Original Research

Effects of Anthracyclines on Aortic Distensibility in Patients with Lymphomas: A Prospective Study

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Introduction: Anthracyclines have been widely used in the treatment of haematological malignancies. Their major adverse effect is cardiomyopathy, but their effect on vascular elasticity has not been completely elucidated. The aim of the present study was to investigate the effects of anthracyclines on aortic elastic properties in patients with lymphomas.

Methods: We studied 70 patients with lymphomas, 37 males (52.9%), age 44 ± 19 years, who were free of any cardiorenal or metabolic comorbidity. Forty-five (64.2%) had a non-Hodgkin lymphoma and the remainder a Hodgkin lymphoma. All participants were evaluated with echocardiography, laboratory and clinical examinations to estimate cardiac function and aortic elasticity in the following study phases: before the administration of anthracyclines (i.e. baseline), after three months, and after the end of treatment.

Results: A progressive decrease in aortic distensibility was observed over the three phases of the study (2.48 ± 0.2 vs. 2.41 ± 0.18, vs. 2.36 ± 0.23, $10^{-6}\cdot dyn^{-1}\cdot cm^2$; $p<0.016$ for all comparisons). A statistically significant decrease in left ventricular ejection fraction was also observed between baseline and final follow up. Significant negative predictors of aortic distensibility at final follow up were baseline age, systolic blood pressure, left atrial diameter, and left ventricular ejection fraction.

Conclusions: Anthracycline therapy decreases aortic distensibility in patients with lymphomas.

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Anthracyclines are potent chemotherapeutic agents with wide applications in cancer therapy and haematological malignancies such as leukaemia, Hodgkin and non-Hodgkin lymphomas, because of their broad efficacy and generally acceptable tolerability.[1] However, anthracycline administration has been correlated with a high rate of cardiotoxicity that has limited their application. The anthracyclines generally used are doxorubicin, epirubicin, pirarubicin, and idarubicin. All anthracyclines are cardiotoxic to varying degrees, depending on the dosage, method of administration, age, cardiovascular history, previously administered cytotoxic agents and concomitant therapy.[2]

It is well established that dysfunction of the left ventricle (LV) is associated with the development of congestive heart failure, and an increased risk of sudden death. Death from cardiac causes can occur weeks or months after anthracycline therapy.[3-7] The pathophysiological mechanism of cardiotoxicity involves the generation of reactive oxygen species via different pathways, including redox-cycling, iron complexation, chaotropic effects in mitochondria and the consequent uncou-

pling of electron transport.[8,9] Consequently, adding anthracyclines to cardiac preparations increases the formation of reactive oxygen species. All the above have been documented using various oxidative stress markers.[8-11]

Aortic stiffness has emerged as an important and independent risk factor for cardiovascular disease, depicting the impairment of aortic elastic properties with repercussions for atherosclerosis development.[12] Aortic distensibility (AoD) is an elasticity index of the ascending aorta, along with aortic pulse wave velocity and augmentation index. AoD also represents a simple and reproducible marker of subclinical atherosclerosis.

The aim of the present study was to investigate whether treatment with anthracyclines in patients with lymphomas impacts negatively on aortic elastic properties and cardiac performance.

Methods

The study protocol was reviewed and approved by the Research Ethics Committee of our hospital. It conformed to the ethical guidelines of the Declaration of Helsinki[13] and to the principles for medical research involving human subjects. Informed consent was obtained from all patients.

One hundred and twenty-three consecutive patients with lymphoma who had been scheduled to receive anthracycline-based chemotherapy were initially recruited for the study. We subsequently excluded patients who had a history of myocardial infarction (n=3), heart failure (n=1), diabetes mellitus (n=7), or renal failure (n=6). We also excluded patients who were under treatment with b-blockers or blockers of the renin–angiotensin system (n=20). Finally, 86 patients were eligible to participate. However, 12 were either lost to follow up or did not adhere to the follow-up visits, while 4 died early from their haematological malignancy. The final study population was comprised of 70 patients who adhered to the study protocol. None of them had previously received radiotherapy or chemotherapy treatments.

Baseline

Patients were instructed to pursue the scheduled therapeutic protocol of chemotherapy for their malignancy according to the usual practice. At baseline (T0), after a detailed clinical examination, patients underwent serial measurements of AoD, LV ejection fraction and additional measures of cardiac performance, as well as of cardiac troponin I, brain natriuretic peptide (BNP), and creatinine kinase-MB (CKMB). Blood pressure was measured with a mercury sphygmomanometer according to guidelines.[14]

Follow-up

Patients underwent the same clinical and laboratory workup as in the baseline period (T0). Follow-up visits were performed at 3 months from the baseline visit (T1) and after the end of treatment (T2). During follow up, patients received doxorubicin as anthracycline treatment. The cumulative dose of doxorubicin was 150-200 mg/m^2 at T1 and 300-400 mg/m^2 at T2. In addition to doxorubicin, patients with Hodgkin lymphoma received bleomysin, vinblastine, and dacarbazine, while patients with non-Hodgkin lymphoma further received cyclophosphamide, vincristine and methylprednisolone.

Measures

The echocardiographic study and measurement of AoD were performed by a senior echocardiographer at Laikon Hospital using a Hewlett Packard Sonos ultrasound system 1500 (Philips) with a 2-5 MHz transducer. Procedures were carried out according to guidelines.[15] The echo operator was blinded to the clinical status of the examinees. AoD was determined non-invasively, based on the relation between changes in aortic diameter and pressure within each cardiac cycle. In brief, each patient was placed in the mild left recumbent position and the ascending aorta was recorded at a level of 3 cm above the aortic valve. The M-mode tracings were guided by the two-dimensional echocardiogram in the parasternal long-axis view.[16] Internal aortic diameters were measured by means of a calliper in systole and diastole as the distance between the trailing edge of the anterior aortic wall and the leading edge of the posterior aortic wall. Systolic aortic diameter was measured as the maximal anterior motion of the aorta and diastolic diameter at the peak of the QRS complex on the simultaneously recorded electrocardiogram. Ten consecutive cardiac beats were measured routinely and averaged.[16,17] AoD was finally calculated according to the following formula:[16]

$$\text{Aortic distensibility} = \frac{2\Delta D}{Dd\,(Ps-Pd)}\ 10^{-6}\cdot dyn^{-1}\cdot cm^2$$

where ΔD is the change in the aortic diameter between systole and diastole, Dd the aortic diameter in diastole, Ps the systolic arterial blood pressure, and Pd the diastolic arterial blood pressure.

Venous blood samples were obtained from all individuals for the evaluation of serum creatinine, fasting low-density lipoprotein cholesterol and glucose. Blood samples were collected in EDTA tubes and measured according to established methods. To measure serum BNP, cardiac troponin I, and CKMB, commercially available fluorescence immunoassay panels (Triage profiler SOB panel, San Diego, CA, USA) were used. Kidney function was estimated in terms of glomerular filtration rate, according to the Cockcroft–Gault formula.

Statistical analysis

The SPSS statistical package release 14.0 (SPSS Inc.) was used for all the statistical analyses. All of the descriptive continuous variables are presented as means and SDs, since their distribution was normal (Shapiro–Wilk test). Categorical variables are presented as absolute and relative (percentages) frequencies. Significant differences between the groups were determined using Student's independent samples t-test, after checking for equality of variances using Levene's test and ANOVA. To adjust for the inflation of type I error because of the high number of multiple comparisons, we used the Bonferroni correction. Because there were 3 groups (i.e. T0, T1, T2), the differences were considered significant if the p-value was <0.016. Conventional linear multivariate regression analysis was used to examine the significant determinants of the AoD at final follow up. The candidate explanatory variables entered into the model were age, sex, body mass index, systolic and diastolic blood pressure (BP), heart rate, type of lymphoma, LV ejection fraction, low-density lipoprotein cholesterol, glomerular filtration rate and cardiac markers. Values of $p<0.05$ were considered statistically significant.

Results

Seventy consecutive patients of Hellenic origin (males n=37, 53%) aged 44 ± 19 years were studied. Among them, 45 (64.2%) presented with a non-Hodgkin lymphoma and the remainder had a Hodgkin lymphoma. The demographic and clinical characteristics of the study population at baseline are depicted in Table 1. Patients with non-Hodgkin as compared to those with Hodgkin lymphoma were older, demonstrated high-

Table 1. Demographic and clinical characteristics of the study population at baseline according to the type of lymphoma.

	Non-Hodgkin	Hodgkin	p
Age (years)	52.09 ± 16.66	27.83 ± 9.19	<0.0001
Males, n (%)	23 (51%)	13 (52%)	0.989
BMI, kg/m^2	27.36 ± 4.26	26.34 ± 5.96	0.202
Current smoking, n (%)	5 (11%)	3 (12%)	0.864
SBP, mmHg	124.47 ± 19.50	116.19 ± 14.33	0.005
DBP, mmHg	78.61 ± 12.54	75 ± 11.29	0.067
HR, bpm	75.1 ± 12	78.6 ± 13.56	0.345
Glucose, mg/dL	91 ± 11.58	89 ± 12.65	0.687
GFR mL/min	96.32 ± 6.8	98.65 ± 5.7	0.468
LDL, mg/dL	98.53 ± 6.32	102 ± 9.35	0.345
LVDD, mm	51.74 ± 2.97	51.4 ± 3.55	0.221
LVSD, mm	33.81 ± 2.91	33.18 ± 3.63	1.511
EF, %	59 ± 4.5	60.42 ± 5.38	0.068
E/A	0.925 ± 0.23	1.179 ± 0.18	<0.0001
DT, ms	178.35 ± 13.43	168.41 ± 10.76	<0.0001
LA, mm	37.87 ± 7.55	34.70 ± 5.7	0.005
AoD, $10^{-6}\cdot dyn^{-1}\cdot cm^2$	2.33 ± 0.17	2.55 ± 0.13	<0.0001
BNP, pg/mL	24.26 ± 0.81	20.69 ± 50.35	0.579
CBMB, ng/mL	1.09 ± 3.9	0.77 ± 3.35	0.568
cTnI, ng/mL	0.001 ± 0.009	0.003 ± 0.015	0.28

BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; HR – heart rate; GFR – glomerular filtration rate; LDL – low-density lipoprotein cholesterol; LVDD – left ventricular diastolic dimension; LVSD – left ventricular systolic dimension; EF – left ventricular ejection fraction; E/A – ratio between early (E) and late (atrial - A) ventricular filling velocity; DT – deceleration time; LA left atrium; AoD – aortic distensibility; BNP – brain natriuretic peptide; CKMB – creatinine kinase-MB; cTnI – cardiac troponin I.

er levels of systolic BP, more impaired LV diastolic function, enhanced left atrial diameter, and lower AoD by almost 10%. There was no significant association between the two groups regarding sex, body mass index, heart rate, diastolic BP, or the studied cardiac markers.

During the whole follow-up period no significant changes were observed in kidney function and metabolic parameters. In the whole study population there was a progressive decline in ejection fraction ($p<0.0001$) and AoD ($p=0.002$) from baseline to final follow up. Left atrial diameter, diastolic function, and bloodstream measurements of cardiac function did not differ from baseline to either interim or final follow-up times (Table 2). Although no significant changes in LV ejection fraction were observed from baseline during 3 months of follow up, AoD demonstrated significant changes over this period.

Significant determinants of final follow-up AoD are presented in Table 3. A higher (i.e. less impaired) AoD was associated with younger age, better LV ejection fraction, smaller left atrial diameter, and lower systolic BP. The type of lymphoma did not predict AoD in this setting. Figure 1 depicts the progressive deterioration of AoD from baseline to final follow up.

Discussion

This study provides prospective evidence that the treatment of lymphomas with doxorubicin is associated with impaired elastic properties of the aorta, independently of the progressive reduction in LV ejection fraction, during treatment with anthracyclines. The type of lymphoma does not correlate with the magnitude of aorta impairment. Finally, the emergent aortic injury develops progressively over time from the first anthracycline administration, suggesting a dose-dependent effect.

Cardiotoxicity following anthracycline administration is dose-dependent, and the risk of cardiovascular death is almost 8 times higher than that of the normal population, even 25 years after therapy.[18] In one retrospective series,[19] a 7.5% incidence of clinical cardiomyopathy was recorded at a cumulative doxorubicin dose of 550 mg/m^2. This incidence rises to 20% when careful prospective observations are made, including serial determinations of LV ejection fraction at follow up.[20] These observations led to cumulative dose limitations of doxorubicin (<450-500 mg/m^2) given as a bolus every three weeks. Although dose limitation strategies reduce the incidence of anthracycline-related cardiac events,[21] the risk is also associated with underlying cardiovascular comorbidi-

Table 2. Variation of cardiovascular markers during the follow-up period

	T0	T1	T2
EF, %	61.36 ± 4.81	59.43 ± 5.31	57.65 ± 4.73*
AoD, $10^{-6}\cdot dyn^{-1}\cdot cm^2$	2.48 ± 0.20	2.41 ± 0.18‡	2.36 ± 0.23*,†
LA, mm	34.86 ± 8.61	36.5 ± 6.56	38.08 ± 4.2
E/A	1.02 ± 0.24	1.02 ± 0.26	1.03 ± 0.24
CKMB, ng/mL	0.72 ± 1.16	0.54 ± 1.13	1.64 ± 5.89
cTnI, ng/mL	0.003 ± 0.014	0.00 ± 0.00	0.002 ± 0.012
BNP, pg/mL	22.81 ± 30.54	20.07 ± 31.93	24.51 ± 51.52

$^{*}p<0.016$ for comparisons between T1 and T3; $^{\dagger}p<0.016$ for comparisons between T2 and T3; $^{\ddagger}p<0.016$ for comparisons between T1 and T2.
EF – left ventricular ejection fraction; AoD – aortic distensibility; LA – left atrium; E/A – ratio between early (E) and late (atrial - A) ventricular filling velocity; CKMB – creatinine kinase-MB; cTnI – cardiac troponin I; BNP – brain natriuretic peptide.

Table 3. Multivariate determinants of aortic distensibility in the whole study population: stepwise regression model. Variables entered into the model that were found to have no significant association with the outcome were: type of lymphoma, diastolic blood pressure and heart rate. R^2 adjusted=72%.

Determinants	B (SE)	Beta	95% confidence interval for B		p
			Lower	Upper	
Age, years	-0.007 (0.001)	-0.650	-0.008	-0.006	<0.0001
EF, %	0,009 (0.002)	0,225	0,005	0,012	<0.0001
SBP, mmHg	-0.003 (0.001)	-0.207	-0.005	-0.002	<0.0001
LA, mm	-0.004 (0.001)	-0.126	-0.006	-0.001	0.011

SE – standard error; EF – left ventricular ejection fraction; SBP – systolic blood pressure; LA left atrium.

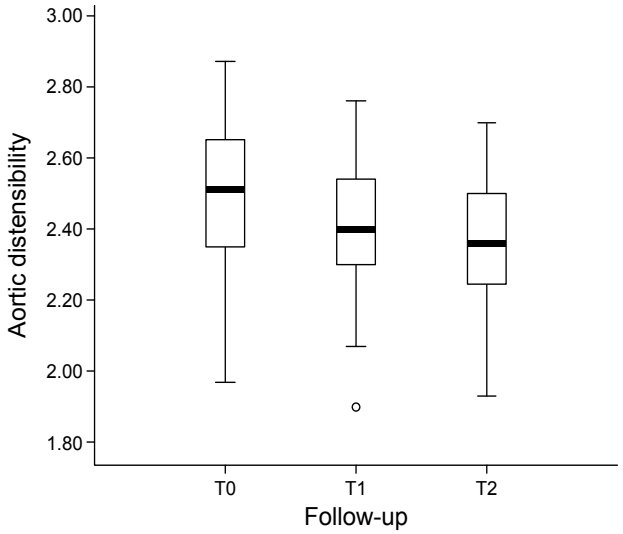

Figure 1. Aortic distensibility (in 10^{-6}·dyn^{-1}·cm^2) during the follow-up period. Aortic distensibility decreased significantly from baseline to the end of follow up ($p<0.0001$), while there were no significant changes between baseline (T0) and after three months of anthracycline therapy (T1; $p=0.059$), or between T1 and the end of follow up (T2; $p=0.107$).

ties.[22] In our present study, despite the fact that the cumulative dose of doxorubicin was lower than the dose deemed cardiotoxic, there was still a significant combined decrease in AoD and LV ejection fraction.

Decreased AoD was found to be associated with ageing, LV ejection fraction, increased left atrial dimension, and systolic BP. Interestingly, the AoD decline was significant even in the early period of follow up, but the reduction was still ongoing in the later follow-up period. By contrast, there was no significant increase in plasma levels of biochemical markers of cardiac injury and dysfunction (i.e. cardiac troponin I, CK-MB, and BNP) and their levels were not abnormal at any phase of the study.

Although the mechanisms of anthracycline's toxicity to the myocardial tissue have been extensively reviewed in the literature,[23-26] it is still unclear how anti-malignancy treatment with anthracyclines may cause the great vessels' elastic properties to deteriorate. Nuclear cellular actions of anthracyclines may adversely modulate collagen turnover and vascular wall homeostasis. These actions could be summarised as the suppression of transcription factors that regulate cell survival, damage to nuclear DNA, increased release of free radicals secondary to the formations of Fe^{3+}-doxorubicin complexes,[27,28] and induction of enhanced oxidative stress with inflammatory repercussions on the vessel wall. Over and above these potential direct effects of anthracycline-based therapy on the vascular wall, the impairment of elastic properties may also be accelerated by traditional partners of vascular ageing, which was found to be significant in the present study, and most importantly ageing and systolic BP.

We acknowledge some limitations in our study. First, all patients were treated with doxorubicin; thus the results could not be extended to other anthracycline-based therapies. Second, we did not account for the potential adverse effects of drugs used as adjunctive therapy to doxorubicin. Third, we did not perform measurements of alternative measures of aortic elastic properties, such as pulse-wave velocity or augmentation index. The estimation of such measures might have provided a more integrated approach to vascular deterioration. However, among the strengths of our study is the recruitment of real-world lymphoma patients, who were free of cardiovascular comorbidities and were not taking drugs that could have a confounding effect on aortic elastic properties.

Clinical perspectives

Our findings suggest that antineoplastic treatment with doxorubicin may impair cardiovascular coupling, because apart from myocardial dysfunction, aortic properties also deteriorate. This might represent a further possible mechanism of the cardiovascular complications frequently observed in such patients. In hypertensive patients treated with doxorubicin, drugs blocking the renin–angiotensin system may be preferred over other drug classes, not only for their potential to reduce blood pressure, but also because of their antistiffening properties in the aorta.[29,30] This might also be an area for investigation in future studies, not only in hypertension associated with malignancy under chemotherapy, but even in normotensive patients.

We conclude that doxorubicin adversely affects elastic aortic properties in tandem with cardiac performance, independently of traditional confounders, in patients with lymphomas who are free of cardiovascular disease. This finding opens a new window for the impact of anthracyclines on vascular physiology, over and above its negative effect on cardiac performance.

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