c. QT dispersion ☐1 Yes ☐0 No
   d. Coronary artery disease (heart attack, chest pain) ☐1 Yes ☐0 No
   e. Arrhythmia ☐1 Yes ☐0 No

3. Diagnosis method
   a. Based of symptoms (please specify _____) ☐1 Yes ☐0 No
   b. ECHO (Findings at diagnosis:_____) ☐1 Yes ☐0 No
   c. ECG (Findings at diagnosis:_____) ☐1 Yes ☐0 No
   d. Biomarkers ☐1 Yes ☐0 No

(Type of markers / Result at diagnosis: _____/_____)

4. Cardiac function monitoring prior to anthracycline use ☐1 **Yes** ☐0 **No**
   a. ECHO ☐1 Yes ☐0 No
   b. ECG (Findings:_____) ☐1 Yes ☐0 No
   c. Biomarkers ☐1 Yes ☐0 No

(Type of markers / Result at diagnosis: _____/_____)

5. ECHO & ECG findings

	Date / Items					
ECHO	IVS					
	LVEDd					
	LVESd					
	LVPW					
	FS%					
	EF% (mode)					
	LA					
	Aorta root					
	Comments					
ECG	Date / Items					
	Rate					
	PR					
	QRSD					
	QT/QTc					
	P / QRS / T					
	Comments					

Radiotherapy

1. Date: **from** __ __/__ __/__ __ __ __ (DD/MM/YYYY) **to** __ __/__ __/__ __ __ __ (DD/MM/YYYY)
2. Site
3. Dose _____ Gy
4. Fractions _____

1. Date: **from** __ __/__ __/__ __ __ __ (DD/MM/YYYY) **to** __ __/__ __/__ __ __ __ (DD/MM/YYYY)
2. Intent
   a. Palliative ☐1 Yes ☐0 No
   b. Radical ☐1 Yes ☐0 No
3. Site
4. Technique
   a. Direct ☐1 Yes ☐0 No
   b. Par. Pair ☐1 Yes ☐0 No
   c. Wedged pair ☐1 Yes ☐0 No
   d. _____Fields ☐1 Yes ☐0 No
   e. Others: (please specify):_____ ☐1 Yes ☐0 No
5. Energy mode _____ mV / MeV*
6. Dose _____ Gy
7. Fractions _____

1. Date: **from** __ __/__ __/__ __ __ __ (DD/MM/YYYY) **to** __ __/__ __/__ __ __ __ (DD/MM/YYYY)
2. Intent
   a. Palliative ☐1 Yes ☐0 No
   b. Radical ☐1 Yes ☐0 No
   c. Boost ☐1 Yes ☐0 No
3. Site _____
4. Technique
   f. Direct ☐1 Yes ☐0 No
   g. Par. Pair ☐1 Yes ☐0 No
   h. Wedged pair ☐1 Yes ☐0 No
   i. _____Fields ☐1 Yes ☐0 No
   j. Others: (please specify):_____ ☐1 Yes ☐0 No
5. Energy mode _____ mV / MeV*
6. Dose _____ Gy
7. Fractions _____

Visit after AIC diagnosis ☐ **In-patient** ☐ **Outpatient**

1. Visit number _____

2. Purpose of visit
   - ☐ Malignancy care
   - ☐ Cardiotoxicity care
   - ☐ Others : _____

3. Visit date __ __/__ __/__ __ __ __ (DD/MM/YYYY)

4. Discharge date ☐ Not applicable __ __/__ __/__ __ __ __ (DD/MM/YYYY)

5. Duration of hospital stay ☐ Not applicable _____ days

6. Type of ward ☐ Not applicable
   - ☐ Intensive care
   - ☐ Normal ward
   - ☐ Others : _____

7. Seen by ☐ Consultant ☐ Specialist ☐ Medical officer ☐ House officer ☐ Others _____

8. Investigations

	Investigations	Date(s) of investigations	Number of investigations
1.	Echo		
2.	ECG		
3.	Renal Profile		
4.	Liver Profile		
5.	CK		
6.	CKMB		
7.	Troponin T		
8.	Troponin I		
9.			
10.			

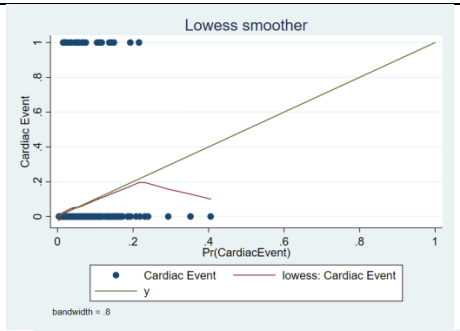
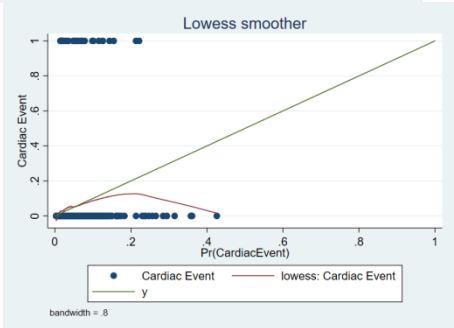
9. Pharmacological management

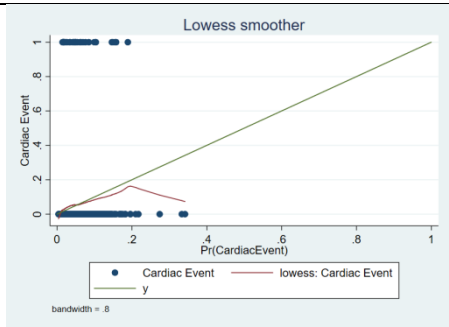
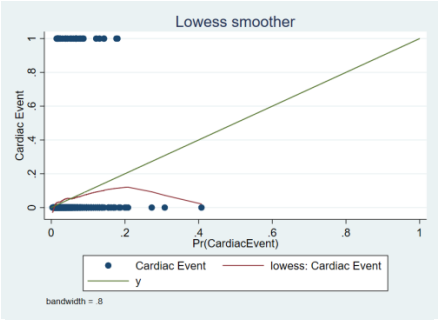
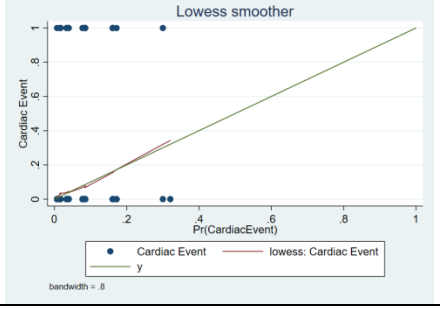
	Drug (strength)	Regimen (dose, frequency)	Start date	End date	Total pills
1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					
9.					
10.					
11.					
12.					

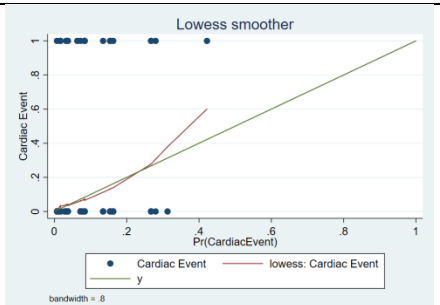
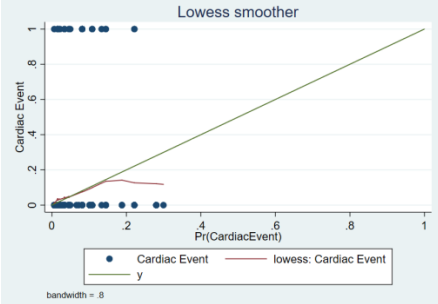
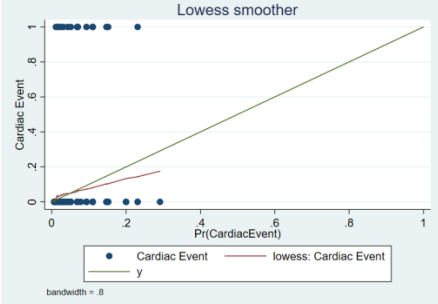
10. Non-pharmacological management ☐ Yes _____ ☐ No

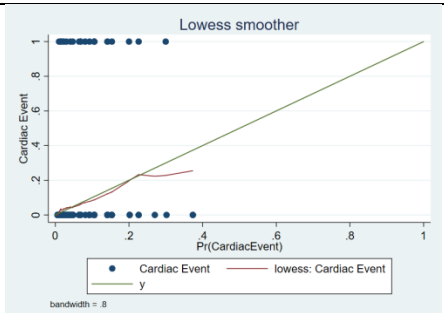
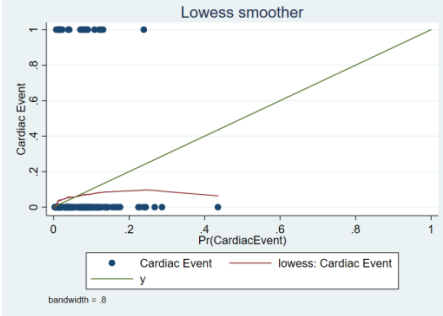
Appendix 6: Performance of potential models

Ten of fifty-one models have AUROC of 0.75 or more (rounded to two decimal points). Model 5 was selected as final model based on the AUROC and calibration slope.

Model	Description	R-squared	AUROC	Hosmer-Lemeshow test	Calibration slope
1	Age + Cardio-protective agent + Haematology + Cyclophosphamide & Trastuzumab	0.0412	0.7508	0.0151	
2	Age + ACEi + Haematology + Cyclophosphamide & Trastuzumab	0.0446	0.7534	0.0002	

Model	Description	R-squared	AUROC	Hosmer-Lemeshow test	Calibration slope
3	Age + Cardiac comorbid + Haematology + Cyclophosphamide & Trastuzumab	0.0356	0.751	0.0020	
4	Age + Haematology + Cyclophosphamide & Trastuzumab	0.0333	0.7472	0.0002	
5	**Age50 + Cardio-protective agent + Haematology + Cyclophosphamide & Trastuzumab**	**0.0384**	**0.7479**	**0.8178**	

Model	Description	R-squared	AUROC	Hosmer-Lemeshow test	Calibration slope
6	Age50 + Number of cardiac comorbid + Haematology + Cyclophosphamide & Trastuzumab	0.0454	0.7457	0.5998	
7	Age50 + Chinese + ACEi + Haematology + Cyclophosphamide & Trastuzumab	0.0462	0.7487	0.2456	
8	Age50 + Chinese + Cardiac Comorbid + Haematology + Cyclophosphamide & Trastuzumab	0.038	0.75	0.5049	

Model	Description	R-squared	AUROC	Hosmer-Lemeshow test	Calibration slope
9	Age50 + Chinese + Cardiac Comorbid + Haematology + Cyclophosphamide & Trastuzumab + breast cancer	0.0402	0.7529	0.3541	
10	Age50 + Haematology + Creatinine clearance + Cyclophosphamide & Trastuzumab	0.0354	0.754	0.9932	

Age, age as continuous covariate; Age50, age as 50 or more years

Appendix 7: Qualitative survey form

Content and usability of a 4-factors ACT prediction model

Dear Consultants, Specialists and Pharmacists,

We developed and validated a prediction model to predict individual risk for anthracycline-induced cardiotoxicity (ACT), based on demographic and clinical characteristics . The prediction model was developed from a total of 2034 cancer patient in Malaysia consists of 4 factors: Age, cancer diagnosis, use of cardio-protective agents (beta blocker, angiotensin converting enzyme inhibitor, angiotensin II receptors blocker) and concomitant use of cyclophosphamide and trastuzumab. You may access and download to try out the excel format of the prediction model via this link: https://drive.google.com/open?id=1DAkRJZ86_MbMzOTTRI7Q47j5MyMD6dOX

We would like to get your expert opinions on the content and usability of the 4-factors ACT prediction model by answering the following 7 questions which will take approximately 10 minutes.

Please contact me should you prefer a face-to-face or video conference interview or additional information about the research.
Thank you.

Leong Siew Lian
PhD candidate
Monash University Malaysia
Mobile: 012 8817198
Email: Siew.Leong@monash.edu

1. **Email address** *

2. **Position**
   Check all that apply.

	Please choose one
Consultant	
Specialist	
Pharmacist	

Content and usability questions

These are open ended questions.

3. **1. When starting a patient on anthracycline chemotherapy, do you have any scoring system that you use in your clinical practice to assess patient's condition/risk for cardiotoxicity and how frequent you use them?**

4. **2. What are the criteria of these scoring systems that encourage your usage?**

5. **3. What are the factors that you will consider in making above decision?**

Please download and try out the excel format of the prediction model at https://drive.google.com/open?id=1DAkRJZ86_MbMzOTTRI7Q47j5MyMD6dOX before attempting the following questions.

6. **4. How do you find the practicality of the attached ACT prediction model?**

7. **5. How possible you will adopt it your clinical practice and why?**

8. **6. How available are the factors in the attached ACT prediction model?**

9. **7. What improvement will you suggest for the ACT prediction model?**

Thank you.

☐ Send me a copy of my responses.

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