Retinal Vessel Analysis as a Novel Screening Tool to Identify Childhood Acute Lymphoblastic Leukemia Survivors at Risk of Cardiovascular Disease

Azanan, Mohamad S.; Chandrasekaran, Sudhashini; Rosli, Erda S.; Chua, Ling Ling; Oh, Lixian; Chin, Tong Foh

Published in:
Journal of Pediatric Hematology/Oncology

DOI:
10.1097/MPH.0000000000001766

Publication date:
2020

Document Version
Peer reviewed version

Retinal vessel analysis as a novel screening tool to identify childhood leukemia survivors at risk of cardiovascular disease

Running title: **Retinal vessel analysis of cancer survivors** (max 40 characters)

Mohamad Shafiq Azanan, PhD[1, 2], Sudhashini Chandrasekaran, MD[3], Erda Syerena Rosli, MS[2], Chua Ling Ling, BS[2], Oh Lixian, BS[1, 2], Chin Tong Foh, BS[2], Yap Tsiao Yi, MD[1,4], Revathi Rajagopal, MD[1,4], Reena Rajasuriar, PhD[5], Tom MacGillivray, PhD[6], Emanuele Trucco, PhD[7], Norlina Ramli, MD[3], Tengku Ain Fathlun Kamalden, MD, D.Phil[3], Hany Ariffin, MD, PhD[1,2,4]

[1] Department of Paediatrics, Faculty of Medicine, University of Malaya, Kuala Lumpur
[2] Pediatric Oncology Research Laboratory, University of Malaya, Kuala Lumpur
[3] University of Malaya Eye Research Centre, Department of Ophthalmology, University of Malaya, Kuala Lumpur
[4] Division of Pediatric Hematology-Oncology and Bone Marrow Transplantation, University of Malaya Medical Centre, Kuala Lumpur
[5] Department of Pharmacy, Faculty of Medicine, University of Malaya, Kuala Lumpur
[6] VAMPIRE Project, Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, United Kingdom
[7] VAMPIRE project, Computing (SSEN), University of Dundee, Dundee, UK

Corresponding Author:
Tengku Ain Kamalden
University of Malaya Eye Research Centre, Department of Ophthalmology, University of Malaya, 50603 Kuala Lumpur, Malaysia
Email: taftkamalden@um.edu.my
Telephone:

Research support: Establishment of the Childhood Cancer Survivor Cohort at University of Malaya was supported by the Ministry of Education, Malaysia (UM.C/HIR/MOHE/MED/12). This study was partially funded by University Of Malaya Research Grant (UMRG 049-17HTM). The VAMPIRE team acknowledges the support of EPSRC grant EP/M005976/1 “Multimodal retinal biomarkers for vascular dementia”.

Disclaimers: The authors indicated no potential conflicts of interest

Author contributions:

Mohamad Shafiq Azanan: Research investigation, data analysis and curation, data interpretation, manuscript writing, editing, data visualization, manuscript review and review and approval of final manuscript. **Sudhashini Chandrasekaran:** Research investigation, data analysis and curation, data interpretation, manuscript review and review and approval of final manuscript. **Erda Syerena Rosli:** Research investigation, manuscript review and review and approval of final manuscript. **Chua Ling Ling:** Research investigation, manuscript review and review and approval of final manuscript. **Oh Lixian:** Research investigation, manuscript review and review and approval of final manuscript. **Chin Tong Foh:** Research investigation, manuscript review and review and approval of final manuscript. **Yap Tsiao Yi:** Study resources, manuscript review and review and approval of final manuscript. **Revathi Rajagopal:** Study resources, manuscript review and review and approval of final manuscript. **Reena Rajasuriar:** Project supervision, manuscript review and review and approval of final manuscript. **Tom MacGillivray:** Research investigation, manuscript writing, editing, manuscript review and review and approval of final manuscript. **Emanuele Trucco:** Research investigation, manuscript writing, editing, manuscript review and review and approval of final manuscript. **Norlina Ramli:** Project supervision,

manuscript review and review and approval of final manuscript. **Tengku Ain Fathlun Kamalden:** Guarantor of all content, project supervision, data interpretation, manuscript writing, manuscript review and review and approval of final manuscript. **Hany Ariffin:** Project supervision, funding acquisition, data interpretation, manuscript writing, manuscript review and review and approval of final manuscript.

Precis:

Childhood cancer survivors possess a higher risk for cardiovascular disease (CVD) compared to age-matched peers. Retinal vessel analysis could be used to identify survivors at risk of CVD.

ABSTRACT

Background

Microvascular endothelial dysfunction is central to the pathogenesis of CVD. The eye offers direct access for endothelial health assessment via the retinal microvasculature. Using rigorous software analysis, we investigated whether image-based retinal vessel analysis is a feasible method of assessing endothelial health in childhood cancer survivors (CCS).

Methods

Cardiovascular risk factors (CRF) were estimated using the 30-year Framingham Heart Study Risk Score in 78 survivors of childhood leukaemia (median age: 26, IQR 23-29; median years from diagnosis: 19, IQR 14-22) and 78 healthy controls (median age: 24, IQR 23-27). Radial arterial stiffness was measured using pulse wave analyser, while endothelial activation markers were measured by soluble intercellular adhesion molecule 1 (sICAM-1) and soluble vascular cell adhesion molecule 1 (sVCAM-1). Retinal fundus

images were analysed on Vascular Assessment and Measurement Platform for Images of the REtina (VAMPIRE) software for central retinal artery/vein equivalents (CRAE/CRVE) and arteriolar-venular ratio (AVR).

Results

CCS had higher CRF ($p<0.0001$), arterial stiffness ($p<0.001$) and sVCAM-1 ($p=0.002$) compared to controls. Survivors also had significantly higher CRVE ($p=0.022$) whilst AVR was significantly lower ($p=0.042$) than in controls, compatible with endothelial dysfunction. Survivors with intermediate CRF exhibited lower CRAE compared to survivors with low CRF ($p=0.033$). In a regression model, CRAE of the survivors was negatively associated with CRF (ß=-0.381, $p=0.037$) along with covariates (age, gender and smoking status).

Conclusions

CCS had evidence of endothelial dysfunction and increased risk of cardiovascular disease compared to controls. Retinal vessel analysis is comparable to pulse wave analysis and may be utilized as a robust screening tool for detecting CCS at risk of developing CVD.

Keywords: Inflammation, retinal vessels, cardiovascular diseases, cancer survivors

Number of text pages: 22
Number of tables: 2
Number of figures: 5

INTRODUCTION

Late-effects of therapy with significant health impact especially cardiovascular disease (CVD) are a major concern in childhood cancer survivors (CCS).[1] Cohort studies from the United States as well as Europe have shown that young adult survivors of childhood cancer have an up to 7-fold higher risk of death from cardiac causes compared to age-, and sex-matched members of the general population.[2-4] Impaired endothelial function is postulated as the initial step in the pathogenesis of atherosclerosis.[5] Various manifestations of cardiovascular disease such as cardiomyopathy and vasculopathy are believed to be the downstream complications driven by atherosclerosis.[6-8] The advancement of pathophysiological insights in CVD has allowed sensitive detection of biomarkers and non-invasive *in vivo* detection of a pre-atherosclerotic signature through evaluation of endothelial dysfunction.[9-11]

CCS, especially those with prior exposure to anthracyclines, exhibit preclinical vasculopathy, marked by increased arterial stiffness and endothelial dysfunction.[12] Two studies assessing vascular endothelial function by brachial artery reactivity, also demonstrated that CCS have impaired vascular endothelial function compared to their cancer-free counterparts.[13,14]

There is increasing appreciation that coronary microvascular dysfunction plays a crucial role in coronary heart disease[15] although difficulties in studying the coronary microcirculation directly has impeded our understanding regarding this connection. The eye offers direct access to the retinal microvasculature, thus through high-quality imaging and assistance of reliable, validated computer software, the retinal

vasculature may be non-invasively evaluated.[16] Using the VAMPIRE software, as with other similar analysis platforms such as IVAN, SVA-T and SIVA, retinal vessels can be analysed as biomarkers of systemic conditions such diabetes[17,18], hypertension[19], cardiovascular disease[20,21] as well as neurodegenerative conditions such as Alzheimer's disease.[22] Multiple studies have shown that changes in retinal vessel diameter are associated with risk of cardiovascular disease (CVD)[23-25] and stroke mortality[26,27], independent of traditional risk factors.

In this study, we assessed vascular endothelial health in a cohort of CCS and investigated the association of these changes with cardiovascular risk factors, with the aim of finding novel screening tools for CVD in adult survivors of childhood cancer.

METHODS

Participants

Young adult survivors of childhood leukemia were recruited into this study. The inclusion criteria were: (i) individuals aged 18–35 years, (ii) 5 or more years since completion of leukemia treatment, (iii) no history of hematopoietic stem cell transplantation, (iv) no acute illness or pregnancy at the point of recruitment, and (v) did not receive a vaccination in the 6 months prior to recruitment. Age-matched volunteer controls were recruited amongst subjects' siblings, partners and hospital staff. All participants gave written consent to enrol in the study. The study protocol was approved by the hospital research ethics board (MREC 2014/1093.65) and followed the principles of the Helsinki Declaration. A standardized questionnaire was used to collect demographic details and smoking history. Physical examination as well as biochemical screening were performed on all participants while a detailed history

of cancer treatment was obtained from medical records by two independent researchers to ensure accuracy.

Biochemical parameters

Blood glucose, serum lipid profile and high-sensitivity C-reactive protein (hsCRP) levels were measured by the hospital's diagnostic laboratory within 4 hours of venepuncture.

Risk factors for cardiovascular disease

Risk for developing cardiovascular disease within 30 years was estimated using the Framingham Heart Study Risk Score.[28] Using this assessment tool, the Cardiovascular Risk Factor (CRF) score was calculated using the following parameters: gender, age, systolic blood pressure, usage of antihypertensive agents, smoking status, presence of diabetes mellitus, serum total cholesterol and high-density lipoprotein (HDL).[28] 'Full' CRF is defined as risk of getting coronary death, myocardial infarction, coronary insufficiency, angina, transient ischemic attack, periphery artery disease, heart failure and strokes including ischemic and haemorrhagic stroke.[28]

Pulse wave analysis

Arterial stiffness was inferred through measuring the augmentation index (AIx) of the brachial artery using a cuff based pulse wave analyser, SphygmoCor XCEL PWA (AtCor Medical, Sydney, Australia). AIx is the difference between the early systolic

pressure and the late systolic peak of the central aortic waveform, expressed as a percentage of pulse pressure. Measurements were taken from a partially inflated brachial cuff and the brachial artery waveforms were calibrated using cuff-measured systolic and diastolic pressures. The arterial pulse waves were processed by the system software using a validated transfer factor as previously described.[29]

Retinal vessel caliber analysis

Each subject underwent eye fundus screening and retinal digital photography at the Ophthalmology out-patient clinic in University of Malaya Medical Centre. Prior to photography, 0.5% proparacaine hydrochloride plus 1% tropicamide eye drops were instilled into each eye for pupillary dilation. Colour retinal digital images (35 degrees field of view) were obtained using a TopCon digital fundus camera (TRC-50DX). For standardisation, only images from the left eye were analysed in this study.

Retinal vessel calibre parameters were measured using the VAMPIRE (Vessel Assessment and Measurement Platform for Images of the REtina) software suite (version 3.0; Universities of Edinburgh and Dundee, UK).[16,30,31] Images of insufficient quality or low contrast were excluded from the analysis by the software operators. The assessors were blinded to the subjects' clinical information.

Following convention, we used optic disc (OD) centered images for analysis.[32,33] The software first automatically detects the centre of the OD and of the macula using validated algorithms, reported elsewhere.[30,31] This establishes a reference frame delineating the standard zones A, B and C, i.e., circular sectors (annuli) concentric with the OD, delimited by circles of radii 0.5 and 1 OD diameters (A), 1 and 1.5 (B),

1.5 and 2 (C) . Next, vessels were automatically segmented and classified as arteries or veins. To maximize accuracy, a software operator performed manual corrections to rectify possible software errors, ~~e.g.~~ i.e. OD and macula location, vessel labels (A/V) ~~or~~ and vessel selected for CRAE ~~and~~ / CRVE calculation. CRAE and CRVE were calculated according to the Parr and Hubbard formula[34] and allied protocols, selecting 6 main arteries and veins in Zone B wherever possible. Local vessel widths were estimated using a validated algorithm[35] as the mean of 7 measures taken every 3 pixels along the zone-B vessel segment. Width estimates with high standard deviation (those in the extreme 10 percentiles) were discarded as unreliable.

Endothelial activation markers

Endothelial activation markers, soluble intercellular adhesion molecule 1 (sICAM-1) and soluble vascular cell adhesion molecule 1 (sVCAM-1) were measured by BD Cytometric Bead Array (CBA) immunoassay (BD Biosciences, Breda, The Netherlands). Individual blood plasma concentration (1:256) was derived from standard curves generated using purified ICAM-1 and VCAM-1 provided by the manufacturer. Samples were then incubated with the beads for each marker. Analysis was performed by flow cytometer using FCAP Array version 1 software.

Statistical analysis

Comparison between groups for continuous variables was made using the Mann-Whitney U test. Analysis of covariance (ANCOVA) and partial correlation were performed to investigate the relationship of endothelial dysfunction markers (AIx, sICAM-1 and sVCAM-1) and retinal vessel parameters (CRAE, CRVE and AVR) with Framingham's 30-year full CVD risk, after adjusting for age. Correlation among

continuous parameters within CCS was calculated using Spearman's correlation. Multiple linear regression was used to study the relationship of endothelial dysfunction and retinal vessel parameters with Framingham's 30-year full CVD risk. All analyses were performed using the IBM SPSS Statistics version 20 and figures were generated using GraphPad Prism 7.

RESULTS

Subjects

Seventy-eight asymptomatic young adult survivors of childhood acute lymphoblastic leukemia (ALL) were recruited. Clinical characteristics of subjects, including treatment history for the survivors, are shown in Table 1. The median age at recruitment for survivors and controls are 26 and 24 years old respectively. As ALL treatment protocols were risk-stratified according to individual disease biology, subjects did not all receive uniform therapy. Of note, the number of subjects who received anthracyclines, alkylating agents and radiotherapy were 72%, 73% and 49% respectively.

Cardiovascular disease risk factor (CRF)

CRF of the subjects based on clinical parameters is shown in Figure 1. Overall, survivors had higher blood glucose ($p=0.004$), total serum cholesterol ($p=0.011$), triglyceride ($p<0.001$), LDL ($p=0.005$) and hsCRP ($p=0.007$) when compared to controls. Similarly, systolic ($p<0.001$) and diastolic ($p=0.001$) blood pressure of survivors were significantly elevated compared to controls. Framingham Heart Study Risk Score was used to predict 30-year CRF in both leukemia survivors and controls

(Fig. 2). Survivors had a higher 30-year Framingham Heart Study Risk Score for full CVD compared to controls ($p<0.0001$).

Arterial stiffness and markers of endothelial activation

Arterial stiffness of survivors as measured through augmentation index (AIx) was significantly higher in survivors than controls ($p<0.001$, Figure 3A). Survivors had significantly increased levels of the endothelial activation marker, sVCAM-1 ($p=0.002$), but not sICAM-1 ($p=0.090$) compared to controls (Fig. 3B-C).

Retinal vessel caliber analysis

Retinal vessel caliber analysis showed that central retinal vein equivalent (CRVE) was significantly higher in survivors ($p=0.022$, Fig. 4B), while central retinal artery equivalent (CRAE) was comparable in survivors compared to controls ($p=0.511$, Fig. 4A). Consequently, the arterio-venous ratio (AVR=CRAE/CRVE) of survivors was significantly lower than in controls ($p=0.042$, Fig. 4C).

Association of endothelial dysfunction markers and retinal vessel caliber analysis with cardiovascular risk factors

The association of arterial stiffness, endothelial activation markers and retinal vessel caliber diameters with Framingham's 30-year full CVD risk score were then analyzed among survivors. Survivors with intermediate CRF showed lower CRAE compared to survivors with low CRF ($p=0.033$, Fig. 5 A) calculated using ANCOVA adjusted for age. Parameters of CRVE, AVR, augmentation index, sVCAM-1 and sICAM-1 were all similar between survivors with intermediate and low CRF (Fig. 5B-F). In

Spearman's correlation, AVR is inversely correlated with sVCAM-1 (r=-0.464, p=0.026). In multiple linear regression analysis, CRAE was independently associated with Framingham's 30-year full CVD risk score, along with covariates of age, smoking status and gender (Table 2).

DISCUSSION

Exposure to cytotoxic agents and radiation have rendered childhood cancer survivors at an increased risk of developing cardiovascular disease, metabolic syndrome, diabetes, hypertension and other conditions as a result of long-term vascular complications.[36-39] In this study, we have shown that clinical biomarkers of CVD are significantly elevated in young adult survivors of childhood leukemia.

Previously, we reported that childhood leukemia survivors had a biological signature of inflammation. In particular, survivors had significantly elevated levels of serum hsCRP.[40] CRP has been shown to be a sensitive biomarker associated with metabolic syndrome, cardiovascular disease and microvascular complications in the general population.[41-43] Additionally, CRP is also associated with albuminuria in individuals with diabetes, which is a known marker of diabetic nephropathy.[44-46]

We hypothesise that inflammation may play an important role in late effects observed among CCS, although the exact pathophysiological mechanisms remain to be elucidated. Therapy-induced damage to normal cells may be associated with systemic chronic inflammation, leading to premature aging and late effects in CCS. Individuals exposed to cancer therapy are at risk of cardiovascular damage.[47] In a Swedish and Danish cohort of breast cancer survivors, the incidence of a major coronary event increased linearly with the mean dose of thoracic radiotherapy (RT) by 7.4% per gray (Gy).[48] It is intriguing that CCS whose exposure to radiotherapy is restricted to the

cranium still exhibit higher cardiovascular disease risk compared to the general population.[49,50] This observation is likely secondary to the endocrinopathies arising from irradiation to the hypothalamic-pituitary system[51], with consequences of hormonal imbalance and development of metabolic syndrome.

Another important component of many childhood cancer treatment protocols are the anthracyclines. In rat models, anthracyclines especially doxorubicin have been shown to cause structural and functional damage to the heart.[52,53] In humans, anthracyclines have been associated with various coronary pathology such as arrhythmia, pericarditis and myocarditis.[2,13,54-56] Recent studies have also shown that anthracyclines have the propensity to induce toxicity in the vascular endothelium[12-14,54], the antecedent event in the etiology of atherosclerosis.

In our study, we have shown that CCS have endothelial dysfunction, concurring with results published by other groups.[57,58] Endothelial dysfunction is a crucial factor in atherosclerosis-related diseases.[10] In our study, we have shown that sVCAM-1 is elevated in CCS compared to controls. The accumulation of inflammatory cells during plaque formation activate endothelial cells that express adhesion molecules such as VCAM-1.[59] Endothelial cell activation is typically induced by proinflammatory cytokines particularly Interleukin-6 and Tumour Necrosis Factor-alpha.[60] This state of endothelial activation and increased inflammation may promote plaques to be more vulnerable to rupture and consequently, to myocardial infarction or acute coronary syndromes.[10]

Retinal vessel analysis~~es~~ support~~s~~ the findings of increased CVD risk among childhood cancer survivors. We found a narrower retinal arteriolar diameter in our leukemia survivor group, but a significantly lower AVR - both parameters have been implicated in increased cardiovascular morbidity and mortality.[61,62] In a large

population-based study analysing 1,900 participants in Rotterdam, each SD decrease in AVR significantly raised the risk of hypertension by 24%.[61] Additionally, we also observed an inverse correlation between AVR and sVCAM-1, a marker which has been shown to be elevated in individuals with arterial stiffness.[63,64]

When analysed independently however, we found a significant inverse relationship between CRAE and CVD risk score among survivors. CRAE was also the sole marker which retained its significance in multiple linear regression analysis. Compared to AVR, generalized retinal arteriolar narrowing is more strongly associated with the risk of hypertension.[61] In a recent Dutch study among overweight and obese children (n=226, median age=13 years), CRAE has been shown to be correlated with cardiovascular risk factors such as plasma glucose and LDL, indicating that retinal vessel analysis parameters may be suitable even in children.[65] Furthermore, we were able to detect significantly higher CRVE in the survivor group in our cohort. Increased retinal venular diameter has been associated with the risk of stroke and cerebral infarction[66], heart diseases[67] and overall increased cardiovascular deaths.[68] Taken together, retinal vessel analysis gives a real-time reflection of systemic microvascular health, and may be used as a predictive tool to identify CCS at risk of cardiovascular disease. Its relative simplicity and affordable infrastructural requirements make retinal vessel analysis a useful addition to late-effects surveillance clinics for cancer survivors.

REFERENCES

1. Armstrong GT, Kawashima T, Leisenring W, et al. Aging and Risk of Severe, Disabling, Life-Threatening, and Fatal Events in the Childhood Cancer Survivor Study. *J Clin Oncol.* 2014;32(12):1218-1227.
2. Armstrong GT, Liu Q, Yasui Y, et al. Late mortality among 5-year survivors of childhood cancer: a summary from the Childhood Cancer Survivor Study. *J Clin Oncol.* 2009;27(14):2328-2338.
3. Kero AE, Jarvela LS, Arola M, et al. Cardiovascular morbidity in long-term survivors of early-onset cancer: a population-based study. *International journal of cancer. Journal international du cancer.* 2014;134(3):664-673.
4. Tukenova M, Guibout C, Oberlin O, et al. Role of cancer treatment in long-term overall and cardiovascular mortality after childhood cancer. *J Clin Oncol.* 2010;28(8):1308-1315.
5. Juonala M, Viikari JS, Kahonen M, et al. Life-time risk factors and progression of carotid atherosclerosis in young adults: the Cardiovascular Risk in Young Finns study. *Eur Heart J.* 2010;31(14):1745-1751.
6. Gidding SS, Rana JS, Prendergast C, et al. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Risk Score in Young Adults Predicts Coronary Artery and Abdominal Aorta Calcium in Middle Age: The CARDIA Study. *Circulation.* 2016;133(2):139-146.
7. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med.* 2005;352(16):1685-1695.
8. Momiyama Y, Adachi H, Fairweather D, Ishizaka N, Saita E. Inflammation, Atherosclerosis and Coronary Artery Disease. *Clin Med Insights Cardiol.* 2014;8(Suppl 3):67-70.
9. Bruyndonckx L, Hoymans VY, Van Craenenbroeck AH, et al. Assessment of endothelial dysfunction in childhood obesity and clinical use. *Oxid Med Cell Longev.* 2013;2013:174782.
10. Constans J, Conri C. Circulating markers of endothelial function in cardiovascular disease. *Clin Chim Acta.* 2006;368(1-2):33-47.
11. Widlansky ME, Gokce N, Keaney JF, Jr., Vita JA. The clinical implications of endothelial dysfunction. *J Am Coll Cardiol.* 2003;42(7):1149-1160.
12. Jenei Z, Bardi E, Magyar MT, Horvath A, Paragh G, Kiss C. Anthracycline causes impaired vascular endothelial function and aortic stiffness in long term survivors of childhood cancer. *Pathol Oncol Res.* 2013;19(3):375-383.
13. Chow AY, Chin C, Dahl G, Rosenthal DN. Anthracyclines cause endothelial injury in pediatric cancer patients: a pilot study. *J Clin Oncol.* 2006;24(6):925-928.
14. Jang WJ, Choi DY, Jeon IS. Vascular endothelial dysfunction after anthracyclines treatment in children with acute lymphoblastic leukemia. *Korean J Pediatr.* 2013;56(3):130-134.
15. Taqueti VR, Solomon SD, Shah AM, et al. Coronary microvascular dysfunction and future risk of heart failure with preserved ejection fraction. *Eur Heart J.* 2018;39(10):840-849.
16. Perez-Rovira A, MacGillivray T, Trucco E, et al. VAMPIRE: Vessel assessment and measurement platform for images of the REtina. *Conf Proc IEEE Eng Med Biol Soc.* 2011;2011:3391-3394.
17. Blindbaek SL, Torp TL, Lundberg K, et al. Noninvasive Retinal Markers in Diabetic Retinopathy: Advancing from Bench towards Bedside. *J Diabetes Res.* 2017;2017:2562759.
18. Rasmussen ML, Broe R, Frydkjaer-Olsen U, et al. Retinal vascular geometry and its association to microvascular complications in patients with type 1 diabetes: the Danish Cohort of Pediatric Diabetes 1987 (DCPD1987). *Graefes Arch Clin Exp Ophthalmol.* 2017;255(2):293-299.
19. Hubbard LD, Brothers RJ, King WN, et al. Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the Atherosclerosis Risk in Communities Study. *Ophthalmology.* 1999;106(12):2269-2280.
20. Heitmar R, Lip GYH, Ryder RE, Blann AD. Retinal vessel diameters and reactivity in diabetes mellitus and/or cardiovascular disease. *Cardiovasc Diabetol.* 2017;16(1):56.

21. Phan K, Mitchell P, Liew G, et al. Association between Retinal Arteriolar and Venule Calibre with Prevalent Heart Failure: A Cross-Sectional Study. *PloS one.* 2015;10(12):e0144850.
22. Csincsik L, MacGillivray TJ, Flynn E, et al. Peripheral Retinal Imaging Biomarkers for Alzheimer's Disease: A Pilot Study. *Ophthalmic Res.* 2018;59(4):182-192.
23. Drobnjak D, Munch IC, Glumer C, et al. Retinal Vessel Diameters and Their Relationship with Cardiovascular Risk and All-Cause Mortality in the Inter99 Eye Study: A 15-Year Follow-Up. *J Ophthalmol.* 2016;2016:6138659.
24. Guo VY, Chan JC, Chung H, et al. Retinal Information is Independently Associated with Cardiovascular Disease in Patients with Type 2 diabetes. *Scientific reports.* 2016;6:19053.
25. Roy MS, Klein R, Janal MN. Relationship of retinal vessel caliber to cardiovascular disease and mortality in African Americans with type 1 diabetes mellitus. *Arch Ophthalmol.* 2012;130(5):561-567.
26. Wong TY, Klein R, Couper DJ, et al. Retinal microvascular abnormalities and incident stroke: the Atherosclerosis Risk in Communities Study. *Lancet.* 2001;358(9288):1134-1140.
27. Mutlu U, Ikram MK, Wolters FJ, Hofman A, Klaver CC, Ikram MA. Retinal Microvasculature Is Associated With Long-Term Survival in the General Adult Dutch Population. *Hypertension.* 2016;67(2):281-287.
28. Pencina MJ, D'Agostino RB, Sr., Larson MG, Massaro JM, Vasan RS. Predicting the 30-year risk of cardiovascular disease: the framingham heart study. *Circulation.* 2009;119(24):3078-3084.
29. Karamanoglu M, O'Rourke MF, Avolio AP, Kelly RP. An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. *Eur Heart J.* 1993;14(2):160-167.
30. Trucco E, Ballerini L, Relan D, et al. Novel VAMPIRE algorithms for quantitative analysis of the retinal vasculature. Paper presented at: 2013 ISSNIP Biosignals and Biorobotics Conference: Biosignals and Robotics for Better and Safer Living (BRC); 18-20 Feb. 2013, 2013.
31. Trucco E, Giachetti A, Ballerini L, Relan D, Cavinato A, MacGillivray T. Morphometric Measurements of The Retinal Vasculature in Fundus Images with Vampire. In: Lim J-H, Ong S, Xiong W, eds. *Biomedical Image Understanding*: John Wiley & Sons Inc.; 2015:91-112.
32. Sumukadas D, McMurdo M, Pieretti I, et al. Association between retinal vasculature and muscle mass in older people. *Arch Gerontol Geriatr.* 2015;61(3):425-428.
33. Mookiah MRK, McGrory S, Hogg S, et al. Towards Standardization of Retinal Vascular Measurements: On the Effect of Image Centering. 2018; Cham.
34. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BE. Revised formulas for summarizing retinal vessel diameters. *Curr Eye Res.* 2003;27(3):143-149.
35. Lupascu CA, Tegolo D, Trucco E. Accurate estimation of retinal vessel width using bagged decision trees and an extended multiresolution Hermite model. *Med Image Anal.* 2013;17(8):1164-1180.
36. Pluimakers VG, van Waas M, Neggers S, van den Heuvel-Eibrink MM. Metabolic syndrome as cardiovascular risk factor in childhood cancer survivors. *Crit Rev Oncol Hematol.* 2019;133:129-141.
37. Felicetti F, D'Ascenzo F, Moretti C, et al. Prevalence of cardiovascular risk factors in long-term survivors of childhood cancer: 16 years follow up from a prospective registry. *Eur J Prev Cardiol.* 2015;22(6):762-770.
38. Bansal N, Amdani SM, Hutchins KK, Lipshultz SE. Cardiovascular disease in survivors of childhood cancer. *Current opinion in pediatrics.* 2018;30(5):628-638.
39. Winther JF, Bhatia S, Cederkvist L, et al. Risk of cardiovascular disease among Nordic childhood cancer survivors with diabetes mellitus: A report from adult life after childhood cancer in Scandinavia. *Cancer.* 2018;124(22):4393-4400.

40. Ariffin H, Azanan MS, Abd Ghafar SS, et al. Young adult survivors of childhood acute lymphoblastic leukemia show evidence of chronic inflammation and cellular aging. *Cancer.* 2017;123(21):4207-4214.
41. Kang ES, Kim HJ, Ahn CW, et al. Relationship of serum high sensitivity C-reactive protein to metabolic syndrome and microvascular complications in type 2 diabetes. *Diabetes Res Clin Pr.* 2005;69(2):151-159.
42. Ridker PM, Bassuk SS, Toth PP. C-reactive protein and risk of cardiovascular disease: evidence and clinical application. *Curr Atheroscler Rep.* 2003;5(5):341-349.
43. Rost NS, Wolf PA, Kase CS, et al. Plasma concentration of C-reactive protein and risk of ischemic stroke and transient ischemic attack: the Framingham study. *Stroke.* 2001;32(11):2575-2579.
44. De Muro P, Lepedda AJ, Nieddu G, et al. Evaluation of Early Markers of Nephropathy in Patients with Type 2 Diabetes Mellitus. *Biochem Res Int.* 2016;2016:7497614.
45. Brantsma AH, Bakker SJ, Hillege HL, et al. Urinary albumin excretion and its relation with C-reactive protein and the metabolic syndrome in the prediction of type 2 diabetes. *Diabetes Care.* 2005;28(10):2525-2530.
46. Mojahedi MJ, Bonakdaran S, Hami M, Sheikhian MR, Shakeri MT, Aiatollahi H. Elevated serum C-reactive protein level and microalbuminuria in patients with type 2 diabetes mellitus. *Iran J Kidney Dis.* 2009;3(1):12-16.
47. Aleman BM, Moser EC, Nuver J, et al. Cardiovascular disease after cancer therapy. *EJC Suppl.* 2014;12(1):18-28.
48. Darby SC, Ewertz M, McGale P, et al. Risk of ischemic heart disease in women after radiotherapy for breast cancer. *N Engl J Med.* 2013;368(11):987-998.
49. Oeffinger KC. Are survivors of acute lymphoblastic leukemia (ALL) at increased risk of cardiovascular disease? *Pediatric blood & cancer.* 2008;50(2 Suppl):462-467; discussion 468.
50. Meacham LR, Chow EJ, Ness KK, et al. Cardiovascular risk factors in adult survivors of pediatric cancer--a report from the childhood cancer survivor study. *Cancer Epidemiol Biomarkers Prev.* 2010;19(1):170-181.
51. de Haas EC, Oosting SF, Lefrandt JD, Wolffenbuttel BH, Sleijfer DT, Gietema JA. The metabolic syndrome in cancer survivors. *Lancet Oncol.* 2010;11(2):193-203.
52. Hershko C, Link G, Tzahor M, et al. Anthracycline toxicity is potentiated by iron and inhibited by deferoxamine: studies in rat heart cells in culture. *J Lab Clin Med.* 1993;122(3):245-251.
53. Kertmen N, Aksoy S, Uner A, et al. Which sequence best protects the heart against trastuzumab and anthracycline toxicity? An electron microscopy study in rats. *Anticancer Res.* 2015;35(2):857-864.
54. Bansal N, Amdani S, Lipshultz ER, Lipshultz SE. Chemotherapy-induced cardiotoxicity in children. *Expert Opin Drug Metab Toxicol.* 2017:1-16.
55. Lipshultz SE, Landy DC, Lopez-Mitnik G, et al. Cardiovascular status of childhood cancer survivors exposed and unexposed to cardiotoxic therapy. *J Clin Oncol.* 2012;30(10):1050-1057.
56. Franco VI, Lipshultz SE. Cardiac complications in childhood cancer survivors treated with anthracyclines. *Cardiol Young.* 2015;25 Suppl 2:107-116.
57. Brouwer CA, Postma A, Hooimeijer HL, et al. Endothelial damage in long-term survivors of childhood cancer. *J Clin Oncol.* 2013;31(31):3906-3913.
58. Krystal JI, Reppucci M, Mayr T, Fish JD, Sethna C. Arterial stiffness in childhood cancer survivors. *Pediatric blood & cancer.* 2015;62(10):1832-1837.
59. Videm V, Albrigtsen M. Soluble ICAM-1 and VCAM-1 as markers of endothelial activation. *Scandinavian journal of immunology.* 2008;67(5):523-531.
60. Liao JK. Linking endothelial dysfunction with endothelial cell activation. *J Clin Invest.* 2013;123(2):540-541.

61. Ikram MK, de Jong FJ, Bos MJ, et al. Retinal vessel diameters and risk of stroke: the Rotterdam Study. *Neurology.* 2006;66(9):1339-1343.
62. McGeechan K, Liew G, Macaskill P, et al. Meta-analysis: retinal vessel caliber and risk for coronary heart disease. *Ann Intern Med.* 2009;151(6):404-413.
63. Kals J, Kampus P, Kals M, et al. Inflammation and oxidative stress are associated differently with endothelial function and arterial stiffness in healthy subjects and in patients with atherosclerosis. *Scand J Clin Lab Invest.* 2008;68(7):594-601.
64. de Faria AP, Ritter AM, Sabbatini AR, et al. Deregulation of Soluble Adhesion Molecules in Resistant Hypertension and Its Role in Cardiovascular Remodeling. *Circ J.* 2016;80(5):1196-1201.
65. Rijks J, Vreugdenhil A, Dorenbos E, et al. Characteristics of the retinal microvasculature in association with cardiovascular risk markers in children with overweight, obesity and morbid obesity. *Scientific reports.* 2018;8(1):16952.
66. Ikram MK, De Jong FJ, Van Dijk EJ, et al. Retinal vessel diameters and cerebral small vessel disease: the Rotterdam Scan Study. *Brain.* 2006;129(Pt 1):182-188.
67. Wang JJ, Liew G, Wong TY, et al. Retinal vascular calibre and the risk of coronary heart disease-related death. *Heart.* 2006;92(11):1583-1587.
68. Wang JJ, Liew G, Klein R, et al. Retinal vessel diameter and cardiovascular mortality: pooled data analysis from two older populations. *Eur Heart J.* 2007;28(16):1984-1992.

Table 1. Demographic of survivors and controls

	Group	
	Survivors	Controls
Number in each group	78	78
Sex, n (%)		
Male	39 (50%)	31 (44%)
Female	39 (50%)	40 (56%)
Age at recruitment, years	26 (23-29)	24 (23-27)
Age at diagnosis, years	5 (4-9)	NA
*BMI, kg/m^2	23.7 (20.7-28.7)	22.1 (20.4-24.9)
Diagnosis, n (%)		
ALL	73 (94%)	NA
AML	5 (6%)	NA
Duration since chemotherapy, years	19 (14-22)	NA
Chemotherapy received		
Antracyclines, n (%)	56 (72%)	NA
Antracycline cumulative dosage, mg/m^2	240 (0-240)	NA
Alkylating agents, n (%)	57 (73%)	NA
Alkylating agent cumulative dosage, mg/m^2	2000 (0-3000)	NA
Radiotherapy received, n (%)	38 (49%)	NA
Radiotherapy dosage, Gy	18 (0-18)	NA
*Smoking status, n (%)		
Smoker	12 (15%)	2 (3%)
Non-smoker	46 (59%)	66 (93%)
Not available	20 (26%)	1 (1%)

Data shown are median (interquartile range, IQR) or n (%).

NA indicates not applicable.

*Variables that are significantly different ($p<0.05$ on Mann Whitney or Chi-square tests) in survivors compared to controls.

Table 2. Association between retinal vessel diameters, endothelial dysfunction markers and covariates of age, gender and smoking status with Framingham's 30-year full CVD risk score calculated using multiple linear regression

	Coefficient, β	95% CI	P-value
AIx, %	-0.015	-0.129 to 0.098	0.780
sVCAM-1, pg/mL	8.181×10^{-6}	-3.689×10^{-5} to -5.325×10^{-5}	0.703
sICAM-1, pg/mL	1.733×10^{-5}	-5.367×10^{-6} to -4.003×10^{-5}	0.124
CRAE, px	-0.381	-0.735 to -0.026	0.037*
CRVE, px	0.234	-0.151 to 0.618	0.213
Age, year	0.830	0.496 to 1.164	<0.001*
Smoking status, smoker	6.444	2.704 to 10.184	0.002*
Gender, male	4.529	1.166 to 7.891	0.012*

Variable were initially checked for collinearity using linear regression and variable with variance inflation factor (VIF) of >10 (AVR) was excluded.

* denotes significant p-value

Figure legends

Figure 1. Clinical cardiovascular risk factor for leukemia survivors and controls. **(A)** Glucose, **(B)** total cholesterol, **(C)** systolic blood pressure, **(D)** diastolic blood pressure, **(E)** triglyceride, **(F)** high-density lipoprotein (HDL), **(G)** low-density lipoprotein (LDL), and **(H)** high-sensitivity C-reactive protein (hsCRP). Survivors had higher blood glucose, total serum cholesterol, blood pressure, LDL and hsCRP, and had lower HDL compared to controls.

Figure 2: 30-year risk of full CVD for leukemia survivors and controls. 30-year Framingham Heart Study Risk Score for full CVD calculated using lipid profile. * denotes significant p-value calculated using Mann-Whitney U-test. Bars represent median and interquartile range.

Figure 3. Arterial stiffness and endothelial activation markers for leukemia survivors and controls. Survivors had higher **(A)** augmentation index (AIx) and **(B)** soluble VCAM-1 (sVCAM-1) compared to controls. Level of **(C)** soluble ICAM-1 (sICAM-1) between survivors and controls were similar. * denotes significant p-values calculated using Mann-Whitney U-test. Bars represent median and interquartile range.

Figure 4. Retinal vessel caliber analysis of leukemia survivors and controls. (A) Central retinal artery equivalent (CRAE) **(B)** central retinal vein equivalent (CRVE) and **(C)** arterio-venous ratio (AVR). Survivors had higher CRVE and lower AVR compared to controls. * denotes significant p-values calculated using Mann-Whitney U-test. Bars represent median and interquartile range.

Figure 5. Relationship of retinal vessel calibre analysis and endothelial dysfunction markers with Framingham's 30-year risk for full CVD. An analysis of covariance (ANCOVA) was utilised to assess differences in retinal vessel calibre parameters: **(A)** CRAE, **(B)** CRVE and **(C)** AVR & endothelial dysfunction markers: **(D)** augmentation index, **(E)** sVCAM-1 and **(F)** sICAM-1; among survivors with low and intermediate risk for Framingham's 30-year CVD risk score, adjusting for significant covariate (age). $P<0.05$ were considered statistically significant and are marked with an asterisk. Bars indicate medians and interquartile ranges.