Early life environment and the developing cardiovascular system

Nikmah Salamia Idris

Early life environment and the developing cardiovascular system
PhD thesis, Utrecht University, the Netherlands, with a summary in Dutch

ISBN

Author	Nikmah S Idris
Cover design by	The Educational Resource Centre, Royal Children's Hospital Melbourne
	The leaves/growth reflect the anatomy of the heart, major vessels, and lungs
Lay out by	Nikmah S Idris
Printed by	Proefschriftmaken.nl || Uitgeverij BOXPress
Published by	Uitgeverij BOXPress, 's-Hertogenbosch

© 2015 Nikmah S Idris, Utrecht, the Netherlands.

Early life environment and the developing cardiovascular system

Omgeving in het vroege leven en het ontwikkelende cardiovasculaire systeem

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor
aan de Universiteit Utrecht op gezag van

de rector magnificus, prof. dr. G.J. van der Zwaan,
ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op
donderdag 26 mei 2015 des middags te 12.45 uur

door
Nikmah Salamia Idris
geboren op 31 februari 1977 te Bandung, Indonesië

Promotoren:	Prof. dr. D.E. Grobbee
Copromotor:	dr. C.S.P.M. Uiterwaal

Contents

Chapter 1	General Introduction	7
Part I.	**Early life nurture and cardiovascular risk**	
Chapter 2	Factors predicting breastfeeding plan of Asian women	13
Chapter 3	Famine in childhood and coronary artery calcification	23
Chapter 4	Physical activity and vascular characteristics in young children	39
Part II.	**Childhood HIV infection and cardiovascular health**	
Chapter 5	Cardiovascular manifestations of HIV Infection in children	57
Chapter 6	Effects of paediatric HIV infection on childhood vasculature	81
Chapter 7	Effects of paediatric HIV infection on electrical conduction of the heart	95
Chapter 8	Cardiac effects of antiretroviral-naïve versus antiretroviral-exposed HIV infection in children	109
Chapter 9	General discussion	
Samenvatting/Summary		125
Acknowledgment		145
Manuscript based on studies presented in this thesis		149
Contributing authors		150
Curriculum vitae		152
Appendices		153

Chapter 1

General introduction

Background and objectives

The dynamics of cardiovascular system development in childhood are still largely unknown. Despite its known sensitivity to small perturbations,[1] how the cardiovascular system evolves and responds to different stimuli and how these impact the future cardiovascular status has not been fully elucidated. In recent years, after Barker and colleagues brought up the Developmental Origin of Adult Health and Diseases (DOHAD) hypothesis,[2] which was based on their observations of the probable association between low birth weight and later cardiovascular diseases, a large number of studies have been done in this area, trying to investigate early life determinants of future cardiovascular disease. Nevertheless, the complete picture is not yet established. A difficulty there is particularly that the cardiovascular system is very dynamic at a young age, continues to develop during childhood and beyond and is altered by various different factors throughout the growth and developmental phases. Studies are also hampered by long observations needed to find the true associations between proposed determinants and the occurrence of actual diseases in later life. Studies of sufficiently long follow-up are very scarce to date. In order to bypass that, usually surrogate markers or proxy outcome measurements for cardiovascular disease are used in childhood studies, or case control type designs are used, each with inherent limitations.

This thesis is basically aimed at constructing pieces of evidence to fill in some the present knowledge gaps. We particularly focus on postnatal possible determinants as relatively little work has been done in that area and because such determinants could have immediate effects on the childhood cardiovascular system, perhaps contributing to risk for clinically manifest diseases in later life. Particular attention is given to childhood conditions that are relevant for low-to-middle income countries (first part). There is currently a strong upsurge in the occurrence of cardiovascular diseases in those regions of the world,[3] while certainly there, many of the determinants, such as child-hood infection, malnutrition, physical activity and breastfeeding issues, have been relatively been untouched by previous studies. We specifically also look at HIV infection (second part) as a model of childhood infection and inflammation that may cause immediate cardiovascular problems in childhood as well as not readily manifest cardiovascular structural and functional changes that may potentiate increased risk of cardiovascular diseases in adulthood. HIV and/or treatment in adults has been recognised to increase cardiovascular disease risk. We have the unique opportunity to look at antiretroviral (ART)-naive HIV infection and the ART-exposed one, and help us tease out the effects of HIV infection itself from combined effects which treatment, which has never been clearly described before.

Our ultimate objective is that the evidence that we provide in this thesis can aid in the design of effective cardiovascular disease prevention. An assumption that we consider reasonable and justifiable is that such prevention should begin in early life if evidence

provides sufficient clues for risk mediation, and knowing that the pathologic (atherosclerotic) process starts in early childhood. Moreover, we consider ultimate applicability in a global setting important, as cardiovascular disease is already one of the three top causes of morbidity and mortality worldwide.[4] Prevention is particularly important in the less affluent regions with limited human, infrastructure, and financial resources for an effective curative measures.

Methods

All studies described in this thesis were embedded in the following different cohorts or observational studies:

1. A pilot/feasibility study of a currently enrolling randomised breastfeeding optimisation trial in Indonesia. Within this study, we investigated determinants of breastfeeding planning in pregnant women.
2. A Prospect-European Prospective Investigation into Cancer and Nutrition (Prospect-EPIC), part of the EPIC, cohort, which enrolled 17 357 healthy 49-year-old to 70-year-old women who underwent breast cancer screening and lived in the Dutch city of Utrecht or the surrounding area between 1993 and 1997. Within this study, we investigated the effects of famine exposure in childhood on coronary artery calcifications on 573 postmenopausal women.
3. An ongoing Wheezing Illnesses Study in Leidsche Rijn (WHISTLER) birth cohort set up in December 2001 in Utrecht, the Netherlands. Briefly, around 2600 healthy neonates born at a gestational age of at least 36 weeks with no major congenital anomalies or neonatal respiratory diseases have been enrolled. In 2007, the study was extended for cardiovascular research questions (WHISTLER-Cardio) and all 5-year-old children were invited for follow-up measurements. Starting in 2010, children who had participated in the measurements at age 5 years were re-invited at the age of 8 years for further follow up. Within this cohort, we studied the effects of physical activity on childhood vascular characteristics.
4. A cohort of 111 HIV infected and 51 healthy children established in June 2013 in Jakarta, Indonesia. HIV infected children (56 newly diagnosed antiretroviral (ART)-naïve and 58 ART-exposed) were recruited from the paediatric HIV clinics of Cipto Mangunkusumo National General Hospital, Koja District General Hospital, and the Indonesian Planned Parenthood Association (IPPA), Jakarta Indonesia. We performed cross-sectional analyses on the effect of ART-naive and ART-exposed HIV infection on childhood cardiac status and potential future cardiovascular risk.

Outlines

This thesis consists of two parts. The first part mainly looks at various childhood exposures that are particularly relevant for low-to-middle income countries and that

may determine the occurrence of cardiovascular diseases in adulthood. These include (1) determinants of breastfeeding planning, with breastfeeding having been previously reported to be implicated in carotid intima-media thickness and vascular distensibility;[5] (2) famine in childhood; and (3) physical activity.

The second part focuses on HIV infection as a model of childhood infection that may cause immediate cardiovascular problems in childhood as well as may potentiate future cardiovascular disease risk. We begin part two by presenting a review on current evidence in this area, highlighting knowledge gaps, which potentially require further research. This review is followed by three chapters describing the effects of ART-naive and ART-exposed HIV infection on vascular characteristics, cardiac structure and function, and heart conduction.

References

1. Srivastava D, Olson EN. A genetic blueprint for cardiac development. Nature. 2000;407:221-6.
2. Barker DJ. Human growth and chronic disease: a memorial to Jim Tanner. Ann Hum Biol. 2012;39:335-41.
3. Yusuf S, Rangarajan S, Teo K, Islam S, Li W, Liu L, et al. Cardiovascular risk and events in 17 low-, middle-, and high-income countries. N Engl J Med. 2014;371:818-27.
4. Kelly BB, Narula J, Fuster V. Recognizing global burden of cardiovascular disease and related chronic diseases. Mt Sinai J Med. 2012;79:632-40.
5. Evelein AM, Geerts CC, Visseren FL, Bots ML, van der Ent CK, Grobbee DE, et al. The association between breastfeeding and the cardiovascular system in early childhood. Am J Clin Nutr. 2011;93:712-8.

Part I

Early life nurture and cardiovascular risk

Chapter 2

Exclusive breastfeeding plan of pregnant South-East Asian women: what encourages them?

Nikmah Salamia Idris
Sudigdo Sastroasmoro
Fatimah Hidayati
Irma Sapriani
Rulina Suradi
Diederick E. Grobbee
Cuno S.P.M Uiterwaal

Breastfeed Med. 2013 Jun;8(3):317-20

Abstract

Objectives

To investigate factors involved in breastfeeding planning of Asian pregnant women.

Methods

A cross-sectional study was conducted on 207 pregnant women visiting the Budi Kemuliaan Hospital, Jakarta between June - Aug 2011. The planned breastfeeding duration and determinants were sought using a standardised self reported questionnaire.

Results

Most subjects had low income (84.1%) and education (79.7%). Women who had been informed about breastfeeding had a higher likelihood to plan longer (> 6 months) breastfeeding (OR 1.97; 95% CI 1.04-3.75; p = 0.04) while women who breastfed previous children over a shorter period had a lower likelihood (OR 0.26; 95% CI 0.11-0.59; p=0.001). Age, low education, first pregnancy, and low income had no association with breastfeeding plans. Working mothers who had to return to work before 6 months and worked for >8 hours/day were less likely to plan longer breastfeeding (OR 0.14; 95% CI 0.02-0.83; p=0.03, OR 0.53; 95%CI 0.17-1.63; p=0.27, respectively) while those intending to express their milk were more likely to breast feed longer (OR 16.85; 95%CI 4.21-67.48, p<0.001).

Conclusions

Planning of prolonged breastfeeding has little to do with maternal age, education, or number of pregnancies. However, mothers who work, previously breastfed for a short period and who are not well informed about breastfeeding tend to plan shorter breastfeeding. Among mothers who work, it is the length of maternal leave and required working hours that determine the plans.

Background

The importance of breastfeeding in early child nutrition is widely acknowledged.[1] Evidence regarding its benefits for future health outcomes is also growing.[2] Breastfeeding has been formally endorsed and promoted globally since the last two decades when WHO and UNICEF launched the Baby-friendly Hospital Initiative (BFHI) in 1991 following the Innocenti Declaration of 1990.[3] The initiative aims at protecting, promoting, and supporting exclusive breastfeeding for 6 months by developing different tools and materials that were implemented universally in more than 152 countries. It also has measurable and proven impact, increasing the likelihood of babies being exclusively breastfed for the first 6 month.[4] A 6-month exclusive breastfeeding compared to a shorter duration has been suggested to result in less gastrointestinal morbidity without adverse effects on growth.[5]

Despite the established benefits and intensive campaigning, breastfeeding rates in Asian countries are still very low. For example, a national survey in 2010 conducted by the Ministry of Health of Indonesia revealed that the percentage of women who give exclusive breastfeeding for the first six month is only 15.3%.[6] A study on the secondary data of national demographic and health survey among Asian countries reported similar rates: Vietnam 15.5%, Timor Leste 30.7%, Philippines 33.7%.[7] The same study suggested several factors that might be associated with non-exclusive breastfeeding, notably are mother's work, first children, low number of antenatal clinic visits, and higher proportions of richer households. However, these studies reflect survey data, and it is important to know about factors that determine women's plans for breastfeeding during their pregnancy as this creates a window of opportunity for intervention and management. Clearly, the planning and lactation instruction about breastfeeding should start then. With a view to future optimisation of breastfeeding it is of relevance to determine whether the women's circumstances particularly determine breastfeeding plans or whether a woman's beliefs, level of information, or aversion against breastfeeding are determining her plans. Specifically, it is still largely unknown which factors encourage giving 6-month exclusive breastfeeding.

We analysed factors influencing a mothers plans for breastfeeding during the phase of pregnancy using data from a feasibility study for a large breastfeeding optimisation trial in Indonesian pregnant women.

Methods

This cross-sectional study was conducted on pregnant women visiting the antenatal clinic Budi Kemuliaan Hospital, Jakarta between June-August 2011. The Budi Kemuliaan Hospital is a private hospital in the Jakarta municipality, specialised in mother and child care with some 7,800 births registered each year, which is among the highest delivery rates in Indonesia. This study was approved by the Ethics Committee of Medical Faculty University of Indonesia. Informed consent was obtained for every subject.

A short questionnaire about identity and demographic characteristics (8 combined open and closed questions), breastfeeding plan and previous practice (6 closed questions), as well as working situation (6 closed questions) was handed out and filled out by the subjects after brief explanation. We emphasised the importance of giving the true answers reflecting their plan instead of 'socially acceptable' ones and told them as researchers that we did not have any specific preference for answers. The questionnaire was first tested on 15 pregnant women to ensure its comprehensiveness and most could answer the questions within 10 to15 minutes. After a pregnant woman filled out the questionnaire, the authors performed a brief review together with the woman and checked for its completeness. We sought for age, pregnancy order, education, income, previous information/education about breastfeeding, previous breastfeeding practice, and working status/situation as the proposed determinants of planning to breast feed for 6 months or longer (Table 1).

Characteristics of the pregnant women were first classified by breastfeeding planning. These characteristics were then evaluated as possible determinants of breastfeeding using a univariable logistic regression model with breastfeeding plan for 6 months (yes/no) as the dependent variable and characteristics as independent variables. Subsequently, the same logistic regression model was used for multivariable analysis. As previous findings from Indonesian survey indicate that working of the mothers might be an important determinant of breastfeeding plans, we subsequently analysed the group of working mothers separately.

Results are expressed as odds ratios and 95% confidence intervals. Intervals not including one, corresponding to p-values of less than 0.05 were considered statistically significant. Statistical analysis was performed using the IBM® SPSS® Statistic Version 19 for Mac computer program.

Results

The characteristics of women intending to breast feed for less than 6 months and of those intending to breast feed six months or longer are shown in Table 2. We included 207 women during the study period with ages ranging from 17 to 45 years; 6 women refused to participate because needed to undergo urgent antenatal evaluation. Most subjects had a low income (84.1%) and education (79.7%) and almost half (43.5%) were working mother.

Table 3 shows that age, low education, first pregnancy, and low income had little or no association with intention to breast feed for 6 months or longer, neither in the univariable nor in the multivariable analysis. Having been informed about breastfeeding showed an almost twice higher likelihood of breastfeeding for 6 months or longer. The results also showed that if a mother had breastfed her previous child for less than 6 month, the probability that she planned a long breastfeeding period for the next child decreased by almost 75%. Working affected breastfeeding plans although not statistically significant.

Table 1. Outcome and determinants

		Definitions
Outcome variables	Exclusive breast feeding	Infants should receive only breast milk and nothing else, with exceptions such as oral rehydration solution, syrups, vitamins, minerals, and medicines. This term included breast feeding by a wet nurse and feeding by expressed milk*
	Plan to breast feed for less than 6 months	Explicit intention to breast feed for less than 6 months or plan of giving early supplemental formula in case the breast milk production was perceived as inadequate
Proposed determinants	Age	Time duration between date of birth and date of inclusion
	Low education	Primary or secondary school graduate
	Low income	Family income was less than IDR 2,960,723/month#
	Having been informed about breast feeding	Had received any previous information or education regarding breast feeding, either from electronic/print media, health professionals, or peer counselors
	Previous breast feeding of less than 6 months	History of exclusive breast feeding for less than 6 months in any previous child
	First pregnancy	The first pregnancy of mothers at the time of study inclusion, excluding previous abortion/terminated pregnancy
	Working mother	Refers to women who are mothers and who work outside of the home for income in addition they perform at home raising their children.
	Work for more > 8 hours/ day	Spend more than 8 hours/day for working outside the house (including time spent for travel between home and work place)

*Definition based on: World Health Organisation (WHO). Indicators for assessing breastfeeding practices: report of an informal meeting. Geneva, Switzerland: WHO; 1991.
#Conforms to 'poor' and 'poorest' economic classification of the World Bank

Table 2. Characteristics of the study population of Indonesian pregnant women according to planned duration of breastfeeding.

Variables	Univariable			Multivariable		
	OR	95% CI	P	OR	95% CI	P
Working mother	0.62	0.35 - 1.11	0.11	0.544	0.29 - 1.01	0.05
Age	0.94	0.94 - 1.05	0.76	0.99	0.93 -1.06	0.83
Low education	0.88	0.43 - 1.78	0.72	0.88	0.41 - 1.92	0.76
First pregnancy	1.17	0.66 - 2.11	0.59	1.27	0.61 - 2.66	0.53
Low income	0.89	0.39 - 2.03	0.78	0.96	0.40 - 2.31	0.93
Informed about breastfeeding	1.7	0.93 - 3.10	0.09	1.97	1.04 - 3.75	0.04
Previous breastfeeding of <6 months	0.26	0.11 - 0.59	0.001			

We further performed a subgroup analysis on 90 working mothers to find out working-associated factors that might determine their breastfeeding plan (Table 4). On multivariable analysis, the length of maternal leave affected breastfeeding plans. If a mother had to return to work before 6 months after delivery then she had one seventh times lower chance to plan breastfeeding for 6 months or longer. Working hours of

more than 8 hours might were also related to breastfeeding plans although not statistically significant. On the other hand, working pregnant mothers who planned to express or pump their milk had a markedly higher chance to breast feed longer.

Table 3. Predictors of breastfeeding plan of more than 6 months

Variables	Breastfeed <6 month N = 130	Breastfeed >6 month N = 77
Age, mean (SD)	27.71 (5.58)	27.47 (4.85)
Working mother (N = 201)	61 (49%)	29 (38%)
1st pregnancy (N = 205)	46 (35%)	30 (39%)
Low education (N = 205)	104 (81%)	61 (79%)
Low income (N = 201)	108 (87%)	66 (86%)
Having been informed about breast feeding (N = 201)	72 (58%)	54 (70%)
Previous breast feeding of <6 month (N = 124)	44 (54%)	10 (23%)

*The multivariable analysis for previous breastfeeding was non-estimable due to small numbers.

Table 4. Predictors of breastfeeding plan of more than 6 months: subgroup analysis in working mothers (N = 90)

Variables	Univariable			Multivariable		
	OR	95% CI	P	OR	95% CI	P
Work hour > 8 hours/day	0.57	0.22 - 1.44	0.23	0.53	0.17 - 1.63	0.27
Return work < 6 month	0.34	0.09 - 1.39	0.13	0.14	0.02 - 0.83	0.03
Job in public places/frequent travel	0.71	0.29 - 1.73	0.89	2.45	0.67 - 9.92	0.18
Intention to express/pump milk	8.64	3.1 - 24.09	<0.001	16.85	4.21 - 67.48	<0.001
Availability of lactation room	1.06	0.44 - 2.59	0.89	0.48	0.14 - 1.70	0.26
Availability of refrigerator	1.52	0.61 - 3.18	0.37	1.36	0.36 - 5.14	0.65
Available time to express milk	2.27	0.68 - 7.55	0.18	2.28	0.47 - 11.10	0.32

Discussion

The findings in this cross sectional study among 207 pregnant Indonesian women suggest that previous breastfeeding duration and extent of receiving information on breastfeeding during antenatal care determines the length of planned breastfeeding period for the next child. In addition, working outside the house predicted the plan for breastfeeding, but particularly the length of maternal leave and working hours affected the planned duration of breastfeeding.

To appreciate these findings some aspects of the study need to be addressed. A limitation of this study is that it only assessed the planning of breastfeeding as an outcome, not the eventual real practice. Although what a pregnant woman plans is likely

to determine her actual behaviour, there is the possibility that women gave ‘socially acceptable’ answers while they did not really mean to or that they changed their minds later. We tried to minimise this bias by giving explanation to the mothers prior to the questionnaire administration, emphasising the importance of giving true answers reflecting their plan and the neutral position of the investigators as to their answers. In addition, to confirm whether they indeed did not intend to breast feed for less than 6 months, we also asked an implicit question regarding their view toward early supplemental formula feeding. However, the inquiry regarding breastfeeding planning during pregnancy is, on the other hand, a strength of this study since early prediction will make early directed intervention more feasible.

To our knowledge there are limited published studies focusing on the evaluation of the determinants of exclusive breastfeeding in Indonesian women. Most studies were part of a multi-purpose national survey. The 1987 National Indonesia Contraceptive Prevalence Survey revealed that household economic level, language, place of delivery, type of birth attendant, mother's education, mother's occupational experience, spouse's education, spouse's occupation, mother's age at the time of birth, parity, child's sex, and desired pregnancy were associated with duration of breastfeeding.[8] Another more recent study also used secondary data of the national demographic survey and found that mother’s work, first children, low antenatal clinic visits, and higher proportion of richest households were associated with non - exclusive breastfeeding.7 Compared to those studies, our findings are different and more pertinent which may have been caused by differences in design, time setting, subject characteristics, and different determinants studied. Other studies regarding the determinants of exclusive breastfeeding, as expected, showed various results.9-11 In fact, one study which tried to pool the determinants of exclusive breastfeeding in five East and South East Asian countries could not reach results because of the large heterogeneity in results. This suggests that determinants of breastfeeding have strong ethnic, cultural and local characteristics. However the heterogeneity can also have been caused by different factors of interest explored by each national survey.

The Indonesian health authorities have expressed an urge for improving the breastfeeding habits of Indonesian women and interventions to achieve that have already been initiated. We believe that for these interventions to be successful, detailed knowledge of what drives pregnant women in their breastfeeding plans is indispensable. It is particularly important to know whether it is issues like information about breastfeeding or whether it is for instance circumstances at work. Such knowledge may fuel campaigns from several angles and strategies. Our findings suggest that more education should be given to pregnant women regardless of their background education or socioeconomic level. This strategy was also found to be successful in other studies.12 Moreover, our findings suggest that there is a concentration of poor breastfeeding habits among women who

used to breast feed for less than 6 months with previous children, they seemed not likely to change the poor practice into longer breastfeeding for the next child. Although we performed a subgroup analysis on a relatively small number of working mothers, our findings do suggest that regulation regarding maternal leave and work should be reviewed to facilitate the success of 6 month exclusive breastfeeding.

In conclusion, planning of prolonged breastfeeding appears to have little relation with maternal age, education level, or number of pregnancies. However, mothers who work, previously gave breastfeeding for a short period and those who are not well informed about breastfeeding tend not to plan longer periods of breastfeeding. Among mothers who work, it is not so much the circumstances at work that determine the plans for breastfeeding, but the length of maternal leave, required working hours, and intention to pump/express milk that determine the plans.

References

1. Kramer MS, Guo T, Platt RW, Shapiro S, Collet JP, Challmers B, et al. Breastfeeding and infant growth: biology or bias? Pediatrics 2002;110;343-7.
2. Evelein AM, Geerts CC, Visseren FL, Bots ML, van der Ent CK, Grobbee DE, Uiterwaal CS. The association between breastfeeding and the cardiovascular system in early childhood. Am J Clin Nutr. 2011;93:712-8.
3. World Health Organization (WHO). Infant and young child nutrition: global strategy on infant and young child feeding. Geneva, Switzerland: WHO; 2002.
4. Kramer MS, Chalmers B, Hodnett ED, Sevkovskaya Z, Dzikovich I, Shapiro S. Promotion of Breastfeeding Intervention Trial (PROBIT): a randomized trial in the Republic of Belarus. JAMA. 2001 Jan 24-31;285:413-20.
5. Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding. Cochrane Database of Systematic Reviews 2002, Issue 1. Art. No.: CD003517. DOI: 10.1002/14651858.CD003517.
6. The Ministry of Health of Indonesia. Riset kesehatan dasar: Riskesdas 2010. Jakarta: The Agency of Research and Development, The Ministry of Health of Indonesia; 2010.
7. Senarath U, Dibley, MJ, Agho KE. Factors Associated With Nonexclusive Breastfeeding in 5 East and Southeast Asian Countries: A Multilevel analysis. J Hum Lact 2010;26:248-57.
8. Iskandar MB, Costello C, Nasution Y. Initiation and duration of breast-feeding in Indonesia. Asia Pac Popul J. 1990 Mar;5:89-112.
9. Ogbuanu CA, Probst J, Laditka SB, Liu J, baek JD, Glover S. Reasons why women do not initiate breastfeeding: a southeastern state study. Women's Health Issues. 2009;19:268-78.
10. Sachdev HP, Mehrotra S. Predictors of exclusive breastfeeding in early infancy: operational implications. Indian Pediatr. 1995 Dec;32:1287-96.
11. Jones JR, Kogan MD, Singh GK, Dee DL, Grummer-Strawn LM. Factors associated with exclusive breastfeeding in the United States. Pediatrics 2011;128:1117–1125.
12. Tylleskär T, Jackson D, Meda N, Engebretsen IMS, Chopra M, Diallo AH, et al. Exclusive breastfeeding promotion by peer counselors in sub-Saharan Africa (PROMISE-EBF): a cluster-randomised trial. Lancet 2011;378:420–27.

Chapter 3

Famine in childhood and postmenopausal coronary artery calcification

Nikmah Salamia Idris
Cuno S.P.M. Uiterwaal
Yvonne T. van der Schouw
Annet F.M. van Abeelen
Tessa J. Roseboom
Pim A de Jong
Annemarieke Rutten
Diederick E. Grobbee
Sjoerd G. Elias

BMJ Open
2013;3:e003818.

Abstract

Background

We have shown a relation between famine exposure in adolescence and an increased risk for clinically manifest cardiovascular disease. Here we assessed if childhood famine exposure does have an effect on coronary calcium deposition, which is strongly associated with atherosclerosis formation. We also secondarily looked at the effect of famine exposure on cardiac valve and aortic calcifications, which potentially could also explain our previous findings.

Methods

In 286 postmenopausal women with individual measurements of World War II famine exposure during childhood (0-18 years old) in the Netherlands, we used CT scans to score coronary (using the Agatston method), aortic and valvular calcifications (both semi-quantitative). Logistic regression was used with coronary Agatston score of >100 or ≤100, valve or aortic calcifications as dependent variable and an indicator for famine exposure as independent variable. These models were also used for confounder adjustment and stratification based on age groups of 0-9 and 10-17 years old.

Results

In the overall analysis, no statistically significant association was found between severe famine exposure in childhood and a high coronary calcium score (OR 1.80, 95%CI 0.87-3.78). However, when looking at specific risk periods, severe famine exposure during adolescence was related to a higher risk for a high coronary calcium score than non-exposure to famine, both in crude (OR 3.47, 95%CI 1.00-12.07) and adjusted analyses (OR 4.62, 95%CI 1.16-18.43). No statistically significant association was found between childhood famine exposure and valve or aortic calcification (OR 1.66, 95%CI 0.69-4.10).

Conclusion

Famine exposure in childhood, especially during adolescence, seems to be associated with a higher risk of coronary artery calcification in late adulthood. However, the association between childhood famine exposure and cardiac valve/ aortic calcification is less clear.

Background

Cardiovascular diseases remain a leading cause of morbidity and mortality. Globally, ischemic heart and cerebrovascular diseases are major causes of death and it has been projected that this condition will not change in the next 20 years, resulting in 20 million deaths per year in 2030.[1] Despite advances in treatment, such diseases still have a significant impact on quality of life[2] and cause great economic burden. As such, effective prevention is essential.

Evidence suggests that many chronic diseases originate from particular events in early life. Since Barker first proposed the developmental origins of health and diseases (DOHAD) hypothesis,[3,4] many studies have indicated that adverse influences, such as undernutrition during growth and development, might result in permanent physiological and metabolic alterations.[5-7] Such alterations may benefit short-term survival, but at the expense of increased risk of chronic diseases later in life.[8] However, the exact critical periods have yet to be defined 9 as to whether they extend beyond foetal life and infancy into childhood and adolescence.

Although studies on the foetal and infant periods are numerous, there is little data on the effect of childhood and adolescent nutritional disturbances on cardiovascular risk. Moreover, most studies evaluated postnatal disturbances as a consequence of prenatal events. As suggested by several large cohort studies,[10-13] growth patterns after birth seem to be associated with a substantial increase in risk for cardiovascular and metabolic disorders.

Undernutrition during childhood and adolescence may have chronic disease consequences. Studies on the Dutch 1944-45 famine in World War II showed that severe famine exposure in adolescence was associated with a 30% increased risk of coronary heart diseases [14] and a 4-5-fold increased risk of diabetes mellitus and/or peripheral arterial disease in older females.[15] Men who experienced severe starvation around the time of puberty were reported to have a 40-60% increased risk of acute myocardial infarction and stroke.[16] However, despite circumstantial evidence showing that childhood famine may lead to a similar ‘catch up fat phenotype’ as underweight newborns,[17] the mechanism by which food deprivation in these periods increases cardiovascular disease risk remains obscure. Since coronary artery calcium deposition has emerged as a strong predictor for cardiovascular diseases[18,19] and likely represents pathologic alterations of the vessel wall underlying clinically manifested disease, 14 we investigated an association between childhood exposure to famine and coronary calcium deposition. As findings implicating the association between cardiac valve or aortic calcification and coronary cardiovascular events are also emerging,[20-22] we also assessed for an association between childhood undernutrition and extra-coronary (valve and aortic) calcification.

Methods

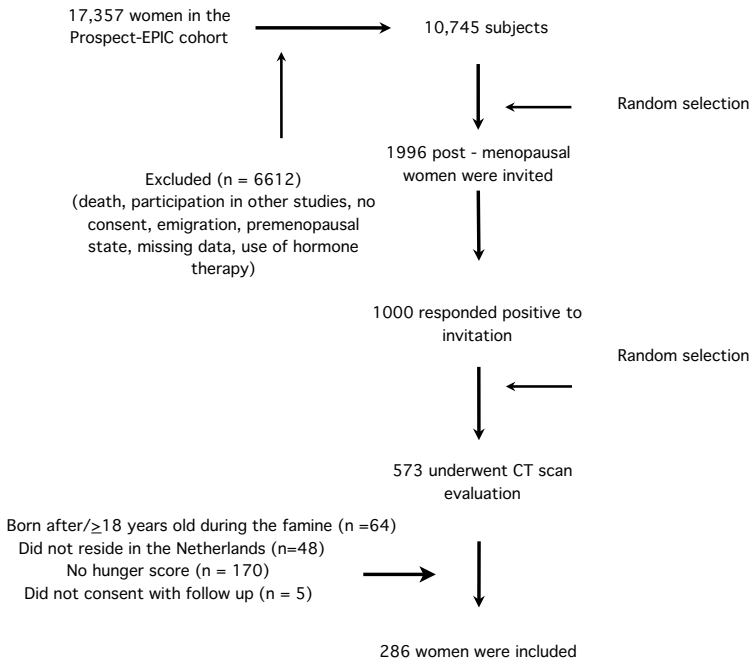
Study population

Our study was a subset of a larger Dutch cohort, Prospect-EPIC, part of the European Prospective Investigation into Cancer and Nutrition (EPIC), as described elsewhere.[23] Prospect-EPIC enrolled 17,357 healthy 49-70 year old women who underwent breast-cancer screening and lived in Utrecht or the surrounding area between 1993 and 1997.

Our subset was comprised of 573 post-menopausal women, who had been evaluated for associations between metabolic and reproductive factors and coronary artery calcium.[24] For our study, pre-menopausal women were excluded (n=1309). After further selection (Figure 1), we obtained coronary CT scans of 573 women. We further excluded women who were born after or aged 18 years or over during the famine (n = 64), resided outside occupied Netherlands during the famine (n = 48), had unavailable hunger scores (n = 170), or did not consent to follow-up (n = 5), leaving 286 women for our analyses. Data was collected from October 2002 until December 2004.

Informed consent was obtained from all women before their inclusion to the study. The study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of the University Medical Centre of Utrecht.

Figure 1. Subjects' recruitment flow



Famine exposure

We employed a general questionnaire to evaluate the subjects' degree of exposure to famine. As detailed elsewhere,[25] the Dutch famine occurred in the winters of 1944-1945 during World War II when banned food transport dramatically reduced food supplies in the western Netherlands, with official daily rations dropping to 400 to 800 kcal. After 6 months of starvation conditions, the Netherlands was liberated, abruptly ending the famine.

The questionnaire was used to collect information on place of residence as well as experiences of hunger and weight loss during the Dutch famine. The latter two questions had answer categories of 'hardly,' 'little,' or 'very much.' Women who answered 'not applicable' or 'I don't know' to either of the two questions were excluded. Famine exposure was then categorised into a three-point score as follows: 'severely exposed' for women who reported having been 'very much' exposed to both hunger and weight loss, 'unexposed' for those who were 'hardly' exposed to either hunger or weight loss, or 'moderately exposed' for all other responses.

Outcome assessment

CT imaging

Non-enhanced CT imaging of the heart was performed using a 16-slice CT scanner (Mx8000 IDT 16; Philips Medical Systems, Best, The Netherlands). During a single breath hold, a prospectively ECG-triggered ('step-and-shoot') CT scan was performed. The scan ranged from the tracheal bifurcation to below the apex of the heart. Scan parameters were 16x1.5-mm collimation, 205-mm field of view, 0.42-s rotation time, 0.28-s scan time per table position, 120 kVp, and 40 to 70 mAs (patient weight 70 kg: 40 mAs; 70 to 90 kg: 55 mAs; 90 kg: 70 mAs). Scan duration was approximately 10 s, depending on heart rate and patient size.

Measurement of calcifications

After CT scan acquisition, coronary artery calcification (CAC) was quantified using Agatston's method 26 on the 1.5 mm slices using software for calcium scoring (Heartbest-CS, EBW; Philips Medical Systems) by a trained scan reader (AR). The continuous score was dichotomised at a cut-off of 100 due to its highly skewed distribution. This cut-off value has been used clinically and scientifically to indicate cardiovascular risk. As published elsewhere,[27] a cut-off above 100 is often referred to as moderate calcification, while a cut-off of 300 reflects heavy calcification. We also performed sensitivity analyses using different cut-off values (0 and 300).

We attempted to ensure reproducibility by having 199 scans read by two independent observers and by having 58 women undergo a second scan within 3 months. The inter-reader and inter-scan agreement was excellent with within-class correlation coefficients greater than 0.95. [28]

Valve and aortic calcification was quantified using the same CT dataset as the CAC evaluation. We divided calcification of the heart valves anatomically into aortic valve leaflet (AVL) and mitral valve leaflet (MVL) findings. The AVL and MVL calcification was graded as 0 (absent), 1 (mild, meaning only one leaflet affected), or 2 (severe, 2 or 3 leaflets affected), as previously described.[20] Due to small numbers of subjects, the grades were further dichotomised into absent, or and present for mild to severe calcification. We also computed assessed composite valve calcification, which was classified as 'present' if calcification was detected in either of the two valves, or 'absent' if no calcification was found in either the mitral or aortic valve.

For the thoracic aorta, we initially categorised the calcification into absent (none detected), mild (≤4 calcified foci or 1 calcification extending over ≥3 slices), moderate (>4 calcified foci or 2 calcifications extending over ≥3 slices), or severe (calcified aorta covering multiple segments). We later simplified this classification into none-to-mild ('low') and moderate-to-severe ('high') calcifications because of the small number of subjects, especially those without calcification. A single reader (PdJ) performed all CT evaluations while blinded for famine exposure status.

Measurement of potential confounders

We collected data on date of birth, cardiovascular disease history, and established risk factors for cardiovascular diseases using questionnaires. Smoking was defined as current, past, or never.

From fasting venous blood specimens, we measured plasma total cholesterol, triglycerides, and glucose using standard enzymatic procedures, and high-density lipoprotein (HDL) cholesterol by direct method (inhibition, enzymatic). Systolic and diastolic blood pressures were measured by an automatic device (DINAMAP XL, Critikon; Johnson & Johnson, Tampa, FL). We took the higher value of either the right or left arm measurement. Height and weight were also measured, and the body mass index was calculated as weight divided by height squared (kg/m2). Body fat distribution was assessed by measuring the waist-to-hip ratio. We defined diabetes mellitus as previously described.[29]

Data analysis

Baseline characteristics were tabulated into famine exposure categories for descriptive purposes and confounding assessment, and differences were tested using Anova, Chi-square or Kruskal-Wallis tests.

We used (adjusted) logistic regression with coronary calcium (Agatston >100 yes/no) as the dependent variable, and an indicator for famine exposure levels as the independent variable. Because age at famine exposure could be an effect modifier[14] we stratified the analysis by age groups, 0-9 years (pre-adolescence) and 10-18 years (adolescence). Since smoking may be a potential effect-modifier as it may increase sensitivity for environmental stressors, we stratified the analysis by smoking status. Adjustments

were made for lipid profile, glucose level, and waist-to-hip ratio for their known strong associations with cardiovascular diseases and possibly with malnutrition. Similar modelling was used to evaluate calcium deposition in the aorta and mitral/aortic valves. Results are expressed as odds ratios and 95% confidence intervals. All analyses were conducted using SPSS version 19.0 for Mac.

Results

Table 1 shows the baseline characteristics of study subjects. Although not statistically significant, subjects exposed to famine were slightly older, and had higher BMI, waist-to-hip ratio, as well as triglyceride levels than the unexposed.

Table 1. Baseline characteristics of the study subjects based on famine exposure level

Variables		Famine exposure			P
		Unexposed n = 139	Moderate n = 103	Severe n = 44	
Age at famine, mean (SD)		7.6 (4.7)	8.9 (5.6)	8.7 (4.3)	0.10
Age category, n(%)	through 9 years	98 (70.5)	64 (62.1)	29 (65.9)	0.39
	10-18 years	41 (29.5)	39 (37.9)	15 (34.1)	
Age at CT scan		67.7 (5.0)	69.0 (5.4)	69.0 (4.7)	0.11
BMI (kg/m^2), mean (SD)		26.4 (4.4)	27.3 (4.6)	27.8 (4.6)	0.12
Waist-to-hip ratio, mean (SD)		0.84 (0.07)	0.86 (0.07)	0.85 (0.06)	0.06
Highest systolic BP (mmHg), mean (SD)		141.5 (20.4)	143.1 (23.4)	145.0 (21.8)	0.63
Highest diastolic BP (mmHg), mean (SD)		75.4 (9.6)	74.8 (9.6)	74.6 (8.2)	0.87
Cholesterol to HDL ratio, mean (SD)		4.5 (1.2)	4.7 (1.4)	4.8 (1.2)	0.43
Glucose level (mmol/L), mean (SD)		5.6 (1.0)	5.8 (1.2)	5.5 (0.5)	0.35
Triglyceride (mmol/L), median (range)		1.0 (0.5-3.8)	1.1 (0.3-3.7)	1.3 (0.5-3.2)	0.15*
Smoking, n (%)	Currently	9 (6.5)	11 (10.7)	3 (6.8)	0.71
	Former	57 (41.0)	45 (43.7)	18 (40.9)	
	Never	75 (52.5)	47 (45.6)	23 (52.3)	
Pack-years smoked in ever/current smokers, median (range)		6.0 (0.05-94)	7.4 (0.05-73.5)	11.5 (0.03-53.8)	0.66
Pack-years smoked, median (range)		0.0 (0.0-94)	0.3 (0.0-73.5)	0.0 (0.0-53.8)	0.57*
Metabolic syndrome, n(%)		15 (10.8)	18 (17.5)	3 (6.5)	0.14

*Kruskal-Wallis test

The association between famine exposure and coronary calcium score is shown in Table 2. The overall analysis, both crude and adjusted, showed that severe famine almost doubled the risk for coronary calcium scores >100 (OR 1.80, 95%CI 0.87-3.78), but not in a statistically significant manner. After stratification for age at famine exposure, those exposed to severe famine as adolescents had higher risks (OR 3.47, 95%CI 1.00-12.07) for coronary calcium scores >100 than unexposed women, which remained significant after adjustment for several variables. Using different cut-off points of the Agatston score (0 and 300) led to similar results (data not shown).

Table 2. The effect of famine exposure on coronary artery calcification as reflected by Agatston score

Famine Exposure		Agatston score (n, %)		Crude			Model 1			Model 2		
		<100 n = 204	>100 n = 78	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
All ages	Unexposed	104 (51.0)	33 (42.3)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	72 (35.3)	29 (37.2)	1.27	0.71-2.27	0.42	1.08	0.58-1.99	0.81	1.00	0.53-1.92	0.99
	Severe	28 (13.7)	16 (20.5)	1.80	0.87-3.78	0.11	1.63	0.76-3.46	0.21	1.74	0.79-3.84	0.17
	P for trend				0.11			0.26			0.24	
Age 0-9 years	Unexposed	78 (51.3)	18 (48.6)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	51 (33.6)	13 (35.1)	1.11	0.50-2.45	0.81	1.08	0.48-2.40	0.86	0.92	0.39-2.18	0.84
	Severe	23 (15.1)	6 (16.2)	1.13	0.40-3.18	0.82	1.06	0.37-3.02	0.92	1.22	0.40-3.70	0.17
	P for trend				0.78			0.88			0.24	
Age 10-17 years	Unexposed	26 (50.0)	15 (36.6)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	21 (40.4)	16 (39.0)	1.32	0.53-3.28	0.55	1.07	0.41-2.80	0.89	1.00	0.53-1.92	0.99
	Severe	5 (9.6)	10 (24.4)	3.47	1.00-12.07	0.05	3.65	1.01-13.31	0.04	4.62	1.16-18.43	0.03
	P for trend				0.07			0.09			0.06	

Model 1: Adjusted for age and pack-years smoked
Model 2: Adjusted for age, pack-years smoked, BMI, glucose, triglyceride, waist-to-hip ratio, systolic BP, cholesterol-to-HDL ratio
OR = odds ratio, 95%CI = 95% confidence interval, ref = reference category

Neither overall nor age-specific analyses indicated associations between famine exposure and calcification of mitral and/or aortic valves (Table 3). Table 4 shows no statistically significant association between famine exposure and aortic calcification, although famine in young children (between 0-9 years of age) may later increase aortic calcium deposition (OR 2.64; 95%CI 0.69-4.10, P = 0.10).

Stratification based on smoking status did not modify the overall results at any calcification site (data not shown).

Discussion

Our study shows that severe famine exposure during childhood, especially in adolescence, is associated with a 3-5-fold risk increase to have at least moderate coronary artery calcification in later life compared to non-exposure to famine. Although not statistically significant, famine exposure also tended to increase the risk of moderate-to-severe aortic calcification by approximately 60%, which was more pronounced in severe exposure to famine before the age of 9 years. Famine exposure was not related to mitral or aortic valve calcification.

To appreciate these findings some study aspects need to be addressed. Although unique for the combined availability of individual famine exposure data and thoracic CT images, the sample size was relatively small, limiting the statistical power. It is possible that effects that would have been seen a larger study were missed, such as the effect

of famine on extra-coronary calcification. Nevertheless, we found an association between famine in adolescence and coronary artery calcification. Also, our study population was restricted to post-menopausal women, so that generalising to men or to younger women remains speculative. However, our findings are in line with a previous report on the association between famine exposure in adolescence and late adulthood cardiovascular events,[14] providing further insight into the possible underlying mechanism.

Table 3. The effect of famine exposure on aortic and/or mitral valve calcification

Famine Exposure		Valve calcification (n, %)		Crude			Model 1			Model 2		
		No n = 206	Yes n = 80	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
All ages	Unexposed	102 (49.5)	37 (46.3)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	71 (34.5)	32 (40.0)	1.24	0.71-2.18	0.45	1.04	0.57-1.88	0.90	0.98	0.53-1.81	0.94
	Severe	33 (16.0)	11 (13.8)	0.92	0.42-2.00	0.83	0.78	0.35-1.76	0.35	0.68	0.29-1.58	0.37
	P for trend				0.92			0.65			0.44	
Age 0-9 years	Unexposed	74 (50.0)	24 (55.8)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	51 (34.5)	13 (30.2)	0.79	0.37-1.69	0.54	0.72	0.32-1.58	0.41	0.67	0.30-1.54	0.35
	Severe	23 (15.5)	6 (14.0)	0.80	0.29-2.21	0.67	0.63	0.22-1.78	0.38	0.56	0.18-1.71	0.56
	P for trend				0.56			0.30			0.23	
Age 10-17 years	Unexposed	28 (48.3)	13 (35.1)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	20 (34.5)	19 (51.4)	2.05	0.82-5.08	0.12	1.66	0.64-4.30	0.99	1.65	0.52-4.65	0.34
	Severe	10 (17.2)	5 (13.5)	1.08	0.31-3.79	0.91	1.06	0.29-3.92	0.93	0.79	0.20-3.16	0.74
	P for trend				0.53			0.66			0.37	

Table 4. The effect of famine exposure on aortic calcification

Famine Exposure		Aortic calcification* (n, %)		Crude			Model 1			Model 2		
		Low n = 209	High n = 77	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
All ages	Unexposed	108 (51.7)	31 (40.3)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	71 (34.0)	32 (41.6)	1.57	0.88-2.80	0.11	1.23	0.65-2.34	0.53	1.17	0.58-2.37	0.65
	Severe	30 (14.4)	14 (18.2)	1.63	0.77-3.44	0.20	1.39	0.61-3.15	0.43	1.66	0.69-4.10	0.26
	P for trend				0.12			0.39			0.28	
Age 0-9 years	Unexposed	85 (53.1)	13 (41.3)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	53 (33.1)	11 (35.5)	1.36	0.57-3.25	0.49	1.23	0.50-3.07	0.65	1.05	0.39-2.86	0.92
	Severe	22 (13.8)	7 (22.6)	2.08	0.74-5.84	0.16	1.68	0.57-4.91	0.35	2.64	0.82-8.52	0.10
	P for trend				0.17			0.35			0.16	
Age 10-17 years	Unexposed	23 (46.9)	18 (39.1)	Ref	Ref		Ref	Ref		Ref	Ref	
	Moderate	18 (36.7)	21 (45.7)	1.49	0.62-3.60	0.38	1.28	0.51-3.24	0.61	1.34	0.45-4.30	0.60
	Severe	8 (16.3)	7 (15.2)	1.12	0.34-3.67	0.85	1.07	0.31-3.73	0.92	0.91	0.21-3.96	0.90
	P for trend				0.65			0.79			0.92	

Model 1: Adjusted for age and pack-years smoked
Model 2: Adjusted for age, pack-years smoked, BMI, glucose, triglyceride, waist-to-hip ratio, systolic BP, cholesterol-to-HDL ratio
OR = odds ratio, 95%CI = 95% confidence interval, ref = reference category

Despite a possible association between pre-menopausal metabolic factors and both coronary and aortic calcifications, [30,31] we chose not to adjust our analysis for these factors. As pre-menopausal and postmenopausal metabolic factors are strongly correlated,[30] we believe that adjusting for both factors is not necessary and would even be misleading. By adjusting for post-menopausal factors, we indirectly took the pre-menopausal and the actual metabolic states at the time of calcium quantification into account. As previously shown, some traditional risk factors, such as lipid profiles,[32] are exaggerated by menopause, so that accounting for these factors at the postmenopausal stage is important. However, the age at menopause itself seems not to be associated with coronary calcification.[24]

The choice of Agatston score of 100 as an arbitrary cut-off to rank the severity of coronary calcification might be a limitation of our study. However, despite ongoing controversies on the reliability of a particular cut-off to truly differentiate the risks of having cardiovascular diseases,[33,34] we used the most commonly applied cut-off. A score above 100 has repeatedly been reported to be associated with an approximately 3-7-fold increased risk of coronary events or cardiovascular death,[35,36] therefore, our choice of cut-off seems justifiable. Moreover, we found that using different cut-offs did not lead to different results.

A major strength of our study was the use of the unique circumstances of the Dutch famine. As previously described,[25] the Dutch famine occurred within an approximately 6-month period between the end of 1944 and mid-1945, when food supplies in the Netherlands acutely and dramatically dropped to 400-800 kcal/day due to food transport bans and severe winter weather. After that period, the situation quickly improved, abruptly ending the famine. This situation allowed us to investigate the effects of post-natal undernutrition on health as a 'natural experiment,' rather than purely observational and to study the well-documented, acute nature of the undernutrition.

Although the measurement of famine exposure by recall may have some drawbacks related to its subjective nature and propensity of misclassification, we believe that if anything had occurred, it would have happened at random and only underestimate the observed effects. On the other hand, the use of individual data on famine exposure rather than grouping populations according to place of residence or time is a strength of our study since we used a more precise exposure measurement. As described previously, our exposure classification agrees with rationing practices at that time, in which individual calorie amounts were based on age. Young children (1-3 years) were relatively protected from the famine and received about 50%, whereas adults received about 25% of the distributed calorie amounts at the start of the famine. [25] These historical facts are reflected by our data, such that the older the women were at the start of the famine, the higher the proportion of women who reported having been exposed to famine. This may be considered in support of the quality of our exposure

data. We performed blinded and objective measurements of the calcium content using the CT scan, so that differential scan measurement by exposure knowledge is excluded as an explanation of our findings.

Coronary artery calcifications are thought to be a reflection of the burden of intima lesions; hence, coronary atherosclerosis.[37] For aortic calcifications, it is not clear that these always reflect atherosclerosis.[38] Most pathological studies found a substantial amount of aortic calcifications located in the tunica media and not the intima. Data even suggest that media calcifications develop earlier and more extensively in the aorta compared to intima calcifications.[39] Also for valve calcifications, the biology is now thought to be more on longstanding mechanical stress than inflammation.[40] Changes in valve tissue have been observed in persons who do not exhibit features of atherosclerosis, indicating that early stages of cardiac valve calcification involve mechanisms different from coronary artery calcification.[40]

To our knowledge, there has been no previous study addressing an association between childhood famine exposure and vascular or valve calcifications in later life. Most studies linking malnutrition and coronary or valve calcification were conducted on patients with end-stage renal disease.[41] Such patients are reported to experience a malnutrition-inflammation-atherosclerosis/calcification (MIAC) syndrome, which is a strong predictor for cardiovascular death. Although a full assessment of the mechanisms underlying the association between undernutrition and coronary artery or valve calcification is beyond the scope of our study, the findings in patients with renal diseases suggest that a similar interaction between malnutrition, increased levels of pro-inflammatory cytokines, and calcification or atherosclerosis may play a role.[42,43] This suggests that the protective effect of caloric restriction in adulthood and animals on atherosclerosis and metabolic syndromes[44,45] may not be readily translated to undernutrition in early life. Caloric restriction in different periods of life probably has different effects on atherosclerosis development, as suggested by findings from two studies on the Dutch famine which showed that famine exposure during adolescence increased the risk for cardiovascular events,[14] whereas prenatal famine exposure did not.[46]

Our findings suggest that the critical period determining future development of cardiovascular diseases may extend beyond foetal life and infancy. Previous studies have demonstrated the role of early life nutrition in the development of chronic diseases in adulthood. For example, the Biafran famine study showed that foetal-infant undernutrition is associated with an increased risk of hypertension and impaired glucose tolerance in adulthood,[47] similar to what was found in the Chinese great famine study.[48] Although few studies have evaluated the effect of post-natal events per se on health in later life, it seems that maintaining balanced nutrition in later childhood is also crucial, as suggested by the detrimental effects of weight fluctuation or 'yoyo' dieting in young adulthood on coronary heart disease.[17] Our study only addresses one of the possible

mechanisms of the development of atherosclerosis and cardiovascular diseases by childhood famine exposure. The findings are consistent with previous finding of the association between famine exposure in adolescence and clinical cardiovascular disease. Hence, our findings warrant further studies into the role of calcium deposition in the relationship between acute famine and later life cardiovascular disease.

In conclusion, famine exposure in childhood, especially during adolescence, may be associated with a higher risk of coronary artery calcification in late adulthood. There seems to be no clear association between childhood famine exposure and cardiac valve or aortic calcification.

Funding statement

The original study for which coronary calcium was measured was supported by grant 2100.0078 from the Netherlands Organisation for Health Research and Development.

References

1. Hughes BB, Kuhn R, Peterson CM, Rothman DS, Solorzano JR, Mathers CD, et al. Projections of global health outcomes from 2005 to 2060 using the international futures integrated forecasting model. Bull World Health Organ. 2011;89:478-86.
2. Djärv T, Wikman A, Lagergren P. Number and burden of cardiovascular diseases in relation to health-related quality of life in a cross-sectional population-based cohort study. BMJ Open. 2012;2:e001554..
3. Barker DJP, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet. 1986;327:1077-81.
4. Barker DJP, Osmond C, Simmonds SJ, Wield GA. The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. BMJ. 1993;306:422-6.
5. Stein C, Fall C, Kumaran K, Osmond C, Cox V, Barker D. Fetal growth and coronary heart disease in South India. Lancet. 1996;348:1269-73.
6. Canani RB, Costanzo MD, Leone L, et al. Epigenetic mechanisms elicited by nutrition in early life. Nutr Res Rev. 2011;24:198-205.
7. D Leon DA, Lithell HO, Vâgerö D, Koupilová I, Mohsen R, Berglund L, et al. Reduced fetal growth rate and increased risk of death from ischaemic heart disease: cohort study of 15 000 Swedish men and women born 1915-29. BMJ. 1998;317:241-45.
8. Hales CN, Barker DJP. The thrifty phenotype hypothesis: type 2 diabetes. British Med Bull. 2001;60:5-20.
9. Attig L, Gabory A, Junien C. Early nutrition and epigenetic programming: chasing shadows. Curr Opin Clin Nutr Metab Care. 2010;13:284-93.
10. Berends LM, Fernandez-Twinn DS, Martin-Gronert MS, Cripps RL, Ozanne SE. Catch-up growth following intra-uterine growth-restriction programmes an insulin-resistant phenotype in adipose tissue. Int J Obes (Lond). 2012 Dec 11.
11. Evelein AM, Visseren FL, van der Ent CK, Grobbee DE, Uiterwaal CS. Excess early postnatal weight gain leads to thicker and stiffer arteries in young children. J Clin Endocrinol Metab. 2013 Jan 2.
12. Fall CH, Sachdev HS, Osmond C, Lakshmy R, Biswas SD, Prabhakaran D, et al. Adult metabolic syndrome and impaired glucose tolerance are associated with different patterns of BMI gain during infancy: data from the New Delhi birth cohort. Diabetes Care. 2008;31:2349-56.
13. Norris SA, Osmond C, Gigante D, Kuzawa CW, Ramakrishnan L, Lee NR, et al. Size at birth, weight gain in infancy and childhood, and adult diabetes risk in five low- or middle-income country birth cohorts. Diabetes Care. 2012;35:72-9.

14. van Abeelen AFM, Elias SG, Bossuyt PMM, Grobbee DE, van der Schouw YT, Roseboom TJ, et al. Cardiovascular consequences of famine in the young. Eur Heart J. 2012;33:538-45.
15. Portrait F, Teeuwiszen E, Deeg D. Early life undernutrition and chronic diseases at older ages: the effects of the Dutch famine on cardiovascular diseases and diabetes. Soc Sci Med. 2011;73:711-718.
16. Sparén P, Vågerö D, Shestov DB, Plavinskaja S, Parfenova N, Hoptiar V, et al. Long term mortality after severe starvation during the siege of Leningrad: prospective cohort study. BMJ. 2004;328:11.
17. Dulloo AG, Jacquet J, Seydoux J, Montani JP. The thrifty 'catch-up fat' phenotype: ts impact on insulin sensitivity during growth trajectories to obesity and metabolic syndrome. Int J Obes (Lond). 2006;30 (Suppl 4):S23-35.
18. Kondos GT, Hoff JA, Sevrukov A, Daviglus ML, Garside DB, Devries SS, et al. Electron-beam tomography coronary artery calcium and cardiac events: a 37-month follow-up of 5635 initially asymptomatic low- to intermediate-risk adults. Circulation. 2003;107:2571-6.
19. Graham G, Blaha MJ, Budoff MJ, Rivera JJ, Agatston A, Raggi P, et al. Impact of coronary artery calcification on all-cause mortality in individuals with and without hypertension. Atherosclerosis. 2012;225:432-437.
20. Gondrie MJ, van der Graaf Y, Jacobs PC, Oen AL, Mali WP, PROVIDI Study Group. The association of incidentally detected heart valve calcification with future cardiovascular events. Eur Radiol. 2011;21:963-73.
21. Owens DS, Budoff MJ, Katz R, Takasu J, Shavelle DM, Carr JJ, et al. Aortic valve calcium independently predicts coronary and cardiovascular events in a primary prevention population. JACC Cardiovasc Imaging. 2012;5:619-25
22. Budoff MJ, Nasir K, Katz R, Takasu J, Carr JJ, Wong ND, et al. Thoracic aortic calcification and coronary heart disease events: the multi-ethnic study of atherosclerosis (MESA). Atherosclerosis. 2011;215:196-202.
23. Boker LK, van Noord PA, van der Schouw YT, Koot NV, Bueno de Mesquita HB, Riboli E, et al. Prospect-EPIC Utrecht: study design and characteristics of the cohort population. European prospective investigation into cancer and nutrition. Eur J Epidemiol. 2001;17:1047-53.
24. Atsma F, Bartelink ML, Grobbee DE, Rutten A, Bots ML, Prokop M, et al. Reproductive factors, metabolic factors, and coronary artery calcification in older women. Menopause. 2008;15:899-904.
25. Burger G, Sandstead H, Drummond J. Malnutrition and starvation in western Netherlands, September 1944 to July 1945. Part I and II. The Hague: General State Printing; 1948.
26. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte Jr. M, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol. 1990;15:827-32.

27. Hecht HS. Subclinical atherosclerosis imaging comes of age: coronary artery calcium in primary prevention. Curr Opin Cardiol. 2012;27:508-14.
28. Sabour S, Atsma F, Rutten A, Grobbee DE, Mali W, Prokop M, et al. Multi detector-row computed tomography (MDCT) had excellent reproducibility of coronary calcium measurements. J Clin Epidemiol. 2008;61:572-79.
29. van Abeelen AF, Elias SG, Bossuyt PM, Grobbee DE, van der Schouw YT, Roseboom TJ, et al. Famine exposure in the young and the risk of type 2 diabetes in adulthood. Diabetes. 2012;61:2255-60.
30. Matthews KA, Kuller LH, Chang Y, Edmundowicz D. Premenopausal risk factors for coronary and aortic calcification: a 20-year follow-up in the healthy women study. Prev Med. 2007;45:302-8.
31. Kuller LH, Matthews KA, Sutton-Tyrrell K, Edmundowicz D, Bunker CH. Coronary and aortic calcification among women 8 years after menopause and their premenopausal risk factors: the healthy women study. Arterioscler Thromb Vasc Biol. 1999;19:2189-2198.
32. Torng PL, Su TC, Sung FC, Chien KL, Huang SC, Chow SN, et al. Effects of menopause and obesity on lipid profiles in middle-aged Taiwanese women: the Chin-Shan community cardiovascular cohort study. Atherosclerosis. 2000;153:413-421.
33. Nucifora G, Bax JJ, van Werkhoven JM, Boogers MJ, Schuijf JD. Coronary artery calcium scoring in cardiovascular risk assessment. Cardiovasc Ther. 2011;29:e43-5.
34. Tota-Maharaj R, Blaha MJ, McEvoy JW, Blumenthal RS, Muse ED, Budoff MJ, et al. Coronary artery calcium for the prediction of mortality in young adults 75 years old. Eur Heart J. 2012;33:2955-62.
35. Polonsky TS, McClelland RL, Jorgensen NW, Bild DE, Burke GL, Guerci AD, et al. Coronary artery calcium score and risk classification for coronary heart disease prediction. JAMA. 2010;303:1610-6.
36. Erbel R, Möhlenkamp S, Moebus S, Schmermund A, Lehmann N, Stang A, et al. Coronary risk stratification, discrimination, and reclassification improvement based on quantification of subclinical coronary atherosclerosis: the Heinz Nixdorf recall study. J Am Coll Cardiol. 2010;56:1397-406.
37. Sage AP, Tintut Y, Demer LL. Regulatory mechanisms in vascular calcification. Nat Rev Cardiol. 2010;7:528-36.
38. Kälsch H, Lehmann N, Berg MH, Mahabadi AA, Mergen P, Möhlenkamp S, et al. Coronary artery calcification outperforms thoracic aortic calcification for the prediction of myocardial infarction and all-cause mortality: the Heinz Nixdorf recall study. Eur J Prevent Cardiol. 2013 March 06.
39. Amann K. Media calcification and intima calcification are distinct entities in chronic kidney disease. Clin J Am Soc Nephrol. 2008;3:1599-605.

40. Karwowski W, Naumnik B, Szczepanski M, Mysliwiec M. The mechanism of vascular calcification - a systematic review. Med Sci Monit. 2012;18:RA1-11.
41. Srivaths PR, Silverstein DM, Leung J, Krishnamurthy R, Goldstein SL. Malnutrition-inflammation-coronary calcification in pediatric patients receiving chronic hemodialysis. Hemodial Inter. 2010;14:263-9.
42. Stenvinkel P, Heimbürger O, Lindholm B, Kaysen GA, Bergström J. Are there two types of malnutrition in chronic renal failure? Evidence for relationships between malnutrition, inflammation and atherosclerosis (MIA syndrome). Nephrol Dial Transpl. 2000;15:953-60.
43. Wang AY, Woo J, Lam CW, Wang M, Chan IH, Gao P, et al. Associations of serum fetuin-A with malnutrition, inflammation, atherosclerosis and valvular calcification syndrome and outcome in peritoneal dialysis patients. Nephrol Dial Transplant. 2005;20:1676-85.
44. Choi KM, Han KA, Ahn HJ, Lee SY, Hwang SY, Kim BH, et al. The effects of caloric restriction on fetuin-A and cardiovascular risk factors in rats and humans: randomized controlled trial. Clin Endocrinol (Oxf). 2012 Oct 15.
45. Fontana L, Meyer TE, Klein S, Holloszy JO. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. Proc Natl Acad Sci U S A. 2004;101:6659-63.
46. Lumey LH, Martini LH, Myerson M, Stein AD, Prineas RJ. No relation between coronary artery disease or electrocardiographic markers of disease in middle age and prenatal exposure to the Dutch famine of 1944-5. Heart. 2012;98:1653-59.
47. Hult M, Tornhammar P, Ueda P, Chima C, Bonamy AK, Ozumba B, et al. Hypertension, diabetes and overweight: looming legacies of the biafran famine. PLoS One. 2010;5:e13582. 10.1371/journal.pone.0013582.
48. Wang PX, Wang JJ, Lei YX, Xiao L, Luo ZC. Impact of fetal and infant exposure to the Chinese great famine on the risk of hypertension in adulthood. PLoS One. 2012;7:e49720.

Chapter 4

Effect of physical activity on vascular characteristics in young children

Nikmah S. Idris, MD
Annemieke M.V. Evelein
Caroline C. Geerts
Sudigdo Sastroasmoro
Diederick E. Grobbee
Cuno S.P.M. Uiterwaal

Eur J Prev Cardiol. 2014 Feb 13. (Epub ahead of print)

Abstract

Objectives

Physical activity has long been proposed as an important modifiable cardiovascular risk factor in adults. We assessed whether physical activity already has an effect on childhood vasculature.

Methods

In the Wheezing-Illnesses-Study-in-Leidsche-Rijn birth cohort, we performed vascular ultrasound to measure carotid intima-media thickness (cIMT) and functional properties (distensibility, elastic modulus) at 5 and 8 years of age. Child typical physical activities were inquired using a questionnaire completed by parents. Linear regression was used with physical activity level, expressed as a standardised value of time-weighted metabolic equivalent (MET) as the independent variable and vascular properties as dependent variables with further confounder adjustment and evaluation for possible body mass index (BMI) and gender effect modifications

Results

In 595 5-year-old children and in 237 of those who had reached the age of 8 years, we did not find statistically significant associations between total time-weighted MET and each vascular parameter, neither in pooled nor stratified analysis. However, sport activities were associated with thinner cIMT (-3.20 micrometers/SD, 95%CI -6.34;-0.22, P = 0.04) at 5 years of age; a similar pattern was seen for organised sport. This effect was strongest in children in the highest BMI tertile (-5.38 micrometers/SD, 95%CI -10.54;-0.19, P = 0.04). At the age of 8 years, higher sport level tended to be associated with higher vascular distensibility (2.64 kPa x 103/SD, 95%CI -0.18; 5.45, P = 0.07) although this was not statistically significant.

Conclusions

Sport activity may have beneficial effects on arteries of young children, particularly those with higher relative body weight.

Background

Cardiovascular disease (CVD) is a global leading cause of mortality and morbidity.[1] Despite the identification and measures directed to modify multiple risk factors, the prevalence is still high and even increasing in some countries. As such increase is most likely due to adverse changing of risk factor levels, particularly modifiable risk factors need to be explored and intervened at the right age.

Increasingly sedentary lifestyles and lower tendency to exercise have been proposed to significantly contribute to the high CVD incidence.[2] Evidence in adults is quite firm that active lifestyle reduces cardiovascular risk factor levels and mortality[3,4] thus regular moderate-intensity aerobic physical activity has been recommended.[5] Although the beneficial effects of physical activity were initially thought to occur by modification of classical risk factors, this only explains about half of the risk reduction associated with exercise,[6] implicating that physical activity may also have direct effects on vascular walls.[7] It is important to further investigate these effects, particularly the timing of onset, because pre-atherosclerotic changes start as early as in young childhood.[8,9] If indeed physical activity is beneficial for the childhood vasculature, enhancing physical activity may interrupt already ongoing pre-atherosclerotic changes. Since lack of physical activity is principally modifiable, for instance through parents, school, sports, or internet-based education,[10] and is probably best preserved on the long run if adopted at a young age,[11] it might become a very important tool in early life CVD prevention. Even if there would be little direct effect on the childhood vessels, an active lifestyle is associated with lower risk of later life chronic diseases.

Data regarding the effect of physical activity on cardiovascular risk at a young age is scarce. A recent longitudinal study on adolescents showed that physical activity is favourably associated with endothelial function and aortic intima-media thickness (IMT).[12] A Chinese study also reported an association between physical activity and cardiovascular risk scores in children.[13] As CVD manifests in later life, studies in the young can only be performed with outcomes such as risk factor levels or vascular characteristics as an estimate of early life vascular damage. It is unknown whether physical activity already alters the early childhood arterial wall. Variations in physical activity levels have been observed as early as in preschool age[14,15] and it might be from then on that activity has an effect on the developing cardiovascular system. Therefore, we conducted a study to assess whether physical activity level already determines the arterial wall structure and function of young children.

Methods

Study design and population

This study is a part of the ongoing WHeezing Illnesses STudy in LEidsche Rijn (WHISTLER) birth cohort set up in December 2001 in Utrecht, the Netherlands.[16] Briefly,

Table 1. Baseline characteristics of parents and study subjects at ages 5 and 8 years, by quartiles of MET score.

Variables		Time-weighted MET score at age 5 years (N = 595)					Time-weighted MET score at age 8 years (N = 237)				
		Q1 n = 147 <4059	Q2 n = 151 4059 - <6055	Q3 n = 148 6055 - <8676	Q4 n = 149 ≥8676	p	Q1 n = 59 <4311	Q2 n = 60 4311 - <6756	Q3 n = 59 6756 - <9908	Q4 n = 59 >9908	p
Age (years), mean (SD)		5.4 (0.3)	5.4 (0.2)	5.4 (0.3)	5.4 (0.3)	0.31	8.2 (0.5)	8.2 (0.4)	8.0 (0.4)	8.0 (0.4)	0.22
Male gender (%)		62 (42.2)	69 (45.7)	72 (48.6)	84 (56.4)	0.02	15 (25.4)	25 (41.7)	27 (45.8)	32 (54.2)	0.01
Low/middle education (%)*		53 (35.9)	49 (32.4)	54 (36.4)	70 (46.9)	0.07	24 (49.0)	19 (34.5)	20 (39.2)	28 (56)	0.12
Western ethnicity (%)		130 (88.4)	140 (92.8)	140 (94.3)	127 (84.9)	0.06	43 (82.7)	49 (92.5)	49 (94.2)	46 (92)	0.19
BMI (kg/m^2), mean(SD)		15.3 (1.6)	15.1 (1.3)	15.2 (1.1)	15.2 (1.5)	0.60	16.2 (1.7)	15.5 (1.6)	15.8 (1.8)	16.1 (2.1)	0.13
Waist circumference (cm)		53.3 (4.2)	52.6 (3.6)	53.1 (3.1)	52.9(3.7)	0.37	58.8 (5.1)	57.5 (4.2)	58.1 (5.4)	58.7 (5.7)	0.50
Maternal BMI (kg/m^2), mean(SD)		25.1 (4.7)	24.6 (3.5)	24.6 (3.9)	25.1 (4.8)	0.65	24.4 (4.0)	24.2 (3.6)	24.6 (3.1)	24.5 (4.7)	0.96
Paternal BMI (kg/m^2), mean(SD)		25.6 (3.3)	25.2 (2.9)	25.2 (3.2)	25.4 (3.1)	0.66	25.2 (2.8)	25.8 (3.4)	25.2 (2.6)	27.2 (5.8)	0.03
Maternal smoking during pregnancy (%)		7 (4.8)	7 (4.6)	6 (4.1)	17 (11.4)	0.07	3 (5.1)	2 (3.3)	1 (1.7)	7 (12.5)	0.06
Postnatal parental smoking (%)	One	32 (21.8)	35 (23.2)	31 (20.9)	38 (25.5)	0.59	9 (16.7)	5 (10)	14 (25)	9 (18.8)	0.44
	Both	11 (7.5)	8 (5.2)	8 (5.4)	16 (10.7)		4 (7.4)	4 (8)	3 (5.4)	6 (12.5)	
Systolic BP (mmHg)#		105.9 (7.4)	103.7 (6.9)	105.0 (7.3)	105.9 (8.2)	0.04	109.4 (7.5)	106.9 (9.0)	107.1 (9.3)	107.5 (7.6)	0.36
Diastolic BP (mmHg)#		54.9 (7.4)	53.2 (6.3)	53.7 (7.1)	54.8 (7.1)	0.09	55.6 (5.6)	55,9 (6.3)	54.2 (7.4)	55.8 (6.3)	0.44

*n = 205 (8 year old children); #n = 591 (5 year old children); MET = metabolic equivalent; BMI = body mass index; Q = quartile

around 2,600 healthy neonates born at a gestational age of at least 36 weeks with no major congenital anomalies or neonatal respiratory diseases had been enrolled currently. In 2007, the study was extended for cardiovascular research questions (WHISTLER-Cardio) and all 5 year old children were invited for follow up measurements. Starting in 2010, children who had participated in the measurements at age 5 years were re-invited at the age of 8 years for further follow up.

At the time of data analysis (August 2012), there were 1539 and 445 children who had reached the age of 5 and 8 years respectively. Among those who had reached five years of age, 684 (44.3%) children had attended the 5-year-old visit, whereas 244 (15.8%) could not be contacted, 400 (25.9%) declined, 169 (10.9%) had not decided, and 42 (2.7%) agreed but had not come yet. Of the 684 children who had accomplished the 5-year follow up visit, physical activity could be inquired in 655 (95.8%) subjects and vascular measurements were successful in 595 (87.0%).

Among 445 children who had reached the age of 8 years, 237 completed the 8-year old visit and had successful vascular measurement. Children who did not accomplish the visit were those who were lost to follow up (n = 24), declined (n=67), had not decided or contacted (n=100), or agreed but did not visit yet (n=17). This study was approved by the Paediatric Medical Ethical Committee of the University Medical Centre Utrecht. Parents gave informed consent for participating in the study.

Physical activity measurement

A parent-filled questionnaire was applied at 5 years and 8 years of age to inquire for types and duration of child activities in a typical week. Open questions were used to obtain information about the duration of listed non-sport activities, including travel to and from school (cycling or walking), free-time outdoor play, free time indoor play, free time walking, free time cycling as well as type of free time sports and its corresponding duration.

For each activity, metabolic equivalents (MET), a physiological measure expressing the energy cost, were assigned based on the Compendium of Energy Expenditures for Youth, which provides the most up-to-date collation of energy costs for youth.[17] In this Compendium, specific MET values for a particular activity were graded further according to the activity intensity, whether it was of light, moderate, or high effort. Since we did not specifically inquire for activity intensity in the questionnaire, unless the answers indicated organised intensive activities, we assumed that activities were done with moderate effort, as vigorous activities were unlikely at this age.[18] Metabolic equivalent is defined as the ratio of metabolic rate (and therefore the energy consumption rate) during a specific physical activity to a reference metabolic rate, set by convention to 3.5 ml O2·kg−1·min−1 or equivalently 1 kcal·kg−1·h−1 or 4.184 kJ·kg −1·h−1. Originally, 1 MET was defined as the resting metabolic rate obtained during quiet sitting.[19]

After we assigned the MET value for each activity, the specific MET was multiplied by duration in minutes to obtain a time-weighted MET score as a measure of physical activity level. The physical activity level in a typical week was calculated by summing the time-weighted MET scores of all activities done by a subject in a week. As an illustration, if a child was completely in quiet sitting during its 10-hour (600 minute) awake period per day, then the time-weighted MET in a week for that child for the given awake period would be 4200 (1 MET x 600 x 7). Besides calculating time-weighted MET for whole and sport activities reported by parents, we also looked specifically at organised sport usually done in regular or club setting.

Vascular measurement

The vascular condition of the far wall of the right common carotid artery was studied using an ultrasound with high-resolution echo-tracking technology (Art. Lab, Esaote, Italy ®). The detailed technique and reproducibility have been described elsewhere.[20] In short, this technology gives access to all major mechanical parameters for 4-cm arterial segments: diastolic diameter d, the change in diameter, distension as a function of time, and carotid intima media thickness (cIMT). All measurements were performed by two of the investigators (AMVE, CCG) and a trained research nurse, who were blinded to child physical activity and confounders.

During ultrasonography, blood pressure was recorded twice in the brachial artery with a semiautomatic oscillometric device using a cuff appropriate to the arm size (DINAMAP; Criticon, Tampa, FL). Both values were averaged (Appendix) to estimate common carotid artery local pulse pressure, assuming mean arterial pressure minus diastolic blood pressure constant throughout the large arterial tree. Averages of cIMT, lumen diameter, and distension over every session per individual were used to assess the elastic properties of the artery as a hollow structure (distensibility coefficient) and of the wall (elastic modulus). (See the formulas and units in Appendix)

cIMT measurement was successful in 595 of 655 (90.8%) 5 year old and in 237 (100%) 8 year old children, respectively. The distensibility measurement was successful in 507 (85.2%) 5 year old and 227 (95.8%) 8 year old children, respectively.

Measurement of potential confounders and effect modifiers

Age, sex, pre- and post-natal smoking exposure, ethnicity, and socioeconomic status reflected by education, were a priori set as possible confounders. Associations between these variables and both physical activity and vascular characteristics are biologically plausible and some had been explained,[21-23] whereas educational level may be a summary of other lifestyle-associated confounders, such as diet.

We considered a priori that gender potentially modifies the association between physical activity and vascular characteristics. Sex-differences in cardiovascular risk are well known in adults and there is evidence that males and females indeed follow

divergent trajectories in the development of cardiovascular risk.[24,25] In addition, we thought that body mass index (BMI), parental BMI, and blood pressure can potentially act as confounders or as intermediary variables, and therefore were tested in the regression models. Several studies have shown that BMI and blood pressure influence the vasculature,[26] while physical activity has a direct effect on these variables. Body mass index was considered as a potential effect modifier as heavier children are likely to have thicker vessel wall,[26] so that if indeed there was any effect of physical activity, it would probably be more easily seen in this group of children.

We collected information regarding age, sex, ethnicity, educational levels, smoking exposure, parental body weight and height using a questionnaire, which was filled by parents at home before each visit. During the visits, investigators reviewed the questionnaire together with parents to scan for missing information and clarify unclear items. BMI was calculated as body weight (kg)/height (m)2 measured during the visits at 5 and 8 years of age. The same calculation applied for parental BMI.

Data analysis

We first scanned for missing data, which was mostly found in the “free time indoor play” variable at age 5 years (12.3%). Multiple imputations using linear regression were done for missing duration of activities and covariates. After imputation, the time weighted METs for whole activities, sport, and organised sport were calculated. The distribution of time-weighted MET was assessed, from which mean, standard deviation, and standardised value (z-score) were derived.

We then described baseline characteristics at the ages of 5 and 8 years across quartiles of time-weighted MET and used means, medians, or proportions as appropriate. For both ages, we performed univariable and multivariable linear regression analyses to evaluate the effect of physical activity on vascular parameters (cIMT, distensibility coefficient, and elastic modulus), which were expressed as standardised values (z-scores) for the purpose of effect size comparability. We also evaluated possible gender and BMI effect modification by testing the interactions in separate regression models and stratifying (tertiles of BMI) the analysis.

Findings are expressed as linear regression coefficients, 95% confidence intervals, and corresponding p-values. A 95% confidence interval not including zero, corresponding to a p-values of <0.05 was considered statistically significant. All analyses were conducted using SPSS version 19.0 for Mac.

Results

Subjects’ baseline characteristics at the age of 5 and 8 year across quartiles of time-weighted MET score are described in Table 1. Among the 5 year and 8 year olds, higher proportions of male sex, low-to-middle socioeconomic group, and prenatal smoking exposure were found in the higher quartiles of physical activity. Eight-year

old children with relatively high physical activity levels also showed higher paternal BMI. In terms of blood pressure, the 5 year old children at the extremes of physical activity levels tended to have higher blood pressures than those with moderate activity, but this was only statistically significant for the systolic blood pressure.

Table 2 shows the association between time-weighted MET z-score and cIMT, arterial distensibility, and elastic modulus in 5 year olds. Neither crude nor adjusted analyses showed statistically significant associations between total time-weighted MET z-score and all vascular parameters. A statistically significant interaction between total time-weighted MET and BMI was found in the regression model, whereas in the stratified analysis based on tertiles of BMI, we did not find any statistically significant association within strata (Figure 1).

When we looked at sport activities only, there was a statistically significant adjusted association between higher sport activity level at 5 years of age and thinner cIMT (-3.20 micrometer/SD, 95%CI -6.34;-0.22, p = 0.04). This effect seemed stronger in children with higher (3rd tertile) BMI levels (-5.38 micrometers/SD, 95%CI -10.54;-0.19, p = 0.04) (Figure 1). The analysis of organised regular sport showed a similar pattern as sport in general. No effect modification by gender was found for the associations of both total and sport time-weighted METs and vascular characteristics.

The association between physical activity level and the vasculature at 8 years of age is shown in Table 3. Overall, no association was found between the total-, sport, or organised sport activities and the vascular parameters both in crude and adjusted analyses. BMI- and sex-stratified analysis also showed no major differences for any vascular characteristic between the BMI groups or gender, respectively. However, although not statistically significant, total physical activities tended to be associated with thinner cIMT in boys (-7.25 micrometer/SD, 95%CI -16.72;1.82, p = 0.10), but not in girls (2.93 micrometer/SD, 95%CI -4.29;10.15, p = 0.43). A consistent effect direction was also found in the crude and adjusted associations between sport- or organised sport activities and vascular distensibility at 8 years of age, although these were not statistically significant (Table 3). The effect of organised sport on vascular distensibility (4.59 MPa-1/SD, 95%CI -2.29;11.48, p = 0.19) and elastic modulus (-19.63 kPa/SD, 95% CI -42.43;3.17, p =0.09) tended to be stronger in children within the 3rd BMI tertile group, but this was not statistically significant.

Discussion

In this study, we found that total physical activity did not have an independent effect on the early childhood vasculature. However, higher sport activity level was associated with thinner cIMT at 5 years of age, which was more prominent in children with higher BMI level. Higher sport activity level also seemed associated with higher vascular

distensibility at the age of 8 years, especially in children with higher BMI, but none of these associations were statistically significant.

Table 2. Association between physical activity level and vascular characteristics at age 5 years (imputed set)

		cIMT (micrometer)			Distensibility (MPa^{-1})			Elastic Modulus (kPa)		
		RC	95% CI	p	RC	95% CI	p	RC	95% CI	p
Total time-weighted MET (z-score)	Crude	1.06	-2.32;4.45	0.54	0.27	-2.42;2.96	0.84	0.52	-4.45;5.31	0.84
	Model 1[a]	0.73	-2.67;4.13	0.67	0.38	-2.30;3.07	0.78	0.39	-4.57;5.34	0.88
	Model 2[b]	0.81	-2.58;4.19	0.64	0.41	-2.25;3.07	0.76	0.38	-4.49;5.26	0.88
	Model 3[c]	0.67	-2.76;4.10	0.70	0.55	-2.12;3.22	0.69	0.06	-4.82;4.93	0.99
	Model 4[d]	0.36	-3.09;1.81	0.84	0.93	-1.52;3.39	0.46	-0.31	-5.02;4.40	0.90
Sport time-weighted MET (z-score)	Crude	-3.05	-6.11;0.02	0.05	0.40	-1.87;2.67	0.73	-0.25	-4.55;4.05	0.91
	Model 1[a]	-3.21	-6.27;-0.15	0.04	0.44	-1.84;2.71	0.71	-0.26	-4.56;4.03	0.91
	Model 2[b]	-3.23	-6.28;-0.18	0.04	0.41	-1.86;2.67	0.73	-0.32	-4.58;1.95	0.89
	Model 3[c]	-3.12	-6.17;-0.06	0.04	0.40	-1.86;2.67	0.73	-0.17	-4.43;4.09	0.94
	Model 4[d]	-3.20	-6.34;-0.22	0.04	0.33	-1.84;2.51	0.77	-0.19	-4.31;4.27	0.99
Organized sport time-weighted MET (z-score)	Crude	-2.10	-5.24;1.03	0.19	0.21	-2.10;2.51	0.86	-0.06	-4.51;4.40	0.98
	Model 1[a]	-2.37	-5.50;0.76	0.14	0.39	-1.92;2.69	0.74	-0.40	-4.85;4.05	0.86
	Model 2[b]	-2.50	-5.63;0.62	0.12	0.41	-1.89;2.71	0.72	-0.64	-5.06;1.77	0.77
	Model 3[c]	-2.43	-5.54;0.72	0.13	0.56	-1.74;2.86	0.64	-0.71	-5.12;3.69	0.75
	Model 4[d]	-2.68	-5.81;0.46	0.09	0.29	-1.92;2.50	0.80	-0.02	-4.31;4.27	0.99

Notes: RC = linear regression coefficient; cIMT = carotid artery intima media thickness; MET = metabolic equivalent; CI = confidence interval

[a] Model 1: adjusted for age, sex

[b] Model 2: adjusted for age, sex, BMI

[c] Model 3: adjusted for age, sex, parental (mother and father) BMI, prenatal & postnatal smoking exposure, ethnicity, socioeconomic status.

[d] Model 4: adjusted for age, sex, parental (mother and father) BMI, prenatal & postnatal smoking exposure, ethnicity, socioeconomic status, systolic blood pressure, diastolic blood pressure

Figure 1. BMI-stratified effects of total and sport time-weighted MET on carotid intima media thickness of 5 year old children

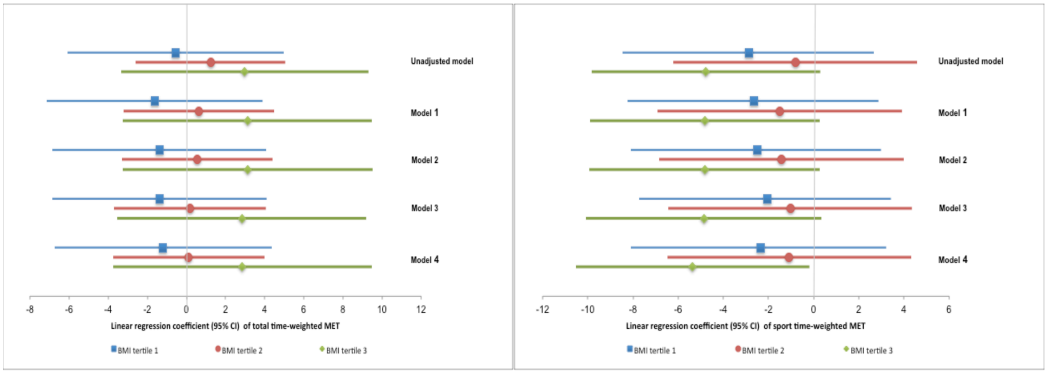


Table 3. Association between physical activity level and vascular characteristics at age 8 years.

		cIMT (micrometer)			Distensibility (MPa^{-1})			Elastic Modulus (kPa)		
		RC	95% CI	P	RC	95% CI	P	RC	95% CI	P
Total time-weighted MET (z-score)	Crude	0.62	-4.38;5.63	0.81	0.25	-2.76;3.26	0.87	-2.60	-10.48;5.30	0.52
	Model 1[a]	0.61	-4.41;5.72	0.82	0.61	-2.43;3.66	0.69	-3.83	-11.74;4.07	0.34
	Model 2[b]	0.35	-4.84;5.54	0.90	0.71	-2.26;3.70	0.64	-4.13	-11.83;3.57	0.29
	Model 3[c]	-1.84	-8.07;4.40	0.56	0.02	-3.40;3.43	0.99	-1.62	-10.60;7.35	0.72
	Model 4[d]	-1.50	-7.72;4.73	0.64	-0.10	-3.44;3.25	0.95	-1.39	-10.36;7.57	0.76
Sport time-weighted MET (z-score)	Crude	0.44	-2.07;2.94	0.86	2.12	-0.92;5.15	0.18	-4.07	-8.24;0.10	0.84
	Model 1[a]	0.48	-2.08;3.04	0.85	2.88	1.31;4.46	0.06	-6.36	-14.50;1.79	0.12
	Model 2[b]	0.20	-2.37;2.76	0.94	2.79	-0.25;5.84	0.07	-6.09	-14.04;1.87	0.10
	Model 3[c]	-1.27	-6.72;4.18	0.65	2.52	-0.54;5.58	0.11	-5.30	-13.48;2.88	0.20
	Model 4[d]	-1.14	-6.58;4.29	0.68	2.68	-0.29;5.65	0.08	-5.54	-13.67;2.58	0.18
Organized sport time-weighted MET (z-score)	Crude	0.60	-4.31;5.50	0.81	1.92	-0.91;4.76	0.18	-4.00	-11.48;3.46	0.29
	Model 1[a]	0.76	-4.33;5.64	0.77	2.80	-0.12;5.71	0.06	-6.60	-14.25;1.05	0.09
	Model 2[b]	0.71	-4.39;5.81	0.78	2.58	-0.29;5.45	0.08	-5.96	-13.50;1.53	0.12
	Model 3[c]	-0.51	-5.97;4.96	0.86	2.46	-0.45;5.35	0.10	-5.55	-13.30;2.19	0.16
	Model 4[d]	-0.53	-5.97;4.91	0.85	2.64	-0.18;5.45	0.07	-5.85	-13.55;1.86	0.14

Notes: RC = linear regression coefficient; cIMT = carotid artery intima media thickness; MET = metabolic equivalent; CI = confidence interval

[a] Model 1: adjusted for age, sex

[b] Model 2: adjusted for age, sex, BMI

[c] Model 3: adjusted for age, sex, parental (mother and father) BMI, prenatal & postnatal smoking exposure, ethnicity, socioeconomic status.

[d] Model 4: adjusted for age, sex, parental (mother and father) BMI, prenatal & postnatal smoking exposure, ethnicity, socioeconomic status, systolic blood pressure, diastolic blood pressure

Measuring physical activity using subjective methods is our study limitation. The completeness and accuracy of information may have been influenced by how parents perceived the questions. The accuracy-practicality trade-off presents a challenge in assessing physical activity in children. Questionnaires have been considered the most feasible low-cost method in moderate-to-large scale studies with acceptable correlation with objective physical activity measurement.[27] Moreover, we did not intend to precisely capture all physical activities performed, but to rank them based on standardised questions incorporating the most common and routine activities around 5 and 8 years of age.

Missing information in physical activity duration data as the component variable to calculate time-weighted MET was another challenge. This again underlines the difficulties in measuring physical activity in children, especially because most children's time is spent on unstructured activities, which are difficult to assess, even by parents. We were also not able to measure the intensity of activities. However, we believe that if there were under or overestimation of physical activity, it would probably have occurred at random and only diminished the effect size. To handle missing data, we have applied multiple

imputations, which is currently considered the most preferable approach.[28,29] To our knowledge, this is the first study to evaluate the effect of physical activity on the vasculature at very young age with repeated vascular measurements using an automated device, so that measurement bias is unlikely.

Thickening of cIMT and corresponding less vascular elasticity is a surrogate marker of pre-clinical atherosclerosis and is strongly associated with increased CVD risk.[30,31] Both in asymptomatic adults and patients with or at increased risk for CVD, the cardio-protective effects of exercise, including direct effects on vascular function and dimension, have been consistently reported.[7] A large population-based trial in adults has shown a relationship between higher physical activity and less atherosclerosis, but only in men.[32] A study in adolescents has also shown favourable effects of physical activity on aortic IMT and flow-mediated dilation.[12] In children, the beneficial effects of physical activity on various cardio-metabolic risk factors, such as waist circumference, blood pressure, lipid profiles, and insulin levels have also been demonstrated.[33]

A number of studies have investigated the possible mechanisms of how physical activity affects the vasculature. It is hypothesised that exercise training is associated with an outward remodelling of the arterial lumen and decreased wall thickness in favour of lower cardiovascular risk.[7] The beneficial effect could be mediated by increased vascular shear stress and therefore nitric oxide bioavailability during regular physical activity, leading to vasodilatation. Regular exercise also has been associated with better angiogenesis, arteriogenesis, and antioxidant capacity of the vascular wall.[34,35] However, we have studied the vasculature in early life when there is neither vigorous exercise nor large physical activity variation between children. We did not find any substantial relation between total activity, which is probably largely restricted to leisure activity, and the vasculature of the 5 year olds but the effect was seen in sports that likely represent activities of higher intensity at this age. In fact, effect estimates at this age were small, certainly as compared to other influences, such as body size, smoking exposure,[23] early life growth,[36] and breastfeeding.[20] We assume that with increasing age, the variation between children in activity types and intensity would gradually increase. At 8 years of age, there was a tendency of lower cIMT with increasing activity level in boys and higher distensibility with more sports although we were not able to estimate them statistically robustly, probably due to relatively small sample size.

Knowing when in life physical activity first exerts effects on cardiovascular risk and in what direction could direct preventive measures. This study shows that in the very young, sport activities may already exert their initial beneficial effects on the vessel wall and therefore should be encouraged. This especially seems relevant for children with higher BMI level given the tendency of more prominent effects in these heavier children. The fact that we were not able to show the effects of total activity involving leisure activities at these young ages does not in our view justify neglect of preventive

measures involving this kind of activities. Although not studied here, physical activity in general modifies risk factor levels in children,[33] which by itself could have beneficial vascular effects later in life,[37] including in adolescence.[12] Active lifestyles adopted in childhood are also most likely to be preserved into adulthood[11] and has preventable effect on many chronic diseases.

In conclusion, total physical activity has little independent effect on the early childhood vasculature. It is sport activity that seems to matter, in which it is associated with thinner carotid intima media thickness around 5 years of age and may lead to more distensible vasculature in pre-puberty, particularly in those with higher body mass index.

Acknowledgement

We gratefully acknowledge all the parents and children who participated, Liesbeth van der Feltz-Minkema for her dedicated assistance, Myriam Olling-de Kok for secretarial support, Jildou Zwerver for data management, and the Vascular Imaging Center for assistance in the vascular measurements.

References

1. Cardiovascular diseases, fact sheet no. 317 [Internet].: World Health Organization; 2009; cited 12 Dec 2012]. Available from: http://www.who.int/mediacentre/factsheets/fs317/en/index.html.
2. Lee I, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT, et al. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet. 2012;380:219-2.
3. Blair SN, Morris JN. Healthy hearts--and the universal benefits of being physically active: physical activity and health. Ann Epidemiol. 2009;19:253-6.
4. Barengo NC, Hu G, Lakka TA, Pekkarinen H, Nissinen A, Tuomilehto J. Low physical activity as a predictor for total and cardiovascular disease mortality in middle-aged men and women in Finland. Eur Heart J. 2004;25:2204-11.
5. Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin BA, et al. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Circulation. 2007;116:1081-93.
6. Mora S, Cook N, Buring JE, Ridker PM, Lee IM. Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms. Circulation. 2007;116:2110-8.
7. Thijssen DH, Cable NT, Green DJ. Impact of exercise training on arterial wall thickness in humans. Clin Sci (Lond). 2012;122:311-22.
8. Norman M, Bonamy AK. Aortic wall thickening in utero. Lancet. 2005;365:1444-6.
9. Natural history of aortic and coronary atherosclerotic lesions in youth. Findings from the PDAY study. Pathobiological determinants of atherosclerosis in youth (PDAY) research group. Arterioscler Thromb Vasc Biol. 1993;13:1291-8.
10. Davies CA, Spence JC, Vandelanotte C, Caperchione CM, Mummery WK. Meta-analysis of internet-delivered interventions to increase physical activity levels. Int J Behav Nutr Phys Act. 2012;9:52,5868-9-52.
11. Telama R, Yang X, Viikari J, Valimaki I, Wanne O, Raitakari O. Physical activity from childhood to adulthood: a 21-year tracking study. Am J Prev Med. 2005;28:267-73.
12. Pahkala K, Heinonen OJ, Simell O, Viikari JS, Ronnemaa T, Niinikoski H, et al. Association of physical activity with vascular endothelial function and intima-media thickness. Circulation. 2011;124:1956-63.
13. Kong AP, Choi KC, Li AM, Hui SS, Chan MH, Wing YK, et al. Association between physical activity and cardiovascular risk in Chinese youth independent of age and pubertal stage. BMC Public Health. 2010;10:303,2458-10-303.
14. Bornstein DB, Beets MW, Byun W, McIver, K. Accelerometer-derived physical activity levels of preschoolers: a meta-analysis. J Sci Med Sport. 2011;14:504-11.

15. Vorwerg Y, Petroff D, Kiess W, Bluher, S. Physical activity in 3-6 year old children measured by SenseWear pro(R): direct accelerometry in the course of the week and relation to weight status, media consumption, and socioeconomic factors. PLoS One. 2013;8:e60619.
16. Katier N, Uiterwaal CS, de Jong BM, Kimpen JL, Verheij TJ, Grobbee DE, et al. The Wheezing Illnesses Study Leidsche Rijn (WHISTLER): Rationale and design. Eur J Epidemiol. 2004;19:895-903.
17. Ridley K, Ainsworth BE, Olds TS. Development of a compendium of energy expenditures for youth. Int J Behav Nutr Phys Act. 2008;5:45,5868-5-45.
18. Dessing D, Pierik FH, Sterkenburg RP, van Dommelen P, Maas J, de Vries SI. Schoolyard physical activity of 6-11 year old children assessed by GPS and accelerometry. Int J Behav Nutr Phys Act. 2013;10:97-105.
19. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: An update of activity codes and MET intensities. Med Sci Sports Exerc. 2000;32(9 Suppl):498-504.
20. Evelein AM, Geerts CC, Visseren FL, Bots ML, van der Ent CK, Grobbee DE, et al. The association between breastfeeding and the cardiovascular system in early childhood. Am J Clin Nutr. 2011;93:712-8.
21. Nagaya T, Yoshida H, Takahashi H, Kawai M. Cigarette smoking weakens exercise habits in healthy men. Nicotine Tob Res. 2007;9:1027-32.
22. Kaczynski AT, Manske SR, Mannell RC, Grewal K.. Smoking and physical activity: a systematic review. Am J Health Behav. 2008;32:93-110.
23. Geerts CC, Bots ML, van der Ent CK, Grobbee DE, Uiterwaal CS. Parental smoking and vascular damage in their 5-year-old children. Pediatrics. 2012;129:45-54.
24. Li S, Chen W, Srinivasan SR, Tang R, Bond MG, Berenson GS. Race (black-white) and gender divergences in the relationship of childhood cardiovascular risk factors to carotid artery intima-media thickness in adulthood: the Bogalusa heart study. Atherosclerosis. 2007;194:421-5.
25. Peterson M, Liu D, IglayReger HB, Saltarelli W, Visich P, Gordon PM. Principal component analysis reveals gender-specific predictors of cardiometabolic risk in 6th graders. Cardiovasc Diabetol. 2012;11:146.
26. Moore XL, Michell D, Lee S, Skilton MR, Nair R, Dixon JB, et al. Increased carotid intima-media thickness and reduced distensibility in human class III obesity: independent and differential influences of adiposity and blood pressure on the vasculature. PLoS One. 2013;8:e53972.
27. Loprinzi PD, Cardinal BJ. Measuring children's physical activity and sedentary behaviors. J Exerc Sci Fit. 2011;9:15-23.
28. Donders AR, van der Heijden GJ, Stijnen T, Moons KG. A gentle introduction to imputation of missing values. J Clin Epidemiol. 2006;59:1087-91.

29. Janssen KJ, Donders AR, Harrell FE Jr, Vergouwe Y, Chen Q, Grobbee DE, et al. Missing covariate data in medical research: to impute is better than to ignore. J Clin Epidemiol. 2010;63:721-7.
30. van den Oord SC, Sijbrands EJ, Ten Kate GL, van Klaveren D, van Domburg RT, van der Steen AF, et al. Carotid intima-media thickness for cardiovascular risk assessment: systematic review and meta-analysis. Atherosclerosis. 2013 Jan 25.
31. Iwakiri T, Yano Y, Sato Y, Hatakeyama K, Marutsuka K, Fujimoto S, et al. Usefulness of carotid intima-media thickness measurement as an indicator of generalized atherosclerosis: findings from autopsy analysis. Atherosclerosis. 2012;225:359-62.
32. Sternfeld B, Block C, Quesenberry CP Jr, Block TJ, Husson G, Norris JC, et al. Improving diet and physical activity with ALIVE: a worksite randomized trial. Am J Prev Med. 2009;36:475-83.
33. Ekelund U, Luan J, Sherar LB, Esliger DW, Griew P, Cooper A; International Children's Accelerometry Database (ICAD) Collaborators. Moderate to vigorous physical activity and sedentary time and cardiometabolic risk factors in children and adolescents. JAMA. 2012;307:704-12.
34. Whyte JJ, Laughlin MH. The effects of acute and chronic exercise on the vasculature. Acta Physiologica. 2010;199:441-50.
35. Schmidt W, Endres M, Dimeo F, Jungehulsing GJ. Train the vessel, gain the brain: physical activity and vessel function and the impact on stroke prevention and outcome in cerebrovascular disease. Cerebrovasc Dis. 2013;35:303-12.
36. Evelein AM, Visseren FL, van der Ent CK, Grobbee DE, Uiterwaal CS. Excess early postnatal weight gain leads to thicker and stiffer arteries in young children. J Clin Endocrinol Metab. 2013 Feb;98:794-801.
37. Kvaavik E, Klepp KI, Tell GS, Meyer HE, Batty GD. Physical fitness and physical activity at age 13 years as predictors of cardiovascular disease risk factors at ages 15, 25, 33, and 40 years: extended follow-up of the Oslo youth study. Pediatrics. 2009 Jan;123:e80-6.

Part II

Childhood HIV infection and cardiovascular health

CHAPTER 5

Cardiovascular manifestations of HIV infection in children

Nikmah S. Idris
Diederick E. Grobbee
David Burgner
Michael M.H. Cheung
Nia Kurniati
Sudigdo Sastroasmoro
Cuno S.P.M. Uiterwaal

Eur J Prev Cardiol. 2014 Nov 14. [Epub ahead of print]

Abstract

Background

HIV infection in children is now considered as a chronic condition, in which various non-infectious complications may occur, including those affecting the developing cardiovascular system. As children are expected to survive well into adulthood, understanding childhood as well as potential future cardiovascular complications is of major importance.

Methods

We reviewed published literature on childhood cardiac manifestations and longer term effects of paediatric HIV infection on the cardiovascular system. Evidence gaps that should be prioritised in research are highlighted.

Results

Through poorly understood mechanisms, HIV infection may cause various cardiac complications already manifesting in childhood, such as structural and functional myocardial derangements, pulmonary hypertension, pericardial effusion, and possibly endocarditis. Evidence indicates that HIV infection in children also has unfavourable effects on the vasculature and cardiovascular biomarkers, such as increased intima-media thickness and decreased flow-mediated dilation, a marker of endothelial function. However, studies are small and predominantly include ART-treated children, so that it is difficult to differentiate between effects of HIV infection per se and ART treatment, reported in adults to have cardiovascular side effects.

Conclusion

HIV infection in children may greatly impact the cardiovascular system, including effects on the heart, which tend to manifest early in childhood, and on the vasculature. The underlying mechanisms, essential for targeted prevention, are poorly understood. Current evidence largely stems from research in adults. However, as modes of infection, immune maturity, growth and development, and treatment are markedly different in children, specific paediatric research, accounting for the complex interplay of normal growth and development, HIV infection and treatment is clearly warranted.

Glossary terms

Human immunodeficiency virus (HIV), an RNA virus that chronically infects the host and causes life-threatening immune dysfunction, particularly of cell-mediated immunity. It typically infects CD4+ cells, which play a central role in the immune response to most pathogens.

Acquired immunodeficiency syndrome (AIDS), a disease caused by infection with human immunodeficiency virus (HIV).

AIDS defining illness/condition, any HIV-related illness included in the Centres for Disease Control and Prevention's (CDC) list of diagnostic criteria for AIDS, including opportunistic infections that are life threatening in children with HIV.

CD4 (cluster designation 4), a symbol for a glycoprotein expressed on the surface of helper T lymphocytes and other immune cells. CD4 serves as a receptor for HIV envelope glycoprotein gp120. Measurement of absolute count or percentage of CD4+ cells is a standard way to characterise the severity of HIV related immunodeficiency; in children, this is defined by a specific age-related cut off. For example, severe or stage 4 disease is defined if absolute counts are less than 200 per mm3 or %CD4 <15% for children older than 5 years, %CD4+ <15 for age 36-59 months, <20 for age of 12-35 months, and <25 for age younger than 11 months).

Viral load, a measure of the severity of a viral infection, which can be calculated by estimating the amount of virus in an involved body fluid, for example given as RNA copies per millilitre of blood plasma.

Antiretroviral therapy (ART), medications for the treatment of HIV infection. Five major classes of ART are currently available: (1) nucleoside/nucleotide analogue reverse transcriptase inhibitors (NRTIs/NtRTIs); (2) non-nucleoside reverse transcriptase inhibitors (NNRTIs); (3) protease inhibitors (PIs); (4) entry and fusion inhibitors; and (5) integrase inhibitors. The regimen given to children, also defined as highly active ART (HAART), consists of three drugs from at least two major classes.

Background

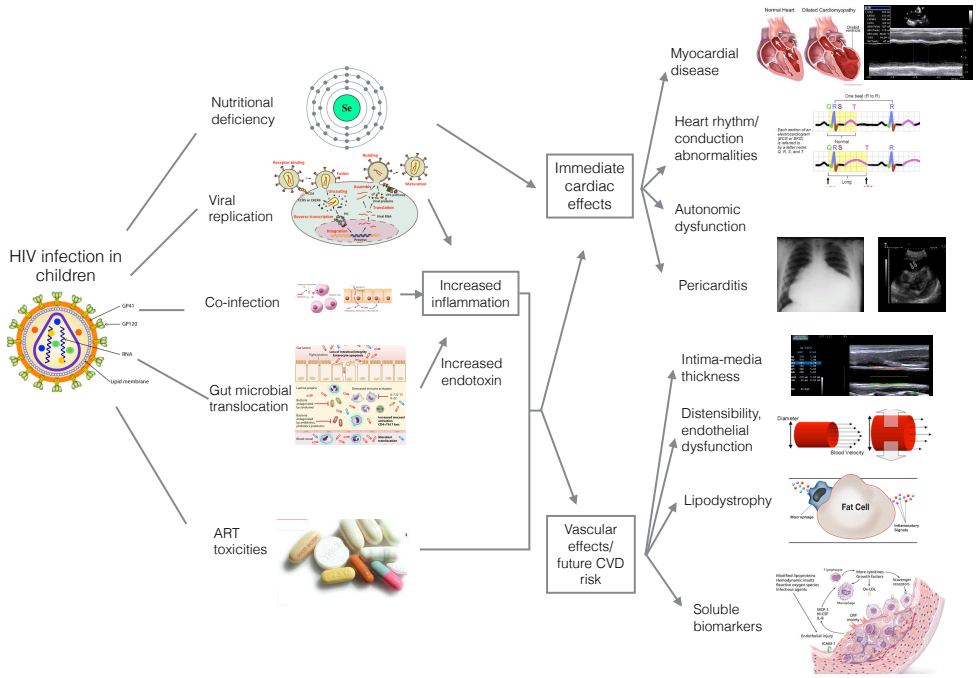
The global Human Immunodeficiency Virus (HIV) epidemic has been ongoing for almost three decades. Despite treatment advances, HIV remains one of the greatest health challenges worldwide. Although the global number of newly infected patients is declining, local epidemics continue to occur. Globally, there are approximately 3.3 million HIV-infected children, with an estimated 1700 new infections per day, of which more than 90% occur in low and middle income countries.[1] HIV infections in children are almost exclusively vertically acquired, either in utero, at delivery, or with breast feeding. Once infection is established in children, the current best practice is to commence antiretroviral treatment (ART) as early as possible to restore immune function and improve survival.

Anti-retroviral therapy (ART) has improved survival substantially, such that HIV infection is now considered a chronic condition. However both HIV infection per se and its treatment have been suggested to be associated with significant cardiovascular complications.[2] Through incompletely understood mechanisms, HIV infection may damage the heart and vasculature. In adults, this is reflected in higher risks of atherosclerosis,[3] cardiovascular events, and heart failure.[4] In children, cardiovascular effects of HIV infection may already manifest early in life and/or impact on the risk of later cardiovascular disease (Figure 1). Immediate clinical manifestations in childhood mostly involve the heart, whereas future cardiovascular risk is largely related to the effects of HIV infection on the vasculature. As ART-treated HIV-infected children are expected to survive well into adulthood, understanding childhood as well as potential future cardiovascular complications is of major importance. Here, we review the current evidence regarding immediate and longer term effects of paediatric HIV infection on the cardiovascular system. We highlight evidence gaps and areas that should be prioritised in research.

Methods

Relevant published literature was initially retrieved by electronic searching in PubMed and EMBASE using predefined keywords (see Table 1). We initially started with a broad search on possible cardiovascular complications and further refined the search according to particular cardiovascular issues. Bibliographies of the retrieved articles were further traced to obtain additional relevant articles. The search was initially focused on studies involving paediatric populations and further expanded to adults if there was limited evidence in children for a particular topic. Neither publication year nor language limits were applied. The summary of key studies is presented in Table 2.

Figure 1. Proposed cardiovascular effects of HIV infection in children. (Some illustrations are reproduced with permissions from several sources). ART: antiretroviral therapy; CVD: cardiovascular disease.



Effects of paediatric HIV infection on the heart

Paediatric HIV infection may affect the myocardium, endocardium, pericardium, conduction system, and the coronary vessels.[5] Abnormalities include dilated cardiomyopathy, diastolic dysfunction, pericardial effusion, pulmonary hypertension, non-bacterial thrombotic endocarditis, arrhythmia, autonomic dysfunction, lymphoma, as well as secondary complications, such as infective endocarditis.[6] Dilated cardiomyopathy and pulmonary hypertension are common, whereas endocarditis, lymphoma, significant arrhythmia and pericardial effusion are rare.

In the pre- and early ART era, subclinical cardiac abnormalities in early childhood were frequent, persistent, and often progressive.[7] Almost one-third of children developed serious cardiac events following an AIDS diagnosis.[8] The likelihood of developing chronic cardiac disease increased with age.[9] Cardiomegaly was found in over half of HIV-infected children and correlated with increased left ventricular mass, clinical symptoms, and pericardial effusion.[10] Serious cardiac events (congestive heart failure, hypotension, severe dysrhythmia, cardiac tamponade, cerebrovascular accident, and cardiac arrest) typically occurred between 8 months and 5 years of age.[8]

Table 1. Keywords used in literature searching

Determinant		HIV OR 'human immunodeficiency virus' OR AIDS OR 'acquired immune deficiency syndrome' OR 'HIV-infected' OR 'HIV-positive'
Outcomes	General search	cardiac OR heart OR vascular OR cardiovascular OR atherosclerosis OR 'ischemic heart disease' OR 'coronary heart disease'
	Heart failure/ dysfunction	heart failure' OR 'cardiac failure' OR 'ventricular function' OR 'heart function' OR 'cardiac function' OR 'systolic function' OR 'systolic dysfunction OR 'diastolic function' OR ' diastolic dysfunction' OR cardiomyopathy OR myocarditis
	Pulmonary hypertension	pulmonary hypertension' OR 'pulmonary arterial hypertension'
	Rhythm/ECG abnormalities	arrhythmia OR dysrhythmia OR ECG OR electrocardiogram OR electrocardiography OR autonomic OR autonome
	Endocarditis	endocarditis OR vegetation
	Pericardial effusion	pericardium OR pericardial OR 'pericardial effusion' OR pericarditis
	Vascular structure/ function	intima-media thickness OR distensibility OR 'arterial stiffness' OR endothelial OR 'elastic modulus' OR 'flow mediated dilatation' OR 'flow-mediated dilatation' OR FMD OR 'pulse wave velocity'
	Lipodystrophy	lipodystrophy OR lipoatrophy OR lipohypertrophy OR fat OR cholesterol
	Soluble biomarkers	(marker AND vascular) OR (marker AND cardiovascular) OR 'cardiovascular biomarker' OR 'cardiovascular marker' OR 'vascular biomarker' OR 'vascular marker'
Domain*		children OR child OR pediatric OR paediatric

*keywords were opt out if no relevant paediatric study retrieved on the first attempt.

With the introduction of ART, the incidence of clinically relevant early cardiovascular complications decreased significantly.[11] ART appears to be cardioprotective and preserve left ventricular (LV) fractional shortening (FS). For example the prevalence of cardiomyopathy reduced from 44% of children in the pre-ART era to 3.7% with ART. Whether these effects are direct, by reduction of HIV-related cardiac damage, or indirect, through improving overall health and nutrition, is unclear. Notably, asymptomatic structural abnormalities in childhood on ART remain common,[12] and may lead to clinical manifestations in later life.

Despite demonstrable cardiac abnormalities in HIV-infected children, especially in the pre-ART era,[10] underlying mechanisms remain poorly understood. Various causes have been proposed, largely based on adult studies. Although each type of cardiac pathology may have specific causes (as discussed below), general effects on children's hearts may occur through HIV-induced inflammatory mediators (e.g. cytokines or proteolytic enzymes), co-infection, nutritional deficiencies, autoimmune response to infecting pathogens, ART cardiotoxicity, and/or direct viral cell invasion.[5] HIV-associated cardiac pathology is likely to be multifactorial. Direct HIV infection of cardiomyocytes in children is unusual and indirect mechanisms are therefore probably more important.[13]

Heart muscle disease, either dilated cardiomyopathy or isolated left or right ventricular dysfunction, is the most important and best studied cardiac manifestation of HIV

infection in children. Cardiomyopathy increases mortality of HIV-infected children up to six-fold, independent of CD4 count, gender, age, and HIV clinical stage.[8] In settings where ART is readily available, the incidence of clinical cardiomyopathy has reduced to 4%,[11] but remains relatively common in settings with limited access to ART. Moreover, the cardio-protective effect of ART may be finite, as HIV-infected ART-exposed children show a significant decline in LV contractility at 10 years of follow-up that is equivalent to that observed in HIV-infected but less ART exposed children.[14]

Myocardial injury

Myocarditis is probably the most important cause of HIV-associated cardiomyopathy. Recently, myocarditis has been identified in 44% of adults with HIV-associated cardiomyopathy.[15] A variety of pathogens (including cytomegalovirus, *Mycobacteria sp*, toxoplasma, *Candida sp*, histoplasma, and *Staphylococcus aureus*) have all been identified in the myocardium of HIV-infected patients, but the clinical significance is unclear; myocyte necrosis or inflammation is very rarely observed in surrounding tissue, suggesting that secondary infection is unlikely to cause myocarditis directly. In adults, most opportunistic pathogens are identified only by PCR, indicative of a low pathogen load. PCR is also unable to differentiate viable or actively dividing pathogens.[5]

Myocardial cells may be damaged through reservoir cells located in myocardial and brain interstitial tissue, which may harbour viruses for extended periods and chronically release cytotoxic cytokines, including tumour necrosis (TNF)-alpha, interleukin (IL) 6, IL-1, and transforming growth factor-beta, resulting in tissue damage.[5] TNF-alpha alters intracellular calcium homeostasis and induces nitric oxide synthesis, which can injure myocardial cells and produce negative inotropic effects. TNF-alpha induced apoptosis and myocardial diffuse regressive alterations (thinning and waving cardiomyocytes with increase of lipofuscin pigment granules) have been observed in HIV-infected patients with biventricular dilatation.[16] This mechanism may also partly explain the increased risk of congestive heart failure in HIV-infected children with encephalopathy, as reservoir cells may persist in the cerebral cortex even with ART.[17]

The effects of HIV on myocardial growth and function are believed to be immunologically mediated, as children with AIDS receiving monthly intravenous immunoglobulin therapy have more normal LV structure and function.[18] HIV-infected adults have elevated cardiac autoantibodies (anti-alpha myosin autoantibodies) compared to HIV-negative individuals and the antibody titre is even higher in HIV-infected adults with heart muscle disease.[19]

HIV-infected children are prone to nutritional deficiency and electrolyte imbalance from poor intake, malabsorption, and diarrhoea. A lower body mass index is an independent risk factor for cardiomyopathy.[20] Deficiency of trace elements, especially selenium, may be linked to cardiomyopathy in HIV uninfected children and this may be relevant in HIV-infected children.[21] Low levels of vitamin B12, carnitine, growth hormone, and thyroid

hormone may occur in HIV infection, and are also associated with LV dysfunction in children.[14] The role of trace element deficiency in HIV-associated cardiomyopathy has not been studied in children.

Antiretroviral therapy improves short term LV function in HIV-infected children,[22] but some classes of ART may induce ventricular dysfunction, possibly through myocyte injury. Zidovudine has been reported to increase the risk of cardiomyopathy up to almost seven-fold, especially for children with low nadir CD4 percentage (<15%), although the findings are controversial.[22] In animals, zidovudine induces diffuse destruction of cardiac mitochondrial ultrastructures and inhibits mitochondrial DNA replication, which hampers growth, reduces myocardial energy production, increases apoptosis, and inhibits repair mechanisms.[23] In animal models, protease inhibitors such as lopinavir and ritonavir, interfere with myocardial ubiquitin proteasome system, contributing to long term cardiac contractile dysfunction.[24]

Ventricular function

There are two types of HIV-associated cardiomyopathy; LV dilatation with a reduced ratio of LV wall thickness to end-systolic dimension, and dilatation with concentric hypertrophy, such that the ratio of wall thickness to end-systolic dimension is unchanged or increased.(14) Both may manifest as LV systolic dysfunction, which clinically varies from asymptomatic to overt heart failure, and probably diastolic dysfunction.[14]

HIV-infected children who are not or minimally exposed to ART show progressive deterioration of LV structure and function in the first 3 years of life, resulting in chronically low LV function and elevated LV mass.[23] Even when treated, HIV-infected children have lower fractional shortening and ejection fractions than healthy children. About half of HIV-infected African children had LV systolic dysfunction.[25] Among ART-treated children, older age at treatment initiation, a regimen containing zidovudine, and a nadir CD4 % < 15% were independent predictors of cardiomyopathy.[22]

Left ventricular systolic dysfunction may be more subtle, with prolonged ART duration (>10 years) and only detectable by strain echocardiography that measures myocardial deformation in circumferential, radial, and longitudinal directions.[26] HIV-infected children and young adults may have impaired longitudinal strain and strain rate despite normal systolic function and ejection fraction, though available data are based on unadjusted analysis.[26, 27]

Diastolic dysfunction is less well-characterised in HIV-infected children, although it may be the first sign of underlying cardiac disease. Almost half of HIV-infected, particularly older, adults with higher blood pressure and heart rate (both ART naive and exposed) have mild diastolic dysfunction,[28] mostly manifested as a lower E/A (early passive filling/atrial contraction) ratio, indicating impaired relaxation.[28] After adjustment for age

and hypertension, HIV-infected adults still have a 2.4 fold increased risk for diastolic dysfunction compared to uninfected subjects.[28] It is unclear if diastolic dysfunction results from HIV infection itself, ART, and/or co-morbidities although affected subjects tended to have lower CD4 count and/or a longer duration of NRTI use.[28] Data from children are very limited. Left or right ventricular diastolic dysfunction, predominantly of restrictive pattern (high E/A ratio), has been found in around 40% and 25% of clinically stable HIV infected children, respectively.[29] Its relation with HIV treatment status has not been clearly demonstrated, but ART may play a role as HIV-exposed but uninfected infants who have been exposed to prophylactic ART have lower early diastolic annular velocity than controls.[30]

Pulmonary arterial hypertension

Data on pulmonary arterial hypertension in children are scarce and mostly extrapolated from adults with HIV, in whom the prevalence is 0.5% to 5% with a slight female predominance,[31] a likely underestimation as most reports are limited to symptomatic patients.[32] In low-to-middle income countries, the prevalence among adults is probably much higher, ranging from 8% to 13% in the ART era.[31] In fact, HIV infection presents in one third of adults with pulmonary arterial hypertension in Africa. [33] Autopsy data indicate that pulmonary arterial hypertension is caused by replacement of normal endothelial structure by arteriopathic remodelling with intimal fibrosis.[34] The mechanism is largely unknown but may involve HIV-related proteins (Nef, Tat, and Gp-120 protein), HIV-induced inflammation, co-infections (particularly human herpes virus 8, hepatitis C, and hepatitis B) and heightened inflammation due to exposure to microbial products, thromboembolic events, and genetic susceptibility through certain human leukocyte antigens, such as HLA-DR6 and HLA-DR54.(34) The role of ART in the pathogenesis and severity of PAH is also uncertain.[31] Clinically, patients often present with dyspnea, whereas chest pain or discomfort, cough, haemoptysis, dizziness or syncope are less common.[35] Children may also have right-sided heart failure, with facial oedema, hepatomegaly, or oedema of lower extremities. Most adults with HIV show cardiomegaly, pulmonary arterial enlargement, dilated right ventricle, and tricuspid regurgitation. In adults, HIV-associated pulmonary hypertension is best managed with pulmonary hypertension-specific drugs (endothelin receptor antagonists, prostaglandin analogs, and phosphodiesterase 5 inhibitors) and ART, which together improve survival, compared to ART alone.(34) General measures such as supplemental oxygen may also be beneficial. Evidence in children, although circumstantial, is similar.[36,37]

Pericardial effusion

In the pre-ART era, pericardial effusion occurred in 11% of HIV infected children. The current incidence in developed countries is 0.5%,[14] but pericardial effusion accounts for 12% of cardiac manifestations in HIV-infected children in Africa. The effusion is

usually clinically insignificant and drainage is rarely required but should be closely monitored and evaluated for opportunistic infection.[38] Pericardial effusion with HIV in low-resource settings is probably associated with the high endemicity of tuberculosis, as the non-HIV infected incidence is similar.[38] It often occurs concurrently with peritoneal and pleural effusions and may reflect a direct effect of HIV(39) and/or co-infection with tuberculosis; HIV infected children are at greatly increased risk for tuberculosis.[40]

Endocarditis

There is scant evidence in children of other cardiac manifestations reported in HIV-infected adults, such as infective endocarditis, nonbacterial thrombotic endocarditis, and cardiovascular malignancy. Infective endocarditis has been reported as the first presentation of AIDS in early infancy.[41] Neither cardiac malignancy nor nonbacterial thrombotic endocarditis have been reported in paediatric populations.

Electrophysiologic abnormalities

Between 50 to 93% of HIV-infected children show electrocardiographic abnormalities, including voltage abnormalities, conduction defects, and dysrhythmias.[42] Most abnormalities are benign and rarely require treatment. In HIV-infected children, 24-hour Holter monitoring suggests that 6% have some form of dysrhythmia, including second-degree atrioventricular block or supraventricular or ventricular tachycardia, but third-degree AV block, atrial fibrillation, and atrial flutter were not observed.[42] More rarely, junctional rhythm, first degree AV block, premature atrial or ventricular contraction, were observed. HIV-infected children tend to have a prolonged QT interval, particularly with older ages.[42] Prolonged QT interval may predispose to ventricular arrhythmias, particularly torsades de pointes. In HIV-infected adults, it is associated with hyperlipidaemia, diastolic dysfunction or other cardiac conditions, older age, female sex, ethnicity, and diabetes mellitus.[43] ART, particularly PI, also appears to prolong QTc.[44] Autonomic nervous system disturbances and sudden death have also been rarely reported in HIV-infected children.[45] The immune and autonomic nervous systems are functionally related and sympathetic and parasympathetic nerves influence the development and proliferation of CD4+ cells. Cardiac autonomic dysfunction correlates with adverse immunological status of HIV-infected children. Abnormalities include attenuation of parasympathetic-mediated circadian rhythm heart rate variability and impairment of night time QTc interval lengthening.[46]

HIV infection, childhood vasculature, and future risk of ischemic vascular disease

The importance of vascular pathology in HIV-infected patients is increasingly recognised. Adults with HIV infection have a 1.5-1.7 fold higher risk of acute myocardial infarction than the general population[47] and the risk remains 40% higher even when HIV-1 RNA levels are controlled to under 500 copies/ml.[48] Low grade chronic inflammation is a central pathogenic mechanism in atherosclerosis and is a mechanism by which HIV infection may accelerate atherosclerosis and increase cardiovascular disease (CVD) risk.[49] Autopsy data show that vasculopathy occurs earlier and with greater severity in HIV-infected individuals, both in the coronary arteries and large vessels. This may be mediated by immunodeficiency, increased circulating endotoxin, or direct induction of pro-thrombotic and pro-inflammatory changes in the vessel wall. In addition, it may reflect toxicity of ART, particularly the protease inhibitors,[50] which may induce lipodystrophy, dyslipidaemia, and impaired glucose metabolism, each established independent CVD risk factors. Circumstantial evidence indicates that HIV infection in children also has unfavourable effects on the vasculature and cardiovascular biomarkers. Compared to uninfected subjects, older HIV-infected children with long term exposure to ART have increased intima-media thickness (IMT), a non-invasive ultrasound marker of atherosclerosis, and decreased flow-mediated dilation (FMD), a marker of endothelial function.[51] However, studies are small and predominantly include ART-treated children, so that it is difficult to differentiate between effects of HIV infection per se and treatment.

The pathophysiology of accelerated atherosclerosis in HIV-infected patients is complex, multifactorial, and not well understood.[52] The underlying mechanisms are probably different in ART-naive and -exposed patients.[53] In ART-naive patients, HIV infection may promote atherosclerosis through immune activation, chronic inflammation, coagulation disorders, and/or lipid disturbances.[53] HIV replication and lymphocyte/monocyte activation is associated with release of inflammatory cytokines and early endothelial dysfunction, which may be driven by HIV persistence, damage of mucosal lymphatic tissue associated with microbial translocation,[54] and co-infection. Endothelial injury was shown to occur in HIV-infected subjects due to adhesion molecules, HIV Tat protein, and related angiogenic effects.[53] Independent of traditional cardiovascular risk factors, the HIV also induces pro-thrombotic and pro-coagulation activity associated with high levels of circulating endothelial cells and micro particles from endothelium and platelets. Normal restoration mechanisms after an injury to blood vessels also appear to be impaired with HIV infection. In addition, HIV infection in adults may, independent of ART, be associated with dyslipidaemia, including high triglycerides and decreased high-density lipoprotein cholesterol.[55]

Although pro-atherosclerotic mechanisms in treatment-naive HIV patients are probably partly attenuated by treatment, ART exposed HIV patients may still have a higher cardiovascular risk than the general population. ART induced immune activation, chronic inflammation, and toxicity are suggested mechanisms.[52] It is increasingly apparent that chronic immune activation and inflammation may promote atherosclerosis and increased arterial stiffness in the absence of residual viral replication.[56] A critical but unanswered question is whether suppression of immune activation decreases cardiovascular risk, especially if ART is commenced early in childhood.

The contribution of ART to atherosclerosis is a burgeoning field. Protease inhibitor exposure is consistently associated with increased cardiovascular risk, probably through its induction of adipocyte dysfunction with disturbed lipid and glucose metabolism, leading to lipodystrophy syndrome.[57] The association between NRTI or NNRTI and cardiovascular risk remains controversial; some drugs in these classes are associated with insulin resistance (stavudine, zidovudine) or dyslipidemia (didanosine, stavudine; efavirenz, nevirapine) to varying degrees.[52] Thus ART could have beneficial or detrimental effects on cardiovascular risk and the net effect is unknown, particularly in children.

Vascular structure and function

Most studies investigating the association between HIV infection and childhood vascular characteristics have enrolled HIV-infected children exposed to ART. Carotid IMT appears to be dynamic in children on ART, as following treatment duration, carotid intima-media thickness (cIMT) may regress to levels similar to those of healthy children, or increase with prolonged treatment.[58] With respect to vascular function, few studies have shown reduced FMD, distensibility, and increased arterial wall stiffness in HIV-infected children compared to non-infected children. The causes of structural and functional vascular changes in HIV-infected children are largely unknown. Chronic inflammation, immune activation, and treatment have been suggested but evidence is scant. A small study did not reveal an association between immune activation markers (HLA-DR+ and CD38 expression) and increased cIMT, despite higher levels of immune activation in ART-exposed HIV-infected children.[59] Associations between high sensitivity C-reactive protein (hsCRP), CD4 level, viral load, and cIMT are also inconclusive.[60] Moreover, as most data are from ART-exposed children, it is not possible to differentiate between the effects of HIV infection and treatment. However, there is evidence that PI treatment in children is associated with increased cIMT and lower FMD than children unexposed to PI or unexposed to any ART.[60]

Lipodystrophy

Up to half of HIV-infected children develop lipodystrophy, especially with long-term exposure to the NRTI stavudine, which is now rarely used in developed countries.[61] Lipodystrophy is characterised by at least one of the following two conditions: (1)

peripheral lipoatrophy (LA) or loss of fat in the face, arms, legs, neck, and buttocks; (2) central lipohypertrophy (LH) or fat accumulation in the abdomen/trunk, breast, and over the dorsocervical spine.[61] Lipodystrophy is often, but not always, accompanied by metabolic derangements, such as insulin resistance or dyslipidaemia. Lipodystrophy is diagnosed with a clinical score and, particularly for research purposes, diagnosis often involves objective fat mass quantification by skin-fold thickness, dual-energy X-ray absorptiometry, magnetic resonance imaging, and metabolic abnormalities.

The mechanisms by which fat redistribution occurs in HIV-infected children are incompletely understood. It is suggested that the mechanisms for lipoatrophy and lipohypertrophy differ but how they result in certain phenotypes, either LA dominant, LH dominant, or mixed patterns is unknown. Lipoatrophy is probably caused by mitochondrial DNA toxicity due to NRTI exposure, and possibly HIV infection itself, leading to mitochondrial depletion, respiratory chain dysfunction, decreased ATP and lipogenesis as well as increased fat apoptosis.[62] Lipohypertrophy and dyslipidaemia are often linked to a sequence homology between HIV-1 protease and both cytoplasmic retinoic acid-binding protein type 1 (CRABP-1) and low-density lipoprotein receptor-related protein (LDLR-RP).[62] Drugs that have a high affinity to HIV-1 protease are most likely to also inhibit CRABP-1 and LDL-RP, resulting in fat storage depletion and increased apoptosis with subsequent increased fat release into the circulation, together with a failure of hepatocytes and endothelial cells to remove the circulating lipids efficiently. There is some evidence that disturbed insulin sensitivity and lipid metabolism in HIV-infected children is mediated by low levels of adiponectin, an adipocytokine that controls insulin sensitivity,[63] and by ART-induced adipocyte dysfunction, which increases pro-inflammatory mediator secretion and limits fat storage capacity.[64] Lipodystrophy is associated with poor self-image and non-compliance with treatment, and has been suggested to increase cardiovascular risk in HIV-infected adults.[65] Whether HIV-children with lipodystrophy also have an increased risk of premature atherosclerosis is unclear.

Cardiovascular biomarkers

The effects of HIV infection in children on the vasculature may also be reflected in changes in CVD risk soluble biomarkers, HIV replication, increased microbial translocation due to permanent mucosal lymphatic damage, and the presence of co-pathogens are proposed to induce release of pro-inflammatory mediators, coagulation markers, tissue factors, leading to vascular injury and endothelial dysfunction.[66] Increased levels of CRP, IL-6, and D-dimer (a coagulation marker) are reported in HIV-infected adults and predict CVD and all-cause mortality.[67] These increased levels persist despite ART and undetectable HIV RNA.[68] Higher viral load is associated with higher levels of TNF-α and soluble vascular adhesion molecule (sVCAM, a marker of endothelial activation). Reduced CD4 counts are correlated with increased IL-6.[69] Findings from HIV-

infected children, mostly with long-term ART exposure, are inconclusive. Some studies indicate increased sVCAM and fibrinogen levels, but not higher CRP or IL-6 levels in HIV-infected children compared to healthy or exposed-uninfected children,[70] but findings are inconsistent.[71] It is likely that the complex interactions between metabolic status, inflammation and infection burden affect biomarker profile, which is dynamic. Unfavourable lipid profiles are positively associated with IL-6, monocyte chemoattractant protein (MCP-1), fibrinogen, and P- and E-selectin, while increased HIV viral load is correlated with increased markers of inflammation (MCP-1 and CRP) and endothelial dysfunction (soluble intercellular adhesion molecule (sICAM), and sVCAM).[70]

Accelerated inflammation in HIV-infected subjects is also suggested to result from increased microbial translocation from the gastrointestinal tract with a high level of circulating endotoxin (lipopolysaccharidase ,LPS), from gut microbiota.[72] HIV-infected adults have a higher levels of circulating LPS, while LPS is associated with increased level of triglycerides and LDL, lower insulin sensitivity, and a higher Framingham risk score. There are no published data for HIV-infected children on the association between LPS and cardiovascular risk factor levels.

Implications for patient care

Given increasing evidence of cardiovascular involvement in HIV infection in children, evaluation for possible cardiovascular complications should be emphasised in the care of HIV-positive children to allow early detection and effective management.[73] Cardiac abnormalities probably need to be screened regularly using a sensitive tool, such as strain echocardiography to detect systolic dysfunction.[27] As HIV infected children may have an increased baseline CVD risk, management of traditional risk factors, such diet, physical inactivity, tobacco exposure, blood pressure, lipid, and obesity is also very important.[74] Imaging biomarkers, such as IMT and/or FMD measurements, may also be useful to identify early changes and stratify CVD risk. Given that ART has been implicated to play a role in the development of cardiovascular morbidities, there might be a need to modify treatment regimen in the future if evidence warrants it.

Conclusions

HIV infection in children has significant consequences for the cardiovascular system, including effects on the heart, which tend to manifest early in childhood, and on the vasculature. The underlying mechanisms, essential for targeted prevention, are poorly understood. Current evidence on HIV infection and cardiovascular disease largely stems from research in adults. However, modes of infection, immune maturity, growth and development, and treatment are markedly different in children and specific paediatric research, which accounts for the complex interplay of normal growth and development, HIV infection and ART, is clearly warranted.

Table 1. Summary of key studies on cardiovascular manifestations of HIV infection in children

CV issue/ disease	Authors	Year	Country	Study design	Sample size	HIV-positive	HIV-negative	Key findings
Cardiac structure (pathology study)	Kearney et al	2003	US	Postmortem study, correlated with clinical findings	30	30*	-	Postmortem cardiomegaly was found in 53% of HIV-infected children and correlated with high LV mass on echocardiography.
LV structure/ function	Lipshultz et al	2002	US	Longitudinal cohort of infants born to HIV-positive mothers	546	93*	463 (HIV-exposed uninfected)	Vertically-transmitted HIV-1 infection is associated with lower LV fractional shortening and higher LV mass relative to those exposed uninfected.
	Ige et al	2012	Nigeria	Cross-sectional study	300	150*	150	LV systolic dysfunction is more frequent in HIV-infected than healthy children (50% vs. 3.3%).
	Lipshultz et al	2013	US	Cross sectional study; comparisons with historical cohort (pre-HAART) and HIV exposed uninfected group.	584	325 (HAART), 70 (pre-HAART)	189	HIV-infected children exposed to long-term HAART have better LV systolic function than children in pre-HAART era.
Cardiac function (strain)	Sims et al	2012	US	Cross sectional study; analysis on stored echo images of 2-29 year old HIV-infected subjects and age/sex-matched controls.	56	28 (treated)	28	Despite normal ejection fraction and fractional shortening, global longitudinal and circumferential strain rate, as well as radial strain rate, were significantly impaired in the HIV-infected group compared with controls.

CV issue/ disease	Authors	Year	Country	Study design	Sample size	HIV-positive	HIV-negative	Key findings
	Al Naami et al	2014	US	Cross sectional study on 15 children and 25 young adults with HIV infection. Controls were patients referred for murmur, chest pain, or suspected ischemic heart disease	95	40 (mean age 21 years)	55 (mean age 17 years)	HIV infected children and young adults have lower longitudinal systolic cardiac strain rate as compared to uninfected subjects (unadjusted analysis). Normal ejection fraction might be attributed to preserved circumferential myocardial deformation.
Type of cardiac clinical manifestations	Luginbuhl et al	1993	US	Historical cohort of children evaluated in 1984-1991	81	81	-	Dysrhythmia, mostly atrial ectopic, occurs in 25% of patients, unexpected cardiac arrest in 9%, chronic congestive heart failure in 10%, and transient depressed LV systolic function in 10%. The presence of encephalopathy or EBV infection appears to identify children at high risk for adverse cardiac outcomes.
	Brown et al	2005	South Africa	Cross sectional study	171	75*	96	LV systolic function is the most common abnormality in HIV children. Pericardial effusion occurred in 19% of HIV-infected and 10% of uninfected children.
Diastolic dysfunction	Cade et al	2012	US	Cross-sectional study	60	-	30 HIV and ART exposed, 30 HIV unexposed	HIV-negative pre-adolescent children exposed to ART in utero and postpartum have lower values of LV diastolic function and LV mass than healthy children.
Pulmonary hypertension**	Janda et al	2010	US	Systematic review on adult studies	154 case reports; 13 cohort, 1 case series, 2 case control studies			HIV-related pulmonary hypertension is a rare entity with features similar to those of idiopathic PAH. There is some limited evidence on the benefit of HAART, bosentan, or prostaglandin therapy.
	Stewart et al	2011	South Africa	Cross-sectional analysis on adult registry of heart failure	5328	?		33% of adult cases of pulmonary hypertension are related to concurrent HIV infection.

CV issue/ disease	Authors	Year	Country	Study design	Sample size	HIV-positive	HIV-negative	Key findings
	Wong et al	2006	Malaysia	Case report	1	1	-	Sildenafil may be beneficial for HIV-related pulmonary hypertension.
	Nunes et al	2003	France	Case series of 82 adults	82	82 (ART treated)	-	CD4 lymphocyte count is an independent predictor of survival in patients with HIV-associated PAH.
Endocarditis	van Doorn et al	1998	London	Case report	1	1	-	Endocarditis may present in HIV-infected children with structurally normal hearts.
ECG abnormalities	Saidi et al	2000	US	Prospective cohort with annual ECG and Holter monitoring studies.	761	298	463	Rhythm disturbances are rare in HIV-infected children. The incidence of 1st degree AV block or PVC is around 8%, but complete block or ventricular tachycardia is very rare. Corrected QTc interval is significantly higher in HIV-infected children but still within normal limits.
Cardiac autonomic function	Benchimol-Barbosa	2009	Brazil	Cross-sectional analysis on HIV-infected preschool children with preserved LV systolic function	35	35	-	Parasympathetic-mediated autonomic dysfunction correlates with immune status. Both 24-hour RR interval variability and circadian QTc interval adaptation seems to be impaired in HIV-infected children.
Arterial stiffness	Bonnet et al	2004	France	Cross sectional study	73	49*	24	Arterial compliance, distensibility, and endothelium-dependent dilation are reduced in HIV-infected children, while wall stiffness is higher compared to healthy controls. No significant difference in carotid intima-media thickness observed.
IMT	Ross	2010	US	Prospective cohort with measurement at baseline and 48 weeks	72	35* (mostly on ART with undetectable HIV-RNA)	37	cIMT was higher in HIV-infected children at baseline but similar at 48 weeks as compared to healthy controls.
	Sainz et al	2014	Spain	Cross sectional analysis on an HIV cohort	300	150*	150	IMT was thicker in HIV-infected children compared to healthy controls. Increased IMT was related to a low nadir CD4 but not with inflammatory, immune activation (CD38+HLA-DR+) or immune senescence markers (CD28-CD57+).

CV issue/ disease	Authors	Year	Country	Study design	Sample size	HIV-positive	HIV-negative	Key findings
IMT/FMD	Charakida et al	2005	UK	Cross-sectional study	142	83 (56 had taken ART, 27 never treated)	59	HIV infection in childhood is associated with thicker IMT and lower FMD, and most pronounced in children exposed to PI therapy.
Lipodystrophy	European Paediatric Lipodystrophy Group	2004	UK	Cross sectional study on children 3 years old or above.	477	477* (mostly on ART, median treatment time is 4.7 years)	-	Approximately a quarter of HIV-infected children have signs of lipodystrophy. Independent predictors are female gender, ever use of PI and stavudine.
	Vigano et al	2003	Italy	Prospective cohort with measurement at baseline and after 12-months of protease inhibitor (PI) based HAART	93	37	54	Rate of DXA-detectable lipodystrophy at baseline and 12-months after starting PI based HAART are 70% and 84%, respectively. Mixed lipodystrophy and central fat accumulation are the most common phenotypes. Duration of HAART influences visceral adiposity and peripheral fat loss.
	Vigano et al	2011	Italy	Cross sectional study	208	36	171	Low adiponectin concentration is associated to central fat and mixed lipohypertrophy and to signs of insulin resistance in HIV infected children and adolescents.
CV soluble biomarkers	Miller et al	2012	US	Cross sectional analysis on an HIV cohort	366	226*	140 (HIV-exposed, uninfected)	HIV-infected children have higher lever of vascular dysfunction biomarkers (MCP-1, fibrinogen, sICAM, sVCAM) than HIV-exposed uninfected children. Unfavourable lipid levels and active HIV replication are associated with higher biomarker levels.
CV soluble biomarkers and IMT	Ross et al	2010	US	Cross sectional analysis on an HIV cohort	57	27 (96% treated)	30	HIV-positive children have higher hs-CRP compared to healthy children, but similar cIMT, adhesion molecule and other pro-inflammatory marker levels. The hs-CRP level was associated with cIMT.

*No disentanglement between ART-treated and untreated HIV-infected children in the analysis; **Most evidence comes from adult studies.

References

1. UNAIDS. Global report: UNAIDS report on the global AIDS epidemic 2012. Geneva: UNAIDS, 2012.
2. Chakraborty R. HIV-1 infection In children: a clinical and immunologic overview. Curr HIV Res. 2005;3:31-41.
3. Post WS, Budoff M, Kingsley L, Palella FJ, Jr., Witt MD, Li X, et al. Associations between HIV infection and subclinical coronary atherosclerosis. Ann Intern Med. 2014;160:458-67.
4. Butt AA, Chang C, Kuller L, Goetz MD, Leaf D, Rimlan D, et al. Risk of heart failure with human immunodeficiency virus in the absence of prior diagnosis of coronary heart disease. Arch Intern Med. 2011;171:737-43.
5. Barbaro G, Fisher SD, Lipshultz SE. Pathogenesis of HIV-associated cardiovascular complications. Lancet Infect Dis. 2001;1:115-24.
6. Fisher SD, Lipshultz SE. Epidemiology of cardiovascular involvement in HIV Disease and AIDS. Ann New York Academy Sci. 2001;946:13-22.
7. Lipshultz SE, Easley KA, Orav EJ, Kaplan S, Starc TJ, Bricker JT, et al. Cardiovascular status of infants and children of women infected with HIV-1 (P2C2 HIV): a cohort study. Lancet. 2002;360:368-73.
8. Al-Attar I, Orav EJ, Exil V, Vlach SA, Lipshultz SE. Predictors of cardiac morbidity and related mortality in children with acquired immunodeficiency syndrome. J Am College Cardiol. 2003;41:1598-605.
9. Keesler MJ, Fisher SD, Lipshultz SE. Cardiac Manifestations of HIV Infection in Infants and Children. Ann N Y Acad Sci. 2001;946:169-78.
10. Kearney DL, Perez-Atayde AR, Easley KA, Bowles NE, Bricker JT, Colan SD, et al. Postmortem cardiomegaly and echocardiographic measurements of left ventricular size and function in children infected with the human immunodeficiency virus: the Prospective P2C2 HIV Multicenter Study. Cardiovasc Pathol. 2003;12:140-8.
11. Lipshultz SE, Williams PL, Wilkinson JD, Leister EC, Van Dyke RB, Shearer WT, et al. Cardiac status of children infected with human immunodeficiency virus who are receiving long-term combination antiretroviral therapy: results from the adolescent master protocol of the multicenter pediatric HIV/AIDS cohort study. JAMA Pediatr. 2013;167:520-7.
12. Mondy KE, Gottdiener J, Overton ET, Henry K, Bush T, Conley L, et al. High prevalence of echocardiographic abnormalities among HIV-infected persons in the era of highly active antiretroviral therapy. Clin Infect Dis. 2011;52:378-86.
13. Currie PF, Boon NA. Immunopathogenesis of HIV-related heart muscle disease: current perspectives. AIDS (London, England). 2003;17 Suppl 1:S21-8.
14. Lipshultz SE, Miller TL, Wilkinson JD, Scott GB, Somarriba G, Cochran TR, et al. Cardiac effects in perinatally HIV-infected and HIV-exposed but uninfected

children and adolescents: a view from the United States of America. J Int AIDS Soc. 2013;16:18597.
15. Shaboodien G, Maske C, Wainwright H, Smuts H, Ntsekhe M, Commerford PJ, et al. Prevalence of myocarditis and cardiotropic virus infection in Africans with HIV-associated cardiomyopathy, idiopathic dilated cardiomyopathy and heart transplant recipients: a pilot study: cardiovascular topic. Cardiovasc J Afr. 2013;24:218-23.
16. Pozzan G, Pagliari C, Tuon FF, Takakura CF, Kauffman MR, Duarte MIS. Diffuse-regressive alterations and apoptosis of myocytes: possible causes of myocardial dysfunction in HIV-related cardiomyopathy. Int J Cardiol. 2009;132:90-5.
17. Cooper ER, Hanson C, Diaz C, Mendez H, Abboud R, Nugent R, et al. Encephalopathy and progression of human immunodeficiency virus disease in a cohort of children with perinatally acquired human immunodeficiency virus infection. Women and Infants Transmission Study Group. J Pediatr. 1998;132:808-12.
18. Lipshultz SE, Orav EJ, Sanders SP, Colan SD. Immunoglobulins and left ventricular structure and function in pediatric HIV infection. Circulation. 1995;92:2220-5.
19. Currie PF, Goldman JH, Caforio AL, Jacob AJ, Baig MK, Brettle RP, et al. Cardiac autoimmunity in HIV related heart muscle disease. Heart. 1998;79:599-604.
20. Lemmer CE, Badri M, Visser M, Mayosi BM. A lower body mass index is associated with cardiomyopathy in people with HIV infection: evidence from a case comparison study. S Af Med J. 2011;101:119-21.
21. Constans J, Sire S, Sergeant C, Simonoff M, Ragnaud JM. [Dilated cardiomyopathy and selenium deficiency in AIDS. Apropos of a case]. Rev Med Interne. 1997;18:642-5.
22. Patel K, Van Dyke RB, Mittleman MA, Colan SD, Oleske JM, Seage GR, 3rd, et al. The impact of HAART on cardiomyopathy among children and adolescents perinatally infected with HIV-1. AIDS (London, England). 2012;26:2027-37.
23. Dubé MP, Lipshultz SE, Fichtenbaum CJ, Greenberg R, Schecter AD, Fisher SD, et al. Effects of HIV Infection and Antiretroviral Therapy on the Heart and Vasculature. Circulation. 2008;118:e36-e40.
24. Reyskens KMSE, Fisher T-L, Schisler JC, O'Connor WG, Rogers AB, Willis MS, et al. Cardio-Metabolic Effects of HIV Protease Inhibitors (Lopinavir/Ritonavir). PLoS ONE. 2013;8:e73347.
25. Ige OO, Oguche S, Bode-Thomas F. Left ventricular systolic function in Nigerian children with Human Immunodeficiency Virus infection. Congenital Heart Dis. 2012;7:417-22.
26. Sims A, Frank L, Cross R, Clauss S, Dimock D, Purdy J, et al. Abnormal Cardiac Strain in Children and Young Adults with HIV Acquired in Early Life. J Am Soc Echo. 2012;25:741-8.

27. Al-Naami G, Kiblawi F, Kest H, Hamdan A, Myridakis D. Cardiac mechanics in patients With Human Immunodeficiency Virus: a study of systolic myocardial deformation in children and young adults. Pediatr Cardiol. 2014;35:1046-51.
28. Hsue PY, Hunt PW, Ho JE, Farah HH, Schnell A, Hoh R, et al. Impact of HIV infection on diastolic function and left ventricular mass. Circ Heart Fail. 2010;3:132-9.
29. Silva ML, Nassar SM, Silva AP, Ponce LL, Pires MM. Biventricular diastolic function assessed by Doppler echocardiogram in children vertically infected with human immunodeficiency virus. J Pediatr (Rio J). 2014;90:403-7.
30. Cade WT, Waggoner AD, Hubert S, Krauss MJ, Singh GK, Overton ET. Reduced diastolic function and left ventricular mass in HIV-negative preadolescent children exposed to antiretroviral therapy in utero. AIDS (London, England). 2012;26:2053-8.
31. Bloomfield GS, Khazanie P, Morris A, Rabadan-Diehl C, Benjamin LA, Murdoch D, et al. HIV and noncommunicable cardiovascular and pulmonary diseases in low- and middle-income countries in the ART era: what we know and best directions for future research. J Acquir Immune Defic Syndr. 2014;67(Suppl 1):S40-S53.
32. Mirrakhimov AE, Ali AM, Barbaryan A, Prueksaritanond S. Human Immunodeficiency Virus and pulmonary arterial hypertension. ISRN Cardiol. 2013;2013:903454.
33. Stewart S, Mocumbi AO, Carrington MJ, Pretorius S, Burton R, Sliwa K. A not-so-rare form of heart failure in urban black Africans: pathways to right heart failure in the Heart of Soweto Study cohort. Eur J Heart Fail. 2011;13:1070-7.
34. Cicalini S, Almodovar S, Grilli E, Flores S. Pulmonary hypertension and human immunodeficiency virus infection: epidemiology, pathogenesis, and clinical approach. Clin Microbiol Infect. 2011;17:25-33.
35. Janda S, Quon BS, Swiston J. HIV and pulmonary arterial hypertension: a systematic review. HIV Med. 2010;11:620-34.
36. Wong AR, Rasool AH, Abidin NZ, Noor AR, Quah BS. Sildenafil as treatment for Human Immunodeficiency Virus-related pulmonary hypertension in a child. J Paediatr Child Health. 2006;42:147-8.
37. Nunes H, Humbert M, Sitbon O, Morse JH, Deng Z, Knowles JA, et al. Prognostic factors for survival in human immunodeficiency virus-associated pulmonary arterial hypertension. Am J Respir Crit Care Med. 2003;167(10):1433-9.
38. Brown SC, Schoeman CJ, Bester CJ. Cardiac findings in children admitted to a hospital general ward in South Africa: a comparison of HIV infected and uninfected children. Cardiovasc J South Afr. 2005;16(4):206-10.
39. Rapose A, Sarvat B, Sarria JC. Immune reconstitution inflammatory syndrome presenting as pericarditis and pericardial effusion. Cardiology. 2008;110(2):142-4.

40. Matthews K, Ntsekhe M, Syed F, Scriba T, Russell J, Tibazarwa K, et al. HIV-1 infection alters CD4+ memory T-cell phenotype at the site of disease in extrapulmonary tuberculosis. Eur J Immunol. 2012;42:147-57.
41. van Doorn CA, Yates R, Tsang VT. Endocarditis as the first presentation of AIDS in infancy. Arch Dis Child. 1998;79(2):179-80.
42. Saidi AS, Moodie DS, Garson A, Jr., Lipshultz SE, Kaplan S, Lai WW, et al. Electrocardiography and 24-hour electrocardiographic ambulatory recording (Holter monitor) studies in children infected with human immunodeficiency virus type 1. The Pediatric Pulmonary and Cardiac Complications of Vertically Transmitted HIV-1 Infection Study Group. Pediatr Cardiol. 2000;21(3):189-96.
43. Moreno T, Perez I, Isasti G, Cabrera F, Santos J, Palacios R. Prevalence and factors associated with a prolonged QTc interval in a cohort of asymptomatic HIV-infected patients. AIDS Res Hum Retroviruses. 2013;29:1195-8.
44. Hunt K, Hughes CA, Hills-Nieminen C. Protease inhibitor-associated QT interval prolongation. Ann Pharmacother. 2011;45:1544-50.
45. Luginbuhl LM, Orav EJ, McIntosh K, Lipshultz SE. Cardiac morbidity and related mortality in children with HIV infection. JAMA. 1993;269:2869-75.
46. Benchimol-Barbosa PR. Circadian cardiac autonomic function in perinatally HIV-infected preschool children. Braz J Med Biol Res. 2009;42:722-30.
47. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. J Clin Endocrinol Metab. 2007;92:2506-12.
48. Freiberg MS, Chang CC, Kuller LH, Skanderson M, Lowy E, Kraemer KL, et al. HIV infection and the risk of acute myocardial infarction. JAMA Intern Med. 2013;173:614-22.
49. Maniar A, Ellis C, Asmuth D, Pollard R, Rutledge J. HIV infection and atherosclerosis: evaluating the drivers of inflammation. Eur J Prev Cardiol. 2013;20:720-8.
50. Mary-Krause M, Cotte L, Simon A, Partisani M, Costagliola D. Increased risk of myocardial infarction with duration of protease inhibitor therapy in HIV-infected men. AIDS (London, England). 2003;17:2479-86.
51. Bonnet D, Aggoun Y, Szezepanski I, Bellal N, Blanche S. Arterial stiffness and endothelial dysfunction in HIV-infected children. AIDS (London, England). 2004;18:1037-41.
52. Boccara F, Lang S, Meuleman C, Ederhy S, Mary-Krause M, Costagliola D, et al. HIV and coronary heart disease: time for a better understanding. J Am Coll Cardiol. 2013;61:511-23.
53. Baker JV, Lundgren JD. Cardiovascular implications from untreated human immunodeficiency virus infection. Eur Heart J. 2011;32:945-51.

54. Troseid M, Manner IW, Pedersen KK, Haissman JM, Kvale D, Nielsen SD. Microbial translocation and cardiometabolic risk factors in HIV infection. AIDS Res Hum Retroviruses. 2014;30.
55. El-Sadr WM, Mullin CM, Carr A, Gibert C, Rappoport C, Visnegarwala F, et al. Effects of HIV disease on lipid, glucose and insulin levels: results from a large antiretroviral-naive cohort. HIV Med. 2005;6:114-21.
56. Ross AC, Rizk N, O'Riordan MA, Dogra V, El-Bejjani D, Storer N, et al. Relationship between inflammatory markers, endothelial activation markers, and carotid intima-media thickness in HIV-infected patients receiving antiretroviral therapy. Clin Infect Dis. 2009;49:1119-27.
57. Vigano A, Mora S, Testolin C, Beccio S, Schneider L, Bricalli D, et al. Increased lipodystrophy is associated with increased exposure to highly active antiretroviral therapy in HIV-infected children. J Acquir Immune Defic Syndr. 2003;32:482-9.
58. Ross AC, Storer N, Ann O'Riordan M, Dogra V, McComsey GA. Longitudinal Changes in Carotid Intima-Media Thickness and Cardiovascular Risk Factors in Human Immunodeficiency Virus-Infected Children and Young Adults Compared With Healthy Controls. Pediatr Infect Dis J. 2010;29:634-8.
59. Sainz T, Alvarez-Fuente M, Novarro ML, Diaz L, Rojo P, Blazquez D, et al. Subclinical atherosclerosis and markers of immune activation in HIV-infected children and adolescents: the CaroVIH study. J Acquir Immune Defic Syndr. 2014;65:42-9.
60. Charakida M, Donald AE, Green H, Storry C, Clapson M, Caslake M, et al. Early structural and functional changes of the vasculature in HIV-infected children: impact of disease and antiretroviral therapy. Circulation. 2005;112:103-9.
61. European Paediatric Lipodystrophy Group. Antiretroviral therapy, fat redistribution and hyperlipidaemia in HIV-infected children in Europe. AIDS (London, England). 2004;18:1443-51.
62. Barlow-Mosha L, Eckard AR, McComsey GA, Musoke PM. Metabolic complications and treatment of perinatally HIV-infected children and adolescents. J Int AIDS Soc. 2013;16:18600.
63. Vigano A, Zuccotti GV, Cerini C, Stucchi S, Puzzovio M, Giacomet V, et al. Lipodystrophy, insulin resistance, and adiponectin concentration in HIV-infected children and adolescents. Curr HIV Res. 2011;9:321-6.
64. Krause JC, Toye MP, Stechenberg BW, Reiter EO, Allen HF. HIV--associated lipodystrophy in children. Pediatric endocrinology reviews : PER. 2005;3:45-51.
65. Behrens GM, Meyer-Olson D, Stoll M, Schmidt RE. Clinical impact of HIV-related lipodystrophy and metabolic abnormalities on cardiovascular disease. AIDS (London, England). 2003;17 Suppl 1:S149-54.
66. Monsuez JJ, Charniot JC, Escaut L, Teicher E, Wyplosz B, Couzigou C, et al. HIV-associated vascular diseases: structural and functional changes, clinical implications. Int J Cardiol. 2009;133:293-306.

67. Baker JV, Neuhaus J, Duprez D, Kuller LH, Tracy R, Belloso WH, et al. Changes in inflammatory and coagulation biomarkers: a randomized comparison of immediate versus deferred antiretroviral therapy in patients with HIV infection. J Acquir Immune Defic Syndr. 2011;56:36-43.
68. Neuhaus J, Jacobs DR, Jr., Baker JV, Calmy A, Duprez D, La Rosa A, et al. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. J Infect Dis. 2010;201:1788-95.
69. Lacerda HR, Correia Falcao MD, de Albuquerque VM, Zirpoli JC, Miranda-Filho DD, Pessoa Militao de Albuquerque MD, et al. Association of inflammatory cytokines and endothelial adhesion molecules with immunological, virological, and cardiometabolic disease in HIV-infected individuals. J Interferon Cytokine Res. 2013;34:385-93.
70. Miller TL, Borkowsky W, DiMeglio LA, Dooley L, Geffner ME, Hazra R, et al. Metabolic abnormalities and viral replication are associated with biomarkers of vascular dysfunction in HIV-infected children. HIV medicine. 2012;13:264-75.
71. Ross AC, O'Riordan MA, Storer N, Dogra V, McComsey GA. Heightened inflammation is linked to carotid intima-media thickness and endothelial activation in HIV-infected children. Atherosclerosis. 2010;211:492-8.
72. Pedersen KK, Pedersen M, Troseid M, Gaardbo JC, Lund TT, Thomsen C, et al. Microbial translocation in HIV Infection is associated with dyslipidemia, insulin resistance, and risk of myocardial infarction. J Acquir Immune Defic Syndr. 2014;64:425-33.
73. Monge MC, Samples CL. Primary care for children and adolescents living with HIV. Curr Opin Pediatr. 2012;24(4):453-61.
74. National Heart Lung and Blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. 2014 9 Oct 2014. Available from: http://www.nhlbi.nih.gov/health-pro/guidelines/current/cardiovascular-health-pediatric-guidelines/summary.htm#content.

CHAPTER 6

Effects of paediatric HIV infection on childhood vasculature

Nikmah S. Idris
Diederick E. Grobbee
David Burgner
Michael MH Cheung
Nia Kurniati
Cuno S.P.M. Uiterwaal

Submitted

Abstract

Background

HIV infection may alter childhood vascular properties and determine future cardiovascular risk. Whether vascular changes are associated with HIV infection per se or antiretroviral (ART) treatment is unknown. We investigated the effects of ART-naive or ART-exposed HIV infection in children on childhood vascular characteristics.

Methods

We performed vascular ultrasound to measure carotid intima media thickness (cIMT), distensibility, and elastic modulus on 114 children with perinatally-acquired HIV infection (56 ART-naive, 58 ART treated) and 51 healthy children in Jakarta, Indonesia. Children also underwent clinical and blood examinations. We used general linear modelling to estimate associations between HIV infection/treatment status and vascular characteristics with adjustment for confounders or possible mediators.

Results

Vascular measurements were successful in 42 ART-naïve HIV infected (median age 4.0 years (min 0.4 – max 11.5); 53 ART-treated HIV infected, 5.7 years (0.6 – 12.2), median ART duration 2.4 years (0.1-9.9); and 48 healthy children, 6.5 years (2.4 – 14.0). The ART-naïve HIV infected had thicker cIMT (difference 70.4 μm, 95%CI 32.1 to 108.7, p <0.001), adjusted for age, sex, socioeconomic status, parental smoking, body mass index, systolic and diastolic blood pressure, LDL-cholesterol, and HbA1c. Adding hs-CRP level did not alter this (71.6 μm, 31.9 to 111.2, p = 0.001). The ART-treated HIV infected had similar cIMT to healthy children. Distensibility and elastic modulus were not significantly different between HIV infected, either ART-naïve or -exposed, and healthy children.

Conclusion

ART-naive HIV infection in children appears to thicken cIMT, independent of inflammation level, while ART-exposed HIV infection is associated with similar vascular properties as healthy children.

Background

Cardiovascular diseases (CVD) remain the number one cause of death worldwide. The development of CVD has been suggested to start in early life with inflammation playing a central role in the atherosclerosis formation.[1, 2] Human immunodeficiency virus (HIV) infection is a model for chronic inflammatory state, which may potentiate the development of atherosclerosis. In fact, vascular pathological changes in HIV-infected patients are increasingly recognised, particularly in adults. HIV-infected adults are reported to have a 1.5-1.7 fold higher risk of acute myocardial infarction than the general population.[3-5] Also, autopsy studies revealed that large vessel and coronary artery vasculopathy occurs earlier and with greater severity in HIV infected populations. The mechanisms underlying the effect of HIV infection on accelerated atherosclerosis remain speculative[6, 7] and may be associated with viral replication, co-infections, and increased microbial translocation, inducing release of pro-inflammatory and pro-thrombotic mediators.(8) Vascular changes may also reflect toxicity of antiretroviral therapy (ART), particularly protease inhibitors (PI), which, through or independent of lipodystrophy, may induce dyslipidaemia and impaired glucose metabolism, each established CVD risk factors.[7, 9]

It has been indicated that HIV infection in children also has unfavourable effects on the vasculature. Compared to non-HIV subjects, HIV-infected children with long-term exposure to ART were reported to have increased intima-media thickness (IMT),[10, 11] and decreased flow-mediated dilation.[12] However, studies are small and mostly only included ART-treated children, so that it is difficult to differentiate the effects of HIV infection per se from treatment. Given the continuously increased incidence of HIV worldwide, including in children, and the possible association between HIV and increased cardiovascular risk, it is important to find out whether HIV does induce vascular changes at a very young age and to differentiate the effect of HIV infection itself from treatment.[13] Here, we investigated whether HIV infection naïve or exposed to ART does already have an effect on childhood vascular wall characteristics.

Methods

Study design and population

This study involved a cross sectional measurement on HIV infected child cohorts established from June 2013 to assess cardiovascular effects of HIV infection in children. One hundred and fourteen HIV-infected children aged 0-18 years were enrolled from the paediatric HIV clinics of Cipto Mangunkusumo National General Hospital, Koja District General Hospital, and the Indonesian Planned Parenthood Association (IPPA), Jakarta, Indonesia. HIV-infected children were classified into two groups: the ART-naïve HIV infected (56 children), consisting of 49 children who had never received or within the first week of antiretroviral (ART) treatment and 7 children who had been off treatment for at

least 5 years; and the ART-exposed HIV infected, comprising 57 children who had been receiving ART at enrolment. Children suggestive to have non-vertically acquired HIV (HIV-negative parents and previous blood transfusion) were excluded.

We also recruited 51 healthy children with the same age range from the area around the Cipto Mangunkusumo hospital. We invited healthy children by directly approaching parents and community leaders in the neighbourhood and giving leaflets explaining the background, purpose, and brief study procedures. Of the total of 55 children invited, 3 refused to participate and 1 was excluded due to dysmorphic features suggestive of a syndrome.

This study complied with the Declaration of Helsinki and has been approved by the Ethics Committee of Faculty of Medicine University of Indonesia. Written informed consent has been obtained from children's guardians.

Exposure measurement

We established HIV infection in children based on the WHO diagnostic criteria,[13] in which below 18 months of age, diagnosis was established based on a positive HIV DNA or RNA PCR examination, whereas for those aged above 18 months, it was defined by positive HIV antibodies. To indicate disease severity, we further classified children based on the WHO clinical and immunological staging. For clinical staging, classified as asymptomatic (stage 1), mild (stage 2), advance (stage 3), and severe (stage 4), we applied the criteria using patients' actual conditions at the time of vascular measurement. The CD4+ cell percentage level was used to define immunological staging into mild, advanced, and severe HIV according to the age-specific cut off of the WHO criteria.[14] The CD4+ cell levels were obtained from routine clinical data within 3 months of vascular measurement in which the tests were performed at the certified pathology laboratories of Cipto Mangunkusumo or Koja Hospital. Viral load examination was not routinely done in our HIV care setting due to financial constraint and only selectively performed on particular subjects as indicated by treating physicians.

Vascular measurement

We studied the vascular condition of the far wall of the right common carotid artery using an ultrasound with high-resolution echo tracking technology (MyLab One; Esaote, Italy). The technique principles and reproducibility have been described elsewhere.[14,15] In brief, this technology gives access to real time automatic measurements of all major mechanical parameters for 2 to 3 cm arterial segments: diastolic diameter d, the change in diameter, distension as a function of time, and carotid intima media thickness (cIMT). All measurements were performed by the clinical investigator (NSI) and a trained technician, who were blinded to information of potential confounders. Blinding of HIV status was not possible, particularly for the ART-naive HIV-infected subjects as their devastating clinical appearance was obvious. Inter-observer

agreement was checked by calculating intraclass correlation coefficient using two-way mixed effects model, revealing a coefficient of 0.86 (95% CI 0.51 – 0.97).

During ultrasonography, blood pressure was recorded three times in the right brachial artery with a semiautomatic oscillometric device using a cuff appropriate to the arm size (Dinamap; Criticon, Tampa, FL, USA). We averaged these values to estimate common carotid artery local pulse pressure, assuming mean arterial pressure minus diastolic blood pressure constant throughout the large arterial tree. Averages of cIMT, lumen diameter, and distension over every session per individual were used to assess the elastic properties of the artery as a hollow structure (distensibility coefficient) and of the wall (elastic modulus). Of 165 children included, vascular ultrasound data were available for 143 subjects (42 ART-naïve, 53 ART-exposed HIV infected, and 48 healthy children); however distensibility measurement, requiring children to be in a very settled condition, was only successfully obtained in 98 children. Reasons for unsuccessful vascular measurement were unsettled children, very young age (below 3 years old) and/or unstable clinical condition. Among the 42 ART-naïve HIV infected children with vascular measurement, three children once received ART in the past for less than 4 months but then had been lost to follow up and off treatment for at least 5 years before they presented to our hospital at study enrolment.

Measurement of potential confounders and intermediary variables

We a priori set age, sex, socioeconomic status, body mass index, maternal and second hand smoking exposure after birth, systolic and diastolic blood pressures, as well as LDL cholesterol and HbA1c levels as a marker of diabetes a priori as potential confounders. These are established cardiovascular disease risk factors[16, 17] and likely to be associated with HIV infection and/or its treatment as well. The LDL cholesterol and HbA1c levels may also act as intermediary variables, particularly in treatment exposed HIV-infected children as the associations between ART and lipid or glucose metabolism have been frequently reported. As heightened inflammation may mediate the effect of HIV infection on childhood vasculature,[8, 18] we considered high sensitivity-C-reactive protein (hsCRP) level as a possible intermediary variable.

Data on age, sex, socioeconomic status, and smoking exposure were obtained using a standard questionnaire filled by parents or caregivers. Serum lipid, HbA1c, and hs-CRP levels were determined at the Cipto Mangunkusumo Clinical Pathology Laboratory, Jakarta.

Data analysis

Baseline characteristics, expressed as mean, median, or proportion as appropriate, are described by HIV status. The distributions of categorical and numerical variables across HIV status were statistically evaluated using the Chi-square and oneway ANOVA or Kruskal-Wallis tests, respectively.

To investigate the effects of HIV infection on vascular characteristics, we performed univariable and multivariable general linear modelling with HIV status as a fixed factor and vascular characteristics (cIMT, distensibility, and elastic modulus) as separate dependent variables. The same models were used for adjustment for the above mentioned potential confounders. To see whether inflammation explains effects of HIV infection on the vasculature, we added hsCRP in the final model. We performed sensitivity analyses, first by excluding 4 'ART-naïve' HIV infected children who once had a brief ART in the past and secondly, by excluding 4 ART-exposed HIV-infected children who had been receiving ART for less than 6 months

Findings are presented as crude and adjusted mean difference with 95% confidence interval and corresponding p-values, which are also displayed in graphs. A 95% confidence interval not including zero, corresponding to a p-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 19 for Mac.

Results

Baseline characteristics are shown in Table 1. ART-naïve HIV-infected patients were younger and had smaller body size than the other groups. HIV-infected children, both treated and untreated, came from lower socioeconomic groups than healthy children, were more heavily exposed to parental smoking, both during pregnancy and after birth, more rarely being breastfed, and more often had a family history of CVD. The median duration of treatment in ART-exposed group was 2.9 (0.1 – 9.9) years. Only 5 treated HIV infected children received a regimen containing PI drugs.

In terms of clinical and laboratory characteristics related to CVD risk, despite lower waist circumference, HIV infected children, particularly the treated ones, had a slightly higher waist-to-hip ratio compared to healthy children (p for difference < 0.001). ART exposed children had the highest mean total and LDL cholesterol levels and a lower mean HDL level than healthy children. ART-naïve HIV infected children, having the poorest nutritional status among others, had the lowest mean total, HDL, LDL cholesterol, and HbA1c levels. In terms of morbidities, ART-naïve HIV children were in more severe clinical stages with lower CD4 cell counts than the ART-exposed group.

Effects of untreated and treated HIV infection on vascular characteristics are described in Table 2. On crude analysis, HIV infection showed 36.1 μm thicker cIMT (p=0.02), and more prominent after confounder adjustment (difference 70.4 μm, p = <0.001). This finding remained after further adjustment for hs-CRP level (difference 71.6 μm, p = <0.001). Analysis restricted to 38 children who had not received ART at all showed similar but slightly more prominent findings (Table 2). There was no statistically significant difference in cIMT between ART-exposed HIV infected children and healthy children, neither in crude (difference -17.1 μm, p =0.21) nor in adjusted analysis (difference -14.2 μm, p = 0.37). In terms of vascular distensibility and elastic modulus, we did not find any statistically significant difference between ART-naive or ART-exposed HIV-infected children and healthy children. Analysis excluding children who had been receiving treatment for less than 6 months showed similar results (data not shown).

Table 1. Baseline characteristics of study subjects by HIV status

Characteristics	ART-naïve HIV n = 42	ART-exposed HIV n = 53	Non-HIV N = 48	P-value
Age (years)*, median (range)	4.0 (0.4 – 11.5)	5.7 (0.6 – 12.2)	6.5 (2.4 – 14.0)	0.001
Male sex, n (%)	19 (45)	23 (55)	26 (54)	0.92
Low socioeconomic status, n (%)	18 (44)	30 (57)	10 (21)	0.005
Parental smoking after birth, n (%)				
Mother	3 (8)	7 (14)	0 (0)	0.03
Father/other household member	34 (81.0)	46 (87)	32 (67)	0.04
Parental smoking in pregnancy, n (%)				
Mother	6 (16)	9 (16)	1 (2)	0.04
Father/other household member	33 (79)	44 (83)	33 (69)	0.23
Ever breastfed, n (%)	28 (74)	38 (73)	47 (98)	0.002
Family history of CVD	14 (36)	15 (29)	3 (7)	0.003
Body weight (kg)	13.4 (7.0)	17.0 (5.8)	22.7 (9.7)	<0.001
Body height (cm)	95.4 (19.1)	105.9 (16.0)	116.7 (18.2)	<0.001
Body mass index (kg/m^2)	14.1 (2.3)	15.4 (2.4)	15.9 (2.9)	0.01
Waist circumference (cm)	48.7 (10.2)	51.1 (6.6)	55.3 (8.8)	0.003
Waist-to-hip ratio	1.02 (0.08)	1.03 (0.08)	0.98 (0.05)	0.001
Mid upper arm circ. (cm)	13.7 (3.5)	14.7 (2.6)	17.0 (3.1)	<0.001
Systolic BP (mmHg),	100.6 (11.4)	99.7 (10.3)	102.1 (9.6)	0.52
Diastolic BP (mmHg)	63.0 (9.6)	60.4 (7.6)	60.9 (7.4)	0.35
Total cholesterol (mmol/l), n = 121	3.3 (0.8)	4.4 (0.9)	4.3 (0.7)	<0.001
HDL cholesterol (mmol/l)	0.7 (0.3-1.5)	1.2 (0.2-2.3)	1.3 (0.6-1.9)	<0.001#
LDL cholesterol (mmol/l)	1.9 (0.6)	2.7 (0.8)	2.6 (0.6)	<0.001
HbA1c level (%), n = 130	0.05 (0.04 – 0.06)	0.06 (0.04 – 0.07)	0.06 (0.04 – 0.07)	0.35
hs-CRP, median (range), nmol/l	39 (0 –2759)	47 (3.7-5507)	4.7 (0-155)	<0.001#
Chronic infection (ever)*	37 (93)	28 (62)	0 (0)	<0.001
Severe infection**	17 (53)	4 (11)	0 (0)	<0.001
HIV clinical stage 3 or 4	38 (93)	28 (68)	-	0.001
CD4 absolute level (n= 58)	286 (2-1499)	1217 (4 – 2301)	-	0.001
CD4 % (n = 67)	9.5 (0-35)	30 (4-79)	-	0.002
Viral load (n = 20)	270 (203 – 5840)	544 (0 – 1.2*10^6)		
CIMT (micrometre), n = 143	449.9 (71.0)	396.8 (67.2)	413.9 (62.5)	***
Distension (MPa-1), n = 97	69.6 (22.0)	67.5 (16.7)	64.7 (16.2)	***
Elastic modulus (kPa)	198.8 (73.0)	223.1 (63.8)	217.8 (55.6)	***

Notes: #Kruskal-Wallis test; Values are means (SD) unless otherwise indicated.
*Chronic infection includes tuberculosis, otitis media, and persistent diarrhoea.
**Severe infection includes sepsis, central nervous system infection, pyelonephritis, and pneumonia.
***Tests for differences provided in table 2.

Table 2. Association between HIV infection and vascular characteristics

			cIMT (micrometer)			Distensibility (MPa-1)			Elastic modulus (kPa)		
			Mean difference	95%CI	p	Mean difference	95%CI	p	Mean difference	95%CI	p
Crude	Non-HIV		Ref			Ref			Ref		
	ART-naïve HIV	38 never ART	36.0	6.9 to 65.1	0.02	6.4	-3.3 to 16.2	0.19	-28.0	-61.5 to 5.5	0.1
		All 42	36.1	8.2 to 64.0	0.01	4.9	-4.3 to 14.2	0.29	-19.0	-52.0 to 14.0	0.3
	ART-exposed HIV		-17.1	-43.4 to 9.3	0.21	2.8	-5.6 to 11.3	0.51	5.3	-24.4 to 35.0	0.7
Model 1	Non-HIV		Ref			Ref			Ref		
	ART-naïve HIV	38 never ART	41.0	9.8 to 72.1	0.01	4.6	-6.0 to 15.3	0.39	-13.7	-49.8 to 22.3	0.5
		All 42	38.8	9.6 to 68.0	0.01	3.3	-6.6 to 13.1	0.51	-4.6	-38.9 to 29.6	0.8
	ART-exposed HIV		-18.8	-46.1 to 8.5	0.18	1.9	-7.2 to 11.0	0.7	15.5	-15.7 to 46.7	0.3
Model 2	Non-HIV		Ref			Ref			Ref		
	ART-naïve HIV	38 never ART	62.3	28.1 to 96.5	<0.001	1.7	-8.8 to 12.2	0.74	-12.3	-51.7 to 27.2	0.5
		All 42	58.2	26.6 to 89.8	<0.001	-1.1	-10.7 to 8.5	0.82	3.0	-34.3 to 40.3	0.9
	ART-exposed HIV		-8.8	-37.4 to 19.7	0.54	-1.2	-9.9 to 7.5	0.78	23.8	-9.3 to 57.0	0.2
Model 3	Non-HIV		Ref			Ref			Ref		
	ART-naïve HIV	38 never ART	77.1	36.5 to 117.7	<0.001	7.1	-3.2 to 17.4	0.17	-41.8	-77.5 to -6.1	0
		All 42	70.4	32.1 to 108.7	<0.001	4.8	-5.0 to 14.5	0.33	-28.0	-62.5 to 6.5	0.1
	ART-exposed HIV		-14.2	-45.6 to 17.2	0.37	-0.7	-8.6 to 7.2	0.86	23.3	-4.7 to 51.4	0.1
Model 4	Non-HIV		Ref			Ref			Ref		
	ART-naïve HIV	38 never ART	78.8	36.7 to 120.9	<0.001	8.6	-2.0 to 19.3	0.11	-48.5	-85.2 to -11.8	0
		All 42	71.6	31.9 to 111.2	<0.001	6.0	-4.1 to 16.0	0.24	-33.1	-66.6 to 2.3	0.1
	ART-exposed HIV		-17.9	-45.7 to 18.7	0.37	-0.05	-8.1 to 8.0	0.99	20.5	-7.9 to 48.9	0.2

Notes:

Model 1: adjusted for age, gender, socioeconomic status;

Model 2: Model 1 + parental smoking after birth, BMI, systolic blood pressure, diastolic blood pressure;

Model 3: Model 2 + diastolic blood pressure LDL cholesterol, HbA1c;

Model 4: Model 3 + hs-CRP

Discussion

We have found that ART-naive HIV-infected children had thicker cIMT than healthy children, whereas ART-exposed children did not. These findings could not be fully explained by the level of inflammation as reflected in the hs-CRP level.

We had the unique opportunity to investigate the effects of treatment naive HIV infection in children, but full exploration of the influence of disease severity was limited by our sample size and incomplete CD4 count data. This study was done in a limited resource care setting, where the CD4 count assay is not routinely performed in practice

despite being recommended as an objective measure to evaluate immunodeficiency state and to monitor disease progression. Stratifying the analysis based on clinical staging was also not possible because most ART-naive HIV infected children presented in advanced to severe stages, while the ART-exposed ones were in milder stages, leaving small numbers of children in certain groups. However, although combined with the effects of treatment, comparing untreated and treated children roughly reflects the HIV infection severity itself, in which we did show a substantial difference in their vascular characteristics. Distensibility and the corresponding elastic modulus measurements could be obtained in the majority but not all of the sample, resulting in limited sample size to obtain robust findings.

The fact that we have looked separately at the ART-naive and ART-exposed HIV infected children is particularly important. Most studies reporting on vascular characteristics in HIV-infected children were done in the HAART era, in which the majority of subjects were already being treated,[10,11, 9] making differentiation of the effects of HIV infection per se from treatment difficult. As far as we know, there has only been one study[20] investigating vascular characteristics in both untreated and treated HIV-infected children as a pooled group compared to healthy subjects. In that study, within HIV group comparisons of untreated, non PI-, and PI-treated subjects were described. However, we believe that direct comparisons of vascular characteristics of each ART-naive or ART-exposed HIV infected group to healthy do provide a direct delineation of effects of HIV infection per se from treatment. Moreover, Charakida et al. [20] studied children of older age (median age 10-11 years old), among whom the untreated probably represented specific fitter HIV-infected children that had been able to survive the infection without treatment. Since in vertically-acquired HIV infection, children's ages reflect the duration of treatment exposures,[21] our involvement of relatively young children offers new insight on whether short-to-medium duration of HIV infection already affects the developing vasculature, although we also probably missed the most severe HIV infections in deceased children.

Our study showed that HIV infection itself does have major effects on childhood cIMT, even in the first few years of infection. Although it is still unclear whether this thicker childhood cIMT would persist into adulthood and therefore increased CVD risk, IMT has been suggested to represent the cumulative burden of adverse influences operating from the earliest stages of disease[22] and may serve as the best available marker of preclinical atherosclerosis.[23] Our findings support a previous observation in adults of increased occurrence of myocardial infarction after the cessation of ART, indirectly highlighting the role of HIV replication and dysimmunity in the pathogenesis of CVD in this population.[8, 24] However, in our view, the finding of thicker cIMT in the untreated HIV children should be interpreted cautiously, as to whether this translates directly to increased risk of future cardiovascular events. Although we do not know the exact nature of this HIV related

thickening of cIMT is unclear, we do know that the magnitude of this thickening equals an effect size that is not found for other determinants in childhood.[15, 25-27] Multiple factors, including antiretroviral treatment, may cause dynamic alterations in the vascular characteristics as indirectly shown in our study by the fact that our ART-exposed HIV-infected children did not have significantly different cIMT compared with healthy children, which contrasts with most previous findings.[10,11,19,,20] The relative impact of disease, treatment, and possible underlying risk factors remains hard to disentangle among ART-exposed HIV-infected population. ART may attenuate pro-atherosclerotic mechanisms exerted by HIV infection itself, but there are also concerns of toxicities on cardiovascular system, in which their relative importance may be modified by treatment duration. Previous studies demonstrated that among ART-treated subjects, cIMT did change with time, showing a decrease to a level similar to that of healthy children at some point before increasing again as the treatment continues.[28, 29] As our study participants had been treated for shorter durations than those in previous studies, this may explain the absence of cIMT difference between our ART-exposed HIV infected children and the healthy reference group. To confirm this hypothesis, further follow up studies are required.

With regard to distensibility and elastic modulus, we did not find any differences between HIV-infected, either ART-naïve or -exposed and healthy children, which may be caused by limited numbers of measurements. Importantly, the measurement was selectively not able to be obtained in smaller children, who comprised the largest proportion of ART-naive HIV- infected group as well as in those with rapid heart rate and unstable clinical condition, indicating a more severe HIV stage. Given these issues, our findings regarding arterial stiffness should be interpreted cautiously and needs to be confirmed in larger studies. There have been very few studies looking at the elastic properties of arterial wall in treated HIV-infected children[12] or adults,[30] showing less distensible vasculature, reflected in decreased flow mediated dilation and higher pulse wave velocity, in HIV-infected populations. Even without residual viral replication, it has been shown that HIV infection in ART-exposed subjects may promote arterial stiffness.[12]

Our finding that HIV infection itself, apart from treatment, causes alterations in childhood vascular structure may be due to viral replication or persistence, co-pathogens, or continuous gut microbial translocation, which induce immune activation and chronic inflammation associated with pro-inflammatory cytokine release, early vascular dysfunction, pro-thrombotic and pro-coagulation activities, and dyslipidaemia. Moreover, there is circumstantial evidence, largely based on studies investigating the blood-brain barrier in HIV infected adults,[31] that endothelial cells, including the coronary,[32] are not impenetrable to the virus although most infection will be abortive. Mechanisms involved in ART-treated HIV-infected subjects are thought to be different than the untreated ones.[7] In treated subjects, besides chronic inflammation and immune activation, ART toxicities, particularly the PIs, may play a role, probably by causing

adipocyte dysfunction with further derangement of lipid and glucose metabolism, leading to lipodystrophy syndrome. In our study, hs-CRP as an established inflammatory and cardiovascular biomarkers,[33] could not fully explain the associations between ART-exposed HIV infection and thicker cIMT. This may suggest that mechanisms other than chronic inflammation may play a more dominant role. Alternatively, hs-CRP may not a reliable inflammatory marker in this special paediatric population with immune system derangements and possible low capacity to produce some inflammatory cytokines required in hs-CRP production. In line with our findings, previous studies linking hs-CRP and CVD surrogates in HIV-infected children or adolescents remained inconclusive[20,28,34] although in adults, hs-CRP has been shown to predict CVD and all-cause mortality.[35, 36]

Although the translation of thicker cIMT in ART-naive HIV-infected children into an increased future CVD risk remains speculative, we believe that our findings should raise awareness of the importance of more intensive early preventive measures of CVD, particularly in this specific population. Notably, the effects that we show are substantial, with a difference in cIMT between ART-naïve and healthy children explaining almost a standard deviation. Moreover, the findings could probably be generalisable to other childhood chronic infection or inflammatory conditions and highlight their possible roles in the life course development of atherosclerosis. The present report is based on cross-sectional associations. Further longitudinal follow up should explore the complex interplay between HIV infection, treatment, and background individual risk profiles, and to find out the association between the alterations in childhood vasculature and the occurrence of CVD in adulthood. Specifically, the role of biomarkers related to vascular damage, fat metabolism, or inflammation in relation with vascular changes in HIV-infected children seems to warrant further investigation.

In conclusion, our findings suggest that ART-naïve HIV infection is associated with thicker cIMT independent of the degree of inflammation, whereas ART-exposed HIV infected children have similar vascular properties compared to healthy ones.

Acknowledgement

The authors gratefully acknowledge Septiani Madonna Gultom, Hanitya Dwi Ratnasari, and (the deceased) Weni Tenlima for their dedicated assistance in data collection and Dra. Diyah Kristanty, M. Biomed and Enny Hasrini for organizing the laboratory assays. We also thank the medical/nursing staff of Cipto Mangunkusumo and Koja hospitals; Edi Sugiarto, Sri Yani, and Santi Sardi from the Indonesian Planned Parenthood Association Clinic (an associate member of International Planned Parenthood Federation); all patients and families for their invaluable contribution in this project. We greatly appreciated Esaote Europe B.V. the Netherlands for their in kind support in providing the ultrasound machine for vascular measurement in this study.

References

1. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med. 1999;340:115-26.
2. Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. Nat Med. 2011;1410-1422.
3. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. J Clin Endocrinol Metab. 2007;92:2506-12.
4. Lang S, Mary-Krause M, Cotte L, Gilquin J, Partisani M, Simon A, et al; French Hospital Database on HIV-ANRS CO4. Increased risk of myocardial infarction in HIV-infected patients in France, relative to the general population. AIDS (London, England). 2010;24:1228-30.
5. Armah KA, Chang C-CH, Baker JV, Ramachandran VS, Budoff MJ, Crane HM, et al; Veterans Aging Cohort Study (VACS) Project Team. Prehypertension, hypertension, and the risk of acute myocardial infarction in HIV-infected and -uninfected veterans. Clin Infect Dis. 2014;58:121-9.
6. Barbaro G, Fisher SD, Lipshultz SE. Pathogenesis of HIV-associated cardiovascular complications. Lancet Infect Dis. 2001;1:115-24.
7. Boccara F, Lang S, Meuleman C, Ederhy S, Mary-Krause M, Costagliola D, et al. HIV and coronary heart disease: time for a better understanding. J Am Coll Cardiol. 2013;61:511-23.
8. Baker JV, Lundgren JD. Cardiovascular implications from untreated human immunodeficiency virus infection. Eur Heart J. 2011;32:945-51.
9. Prendergast AJ. Complications of long-term antiretroviral therapy in HIV-infected children. Arch Dis Child. 2013;98:245-6.
10. McComsey G, O'Riordana M, Hazenc S, El-Bejjanid D, Bhatte S, Brennanc ML, et al. Increased carotid intima media thickness and cardiac biomarkers in HIV infected children. AIDS. 2007;21:921-7.
11. Sainz T, Alvarez-Fuente M, Novarro ML, Diaz L, Rojo P, Blazquez D, et al; Madrid Cohort of HIV-infected children and adolescents integrated in the Pediatric branch of the Spanish National AIDS Network (CoRISPE). Subclinical atherosclerosis and markers of immune activation in HIV-infected children and adolescents: the CaroVIH study. J Acquir Immune Defic Syndr. 2014;65:42-9.
12. Bonnet D, Aggoun Y, Szezepanski I, Bellal N, Blanche S. Arterial stiffness and endothelial dysfunction in HIV-infected children. AIDS (London, England). 2004;18:1037-41.
13. Idris NS, Grobbee DE, Burgner D, Cheung MM, Kurniati N, Sastroasmoro S, et al. Cardiovascular manifestations of HIV infection in children. Eur J Prev Cardiol. 2014.

14. World Health Organization. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. Geneva: WHO Press; 2007.
15. Evelein AM, Geerts CC, Visseren FL, Bots ML, van der Ent CK, Grobbee DE, et al. The association between breastfeeding and the cardiovascular system in early childhood. Am J Clin Nutr. 2011;93:712-8.
16. Eichler K, Puhan MA, Steurer J, Bachmann LM. Prediction of first coronary events with the Framingham score: a systematic review. Am Heart J. 2007;153:722-31, 31.e1-8.
17. Dent TH. Predicting the risk of coronary heart disease I. The use of conventional risk markers. Atherosclerosis. 2010;213:345-51.
18. Ross AC, O'Riordan MA, Storer N, Dogra V, McComsey GA. Heightened inflammation is linked to carotid intima-media thickness and endothelial activation in HIV-infected children. Atherosclerosis. 2010;211:492-8.
19. Giuliano ICB, Freitas SFT, de Souxa M, Caramelli B. Subclinic atherosclerosis and cardiovascular risk factors in HIV-infected children: PERI study. Coronary Artery Dis. 2008;19:167-72,.
20. Charakida M, Donald AE, Green H, Storry C, Clapson M, Caslake M, et al. Early structural and functional changes of the vasculature in HIV-infected children: impact of disease and antiretroviral therapy. Circulation. 2005;112:103-9.
21. Chakraborty R. HIV-1 infection In children: a clinical and immunologic overview. Curr HIV Res. 2005;3:31-41.
22. Wang TJ. Assessing the role of circulating, genetic, and imaging biomarkers in cardiovascular risk prediction. Circulation. 2011;123:551-65.
23. Jarvisalo MJ, Jartti L, Nanto-Salonen K, Irjala K, Ronnemaa T, Hartiala JJ, et al. Increased aortic intima-media thickness: a marker of preclinical atherosclerosis in high-risk children. Circulation. 2001;104:2943-7.
24. El-Sadr WM, Mullin CM, Carr A, Gibert C, Rappoport C, Visnegarwala F, et al. Effects of HIV disease on lipid, glucose and insulin levels: results from a large antiretroviral-naive cohort. HIV Med. 2005;6:114-21.
25. Geerts CC, Evans GW, Grobbee DE, Uiterwaal CS. Parental smoking and vascular damage in young adult offspring: is early life exposure critical? The atherosclerosis risk in young adults study. Arterioscler Thromb Vasc Biol. 2008;28:2296.302.
26. Geerts CC, Evelein AM, Bots ML, van der Ent CK, Grobbee DE, Uiterwaal CS. Body fat distribution and early arterial changes in healthy 5-year-old children. Ann Med. 2012;44:350-9.
27. Idris NS, Evelein AM, Geerts CC, Sastroasmoro S, Grobbee DE, Uiterwaal CS. Effect of physical activity on vascular characteristics in young children. Eur J Prev Cardiol. 2014 Feb 13.

28. Ross AC, Storer N, Ann O'Riordan M, Dogra V, McComsey GA. Longitudinal changes in carotid intima-media thickness and cardiovascular risk factors in human immunodeficiency virus-infected children and young adults compared with healthy controls. Pediatr Infect Dis J. 2010;29:634-8.
29. Ross Eckard A, O'Riordan MA, Storer N, McComsey GA. Long-term changes in carotid intima-media thickness among HIV-infected children and young adults. Antivir Ther. 2014;19:61-8.
30. van Vonderen MG, Hassink EA, van Agtmael MA, Stehouwer CD, Danner SA, Reiss P, et al. Increase in carotid artery intima-media thickness and arterial stiffness but improvement in several markers of endothelial function after initiation of antiretroviral therapy. J Infect Dis. 2009;199:1186-94.
31. Liu NQ, Lossinsky AS, Popik W, Li X, Gujuluva C, Kriederman B, et al. Human immunodeficiency virus type 1 enters brain microvascular endothelia by macropinocytosis dependent on lipid rafts and the mitogen-activated protein kinase signaling pathway. J Virol. 2002;76:6689-700.
32. Gujuluva C, Burns AR, Pushkarsky T, Popik W, Berger O, Bukrinsky M, et al. HIV-1 penetrates coronary artery endothelial cells by transcytosis. Mol Med. 2001;7:169-76.
33. Ridker PM. High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. Circulation. 2001;103:1813-8.
34. Sainz T, Diaz L, Navarro ML, Rojo P, Blazquez D, Ramos JT, et al; Madrid Cohort of HIV-infected children and adolescents, integrating the Pediatric branch of the National AIDS Research Network of Spain (CORISPE). Cardiovascular biomarkers in vertically HIV-infected children without metabolic abnormalities. Atherosclerosis. 2014;233:410-4.
35. Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, et al; INSIGHT SMART Study Group. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med. 2008;5:e203.
36. Triant VA, Meigs JB, Grinspoon SK. Association of C-reactive protein and HIV infection with acute myocardial infarction. J Acquir Immune Defic Syndr. 2009;51:268-73.

CHAPTER 7

Effects of paediatric HIV infection on electrical conduction of the heart

N Nikmah S. Idris
Michael MH Cheung
Diederick E. Grobbee
David Burgner
Nia Kurniati
Mulyadi M Djer
Cuno S.P.M. Uiterwaal

Submitted

Abstract

Background

Heart conduction disorders, such as prolongation of QT interval, have been reported in HIV-infected adults. However, little is known about such disorders in paediatric HIV, particularly in relation to delineation of the effects of HIV infection from treatment.

Methods

This cross-sectional study involved 114 perinatally-acquired HIV-infected (and 51 healthy age-matched children in Jakarta, Indonesia. On 12-lead ECGs, which were available for 129 children (37 antiretroviral treatment (ART) naïve and 42 ART-exposed HIV infected; 50 healthy), we measured cardiac conduction parameters: PR, QRS, and QTc (corrected using the Bazett's formula) intervals. The associations between HIV infection/treatment status and ECG intervals were evaluated using general linear modelling with further adjustment for potential confounders or explanatory variables. Findings are presented as (adjusted) mean differences between each of the two HIV groups and healthy children.

Results

Compared to healthy children, QTc intervals were longer in ART-naïve (difference 18.2 milliseconds, 95%CI 7.0 to 29.3) and, to greater extent, in ART-exposed HIV infected children (difference 28.9 milliseconds, 19.3 to 38.5). Following adjustment for RR interval, age, and height, prolongation of PR interval was seen only in ART-naïve HIV infected children, (difference 12.9 milliseconds, 2.4 to 23.3). Cardiac mass/function, high sensitive C reactive protein, lipid and HbA1c levels, systolic and diastolic blood pressures, or postnatal parental smoking exposure did not affect these associations. No difference in QRS interval was observed between the groups.

Conclusion

Prolongation of QTc interval occurs in ART-naive HIV infected children and, to a greater extent, in the ART-exposed ones. In addition, ART-naive HIV infected children have a longer PR interval than healthy children.

Background

Perinatally acquired HIV infection is one of the most devastating diseases in childhood which may affect any organ, including the cardiovascular system.[1] HIV infection may be associated with pathological changes in various cardiac structures, including the myocardium, endocardium, and pericardium with diverse manifestations from asymptomatic presentation, overt heart failure, to sudden death.[2]

Conduction abnormalities have been infrequently reported in children with HIV. An autopsy study on children with acquired immune deficiency syndrome showed that various pathologies may occur throughout the conduction system, including vasculitis, myocarditis, or fragmentation of His bundle with fibrosis.[3] In adults, HIV infection is associated with a 20% prevalence of prolonged QTc (RR corrected QT) interval.[4] A prolonged QTc potentially induces fatal ventricular arrhythmia, in particular *torsade de pointes*, which has been reported in HIV infected adults,[5] and predicts mortality both in healthy [6] and high-risk populations, including those with previous myocardial infarction, diabetes mellitus,[7] or pulmonary hypertension.[8] The development of QTc prolongation in HIV-infected adults may be associated with hyperlipidaemia, cardiac conditions, increasing age, female sex, ethnicity, and diabetes mellitus. The use of antiretroviral treatment (ART), particularly the protease inhibitors may additionally increase risk.[9]

Determining the independent effects of HIV infection per se from ART on the cardiac conduction is difficult and has never been thoroughly studied before, although a US based birth cohort[10] revealed QTc prolongation in HIV- infected children compared to children who were perinatally HIV-exposed but uninfected. Delineation of the effects of HIV infection from ART is also important from a clinical perspective, guiding both initial ART choices and providing a framework for ongoing follow-up of HIV-infected children as they transition to adult care. Therefore, we aimed to investigate the effect of ART-naive and ART-exposed HIV infection on cardiac electrical conduction in children.

Methods

Study design and population

This was a cross-sectional analysis involving 114 HIV infected and 51 healthy children enrolled in an ongoing cohort study established in June 2013 to evaluate cardiovascular complications of HIV infection in children. Of these children, electrocardiograms were available for 129 children, of whom 37 were ART-naïve, 42 ART-exposed, and 50 healthy children. Of 36 children who did not have the ECG measurements, 9 died (of whom 5 were ART-naive infected children), 2 had a very poor quality recording, 3 moved out of town, and for the remaining, children refused to have the examination.

HIV infected children were recruited from the paediatric HIV clinics of Cipto Mangunkusumo National General Hospital, Koja District General Hospital, and the

Indonesian Planned Parenthood Association (IPPA), Jakarta Indonesia. Healthy children of the same age range were enrolled from the same geographical area. We invited healthy children by directly approaching parents and community leaders in the neighbourhood and giving written explanation in the form of leaflets about the background, purpose, and brief procedures of the study. Of the total of 55 children approached, three refused to participate and one was excluded due to dysmorphic features suggestive of a syndrome. HIV-infected children were classified into two groups: the ART-naïve HIV infected (56 children), consisting of 49 children who had never received or were within the first week of initiation of antiretroviral (ART) treatment and 7 children who had been off ART for at least 5 years; and the ART-exposed HIV infected, comprising 57 children who had been receiving ART at enrolment. Among ART treated children, 2 children had recently commenced treatment in our hospital at enrolment after previously managed at another hospital with potential compliance issue. We excluded children with conditions suggestive of non-vertically acquired HIV (HIV-negative parents and history of blood transfusion).

Ethical approval was obtained from the Ethics Committee, Faculty of Medicine University of Indonesia and University of Melbourne. Informed consent was obtained from all participating families.

Exposure measurement

We established the diagnosis of HIV infection based on the WHO criteria.[11] In short, in children aged less than 18-months, diagnosis was made by a positive HIV DNA or RNA PCR, whereas for those aged above 18 months, diagnosis was confirmed by positive HIV serology. To measure disease severity, we further classified children based on the WHO clinical and immunological staging. For clinical staging, we applied the criteria using patients' actual condition at the time of ECG measurement. The CD4 cell percentage, used to define immunological staging, was obtained from routine clinical data within 3 months of ECG measurement .Viral load examination was not routinely performed in our clinical setting due to financial constraints and is only performed as indicated by treating physicians.

ECG measurement

We performed 12-lead ECG examinations on children in supine position with the speed set at 25 mm/second and the gain at 10 mm/mV. The ECG was interpreted manually using a calibrated ruler by a single investigator (NSI) blinded to HIV infection or treatment status. Twenty randomly selected recordings were separately measured by two paediatric cardiologists (MC, MMD) for validation. Conduction (depolarisation and repolarisation) parameters, including PR, QRS, and QT intervals, were measured as an average of three beats in lead II in most patients and in lead V5 in 5 patients due to low voltage recordings in lead II. The PR interval, reflecting atrial and atrioventricular nodal depolarisation was defined as the distance from the beginning of the P wave to the beginning of the first deflection of QRS complex, and was measured in the shortest RR

interval. The QT intervals, representing total depolarisation repolarisation time, were measured after the shortest RR interval. The QT interval was defined as starting at the beginning of the first deflection of QRS complex and lasted until the end of the T wave (QT). To determine the end of the QT interval, we applied the tangential method by drawing a tangent against the steepest part of the end of the T wave and determine the point where the tangent and the baseline cross (see appendix).[12] The QT interval were corrected for heart rate using Bazett's formula ($QTc = QT/\sqrt{RR}$), while the PR interval was corrected in the statistical model for the corresponding RR interval. All interval measurements are presented in milliseconds.

Of the available 129 electrocardiograms, 124 and 123 were analysable for QT and PR interval, respectively. Five electrocardiograms was not analysable for QT interval due to right bundle branch block and non-specific ST/T wave changes, whereas six were not possible for PR interval measurement due to low voltage P wave.

Measurement of potential confounders and intermediary variables

We a priori included age and body mass index (BMI) as potential confounders in the association between HIV infection status and cardiac electrical conduction. In addition to their association with HIV infection status, both variables are likely related to cardiac conduction time, to which some of the associations have been reported previously.[13] As cardiac conditions (mass and function)[14] as well as inflammation may mediate the effect of treated or untreated HIV infection on heart ECG intervals, we considered left ventricular (LV) mass, ejection fraction, and high sensitive C reactive protein (hs-CRP) level as possible intermediary variables. Left ventricular mass index and ejection fraction were measured by echocardiographic M-mode and biplane Simpson methods, respectively, using standard techniques.[15] In addition, as dyslipidaemia, diabetes, blood pressure, and smoking exposure have been reported to be associated with ECG interval parameters, including in HIV infected populations,[16] we also explored the possible explanatory roles of these variables on the associations between ART-naïve or ART-exposed HIV infection in children and cardiac conduction.

Data on age and smoking exposure were obtained using a standard questionnaire filled by parents or caregivers, whereas BMI was calculated from body weight and height obtained by standard measurements (NCHS) at the time of study enrolment. Laboratory values, including lipid, HbA1c, and hs-CRP levels were analysed at Cipto Mangunkusumo Clinical Pathology Laboratory, Jakarta. As some medications have been implicated in the ECG interval abnormalities,[17] we also recorded the medication use by our study participants at the time of assessment. Based on previous literature we considered macrolides, trimethoprim, azole antifungals, antihistamine,[17] and protease inhibitors[18,19] to potentially prolong cardiac conduction, in particular the QTc interval.

Data analysis

We described baseline characteristics as mean, median, or proportion as appropriate based on child HIV status. The distributions of categorical and numerical variables across the HIV status groups were statistically evaluated using the Chi-square and one-way ANOVA, or Kruskal-Wallis tests, respectively.

To investigate the effects of HIV infection on heart conduction parameters, we performed univariable and multivariable general linear modelling with HIV status as a fixed factor and ECG interval parameters (PR, QRS, and QTc intervals) as separate dependent variables. The same models were used for adjustment for the following potential confounders: age and body mass index. For PR interval, we initially adjusted for RR-interval before performing the adjustment for potential confounders. To explore the role of various potential explanatory variables, we made graphical presentations of the effects of adding each of these variables into the confounder-adjusted model. A graphical presentation was also made to explore the influence of particular type of drugs on ECG interval parameters in ART-exposed as well as ART-naïve HIV infected children.

Findings are presented as crude and adjusted mean differences with 95% confidence intervals and corresponding p-values. A 95% confidence interval not including zero, corresponding to a p-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 19 and RStudio 0.98.507 for Mac.

Results

Baseline characteristics of study subjects are described in Table 1. Children with HIV infection, both naïve or exposed to ART, came from lower socioeconomic status and were more heavily exposed to parental smoking both during pregnancy and after birth. ART-naïve HIV-infected patients were younger and had smaller body size and the lowest LV mass. Despite similar BMI with healthy children, the ART-exposed HIV-infected group had the highest LV mass. With respect to cardiac function, HIV-infected children, both ART-naïve and -exposed, tended to have lower ejection fraction than the healthy ones.

ART exposed HIV-infected children had the highest LDL cholesterol and lower HDL levels compared to healthy children, however no statistically significant difference in HbA1c level was observed between the groups. With regard to disease severity, ART-naïve HIV infected children were in more severe clinical and immune deficient stages than the ART-exposed children. The median duration of treatment in ART-exposed group was 2.4 (0.1 – 9.9) years. Only 5 ART-exposed HIV infected children received a regimen containing protease inhibitor drugs. At the time of ECG measurement, around one-third of ART-naïve and ART-exposed HIV-infected children took medications known to potentially affect QTc interval.

Table 2 summarises the effects of ART-naïve or ART-exposed HIV infection on cardiac electrical conduction. Compared to healthy children, QTc intervals were longer in ART-naïve and, to greater extent, in ART-exposed HIV infected children as shown in the crude

(mean difference 14.0 (p = <0.01) and 26.2 milliseconds (p<0.01), respectively) as well as final adjusted analysis (difference 18.3 (p = <0.01) and 28.9 milliseconds (p <0.01), respectively). These associations could not be explained by cardiac mass/function, hs-CRP level, lipid and HbA1c levels, systolic and diastolic blood pressures, nor by postnatal parental smoking exposure (Figure 1). There were 6 children (1 ART-naïve and 5 ART-exposed) who had QTc intervals of more than 470 milliseconds. In terms of PR interval, prolongation was seen only in ART-naïve HIV infected children after adjustment for RR interval, age, and height (difference = 12.9 milliseconds, p = 0.02). This finding was not substantially explained by cardiac conditions, hs-CRP, LDL-cholesterol, or HbA1c levels, blood pressures, or parental smoking exposure (Figure 2). Analysis restricted to 32 children who had never received any ART did not substantially affect the results (Table 2).

Table 1. Baseline characteristics of study subjects

Characteristics	ART-naïve HIV n = 37	ART-exposed HIV n = 42	Non-HIV n = 50	P-value
Age (years)*, median (range)	4.8 (0.4 – 12.5)	6.1 (1.3 – 12.2)	6.4 (2.4 – 14.0)	0.39
Male gender, n (%)	16 (49)	21 (50)	23 (46)	0.93
Low socioeconomic, n (%)	14 (44)	25 (60)	10 (20)	0.001
Parental smoking after birth, n (%)				
Mother	3 (79)	5 (12)	0 (0)	0.04
Father/other household member	27 (82)	35 (83)	33 (66)	0.10
Parental smoking in pregnancy, n (%)				
Mother	6 (19)	7 (18)	1 (2.0)	0.02
Father/other household member	26 (79)	34 (81)	34 (68)	0.30
Body weight (kg)	15.0 (8.4)	18.8 (5.4)	22.4 (9.6)	0.001
Body height (cm)	99.2 (22.5)	109.1 (14.0)	116.3 (17.8)	<0.001
Body mass index (kg/m2)	14.5 (2.2)	15.5 (1.9)	15.9 (2.8)	0.04
Systolic BP (mmHg)	101.7 (11.2)	99.6 (9.7)	102.4 (9.7)	0.42
Diastolic BP (mmHg)	63.7 (10.1)	60.4 (7.5)	61.3 (7.4)	0.26
Total cholesterol (mmol/L)	3.3 (0.9)	4.5 (0.9)	4.4 (0.7)	<0.001
LDL cholesterol (mmol/L)	2.0 (0.7)	2.6 (0.7)	2.6 (0.6)	<0.001
HbA1c level	0.05 (0.04 – 0.06)	0.05(0.04 – 0.07)	0.06 (0.04 – 0.07)	0.28
hs-CRP, median (range), nmol/L	36 (0 –2759)	47 (3.7-1227)	4.7 (0-155)	<0.001#
HIV clinical stage 3 or 4	27 (84.4)	8 (47.4)		<0.001
CD4 absolute level (n= 71)	338 (10-2716)	1062 (4 – 2301)	-	<0.001
CD4 % (n = 67)	10 (0-41)	30 (4-79)	-	<0.001
Viral load (n = 23)	2070 (203 – 47914)	317 (0 – $1.2*10^6$)		
LV EF (%), median (range)	64.1 (24.8 – 80.5)	63.1 (29.3 – 82.4)	67.4 (58.6 to 80.9)	0.04
LV mass (gram)	41.8 (16.3)	55.9 (18.6)	49.8 (17.3)	0.001
Use of QT prolonging drugs*	16 (28.6)	20 (34.5)	0 (0)	
Pulse rate (/min)	124 (26)	119 (22)	115 (19)	0.14
QTc interval (milliseconds), n = 124	402.9 (25.7)	415.0 (23.7)	388.9 (20.7)	**
PR interval (milliseconds), n = 123	131.0 (25.4)	126.1 (24.9)	122.7 (19.7)	**
QRS interval (milliseconds), n = 125	68.2 (17.3)	65.7 (10.5)	66.1 (11.4)	**

#Kruskal Wallis test; Values are means (SD) unless otherwise indicated.
*macrolides, cotrimoxazole, azole antifungals, antihistamine, efavirenz
**See table 2 for statistical analysis

Table 2. Association between HIV infection and cardiac conduction parameters

			QRS interval (milliseconds)			QTc interval (milliseconds)			PR interval (milliseconds)		
			Mean difference	95%CI	P	Mean difference	95%CI		Mean difference	95%CI	P
Crude	Non-HIV		Ref			Ref			Ref		
	ART-naïve HIV	All 37	2.9	-2.6 to 8.3	0.31	14.0	3.8 to 23	<0.01	8.3	-2.1 to 18.6	0.12
		32 never ART	2.5	-3.2 to 8.1	0.39	11.5	0.4 to 22.5	0.04	4.7	-6.1 to 15.5	0.39
	ART-exposed HIV		-0.4	-5.7 to 4.8	0.9	26.2	16.7 to 35.6	<0.01	3.4	-6.1 to 12.9	0.49
									Ref*		
									9.2	-1.1 to 19.6	0.08
									6.7	-4.3 to 17.5	0.23
									4.1	-5.4 to 13.6	0.40
Model 1	Non-HIV		Ref			Ref			Ref**		
	ART-naïve HIV	All 37	4.6	-0.8 to 10.1	0.10	16.9	6.7 to 27.1	<0.01	10.5	-0.03 to 21.0	0.05
		32 never ART	5.2	-0.7 to 11.0	0.09	15.7	4.5 to 27.0	<0.01	7.8	-3.6 to 19.2	0.18
	ART-exposed HIV		0.1	-5.0 to 5.2	0.97	27.1	17.8 to 36.3	<0.01	4.2	-5.3 to 13.9	0.42
Model 2	Non-HIV					Ref			Ref***		
	ART-naïve HIV	All 32	5.0	-1.0 to 11.0	0.10	18.2	7.0 to 29.3	<0.01	12.9	2.4 to 23.3	0.02
		32 never ART	4.6	-2.0 to 11.1	0.17	17.0	4.7 to 29.3	<0.01	13.3	1.6 to 24.9	0.03
	ART-exposed HIV		0.2	-5.1 to 5.5	0.94	28.9	19.3 to 38.5	<0.01	4.3	-4.7 to 13.3	0.35

Model 1: adjusted for age; Model 2: adjusted for age, BMI;
* Adjusted for RR interval; **Model 1 + adjusted for RR interval; ***Model 2 + adjusted for RR interval

Figure 1. Influence of possible explanatory variables on mean difference (95% CI) of QTc interval

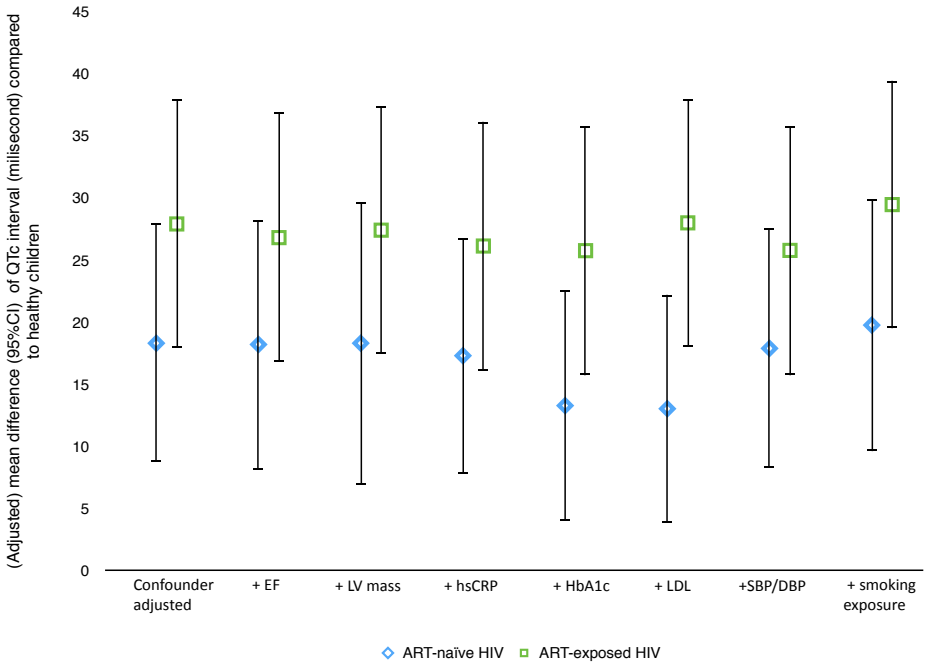
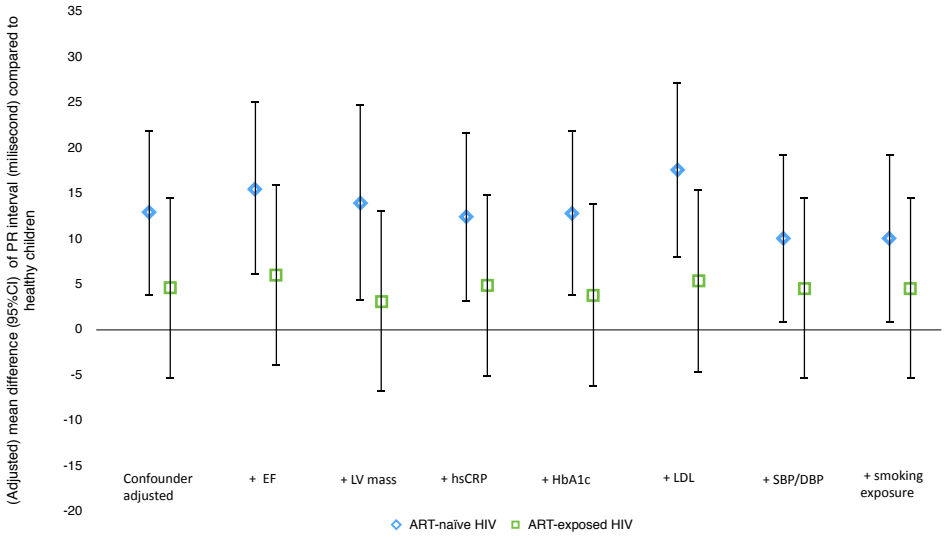


Figure 2. Influence of possible explanatory variables on mean difference (95% CI) of PR interval



The influence of drugs known to be associated with QT prolongation among HIV infected children is shown in Figure 3. In the ART-exposed HIV infected group, children who used protease inhibitor tended to have longer QTc interval compared to those who did not, whereas the use of other QT prolongation drugs seemed to have little and inconsistent effect in the ART-exposed and ART-naïve HIV infected subjects. No statistical association was explored due to limited numbers of children who used protease inhibitor or other QT prolongation drugs.

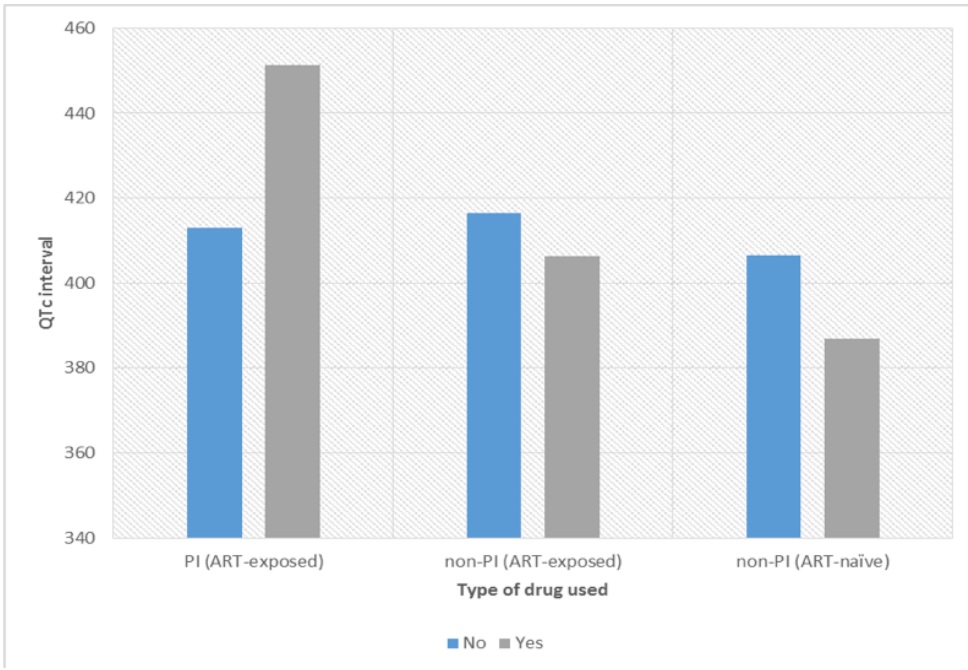


Figure 3. QTc interval based on the use of QT–prolonging drugs in HIV-infected children

*Non-protease inhibitor drugs: macrolides, co-trimoxazole, azole antifungals, antihistamine, efavirenz

Discussion

This study demonstrates that HIV infection in children is associated with a prolongation of QTc interval both in ART-naive and, to greater extent, in ART-treated children, compared to uninfected controls. In ART-naive infected children only there was a longer PR intervals. Neither cardiac conditions, the extent of inflammation, lipid or HbA1c levels, blood pressure, nor parental smoking exposures appeared to mediate these associations.

We were unable to obtain ECG measurements for all children enrolled to this study. Of these, almost one quarter was due to the death of the patients, particularly in the ART-naive HIV infected group. We may therefore have unavoidably missed the effects

of the most severe form of untreated HIV infection on the cardiac conduction system, possibly resulting in an underestimation of the findings in the total population.

In this study, we only performed standard ECG at a single time point rather than Holter ambulatory recordings. Although ambulatory monitoring would be particularly informative in capturing ECG interval variations and relate it with pharmacokinetic data, this was beyond the scope of our study. Moreover, in terms of conduction abnormalities, a standard ECG recording remains the cornerstone for diagnostic purposes and is the only practical option, especially in resource-limited settings. Holter monitoring has many potential problems, including changes in ECG morphology due to postural variations and multiple artefacts due to motion.[20] In addition the added value of QT interval variability recorded by ambulatory ECG in predicting significant ventricular arrhythmia remains controversial.[21]

To our knowledge, this is the first study delineating the effects of ART-naive from ART-exposed paediatric HIV infection on cardiac electrical conduction. A previous larger study[10] with 24-hour Holter ECG monitoring did not differentiate the possible effects of ART. despite showing similar results of QTc interval prolongation in HIV-infected compared to HIV-exposed uninfected children. Determining the differential effects of HIV infection per se from treatment is important from both etiological and clinical perspectives as it may have repercussions on treatment strategies. We demonstrated differences in the effects of ART-treated and untreated HIV infection in children on heart conduction. Even though they shared common effects on QTc prolongation, the magnitude differed.

We found QTc prolongation in both ART-naive and to a greater degree in ART-exposed HIV infected children. In the setting of similar QRS interval with healthy children, this indicates that HIV infected children may have prolonged repolarisation. Based on these findings, we may infer that HIV infection in children itself, independent of age and nutritional status, does have some effects on QTc interval and ART or the interaction between ART and the disease is likely to augment these effects. Prolongation of QTc interval has also been reported in HIV-infected adults and is suggested to be associated with duration of HIV infection.[22] However, since our finding remained after adjusting for age, which in children reflects the duration of perinatally acquired HIV infection, the effects in this population may be independent of the duration of infection exposure. Furthermore, as these associations are not explained by cardiac conditions, inflammation, plasma lipids, blood pressure, or smoking exposure, HIV infection or its treatment may have a direct effect on the autonomic cardiac regulation or cardiac conduction pathway, although the possibility of electrolyte abnormalities should also be considered. A recent animal study showed that HIV-1 trans-activator of transcription (Tat, fragment 1-86) induced an increase in cytosolic calcium concentration in cardiac-projecting parasympathetic neurons of nucleus ambiguous, triggering prolonged bradycardia, which consequently may be involved in the QT interval prolongation.[23] The

fact that we also found greater prolongation of QT interval in ART-exposed HIV-infected children despite a low rate use of protease inhibitors, warrants further exploration of the effects of non-protease inhibitor drugs on the cardiac conduction system, in interaction with HIV infection.

We found that ART-naive HIV infected children had longer PR intervals than healthy children, which has not been reported previously. It has been recognised that a longer PR interval may indicate increased predisposition to first-degree atrioventricular (AV) block and is associated to a 20-30% increased risk of atrial fibrillation,[24] despite controversies regarding the prognostic significance of PR interval prolongation in healthy general population.[25] In our ART-naive HIV infected cohort, the occurrence of longer PR interval may be associated with underlying cardiac pathology or pulmonary hypertension causing dilatation of the heart atria, unfortunately we did not measure atrial size in this current study.

Although the mean values of QTc and PR intervals in our HIV-infected study population are still below the various cut off values for congenital long QT syndrome in children (QTc interval of 450 to 480 milliseconds)[26] or for first degree AV block, our findings may be of clinical importance. Firstly, some individual HIV-infected children in our cohort reached these thresholds. Furthermore, in HIV infected children the association between longer QTc and PR intervals with clinical outcomes, such as mortality or sudden death, warrants further investigation. As demonstrated by ECG findings in HIV-infected adults, our findings may reflect the initial phase of a pathologic process that progresses as the duration of HIV infection and treatment increases, so that ECG examinations should be applied routinely when monitoring cardiac involvement in HIV infected children.

In conclusion, both ART-naive and, to greater extent, ART-exposed HIV infections in children are associated with prolongation of QTc interval, whereas longer PR interval only occurs in ART-naive HIV-infected children. These associations are unlikely mediated by cardiac structural abnormality or systolic function, level of inflammation, lipid or glucose metabolism, blood pressures, nor parental smoking exposures.

Acknowledgment

The authors gratefully acknowledge Septiani Madonna Gultom, Hanitya Dwi Ratnasari, Gina Puspita for their dedicated assistance in data collection and Rik Hopmans, MSc (Eindhoven University of Technology) for his support in data analysis with R statistical program. We also thank the medical/nursing staff of Cipto Mangunkusumo and Koja hospitals; Edi Sugiarto, Sri Yani, and Santi Sardi from the Indonesian Planned Parenthood Association Clinic (an associate member of International Planned Parenthood Federation); all patients and families for their invaluable contribution in this project.

References

1. Sims A, Hadigan C. Cardiovascular complications in children with HIV infection. Curr HIV/AIDS Rep. 2011;8:209-14.
2. Idris NS, Grobbee DE, Burgner D, Cheung MM, Kurniati N, Sastroasmoro S, et al. Cardiovascular manifestations of HIV infection in children. Eur J Prev Cardiol. 2014.
3. Bharati S, Joshi VV, Connor EM, Oleske JM, Lev M. Conduction system in children with acquired immunodeficiency syndrome. Chest. 1989;96:406-13.
4. Sani MU, Okeahialam BN. QTc interval prolongation in patients with HIV and AIDS. J Natl Med Assoc. 2005;97(12):1657-61.
5. Kocheril AG, Bokhari SA, Batsford WP, Sinusas AJ. Long QTc and torsades de pointes in human immunodeficiency virus disease. Pacing Clin Electrophysiol. 1997;20:2810-6.
6. Lindekleiv H, Wilsgaard T, Macfarlane PW, Lochen ML. QT interval and the risk of myocardial infarction and all-cause death: a cohort study. J Cardiovasc Electrophysiol. 2012;23(8):846-52.
7. Cox AJ, Azeem A, Yeboah J, Soliman EZ, Aggarwal SR, Bertoni AG, et al. Heart rate-corrected QT interval is an independent predictor of all-cause and cardiovascular mortality in individuals with type 2 diabetes: the Diabetes Heart Study. Diabetes care. 2014;37:1454-61.
8. Rich JD, Thenappan T, Freed B, Patel AR, Thisted RA, Childers R, et al. QTc prolongation is associated with impaired right ventricular function and predicts mortality in pulmonary hypertension. Int J Cardiol. 2013;167:669-76.
9. Singh P, Hemal A, Agarwal S, Kumar D. Cardiac Manifestations in HIV Infected Children. Indian J Pediatr. 2014.
10. Saidi AS, Moodie DS, Garson A, Jr., Lipshultz SE, Kaplan S, Lai WW, et al. Electrocardiography and 24-hour electrocardiographic ambulatory recording (Holter monitor) studies in children infected with human immunodeficiency virus type 1. The Pediatric Pulmonary and Cardiac Complications of Vertically Transmitted HIV-1 Infection Study Group. Pediatr Cardiol. 2000;21:189-96.
11. World Health Organization. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. Geneva: WHO Press; 2007.
12. Lepeschkin E, Surawicz B. The measurement of the Q-T interval of the electrocardiogram. Circulation. 1952;6:378-88.
13. Nasir JM, Rubal BJ, Jones SO, Shah AD. The effects of body mass index on surface electrocardiograms in young adults. J Electrocardiol. 2012;45:646-51.
14. Soliman EZ, Shah AJ, Boerkircher A, Li Y, Rautaharju PM. Inter-relationship between electrocardiographic left ventricular hypertrophy and QT prolongation as predictors of increased risk of mortality in the general population. Circ Arrhythm Electrophysiol. 2014;7:400-6.

15. Lopez L, Colan SD, Frommelt PC, Ensing GJ, Kendall K, Younoszai AK, et al. Recommendations for quantification methods during the performance of a pediatric echocardiogram: a report from the pediatric measurements writing group of the american society of echocardiography pediatric and congenital heart disease council. J Am Soc Echocardiogr. 2010;.23:465-95.
16. Reinsch N, Buhr C, Krings P, Kaelsch H, Neuhaus K, Wieneke H, et al. Prevalence and risk factors of prolonged QTc interval in HIV-infected patients: results of the HIV-HEART study. HIV Clin Trials. 2009;10:261-8.
17. Yap YG, Camm AJ. Drug induced QT prolongation and torsades de pointes. Heart. 2003;89:1363-72.
18. Anson BD, Weaver JG, Ackerman MJ, Akinsete O, Henry K, January CT, et al. Blockade of HERG channels by HIV protease inhibitors. Lancet. 2005;365:682-6.
19. Ly T, Ruiz ME. Prolonged QT interval and torsades de pointes associated with atazanavir therapy. Clin Infect Dis. 2007;44:e67-8.
20. Christiansen JL, Guccione P, Garson A, Jr. Difference in QT interval measurement on ambulatory ECG compared with standard ECG. Pacing Clin Electrophysiol. 1996;19:1296-303.
21. Puljevic D, Smalcelj A, Durakovic Z, Goldner V. QT dispersion, daily variations, QT interval adaptation and late potentials as risk markers for ventricular tachycardia. Eur Heart J. 1997;18:1343-9.
22. Fiorentini A. QTc interval prolongation in HIV-infected patients: a case–control study by 24-hour Holter ECG recording. BMC Cardiovascular Disorders. 2012;12:124.
23. Brailoiu E, Deliu E, Sporici RA, Benamar K, Brailoiu GC. HIV-1-Tat excites cardiac parasympathetic neurons of nucleus ambiguus and triggers prolonged bradycardia in conscious rats. Am J Physiol Regul Integr Comp Physiol. 2014;306:R814-22.
24. Nielsen JB, Pietersen A, Graff C, Lind B, Struijk JJ, Olesen MS, et al. Risk of atrial fibrillation as a function of the electrocardiographic PR interval: results from the Copenhagen ECG Study. Heart Rhythm. 2013;10:1249-56.
25. Cheng S, Keyes MJ, Larson MG, McCabe EL, Newton-Cheh C, Levy D, et al. Long-term outcomes in individuals with prolonged PR interval or first-degree atrioventricular block. JAMA. 2009;301:2571-7.
26. Hofman N, Wilde AAM, Kääb S, van Langen IM, Tanck MWT, Mannens MMAM, et al. Diagnostic criteria for congenital long QT syndrome in the era of molecular genetics: do we need a scoring system? Eur Heart J. 2007;28:575-80.

Chapter 8

Cardiac effects of antiretroviral-naïve versus antiretroviral-exposed HIV infection in children

Nikmah S. Idris
Michael MH Cheung
Diederick E. Grobbee
David Burgner
Nia Kurniati
Cuno S.P.M. Uiterwaal

Submitted

Abstract

Background

Cardiac involvement in HIV infected children has been frequently reported, but whether this is due to HIV infection itself or to antiretroviral treatment (ART) is unknown.

Methods

This cross sectional study involved 114 perinatally-acquired HIV-infected (56 ART-naive, 58 ART-exposed) and 51 healthy children in Jakarta, Indonesia. Echocardiography was performed to measure dimensions of the left ventricle (LV) and systolic functions. We applied general linear modelling to evaluate the associations between HIV infection/treatment status and cardiac parameters with further adjustment for potential confounders or explanatory variables. Findings are presented as (adjusted) mean differences between each of the two HIV groups and healthy children, with 95% confidence intervals and p values.

Results

Compared to healthy children, ART-naïve HIV-infected children did not show significant differences in age-and-height adjusted cardiac dimensions apart from larger LV internal diameter (difference 2.0 mm, 95%CI 0.2 to 3.7), whereas ART exposed HIV infection showed thicker LV posterior walls (difference = 1.1 mm, 95%CI 0.5 to 1.6), larger LV internal diameter (difference = 1.7 mm, 95%CI 0.2 to 3.2) and higher LV mass (difference = 14.0 g, 7.4 to 20.5). With respect to systolic function, reduced LV ejection fraction was seen in both ART-naïve HIV infected (adjusted difference = -6.7%, -11.4 to -2.0) and, to a lesser extent, in ART-exposed HIV infected children (difference = -4.5%, -8.5 to -0.4). Inflammation level partially explained most associations in ART-exposed HIV-infected, but few, if any, for decreased function in the ART-naive ones, whereas lower haemoglobin appeared to account for chamber dilation in both groups and reduced function, mainly in ART-exposed children.

Conclusion

ART-naive HIV infected children have a substantial decrease in cardiac systolic function, whereas the ART-exposed have thicker ventricular walls with larger internal diameter and higher mass, but less functional impairment.

Background

HIV infection in children remains an important global health challenge, not only because the incidence is still rising in some parts of the world,[1] but also due to its systemic and long term consequences. Cardiac involvement is one of HIV infection complications that raises much attention as it may manifest early with fatal consequences in childhood or remain clinically silent until adulthood with cumulative exposure to additional risk factors.[2]

Reports about cardiac abnormalities in HIV-infected children have come from both the pre- and post- highly-active antiretroviral treatment (ART) era, when studies mainly differed in the type of study population, whether focusing on untreated or treated subjects.[3] Despite being frequently reported, the exact mechanisms underlying cardiac pathology in HIV-infected children are not clearly understood, including the differentiation of HIV infection effects from treatment. Such knowledge may be obtained by studying measurements in the pre- and post- highly active ART era, but historical comparisons usually leave comparability concerns. Also, studies on the effects of ART on the cardiovascular system rarely involved populations that had not been exposed to any single ART at all,[4] making effect differentiation difficult. Concerns may also arise as some studies reported unadjusted findings,[5] while using convenience samples as references that despite clinical indications, had no anomalies on echocardiography. Furthermore, although the ART introduction has resulted in a dramatic decrease in overt cardiac complications, such as cardiomyopathy, subclinical structural and functional changes may still exist despite treatment.[6] It remains unclear whether this arises from HIV infection itself or due to the complex interplay between HIV infection and ART. Such delineation would only be possible by making direct comparisons of cardiac effects between ART-naïve or ART-exposed HIV infected and healthy children. Here, we investigated the effects of perinatally acquired ART-naïve and ART-exposed HIV infection on childhood cardiac structures and functions through comparisons with healthy children.

Methods

Study design and population

We performed a cross-sectional analysis on 114 HIV infected and 51 healthy children enrolled in an ongoing cohort established in June 2013 in Jakarta, Indonesia. HIV infected children (56 ART-naïve and 58 ART-exposed) were recruited from the paediatric HIV clinics of Cipto Mangunkusumo National General Hospital, Koja District Hospital, and the Indonesian Planned Parenthood Association (IPPA), Jakarta Indonesia. The ART-naïve HIV infected group included 49 children who had never received or were within the first week of antiretroviral therapy (ART) initiation and 7 children who had been off treatment for at least 5 years with previous treatment of less than 3 months. Among ART-treated children, two had just started treatment in our clinic after previous management in another hospital with potential compliance issues. We excluded children with conditions suggestive of non-vertically acquired HIV (HIV-negative parents and previous blood transfusion).

Table 2. Effects of HIV infection on cardiac structures

			Crude			Model 1			Model 2		
			Mean difference	95%CI	P	Mean difference	95%CI	P	Mean difference	95%CI	P
LVPWd (mm)	Non-HIV		Ref			Ref					
	ART-naïve HIV	All 48	-0.25	-0.83 to 0.33	0.40	0.14	-0.44 to 0.74	0.63	0.43	-0.21 to 1.08	0.18
		44 Never ART	-0.34	-0.93 to 0.25	0.26	0.13	-0.50 to 0.75	0.69	0.44	-0.23 to 1.12	0.20
	ART-exposed HIV		0.67	0.10 to 1.24	0.02	0.86	0.30 to 1.41	<0.001	1.07	0.50 to 1.65	<0.001
LVIDd (mm)	Non-HIV		Ref			Ref			Ref		
	ART-naïve HIV	All 48	-2.45	-4.53 to -0.37	0.02	0.21	-1.53 to 1.94	0.82	1.98	0.24 to 3.72	0.03
		44 never ART	-3.11	-5.26 to -0.96	0.005	0.11	-1.77 to 2.0	0.91	2.08	0.17 to 0.40	0.03
	ART-exposed HIV		-0.70	-2.71 to 1.31	0.50	0.35	-1.25 to 1.95	0.67	1.69	0.15 to 3.23	0.03
RWT	Non-HIV		Ref			Ref			Ref		
	ART-naïve HIV	All 48	0.03	-0.02 to 0.07	0.24	0.02	-0.03 to 0.06	0.52	0.01	-0.04 to 0.06	0.64
		44 never ART	0.02	-0.03 to 0.07	0.44	0.01	-0.04 to 0.06	0.74	0.004	-0.06 to 0.06	0.90
	ART-exposed HIV		0.06	0.02 to 0.10	0.007	0.06	0.01 to 0.10	0.01	0.05	0.06 to 0.10	0.03
LV mass (gram)	Non-HIV		Ref			Ref			Ref		
	ART-naïve HIV	All 48	-6.96	-14.98 to 1.05	0.09	0.41	-6.62 to 7.43	0.91	3.83	-3.28 to 10.93	0.29
		44 never ART	-8.19	-16.32 to -0.05	0.049	2.46	-4.46 to 9.37	0.48	6.86	0.09 to 13.64	0.047
	ART-exposed HIV		8.39	0.42 to 16.36	0.04	11.88	5.12 to 18.65	<0.001	13.99	7.44 to 20.54	<0.001

Note: Model 1: adjusted for age; Model 2: adjusted for age, and height
LVPWD = left ventricular posterior wall thickness at end-diastole, LVIDd = left ventricular internal diameter at end-diastole, RWT = relative wall thickness systolic excursion

Healthy children with the same age range were invited specifically for this research, from the area around the Cipto Mangunkusumo hospital. We directly approached parents and community leaders and provided leaflets explaining the purpose and procedures of the study. Of the total of 55 children invited, 3 declined and 1 was excluded due to dysmorphic features suggestive of a syndrome.

Measurement of exposure

HIV infection diagnosis was established based on the WHO 2007 criteria. Under 18 months of age, diagnosis was established based on a positive HIV DNA or RNA PCR examination, whereas for those aged 18 months or older, it was defined by positive HIV antibodies. We classified disease severity based on the WHO clinical and immunological staging criteria[7] applied at the time of echocardiography. The CD4 cell level to define immunological staging was obtained from routine clinical data within 3 months of enrolment. Viral load examination was not routinely done in our HIV care setting due to financial constraints and only selectively performed as indicated by treating physicians.

Echocardiography measurement

To measure cardiac structural and functional parameters, we performed standard (B-mode, M mode) echocardiography, following the recommendation of the American Society of Echocardiography.[8] Children were assessed in supine position using a Phillips Sonos 4500 machine with 7 and 12 MHz phase array transducers and simultaneous reference ECG recordings. Using M-mode, the following cardiac dimensions were measured at parasternal long axis view: left ventricular posterior wall thickness at end-diastole (LVPWd), internal diameter at end-diastole (LVIDd), and mass (LV mass); the same approach was used to measure LV fractional shortening (FS) as a systolic function parameter, reflecting degree of LV diameter shortening between end-diastole and end-systole. Relative wall thickness (RWT) was calculated as LVPWd divided by ½ LVIDd.[9] Other functional parameters sought were LV ejection fraction (LVEF, the fraction of blood volume pumped out of the LV at each heartbeat, calculated as the ratio of stroke volume over LV end-diastolic volume measured using the Simpson biplane method), and tricuspid annular plane systolic excursion (TAPSE), indicating right ventricular systolic function. All parameters were measured in 3 to 4 cardiac cycles and the averages were taken.

Echocardiograms were available for 153 children (48 ART-naïve and, 54 ART-exposed HIV infected, 51 healthy). Examinations were performed by one of the investigators (NSI) for the first 134 children and two trained cardiology fellows in further subjects, in whom results were validated offline on stored digital loops. The intraclass coefficient correlation from two-way mixed modelling was 86.7 (95%CI 79.0 to 91.7). All procedures were done

in the Echocardiography Laboratory of Department of Child Health University of Indonesia – Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia.

Measurement of potential confounders and intermediary variables

Age and height were a priori set as potential confounders in the association between HIV infection status and cardiac parameters. Besides being associated with HIV infection status, both are associated with cardiac dimensions as well as functions.[10] Height as an indicator of nutritional status, may also act as mediator as HIV infection likely affects nutritional status, by which the heart might be impaired. As inflammation, assessed by hsCRP level,[11] blood pressure, or haemoglobin level may mediate the effect of ART-naive or ART-exposed HIV infection on cardiac structures and functions, we explored their possible roles as mediators.

We obtained data on age and smoking exposure using a standard questionnaire filled by parents or caregivers, whereas body weight and height were measured using a standard method.[12] Laboratory analyses for haemoglobin and hsCRP levels were performed at Cipto Mangunkusumo Clinical Pathology Laboratory, Jakarta; personnel were blinded to HIV infection/treatment status and other clinical data.

Data analysis

Baseline characteristics are described as mean, median, or proportion as appropriate and differences across HIV status groups were tested using Chi-square, oneway ANOVA, or Kruskal-Wallis tests, respectively.

To investigate the effects of HIV infection on cardiac structures/functions, we performed univariable and multivariable general linear modelling with HIV status as a fixed factor and cardiac parameters (LVPWd, LVIDd, LV mass, RWT, EF, FS, TAPSE) as separate dependent variables. The same models were used for adjustment for age and height as potential confounders. We considered height a better indicator of growth and body size than weight as it is less affected by acute changes, such as volume status or other pathology(ascites, organ enlargement) that may accompany HIV infection. To describe the potential explanatory roles of hsCRP level, systolic and diastolic blood pressures, and haemoglobin level, we graphically presented effects of adding each of these to the confounder adjusted model. We also did sensitivity analyses by only including children who had never received ART at all (Table 2 and 3) and by excluding 2 ART-exposed children previously managed in another hospital.

Findings are presented as crude and adjusted mean differences with 95% confidence intervals and corresponding p-values. A 95% confidence interval not including zero, corresponding to a p-value of <0.05 was considered statistically significant. All analyses were performed using SPSS version 19 and RStudio 0.98.507 for Mac.

Results

Table 1 shows baseline characteristics of study subjects. Children with HIV infection, both ART-naïve or -exposed, came from lower socioeconomic status. ART-naïve HIV-infected patients were younger and had smaller body size among others. Compared to ART-exposed HIV-infected children, ART-naïve children were in more severe clinical and immunodeficiency states, and had the lowest mean haemoglobin levels among others. The median duration of treatment in ART-exposed group was 2.2 (0.1 – 9.9) years and only 5 children had a regimen containing PI drugs.

Table 1. Baseline characteristics of study subjects

Characteristics	ART-naïve HIV n = 48	ART-exposed HIV n = 54	Non-HIV N = 51	P-value
Age (years)*, median (range)	3.6 (0.6 – 11.4)	5.4 (0.6 – 12.2)	6.4 (2.4 – 14.0)	<0.001
Male gender, n (%)	22 (46)	25 (46)	24 (47)	0.99
Low socioeconomic status, n (%)	20 (43)	31 (57)	10 (20)	0.001
Parental smoking exposure after birth, n (%)				
Mother	4 (9)	6 (11)	0 (0)	0.06
Father/other household member	36 (75.0)	47 (87)	34 (67)	0.047
Parental smoking exposure in pregnancy, n (%)				
Mother	7 (16)	8 (15)	1 (2.0)	0.04
Father/other household member	36 (75)	45 (83)	35 (69)	0.21
Family history of CVD	15 (33)	14 (26)	3 (6)	0.004
Body weight (kg), n = 149	12.9 (7.0)	17.4 (6.1)	22.5 (9.5)	<0.001
Body height (cm), n = 145	92.7 (19.3)	104.8 (16.9)	116.5 (17.7)	<0.001
Body mass index (kg/m2), n = 145	14.3 (2.4)	15.4 (2.4)	15.9 (2.8)	0.01
Systolic BP (mmHg), n = 141	101.0 (11.2)	99.9 (10.5)	102.3 (9.6)	0.51
Diastolic BP (mmHg), n = 141	62.5 (9.7)	60.7 (7.7)	61.1 (7.4)	0.5
Hb (mg/dL) level, n = 142	10.9 (6.9 – 19.8)	11.5 (4.2 – 14.1)	12.7 (10.2 – 14.6)	<0.001
hs-CRP, median (range), nmol/L	37 (0 –2759)	48 (371-5507)	5 (0-155)	<0.001#
HIV clinical stage 3 or 4	43 (94)	30 (60)		<0.001
CD4 absolute level (n= 71)	350.5 (2-2716)	1188 (4 – 2301)	-	<0.001
CD4 % (n = 67)	10 (0-49)	30 (4-79)	-	<0.001
Viral load (n = 23)	320 (59 – 47914)	544 (0 – $1.2*10^6$)		
LVPWd (mm)	5.8 (1.4)	6.7 (1.6)	6.0 (1.4)	**
LVIDd (mm)	31.0 (6.3)	32.7 (4.9)	33.4 (4.1)	**
LV mass (gram)	43.2 (18.6)	58.5 (20.3)	50.1 (17.2)	**
LV mass index (gram/m2)	74.2 (26.7)	84.1 (25.5)	62.3 (12.9)	<0.001
RWT	0.38 (0.12)	0.42 (0.12)	0.36 (0.09)	**
LVEF (%)	61.3 (13.2)	63.1 (9.7)	67.7 (5.4)	**
LVFS (%)	33.4 (13.2)	33.5 (9.7)	36.0 (5.4)	**
TAPSE (mm)	16.5 (3.5)	18.9 (4.2)	20.2 (3.3)	**

Note: LVPWd = left ventricular posterior wall thickness at end-diastole; LVIDd = left ventricular internal diameter at end-diastole; RWT = relative wall thickness; LV mass = left ventricular mass; LVEF = left ventricular ejection fraction; LVFS = left ventricular fractional shortening; TAPSE = tricuspid annular plane systolic excursion.
#Kruskal Wallis test; Values are means (SD) unless otherwise indicated. **See Table 2 and 3

Table 2 shows effects of ART-naïve and ART-exposed HIV infection on cardiac structures. While ART-naïve HIV-infected children tended to have smaller LV mass (difference -7.0g, p=0.09), the difference disappeared after adjustment for age and height. ART-naïve HIV-children seemed to have smaller LVIDd (difference -2.5mm, p=0.02), but after being adjusted for age and height, they actually had larger LVIDd than healthy children (difference = 2.0mm, p=0.03). This larger LVIDd seemed to be explained by inflammation (Figure 1a) and haemoglobin levels. There was no difference in posterior wall thickness in end-diastolic (LVPWd) between ART-naïve HIV infected and healthy children (Table 2).

ART exposed HIV infected children had thicker LV posterior walls (LVPWd), both in crude (0.7mm, p=0.02) and adjusted (1.1mm, p <0.001) analyses, and higher LV mass (crude 8.4g, p=0.04; adjusted 14.0g, p<0.001). The RWT also tended to be higher in ART-exposed HIV infected children both on crude (difference 0.06, p=0.007) and adjusted analyses (difference = 0.05, p=0.03). On adjusted analysis, they also had larger LVIDd (1.7mm, p=0.03). Inflammation level seemed to partially explain the effects of ART-exposed HIV infection in children on the observed structural changes, while haemoglobin level appeared to account for larger internal diameter only (Figure 1a).

In terms of left ventricular systolic function (Table 3), ART-naïve HIV infected children had lower LVEF than healthy children (adjusted difference -6.7%, p=0.006); ART-exposed HIV infected children showed this association to a lesser degree (adjusted difference -4.5%, p = 0.03). ART-naïve children tended to have lower FS (-2.5%, p=0.06) and poorer RV systolic function as reflected in lower TAPSE (-3.6mm, p<0.001), but not after adjustment for age and height (-1.3%, p=0.40 and -0.6mm, p=0.42 for FS and TAPSE, respectively). Degree of inflammation seemed to explain reduced LVEF in ART-exposed HIV-infected children, but not in the ART-naïve. In contrast, haemoglobin level explained decreased systolic function in both ART-naïve and, to a larger extent, the ART-exposed HIV infected children (Figure 1b).

Analyses excluding ART-naïve HIV infected children who had a brief period of treatment in the past (Table 2 and 3) or 2 ART-exposed children with potential previous compliance issue (data not shown) revealed similar findings.

Table 3. Effects of HIV infection on cardiac functions

			Crude			Model 1			Model 2		
			Mean difference	95%CI	P	Mean difference	95%CI	P	Mean difference	95%CI	P
LVEF (%)	Non-HIV		Ref			Ref			Ref		
	ART-naïve HIV	All 48	-6.42	-10.35 to -2.49	0.002	-7.07	-11.33 to -2.81	0.001	-6.68	-11.41 to -1.95	0.006
		44 Never ART	-6.10	-10.00 to -2.12	0.002	-6.86	-11.16 to -2.55	0.002	-6.18	-10.97 to -1.40	0.01
	ART-exposed HIV		-4.66	-8.41 to -0.90	0.02	-4.92	-8.74 to -1.10	0.01	-4.46	-8.49 to -0.42	0.03
LVFS (%)	Non-HIV		Ref			Ref			Ref		
	ART-naïve HIV	All 48	-2.54	-5.21 to 0.13	0.06	-2.14	-4.95 to 0.67	0.13	-1.30	-4.32 to 1.72	0.40
		44 Never ART	-3.14	-5.88 to -0.40	0.03	-2.98	-5.95 to -0.01	0.05	-2.33	-5.55 to 0.88	0.15
	ART-exposed HIV		-2.47	-5.09 to 0.16	0.07	-2.27	-4.93 to 0.39	0.10	-1.78	-4.51 to 0.95	0.20
TAPSE (mm)	Non-HIV		Ref			Ref			Ref		
	ART-naïve HIV	All 48	-3.64	-5.15 to -2.12	<0.001	-2.20	-3.63 to -0.77	0.003	-0.61	-2.17 to 0.79	0.42
		44 Never ART	-3.64	-5.22 to -2.05	<0.001	-1.77	-3.29 to -0.25	0.02	0.20	-1.36 to 1.75	0.80
	ART-exposed HIV		-1.26	-2.73 to 0.22	0.10	-0.71	-2.04 to 0.63	0.30	0.20	-1.11 to 1.53	0.77

Note: Model 1: adjusted for age; Model 2: adjusted for age and height
LVEF = left ventricular ejection fraction, LVFS = left ventricular fractional shortening, TAPSE = tricuspid annular plane systolic excursion

Figure 1a. Influence of potential explanatory variables on cardiac structural estimates in ART-naive and ART-exposed HIV infected children

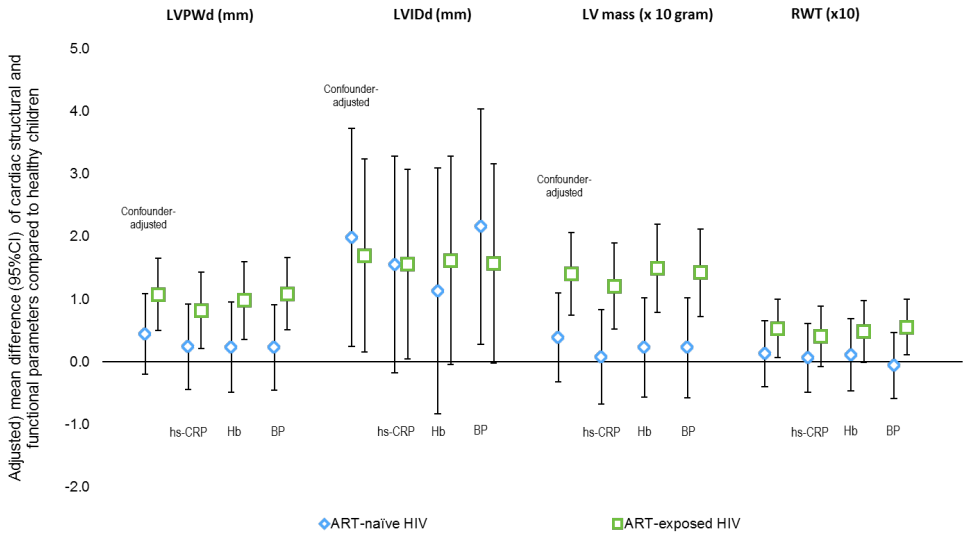
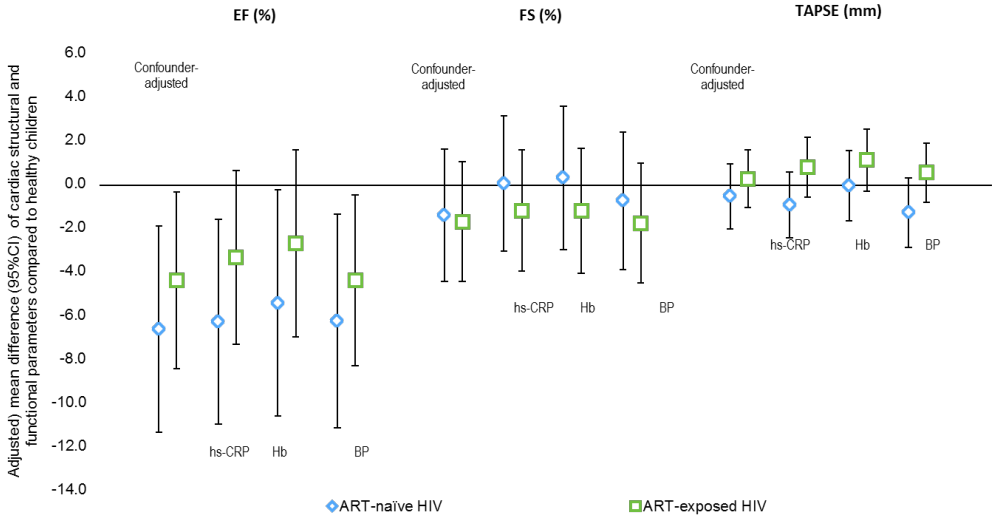


Figure 1b. Influence of potential explanatory variables on cardiac functional estimates in ART-naive and ART-exposed HIV infected children



Note: LVPWd = left ventricular posterior wall thickness at end-diastole; LVIDd = left ventricular internal diameter at end-diastole; LV mass = left ventricular mass; EF = ejection fraction; FS = fractional shortening; TAPSE = tricuspid annular plane systolic excursion; smoking = parental smoking exposure; BP = systolic and diastolic blood pressure.

Discussion

This study shows that ART-naive HIV infected children had markedly lower LV ejection fraction with chamber dilation than healthy children. ART-exposed HIV infection showed structural changes with thicker LV posterior wall, chamber dilation, and higher LV mass, and a lesser degree of decreased systolic function. Degree of inflammation seemed to explain effects of ART-exposed HIV infection on both structural and functional changes, except for the higher LV mass, while for the ART-naïve, it partially explained the larger LV internal diameter, but provided little explanation for the decreased systolic function. Lower haemoglobin level appeared to account for larger internal diameter in both ART-naïve and ART-exposed and decreased systolic function particularly in ART-exposed HIV infected children.

Previous studies evaluating cardiac involvement in HIV infection in children involved larger sample size[5] than ours or used more refined techniques, such as strain analysis.[6] However, those studies, whether in the pre- or post- highly active – ART era, typically looked at either naive[6] or ART exposed populations.[3] and one including both,[13] did not clearly delineate between ART-naive or ART-exposed HIV infection. We had the unique opportunity to study the cardiac status in both ART-naïve and ART-exposed HIV infected children, within the same time frame in the post highly active ART era rather than making a historical comparison. In Indonesia, universal HIV screening in pregnancy is unfortunately not widely applied, resulting in inadequate prevention of mother-to-child transmission and late identification of new paediatric cases. Our design and analytical approach disentangles the effects of ART-naïve from ART-exposed HIV infection. Our use of dedicated healthy children as a reference, rather than published normal values,[14] hospital records of normal echocardiograms,[6] or HIV-exposed uninfected children[13] is a particular strength. With patients referred for echocardiography who showed no cardiac anomalies[6] as reference, the indication for echocardiography may still bias findings. Although some previous studies applied population matching or restrictions, the mandatory corresponding conditional analyses were not applied, and to our understanding none were appropriately adjusted for confounding. Finally, we explored several possible explanatory analyses, which to our knowledge has never been done previously.

We only addressed basic parameters of cardiac structures and functions in HIV-infected children and had not yet looked at the occurrence of cardiac disease entities, which are probably more relevant for paediatric clinical practice.[5] However, our approach may serve as a starting point in analysing fundamental changes in cardiac structures and functions preceding the occurrence of such entities. This involves consideration of certain slightly different definitions and cut offs, such as different criteria to define cardiomyopathy which may not have direct prognostic relevance.

We found that ART-naïve HIV infected children had compromised ventricular systolic function with chamber dilatation, while the ART-exposed children had less functional impairment despite thicker LV posterior wall, larger internal diameter, and higher mass. This was never explicitly reported before as in most studies, ART-naïve and ART-exposed HIV-infected children were pooled into one group, showing various cardiac structural changes, such as hypertrophic or abnormal ventricular mass,[15] chamber dilatation, as well as systolic or diastolic dysfunctions.[5] Our findings imply that ART, either by controlling viral replication or exerting direct cardio protective effects, may improve cardiac functions as was shown by following up HIV-infected children who switched from no or single ART to highly active three drug ART regimens.[4] Thicker ventricular wall, chamber dilatation, and higher mass in ART-exposed HIV infected children may reflect treatment-induced compensatory mechanisms to bear the extra load,[16] by which the systolic function was relatively preserved or, on the other hand, effects of the interaction between treatment and residual HIV infection that may be disadvantageous in the long term, necessitating further follow up. Previous findings about LV mass and ventricular diastolic dimensions were inconclusive as recent studies suggested that ART, either given for prevention in foetal/early postnatal life of HIV-exposed uninfected infants or as a treatment, was associated with reduced LV mass and septal thickness[17, 18] but earlier findings, including an autopsy study, showed a progressive increase of LV mass and end-diastolic dimension in mostly severely symptomatic children who were in majority (60-90%) treated, either by zidovudine only or combination therapy.[19] These differences may occur because of different study populations, comparisons, and analytical approaches, including the use of adjusted measures for nutritional status, which in our view may have a strong association with both HIV infection status and cardiac structures and which we therefore accounted for. We infer that higher LV mass, chamber dilatation, and thicker ventricular wall among our ART- HIV-infected subjects results from the interaction between highly-active ART and partially controlled HIV infection, as around 60% of them were still in a severe-to-advanced clinical stage.

Mechanisms underlying cardiac involvement in HIV-infected children remain poorly understood. Multiple factors may be involved, including HIV-induced cytokine or proteolytic enzyme release, co-infection, nutritional deficiency, autoimmune response to infecting pathogens, ART cardio toxicity, or direct viral cell invasion.[20] Our findings suggest that HIV infection itself, by whatever mechanisms, first cause functional cardiac changes by compromised systolic function. Subsequent treatment, probably by controlling HIV replication, seems to partially restore cardiac function, which might be at the expense of inducing structural abnormality. The suboptimal LVEF that was still observed in our ART-treated HIV infection subjects may indicate residual damage due to previously untreated infection. Previous studies investigating cardiac involvement in HIV-infected subjects rarely, if at all, explored potential mechanisms underlying the

reported associations. Based on our findings, degree of inflammation may mediate the effects of ART-exposed HIV infection on thicker posterior wall and slightly reduced LVEF, but provides less explanation for the effects of ART-naive HIV infection on decreased LVEF. On the other hand, lower haemoglobin seemed to account for chamber dilation in both groups and lower LVEF, particularly in ART-exposed HIV infected children. Although family history of CVD was more frequent in our HIV-infected subjects, we believe that it reflects a clustering of high risk behaviours, such as smoking, rather than a causal relation with the observed cardiac structural or functional changes.

From the perspective of clinical practice, our findings underline the detrimental cardiac effects of ART-naïve HIV infection, which should raise awareness to provide early treatment and more elaborate care for this kind of patients. As ART does not completely solve the cardiac problems and even may have long term disadvantageous effects, a regular follow up of cardiac status may be required for HIV-infected children. This is of particular importance as increased wall thickness and depressed systolic function have been reported as a significant predictor for mortality in HIV-infected children independent of CD4 cell count.[21]

In conclusion, ART-naive HIV infected children have a substantial decrease in cardiac systolic functions accompanied by chamber dilatation. In ART-exposed HIV infected children, thicker ventricular walls with higher mass and chamber dilatation dominate, with less functional impairment.

Acknowledgement

The authors gratefully acknowledge Septiani Madonna Gultom, Hanitya Dwi Ratnasari, the deceased Weni Tenlima for their dedicated assistance in data collection and Rik Hopmans, MSc (Eindhoven University of Technology) for his support in data analysis with R statistical program. We also thank Dra. Diyah Kristanty, M. Biomed and Enny Hasrini for organizing the laboratory assays and Dr. Indah Kartika Murni and Dr. Veny Kartika for helping with the echocardigraphy measurement. Our great appreciation for the medical/nursing staff of Cipto Mangunkusumo and Koja hospitals; Edi Sugiarto, Sri Yani, and Santi Sardi from the Indonesian Planned Parenthood Association Clinic (an associate member of International Planned Parenthood Federation); all patients and families for their invaluable contribution in this project.

References

1. UNAIDS. Global report: UNAIDS report on the global AIDS epidemic 2012. Geneva: UNAIDS, 2012.
2. Lipshultz SE, Easley KA, Orav EJ, Kaplan S, Starc TJ, Bricker JT, et al. Cardiovascular status of infants and children of women infected with HIV-1 (P2C2 HIV): a cohort study. Lancet. 2002;360:368-73.
3. Luginbuhl LM, Orav EJ, McIntosh K, Lipshultz SE. Cardiac morbidity and related mortality in children with HIV infection. JAMA. 1993;269:2869-75.
4. Plebani A, Esposito S, Pinzani R, Fesslova V, Bojanin J, Salice P, et al. Effect of highly active antiretroviral therapy on cardiovascular involvement in children with human immunodeficiency virus infection. Pediatr Infect Dis J. 2004;23:559-63.
5. Starc TJ, Lipshultz SE, Kaplan S, Easley KA, Bricker JT, Colan SD, et al. Cardiac complications in children with human immunodeficiency virus infection. Pediatric Pulmonary and Cardiac Complications of Vertically Transmitted HIV Infection (P2C2 HIV) Study Group, National Heart, Lung, and Blood Institute. Pediatrics. 1999;104:e14.
6. Sims A, Frank L, Cross R, Clauss S, Dimock D, Purdy J, et al. Abnormal cardiac strain in children and young adults with HIV acquired in early life. J Am Soc Echo. 2012;25(7):741-8.
7. World Health Organization. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. Geneva: WHO Press; 2007.
8. Lopez L, Colan SD, Frommelt PC, Ensing GJ, Kendall K, Younoszai AK, et al. Recommendations for quantification methods during the performance of a pediatric echocardiogram: a report from the pediatric measurements writing group of the american society of echocardiography pediatric and congenital heart disease council. J Am Soc Echo.23:465-95.
9. Gaasch WH, Zile MR. Left ventricular structural remodelling in health and disease. J Am Coll Cardiol. 2011;58:1733-1740.
10. Graham TP, Jr., Jarmakani JM, Canent RV, Jr., Morrow MN. Left heart volume estimation in infancy and childhood. Reevaluation of methodology and normal values. Circulation. 1971;43:895-904.
11. Barbaro G, Fisher SD, Lipshultz SE. Pathogenesis of HIV-associated cardiovascular complications. Lancet Infect Dis. 2001;1:115-24.
12. National Health and Nutrition Examination Survey (NHANES). Anthropometry Procedures Manual 2007. Available from: http://www.cdc.gov/nchs/data/nhanes/nhanes_07_08/manual_an.pdf.
13. Lipshultz SE, Williams PL, Wilkinson JD, Leister EC, Van Dyke RB, Shearer WT, et al. Cardiac status of children infected with human immunodeficiency virus who are receiving long-term combination antiretroviral therapy: results from the

adolescent master protocol of the multicenter pediatric HIV/AIDS cohort study. JAMA Pediatr. 2013;167:520-7.

14. Cunha Mdo C, Siqueira Filho AG, Santos SR, Abreu TF, Oliveira RH, Baptista DM, et al. AIDS in childhood: cardiac involvement with and without triple combination antiretroviral therapy. Arq Bras Cardiol. 2008;90(1):11-7.
15. Kearney DL, Perez-Atayde AR, Easley KA, Bowles NE, Bricker JT, Colan SD, et al. Postmortem cardiomegaly and echocardiographic measurements of left ventricular size and function in children infected with the human immunodeficiency virus: The Prospective P2C2 HIV Multicenter Study. Cardiovasc Pathol. 2003;12:140-8.
16. Lorell BH, Carabello BA. Left ventricular hypertrophy: pathogenesis, detection, and prognosis. Circulation. 2000;102:470-9.
17. Lipshultz SE, Shearer WT, Thompson B, Rich K, Cheng I, Orav EJ, et al. Cardiac effects of antiretroviral therapy in HIV-negative infants born to HIV-positive mothers: NHLBI CHAART-1 (National Heart, Lung, and Blood Institute Cardiovascular Status of HAART Therapy in HIV-Exposed Infants and Children cohort study). J Am Coll Cardiol. 2011;57:76-85.
18. Lipshultz SE, Shearer WT, Thompson B, Rich K, cheng I, Orav EJ, et al. Antiretroviral therapy (ART) cardiac effects in HIV-infected children: the multicenter NHLBI cardiac highly active antiretroviral therapy (CHAART-II) study. Circulation. 2009;120:S909-10.
19. Lipshultz SE, Easley KA, Orav EJ, Kaplan S, Starch TJ, Bricker JT et al. Left ventricular structure and function in children infected with human immunodeficiency virus: the Prospective P2C2 HIV Multicenter Study. Circulation. 1998;97:1246-56.
20. Currie PF, Boon NA. Immunopathogenesis of HIV-related heart muscle disease: current perspectives. AIDS (London, England). 2003;17 Suppl 1:S21-8.
21. Lipshultz SE, Easley KA, Orav EJ, Kaplan S, Starc TJ, Bricker JT, et al. Cardiac dysfunction and mortality in HIV-infected children: the Prospective P2C2 HIV Multicenter Study. Pediatric Pulmonary and Cardiac Complications of Vertically Transmitted HIV Infection (P2C2 HIV) Study Group. Circulation. 2000;102:1542-8.

Chapter 9

General discussion

The beginning

The development of the cardiovascular system involves complex, precisely orchestrated, series of events, which begin in the very early embryonic life.[1] The process combines multiple cell lineages that, through interactions with the surrounding tissues, become committed to a cardiac destination or to the vascular network. The complex morphogenetic events and the superimposed hemodynamic influences make the cardiovascular system extremely sensitive to subtle perturbations.[1] Although it has been widely acknowledged that small perturbations in the embryonic life may result in life threatening and permanent morbidity, such as readily manifest congenital heart defects, less is known about the influence of various environmental factors on postnatal development of the cardiovascular system.

Although the heart seems to follow a more settled natural course of development with a complete structural formation by the second month of foetal life, the organ still continues to develop after birth. Based on global function, the developing heart appears to be simply a miniature of the adult heart which fills with blood, develops pressure, ejects, and relaxes. Nonetheless, across the development, a child heart actually differs quantitatively and qualitatively in function and structures from the adult heart. For example, adult myocardium is more compliant and has greater active tension than the foetal myocardium. Normally the volume and mass of the heart increase with development through cardiac cell hyperplasia or physiological hypertrophy, which is greatly influenced by workload and mural stress.[2] Various post natal insults which affect mural stress and work load may divert the heart from its normal course of development.

With regard to the formation and development of the vascular system, two main successive processes are involved, which are vasculogenesis, the formation of the first major intra-embryonic blood vessels and vascular plexus from differentiating mesodermal cell precursors, and angiogenesis, the formation of capillaries from preexisting vessels.[3] This vascular network development involves a series of coordinate events of endothelial cell activation, proliferation, migration, alignment, tube formation, branching, and anastomosis. The health of endothelial cells is essential for further normal vascular physiology. As a ‘container’ for blood, endothelial membranes need to remain intact and function normally to present a non-thrombogenic surface for the circulating blood.[4] The endothelium also functions as a selectively permeable barrier, which constantly monitors and integrates blood borne signals through expression of cell surface receptors for various cytokines, growth factors, hormones, as well as bacterial products. Due to these continuous exposures to various signals or insults from early life onwards, endothelial cells are prone to alterations that could be disadvantageous for short- as well as long term cardiovascular health.[5]

Evidence is mounting that atherosclerosis formation starts at a very young age. Diffuse intimal thickening, which is considered an early event in atherogenesis, has been found

in coronary arteries and the aorta from 36 weeks of gestation and the first year of life, respectively.[6] The pathological studies of the Bogalusa Heart Study and the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) have also revealed that even advanced atherosclerotic lesions, such as fibrous plaques can be found in adolescents and young adults.[7] Numerous studies have been done to elucidate early life factors involved in the development of future atherosclerosis and consequently cardiovascular disease (CVD). Work that has been quite fundamental is that of Barker and colleagues, who proposed what is now known as the Developmental Origins of Health and Disease (DOHAD) hypothesis.[8] It suggests that early life insults, such as prenatal undernutrition, may result in permanent physiological and metabolic alterations that probably benefit short term survival but at the expense of increased risk of chronic diseases in later life.

Despite numerous studies confirming or challenging the DOHAD hypothesis and investigating various possible early life determinants of CVD, relatively little is known about the dynamics of cardiovascular system development in the childhood period, particularly on how it progresses, responds, and is being modified by various postnatal environmental factors, which will be discussed here. We will briefly describe how the knowledge of these dynamics may impact preventive and therapeutic health measures to improve cardiovascular health of children, which is not only important for their growth and development period but also for their future CVD risk. The main emphasis will be on implications of this knowledge for global cardiovascular health. Important in that respect is whether there are differences in the aetiology and early life determinants, and therefore prevention or management strategies, of CVD in low-to-middle income countries given differences in disease pattern, child rearing practice, nutrition, and other lifestyle-related habits.

Dynamics of early life cardiovascular development and environmental influence

The development of the cardiovascular system is determined by nature and nurture. Although both early manifest cardiovascular abnormalities and risk of future atherosclerosis seem to have some inheritable components, overall, a purely or major genetic contribution is very unlikely, except for rare conditions.[9] Rather, it appears that genetic factors play a role in providing the basic template for the cardiovascular network which is then shaped by nurture or environmental factors. How cardiovascular phenotypes vary by genetic and environmental influence can be viewed from either one of two ways. First, the genetic component is relatively fixed, such as in a single mutation case, and the environment modify its expression. This is illustrated by the development of congenital heart disease, in which one single gene mutation may give rise to a spectrum of heart malformations, implicating that genomic context, maternal-foetal environment, cardiac biomechanics, and other factors are important influences on the clinical

consequences of mutations. Looking deeper at microstructures, such as the cardiac myofibril gene expression during heart development, the same phenomenon has been observed.[10] Secondly, a particular environmental factor may act in different ways according to the presence of certain genetic allele variations. An example for this is the genetic obesity susceptibility, in which, with the same level of environmental exposure, individuals carrying certain genetic variants are predisposed to higher adult body mass index compared to those not having these particular alleles.[11]

With regard to atherosclerosis formation, the characterisation of genetic roles is even less clear, probably because there would be numerous genes underlying the susceptibility to atherosclerosis in the general population, and that each contributes small effects. Nevertheless, multiple loci significantly associated with coronary artery disease have been unraveled by the genome-wide association studies and some have been replicated in numerous independent and across-ethnicity studies.[12] However, as mentioned above, cardiovascular gene expression is not only governed by genetic factors, but largely by environmental factors, possibly through epigenetic mechanisms, which are currently being extensively studied.[13] Epigenetics refer to heritable traits that are not a consequence of changes in DNA sequence but related to three mechanisms of regulation, which are DNA methylation, histone modification, and noncoding RNA action. It may affect development, differentiation, and disease propensity or expression of the cardiovascular system.[13] Aberrant epigenetic mechanisms may be influenced by environmental factors as well as a variant genetic background.

A substantial number of studies have been done to identify and quantify environmental modifiable influences, which may provide a window of opportunity for prevention. From the latter perspective, environmental influences can be classified into those acting in the pre- or peri conceptional, pregnancy, or infancy or childhood period. Delineations between these periods are not always sharp from a developmental point of view, but at least they are from a care perspective. Notably, generating direct evidence on relations between childhood exposures and later life cardiovascular disease, is challenged by requiring a very long time span for observation, therefore having to resort to animal models, or use surrogate outcome measures.

With regard to pre- or peri conceptional influences and vascular health, much of the evidence is still under construction, such as for the effects of peri-conceptional maternal weight and nutrition. It has been indicated that pre-pregnancy obesity may independently program later offspring obesity, whereas undernutrition also leads to an exaggerated stress response associated with changes in the epigenetic state of the insulin like growth factor 2 (IGF2) in the adrenal glands.[14] That may alter a number of key factors regulating cardiovascular growth and metabolism, increasing the propensity for cardiovascular diseases in later life.[15] Peri-conceptional factors also may cause immediate detrimental effects in the cardiovascular system, as demonstrated in the association between several

cardiac malformations and maternal diabetes or other illnesses,[16] nutritional factors,[17] medications, such as analgesic use around conception,[18] or toxic agent or solvent exposures.[19]

An increasing body of evidence exists for pregnancy-related influences on the development of the cardiovascular system, including the later risk of cardiovascular diseases. Besides the already long recognised effects of particular insults, such as intrauterine infection or nutritional deficiency on gross cardiac malformations, evidence is accumulating for the long-term effects of various factors in pregnancy on cardiovascular health. For example, since Barker first proposed a hypothesis about the links between intrauterine growth restriction and later risk of non-communicable diseases, the associations have been seen across the range of birth size typical for each population.[20] Maternal or second hand smoking exposure in pregnancy has also been indicated to cause vascular intimal-media thickening, which may be associated with increased cardiovascular disease risk in adulthood.

The dynamics for post natal cardiovascular system development, particularly how it may progress towards increased risk of cardiovascular disease in later life, is not completely understood. Postnatal influences and effects of their modification allow for easy observation and quantification. For cardiovascular pathology manifesting in early childhood, there is probably little room for post natal prevention and even none for congenital conditions. However, apart from the macrostructure, almost every other aspect of the cardiovascular system is still developing after birth, providing a window of opportunity to prevent later life cardiovascular disease. Work done in this area has revealed various factors that may influence the development of cardiovascular disease in adulthood, including excess growth or overweight,[21] sedentary lifestyle, smoking exposure,[22] and breastfeeding.[23] This thesis mainly aimed to fill the knowledge gap in this area in order to obtain a better picture about the dynamics of cardiovascular system. We have shown that physical activity, famine in childhood, and infection may exert a substantial influence on the later cardiovascular disease risk. Moreover, our findings have indicated that childhood diseases, in our case HIV infection, may cause immediate changes that manifest early or potentially decrease structural and functional reserve, triggering the risk for future cardiovascular diseases.

Early life prevention and cardiovascular health: some general considerations

The ultimate objective of etiologic studies, which are aimed at finding out why disease occurs, is to assess possible clues for prevention. Prevention is particularly important in the context of cardiovascular disease since its morbidity and mortality remain high despite the availability of multimodal and advanced treatment. As the underlying atherosclerosis formation starts very early in life, it seems rational to argue that cardiovascular disease prevention should begin early in life. Moreover, once learned

young, preventive health measures are probably better sustained through life. It has been suggested that a very low future risk for CVD may be achieved by low CVD risk status maintained to 50 life-years.(24) Preventive health measures applied since early life could also be beneficial for cardiovascular health in childhood itself as some influences, such as infections, already cause clinically manifest pathological alterations in the childhood period (this thesis). If these arguments hold and if there is sufficient knowledge of early life modifiable risk factors, then the next question will be: to which young population should prevention be applied and how?

Particularly if challenged by issues around interventions in early life aimed at prevention of later life cardiovascular disease, awareness of some general prevention concepts is critical.

Basically, there are two types of prevention strategies, an individual approach, targeting only high risk susceptible individuals, and a population approach, which seeks to control the determinants of incidence in the population as a whole.[25] Notably, the definition of 'high risk' in cardiovascular disease is arbitrary. Already decades ago, research in adults has shown that associations between classical risk factors such as blood pressure and blood lipid levels, and cardiovascular disease risk are graded, without thresholds or truncations in the risk distribution.(26) Later it was shown that this also holds for relations between comprehensive risk prediction scores and observed disease occurrence. This specific graded risk relation explains why in an absolute sense, most cardiovascular disease actually occurs in individuals with average levels of risk as they simply comprise the largest part of the population.

The individual or high risk approach is attractive because it tailors to individual needs, substantial individual risk reduction, and thus may result in better motivation and is likely cost-effective. However, considering the graded risk relation, high risk prevention by itself will not eradicate cardiovascular disease in the population.

The population based approach to prevention deals with managing and reducing risk factors, which are not necessarily the 'elevated' ones. This strategy aims to shift the whole distribution of risk exposure in a favorable direction. It is attractive because it addresses the population needs, more radical disease reduction. An important downside is that, except for high-risk members, the benefit in terms of individual disease risk reduction will be small and motivation is an issue.

Finally, an important shift in the thinking about prevention of cardiovascular disease is from levels of separate risk factors towards comprehensive (multivariable) risk, from specifically lowering 'elevated' risk factors to lowering risk. Based on knowledge of graded risk relations, the multivariable character of cardiovascular risk, and of effects of interventions, advocates of such approach are gaining ground. The poly-pill approach

illustrates this reasoning, in which low (prophylactic) doses of anti-hypertensive, anti-cholesterol agents, and aspirin are given regardless of the levels of individual risk factors.

Then what about a high risk approach towards prevention of cardiovascular disease in childhood? A high risk approach requires accurate risk prediction or risk stratification. In adulthood, tools for such prediction are available (Framingham Risk Score, SCORE etc), which are invariably based on combined classical risk factors.[27] However, these usually only allow for 10-year risk predictions, and are derived from research in adults, not children. To our knowledge, there are currently no tools to accurately predict in children in the general population, their risk for later life cardiovascular disease and thus to identify groups of high risk children that would justify targeted interventions. Sufficiently long follow up studies to derive at such tools are lacking to date, and we anticipate that even if they were available they would not provide accurate 50-60 year prediction rules. The reason is that although there is cardiovascular risk factor tracking, there is so much risk factor change over lifetime that it does not automatically translate to a robust tool to predict later disease.[28] Moreover, the currently established associations between commonly used surrogate endpoint measures, such as childhood vascular intimal-media thickness, and later cardiovascular events have not been fully established yet. Exceptions for risk stratification may be relatively rare diseases in childhood that evidently trigger cardiovascular disease risk substantially, such as severe renal disease, diabetes mellitus type 1, and perhaps HIV (this thesis). For such special groups of children closer attention is warranted, probably including elaborate cardiovascular diagnosis. Notably, such children are already under regular health care for their primary conditions, such that there is no need for active screening for that type of high cardiovascular risk. Even though information about genetic susceptibility for certain classical risk factors such as obesity is available, applying genetic screening to spot individuals at risk will raise questions about the accuracy of such lifetime predictions (false positives and negatives) and about cost-effectiveness. Consequently, screening for cardiovascular risk factors in general populations of children likely does not work as well, not only because of cost or resource availability issues, but also for several limitations of individual prevention mentioned above.

With regard to prevention of cardiovascular disease in early life and childhood period, it seems that the population strategy is more feasible and probably more effective for most situations. In our view, preventive measures in childhood need to particularly focus on a population approach through general health measures to shift the whole risk distribution to the left. Knowing that suboptimal nutrition leads to increased risk of future coronary artery calcification (this thesis), for example, may support direct general measures to improve childhood nutrition. Such measures could involve child rearing education, health authorities, health care, food industry, general education institutes, and other parties. Likewise, knowing that physical activity leads to better vascular properties (this thesis) may help support measures aimed at physical exercise for all children regardless of their current risk factor level. In the period from (pre)pregnancy to young adulthood, there are many more

documented risk factors that are in principle modifiable such as pre-gestational maternal overweight, (second hand) smoke exposure in and after pregnancy, breastfeeding versus bottle feeding, and factors that induce childhood overweight. As alluded to above, evidence for direct relations between such factors and manifest cardiovascular disease in later life is not final but highly likely confirmative. Notably, it is reasonable to assume that improving the lifestyle factors discussed here are beneficial not only with respect to later life cardiovascular disease, but to other chronic diseases as well, including some paediatric disorders. A big problem with a population strategy aimed at successful implementation of healthy lifestyles in adulthood is, as discussed above, that personal benefits are usually low in terms of amount of risk reduction, while the challenge of changing life-long habits is extremely difficult to motivate. In pregnancy and early childhood, such strategy can in our view be much more successful. In most health systems, pregnant women and young children are under general care and thus accessible for education of intervention. Certainly for infants, there are no habits to be unlearned. Pregnancy and young infancy are critical periods for normal growth and development. In adulthood, lack of motivation usually arises from having to sustain new lifestyles for a very long time to be effective. In pregnancy, a healthy maternal lifestyle is to be maintained at least only temporary to achieve foetal health benefit, with avoiding (second hand) smoking as an example. In infancy and later childhood, a healthy lifestyle, in principle, can be made an intricate part of regular rearing practices thus requiring little effort to sustain. Finally, although perhaps less relevant for the total population burden of cardiovascular disease, many health professionals and members of the general public have a moral preference for preventing atherosclerosis formation, primordial prevention, in the first place.

Our discussion of some of these general principles of prevention has universal relevance. However, the implications of early life prevention of later life cardiovascular disease vary substantially on a global scale.

A global health view on the study of early life influences on later life cardiovascular disease

Although cardiovascular disease is considered as a global health problem affecting all people worldwide, there are differences in current epidemiological pattern and risk factor level between high income and low-to-middle income countries,[29] which consequently may result in slightly different management strategy to achieve equity of health for all. Currently, cardiovascular disease prevalence and mortality are declining in most high-income countries, while upward trends are escalating in most low to middle income countries, which contribute significantly to the global cardiovascular disease burden. Interestingly, although the level of overall classical risk factor scores is lower in low-to-middle income countries, the likelihood of major or fatal cardiovascular events is higher, and 80% of global cardiovascular deaths now occur in these countries, even at young age.[30] This may indicate that, apart from changes in behaviour and lifestyle associated

with economic transformation, the rising cardiovascular disease burden in low-to-middle income countries also reflects unsatisfactory quality of care, which is associated with limited access, shortages of qualified health professionals, lack of reliable financing systems, and gaps in infrastructure to manage chronic diseases.[30] Economic transformations also may prolong life expectancy to the point where accumulating risk factors over the life course manifests as chronic diseases. This may lead to a more increasing prevalence of cardiovascular disease in low-to-middle income countries in the future as most of the population now comprises of young people.

In the setting of much less access to costly treatment, optimising cardiovascular disease prevention from early life onwards in the less affluent regions is perhaps the best strategy to interrupt the rising incidence and prevalence and to reduce cardiovascular morbidity and mortality. Moreover, preventive efforts are much more likely to be applicable in a large scale setting. There have been examples of wide scale efforts, particularly from developed countries, for CVD risk reduction in children and adolescents, which mainly involved more established risk factors, such as obesity prevention or limitation of smoking or tobacco exposure. However, although cardiovascular risk factors should not be different across the world, there may be some factors that play a greater role in one place than another and probably have been relatively untouched due to scarcity of studies focusing on factors that are less prevalent in the developed world. A recent study shows that although the overall risk factor score is lower in low-to-middle income countries, there are particular risk factors, such as male smoking status that is far more common and probably play a greater role in the development of cardiovascular disease in this part of the world.(29) Therefore, research to characterise early life cardiovascular disease risk factors in low-to-middle income countries is needed to plan the optimal prevention strategy which should start early in life. For example, although breastfeeding has been suggested to be associated with vascular health, it may not be a big issue in high income countries because the rate is already high. However in low-to-middle income countries, where breastfeeding rates may regionally be quite low, it becomes an important issue to be addressed, including by finding out the determinants of breastfeeding rate in these areas (this thesis). Major health problems in children residing in low-to-middle income countries, such as undernutrition, low physical activity, and infection also merit attention since they potentially contribute to later cardiovascular disease besides causing immediate problems in childhood (this thesis). Research conducted locally in low and middle income countries is very important, not only for showing effects in local exposure settings, but also for raising the awareness of local health authorities, which is indispensable for things to change. Moreover, some particular cardiovascular prevention requires (health) authorities' support. An example is the recent work in Europe showing the detrimental effects of air pollution on maternal and foetal ill-health, particularly low birth weight.[31] While regional European differences in air pollution are vast, they are even much bigger in low and middle income countries, which is why we are now engaging in NIH-funded research on that issue in Indonesia. Clearly, changes in

air-pollution exposure, at least for the most vulnerable members of society, can only be brought about by governmental powers, particularly as such changes may well clash with economical reasoning.

Essentially, which prevention strategy should be applied, either high-risk individual or population approaches, may be not very different between high and low-to-middle income countries; the population approach seems to be more applicable and effective for most situations. As an example, individual prevention involving screening of 'high risk' children is in general already quite insensible given the above mentioned reasoning and it is even unrealistic for the low-to-middle-income countries considering the cost and limited resources. In adulthood, it seems most sensible to base prevention of cardiovascular disease on combinations of both types of strategy. We argue that in childhood, the dominance should be on population approaches. The priorities in childhood prevention of cardiovascular disease are probably where there are important differences between the developed and developing world. Since the demography of low-to-middle income countries comprises mostly young people, starting prevention since early life is well worth the effort, also for its intergenerational effects. This could be done by simple measures, such as better promoting healthy lifestyle, including diet and physical activity as well as prevention of communicable diseases in childhood through hygienic measures. Nevertheless, high risk approaches could also still be meaningful for some specific childhood diseases that may act as a cardiovascular risk triggering disease as modelled by HIV infection (this thesis), which also becomes a major issue in the developing world. A concept that seems to have received general acceptance is the so called epidemiological transition, meaning that low and middle income regions in the world not only still suffer traditionally high rates of infectious disease but at the same time now also face increasing chronic disease burdens. Notably, we argue that these apparently different categories of disease might well be related (this thesis). To summarise, like in developed countries, optimal prevention probably involves combination of all possible strategies, but in low and middle income countries population approaches seem most feasible.

Conclusion

The cardiovascular system shows dynamic changes throughout life, particularly in the very first stages of development, thus providing windows of opportunity for disease prevention. Postnatal influences, including childhood nutrition, physical activity, and morbidities appear to shape cardiovascular development, which not only affects childhood cardiovascular status but also may determine future cardiovascular risk in adulthood. With regard to cardiovascular disease prevention, the population strategy to shift the whole distribution of risk factors in a favourable direction regardless the risk status seems to be the most robust approach in both early life and global health setting.

References

1. Srivastava D, Olson EN. A genetic blueprint for cardiac development. Nature. 2000;407:221-6.
2. Sedmera D, Thompson RP. Myocyte proliferation in the developing heart. Dev Dyn. 2011;240:1322-34.
3. Ribatti D, Nico B, Crivellato E. Morphological and molecular aspects of physiological vascular morphogenesis. Angiogenesis. 2009;12:101-11.
4. Aird WC. Endothelium as an organ system. Crit Care Med. 2004;32(Supplement):S271-S9.
5. Aird WC. Endothelial cell heterogeneity and atherosclerosis. Curr Atheroscler Rep. 2006;8:69-75.
6. Nakashima Y, Wight TN, Sueishi K. Early atherosclerosis in humans: role of diffuse intimal thickening and extracellular matrix proteoglycans. Cardiovasc Res. 2008;79:14-23.
7. Newman WP, 3rd, Freedman DS, Voors AW, Gard PD, Srinivasan SR, Cresanta JL, et al. Relation of serum lipoprotein levels and systolic blood pressure to early atherosclerosis. The Bogalusa Heart Study. N Engl J Med. 1986;314:138-44.
8. Barker DJ. Human growth and chronic disease: a memorial to Jim Tanner. Ann Hum Biol. 2012;39:335-41.
9. Stylianou IM, Bauer RC, Reilly MP, Rader DJ. Genetic basis of atherosclerosis: insights from mice and humans. Circulation research. 2012;110:337-55.
10. Zhao W, Liu L, Pan B, Xu Y, Zhu J, Nan C, et al. Epigenetic regulation of cardiac myofibril gene expression during heart development. Cardiovasc Toxicol. 2014.
11. Elks CE, Heude B, de Zegher F, Barton SJ, Clement K, Inskip HM, et al. Associations between genetic obesity susceptibility and early postnatal fat and lean mass: an individual participant meta-analysis. JAMA Pediatr. 2014.
12. Calza L, Manfredi R, Pocaterra D, Chiodo F. Risk of premature atherosclerosis and ischemic heart disease associated with HIV infection and antiretroviral therapy. The Journal of infection. 2008;57:16-32.
13. Loscalzo J, Handy DE. Epigenetic modifications: basic mechanisms and role in cardiovascular disease (2013 Grover Conference series). Pulm Circ. 2014;4:169-74.
14. Zhang S, Rattanatray L, McMillen IC, Suter CM, Morrison JL. Periconceptional nutrition and the early programming of a life of obesity or adversity. Prog Biophys Mol Biol. 2011;106:307-14.
15. Lie S, Sim SM, McMillen IC, Williams-Wyss O, MacLaughlin SM, Kleemann DO, et al. Maternal undernutrition around the time of conception and embryo number each impact on the abundance of key regulators of cardiac growth and metabolism in the fetal sheep heart. J Dev Orig Health Dis. 2013;4:377-90.

16. Loffredo CA, Wilson DP, Ferencz C. Maternal diabetes: an independent risk factor for major cardiovascular malformations with increased mortality of affected infants. Teratology. 2001;64:98-106.
17. Jenkins KJ, Correa A, Feinstein JA, Botto L, Britt AE, Daniels SR, et al. Noninherited risk factors and congenital cardiovascular defects: current knowledge: a scientific statement from the American Heart Association Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. Circulation. 2007;115:2995-3014.
18. Marsh CA, Cragan JD, Alverson CJ, Correa A. Case-control analysis of maternal prenatal analgesic use and cardiovascular malformations: Baltimore-Washington Infant Study. Am J Obstet Gynecol. 2014;211:404 e1-9.
19. Kuehl KS, Loffredo CA. A cluster of hypoplastic left heart malformation in Baltimore, Maryland. Pediatr Cardiol. 2006;27:25-31.
20. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of In utero and early-Life conditions on adult health and disease. The New England journal of medicine. 2008;359:61-73.
21. Raitakari OT, Juonala M, Kahonen M, Taittonen T, Miiki-Torkko N, Jarvisalo MJ, et al. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood. The Cardiovascular Risk in Young Finns Study. JAMA. 290:2277-83.
22. Geerts CC, Evans GW, Grobbee DE, Uiterwaal CS. Parental smoking and vascular damage in yound adult offspring: is early life exposure critical? The atherosclerosis risk in young adults study. Arterioscler Thromb Vasc Biol. 2008;28:2296.302.
23. Evelein AM, Geerts CC, Visseren FL, Bots ML, van der Ent CK, Grobbee DE, et al. The association between breastfeeding and the cardiovascular system in early childhood. Am J Clin Nutr. 2011;93:712-8.
24. Lloyd-Jones DM, Leip EP, Larson MG, D'Agostino RB, Beiser A, Wilson PW, et al. Prediction of lifetime risk for cardiovascular disease by risk factor burden at 50 years of age. Circulation. 2006;113:791-8.
25. Rose G. Sick individuals and sick populations. Int J Epidemiol. 1986;14:32-8.
26. Sachdeva A, Cannon CP, Deedwania PC, Labresh KA, Smith SC, Jr., Dai D, et al. Lipid levels in patients hospitalized with coronary artery disease: an analysis of 136,905 hospitalizations in Get With The Guidelines. Am Heart J. 2009;157:111-7.e2.
27. Eichler K, Puhan MA, Steurer J, Bachmann LM. Prediction of first coronary events with the Framingham score: a systematic review. Am Heart J. 2007;153:722-31, 31.e1-8.
28. Gillman MW, Daniels SR. Is universal pediatric lipid screening justified? JAMA : the journal of the American Medical Association. 2012;307:259-60.

29. Yusuf S, Rangarajan S, Teo K, Islam S, Li W, Liu L, et al. Cardiovascular risk and events in 17 low-, middle-, and high-income countries. N Engl J Med. 2014;371:818-27.
30. Kelly BB, Narula J, Fuster V. Recognizing global burden of cardiovascular disease and related chronic diseases. Mt Sinai J Med. 2012;79:632-40.
31. Pedersen M, Giorgis-Allemand L, Bernard C, Aguilera I, Andersen A-MN, Ballester F, et al. Ambient air pollution and low birthweight: a European cohort study (ESCAPE). Lancet Resp Med. 2013;1:695-704.

Summary

Samenvatting

Samenvatting

De ontwikkeling van het cardiovasculaire system behoort tot de vroegste gebeurtenissen in het menselijk leven. Zoals bij andere aspecten van humane ontwikkeling, wordt deze ontwikkeling bepaald door zowel nature (genetische factoren) als nurture (omgeving). Hoewel relatief veel bekend is over de prenatale ontwikkelingsfasen, is nog grotendeels onbekend hoe het cardiovasculaire systeem reageert op stimuli uit de omgeving na de geboorte. Dit proefschrift beschrijft in essentie hoe verschillende postnatale omgevingsfactoren de dynamiek van het cardiovasculaire systeem beïnvloeden, en wat de mogelijke invloed ervan is op het risico op cardiovasculaire ziekte in de toekomst. Het proefschrift kent twee delen: het eerste deel (hoofdstukken 2, 3 en 4) is gericht op vroege omgevingsfactoren die mogeliik het risico op cardiovasculaire ziekte in de toekomst bepalen; het tweede deel (hoofdstukken 5 tot 8) beschrijft bevindingen over de effecten van HIV infectie bij kinderen op het cardiovasculaire systeem, en toont aan hoe algemene ziekten verregaande gevolgen voor het cardiovasculaire systeem kunnen hebben, niet alleen in de kindertijd zelf, maar mogelijk ook in de latere volwassenheid.

Deel 1 begint met een beschrijving over het plannen van het geven van borstvoeding door Aziatische vrouwen (**Hoofdstuk 2**). Omdat borstvoeding de meest essentiële voeding vormt in het vroege leven en gesuggereerd is dat het vasculaire eigenschappen van kinderen beïnvloedt, beschrijven wij factoren die het plannen gedurende de zwangerschap van het geven van borstvoeding bepalen. Dat is belangrijk omdat de zwangerschap een kritische periode is voor het bewerkstelligen van kennis van en motivatie voor het borst voeden van hun baby's na de geboorte. De resultaten van ons dwarsdoorsnede onderzoek laten zien dat het plannen van langdurig borst voeden weinig te maken heeft met de leeftijd van moeder, haar scholing, aantal zwangerschappen, en juist meer gerelateerd is aan eerdere ervaring met borstvoeding, al dan niet goed geïnformeerd zijn, factoren waarop interventie gericht zou moeten worden. Bij werkende moeders verdient de lengte van verlof en het aantal werkuren aandacht.

In **Hoofdstuk 3**, beschrijven we de relatie tussen doorgemaakte ondervoeding in de kindertijd en verkalkingen in de coronair arteriën bij postmenopauzale vrouwen. In ons Nederlandse cohort werd gevonden dat ondervoeding tijdens de adolescentie gerelateerd is aan een ongeveer vier keer verhoogde kans op het hebben van verkalking in de kransslagaderen van het hart in de postmenopauzale periode. Hoewel deze studie slechts 1 mogelijk mechanisme van atherosclerotische plaque vorming betrof, suggereren de bevindingen dat kritische perioden die de kans op toekomstig hart- en vaatlijden bepalen, niet beperkt zijn tot de foetale en vroege kindertijd.

Hoofdstuk 4 betreft het verband tussen lichamelijke inspanning en vasculaire eigenschappen bij jonge kinderen. Hoewel meer lichamelijke inspanning al lang beschouwd als gunstig voor de algemene gezondheid en het risicofactoren voor hart-en vaatziekten en

sterfte vermindert bij volwassenen, is weinig bekend over de beste leeftijd om ermee te beginnen, specifiek of het al gunstige effecten heeft op het vaatstelsel van jonge kinderen. Onze bevindingen geven aan dat sport activiteit goed is voor het vaatstelsel van kinderen, het is gerelateerd aan dunnere intima media dikte rond het 5e levensjaar en het kan leiden tot soepeler vaten in de pre-puberteit, vooral als dikkere kinderen meer sporten

Deel 2 beschrijft onze bevindingen over de effecten van HIV infectie bij kinderen op de cardiovasculaire gezondheid. Eerst wordt een review gepresenteerd over de huidige kennis van dit onderwerp, en vooral over wat de ontbrekende kennis is en wat mogelijke onderzoeksgebieden zijn (**Hoofdstuk 5)**. Deze review laat zien dat alhoewel de typen van HIV-gerelateerd hartaandoeningen bij het kind grotendeels bekend zijn, het nog altijd moeilijk aan te geven is of deze aandoeningen gewoon een effect van de HIV infectie zelf zijn, of van de HIV behandeling. Ook is het grotendeels onbekend of, zoals bij volwassenen met HIV, HIV bij kinderen al een invloed heeft op risico op hart- en vaatziekten op latere leeftijd. De volgende hoofdstukken beschrijven ons werk dat gericht is op deze lacunes in de kennis, en waarin we een unieke mogelijkheid hadden om onderscheid te maken in de effecten van HIV zelf of de HIV behandeling op het vaatsysteem door niet ART behandelde versus ART behandelde kinderen te bestuderen.

In **Hoofdstuk 6**, tonen wij aan dat ART-naïeve HIV infectie geassocieerd is met dikkere intima media dikte. Hoewel de intima media dikte goed correleert met verhoogd cardiovasculair risico bij volwassenen, is meer onderzoek nodig naar de vraag of dat ook geldt voor vaatkarakteristieken gemeten op kinderleeftijd Naast de potentiele impact op toekomstig cardiovasculair risico, laten wij in **Hoofdstukken 7 en 8** zien dat HIV infectie bij kinderen gerelateerd is aan veranderingen in het elektrische geleidingssysteem van het hart, vooral het QTc interval, maar ook structurele en functionele veranderingen in het hart van kinderen. Een interessante bevinding is dat antiretrovirale behandeling deze veranderingen niet geheel lijkt te doen verdwijnen. Met betrekking tot het QTc interval hadden ART behandelde HIV geïnfecteerde kinderen zelfs meer verlenging van het interval dan de onbehandelde HIV geïnfecteerde. Wat betreft hart structuur en functie suggereren onze bevindingen dat ART onbehandelde HIV infectie gerelateerd is met verminderde systolische functie, terwijl antiretrovirale behandeling, ofwel door controleren van de HIV infectie ofwel door directe effecten van HIV op het hart, de hartfunctie lijkt te verbeteren, maar tevens dat het gerelateerd is aan een dikkere linker ventrikelwand en hogere linker ventrikel massa. Deze structurele veranderingen in ART behandelde HIV infectie bij kinderen kan duiden op een compensatie mechanisme dat onvoordelig kan zijn op de lange termijn en dat vraagt om langere follow-up.

Tot slot in **Hoofdstuk 9** (algemene discussie) bediscussiëren we onze bevindingen in het licht van cardiovasculaire preventie wat vooral belangrijk is in minder ontwikkelde landen met beperkte middelen voor curatieve behandeling. De bevindingen beschreven in dit proefschrift suggereren dan postnatale invloeden, inclusief kindervoeding, lichamelijke activiteit, en kinderziekten, de cardiovasculaire ontwikkeling lijken te beïnvloeden, niet alleen de cardiovasculaire status bij kinderen maar mogelijk ook het cardiovasculaire risico in het latere volwassen leven.

Summary

The development of the cardiovascular system is among the earliest events in human life. As with other human developmental aspects, it is determined by both nature (genetic factors) and nurture (environment). Although the prenatal developmental phases are relatively well known, how the cardiovascular system develops in response to environmental stimuli after birth is largely undiscovered. This thesis basically provides evidence on various postnatal environmental factors that may affect the dynamics of the cardiovascular system, including the potential impact on future cardiovascular disease risk. We outlined the description into two parts: the first part (chapters 2, 3, and 4) mainly focuses on early life environmental factors that may determine future cardiovascular risk; the second part (chapters 5 to 8) provides evidence about the effects of HIV infection in children on the cardiovascular system, showing how general diseases may have a profound cardiovascular involvement, which manifest in childhood and possibly potentiates cardiovascular disease in adulthood.

Part 1 begins with a description about breastfeeding planning among Asian women (**Chapter 2**). As breastfeeding is the most essential nutrition in early life and has been suggested to influence childhood vascular characteristics, we briefly describe factors determining breastfeeding planning in pregnancy. This is important because pregnancy is a critical period for establishing mother's awareness and motivation to breastfeed their babies after birth. The results of our cross sectional study showed that planning of prolonged breastfeeding has little to do with maternal age, education, or number of pregnancies and is more related to previous breastfeeding experience and whether mothers have been well informed or not, which we believe should be the main focus of intervention. Among working mothers, particular attention needs to be directed to the length of maternal leave and required working hours.

In **Chapter 3,** we describe the association between famine in childhood and coronary artery calcification in post menopausal women. In our Dutch cohort, it was found that famine in adolescence is associated with an approximately four times increased risk of having increased coronary artery calcification in the post-menopausal period. Although this study only addressed one of the possible mechanisms of the atherosclerotic plaque formation, the findings suggest that the critical period determining future development of cardiovascular diseases may extend beyond foetal life and infancy.

Chapter 4 addresses the association between physical activity level and vascular characteristics in young children. Even though higher physical activity level has long been considered to be beneficial for general health and has been confirmed to reduce cardiovascular risk factors and mortality in adults, little evidence exists for the best timing of the onset, specifically whether it already exerts beneficial effects on childhood

vasculature. Our findings suggest that sport activity seems to be beneficial for children's arteries, in which it is associated with thinner intima media thickness around 5 years of age and may lead to more distensible vasculature in prepuberty, especially for those with higher body mass index.

Part 2 elaborates our findings about the effects of HIV infection in children on cardiovascular health. We begin with presenting a review on the current evidence of this issue, highlighting knowledge gaps and potential research areas (**Chapter 5)**. The review reveals that although the type of HIV-related childhood cardiac manifestations has been largely known, it is still very difficult to delineate whether the observed manifestations are merely the effects of HIV infection itself or treatment. Moreover, although HIV infection in adults has been shown to increase cardiovascular risk, it is still largely unknown whether the same findings apply to children. The following chapters describe our work aiming to provide some evidence to fill these knowledge gaps, in which we particularly had the unique opportunity to differentiate the effects of HIV itself and HIV treatment on the heart and vasculature, by studying ART-naive versus ART-exposed HIV infection in children.

In **Chapter 6**, we show that ART-naive HIV infection is associated with thicker intima media thickness. Although the intima media thickness correlates well with increased cardiovascular risk in adults, whether this finding translates to higher cardiovascular risk imposed by childhood HIV infection warrants further investigation. Besides this potential impact on future cardiovascular risk, in **Chapters 7 and 8** we showed that HIV infection in children is associated with alterations in the cardiac conduction system, particularly the QTc interval, as well as changes in heart structure and function already manifesting in childhood. Interestingly, antiretroviral treatment does not seem to completely alleviate these observed changes. With regard to the QTc interval, even the ART-exposed HIV infected children were shown to have more prolonged QTc intervals than the ART-naive ones. In terms of cardiac function and structure, our findings suggest that ART-naive HIV infection is associated with decreased systolic function, whereas antiretroviral treatment, either by controlling HIV infection or directly affecting the heart, appears to improve cardiac function but is associated with thicker left ventricular wall and higher left ventricular mass. These structural changes in ART-exposed paediatric HIV infection may reflect a compensatory mechanism, which may be disadvantageous in the long term and therefore necessitates follow up.

Finally in **Chapter 9** (general discussion), we discuss our findings in view of cardiovascular disease prevention, which is particularly important for less developed countries with limited resources for curative measures. Findings described in this thesis suggest that postnatal influences, including childhood nutrition, physical activity, and morbidities, appear to shape cardiovascular development, which not only affects childhood cardiovascular status but also potentially determines cardiovascular risk in later adulthood.

Acknowledgment
Manuscripts based on the studies presented in this thesis
Contributing authors
Curriculum vitae

Acknowledgement

All praises to Allah, the entirely merciful, the especially merciful.

First, I would like to thank all children and guardians for their invaluable contribution in the BRAVO pilot and HIV cardiovascular studies. Also my greatest appreciation to all children and mothers involved the WHISTLER cohort and women of the PROSPECT-EPIC cohort.

Dear Professor dr. D.E. Grobbee, thanks so much for giving me the invaluable opportunity to undertake PhD program in the Julius Centre University Medical Centre Utrecht. It has been one of the best learning experience in my life. Also your strategic advice has always been very useful in guiding my PhD work and professional life.

Dear Professor dr. Cuno, I am very much obliged for all the great and intensive supervision in the past three and half years. Everybody says that doing a PhD is very tedious, which is true, but I did very much enjoy the process. It has always been a joy to work with and learn from you, not only from scientific perspective, but also to learn from your ethos, fairness, punctuality, and commitment (we almost never skipped our weekly Skype meeting for the whole 3 years!).

Professor Sudigdo, I could never thank enough for your endless guidance and support in the past 12 years. Thanks for teaching me, building up my interest in research, and encouraging me to take this PhD program. You are always be one of the best teachers in the world for me.

Dear Michael, thanks so much for your great support and all the enlightening discussions. I greatly appreciate that you are always being efficiently responsive to my inquiries despite your tight schedule. Thanks also for giving me the opportunity to join the paediatric cardiology training in your department, where I can directly learn from your sincere and very reasonable approach in managing patients.

Dear Dave, thanks so much for your innovative ideas and great contribution on the HIV project. It has been very nice to work with such a great yet humble person like you. It is a great honour that you are willing to be the guest member of my PhD committee.

Dear Professor dr. J.Th.A. Knape, thanks for your willingness to chair my PhD defense and your reassuring guidance. To the Utrecht University PhD committee members, Prof.dr. M.L. Bots, Prof.dr. R.A. Coutinho, Prof.dr. F.L.J. Visseren, Prof.dr. C.K. van der Ent, and Prof.dr. P. Reiss, thanks so much for your constructive input for my thesis.

To all my senior colleagues, dr. Sukman, dr. Najib, dr. Mulyadi, dr. Piprim, dr. Rubiana, and fellows in the cardiology department, thanks so much for you kind support and understanding. I also thank the head and all the staff of the Department of Child Health

Faculty of Medicine University of Indonesia/Cipto Mangunkusumo National General Hospital for their support.

My deepest gratitude to the nursing and outpatient clinic staff of Cipto Mangunkusumo Hospital (Ms. Ribby, Ms. Tini, Ms. Tati, Mr. Tomo, and others), Koja Hospital (dr. Riza, dr. Amar, dr. Afaf), and the Indonesian Parenthood Association HIV clinic (Edi Sugiarto, Sri Yani, and Santi Sardi) for their invaluable support for research described in this thesis.

To all colleagues in Budi Kemuliaan Health Institute (dr. Bahar, dr. Irma, dr. Lilik, dr. Maya, dr. Siti, dr. Tri, dr. Suri, all midwives), thanks so much for your continuous support for the BRAVO trial.

Dear Ary, Putri, and Cindy, thanks for being such good friends since we first came to Utrecht. I will surely miss all the cycling (including when we got lost), cooking, and chatting moments!

Dear my PhD and master fellows in UMC Utrecht (Annemieke, Jacobien, Kayode, Mary, Maurice, Mya, Henoch, Tom, Theresa, and other friends), thanks so much for the great friendship and collaboration. To Rik Hopmans, MSc, thanks so much for assisting me to work with R and becoming the problem solver when I got stuck with the syntax.

To the Indonesian community in Utrecht and the Netherlands, friends from PPI Utrecht and SGB, Bang Andy, Mbak Eha, Tante Hetty, Tante Nanda, Om Richard, Tante Umi, and other friends, thanks so much for your hospitality. It was great to have the Indonesian atmosphere that made Utrecht always feels like home.

Dear Donna, Hani, Windy, Riris, Meila, Isni, Ria, ira, Emma, Windy Cahya, Widya, thanks so much for your dedicated assistance and for being the A team for the HIV and BRAVO studies. To the deceased Weni Tenlima, your spirit and resilience always inspired us, may Allah blessed you always.

Dear Mom, thanks so much for always being the best mother and friend for me. Although you often have such enormous activities and issues to tackle, you are always there to support me. Thanks for teaching us to be a tough person, I always admire your resilience, commitment, and integrity.

Dear Papa, I could never thank enough for your sincere love and support, also for being the best grandfather for my children. Your easy going personality and relax attitude towards life always inspire and help me in dealing with all the ups and downs.

Dear my beloved brothers, Mas Sadat and Nassat, thanks so much for your countless support and the joy you have brought throughout the years. I always miss all the moments when we mingle and have chats. Also my deepest thank to my sister in law, Ani, Ratih, Bapak Yoyo, and Ibu Halimah, and all families for their great support and help.

Dear Khaidar, I am very blessed to be surrounded by your amazing love, support, and patience throughout the years. For all the good things, I thank you.

My dearest Arkan and Aisya, you both are the most precious things I have ever had. Thanks so much for always being wonderful children who brighten my days. I always feel grateful and proud for your achievement. Love you gorgeous!

"And if Allah should touch you with adversity, there is no remover of it except Him. And if He touches you with good - then He is over all things competent."

Melbourne, April 3, 2015.

Manuscripts based on the studies presented in this thesis

Idris NS, Sastroasmoro S, Hidayati F, Sapriani I, Suradi R, Grobbee DE, Uiterwaal CS. Exclusive breastfeeding plan of pregnant Southeast Asian women: what encourages them? Breastfeed Med. 2013;8(3):317-20.

Idris NS, Uiterwaal CS, van der Schouw YT, van Abeelen AF, Roseboom TJ, de Jong PA, Rutten A, Grobbee DE, Elias, SG. Famine in childhood and postmenopausal coronary artery calcification: a cohort study. BMJ open. 2013;3:e003818.

Idris NS, Evelein AM, Geerts CC, Sastroasmoro S, Grobbee DE, Uiterwaal CS. Effect of physical activity on vascular characteristics in young children. Eur J Prev Cardiol. 2014 Feb 13. [Epub ahead of print].

Idris NS, Grobbee DE, Burgner D, Cheung MM, Kurniati N, Sastroasmoro S, Uiterwaal, CS. Cardiovascular manifestations of HIV infection in children. Eur J Prev Cardiol. 2014 Nov 14. [Epub ahead of print].

Idris NS, Grobbee DE, Burgner D, Cheung MM, Kurniati N, Uiterwaal CS. Effects of paediatric HIV infection on childhood vasculature. *Submitted.*

Idris NS, Cheung MM, Grobbee DE, Burgner D, Kurniati N, Djer MM, Uiterwaal CS. Effects of paediatric HIV infection on electrical conduction of the heart. *Submitted.*

Idris NS, Cheung MM, Grobbee DE, Burgner D, Kurniati N, Uiterwaal CS. Cardiac effects of antiretroviral naive versus antiretroviral exposed HIV infection in children. *Submitted.*

Contributing authors

Annet F.M. van Abeelen, PhD
Department of Clinical Epidemiology, Academic Medical Centre Amsterdam, The Netherlands

David Burgner, MD, PhD
Department of Paediatrics, University of Melbourne; Murdoch Children Research Institute; Royal Children's Hospital, Parkville, and Department of Paediatrics, Monash University, Victoria, Australia

Michael MH Cheung, MD, MBChB
Department of Paediatrics, University of Melbourne; Murdoch Children Research Institute; Royal Children's Hospital, Parkville, Melbourne, Victoria, Australia

Mulyadi M Djer, MD, PhD
Department of Child Health, Faculty of Medicine University of Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia

Diederick E Grobbee, MD, PhD, FESC
Julius Global Health, Julius Centre for Health Sciences and Primary Care, the University Medical Centre Utrecht, Netherlands

Fatimah Hidayati, MD
Department of Child Health, Faculty of Medicine University of Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia

Pim A de Jong, MD, PhD
Department of Radiology, University Medical Centre Utrecht, The Netherlands

Nia Kurniati, MD, MSc
Department of Child Health, Faculty of Medicine University of Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia

Tessa J. Roseboom, PhD
Department of Clinical Epidemiology, Academic Medical Centre Amsterdam, The Netherlands

Annemarieke Rutten, MD, PhD
Department of Radiology, University Medical Centre Utrecht, The Netherlands

Irma Sapriani, MD
Budi Kemuliaan Health Institute, Jakarta, Indonesia

Yvonne T. van der Schouw, PhD
Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht, The Netherlands

Sudigdo Sastroasmoro, MD, PhD
Centre for Clinical Epidemiology and Evidence Based Medicine, Department of Child Health, Faculty of Medicine University of Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia

Cuno SPM Uiterwaal, MD, PhD
Julius Global Health, Julius Centre for Health Sciences and Primary Care, the University Medical Centre Utrecht, Netherlands

Curriculum vitae

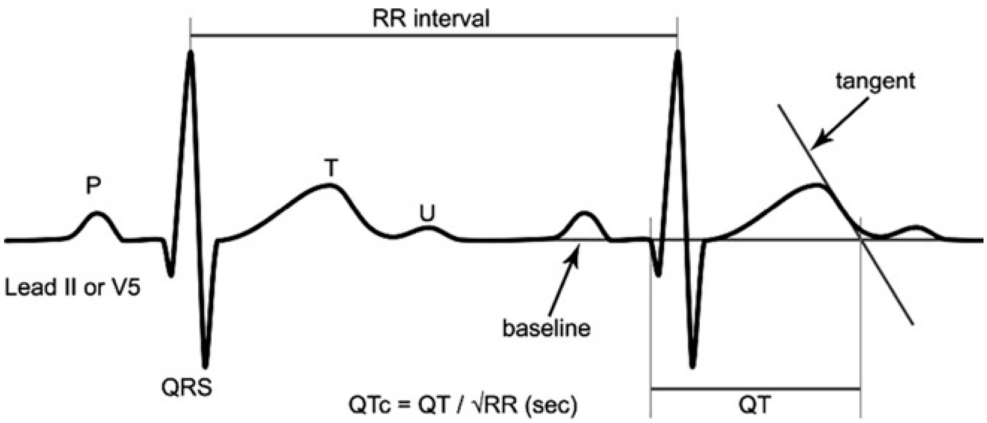
Nikmah Salamia Idris was born on October 31, 1977 in Bandung Indonesia. After graduating with cum laude from her primary medical training in the Padjadjaran University, Bandung, Indonesia in 2003, she worked for two years as the editorial secretary for 'Paediatrica Indonesiana', an official journal of Indonesian Paediatric Society. She started her paediatric clinical training in 2005. While she was a junior paediatric resident, she achieved the highest mark in the Indonesian College of Paediatrics national MCQ and completed her training with the highest mark in the national board exam in 2009. After graduated, she worked as a paediatrician staff at the Cardiology Division Department of Child Health University of Indonesia/Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia. In August 2011, she started to work on research described in this thesis under supervision of Prof. dr. Diederick E. Grobbee, PhD, FESC and Associate Professor dr. Cuno S.P.M. Uiterwaal, PhD. Together with Dr. Uiterwaal, she is also the principal investigator of the BRAVO breast feeding optimisation trial, currently running in Jakarta, Indonesia, in which she managed to randomise approximately 700 pregnant women of the total 1,000 envisioned. In the final year of her PhD, she received a scholarship from the Australia Award to undertake paediatric cardiology fellowship training in Royal Children's Hospital Melbourne Australia in conjunction with master of medicine (paediatrics) in the University of Melbourne under supervision of Associate Professor dr. Michael Cheung, MD, MBChB and Prof dr. David Burgner, PhD who collaborate for the HIV research project described in the thesis. In early 2015, she also managed to obtain a grant from the USAID/NIH (USD 420000 for 3 years) to extend the BRAVO trial for studying the effects of air pollutant exposure in early life on maternal and child health. Per August 2016, she plans to start working as a paediatric cardiology non-invasive imaging fellow in Royal Children's Hospital Melbourne, under supervision of Associate Professor dr. Michael Cheung, MD, MBChB.

APPENDICES

Appendix 1. Vascular measurement terms and equations

Term	Definition	Equation
SBP	Systolic blood pressure in brachial artery (mmHg)	
DBP	Diastolic blood pressure in brachial artery (mmHg)	
MAP	Mean arterial pressure in brachial artery (mmHg)	DBP + (SBP - DBP)/3
cIMT	Carotid intima-media thickness, end-diastolic (mm)	
$d_{systolic}$	Mean end-systolic lumen-IMT diameter (mm)	
$d_{diastolic}$	Mean end-diastolic lumen-IMT diameter (mm)	
Δd	Change in carotid diameter	$d_{systolic} - d$
A	Arterial cross-sectional area (mm^2)	
ΔA	Change in arterial cross-sectional area	$\pi/4 \times [(d + \Delta d)^2 - d^2]$
CF	Conversion factor	$(MAP - DBP)/(d_{mean} - d)$
Δp	Carotid pulse pressure	CF x Δd
DC	Distensibility coefficient (1/MPa)	$(\Delta A/A) / \Delta p = (2 \Delta d \times d + \Delta d^2) / (\Delta p \times d^2)$
EM	Elasticity, Young's modulus (kPa)	(d/IMT)/DC

Appendix 2. ECG intervals and measurement of QT interval using tangential method



Reproduced with permission from Heart Rhythm 2008;5:1015-1018